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Acromegaly : irreversible clinical consequences

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ACROMEGALY

Irreversible consequences

Moniek Wassenaar

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ACROMEGALY
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Part I.

INTRODUCTION

Chapter 1.

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

INTRODUCTION

In this thesis a number of observations is described in acromegalic patients during long-term follow-up with cured or biochemically well-controlled disease. These observations focus on persistent consequences of the disease, which actually represent irreversible effects. In these studies we evaluate the acromegalic arthropathy, bone quality and fractures, colonic abnormalities, and quality of life. In addition, we study genotype-phenotype relationships of the exon-3 deleted growth hormone receptor (d3GHR) polymorphism on long-term clinical outcome in acromegaly.

Acromegaly

An introduction to the disease

Acromegaly is a disease of exaggerated somatic growth and distorted proportions arising from hypersecretion of growth hormone (GH) and insulin-like growth factor I (IGF-I). The clinical characteristics of acromegaly were first described more than 120 years ago by Pierre Marie[1] and are caused by hypersecretion of GH by a pituitary adenoma[2;3]. If GH hypersecretion is present before closure of the growth plates, GH excess leads to a tall stature, this condition is referred to as gigantism rather than acromegaly.

Epidemiology

Acromegaly is a rare condition with a prevalence less than 70 cases per million and annual incidence of 3 to 4 cases per million[4;5]. There are no known geographical and/or sex differences.

Pathogenesis

Both hypersecretion of GH or GH-releasing hormone (GHRH) can lead to acromegaly. Pituitary GH-secreting adenomas are responsible for 98% of acromegaly. In rare conditions, GHRH secreting tumors cause secondary somatotrope hyperplasia and acromegaly. These GHRH producing tumors can arise in the central nervous system (hypothalamic hamartoma, choristoma, and ganglioneuroma) and and in peripheral organs (neuroendocrine tumors).

Familial syndromes associated with GH hypersecretion include multiple endocrine neoplasia type I[6;7], McCune-Albright syndrome[8], and Carney complex[9]. Isolated familial

acromegaly is described with loss of heterozygosity in chromosome 11q13[10] and, recently, low-penetrance germline mutations in the aryl hydrocarbon receptor-interacting protein gene were found in individuals who had familial pituitary adenoma predisposition[11;12].

Exogenous administration of GH to non-GH deficient (GHD) subjects as an athletic performance enhancer[13] or anti-aging treatment[14] has been a growing phenomenon during the last

decade, exposing GH recipients to pathologies similar to those of patients who have endogenous GH hypersecretion. This comes into mind considering the facial appearance of the current governor of California.

Anatomy

The pituitary gland lies within the sella turcica, a recess in the sphenoid bone, close to the hypothalamus and the optic chiasm *(see Figure 1)*. The pituitary weighs approximately 1 gram. It is connected to the hypothalamus consists of the adenohypophysis (80%) and the neurohypophysis (20%). The cell types in the anterior lobe of the pituitary are the somatotropes (50%) which produce GH, lactotropes (20%) which produce prolactin, corticotropes (10%) which produce adenocorticotropic hormone (ACTH), thyrotropes (10%) which produce thyroid stimulating hormone (TSH) and gonadotropes (10%) which produce follicle stimulating hormone (FSH) and luteinizing hormone (LH).

Pituitary tumors, including GH producing adenomas, are staged according to the Hardy-Wilson classification of pituitary tumors, based on the grade of sella turcica enlargement and invasion and suprasellar and parasellar extension. Most microadenomas (diameter < 1 cm) and macroadenomas (diameter > 1 cm) with suprasellar extension have a reasonable chance to be cured by surgery. Tumors invading the sellar floor of those with parasellar extension have a low chance to be cured by surgery.

Most GH producing pituitary tumors produce only GH, although mixed GH and prolactin production is present in 30% of the cases. A minority of GH producing tumors also produce TSH or α-subunits.

GH physiology

GH is a single chain polypeptide hormone that is synthesized, stored, and secreted by somatotrope cells in the pituitary gland. In plasma, GH circulated freely or is bound to GH-binding

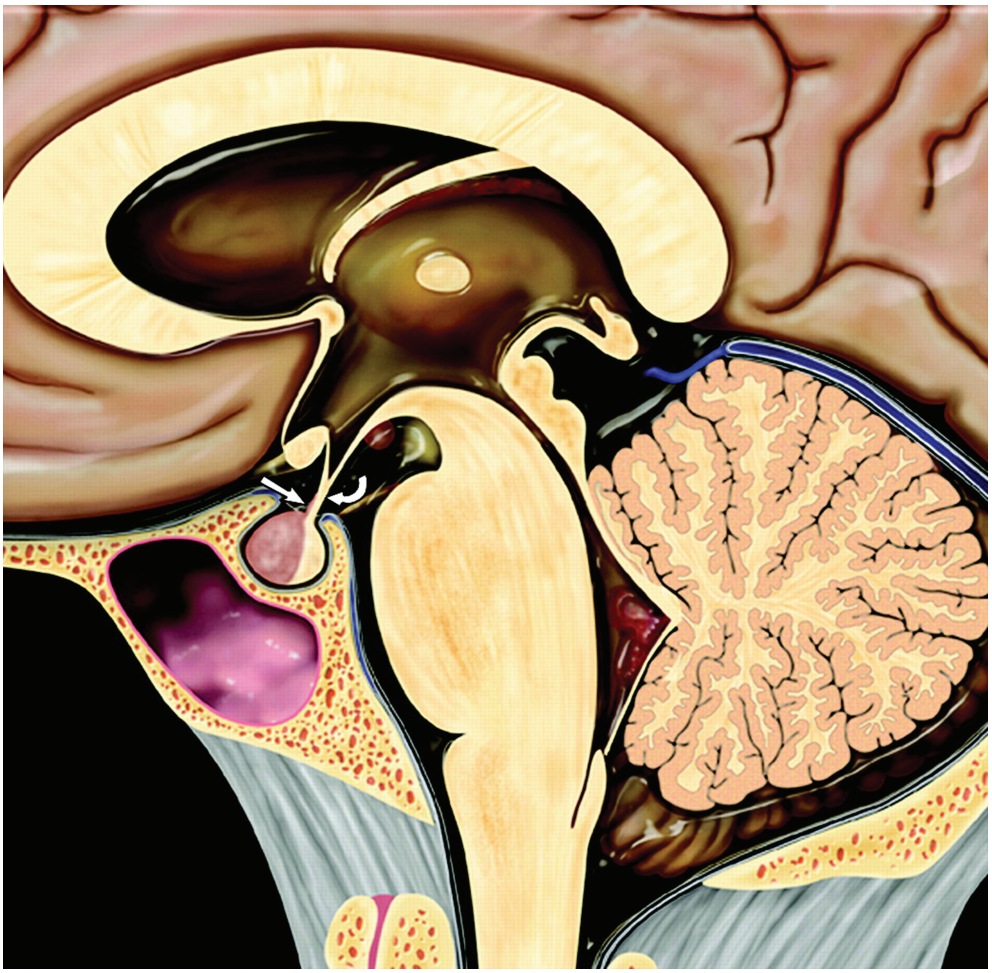


Figure 1. Localization of the pituitary gland: Head and neck digital teaching file. Salt Lake City, UT: Amirsys, 2002.

protein (GHBP). GH is cleared via renal and hepatic mechanisms.

Control of GH secretion is effectuated at the hypothalamic and the pituitary level by the interaction of stimulatory and inhibitory hormones resulting in a diurnal and pulsatile secretion pattern by which the majority of GH is released during sleep, stress and exercise[15;16]. Thyroxin, sex steroids, cortisol, amino acids and fasting also enhance GH secretion. GH secretion is inhibited by meals, glucose, free fatty acids, glucocorticoid excess states and (visceral) adiposity. GH secretion is maximal in the late puberty and thereafter gradually decreases. Women have higher GH production than men[15;17].

The hypothalamic stimulatory (GHRH) stimulates GH gene transcription, GH cell

proliferation and GH release. The hypothalamic inhibitory hormone somatostatin acts via binding to somatostatin receptors and inhibits GH release from the secretory granules in the somatotropes and also inhibits GHRH release. Other negative feedback systems regulating GH secretion are GH at the hypothalamic level, and IGF-I at the hypothalamic and pituitary level. The physiological role of Ghrelin, the native substrate for the GH-releasing peptide (GHRP) receptor, is not fully elucidated, but high GH responses are induced by GHRP or Ghrelin infusion and these act synergistically to GHRH stimuli[15;18-20] *(see Figure 2)*.

GH stimulates the production of IGF-I in many organs, especially by the liver. IGF-I is a polypeptide belonging to the same family of growth factors as insulin. Serum IGF-I concentrations reflect the GH concentrations over 24 hours and, in general, is increased and decreased, when GH concentration is increased or decreased, respectively.

The primary and most obvious clinical effect of GH is the promotion of longitudinal growth. Most effects of GH are mediated via IGF-I[17]. The anabolic actions of GH and IGF-I involve many organs systems throughout life. These actions include stimulation of protein synthesis, increased lipolysis, and inhibition of protein catabolism. GH is involved in bone remodeling, muscle growth, and immunomodulation. GH stimulates the production of IGF-I, IGFBP-3, acid-labile subunit (ALS), and many other growth factors at a local level. GH antagonizes the actions of insulin resulting in glucose intolerance and hyperinsulinemia. In contrast, IGF-I has insulin-like effects by enhancing peripheral glucose uptake.

The GHR and the exon-3 deleted GHR polymorphism

The biological action of GH is mediated by the activation of a cell-surface receptor, the GHR. Without the functional GHR, the final height of patients does not exceed 70% to 80% of normal height. There are different molecular forms of the GHR, reflecting polymorphisms of the GHR gene. Of these GHR polymorphisms, three variants of the GHR, that differ in the presence or absence of exon-3 (GHR_{fl-fl}, GHR_{fl-d3}, and GHR_{d3-d3}), are frequent among the population[21]. The function of exon-3 is unknown, although the deletion is in close proximity to the GH binding site[22]. The loss of exon-3 appears to have little effect on the receptor as GHR_{fl-d3} and GHR_{d3-d3} are stable and functional receptors with no apparent differences in binding activity or internationalization compared with $GHR_{fl-fl.}$ While either allele alone is sufficient for normal growth[23], the presence of at least one deleted allele is thought to confer an increased growth response to GH therapy and *in vitro* associated with increased responsiveness to GH[24].

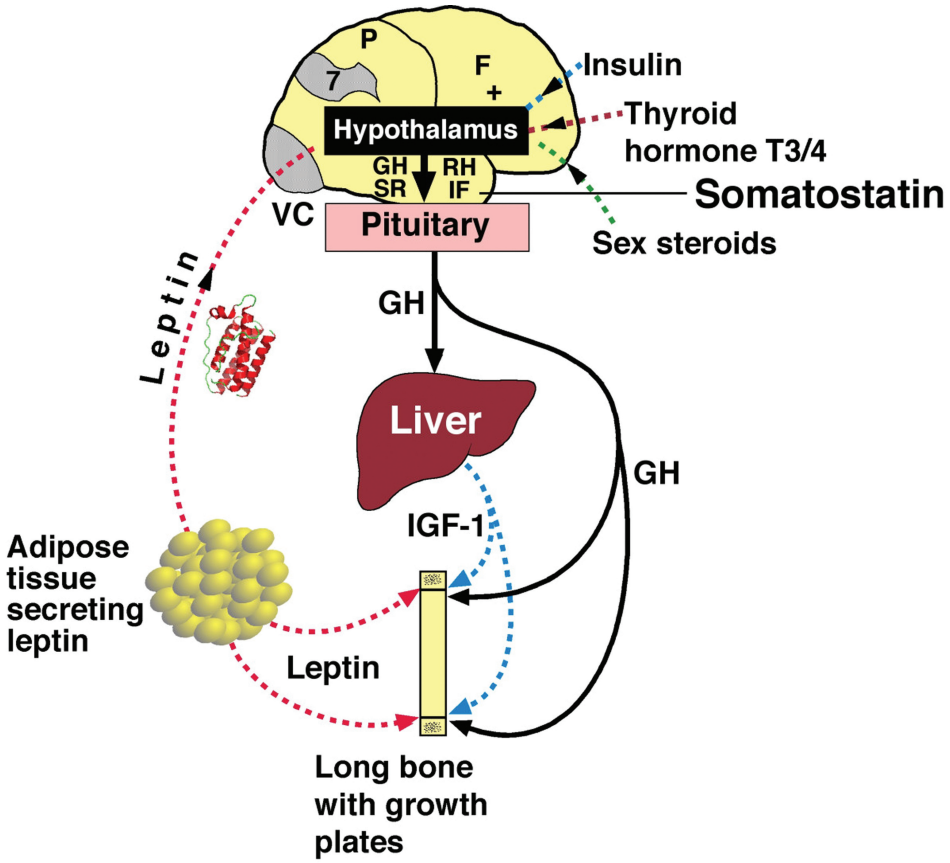


Figure 2. Regulatory mechanisms involved in the secretion of growth hormone: Burwell *et al. Scoliosis* 2009 **4**:24.

Diagnosis

Signs and symptoms

Due to the insidious clinical manifestation of GH excess, acromegaly is a disease with a typically delayed diagnosis, approximately 10 years from the onset of the symptoms[25]. Changes in appearance are the reason to seek medical care in only 13% of the acromegalyic patients[26], even though these changes account for 98% of presenting features[27].

Changes in appearance derive from skeletal growth, and soft-tissue enlargement. Facial changes include enlarged lips and nose and macroglossia which can cause excessive snoring and sleep apnea syndrome, frontal skull bossing and cranial ridges, mandibular overgrowth with prognatism, maxillary widening with teeth separation, jaw malocclusion, and overbite. Increased ring and shoe size are often reported[26].

Arthropathy of the large peripheral and axial joints is a common feature of the disease, occurring in approximately 70 to 80% of patients, even in patients with cured disease. The stature of the patient is characterized by kyphoscoliosis, and weight is increased[28]. Patients suffer often from carpal tunnel syndrome.

Skin thickening, due to accumulation of glucosaminoglycans, is noticed mainly in the face, hands, and feet. Hypertrophy of and hypersecretion by sebaceous and sweat glands result in oily and sweaty skin, respectively. Other symptoms associated with acromegaly are a low voice, tiredness, paresthesias, and hirsutism.

Hyperprolactinemia with or without galactorrhea develops in approximately 30% of patients because of pituitary stalk compression in the case of macro adenomas or mixed secretion of GH and prolactin by the pituitary adenoma[29]. Hypopituitarism ensues by mass compression of normal pituitary tissue by macro adenomas in approximately 40% of patients[30]; amenorrhea or impotence[31] or secondary thyroid[32] or adrenal failure can develop. Other local tumor effects include headache, visual field defects with typical hemianopsia, and sporadically dysfunction of cerebral nerves, especially of the trigeminal, trochlear or abducens nerves.

Important cardiovascular and metabolic manifestations of acromegaly are acromegalic cardiomyopathy, valvular abnormalities[33], hypertension[34-35], type 2 diabetes mellitus, and impaired glucose tolerance. Furthermore, acromegaly is associated with increased prevalence of malignancies, especially of the gastro-intestinal tract[36]. Patients with active acromegaly have a two- to three-fold increased mortality risk due to cardiovascular and respiratory diseases and cancer[5;37-45].

Biochemical markers

Basal plasma GH and IGF-I concentrations are elevated in most cases of acromegaly. However, high GH concentrations can also be found in healthy subjects, owing to the episodic nature of GH secretion, that can fluctuate between undetectable levels and peaks up to 100 μg/L. Therefore, a confirmatory test is required. The glucose tolerance test (GTT) is the golden standard for the diagnosis of GH excess. In healthy controls, after an oral glucose load of 75 grams, the serum GH level is suppressed to low levels. In contrast, in acromegaly, the serum GH concentration is insufficiently suppressed after glucose loading. GH measurements after an GTT are unreliable in patients who have uncontrolled diabetes mellitus or liver or renal diseases, in patients receiving estrogens, or in patients who are pregnant and during late adolescence[46]. In

our centre, the normal suppression of GH after GTT is < 1 mU/L (or < 0.38 μg/L), measured by IFMA, highly sensitive for the 22 kDa protein (Wallac, Turku, Finland).

Imaging

Pituitary MRI with contrast enhancement is most sensitive for determining a pituitary adenoma, with detection limits of tumors as small as 2 mm. MRI can also visualize tumor dimensions, invasiveness and proximity to the optic chiasm. In the rare case when the GH source is extrapituitary, CT, MRI, or both can be used to localize the ectopic source[47].

Treatment

Treatment should aim at managing the tumor mass and GH hypersecretion, to prevent morbidity and increased mortality while preserving normal pituitary function.

Surgery

Transsphenoidal selective adenomectomy is the preferred approach for treating most patients. Serum GH levels are controlled within an hour after complete removal of the GH-secreting adenoma[48]. This approach, in the hands of experienced neurosurgeons, cures the majority of patients who are harboring a well-circumscribed micro adenomas or small macro adenomas[49;50]. Cure rates are approximately 75% for micro adenomas and 50% for non-invasive macro adenomas. The long-term biochemical results of surgery are excellent in most patients with initial cure, although a minority (5-15%) develop (late) recurrence of disease. Life-long follow-up is required because of this chance for recurrence.

Peri-operative mortality is low and there is a low incidence of morbidity postoperatively, including meningitis and cerebrospinal fluid leaks (<1%), transient diabetes insipidus and (partial) hypopituitarism (<10%).

Pharmacotherapy

Somatostatin analogs

Somatostatin analogs are the first-choice pharmacotherapy for acromegalic patients, which can be used as primary or pre-operative treatment or as secondary treatment after unsuccessful surgery. Two formulas are available for treatment of acromegaly: octreotide (Sandostatin ® Novartis) and lanreotide (Somatuline ® Ipsen), which inhibit GH secretion mainly via the

somatostatin receptor subtypes 2 and 5[51]. The half-life of these drugs is increased in comparison with native somatostatin. Both somatostatin analogs are available in depot preparations, using release from microspheres (Sandostatin LAR and Somatuline SR) or an aqueous substance (Lanreotide Autogel). Depot preparations are administered every 4 weeks (Sandostatin LAR or Lanreotide Autogel). Side effects of these drugs are mostly explained by the physiological action of somatostatin. These include bile stone formation, inhibition of insulin secretion and therefore a slight deterioration in glucose tolerance in a minority of patients, and (mostly transient) abdominal pain, diarrhea and nausea.

Reduction of GH and IGF-I levels during treatment with somatostatin analogs is observed in many patients, but control of disease depends on octreotide sensitivity (determined by somatostatin subtype status of the adenoma) and baseline serum GH concentrations. In most studies ~60% of patients are well controlled by somatostatin analog treatment, when used as primary or adjuvant treatment after surgery. Tumor volume reduction of GH adenomas occurs in 20-50% of acromegalic patients during somatostatin analog treatment[52]. Medical pre-treatment before surgery of especially macroadenomas, however, does not clearly improve outcome[53].

There is no contra-indication for long-term (life-long) use of these analogs. However, this is expensive, since treatment with somatostatin analogs costs 10.000-26.000 euro per patient per year.

Growth hormone receptor antagonists

Pegvisomant (® Pfizer) is a pegylated GHR antagonist approved for treatment for acromegaly that interferes with the signaling of the GH receptor, and that inhibits subsequent IGF-I generation. Pegvisomant binds through a high affinity site 1 to one GHR dimer subunit but cannot bind through a mutated site 2 to the second GHR dimer subunit, resulting in failure to initiate subsequent GH signal transduction pathways[54]. Although GH concentrations increase due to a decreased feedback signal, IGF-I is effectively reduced in almost all patients[16]. Pegvisomant is administered via daily subcutaneous injections. Pegvisomant improves insulin sensitivity and glucose tolerance, reflected in reduced fasting serum insulin and glucose levels. Few side effects have been observed, but at present long-term safety data are lacking. A major concern with pegvisomant treatment is the possible growth of the pituitary adenoma due to disrupted feedback systems. However, with the short-term use of this drug and the application of the drug in mostly operated and irradiated patients, tumor growth has been

observed in only a very small number of patients, which may reflect more the aggressive nature of the GH producing adenoma than true side effects of pegvisomant. Another side effect is the development of transient increases of liver enzymes. Pegvisomant is very expensive and cost 28.000-115.000 euros per patient per year.

Dopamine agonists

Bromocriptine and cabergoline have been used as adjuvant therapy for acromegaly[55] and in patients with mixed GH/prolactin producing adenomas. Bromocriptine suppresses serum GH levels to less than 5 μg/L in less than 15% of patients who have acromegaly when used in high doses (up to 20 mg per day). Cabergoline is a long-acting dopamine agonist that reduces serum GH levels to less than 2 μg/L and normalizes IGF-I in approximately 30% of patients. Side effects include gastrointestinal discomfort, transient nausea and vomiting, nasal congestion, dizziness, postural hypotension, headache, and mood disorders[56]. In light of recent studies demonstrating increased incidence of valvular heart disease with high dosis of carbergoline[57;58], dopamine agonists should be prescribed with caution in this patient group already at risk for valvular disease due to the growth hormone excess.

Radiotherapy

Radiotherapy is presently reserved for patients with post-operative persistent or recurrent tumors with resistance to, or intolerance for, medical treatment, although it has been used routinely in the past to treat persistent disease. Conventional external deep X-ray therapy administered by a linear accelerator usually is given in 1.8 Gray (Gy) doses to a maximum accumulating dose of 40 to 50 Gy fractionated in at least 20 sessions. After radiotherapy, the decline in serum GH levels is delayed to ~50% within the first 2 years and 75% after 5 years[59] and dependent on the pre-radiation serum GH concentrations. Remission rates of radiotherapy seem to depend on the extent of surgical intervention (debulking) prior to radiotherapy. The incidence of hypopituitarism increased with the duration of follow-up after radiotherapy and occurs in 60% of the patients during follow-up for 10-15 years[60]. A lower incidence of hypopituitarism is potentially observed when a smaller dose of 20 Gy, instead of 40 Gy, is used[61]. Secondary intracranial tumor formation or visual impairment is not observed when the radiation dose does not exceed 45 Gy and the fractional dose is less than 2.5 Gy[62]. Secondary carcinogenesis is very rare, in the range of less than 2% in 20 years[63].

Another irradiation technique is stereotactic radiosurgery using gamma knife, which

delivers a single tumor-focused radiation fraction. Gamma knife radio-surgery requires precise delineation of the tumor target to allow exact focusing with minimal surrounding tissue exposure, especially to the optic tract. GH decline is faster than with conventional techniques. It is presently unclear whether this technique is associated with decreased incidence of hypopituitarism compared with conventional radiotherapy, but long-term studies are not available[64;65].

Definition of disease control/disease remission

From an international consensus point of view, absolute numbers are used when discussing control rather than cure. Nadir GH levels should be below 1μg/L, preferably less than 0.41μg/L, in the 2 hours after 75-g oral glucose load during the GTT[46]. Age- and gender-adjusted serum IGF-I levels should be within normal ranges. Since IGF-I has a long half-life and stable serum levels, it allows for assessment of disease activity. Circadian periodicity, nutrition, blood glucose levels, steroids, and age effect serum GH and IGF-I levels and have to be taken into account when interpreting IGF-I concentrations[66]. Mean GH concentrations < 2.5 mcg/L are used in many studies as an supportive marker of disease control.

Ideally, both GH and IGF-I values should be obtained to complement evidence for assessing disease activity; however, a discrepancy between abnormal GH levels coexisting with normal IGF-I serum levels is encountered in 30% of patients[67].

Disease recurrence

Recurrences may be re-growth of post-operatively non-detectable tumor remnants or new monoclonal cell expansions. Following surgery recurrences develop in 5 to 15% of patients, in the course of 15 years post-operatively[68]. Following radiotherapy, recurrences are rarely observed. During chronic somatostatin analog treatment, tumor growth is rarely observed.

Patients who have recurrent GH-producing adenomas usually are considered for pharmacotherapy, unless there are clear indications for second surgery.

Osteoarthritis

An introduction to the disease

Osteoarthritis is a slow progressive degeneration of articular cartilage and related changes in the underlying bone at the joint margins[28;69]. Soft-tissue structures in and around the joints are also often affected. These include the synovium that may show signs of inflammation, the surroun-

ding ligaments, which are often lax and bridging muscles which become weak[70].

Epidemiology

Osteoarthritis is the most prevalent joint disorder in the world. The prevalence of osteoarthritis in the general population increases with age and is higher in women than in men, especially among the elderly[71]. The joint groups most often affected by osteoarthritis are the hand, knees, hips, metatarsophalangeal joints, and the apophyseal joints, and intervertebral discs of the spine. The prevalence of radiological osteoarthritis differs per joint site and ranges in a random population in Rotterdam (The Netherlands) from 10% in the hips to 69% in the hands in post-menopausal women[72]. Clinical osteoarthritis is less common than radiological osteoarthritis. Disc degeneration is very common.

The articular manifestations of acromegaly have been recognized since the classical description by Marie in 1886 and are present in most patients with untreated disease[1;73;74]. Both weight and non-weight bearing joints are affected, including shoulders, wrists, knees, hips, and the spine[28;73;75;76]. The long-term effects of acromegaly on arthropathy are not known.

Pathogenesis of osteoarthritis in the general population

In human cartilage, chondrocytes are responsible for the generation of the extra-cellular cartilage matrix and the maintenance of tissue homeostasis[77]. In osteoarthritis, destruction and failure of the extra-cellular matrix takes place, as a result of imbalance in the physiochemical resisting properties of the articular cartilage and applied mechanical stress. This osteoarthritic cartilage degeneration consists of a three-step cellular reaction pattern, not necessarily in sequence. First, chondrocytes activate or deactivate their synthetic-anabolic activity. Second, chondrocytes undergo phenotypic modulation, leading to an altered gene-expression profile of the cells in the diseased tissue. Third, the chondrocytes can die or proliferate in an attempt to compensate for cell loss or in order to increase their synthetic activity[78].

Etiology

Osteoarthritis is a disease with a complex etiology. Systemic factors determine the susceptibility to the impact of local biomechanical factors in developing osteoarthritis as shown in *Figure 3*. Well-known systemic factors include age, female sex, and genetic predisposition[70]. Up till now, it is unknown whether excessive concentrations of circulating GH and/or IGF-I are included

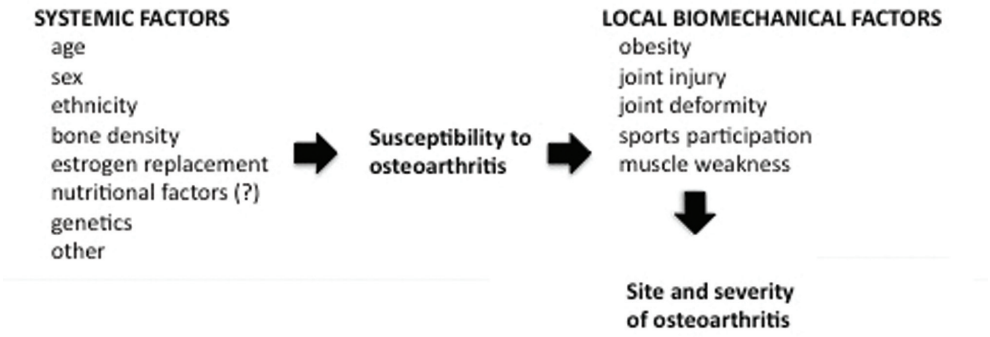


Figure 3. Pathogenesis of osteoarthritis wit putative risk factors: Felson *et al.,* Ann Intern Med 2000; 133(8): 635-46.

in the systemic factors that render patients more susceptible to osteoarthritis. Of the local biochemical factors, physically demanding occupations[79], a history of joint trauma[80], and obesity are factors most commonly associated with osteoarthritis[81].

Diagnosis

Clinically, osteoarthritis is characterized by joint pain, tenderness, limitations of movement, crepitation, and occasional effusion. The golden standard measurement is radiographic examination[82]. There are several radiographic classifications of osteoarthritis, including the classifications according to Kellgren and Lawrence[83] and OARSI[84], which are widely used. Criteria for clinical/symptomatic osteoarthritis were established by the Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology, and the major criterion for classification of osteoarthritis is joint pain on most days of the prior month in combination with structural changes, like radiographic abnormalities or the presence of bony swellings[69].

In osteoarthritis there is a poor association between clinical symptoms and radiological deformities. Since cartilage does not sense pain, cartilage degeneration itself cannot lead to pain. The origin of pain is thought to be due to stimulation of pain receptors in the synovium and surrounding tissues, such as periost, subchondral bone, entheses and tendons.

Treatment

At present, there is no medical treatment available to prevent the onset of osteoarthritis or to cure or delay the structural progression of osteoarthritis. Treatment of osteoarthritis remains

symptomatic aiming at controlling pain and maintaining or improving joint function. In case of persisting severe joint complaints, replacement surgery can be taken into consideration.

Acromegalic artropathy

Two steps are encountered in the pathogenesis of arthropathy in acromegaly. First, elevated GH and IGF-I levels promote growth of the articular cartilage and periarticular ligaments, leading to thickening of the cartilage lining and congestion of the joint space with ensuing limitation in the range of motion. Radiological changes in this phase are joint space widening and periarticular soft tissue hypertrophy. These early changes are, at least partially, reversible upon adequate disease control[73-75;85]. Second, the altered joint geometry results in repeat intra-articular trauma and exuberant reparative reactions, which leads to scar, cyst, and osteophyte formation with further deterioration of joint geometry. At this point, the disease acquires the characteristics and the features of degenerative joint disease[86;87].

OUTLINE OF THIS THESIS

Part II. Osteoarthritis and joint-related problems in acromegaly

Acromegaly is a rare disease, but associated with significant morbidity, including arhropathy. The articular manifestations have been investigated in the past, but mostly in patients with untreated or treated, but still active acromegaly[28;76;88;89]. After treatment resulting in short-term biochemical remission, reversibility without normalization of the joint complications was observed[73-75]. The consequence of long-term cure or biochemical control on joint manifestations was not studied in detail previously. Therefore, in **Chapter 2** we studied the prevalence and characteristics of arthropathy measured by standardized self-reported questionnaires, structured joint assessment, and radiographic joint examination in patients with long-term cure for acromegaly and compared the data with those obtained in controls.

Activation of the GH-IGF-I axis is a key-factor in the development of the degenerative joint disease in acromegaly, since acromegaly is associated with a very early onset of osteoarthritis. Therefore, in **Chapter 3** we aimed to identify parameters of acromegaly at the time of diagnosis associated with manifestations of secondary osteoarthritis in patients with long-term

cure of acromegaly.

The exact pathogenesis of primary osteoarthritis is unknown, but ample evidence suggests that systemic, hormonal, and genetic factors are involved. Female gender is one of the most important risk factors for primary osteoarthritis, especially after the menopause. In contrast, in acromegaly there are no known gender differences in disease prevalence or characteristics[26;90;91]. Therefore, in **Chapter 4** we studied whether the effects of gender and menopause as observed in primary osteoarthritis are also present in patients with secondary osteoarthritis, caused by acromegaly. This study was performed in order to obtain more insight both in pathophysiological processes that play a role in osteoarthritis in acromegaly in particular and in osteoarthritis in general.

Osteoarthritis in acromegaly is associated with osteophytosis but in contrast with primary osteoarthritis not with joint space narrowing *per sé*. Therefore, in **Chapter 5** we compared the radiographic appearances of osteoarthritis in patients with long-term cured acromegaly with a well-described cohort of patients with (generalized) primary osteoarthritis.

Although acromegalic arthropathy shares features with primary osteoarthritis, joint space narrowing is infrequently seen in acromegalic patients[92]. In order to gain more insight on this topic, we compared joint space width of the hand between acromegely and primary osteoarthritis in **Chapter 6**. Our aim was to assess the extend of joint space width, to identify factors associated with joint space width, and to assess the relation between these joint space width and self-reported pain.

Quality of life (QoL) in acromegaly is impaired in patients with untreated and treated disease[93-97], even after long-term biochemical remission[98-100]. Unsubtle and not joint-site specific self-reported joint-complaints predict impaired physical and mental QoL in controlled acromegaly[101]. Therefore, in **Chapter 7,** we studied the impact of joint specific complaints, radiological and clinical osteoarthritis on the different aspects of QoL in patients with long-term cured acromegaly.

Part III. Acromegaly; long-term disease outcome, not joint related

In untreated acromegaly, high circulating concentrations of GH and IGF-I are associated with an increase in bone turnover and in cortical bone mineral density (BMD), but with variable changes in trabecular BMD that have been found to be either increased or decreased[102-106]. We have previously reported normal trabecular BMD measurements in a cross-sectional study of largely eugonadal patients with a mean remission of 10 years after successful treatment of acromegaly[107]. The study in **Chapter 8** aimed to assess whether the changes observed after a mean duration of 10 years after cure of acromegaly were sustained in the longer-term by evaluating BMD 7 years after initial assessment. In addition, we assessed the incidence of vertebral and non-vertebral fractures after long-term remission of acromegely.

Colonic diverticular disease is common in Western and industrialized societies[108] and are most often found in the sigmoid and descending colon[109]. The pathophysiology of colonic diverticular disease is yet incompletely understood, but aging and dietary factors resulting in weakening of the colonic wall are thought to be contributing factors. Marfan's syndrome, a connective tissue disorder, is associated with colonic diverticular disease[110;111] and with heart valve abnormalities[112]. Acromegaly is also associated with heart valve abnormalities[113], possibly due to effects of GH, which has altered the extracellular matrix[114]. Based on these partial similarities between acromegaly and Marfan's syndrome, we hypothesized that acromegaly might be associated with increased prevalence of colonic diverticula. Therefore, in **Chapter 9** we determined whether characteristics of acromegaly, especially GH and/or IGF-I concentrations at diagnosis, were associated with colonic diverticular disease. In addition, we also assessed the prevalence of dolichocolon and colonic polyps.

Part IV. Effects of the exon-3 deleted growth hormone receptor polymorphism

The effect of the deletion of d3GHR has been investigated in several clinical conditions, some focusing on the single or double deletion of exon 3 as one genotype (GHR_{wt} *vs.* GHR_{d3}), and some regarding the single or double deletion of exon 3 as two different genotypes ($GHR_{wt\text{-}wt}$ *vs.* $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$). In children the genotype-phenotype relationships of the GHR polymorphisms can more easily be assessed by growth velocity, which is not possible in adults. Therefore, in **Chapter 10** we focused on the overall effect of the $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ geno-

type on growth in children treated with human GH in a structured meta-analysis.

We hypothesized that the GHR_{d3} genotype would render the patients more susceptible for the long-term complications of acromegaly. Therefore, in **Chapter 11** we determined whether the d3GHR polymorphism may have rendered acromegalic patients more susceptible for the long-term, irreversible effects of GH, by assessing long-term clinical outcome, including body composition, cardiovascular risk factors, the metabolic syndrome, joint-related problems and colon pathology.

Since the first publication on enhanced growth in children with the GHR_{d3} genotype in 2004, several studies assessed the effects of this polymorphism in various clinical conditions. We systematically reviewed all these studies in **Chapter 12**, in order to establish the functional consequences of the exon-3 deleted GHR polymorphism in more detail.

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Part II.

OSTEOARTHRITIS AND JOINT-RELATED PROBLEMS IN ACROMEGALY

Chapter 2.

HIGH PREVALENCE OF ARTHROPATHY, ACCORDING TO THE DEFINITIONS OF RADIOLOGICAL AND CLINICAL OSTEOARTHRITIS, IN PATIENTS WITH LONG-TERM CURE OF ACROMEGALY; A CASE-CONTROL STUDY.

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ABSTRACT

Objective: To evaluate the prevalence and rheumatologic and radiological characteristics of arthropathy in patients after long-term cure of acromegaly in comparison with age-matched controls.

Design: Case-control study.

Patients: We compared 89 patients with adequate biochemical control of acromegaly (mean 14 years) and 67 age-matched controls.

Measurements: Study parameters were the results of symptom-questionnaires, structured physical examination, and radiographs of the spine, hip, knee and hand. The diagnosis of osteoarthritis was based on a) radiological osteoarthritis determined by Kellgren and Lawrence (K&L), and b) clinical osteoarthritis determined by American College of Rheumatology (ACR) criteria. For the radiological comparison with controls a Dutch reference group was used.

Results: Pain/stiffness at ≥1 joint-site was reported by 72% of patients, most frequently of the spine and hands. Radiological osteoarthritis at ≥1 joint-site was present in 99% of patients, most frequently in the spine and hip, and increased at all joint-sites in comparison with controls (odds ratios: 2-20). Despite long-term cure of acromegaly, the characteristic widening of joint-spaces was still present. In addition, severe osteophytosis was present. Representative radiographs of these typical features are included in the manuscript. According to the ACR-criteria, clinical osteoarthritis at ≥1 joint-site was present in 63% of patients, most frequently in the spine and hand. Patients had a higher prevalence of osteoarthritis than controls at all joint-sites according to all scoring methods and at a younger age.

Conclusions: Prior growth hormone excess has irreversible, deleterious late effects on clinical and radiological aspects of joints in patients with long-term cure of acromegaly.

INTRODUCTION

The articular manifestations of acromegaly have been recognized since the classical description by Marie in 1886 and are present in most patients with active disease[1;2]. Both weight and non-weight bearing joints are affected, including shoulders, wrists, knees, hips, and the spine[1;3-5]. Two steps are encountered in the pathogenesis of arthropathy in acromegaly. First, elevated growth hormone (GH) and insulin-like growth factor (IGF-I) levels promote growth of the articular cartilage and periarticular ligaments, leading to thickening of the cartilage lining and congestion of the joint space with ensuing limitation in the range of motion. Radiological changes in this phase are joint space widening and periarticular soft tissue hypertrophy. These early changes are at least partially reversible with adequate disease control[6;1-3]. Second, the altered joint geometry results in repeat intra-articular trauma and exuberant reparative reactions which leads to scar, cyst, and osteophyte formation with further deterioration of joint geometry. At this point, the disease acquires the characteristics and the features of degenerative joint disease[7;8].

The radiological appearance of arthropathy in acromegaly was previously only studied in small non-controlled studies in patients with untreated and treated but active disease [4;5;9;10]. These studies suggested that more severe radiological abnormalities were related to biochemically more active acromegaly and longer disease duration. Limited reversibility of the joint complications was observed after treatment resulting in partial biochemical remission. The effect of short-term successful biochemical treatment with somatostatin (SMS) analogs on cartilage thickness was evaluated by ultrasonography[1-3]. Cartilage thickness decreased after treatment, but did not normalize. It is unclear, whether these findings are associated with decreased arthropathy in the long-term.

We previously reported that a high prevalence of self-reported joint complaints persisted despite successful long-term treatment of acromegaly. These joints problems were an important indicator of impaired quality of life[11-12]. However, the clinical and radiological characteristics of arthropathy in the long-term after normalization of the GH overproduction have never been studied.

The aim of the present study was to perform a structural joint assessment and to document the characteristics of arthropathy and its prevalence in long-term cured acromega-

lic patients, in comparison with controls. The joints were evaluated by different clinical and radiological scoring systems that are well-established for non-acromegalic osteoarthritis and the classification according to American College of Rheumatology (ACR) enabled comparison with age-matched controls.

PATIENTS AND METHODS

Patients

All consecutive patients with acromegaly who were referred for treatment from 1977 onwards to our centre, were collected in a database, and detailed yearly biochemical and clinical follow-up was performed from the onset of treatment. The first treatment option in the majority of patients was transsphenoidal surgery (TPS) performed by a single specialized neurosurgeon. If necessary, adjuvant treatment was given by radiotherapy (prior to 1985) or SMS analogs (from 1985 onwards). From 1998, in some patients primary treatment was given in the form of depot formulations of long-acting SMS analogs. This treatment approach resulted in early postoperative control in 66% and late control in 90% of patients[13].

Disease activity was assessed yearly by oral glucose tolerance tests (except in patients treated with SMS analogs), measurement of fasting serum GH and IGF-I concentrations, and evaluation of other pituitary functions. Remission of acromegaly was defined as a normal glucose suppressed serum GH less than 1.25 (RIA assay untill 1992) or 0.38 μg/liter (IFMA assay from 1992 onwards), serum GH levels less than 1.9 μg/liter (all years) and normal IGF-I levels for age (from 1986 onwards)[13] at yearly follow-up visits[14;15].Treatment decisions were based on these remission criteria during the entire follow-up period. Patients were all seen for follow-up by endocrinologists and not routinely by rheumatologists.

Hypopituitarism was treated promptly with thyroxine, hydrocortisone, testosterone or estrogens (in pre-menopausal women) when deficiencies were documented using appropriate basal hormone and dynamic tests[16].

Protocol

For the present study, 126 consecutive patients, selected from the database on the basis of long-term remission according to a normal IGF-I concentration for age for at least 2 years, were

invited for participation. Each patient was also asked to provide a control person of comparable age (partner, friend or neighbour) to compose a control population with a comparable socio-economic status and level of education derived from the same geographical area. Thirty-seven patients preferred not to participate in the study for various reasons such as illness, travel distance to the outpatients' clinic, lack of time or psychological reasons. A total of 89 patients (71%) and 67 controls were included. The 37 non-participating patients did not differ from the participating patients in age, gender, BMI, duration of disease, pre-treatment GH/IGF-I, type of (primary) treatment, duration of follow-up, and self-reported joint complaints based on an earlier study [12]. The study protocol was approved by the Medical Ethics Committee, and all subjects gave written consent for their participation.

Patients and controls were seen at the outpatient clinic for a single visit. Physical examination was performed by a single physician (MW) trained in structured joint assessment. All patients and controls completed standardized questionnaires (*vide infra)*. Other relevant details of treatment and patients characteristics were derived from the patient records. Conventional radiographs were obtained from the patients and in order to avoid unnecessary radiation exposure to the controls we used available radiological control data from a large epidemiological study from the Netherlands[17]. Blood samples were taken in the post absorptive state to assess actual GH and IGF-I concentrations.

Study parameters

Questionnaires

A standardized questionnaire was completed concerning demographic data, medical history and symptoms and signs of osteoarthritis. The Western Ontario and MAC Master Universities osteoarthritis Index (WOMAC), a questionnaire validated for use in osteoarthritis of the lower limb was used to assess pain, stiffness and disability of the knees and hips. Scores range from 0 to 100, with 0 representing the absence of complaints and 100 as worst score possible. WOMAC scores are presented as a mean score and as percentage of patients or controls with a score ≥ 1[18].

Physical examination and clinical scoring

Distal interphalangeal (DIP), proximal interphalangeal (PIP), metacarpophalangeal (MCP), and first carpometacarpal (CMC1) joints were examined for bony swelling, pain, deformities,

and impaired motion. Internal rotation and flexion of the hips and extension and flexion of the knees was assessed, in combination with both pain and crepitation. The spine was examined for the degree of (lateral) flexion, extension, and pain. Thoracic kyphosis was evaluated using the head-to-wall distance. For this purpose, the patient needed to stand with heels and buttocks touching the wall behind and with the knees straight. The patient was asked to touch the wall with the posterior part of the head, still keeping the chin in the normal position, the distance between the posterior part of the skull at the eye level and the wall is measured in the nearest 0.1 centimeter, using a rigid ruler [19].

The lumbar flexion index was used as range of motion of the lumbar spine. In upright position, two lines were drawn on the back of the patients, one at the level of the sacroiliac joints and the other, following the spine up 10 centimetres rostrally. The patients were asked to bend forward as far as possible and the difference between the two lines was assessed in the nearest 0.1 centimeter, using a rigid ruler [20]. Cylinder grip strength of both hands was assessed to the nearest kilogram, using a cylinder grip meter [21;22]. Other joints investigated for bony swelling, crepitation, and pain were the sternoclavicular, acromio-clavicular and glenohumeral joints, elbows, ankles, and the toes (MTP I-V).

Radiological investigation and radiological scoring

Conventional radiographs of the hands (dorso-volar), knees (Posterior-Anterior [PA] in weight-bearing / semi-flexed and lateral), hips (PA), lumbar (PA and lateral) and cervical spine (Anterior-Posterior [AP] overview, AP transbuccal and lateral) were obtained from all participating patients, following a standard manner with a fixed film-focus distance and fixed joint position. Radiographs of the knees were made in fixed flexion[23]. All radiographic examinations were performed by a single experienced radiology technician. Radiographs were scored by a single experienced musculoskeletal radiologist (HK) according to the Kellgren and Lawrence (K&L) scoring system with the help of the original atlas[24;25]. This is a four scale scoring system with increasing severity based on the presence of osteophytes, joint space narrowing, sclerosis and degenerative cysts.

The intra-reader variability for the different joint sites, scored by the K&L method, assessed by the intra-class correlation coefficient (ICC) was 0.81 for the hands, 0.89 for the knees (femorotibial), 1.00 for the hips, 0.95 for the cervical spine (intervertebral discs and apophyseal joints) and 0.88 for the lumbar spine (intervertebral discs and apophyseal joints). The

intra-reader variability was based on the examination of 10 radiographs, which were selected randomly. The radiographs were blinded for any patient characteristics.

Parameters of acromegalic disease

Disease duration was calculated from the estimated date of onset, using start of signs and symptoms, and facial changes on photographs to the date of normalization of serum IGF-I concentration after transsphenoidal surgery or additional treatment. Duration of remission was calculated from the date of normalization of serum IGF-I concentrations after treatment until start of the present study, supported by the findings during the oral glucose tolerance test. Both surgically and/or irradiation cured patients and patients with controlled disease during SMS analog treatment were collectively referred to as 'in remission'.

Biochemical parameters

Serum GH was measured with a sensitive immunofluorometric assay (IFMA) (Wallac, Turku, Finland), specific for the 22 kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit 0.01 μg/l; intra-assay coefficient of variation (CV) 1.6-8.4% of 0.01-15.38 μg/l) from 1992 onwards. For conversion of μg/l to mU/l, multiply by 2.6. Before 1992, GH was measured by RIA,(Biolab, Serona, Coissins, Switzerland) calibrated against WHO IRP 66/21(detection limit 0.5 mU/L, interassay coefficient of variation < 5%, for conversion from μg/l to mU/l, multiply by 2).

Serum IGF-I concentration (nmol/l) was measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay variation was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/l, respectively. IGF-I levels were expressed as standard deviation (SD) score, using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals [26;27].

The diagnosis of osteoarthritis

We used different definitions for osteoarthritis: 1) a radiological score 2) a combined clinical/radiological score according to the American College of Rheumatology (ACR) criteria and 3) an additional clinical ACR score for hand, hip and knee to enable comparison with our own controls (who did not have radiographs):

1) Radiological osteoarthritis was defined using the K&L scoring system. A K&L score

of ≥2 (mild osteoarthritis) reflects osteoarthritis in a particular joint-site and this was scored for the four major joint sites, the knees (including the femorotibial joints), the hips, the hands (DIP, PIP and CMC1 joints) and the spine (intervertebral discs of the cervical and lumbar spine)[24;26].

2) Using the combined clinical and radiological definition of osteoarthritis, osteoarthritis of the cervical and lumbar spine was present when there was pain and/or stiffness in the cervical or lumbar spine region on most days (>50%) of the prior month in addition to a K&L score of ≥2 in at least one intervertebral disc[28]. Osteoarthritis of the knee was defined as pain and/or stiffness for most days of the prior month and K&L score of ≥2 in the femorotibial joint. Osteoarthritis of the hip was defined as pain and/or stiffness in the groin and hip region in most days of the prior month in addition to K&L score ≥2. Hip and knee replacement surgery as a result of end stage osteoarthritis was scored as osteoarthritis in that particular joint[29;30].

3) The clinical ACR criteria for osteoarthritis of the hand were pain and/or stiffness on most days of the prior month in addition to three of the following four criteria: 1) bony swelling of ≥2 of the 10 selected joints (bilateral DIP joints 2+3, bilateral PIP joints 2+3 and CMC 1 joints), 2) bony swelling of ≥2 DIP joints, 3) <3 swollen MCP joints, and 4) deformity of ≥1 of the 10 selected joints [31]. The clinical ACR criteria for osteoarthritis of the hip were pain in combination with internal rotation of ≥15° and morning stiffness for ≤60 minutes [30]. The clinical criteria for osteoarthritis of the knee were pain, crepitation on physical examination, and morning stiffness ≤30 minutes or morning stiffness ≥30 minutes in combination with bony enlargement [29]. In the present study, age was not taken into account for both the hip and knee classification.

Statistical analysis

SPSS for Windows, version 14.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Data are presented as mean (standard error of the mean (SEM)). Osteoarthritis at each joint site was dichotomized according to the presence or absence of osteoarthritis according to the three different definitions. We used the ANCOVA analysis to compare means of the different groups, corrected for age, gender, and body mass index (BMI). In addition to the control group derived from the environment of the patients, we used available (literature) reference data for the Dutch population from a study reporting normal values for radiological osteoarthritis for specific age groups (n=4842)[17]. These normal data are based on a large epidemiological population study

and are therefore not influenced by selection bias. Binary logistic regression with adjustment for age was used to compare our patients to the literature based reference group.

RESULTS

Patient and treatment characteristics *(Table 1)*

We studied 89 patients and 67 controls with a mean age of 58.3 yrs. All patients were in remission for acromegaly or had controlled disease with SMS analogs for a mean of 14.0 years (range 2-28) after (multimodality) treatment.

Table 1. Clinical characteristics

	Patients n=89	Controls n=67	P-value
Gender (n(%))			0.22
Males	46 (52 %)	28 (42 %)	
Females	43 (48 %)	39 (58 %)	
Age (yr)	58.3 (1.3)	58.2 (1.5)	0.97
BMI (kg/m^2)	28.5 (0.5)	26.2 (0.5)	0.01
Height (meters)	1.76 (1.1)	1.71 (1.1)	0.01
Weight (kilograms)	88.4 (1.9)	76.8 (1.6)	0.00
Waist /Hip ratio	1.1 (0.02)	1.1 (0.01)	0.12
Treatment (n(%)):			
Surgery	48 (54 %)	NA	-
Radiotherapy	19 (21 %)	NA	-
Primary SMS analogs	22 (25 %)	NA	-
Disease duration (yrs)	8.9 (0.8)	NA	-
Duration of remission (yrs)	14.1 (0.8)	NA	-
Fasting GH (ug/l) (median (range))	1.01 (0.08-8.71)	NA	-
IGF-I SD scores	0.58 (0.2)	NA	-
Hypopituitarism (n(%))			
any	29 (33 %)	NA	-
TSH	18 (20 %)	NA	-
LH/FSH*	48 (54 %)	NA	-
ACTH	22 (25 %)	NA	-

Data are shown as mean (SEM), unless mentioned otherwise. *LH/FSH deficiency: includes natural menopause and hypogonadotropic hypogonadism. SEM: standard error of the mean, SD: standard deviation, yrs: years, BMI: body mass index, SMS: somatostatin (analogs).

Twenty-two (25%) patients had received primary SMS analog treatment of whom 18 (20%) in combination with TPS. Twenty patients (23%) received ongoing SMS analog treatment. The mean estimated duration of active disease prior to diagnosis was 8.9 (0.8) years and the mean duration of follow-up since diagnosis was 18.5 (0.8) years. At the time of evaluation, mean serum GH concentrations were 2.37 ± 0.4 μg/l and mean IGF-I SD scores were 0.58 (0.2) SD, reflecting adequate disease control. One or more pituitary hormone deficiencies were present in 33% of the patients. Ninety-five percent of patients were Caucasians and 19% of patients had a positive family history for osteoarthritis, which was comparable to the control population.

Self-reported complaints

On the standardized questionnaire patients reported more pain and/or stiffness than controls at all joint sites, also when corrected for age, sex, and BMI. Pain and/or stiffness were reported for the cervical spine in 61% of patients *vs*. 41% of controls (p=0.01), for the lumbar spine 63% *vs*. 32% (p<0.001), for the hips 58% *vs*. 31% (p<0.001), for the knees 53% *vs*. 24% (p<0.001), and for one or both hands in 81% *vs*. 54% (p<0.001). Only 2 patients were on current non-steroidal anti-inflammatory drugs (NSAID) therapy.

According to the total WOMAC score (≥1), pain, stiffness, or physical impairments of the hips and knees were reported by 81% of patients and by 58% of controls (p<0.001). Mean total WOMAC scores were 17.2 (2.1) in patients and 7.6 (1.8) in controls, respectively (p<0.001).

Self-reported complaints of pain and/or stiffness were not significantly different between younger (<45 yrs) and older (≥65 yrs) acromegalic patients for the spine, hip, and knee, also when corrected for sex and BMI. A remarkable finding was that the younger patients suffered much more from pain and/or stiffness of their hand joints than older patients (100% *vs*. 73%, respectively, p<0.001).

Structured physical examination

The head-to-wall distance was increased in patients compared with controls (2.5 (0.3) *vs*. 1.2 (0.3), p=0.003), indicating increased thoracic kyphosis. Head-to-wall distance was not associated with reported symptoms, like complaints of pain/stiffness. Patients tended to have a lower lumbar flexion index. Bony swelling of the hands, knees, shoulders, elbows, and toes, but not

ankles, was much more frequent in patients compared with controls (*Table 2*), but appeared not necessarily in combination with pain or crepitation.

Table 2: Characteristics on physical examination

	Patients n=89	Controls n=67	Difference (95% CI)	P-value
Head-wall distance (cm)	2.5 (0.3)	1.2 (0.3)	1.4 (0.47 – 2.22)	0.003
Lumbar Flexion Index (cm)	4.0 (0.1)	4.3 (0.1)	-0.37 (-0.72 – -0.02)	0.04
Cylinder grip strength (kg)	35.1 (1.7)	31.9 (1.5)	2.7 (-2.03 – 7.48)	0.26
Bony swellings (n(%))				
Hand	58 (65 %)	23 (35 %)	2.9 (2.0 – 3.8)	< 0.001
Knee	17 (19 %)	3 (5 %)	0.15 (0.04 – 0.26)	0.007
Shoulder	26 (29 %)	1 (2 %)	0.20 (0.10 – 0.30)	< 0.001
Elbow	5 (6 %)	1 (2 %)	0.09 (0.01 – 0.11)	0.04
Ankle	14 (15 %)	7 (10 %)	0.05 (-0.07 – 0.15)	0.35
Big toe (MTP1)	38 (43 %)	7 (10 %)	0.33 (0.19 – 0.46)	< 0.001

Data are shown as mean (SEM). All data were corrected for age, sex and BMI using ANCOVA analysis. SEM: standard error of the mean, CI: confidence interval, cm: centimeter, kg: kilogram, BMI: body mass index.

Radiological osteoarthritis

Patients showed significantly more radiological osteoarthritis at all joint sites when compared with controls after adjusting for age and sex (*Table 3*).

Table 3. Prevalence of radiological osteoarthritis in acromegalic patients compared with a literature based reference group.

	Patients n=89 N (%)	Controls n=4842 N (%)	Odds	95% CI-interval
Cervical spine	82 (92 %)	1921 (40 %)	10.5	4.9-22.1
Lumbar spine	78 (88 %)	1600 (33 %)	20.0	7.2-5.5
DIP	50 (56 %)	1375 (28 %)	1.7	1.1-2.8
PIP	38 (43 %)	482 (10 %)	4.2	2.6-6.8
CMC1	54 (61 %)	684 (14 %)	7.2	4.3-11.4
Hip right	27 (30 %)	177 (4 %)	6.9	4.1-11.5
Hip left	25 (28 %)	155 (3 %)	6.8	4.0-11.4
Knee right	34 (38 %)	551 (11 %)	2.3	1.5-3.8
Knee left	30 (34 %)	537 (11 %)	2.1	1.3-3.3

Data are shown as n(%). All data were corrected for age and sex using ANCOVA analysis. Controls were reference category. DIP: Distal interphalangeal, PIP:proximal interphalangeal, MCP: metacarpophalangeal, CMC1: first carpo-metacarpal, CI: confidence interval.

Figure 1 shows the effect of age on osteoarthritis in patients and controls. Patients showed more osteoarthritis at younger age when compared with controls. Radiological osteoarthritis of the apophyseal joints of the cervical spine was prevalent in almost 100% of patients at the age of 40.

Severe osteophytosis without joint-space narrowing was a typical radiographic characteristic of osteoarthritis seen at all joint sites in patients with long-cured acromegaly. Typical examples are shown in *Figures 2 to 5*.

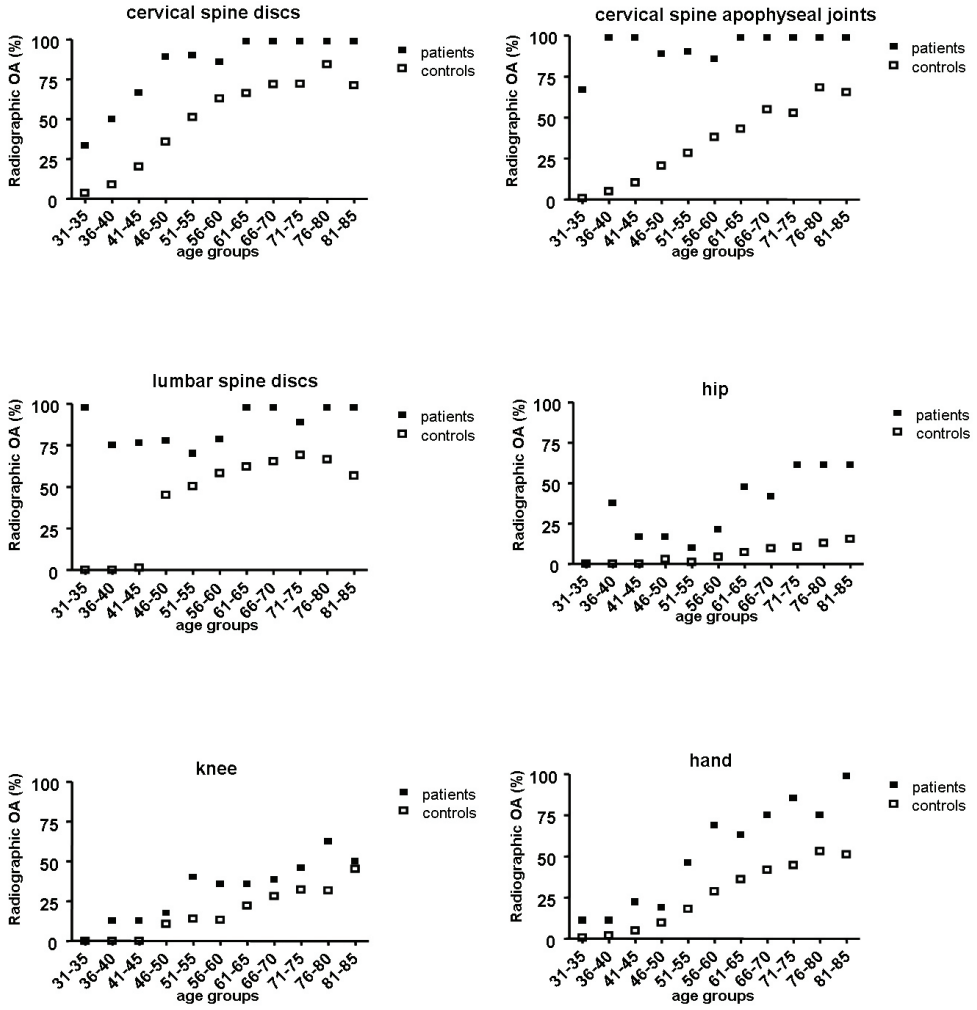


Figure 1. Prevalence of radiological osteoarthritis in relation to age in patients (n=89), compared with reference data for the Dutch population from a study reporting normal values for radiological osteoarthritis for specific age groups (n=4842) [15].



Figure 2. Radiological osteoarthritis of the spine was characterized by osteophytes at the anterior parts of the cervical and lumbar vertebrae which evenly affected the discs and the apophyseal joints. The lower vertebrae were more affected than the upper vertebrae. In this radiograph an enlargement of the Sella Turcica and gross osteophytosis in combination with intervertebral disc space narrowing is prominent. The discs are affected slightly more than the facet joints.



Figure 3. Most patients showed gross acetabular osteophytes but had wide joint spaces, indicative for retained or hypertrophied cartilage (right hip in this radiograph). A minority of patients showed joint space narrowing indicating cartilage loss accompanied by irregularity and sclerosis of subchondral bone (left hip in this radiograph).

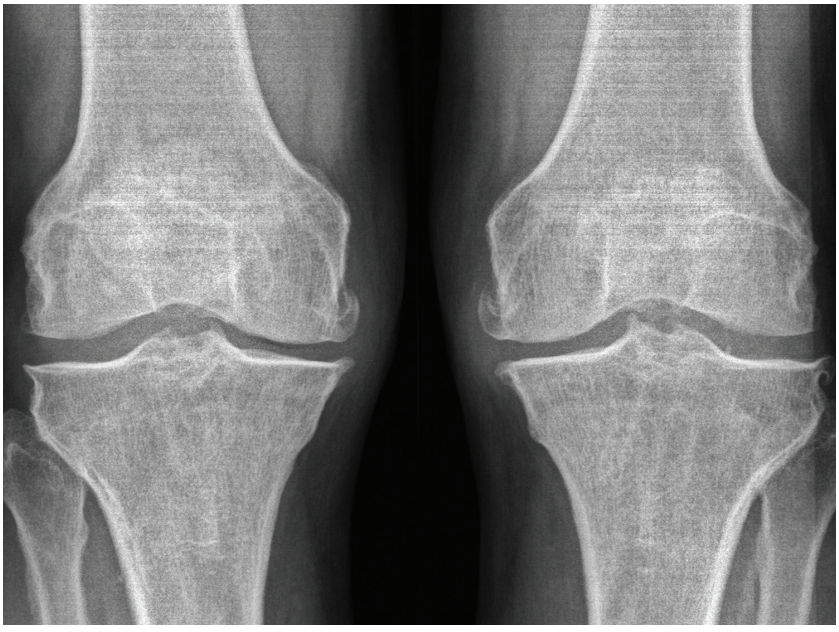


Figure 4. In acromegalic patients, unusually wide joint spaces were common, indicating thickened cartilage, both between the femorotibial and in the patellofemoral joint. The extremely wide joint spaces were combined with severe osteophytosis.



Figure 5. The main feature of osteoarthritis of the hands in acromegalic patients was the heavy tufting of the phalanges, with the terminal phalanges being spade-like in shape. Other features included degenerative changes in the metacarpophalangeal joints, such as subchondral sclerosis and osteophytosis with preservation of the joint space resulting from increased cartilage thickness. Thus, this appearance known from active acromegaly persists even during long-term follow-up.

Clinical osteoarthritis

1) Combined clinical/radiological ACR criteria

There was a high prevalence of spine osteoarthritis (62%), which was already present at young age. The prevalence of hip and knee osteoarthritis was 24% and 26%, respectively (data not shown).

2) Clinical ACR criteria and comparison with controls

Forty-one percent of the patients had clinical hand osteoarthritis. Clinical osteoarthritis was present in 15% of patients at the knee and 18% of patients at the hip. Patients demonstrated significantly more clinical osteoarthritis than controls of the hip, knee, and hand, shown in *Table 4*.

Table 4. Osteoarthritis based on clinical ACR criteria in patients and controls

	Patients n=89	Controls n=67	Difference (95% CI)	P-value
Hip	16 (18 %)	0 (0)	0.19 (0.09 – 0.29)	< 0.001
Knee	13 (15 %)	3 (5 %)	0.11 (0.01 – 0.22)	0.04
Hand	36 (41 %)	11 (16 %)	0.26 (0.12 – 0.41)	0.001

Data are shown as n(%). All data were corrected for age, BMI and sex using ANCOVA analysis. CI: confidence interval.

DISCUSSION

The late effects of acromegaly on arthropathy are striking, also after long-term cure of GH overproduction. This is the first study with a structural clinical and radiological assessment of joint-sites in patients with remission for a mean of 14 years after multimodality treatment for acromegaly. We found evidence for radiological osteoarthritis in at least one joint site in virtually all patients and clinical osteoarthritis according to the ACR criteria in two-third of patients and the prevalence was considerably increased compared with controls. The most prevalent manifestation in our study was axial osteoarthritis, affecting the cervical and lumbar areas, and even at remarkably young ages. The characteristical radiological changes observed in these patients with cured acromegaly consist of the combination of wide joint spaces and severe osteophytosis, which have not been described before.

Previously, acromegalic arthropathy was mainly addressed in patients with either active disease, using various descriptive radiological and clinical definitions and non-structured assessments, reviewed in *Table 5* [4;5;10;32;33] The findings with respect to the impact of reduction of serum GH levels on clinical and radiological arthropathy in previous studies are conflicting. In addition, most of these studies included a short duration of follow-up and included active patients or treated patients that were not cured according to present criteria for cure[1-5;9;10].

Another new and remarkable finding in our study is the preservation of the extremely wide joint spaces, which is a well-known classical characteristic of active acromegaly, in many patients despite long-term remission. These wide joint spaces are indicative of the persistence of thickened cartilage and occurred in combination with severe osteophytes. It is of importance to remark that the K&L score may have underestimated the severity of osteoarthritis in acromegaly due to the presence of these wide joint spaces, because for a K&L score > 2 joint space

narrowing is an obligatory criterion[24]. Thus, there is a discrepancy between the severity of osteophytosis and the lack of joint space narrowing. This observation suggests that also after long-term remission acromegalic arthropathy is different from osteoarthritis. On the other hand, the relation between clinical complaints, radiological osteoarthritis and combined clinical and radiological osteoarthritis in acromegalic patients were comparable with controls in most joints, except for the spine. Remarkably, we observed a discrepancy between the relatively low prevalence of complaints of pain and/or stiffness of the spine (60%) and the high prevalence of osteoarthritis by both physical and radiological examination. Apparently, self reported complaints underestimate the extend of radiological osteoarthritis, even though joint related problems were a main determinant for impaired quality of life in these patients with long term cure of acromegaly [12].

The assumption is that persistent exposure of the tissues to pathologically elevated GH and IGF-I levels induces progressive changes in the joints. Cartilage proliferation occurs unevenly and produces a thickened but mechanically unstable joint surface[1;8]. Laxity of periarticular ligaments and muscle weakness lead to joint instability[8]. Cartilage ulceration over the weight-bearing surfaces occurs and the reparative process deposits excessive amounts of fibrocartilage over the damaged areas. This is followed by development of osteophytes, formation of subchondral cysts and joint space narrowing[8]. Eventually, and after this point independently of the actual levels of GH and IGF-I, the pathophysiological process becomes irreversible and self-perpetuating, with more mechanical trauma causing additional joint deformity that, in turn, leads to yet more structural damage to the articular tissues. Only the early stages in acromegalic arthropathy may be partially (and possibly temporarily) reversible by therapy[1-3]. As soon as significant degenerative changes occur in the affected joints, normalization of GH and IGF-I can stop the continuing cell proliferation, but will not interrupt the vicious cycle of the already altered joint geometry, trauma, scarring, osteophyte formation and further disfigurement of the joint surfaces. Our results support the hypothesis that the second step in the pathogenesis of osteoarthritis in acromegaly is independent of GH and IGF-I.

Some limitations in this study have to be addressed. First, some patients received analgesic and non-steroid anti-inflammatory drugs (NSAID) therapy and/or physiotherapy, and almost 25% of patients received SMS analogs which may have analgesic properties, all these factors might have lessened their joint complaints. However, the clinical course and outcome of osteoarthritis is, as far as we know, not influenced by any type of treatment.

Table 5. Overview of the literature on acromegalic arthropathy

Authors	N	Disease status	Methods	Results/discussion
Bluestone *et al.* 1971	42 pt	42 de novo patients	Clinical records; radiographs	No correlation between joint abnormalities, pain and disease duration/ severity. Back movements maintained despite abnormalities. Effective treatment did not reduce joint problems. No influence of age or disease activity.
Dons *et al.* 1988	90 pt	43 active acromegaly 47 follow-up 5 yrs after RT	Clinical records; radiographs	76% arthropathy Higher baseline GH levels were associated with more severe arthralgias. Cartilage overgrowth predisposes for arthropathy. Progression of arthropathy was independent of fall in GH post-treatment.
Layton *et al.* 1988	19 pt	19 active acromegaly	Treatment by surgery and SMS; clinical examination; radiographs; 2-12 mnt re-assessment intervals	GH reduction: 50% improvement in physical ability, but structural improvement unclear. Radiographs: large joints were affected most. More arthropathy in elderly patients with longer disease duration.
Ezzat *et al.* 1994	500 pt	114 de novo acromegaly 386 not well-controlled	Scoring of most predominant complaints	45% arthropathy
Colao *et al.* 1998	30 pt 18 co	18 active acromegaly 12 cured (2 yrs)	6 months of treatment with SMS; Ultra-sonography; Cartilage thickness: shoulders, wrist and knees	Pre-treatment Cartilage thicker in active patients than in controls. wrist and knee cartilage thicker in active patients than in cured patients Post-treatment: SMS decreased cartilage thickness, no normalization. No difference in cartilage thickness for active and cured patients.
Colao *et al.* 1999	12 pt	12 de novo acromegaly	12 months of treatment with SMS; clinical score for joint pain; ultra-sonography	Decrease in cartilage improved complaints. No normalization of cartilage thickness. No effect of SMS between cured and uncured patients. No effect of disease duration/pre-treatment cartilage thickness.

Colao *et al.* 2003	60 pt 30 co	30 de novo acromegaly 30 cured (3.6 yrs)	12 months of treatment with SMS (in 30 de novo patients); ultrasonography	Pre-treatment: Cartilage thicker in active than in cured patients. Cartilage thickness less in disease duration >10yrs than in <10yrs. Post-treatment: Reduction in cartilage at all joint sites and more reduction in cured disease. No difference for disease duration on cartilage.
Scarpa *et al.* 2004	54 pt 54 co	54 active acromegaly	medical history; radiographs of hip and spine	52% arthropathy in patients, 22% in controls. 68% spinal abnormalities in pts; 28% in controls. More spinal abnormalities with longer disease duration.
Biermasz *et al.* 2005	118 pt	118 long time cured	Self-reported morbidity questionnaire	After long-term remission: 77% arthropathy. Arthropathy not correlated with preoperative GH/IGF-I, disease duration or age. Great impact on QOL.
Miller *et al.* 2008	58 pt	47 cured (duration not mentioned) 11 active acromegaly	QOL questionnaires; Clinical examination	90% self-reported musculoskeletal pain. Arthropathy in ~50%. Impact on QOL.
Current study	89 pt 67 co	89 long-term cured	Questionairs Clinical complaints; rheumatologic examination; radiographs of all joints	99% radiological osteoarthritis. 66% clinical osteoarthritis. Spine and hip most affected. All joint sites in patients significantly more affected with osteoarthritis than in controls. No age difference for spine osteoarthritis in patients.

Pt: patients; co: controls; SMS: somatostatin analogs; QOL: quality of life.

Therefore, the optimal management of acromegalic arthropathy requires further study. In addition, the choice of the control populations is subject to debate. None of the previous studies used control data. Since osteoarthritis is highly prevalent in the general population and age and sex dependent, the use of a control population helps to put the findings in acromegaly into perspective. Although a positive selection bias might present, due to selection of the controls by the patients, this own control cohort provides prevalence data on clinical osteoarthritis of structured joint assessment performed by the same physician. It is of note that the controls had a lower BMI than the patients, and therefore all analysis were adjusted for BMI. Moreover, the

reported prevalences in the control cohort are comparable to other epidemiological studies. Finally, we used the available radiological data from a large Dutch historical reference cohort, since in this cohort a selection bias is unlikely and because of the large group numbers.

In conclusion, our data demonstrate that many patients successfully treated for acromegaly by surgery and/or multimodality therapy and in whom remission was sustained for a mean of 14 years, suffer from extensive osteoarthritis, which is according to clinical and radiological scoring methods, manifest at all joint sites.

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Chapter 3.

PRE-TREATMENT IGF-I CONCENTRATIONS PREDICT RADIOGRAPHIC OSTEOARTHRITIS IN ACROMEGALIC PATIENTS WITH LONG-TERM CURED DISEASE.

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ABSTRACT

Objective: To identify factors influencing the development of osteoarthritis during long-term control of acromegaly, focusing on disease specific parameters, growth hormone (GH) and insulin-like growth factor I (IGF-I) concentrations and duration of disease, adjusted for the well-known determinants of primary osteoarthritis.

Design: Follow-up study.

Methods: We studied 67 patients, with adequate biochemical control of acromegaly for a mean of almost 13 years. Study parameters were the results of radiological assessment of the spine, hip, knee, and hand. Osteoarthritis was defined as radiological osteoarthritis using the scoring system developed by Kellgren and Lawrence (K&L). Correlations between potential factors of influence and osteoarthritis were performed by analysis of covariance and adjusted for age, gender and body mass index (BMI).

Results: Patients with pre-treatment IGF-I standard deviation (SD) scores in the highest tertile had an almost four-fold increased risk for radiological osteoarthritis of the hip when compared with patients in the lowest tertile. After adjustment for age, gender, BMI, and disease duration, pre-treatment IGF-I SD scores predicted radiographic osteoarthritis in all joint sites. Osteoarthritis was not predicted by other factors including pre-treatment GH levels, type of treatment, and duration of follow-up.

Conclusion: This is the first study to document pre-treatment IGF-I concentration as a predictor of radiographic osteoarthritis in acromegalic patients with long-term disease control.

INTRODUCTION

Acromegaly is a chronic disease with insidious onset caused by hypersecretion of growth hormone (GH) from a pituitary adenoma[1-7]. Joint disease is highly prevalent in active acromegaly and is characterized by wide joint spaces due to cartilage hypertrophy in combination with osteophyte formation[4;8;9]. Moreover, after long-term cure of acromegaly joint related complaints were reported by almost 80% of patients[10], and it is assumed that these complaints are based on degenerative joint disease[4-11].

In the general population, degenerative joint disease, like primary osteoarthritis, is a common disease with an estimated prevalence up to 30 to 78% for different joint groups in subjects above 70 years of age[12]. Although the pathogenesis of osteoarthritis is not completely elucidated, it is likely to be a multifactorial disease. Age, gender, body mass index (BMI), genetic factors and biochemical factors, including occupational factors, play a more or less prominent role in the development of primary osteoarthritis[13]. The effects of risk factors may differ between various joint sites. For example, BMI is most strongly associated with knee osteoarthritis and genetic factors with hip and hand osteoarthritis. Hormonal factors may also play a role, but the exact mechanism is not quite clear[13].

It is likely that activation of the GH-IGF-I axis is a key-factor in the development of the degenerative joint disease in acromegaly, since acromegaly is associated with a very early onset of arthropathy[4-10]. However, previous studies with a relatively short period of follow-up during adequate control of acromegaly did not identify disease specific factors predicting arthropathy [4;9;28-30]. Therefore, the aim of the present study was to identify factors influencing the development of degenerative joint disease in patients during long term control of acromegaly, focusing on disease specific parameters, especially on IGF-I and GH concentrations and duration of active disease adjusting for the known predictors of primary osteoarthritis.

PATIENTS AND METHODS

Patients

For the present study, 126 consecutive patients in long-term remission were invited to par-

ticipate. Thirty-seven patients (29%) preferred not to participate in the study for various reasons, including co-morbidities, long travel distance to the outpatient clinic, lack of time, or no willingness to participate because of psychological reasons. Another 22 patients diagnosed before 1986, prior to the introduction of the IGF-I assay were excluded for the present analysis because no IGF-I level at diagnosis was available. Thus, 67 patients were included in the present analysis. The 59 non-included patients were not different from the study population in age, gender, body mass index (BMI), duration of disease, pre-treatment GH/IGF-I, type of primary treatment, duration of follow-up, and self-reported joint complaints, derived from an earlier study[10].

Detailed yearly biochemical and clinical follow-up had been performed from the onset of treatment of acromegaly. The first treatment option in the majority of patients was transsphenoidal surgery performed by a single specialist neurosurgeon. If necessary, adjuvant treatment was given by radiotherapy (prior to 1985) or predominantly somatostatin (SMS) analogs (from 1985 onwards). From 1998, in some patients primary treatment was given in the form of depot formulations of long-acting SMS analogs. Since 2003, pegvisomant is available for treatment-resistant acromegaly.

Disease activity was assessed yearly by oral glucose tolerance tests (except in medically treated patients), measurement of serum GH/IGF-I concentrations, and evaluation of other pituitary functions. Remission of acromegaly was defined as a normal glucose suppressed serum GH less than 0.38 μg/liter, serum GH levels less than 1.9 μg/liter and normal IGF-I levels for age[14]. Patients not meeting these criteria were offered additional treatment.

Eugonadism was defined as normal testosterone concentration in men and by the presence of a normal menstrual cycle at pre-operative and all post-operative evaluations in premenopausal women. Hypogonadism was defined by a testosterone concentration below 8 nmol/l in males, and in females by a low serum oestradiol concentration of less than 70 nmol/L or by the absence of a normal menstrual cycle in premenopausal women. For the purpose of this study, no distinction was made between hypergonadotrophic or hypogonadotrophic hypogonadism in women. Adequately treated hypogonadism was defined as gonadal hormone replacement therapy instituted within a year of onset of hypogonadism, and these patients were not considered hypogonadal in terms of this study. Thyroid stimulating hormone (TSH) deficiency was defined as a thyroxine (free T4) level below the reference range (absolute value <10 pmol/L). Adrenocorticotrophic hormone (ACTH) deficiency was defined as an insuf-

ficient increase in cortisol levels (absolute value <0.55 umol/l) after a corticotrophin releasing hormone test or insulin tolerance test. Hypopituitarism was present in case of clinically relevant hormonal deficiencies in minimal one axis and was treated promptly with thyroxine, hydrocortisone, testosterone or estrogens (in pre-menopausal women).

The study protocol was approved by the Medical Ethics Committee, and all subjects gave written consent for their participation.

Protocol

Sixty-seven patients were seen at the outpatient clinic for a single visit. Physical examination was performed by a single physician (MW) trained in structured joint assessment. Blood samples were taken in the fasting state to assess actual GH and IGF-I concentrations. Other relevant details of treatment and patients characteristics were derived from the patient records. Conventional radiographs were obtained in all patients, according to a standardized protocol (*vide infra)*.

Study parameters

Radiological investigation and radiological scoring

Conventional radiographs of the hands (dorso-volar), knees (Posterior-Anterior (PA) in weight-bearing / fixed flexion[15] and lateral), hips (PA), lumbar (PA and lateral), and cervical spine (Anterior-Posterior (AP) overview, AP transbuccal, and lateral) were obtained from all participating patients, following a standardized manner with a fixed film-focus distance and fixed joint position. All radiographs were performed by a single experienced radiology technician. Radiographs were scored by a single experienced musculoskeletal radiologist (HK) according to the Kellgren and Lawrence (K&L) scale with the help of the original atlas[16-18]. This is a four-point scale scoring system with increasing severity based on the presence of osteophytes, joint space narrowing, sclerosis and degenerative cysts. The intra-reader variability, scored by the K&L method, assessed by the intra-class correlation coefficient (ICC) was 19% for the hands, 11% for the knees (femorotibial), 0% for the hips, 5% for the cervical spine (intervertebral discs and apophyseal joints), and 12% for the lumbar spine (intervertebral discs and apophyseal joints). The intra-reader variability was based on the repeated scoring of 10 radiographs, which were selected randomly. The radiographs were blinded for any patient characteristics.

Parameters of acromegalic disease

Disease duration was calculated from the estimated date of onset, using start of signs and symptoms, and facial changes on photographs to the date of normalization of serum IGF-I concentration after transsphenoidal surgery, or additional treatment. Duration of remission was calculated from the date of normalization of serum IGF-I concentration after treatment until start of the study. Both surgically and/or irradiated cured patients and patients with controlled disease during SMS analog treatment were collectively referred to as 'in remission'.

Biochemical parameters

Serum GH was measured with a sensitive immunofluorometric assay (IFMA) (Wallac, Turku, Finland), specific for the 22 kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit 0.01 μg/l; inter-assay coefficient of variation (CV) 2.0-9.0% of 0.1-15.4 μg/l) from 1992 onwards, and previously with the RIA assay (Biolab/Serono, Coinsins, Switzerland) calibrated against WHO-IRP 66/21, with an inter-assay CV below 5% and a detection limit of 0.19 μg/l. Pretreatment GH concentrations were available from all patients.

From 1986 up till 2005, serum IGF-I concentrations were determined by a radioimmunoassay (RIA) (Incstar; Stillwater, MN, USA) with a detection limit of 1.5 nmol/l and an inter-assay CV below 11%. IGF-I is expressed as SD scores (SD score) for age- and gender-related normal levels determined in the same laboratory[19]. From 2005 onwards serum IGF-I concentration (ng/ml) was measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay CV was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/l, respectively. IGF-I levels were expressed as SD score, using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals[20;21].

The diagnosis of osteoarthritis

For radiographic signs of osteoarthritis we defined a cumulative score indicating severity, for each joint location on the basis of the number of joints with radiographic osteoarthritis based on a K&L score ≥2[16]. Osteoarthritis on the left and right site of the distal interphalangeal (DIP), proximal interphalangeal (PIP), metacarpophalangeal (MCP) and first carpometacarpal (CMC1) joints of the hand, knee, and hip joints respectively, were summarized. The specific

radiographic osteoarthritis score for the hip and knee (range 0-2) represented no, unilateral or bilateral radiographic osteoarthritis. For radiographic hand osteoarthritis a sum score was derived for DIP joints (range 0-8), PIP joints (range 0-8), and CMC1 joints (range 0-2) separately and a sum score for all affected hand joints together (range 0-20). For the cervical and lumbar spine a sum score was derived which was based on the cervical intervertebral discs (0-6; that is, C1/C2-C6/C7) and lumbar intervertebral discs (0-5; that is, L1/L2-L5/S1), respectively.

Statistical analysis

SPSS for windows version 14.0 (SPSS inc., Chicago, IL, USA) was used for data analysis. Data are presented as mean (SEM), unless mentioned otherwise. Radiographic osteoarthritis was scored for each joint at the separate joint sites.

Multinomial logistic regression with adjustments for age, gender, BMI, and disease duration was used to compare the risk on osteoarthritis according to tertiles of pre-treatment IGF-I SD scores. The adjusted ORs and 95% CIs were subsequently transformed to risk ratios (RRs) and corresponding 95% CIs using the approximation formulae described by Zhang *et al.*[22]. Analysis of variance and binary logistic regression analysis with varying adjustments were used when appropriate.

RESULTS

Clinical characteristics (*Table 1*)

We studied 67 patients, 34 male and 33 female with a mean age of 56.8 (1.5) years. BMI and age were comparable in males and females. All patients were in remission for acromegaly or had medically controlled disease for a mean of 12 years after (multimodality) treatment. Sustained biochemical control was maintained since remission, at least for 2 years. Sixteen (23%) patients were still on long-acting SMS analogs.

At the time of diagnosis of acromegaly mean IGF-I SD scores were 7.3 (0.6) SD, and the mean estimated duration of active disease prior to remission was 8.2 (0.8) yrs (range 1 to 45 yrs).

At the time of the current evaluation, the mean estimated duration since diagnosis was 18.5 (1.0) yrs, mean GH level was 0.96 (0.2) μg/l, and mean IGF-I SD was 0.6 (0.2) SD. There

was no gender difference in duration of active disease or duration of remission, serum GH levels, or in IGF-I SD-scores. More female than male patients were hypogonadal, i.e. estrogen deficient, due to natural menopause. There were no gender differences for the other pituitary hormone deficiencies.

Table 1. Clinical characteristics

	Patients (n=67)
Gender (n(%))	
Males	34 (51 %)
Females	33 (49 %)
Age (yr)	56.8 (1.5)
BMI (kg/m^2)	28.3 (0.6)
Height (m)	1.75 (1.3)
Weight (kg)	87.5 (2.2)
Treatment: (n(%))	
Surgery	39 (58 %)
Surgery + RT	6 (9 %)
Surgery + SMS	14 (21 %)
Surgery + RT + SMS	2 (3 %)
Primary SMS	7 (10 %)
Age at diagnosis	43.2 (1.7)
Disease duration (yrs)	8.2 (0.8)
Duration of remission (yrs)	12.4 (0.6)
GH (μg/L)	
Pre-treatment	31.2 (5.2)*
Current	0.96 (0.2)
IGF-I SD scores	
Pre-treatment	7.3 (0.6)
Current	0.6 (0.2)
Hypopituitarism (n(%))	
Hypothyroidism	11 (16 %)
GH deficiency	7 (10 %)
Hypogonadism	31 (46 %)
Hypocortisolism	15 (22 %)

Data are shown as mean and standard error of the mean (SEM), unless mentioned otherwise. N: number, BMI: body mass index, GH: growth hormone, IGF-I: insulin-like growth factor I, SD: standard deviation, RT: radiotherapy (pituitary irradiation), SMS: somatostatin analogs. Mean respective pretreatment GH for IFMA assay (n=49):32.8 (7.7) μg/L and mean GH for RIA assay (n=18): 28.6 (4.1) μg/L.

Risk of developing osteoarthritis according to pre-treatment IGF-I levels

There was a high prevalence of radiographic osteoarthritis (*Table 2)*. This table also shows the relative risks (RR) for having osteoarthritis according to tertiles of pre-treatment IGF-I SD scores. Patients in the highest tertile of IGF-I SD scores had a ~30% increased risk for developing osteoarthritis of the cervical spine (adjusted RR 1.28, 95% confidence interval (CI) 1.01 to 1.31), a four-fold increased risk of developing osteoarthritis of the hip (adjusted RR 3.98, 95% CI 1.69 to 5.01) and a two to almost five-fold increased risk for developing osteoarthritis of the

Table 2. Risk of having osteoarthritis in relation to tertiles of baseline IGF-I levels, expressed in SD scores

Joint site	Tertiles IGF-I SD	Patients with osteoarthritis (%)	Adjusted RR (95% CI)	P-value
Cervical spine	1.69-4.83	76	1	
	4.84-7.39	86	1.23 (0.78 – 1.31)	0.19
	7.40-23.91	91	1.29 (1.07 – 1.31)	0.03
Lumbar spine	1.69-4.83	76	1	
	4.84-7.39	77	0.77 (0.33 – 1.21)	0.61
	7.40-23.91	91	1.22 (0.78 – 1.31)	0.21
Hip	1.69-4.83	19	1	
	4.84-7.39	32	1.72 (0.53 – 3.57)	0.33
	7.40-23.91	57	3.98 (1.69 – 5.01)	<0.01
Knee	1.69-4.83	38	1	
	4.84-7.39	32	1.37 (0.47 – 2.22)	0.49
	7.40-23.91	52	2.15 (1.14 – 2.55)	0.04
DIP	1.69-4.83	45	1	
	4.84-7.39	55	1.53 (0.50 – 2.10)	0.34
	7.40-23.91	60	2.07 (1.09 – 2.21)	0.04
PIP	1.69-4.83	28	1	
	4.84-7.39	46	3.25 (0.89 – 3.56)	0.06
	7.40-23.91	50	3.49 (1.46 – 3.57)	0.02
IP	1.69-4.83	43	1	
	4.84-7.39	43	0.54 (0.11 – 1.52)	0.33
	7.40-23.91	50	1.37 (0.46 – 2.07)	0.47
CMC	1.69-4.83	19	1	
	4.84-7.39	9	0.48 (0.08 – 2.11)	0.38
	7.40-23.91	38	4.76 (1.13 – 5.25)	0.04

N= 67. Tertiles of pre-treatment IGF-I SD scores based on pre-treatment IGF-I SD scores in the whole population. The risks ratios are adjusted for age, sex, BMI, and duration of active disease. Data were analyzed by multinomial logistic regression analysis. DIP: distal interphalangeal (joints), PIP: proximal interphalangeal (joints), IP: interphalangeal (joints), CMC: carpometacarpal (joints), IGF-I; insulin-like-growth factor I, SD: standard deviation, RR: relative risk, CI: confidence interval.

DIP, PIP, and CMC joints, all in comparison to those patients in the lowest tertile of IGF-I SD scores.

There were no significant associations between IGF-I SD scores and osteoarthritis of the knee and lumbar spine, although there was a tendency for an increased risk for osteoarthritis in the higher tertiles.

Pre-treatment IGF-I scores in patients with and without osteoarthritis

The effect of pre-treatment IGF-I SD scores on the degree of radiographic osteoarthritis in separate joint sites is visualized in *Figure 1*.

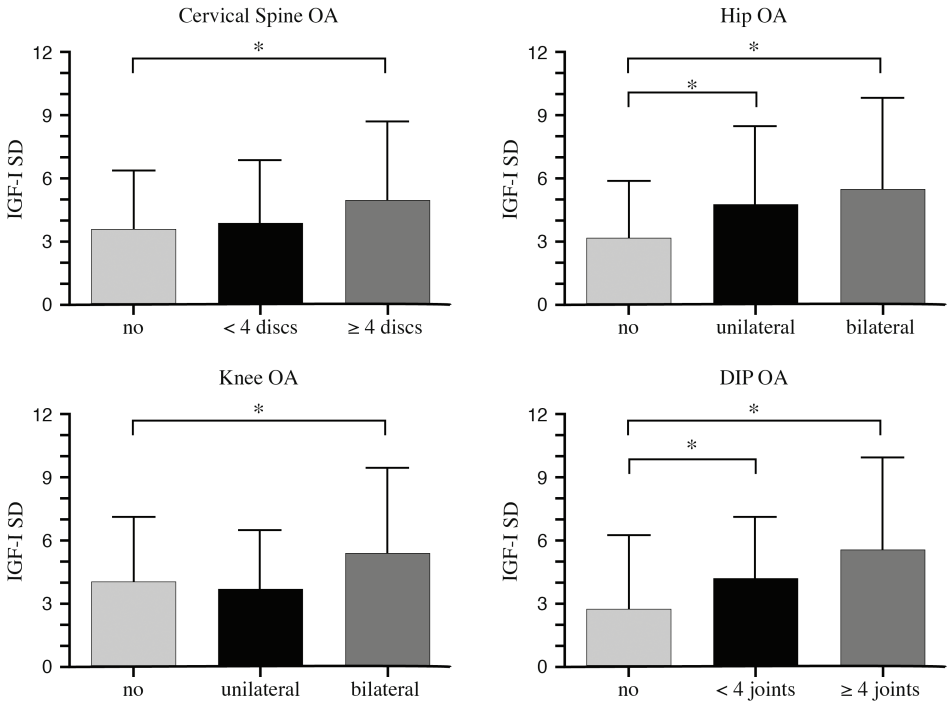


Figure 1. IGF-I SD represent the pre-treatment IGF-I SD scores. * = P<0.05. OA: osteoarthritis, IGF-I: insulin-like growth factor I, SD: standard deviation.

The bars in this figure represent crude values, unadjusted for age, gender, BMI, pre-treatment GH levels, or duration of active disease. The pre-treatment IGF-I SD scores were different in patients with bilateral, but not with unilateral, osteoarthritis of the hip when compared with the IGF-I SD scores of patients without hip osteoarthritis, the mean differences

were 4.78 SD (95% CI 1.89 to 7.67), and 1.49 SD (95% CI -0.03 to 6.03), respectively. Accordingly, bilateral, but not unilateral, osteoarthritis of the knee was significantly associated with higher pre-treatment IGF-I SD scores when compared with patients without knee osteoarthritis, the mean difference was 3.78 SD (95% CI 0.16 to 7.39).

Osteoarthritis in ≥4 intervertebral discs of the cervical spine was associated with higher pre-treatment IGF-I SD scores when compared with patients without osteoarthritis of the spine, the mean difference was 6.16 SD (95% CI 1.03 to 11.28). Patients with osteoarthritis in <4 or ≥4 DIP joints showed higher IGF-I SD scores when compared with patients without osteoarthritis of the DIP joints, the mean differences were respectively 4.47 SD (95% CI 1.21 to 7.73) and 5.36 SD (95% CI 0.39 to 10.32). All mean differences were adjusted for age, gender, BMI, and disease duration. Similar results were seen for PIP, IP and CMC1 joints as well as for the lumbar spine (data not shown).

Development of extensiveness of osteoarthritis in association to pre-treatment IGF-I SD scores

As shown in *Table 3* there was a positive correlation between IGF-I SD scores at the time of diagnosis (pre-treatment IGF-I SD score) and the degree of radiographic osteoarthritis of the hip during long term follow-up. After adjustment for age, gender, and BMI there was a positive correlation between pre-treatment IGF-I SD scores and osteoarthritis of the hip, DIP, IP, and Total Body. In addition, after adjustment for disease duration, age, gender, BMI, pre-treatment IGF-I SD scores significantly correlated with the degree of radiographic osteoarthritis in all investigated joint sites.

Other potential factors of influence

Age at diagnosis showed a positive correlation with osteoarthritis. Patients with a relatively young age at diagnosis showed less osteoarthritis than patients with an older age at diagnosis. The adjusted regression coefficient for total body osteoarthritis was 0.37 (95% CI 0.24 to 0.52). The duration of active disease was not significantly correlated with osteoarthritis. However, after adjustment for pre-treatment IGF-I SD scores there was a significant, negative correlation between osteoarthritis and duration of active disease (adjusted regression coefficient -0.03, 95% CI -0.06, -0.01). There was no significant association between osteoarthritis and pre-treatment GH levels. In addition, the presence or the severity of radiographic osteoarthritis

was not influenced by the type of treatment (*Table 4*), pre-treatment GH levels, duration of follow-up, actual GH and IGF-I concentrations, hypopituitarism, hypogonadism, gender or BMI (P=NS).

Table 3. Regression analysis for IGF-I influencing osteoarthritis, with variable adjustments

	Osteoarthritis in >1 joint (n (%))	**IGF-I SD unadjusted (CI)**	**IGF-I SD adjusted* (CI)**	**IGF-I SD Adjusted** (CI)**
Radiographic osteoarthritis				
Cervical spine disc (0-6)	55 (86 %)	NS	NS	0.10 (0.02-0.18)
Lumbar spine disc (0-5)	53 (83 %)	NS	NS	0.11 (0.02-0.21)
Hip (0-2)	24 (38 %)	0.07 (0.02-0.11)	0.07 (0.03-0.11)	0.07 (0.02-0.12)
Knee (0-2)	27 (42 %)	NS	NS	0.05 (0.00-0.10)
DIP (0-8)	35 (55 %)	NS	0.13 (0.01-0.24)	0.14 (0.01-0.27)
PIP (0-8)	28 (44 %)	NS	NS	0.13 (0.01-0.25)
CMC (0-2)	15 (23 %)	NS	0.04 (0.01-0.08)	0.06 (0.02-0.09)
IP (0-2)	30 (47 %)	NS	NS	NS
Total body † (0-35)	63 (98 %)	NS	0.43 (0.19-0.67)	0.48 (0.21-0.75)

N= 67. Data are presented as regression coefficient with 95% confidence interval if significant. Radiographic osteoarthritis was scored for each joint at the separate joint sites. Correlations between IGF-I SD scores and radiographic osteoarthritis were performed by analysis of covariance. The distribution of the residuals were checked. There were no gross violations of normality.

* Regression coefficient adjusted for age, gender, and BMI.

** Regression coefficient adjusted for age, gender, BMI, and disease duration.

† total body: sum of all radiographic examined joints of the cervical and lumbar spine, hips, knees and hands.

IGF-I: insulin-like growth factor I, SD: standard deviation, n: number, NS: not significant, DIP: distal interphalangeal (joints), PIP: proximal interphalangeal (joints), IP: interphalangeal (joints), CMC: carpometacarpal (joints), CI: confidence interval.

Table 4. The distribution of osteoarthritis according to treatment type, separated for individual joints.

	Surgery	Radiotherapy	SMS	p-value
	N=43	N=8	N=16	
Cervical spine disc	35 (81 %)	7 (88 %)	13 (81 %)	0.78
Lumbar spine disc	36 (83 %)	6 (75 %)	11 (69 %)	0.97
Hip	14 (34 %)	3 (38 %)	7 (44 %)	0.93
Knee	18 (41 %)	3 (38 %)	6 (38 %)	0.82
DIP	23 (53 %)	4 (50 %)	8 (50 %)	0.91
PIP	18 (42 %)	3 (38 %)	7 (44 %)	0.89
CMC	9 (21 %)	2 (25 %)	4 (25 %)	0.76
IP	10 (47 %)	3 (38 %)	7 (44 %)	0.88
Total body †	41 (95 %)	7 (88 %)	15 (94 %)	0.85

Data are expressed as n(%). Osteoarthritis at the different joint sites was dichotomized, according to the presence or absence of osteoarthritis. Data were analyzed with binary logistic regression analyses adjusted for age.
Definitions: Surgery: primary surgical treatment. Radiotherapy: as adjuvant therapy to surgery (no patients underwent primary RT) combined with or without SMS analog treatment. SMS analog treatment: current treatment (primary or additional to prior surgery). † total body: sum of all radiographic examined joints of the cervical and lumbar spine, hips, knees and hands, SMS: somatostatin (analogs).

DISCUSSION

Acromegalic patients with long-term cure or control of disease activity have a high prevalence of osteoarthritis in multiple joints. This study documents that the risk to develop osteoarthritis in these patients is predicted by IGF-I concentrations at the time of initial diagnosis. A four-fold and almost five-fold increase in risk for radiological osteoarthritis of the hip and hand, respectively, was found in patients in the highest tertile of pre-treatment IGF-I SD scores compared with patients in the lowest tertile of pre-treatment IGF-I scores. These associations were not caused by differences in age, gender, or BMI. In addition, the extensiveness of osteoarthritis in all joints, except for the IP joints, was associated with pre-treatment IGF-I SD scores when adjusted for age, gender, BMI, and disease duration.

Osteoarthritis is caused by degeneration of hypertrophied cartilage, which was hypertrophied due to the anabolic effect of elevated GH and IGF-I levels during the active phase of the disease. Osteoarthritis is a multi-factorial disease, with different predictive factors per joint site, like age, gender, and BMI. In general, radiological osteoarthritis of the hip has a specific genetic and geographically prevalence pattern, which suggests the involvement of systemic fac-

tors[13;23]. Moreover, the hip is the joint, which is the least affected by occupational and physical causes of osteoarthritis[13]. Since an effect of BMI and gender could not be identified in our patient group, we assumed that disease specific factors were predictors of secondary osteoarthritis of acromegaly.

IGF-I stimulates chondrocytes to synthesize extracellular matrix components in cartilage. Its action is mediated through the type I IGF receptor[24;25]. The function of IGF-I and its receptor in cartilage formation, both during developmental stages and remodeling of adult cartilage, may be relevant to the etiology of osteoarthritis. IGF-I may also influence osteoarthritis by osteophyte formation[24;25]. In this context, it is interesting to note that Meulenbelt *et al.* observed that the IGF-I allele 3 is associated with radiological osteoarthritis in the general population at any joint site (knee, hip, hand, and spine), and that carriers of the IGF-I 3 allele were predisposed for radiological osteoarthritis at any possible joint[23]. This association was strongest in subjects with radiological osteoarthritis of the hip. In the present study, we extend this observation to patients with acromegaly: the strongest correlation with pre-treatment IGF-I SD scores was demonstrated for the hips. Therefore, in long-term cured patients with acromegaly, the hip is also most prone to secondary osteoarthritis caused by systemic increase of levels of IGF-I during the active phase of the disease and, apparently, this mechanism influences the prevalence of osteoarthritis even after long-term cure.

In the present study we also studied pain and stiffness, but since pain is a subjective finding, we focussed on objective radiological endpoints. Pre-treatment GH or IGF-I SD scores were not associated with pain. Not everyone with an established diagnosis of radiological osteoarthritis report pain and *vice versa* [26]. Pain can be influenced by other factors for example age and gender and can be caused by other musculoskeletal problems.

Two IGF-I assays were used in the follow-up period. However, all pre-treatment IGF-I concentrations were measured with the same RIA assay, and we calculated all pre-treatment IGF-I SD values using own reference data[19]. The GH assay was changed in 1992. For the glucose tolerance tests performed before 1993 we have defined normal suppression of GH < 2.5 mU/L (1.25 mcg/L). However, it is of note that that in follow-up all patients were also controlled according to the GH criteria of the currently used assay and suppressed to below 0.38 mcg/L (on GTT).

Age at diagnosis was positively associated with osteoarthritis after long-term cure. Interestingly, other factors indicating severity of the disease, including pre-treatment GH levels,

disease duration *per sé*, type of treatment, and duration of follow-up, did not predict osteoarthritis. In our study mean serum GH concentrations at diagnosis did not predict osteoarthritis. This may be due to the limitation to accurately measure the actual mean GH concentrations or to the change in assay. However, we repeated the statistical analyses for each GH assay, which did not change our conclusion. It is likely that the serum GH concentration is a main determinant of locally produced IGF-I concentration. In tissue studies locally produced IGF-I was found to be important in the development of osteoarthritis[27].

Our findings are in contrast to the data of previous studies, which studied several potential predictive factors for acromegalic arthropathy, including disease duration, disease severity, pre-treatment GH levels, age of disease onset, disease duration, IGF-I levels, age, and gender[4;9;28-30] (*Table 5*). Only a few studies were able to establish predictive factors. Dons *et al*. identified a predictive effect of baseline GH levels on arthropathy over a 5-year follow-up period of acromegalic patients treated by pituitary irradiation[11]. Layton *et al*. demonstrated a positive predictive effect for disease duration on the severity of arthropathy[9]. The relation between IGF-I and arthropathy was studied in few previous studies, which failed to show a relationship between pretreatment IGF-I levels and arthropathy[28-29]. Potential explanations for the discrepant findings between those studies and the present study are probably related to differences in study design including the inaccuracy of the estimation of disease duration, small sample sizes and differences in disease activity and follow-up duration.

Previously, we documented that disease duration was related significantly to other co-morbidities of acromegaly, including valvular heart disease and survival[28;30]. Interestingly, the negative influences of pre-operative disease activity on survival were strongest in the first 5 years of postoperative follow-up and diminished afterwards[28]. In contrast, arthropathy appears to be irreversible and the effects of previous disease activity remain present even after long-term cure.

Transsphenoidal surgery was the initial treatment for all our patients with acromegaly diagnosed from 1977 onwards and, in case of persistent GH excess, followed by adjuvant therapy in the form of radiotherapy and treatment with SMS analogs. An increasing number of acromegalic patients received initial medical treatment since the introduction of SMS analog depot preparations. However, an effect of initial treatment on osteoarthritis could not be detected.

Table 5. Overview of literature on factors of influence on arthropathy in acromegaly.

				Factors of influence on arthropathy	
Authors	**Date of publication**	**Patient number, Disease status**	**Methods**	**Investigated**	**Identified**
Bluestone *et al.*	1971	42 de novo acromegaly	CR, RG	Duration of active disease Severity of disease	-
Dons *et al.*	1988	43 active acromegaly 47 follow-up 5 yrs after RT	CR, RG	Baseline GH levels	Baseline GH levels
Layton *et al.*	1988	19 active acromegaly	CE, RG	Age of disease onset Pre-treatment GH levels Duration of active disease	Duration of active disease
Ezzat *et al.*	1994	114 de novo acromegaly 386 not well controlled	Scoring of complaints	Not assessed	-
Colao *et al.*	1998	18 active acromegaly 12 cured (2 yrs)	USG	Duration of active disease	-
Colao *et al.*	1999	12 de novo acromegaly	CE, USG	Duration of active disease	-
Colao *et al.*	2003	30 de novo acromegaly 30 cured (3.6 yrs) acromegaly	USG	Duration of active disease	-
Scarpa *et al.*	2004	54 active acromegaly	CR, RG	Age at acromegaly onset Duration of active disease Serum GH levels Serum IGF-I levels Serum Insulin levels	-
Biermasz *et al.*	2005	118 long time cured acromegaly	Self-reported complaints	Age Gender Duration of active disease Serum GH levels Serum IGF-I levels	Female gender
Miller *et al.*	2008	47 cured (duration not mentioned) 11 active acromegaly	CE, QOL questionnaires	Serum GH levels Duration of active disease	-

Current study	-	89 long-term cured acromegaly	CR. CE, RG	Duration of active disease Duration of follow-up Duration since diagnosis Pre-treatment GH levels Pre-treatment IGF-I SD scores Current GH levels Current IGF-I SD scores	Pre-treatment IGF-I SD scores

Pt: patients; co: controls; CR: clinical records; CE: clinical examination; RG: radiographs; USG: Ultra-sonography; QOL: quality of life.

We studied a relatively large acromegalic population with long-term cured disease and a high prevalence of secondary osteoarthritis. Our data demonstrate a predictive role for pre-treatment IGF-I SD scores on radiologic osteoarthritis in patients systemically exposed to high levels of GH and IGF-I for a mean of more than 8 years. This effect was seen at all joint sites. Other disease-specific predictive factors could not be identified. Many subjects cured from acromegaly suffer from subjective complaints due to arthropathy. For instance in a previous study we observed that pain and stiffness at minimal 1 joint-site was reported by 72% of patients. However, radiological manifestations of osteoarthritis at minimal 1 joint-site were present in 99% of patients[31]. This observation indicates that objective radiological manifestations of acromegalic arthropathy are not always reflected in clinical signs of arthropathy. In conclusion, our data demonstrate a predictive role for pre-treatment IGF-I SD scores on osteoarthritis in acromegaly patients with long-term disease control.

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Chapter 4.

OSTEOARTHRITIS IS EQUALLY PREVALENT IN MEN AND WOMEN WITH LONG-TERM CONTROLLED ACROMEGALY.

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Article submitted

ABSTRACT

Objective: Primary osteoarthritis (OA) preferentially affects women. We evaluated whether female gender is also a risk factor for OA development in acromegaly, a disease caused by a growth hormone producing pituitary adenoma.

Design: We conducted a case-control study

Methods: We compared 46 male and 43 female patients (mean: 58 years) with controlled acromegaly for a mean of 14 years. Study parameters were clinical assessment and radiographs of knee, hand and hip. Radiological OA was defined as a Kellgren and Lawrence (KL) score of ≥2. Symptomatic and clinical OA was defined according to the American College of Rheumatology (ACR) criteria combining symptoms with radiological abnormalities or abnormalities at physical examination, respectively. The prevalence of clinical OA was compared with age- and environment-matched controls and the prevalence of radiological OA with a Dutch historical population sample.

Results: Radiological knee, distal interphalangeal joint, and hip OA was seen in 35, 56, and 44% of patients, respectively, and was equally prevalent in women and men. Taking in account symptoms (symptomatic OA) lowered the prevalence but did not change the distribution over the genders. Clinical OA was similarly prevalent in both genders except for hand OA, which was encountered more in women. In comparison with controls and the historical population sample, OA prevalence was increased in women, but even more so in men.

Conclusion: In acromegaly, the prevalence of OA at all joint sites is similar in male and female patients, suggesting that the overproduction of GH and IGF-I overrules the potential role of risk factors, including hormonal factors, in primary OA.

INTRODUCTION

The exact pathogenesis of osteoarthritis (OA) is unknown, but ample evidence suggests that OA is a multifactorial disease[1]. Gender is one of the most important risk factors for primary OA. Women, especially women after 55 years of age, are more frequently affected than men in their knees, hands, and hips, representing the most prevalent subtypes of OA[2-4]. This gender difference in the prevalence of primary OA is not clearly understood, although systemic, hormonal, and genetic factors may play a role[5;6]. Since this gender difference, especially in knee and hand OA, becomes more prominent after the age of 55 years (around the menopause), hormones like estrogens may be of importance in the development of OA[2;3;5-7].

Acromegaly is caused by a growth hormone (GH)-secreting pituitary adenoma. Increased GH concentrations lead to increased insulin-like growth factor-I (IGF-I) secretion by peripheral tissues, especially the liver. Arthropathy is present in 50 to 75% of the patients with active and treated acromegaly[7-12]. In long-standing acromegaly, this arthropathy is mostly characterized as secondary OA and may be the result of cartilage hypertrophy and laxity of ligaments induced by GH and IGF-I excess with secondary damage to the joint[8-13].

In acromegaly, there are no gender differences in disease characteristics, incidence and prevalence[14-16]. It has never been examined whether the risk factors for primary OA, female gender and menopause, are also present in patients with OA due to acromegaly. Since estrogens negatively influence GH-dependent IGF-I production in the liver, it is likely that gender and estrogen status are important in the pathogenesis of GH and IGF-I induced arthropathy.

The aim of this study was to analyze joint site specific gender differences in clinical and radiological characteristics of OA in patients with long-term controlled acromegaly, in order to get more insight both in pathophysiological processes that play a role in OA in acromegaly and in OA in general.

PATIENTS AND METHODS

Patients

All consecutive patients with acromegaly who were referred to our center were collected in a

database. From 1977 onwards, the first treatment option in the majority of patients was transsphenoidal surgery. If necessary, adjuvant treatment was given by radiotherapy (prior to 1985) or predominantly somatostatin analogs (from 1985 onwards). From 1998, some patients received primary somatostatin analog treatment. Disease activity was assessed yearly, and biochemical control was defined as a normal glucose suppressed serum GH <0.38 μg/liter, serum GH levels <1.9 μg/liter and normal IGF-I levels for age[17]. These strict biochemical criteria for control were used from the beginning of the study. This patient cohort is well-characterized and treatment results and biochemical and clinical follow-up results have been described in detail previously[17]. Patients cured or controlled' during somatostatin analog treatment were collectively referred to as 'in remission'.

Hypopituitarism was treated promptly with thyroxine, hydrocortisone, or estradiol/testosterone when deficiencies were documented using appropriate basal hormone and dynamic tests[18]. In men, hypogonadism was defined as a testosterone level below the reference range (8.0 nmol/l) and low or normal luteinizing hormone/follicle-stimulating hormone (LH/FSH) concentrations. Pre-menopausal women were hypogonadal if secondary amenorrhea was present more than 1 year, and post-menopausal women in case of LH/FSH levels below the normal postmenopausal range (LH<10 U/l and FSH <30 U/l).

Study design

For the present study, 126 consecutive patients in long-term biochemical remission were invited for participation. Thirty-seven patients (29%) preferred not to participate in the study for various reasons, including co-morbidities, travel distance, lack of time and psychological reasons. They were not different from the study population in disease characteristics or self-reported joint complaints. Each patient was asked to provide a control person of comparable age (non-relative) to compose a control population with a comparable socioeconomic status, derived from the same geographical area. The study protocol was approved by the Medical Ethics Committee, and all subjects gave written consent for their participation.

Eighty-nine patients (participation rate 71%) and 67 controls participated. They completed questionnaires, underwent a physical examination, hormonal evaluation for actual fasting morning GH/IGF-I concentrations, and conventional radiographs. Radiographs were taken from the patients only to avoid unnecessary radiation exposure to the controls and in the presence of available radiological control data from a large Dutch (n= 4842) historical

population sample, reporting normative values for radiological OA for gender specific 5-year age groups (mean age 56.1 ± 8.7 year, 53% females)[1]. Treatment and patients characteristics were derived from the patient records. The prevalence and radiological characteristics of joint disease were described previously[11]. In this analysis, we focus on the gender difference of OA in acromegaly and controls.

Study parameters

Clinical parameters

A structured standardized questionnaire was completed concerning medical history, and symptoms and signs of OA, i.e. joint-site specific complaints of pain and/or stiffness.

Acromegaly disease duration was defined as the duration between the onset of symptoms and remission (the date that serum IGF-I normalized after treatment[18]. Hypopituitarism was present in case of clinically relevant hormonal deficiencies in ≥1 axis. For this study, we considered estrogen deficiency present in case of LH/FSH deficiency in premenopausal women with prolonged amenorrhea >1 year without adequate replacement therapy and all postmenopausal women.

Physical examination and clinical scoring

Distal interphalangeal joints (DIPJs), proximal interphalangeal joints (PIPJs), first interphalangeal joints (1st IPJs), metacarpophalangeal joints (MCPJs) and first carpometacarpal joints (1st CMCJs) joints were examined for bony and soft-tissue swelling and deformities by a single physician (MW) trained in structured physical examination of the musculoskeletal system and recorded in a standardized manner. The knees were examined for crepitation, bony enlargement and pain on palpation of joint margins. The hips were examined for in- and external rotation.

In order to perform a Doyle index, 20 joint groups were examined for tenderness by pressure on the joint margin or by passive movement of the joint (48 joints; maximal score 144)[19].

Radiological investigation and radiological scoring

Radiographs of the knees (posterior-anterior, weight-bearing, fixed-flexion[20] and lateral), hands (dorso-volar) and hips (posterior-anterior) were acquired by a single experienced radiographer following a standardized manner with a fixed film-focus distance. Radiographs were scored by a

single experienced musculoskeletal radiologist (HK) blinded for patient characteristics, according to the Kellgren-Lawrence atlas (KL)[21]. In the hands the DIPj's, PIPj's, IPj's, and the 1st C-MCj's were scored. The intra-class correlation coefficient (ICC) based on the repeated scoring of 10 randomly selected patients was 0.81 (hands), 0.89 (tibiofemoral joints) and 1.00 (hips).

Definition of OA

We used different definitions for OA:

1. Radiological OA was defined as a KL score of ≥2.
2. Symptomatic OA was defined as pain and stiffness on most days of the preceding month in combination with a KL score of ≥2. For symptomatic hand OA at least 2 hand joints had to be affected [22;23].
3. Clinical OA was scored according to the American College of Rheumatology (ACR) criteria to enable comparison with controls[22-24].

For all definitions a joint prosthesis in the knees and/or hips as a result of end stage OA was included as OA in that particular joint.

Assays

Actual GH and IGF-I concentrations were assessed at the study visit in the fasting state. Serum GH was measured with a sensitive immunofluorometric assay (IFMA) (Wallac, Turku, Finland), specific for the 22 kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit 0.3 μg/l; intra-assay coefficient of variation (CV) 1.6-8.4% of 0.0.1-15.4μg/l). Serum IGF-I concentration (ng/ml) was measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay variation was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/l, respectively. IGF-I levels were expressed as age and gender dependent standard deviation (SD) score, using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals[25;26].

Statistical analysis

SPSS for Windows version 16.0 (SPSS inc., Chicago, IL, USA) was used for data analysis. Data are presented as mean (SD), unless specified otherwise. Comparisons between male and female patients on clinical characteristics were made by analysis of variance and adjusted for age and

BMI. Results were displayed as difference with 95% confidence interval (95% CI) and corresponding p-values.

Symptoms of pain and/or stiffness and OA were dichotomized according to the presence or absence of it. The difference between male and female patients on prevalence of OA and self-reported joint symptoms was analyzed by a binary logistic regression analysis with males as reference category and adjusted for age and BMI. As reference for radiological OA we utilized historical data from the Dutch population from a study reporting normative values for radiological OA[1]. Binary logistic regression analysis with adjustment for 5-year age categories was used to compare our patients with the historical reference group. As reference for clinical OA we utilized the age- and environment-matched controls collected for the present study. Binary logistic regression with adjustment for age and BMI was used for comparison.

When applicable, all analyses were adjusted for age, BMI, estrogen state, disease duration, somatostatin analog treatment, irradiation, hypopituitarism, physical trauma, smoking, and occupational and physical activities.

RESULTS

Clinical characteristics

We studied 46 male and 43 female acromegaly patients. Men were slightly younger than women (55.9±10.7 vs. 60.8±11.9, p=0.04). BMI was comparable between both genders and not different from BMI at times of diagnosis of acromegaly (28.5±4.7 (females) *vs.* 28.9±4.5 (males), p=0.48). The mean duration of active disease was 8.9±7.3 years. All patients were in remission for a mean of 14 years (range 2-28). As treatment for acromegaly 55% of patients received surgery alone, 16% surgery in combination with somatostatin analogs, 7% of patients were treated primarily with somatostatin analogs and 22% received additional radiotherapy. Treatment modalities were not different between the genders. Twenty (23%) patients were currently using somatostatin analogs.

There was no gender difference in duration of active disease, serum GH levels, IGF-I SD-scores at diagnosis and during the evaluation. Because of natural menopause females had a higher prevalence of hypogonadism, i.e. estrogen deficiency, than males (*Table 1*).

Table 1. Clinical characteristics of male and female acromegalic patients with long-term disease control

	Acromegaly patients			Controls from environment	
	Males N=46	Females N=43	Difference (95% CI)	Males (n=28)	Females (n=39)
Age (yrs)	55.9 (10.7)	60.8 (11.9)	-4.9 (-9.75,-0.02)	57.9 (10.1)	58.5 (10.4)
BMI (kg/m²)	28.7 (4.5)	28.3 (4.9)	0.3 (-1.75,-2.35)	26.4 (4.1)	26.0 (4.6)
Menopause					
Pre-menopausal		5 (11 %)			4 (10%)
Post-menopausal		38 (89 %)			35 (90%)
Disease duration	8.1 (5.1)	9.7 (9.1)	0.4 (-3.9, 3.0)	NA	NA
Duration of remission	14.5 (6.5)	13.7 (6.0)	1.3 (-1.8, 4.4)	NA	NA
GH (µg/l)				NA	NA
Pre-treatment	40.2 (49.6)	32.6 (45.8)	3.4 (-17.9, 24.7)		
Current	0.87 (1.77)	0.95 (0.96)	-0.1 (-0.7, 0.5)		
IGF-I SD scores				NA	NA
Pre-treatment	7.9 (4.7)	6.7 (4.7)	1.3 (-1.1, 3.8)		
Current	0.6 (1.6)	0.5 (1.9)	-0.1 (-1.0, 1.4)		
Hypopituitarism	13 (28 %)	16 (37 %)	-0.1 (-0.29, 0.12)	NA	NA

Data are shown as mean (SD), unless mentioned otherwise. Clinical characteristics were compared between males and females by analysis of variance and adjusted for age and BMI, results were displayed as difference (95% CI) en by P-value. NA: not available. There were no differences in BMI and age in controls from the environment. Yr: years, kg/m²: kilograms per square meter, µg/l: micrograms per liter, SD: standard deviation, BMI: body mass index, GH: growth hormone, IGF-I:insuline like growth factor, CI: confidence interval.

From the environment of the patients 67 controls (50 spouses and 17 friends) participated with comparable mean age and gender distribution as the patients (mean age 58.2 years; 58% women). The mean BMI was 26.2 kg/m², which was significantly lower than in the patients (p=0.01). Women and men did not differ on age and BMI in the control population (*Table 1*).

Five-year normative radiographic data from a large Dutch reference population (n= 4842) were utilized.

Radiological OA

Comparison of male and female acromegalic patients

The prevalence (based on KL≥2) and the severity (frequencies of KL 2, 3 and 4) of radiological OA was not different between men and women with long-term cured acromegaly at all

joint sites, even after adjustment, except for the1st CMCJs (*Table 2*). Due to end-stage OA two patients had undergone hip replacement and 2 patients had undergone knee replacement.

Table 2. Gender differences of radiological osteoarthritis in acromegalic patients with long-term disease control.

	Male n=46	Female n=43	Adjusted OR (95% CI)	P-value
Knee	18 (39 %)	13 (30 %)	0.8 (0.32 – 2.10)	0.67
Hand				
DIP	21 (46 %)	29 (67 %)	2.1 (0.67 – 6.81)	0.25
PIP	15 (33 %)	23 (51 %)	1.6 (0.56 – 4.39)	0.39
CMC1	7 (15 %)	17 (38 %)	3.0 (1.04 – 8.81)	0.04
IP	14 (30 %)	24 (53 %)	2.3 (0.82 – 6.38)	0.11
Hip	20 (44 %)	19 (44 %)	0.5 (0.18 – 1.31)	0.19

Data are presented as n (%). Radiological osteoarthritis at each joint site was dichotomized and compared between males and females by binary logistic regression analysis. Odds ratios (OR) were adjusted for age, BMI, physical trauma, smoking and occupational- and physical activities. Males were reference category. DIP: Distal interphalangeal, PIP: proximal interphalangeal, MCP: metacarpophalangeal, CMC1: first carpometacarpal, CI: confidence interval.

Comparison with the Dutch reference population

Radiological OA in acromegaly patients was determined by the same scoring method used in the reference population. Acromegalic patients had a higher chance to have radiological OA at

Table 3. Radiological osteoarthritis in acromegalic patients with long-term disease control compared with a Dutch reference population.

	Males		Females		All	
	Adjusted OR	95% CI	Adjusted OR	95% CI	Adjusted OR	95% CI
Knee right	3.6	1.9-7.1	1.7	0.8-3.3	2.3	1.5-3.8
Knee left	3.6	1.7-6.5	1.4	0.7-2.8	2.1	1.3-3.3
DIP hands	1.7	0.9-3.3	2.0	1.0-4.2	1.7	1.1-2.8
PIP hands	4.5	2.3-8.9	4.7	2.4-9.4	4.2	2.6-6.8
CMC1 hands	7.6	4.0-14.4	8.2	3.9-17.6	7.0	4.3-11.4
Hip right	9.2	4.6-18.6	5.1	2.4-10.8	6.9	4.1-11.5
Hip left	8.7	4.4-18.5	4.9	2.2-10.8	6.7	4.0-11.4

Data represent odds ratios (OR) with 95% confidence intervals (95%CI) derived from binary logistic regression analysis. ORs were adjusted for 5-year age categories. The Dutch reference group was the reference category. DIP: Distal interphalangeal, PIP: proximal interphalangeal, MCP: metacarpophalangeal, CMC1: first carpometacarpal, CI: confidence interval. OR's between male and female acromegalic patients were not significantly different.

all joints (*Table 3*). The odds ratios reflecting the comparison between patients and the reference population were higher for males than for females for the knee and hip, but these gender differences were not significantly.

Symptomatic OA

Comparison of male and female acromegalic patients

Binary logistic regression analysis with adjustments for age, BMI, and factors indicating activity of acromegaly demonstrated no difference in symptomatic knee (17% *vs.* 35%, adjusted OR (95%CI) 0.4 (0.12-1.14)), hip (28% *vs.* 19%, 0.4 (0.12-1.14)), and hand (57% *vs.* 64%, 1.2 (0.45-3.23)) OA between men and women.

Self-reported symptoms

Comparison of pain and stiffness between male and female acromegalic patients

The prevalence of self-reported pain and stiffness was not different between male and female acromegalic patients at the knees (46% *vs.* 58% 1.1 (0.40-2.98)) and hands (76% *vs.* 81% 1.2 (0.47-3.01)), irrespective of adjustment. Only at the hip site, female patients reported more pain and stiffness than male patients (72% *vs.* 46% 2.3 (1.1-4.5)). The Doyle index for male and female patients, 7.0 (12.7) (range 0 to 28) and 7.6 (13.1) (range 0 to 37) respectively, did not differ significantly.

Compared with controls

Fifty-seven percent of female controls reported complaints of pain and stiffness, compared with 31% in male controls (OR: 3.2 (2.1-4.5). Female controls of >55 years, suffered significantly more complaints of pain and stiffness of the hip (56% *vs.* 27% 3.3 (1.7-4.6)) and hand (67% *vs.* 43% OR: 2.9 (1.8-4.0)) than male controls of >55 years. Both male and female acromegalic patients had a significantly higher chance to suffer symptoms of pain and stiffness in the knees, hands and hips when compared with male and female controls after adjustment, with ORs between 2.7 (male hip symptoms and female hand symptoms) up to 13.2 for female hand symptoms (data not shown).

Clinical osteoarthritis

Comparison between male and female acromegalic patients

Clinical knee OA was equally prevalent in male and female patients (15% *vs.* 21%, 1.1 (0.4-1.9). Clinical hand OA was significantly more prevalent in female than in male acromegalic patients (56% *vs*. 26%, 2.9 (1.6-4.1)). In addition, hand OA was more prevalent in both younger and older women compared with males (<55 yrs, p = 0.02 and >55 yrs p = 0.04, respectively). Clinical hip OA was equally prevalent in male and female patients (16% *vs.* 23%, OR 1.3 (0.6-2.1)).

Compared with controls

The prevalence of hip, knee and hand OA in controls was 0%, 5%, and 16%, respectively. Within the controls females >55 years suffered significantly more clinical hand OA (28% *vs.* 12%, p=0.04), than males >55 years. No gender difference could be demonstrated for hip and knee OA.

Table 4. Clinical osteoarthritis in acromegalic patients compared with an age-matched control group derived from the environment from the patient.

	Males			Females			All		
	OA	No OA	Adjusted OR (95% CI)	OA	No OA	Adjusted OR (95% CI)	OA	No OA	Adjusted OR (95% CI)
Knee OA									
patients	6	40		9	34		15	74	
controls	1	27		2	37		3	64	
			4.4 (1.9-22.5)			3.6 (1.3-16.9)			3.7 (1.1-13.6)
Hand OA									
patients	12	34		24	19		36	53	
controls	4	24		7	32		11	56	
			4.8 (1.7-13.5)			3.8 (1.4-16.4)			3.5 (1.6-7.9)
Hip OA									
patients	7	39		10	33		17	72	
controls	0	28		0	39		0	67	
			∞			∞			∞

OA and no OA are represented in numbers. Data represent odds ratios with 95% CI derived from binary logistic regression analysis, with covariates: age, BMI, physical trauma, smoking and occupational- and physical activities. Controls from the patient's environment were the reference category. DIP: Distal interphalangeal, PIP: proximal interphalangeal, MCP: metacarpophalangeal, CMC1: first carpometacarpal, CI: confidence interval.

Both male and female acromegalic patients had a higher chance to have clinical knee or hand OA when compared with the controls at all sites, also after adjustment (*Table 4*). None of the controls had clinical hip OA. The ORs reflecting the comparison between patients and controls were lower for female acromegalic patients than for male acromegalic patients for the knee and hand, however, not significant.

DISCUSSION

Osteoarthritis of the knees, hands and hips is very prevalent in acromegalic patients with long-term disease control. In this study we document that osteoarthritis equally affects male and female patients with acromegaly. The only exception was clinical hand OA and radiological 1stCMC OA, being more prevalent in female patients. The near equal gender distribution of clinical and radiological OA in acromegaly contrasts with the gender difference that is observed in primary OA in which (postmenopausal) women are more frequently affected than men[1].

Patients previously treated for acromegaly have a high prevalence of radiological, symptomatic, and clinical OA despite long term control of GH excess[11]. Elevated GH/IGF-I levels cause cartilage hypertrophy, and laxity of ligaments[8;10;13]. Thereafter, secondary changes result in irreversible damage to the joints, and OA. This second stage may be modulated by factors that also play a role in the development of primary OA[13]. However, our data indicates that, in acromegaly, female gender, which is a risk factor for primary OA[27], is not a risk factor for radiological and clinical OA in most joints, except in the hand.

We hypothesize that the lack of gender difference in acromegaly may be explained by the large effect of GH and IGF-I oversecretion in OA development, overruling other risk factors. However, since estrogens negatively interact with GH induced IGF-I secretion, an alternative explanation is that female patients with acromegaly are relatively protected for pathological changes induced by GH overproduction.

In primary OA, with increasing age women are affected more frequently and with much more severe OA than males[1;2;27;29]. It is suggested that a higher prevalence of OA in older women can, at least partially, be related to post-menopausal estrogen loss[30], and this is supported by epidemiological studies reporting that post-menopausal women taking estrogens have a decreased prevalence and incidence of radiological OA[4;5;31]. Although there is evidence for

estrogen receptors in cartilage and in synovium, a biological role of estrogens in the articular joint has not been established[32-35].

Estrogens have well-known effects on the somatotrope system, affecting both GH[36;37] and IGF-I secretion[38]. In the liver estrogens inhibit GH-dependent IGF-I production. Interestingly, in 1951, estrogens were proposed as the first medical treatment in women with acromegaly[39]. Thus, in acromegaly, both the protective effect of estrogens in itself, as the inhibitory action of estrogens on IGF-I production resulting in less severe IGF-I exposure, may relatively protect women with GH excess during their premenopausal years from OA. However, the ultimate effects of acromegaly on joint pathophysiology appear to be so strong that the prevalence of OA is highly increased in both male and female patients with acromegaly.

Several limitations of this study have to be addressed. First, female acromegalic patients were somewhat older than male patients. The fact that we did not find a gender difference in OA, except for the hand, despite the older age in females further underscores our observation that in acromegaly females are not more affected by OA than men[1]. Acromegalic patients did have a higher BMI than the controls, which is characteristic for the disease; to eliminate a possible effect of the higher BMI we adjusted all analyses for BMI. Unfortunately, normative radiographic data for 5-years age groups were not normalized for BMI, hence we were unable to adjust comparisons for BMI. Third, in the definition of clinical OA self-reported pain is utilized. Since women in general report more pain than men[40], it is unclear whether the more reported pain in the hips and more clinical hand OA in women is the result of more pain *per sé* or of more OA. The hip, more than the hand, is prone to systemic factors influencing the development of OA. The hand is more susceptible for physical and occupational activities. Hip OA is might be the best joint-site to demonstrate a specific effect due to acromegaly, since hip OA is equally common in males and females.

In conclusion, our data demonstrate that secondary OA is equally common in male and female acromegalic patients after long-term cure of disease, with the exception of a higher prevalence of clinical OA of the hands in female patients. These findings are remarkable since primary OA is more common in postmenopausal women than in men.

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Chapter 5.

ARTHROPATHY IN LONG-TERM CURED ACROMEGALY IS CHARACTERIZED BY OSTEOPHYTES WITHOUT JOINT-SPACE NARROWING: A COMPARISON WITH GENERALIZED OSTEOARTHRITIS.

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ABSTRACT

Objective: To compare the distribution of osteophytes and joint space narrowing (JSN) between patients with acromegaly and primary generalized osteoarthritis (OA) to gain insight into the pathophysiological process of growth hormone (GH) and insulin-like growth factor type I (IGF-I) mediated OA.

Design: we conducted a case-control study.

Methods: We utilized radiographs of knee and hip joints of 84 patients with controlled acromegaly for a mean of 14.0 years and 189 patients with primary generalized OA. Hips and knees with doubtful or definite OA (Kellgren-Lawrence (KL) score of ≥1) were compared in the current study. For a semi-quantitative assessment of radiological OA (range 0 to 3) osteophytes and JSN of the medial and lateral tibiofemoral, and hip joints were scored according to the Osteoarthritis Research Society International (OARSI) atlas. Logistic regression analysis was performed with adjustment for age, sex, BMI, and intra-patient effect.

Results: Knee and hip OA in patients with cured acromegaly was characterized by more osteophytosis (odds ratio's (OR) 4.1 to 9.9), but less JSN (OR's 0.3 to 0.5) in comparison with patients with primary OA. Patients with acromegaly and OA had significantly less self-reported functional disability than patients with primary OA ($p<0.001$). Self reported functional disability was associated with JSN rather than with osteophytosis.

Conclusion: Arthropathy due to GH oversecretion results in osteophytosis and to a lesser extend in JSN. This observation suggests that the GH-IGF-I system is mainly involved in bone formation resulting in osteophytosis but may protect against cartilage loss.

INTRODUCTION

Osteoarthritis (OA) is characterized by damage of articular cartilage and changes in both subchondral bone and bone at the joint margins. Clinically OA is based on joint pain, stiffness and disability in addition to osteophyte formation and joint space narrowing (JSN), and more rarely by subchondral sclerosis and subchondral cysts at radiographs. Osteoarthritis is a heterogeneous disorder traditionally classified into primary or secondary OA. The etiology of primary OA is of unknown etiology and secondary OA is the result of an underlying disorder or previous joint damage. Increasing knowledge on risk factors associated with OA has led to the recognition that OA is a multifactorial disease where aging, hormonal, genetic, and other systemic risk factors determine the individual susceptibility for the impact of local biomechanical risk factors [1].

Acromegaly is a chronic, slowly developing disease caused by a growth hormone-secreting pituitary adenoma, resulting in increased concentrations of growth hormone (GH) and insulin-like growth factor-I (IGF-I). A striking and invalidating feature is the presence of secondary OA in 50 to 75% of the patients with active or controlled acromegaly [2-7], which is 4 to 12 fold increased in comparison to the general population [8]. IGF-I, the main mediator of GH action and synthesized in the liver, muscle, adipose tissue, and epiphyseal cartilage is an important anabolic factor for bone and cartilage metabolism. IGF-I can stimulate cell proliferation of cartilage progenitor cells [9;10] and the synthesis of both proteoglycan [9-12165-168] and type II collagen. In addition, IGF-I is involved in osteoblast differentiation and bone formation [13]. Therefore IGF-I might play a role in secondary OA in acromegaly.

The aim of this study was to compare the extent, characteristics, and severity of radiographic OA features, including osteophytes and joint space narrowing, of knees and hips between patients with long-term cured acromegaly and patients with primary generalized OA in order to gain insight into the pathophysiological process of GH and IGF-I mediated OA.

PATIENTS AND METHODS

Study design and patient selection

In this case-control study radiographic OA features of both knee and hip joints in a cohort of patients with OA due to acromegaly and a cohort of controls consisting of patients with primary generalized OA were compared. We studied two cohorts which were collected and followed in the Leiden University Medical Center. The selection criterion for both acromegaly patients and OA controls was the presence of doubtful or definite osteoarthritis at one or more knee and/or hip joints according to a Kellgren and Lawrence (KL) score of 1 or more. This selection criterion was chosen to ensure that all possible OA / OA signs, i.e. also the mildest OA signs, were included.

Acromegaly patients with OA were derived from a previous reported cohort of 89 patients in long-term biochemical remission of acromegaly in which we studied the prevalence of radiographic and symptomatic OA in comparison to age-matched controls [8]. Radiographs of the hip and knee were obtained in all patients. For this study we excluded 5 patients without OA ((KL) score <1) in their hips or knees. Relevant details of treatment and patients characteristics were derived from structured patient records. Fasting blood samples were taken to assess actual GH and IGF-I concentrations.

Controls were derived from the Genetics, Arthrosis, and Progression (GARP) study, a prospective cohort study of 382 patients, aimed at identifying determinants of OA susceptibility and progression. These patients with primary generalized OA consists of sib pairs (sisters and brothers) of Dutch ancestry with symptomatic OA at multiple sites, being multiple sites in the hands or at least two of the following sites: hand, spine (cervical or lumbar), knee, hip. Details of recruitment, selection procedures and inclusion have been described elsewhere [14169]. In the GARP study, patients with secondary OA, familial syndromes with a Mendelian inheritance pattern or shortened life expectancy were excluded. For the present study we randomly selected one patient of each sib pair with doubtful or definite OA (KL score 1 or more) in at least one knee or hip. We chose to use only one sib or proband of each sib-pair, to exclude a family effect. Finally 189 OA patients were included.

The study protocol was approved by the Medical Ethics Committee, and all subjects gave written consent for their participation.

Study parameters

Clinical parameters

Both patient groups completed a standardized questionnaire concerning demographic data and medical history. The functional subscale of the Western Ontario en McMaster Universities Osteoarthitis Index (WOMAC), an OA specific questionnaire, was used to assess functional disability[15]. Scores range from 0 to 100. Higher scores reflect worse function.

In acromegaly patients, the duration of active acromegaly was estimated using start of signs and symptoms, and facial changes to the date of normalization of serum IGF-I concentration after treatment (transsphenoidal surgery alone (52%), combined with somatostatin analogs (21%), radiotherapy (17%) or after primary somatostatin analog treatment (10%). Duration of remission of acromegaly was calculated. The presence of clinically relevant hypopituitarism was recorded.

Radiographic protocol

Radiographs were obtained from all patients with acromegaly at the outpatient clinic of the Leiden University Medical Centre between September and December 2007. Radiographs from the GARP patients were obtained in between August 2000 and March 2003, using the same protocol and radiographer. Conventional radiographs of the knees (posterior-anterior (PA), weight-bearing, fixed-flexion[16]) and hips (PA, supine) were obtained from all participating patients, following a standardized protocol with a fixed film-focus distance and fixed joint position.

Radiographic assessment

For assessment of radiographic OA diagnosis, radiographs were scored by a single experienced musculoskeletal radiologist (HK) according to the KL method with the help of the original atlas [17]. The intra-reader variability as assessed with the intra-class correlation coefficient (ICC), for the knee and hip in acromegaly was 0.89 and 1.00 [14], respectively and in primary OA 0.92 and 0.95, respectively [18].

For a semi-quantitative assessment of radiographic OA severity (range 0 to 3), osteophytes and joint space narrowing of the medial and lateral tibiofemoral, and hip joints were scored by consensus opinion of two experienced readers according to the Osteoarthritis Research Society International (OARSI) atlas [19]. In cases of disagreement, the lower, more

conservative score was adopted. In acromegaly patients, the intra-reader variability was 0.96 and 0.95 for respectively osteophytes and joint space narrowing. In primary OA patients, 0.93 and 0.96 for respectively osteophytes and joint space narrowing [20]. The ICC was based on the repeat scoring of 10% of random selected radiographs.

Assays

Serum GH was measured with a sensitive immunofluorometric assay (IFMA) (Wallac, Turku, Finland), specific for the 22 kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit 0.3 μg/L; intra-assay coefficient of variation (CV) 1.6-8.4% of 0.0.1-15.4μg/L). Serum IGF-I concentration (ng/ml) was measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay variation was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/l, respectively. IGF-I levels were expressed as age and gender dependent standard deviation (SD) score, using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals [21;22125;126].

Statistical analysis

SPSS for Windows version 16.0 (SPSS inc., Chicago, IL, USA) was used for data analysis unless mentioned otherwise.

Patients with a KL≥1 in at least one hip or knee were included. If patients had bilateral hip or knee involvement, both knees and hips were used in the statistical analyses. Osteophytes and joint space narrowing at both the hip and knee were dichotomized according to the presence or absence of it and compared between acromegaly and primary OA. Prevalence of osteophytes and joint space narrowing at the hip and knee in acromegaly *vs*. primary OA was analyzed by logistic regression analysis with adjustments for age, gender, and BMI. To take into account the intra-patient effect the statistical program Stata, version 7.0 (Stata, College Station, TX, USA) was used for analyses.

Linear regression analysis was used for comparison of WOMAC function scores between acromegaly and primary OA. Standard adjustments were made for age, gender, and BMI. Unstandardized beta's (ß) were reported with 95% confidence intervals (95%CI). Additionally ß's (95% CI) were calculated for the association between total score of joint space narrowing (range 0-10) and total osteophyte score (0-6).

RESULTS

Patient and treatment characteristics

We studied 84 patients with acromegaly (52% males and 48% females), and 189 patients with primary generalized OA (20% males and 80% females). As shown in *Table 1*, the mean (SD) age of acromegaly patients was 58.1 (11.7) yrs (range 31 to 83) compared with 60.6 (7.3) yrs (range 43-78) in patients with primary OA (p=ns). Mean (SD) BMI was slightly higher in patients with acromegaly (28.7 (4.7)) than in patients with primary generalized OA (26.8 (4.0)) (p<0.001). When adjusted for sex there was no difference in BMI, height and weight between the groups.

Table 1: Clinical characteristics

	Acromegaly n=84	**Primary OA** n=189
Age (yr)	58.1 (11.7)	60.6 (7.3)
Gender (% female)	48	80
BMI (kg/m²)	28.7 (4.7)	26.8 (4.0) *
Height (cm)	176.0 (6.7)	168.6 (6.3) *
Weight (kilograms)	82.9 (13.1)	73.5 (14.1) †
KL score >1 of the hip		
Unilateral	42 (50 %)	91 (48 %)
Bilateral	38 (45%)	38 (20 %) *
KL score >1 of the knee		
Unilateral	72 (86 %)	137 (72 %)
Bilateral	56 (67 %)	98 (52 %)

Data are shown as mean (SD), unless mentioned otherwise. †: p<0.05, *:p<0.01. OA: osteoarthritis, SD: standard deviation, yrs: years, kg: kilogram, m^2: square meters, cm: centimetres, BMI: body mass index, KL: Kellgren-Lawrence.

In acromegaly patients, the mean estimated duration of active disease prior to remission was 8.7 (7.5) years and the mean estimated duration since the diagnosis was 18.5 (8.3) years. All patients were in remission for a mean of 14 years (range 2 to 28). Sustained biochemical controlled disease was maintained for at least 2 years. The mean IGF-I SD score was 0.58 (1.6) range -7.51, 2.15 SD. Eighteen (21%) patients were treated with somatostatin analogs at the time of evaluation. There were no gender differences in duration of active disease, type of

treatment, duration of remission, serum GH levels, or IGF-I SD-scores at diagnosis and during the evaluation, or in the prevalence of pituitary hormone deficiencies, including LH/FSH deficiency. Because of natural menopause females had a higher prevalence of hypogonadism, i.e. estrogen deficiency, than males.

The distribution of unilateral and bilateral KL≥1 between acromegaly and primary generalized OA was comparable in both males and females.

Osteophytes and joint space narrowing of the hip

As depicted in *Table 2*, 80 (48%) and 129 (34%) hips were scored as KL≥1 in 42 acromegaly and 91 primary OA patients, respectively. In hips with a KL of ≥1, joint space narrowing was observed in 17% of 80 hips in acromegaly and in 54% of 129 hips in primary OA (odds ratio (OR)(95% CI) 0.3 (0.10 to 0.70), whereas osteophytes were observed in 89% and 60% (OR(95% CI) 4.7 (2.51 to 7.80), respectively. Prosthesis of the hip, regarded as an end-stage of OA, was significantly less prevalent in acromegaly than in primary OA (p<0.01).

Table 2. Prevalence of osteophytes and joint space narrowing at the hip in patients with acromegaly *versus* primary OA.

	Hip		
	Acromegaly	Primary OA	Odds ratio (95% CI)
No. of hips with KL≥1	80	129	
JSN (n (%))	14 (17%)*	70 (54%)	0.3 (0.1 to 0.7)
Grade 1	6	54	
Grade 2	6	12	
Grade 3	2	4	
OP	72 (89%)	77 (60%)	4.7 (2.5 to 7.3)
Grade 1	35	54	
Grade 2	20	17	
Grade 3	17	6	
Prosthesis (n)	1	27	0.1 (0.0 to 0.6)

N represents total number of hip joints included. *Data are expressed as number of hip joints with joint space narrowing or osteophytes as scored following the Osteoarthritis Research Society International scoring. JSN: joint space narrowing, OP osteophytes, OA osteoarthritis. Data were analyzed by logistic regression analysis with adjustments for age, gender, and BMI. KL: Kellgren-Lawrence score. Number of hip joints with KL≥1 does not include joint-replacement due to end-stage osteoarthritis.

Figure 1 shows the combined prevalence of JSN and osteophytes of hips with KL≥1 in acromegaly and primary OA. Joint space narrowing without osteophytes at joint level was not present in acromegaly, whereas it reached up to 14% in primary OA. On the other hand, osteophytes without JSN was highly prevalent in acromegaly (72%) and less prevalent in primary OA (18%) (p<0.001) (*see also Figure 2*)

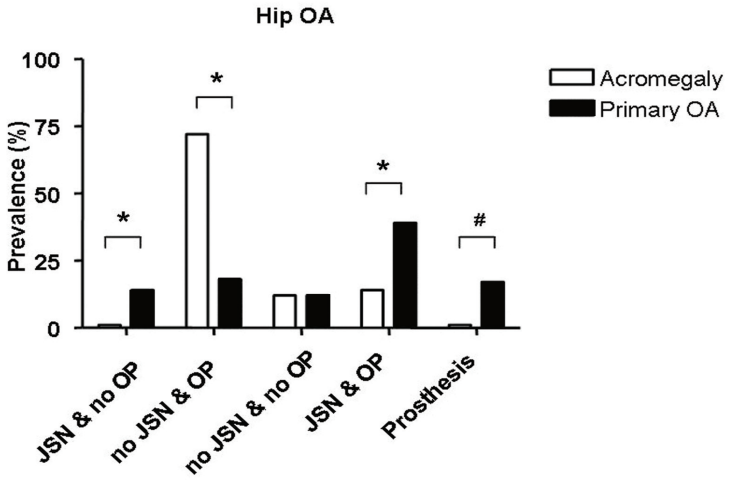


Figure 1. Distribution of joint space narrowing and osteophytes at the hip in patients with acromegaly and primary OA, all with KL≥1. Data are expressed as prevalence in percentage. # p<0.05, *p<0.001,JSN: joint space narrowing, OP: osteophytes, OA: osteoarthritis.

The distribution of JSN and osteophytes of the hip was not different between men and women in both patient groups (data not shown).

Osteophyte and joint space narrowing of the knee

In 72 (86%) acromegaly patients 128 (76%) knees were scored as KL≥1 and in 138 (73%) primary OA patients 237 (63%) knees were scored as KL≥1 (*Table 3*).

Medial knee

Joint space narrowing of the medial knee was less prevalent in acromegaly compared with primary OA (28% *vs.* 37%, respectively (OR (95% CI) 0.5 (0.28 to 1.20)). Femoral and tibial osteophytes were more prevalent in acromegaly compared with primary OA (OR (95% CI) 1.9 (1.31 to 3.80) and 3.8 (2.42 to 6.32), respectively). Severity of JSN and osteophytes (femoral

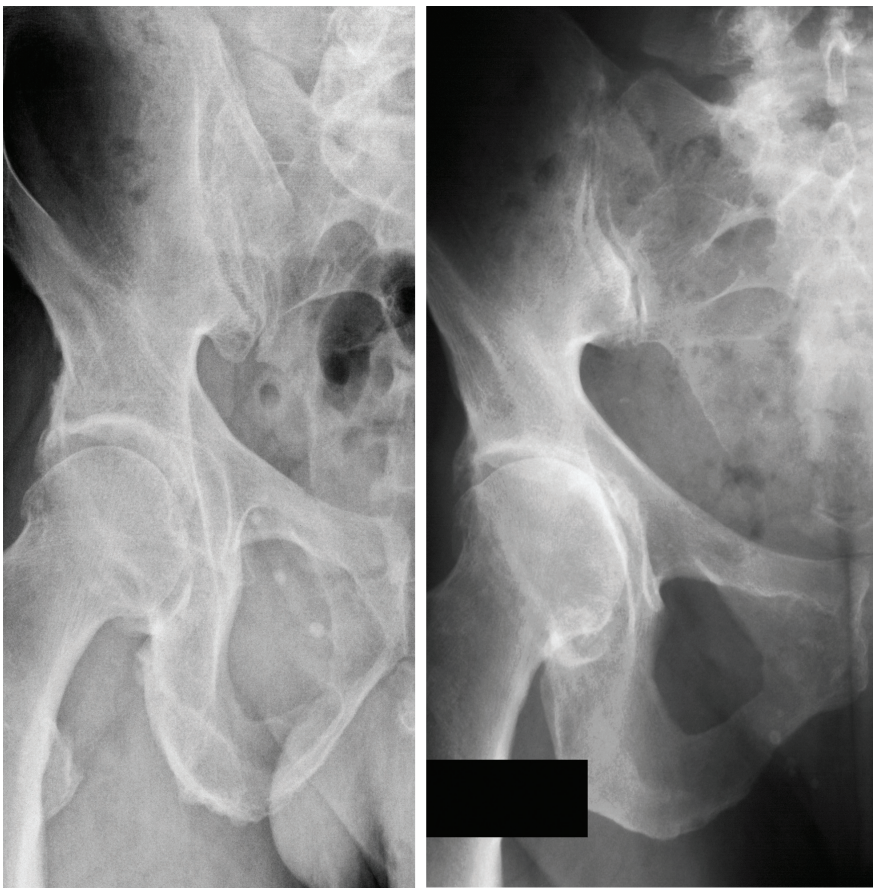


Figure 2. The difference in joint space narrowing of the hip despite a comparable degree of osteophytosis in long-term cured acromegaly (left) and primary generalized OA (right).

and tibial), and the prevalence of knee joint prosthesis were comparable between both patient groups (p=0.72, 0.69, 0.24, and 0.91, respectively).

Figure 3A shows the combined prevalence of JSN and osteophytes of the medial knee in knees with KL≥1 separate for acromegaly and primary OA. Joint space narrowing without osteophytes at joint level was less prevalent in acromegaly compared with primary OA (p=0.003). Osteophytes without JSN was more prevalent in acromegaly compared with primary OA (p<0.001).

Table 3. Prevalence of osteophytes and joint space narrowing at the knee in patients with acromegaly *vs.* primary OA.

	Medial knee			Lateral knee		
	Acro-megaly	Primary OA	Odds ratio (95% CI)	Acro-megaly	Primary OA	Odds ratio (95% CI)
No. of knees KL≥1	128	237		128	237	
JSN n(%) *	37 (28%)	89 (37%)	0.5 (0.3 to 1.2)	7 (5%)	27 (11%)	0.6 (0.4 to 1.6)
Grade 1	20	62		5	16	
Grade 2	13	22		1	7	
Grade 3	4	5		1	4	
Femoral OP	41 (31%)	36 (15%)	1.9 (1.3 to 3.8)	36 (27%)	18 (7%)	4.1 (2.7 to 7.9)
Grade 1	23	24		27	14	
Grade 2	12	10		5	4	
Grade 3	6	2		4	-	
Tibial OP	82 (63%)	69 (29%)	3.8 (2.4 to 6.3)	87 (66%)	41 (17%)	9.9 (5.8 to 17.9)
Grade 1	57	68		64	36	
Grade 2	17	1		18	4	
Grade 3	8	-		5	1	
Prosthesis	3	4	1.4 (0.4 to 7.9)	3	4	1.4 (0.2 to 8.0)

N represents total number of knee joints included. *Data are expressed as number of medial or lateral knees with joint space narrowing and osteophytes as scored following the Osteoarthritis Research Society International scoring. JSN: joint space narrowing, OP: osteophytes, OA: osteoarthritis. Data were analyzed by logistic regression analyses with adjustments for age, gender, and BMI. KL: Kellgren-Lawrence score. Number of knee joints KL≥1 does not include joint-replacement due to end-stage osteoarthritis.

Lateral knee

Joint space narrowing of the lateral knee was equally prevalent in acromegaly and primary OA. Femoral and tibial osteophytes were more prevalent in acromegaly compared with primary OA (OR (95% CI) 4.1 (2.68 to 7.85), and 9.9 (5.75 to 17.85), respectively). Severity of JSN was less in acromegaly compared with primary OA (p=0.03). Severity of osteophytes was comparable between both patient groups (p=0.58).

Figure 3B shows the combined prevalence of JSN and osteophytes of the lateral knee in knees with KL≥1. Joint space narrowing without osteophytes at joint level was almost not seen in both patient groups.

Figure 3A

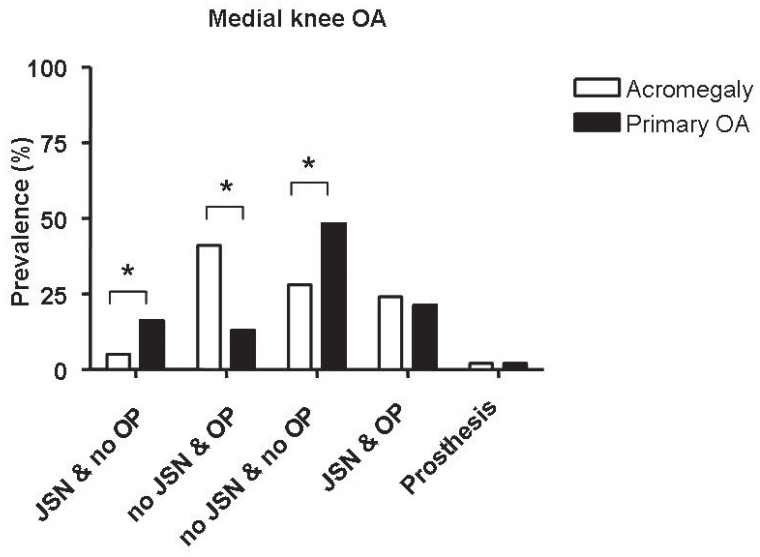


Figure 3B

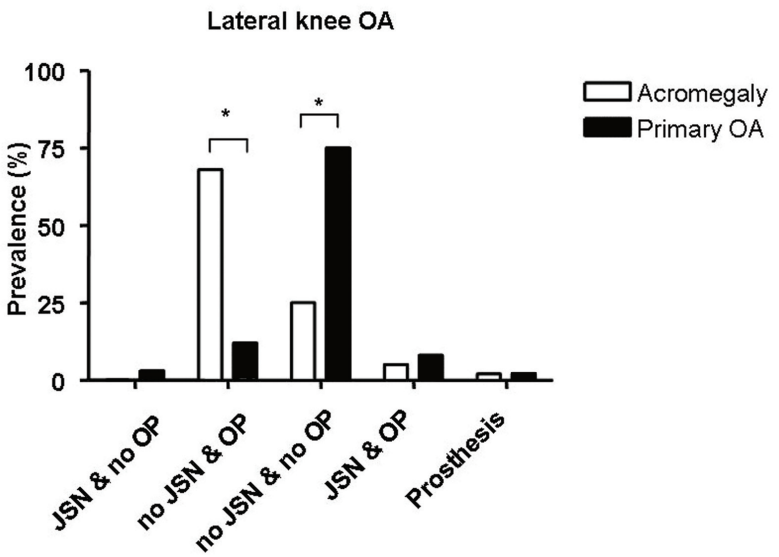


Figure 3A and 3B. Distribution of joint space narrowing and osteophytes at the medial and lateral knee in patients with acromegaly and primary OA, all with KL≥1. Data are expressed as prevalence in percentage. # $p<0.05$, JSN: joint space narrowing, OP: osteophytes, OA: osteoarthritis.

In accordance with the observations for the medial knee, patients with acromegaly had a higher prevalence of OA features of the lateral knee (based on JSN, osteophytes, or a combination of both) (75%) compared with primary OA (25%), p<0.001 (*see also Figure 4*).

The distribution of JSN and osteophytes of the medial and lateral knee was not different between men and women in both patient groups (data not shown).

Functional disability

Acromegaly patients scored significantly better on the WOMAC function subscale than patients with primary OA (18.5 ± 18.6 vs. 28.6 ± 21.9, p<0.001) and this remained significant after adjustment for age, gender, and BMI. The maximum WOMAC function subscale score in acromegaly was 69.1 and in primary OA 94.2.

For the total osteophyte score (range 0-10) and total JSN score (range 0-6) of the hips and knees, linear regression analysis demonstrated that JSN (unstandardized beta (ß) 3.8, 95% CI 0.29 to 5.81), not osteophytosis (ß 0.7, 95% CI -1.35 to 2.74) was positively correlated with WOMAC scores, adjusted for age, gender, and BMI.

DISCUSSION

The OA phenotype in secondary OA present in patients with long-term cured acromegaly differs from that in patients with primary OA with generalized nature. In comparison with primary generalized OA, secondary OA in hips and knees in patients with long-term cured acromegaly was characterized predominantly by osteophytes and these osteophytes were observed frequently without JSN indicating preservation of articular cartilage in these joints. Patients with acromegaly reported less functional disability of the hips and knees than patients with primary generalized OA.

Osteophyte formation was the predominant finding in acromegalic patients. Although a common feature of OA, the clinical relevance and pathophysiology of osteophytes is not fully understood [23170]. Osteophyte formation in OA may be seen as an attempt of repair by broadening the joint surface and stabilizing the degenerating joint. Otherwise it might be the result of an altered internal joint milieu resulting in chondrogenesis of precursor cells in the

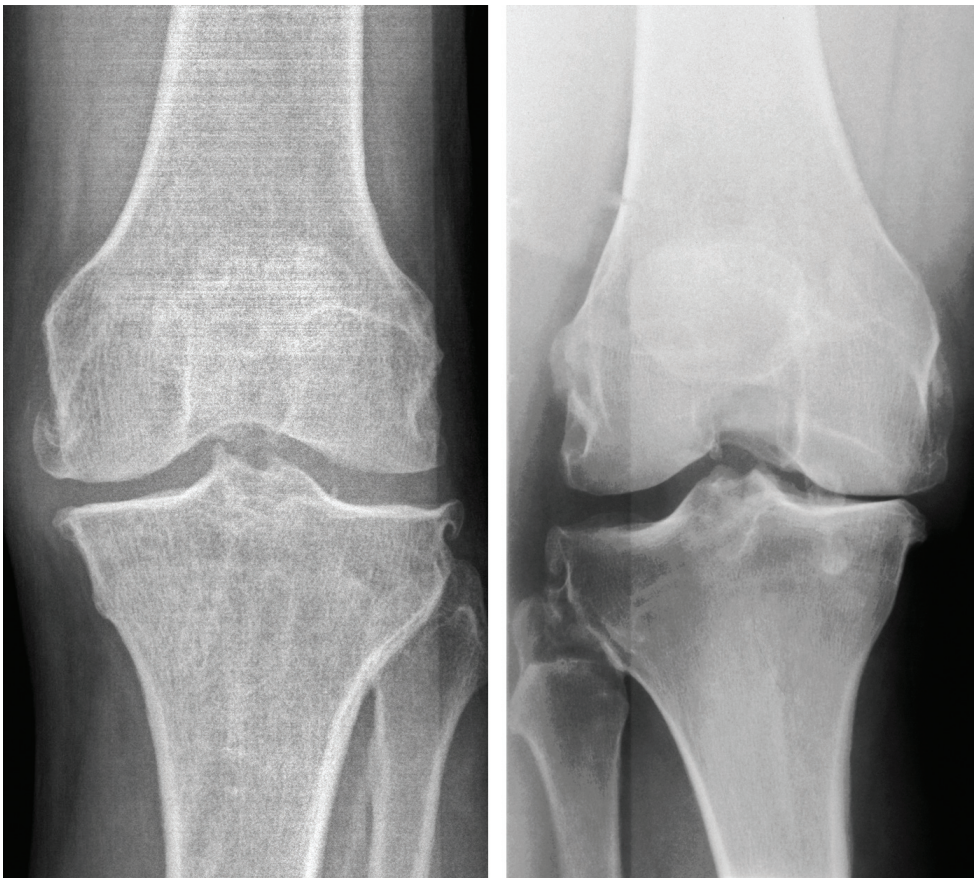


Figure 4. The difference in joint space narrowing of the medial knee despite a comparable degree of osteophytosis in long-term cured acromegaly (left) and primary generalized OA (right).

periosteum and synovial lining. Further elucidation of factors involved in osteophyte formation will improve the understanding of homeostasis in OA joints. The present observation that in well-controlled acromegalic patients, who had pathologically elevated levels of circulating GH and IGF-I in the past, predominantly osteophytosis without JSN is seen suggests that GH and/or IGF-I are implicated in the initiation of osteophyte formation. This is in line with findings from Okazaki *et al*. who demonstrated in mice that the elevation of GH stimulated IGF-I expression in the synovial cells and peripheral cell adjacent to the articular cartilage seemed to lead to the initiation of osteophyte formation. IGF-I was thought to stimulate both cell proliferation and early differentiation in chondrogenesis during osteophyte formation in an autocrine and/or paracrine fashion. This might suggest that other factors seemed to be directly

involved in the osteophyte formation rather than simply being a secondary feature following OA changes [24].

Arthropathy in acromegaly starts during the active phase of the disease with hypertrophy of cartilage and soft-tissue, resulting in typical findings at radiological examination, i.e. widening of joint spaces and peri-articular soft-tissue hypertrophy [2586]. Several studies showed that, despite hypertrophy and cartilage repair that are known to be stimulated *in vivo* by GH and IGF-I [26;27], premature degeneration occurs as a result of poor perfusion and minor trauma [5]. However, in our acromegalic patients, studied after long-term remission of the disease, articular cartilage seems to be maintained, as reflected by lack of JSN, despite severe osteophytes and to be independent of the type of treatment or duration of active disease.

Despite the severe OA in acromegaly, joint-replacement surgery as end-stage of OA is apparently less frequently performed than in patients with primary generalized OA. Patients with acromegaly showed better WOMAC function scores than patients with primary generalized OA, despite a higher prevalence of osteophytes. The preserved joint spaces demonstrated in the hip and knee in acromegaly patients may protect against pain caused by osteophytes and, therefore, prevent acromegaly patients from a decrease in functional capability. The percentage of males was higher in the patients with acromegaly when compared with the patients with primary generalized OA. Since women in general report more pain (leading to functional impairment) than men [28164], the more frequently reported functional impairments in primary generalized OA compared with acromegaly might be the result of more pain and/or a gender effect [28]. Nonetheless, the differences in WOMAC scores persisted despite adjustment for gender, implicating that acromegaly is associated with less functional impairment than primary generalized OA.

In this study patients were selected on the basis of a KL score of ≥1 at the knee and hip joint. Our research question was to study the distribution of OA features rather than the prevalence of OA. As a result of this, patients and controls differed in baseline characteristics and risk factors for OA, such as gender distribution and BMI. The relative number of males was higher in the acromegaly group than in the primary generalized OA group, because acromegaly is equally prevalent in men and women, whereas primary generalized OA is more prevalent in (post-menopausal) women. We repeated all analyses separately for both males and females and this did not influence our results, we therefore decided to report just on the whole group, with adjustment for gender. In addition, acromegaly patients had a slightly higher BMI than patients

with primary generalized OA. To prevent any potential bias by these differences in patient characteristics, we adjusted all analyses for age, gender, and BMI.

Radiographs of the patients with primary generalized OA were obtained several years before radiographs of acromegaly patients. However, all radiographs were performed by the same experienced radiographer following the same protocol, and analyzed by the same experienced musculoskeletal radiologist. Joint space widening *per sé* was not scored, and we were therefore unable to differentiate between preserved or widened joint spaces. However, quantitative measurement of joint space widening would not have provided us with information about the quality of the cartilage, and therefore future MRI studies will be helpful.

In conclusion, acromegaly patients with biochemical cured disease for a mean of 14 years still show signs of the anabolic effects of GH and IGF-I. Osteophyte formation was observed without the degenerative changes in the articular cartilage that are commonly seen in primary generalized OA. This evidence suggests that other factors seemed to be directly involved in the osteophyte formation rather than simply being a secondary feature following OA changes. In addition, the GH and IGF-I system may protect against JSN.

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Chapter 6.

WIDE JOINT SPACES IN HAND RADIOGRAPHS OF LONG-TERM CONTROLLED ACROMEGALY PATIENTS ASSESSED BY AUTOMATED IMAGE ANALYSIS IN COMPARISON WITH HEALTHY CONTROLS; IN RELATION TO DISEASE ACTIVITY AND SYMPTOMS.

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ABSTRACT

Background: Acromegalic arthropathy is an invalidating complication of acromegaly and although it shares features with primary osteoarthritis, joint space narrowing is infrequently seen in acromegalic patients. The late effects of acromegaly on hand joints have not been characterized.

Aim: Three aims of the present study were 1) comparing joint space width of hand joints between patients with long-term controlled acromegaly and age- and gender-matched controls, 2) identification of factors associated with joint space width, and 3) assessment of the relation between joint space abnormalities and complaints of joint pain in acromegaly patients.

Methods: Cross-sectional study in 89 patients (age 58 ± 12 yr, 49% women) with long-term controlled acromegaly and 471 controls without hand symptoms (age 46 ± 12, 42% women). Radiological joint space width of hand joints were measured by automated image analysis.

Results: Patients with long-term remission of acromegaly had 20-24% wider mean joint spaces at the MCP, PIP and DIP-joints ($p < 0.001$). Mean joint space width of all hand joints exceeded the 95th percentile of controls in 64% of patients. Only 9% of patients had mean joint spaces below the 50th percentile of controls. Higher GH and IGF-I concentrations at the time of diagnosis were associated with larger joint space width at the MCP-joints. Duration of follow-up of acromegaly was not related to joint space. In males, not in females, increased joint spaces were associated with more self-reported pain ($P=0.02$).

Conclusion: The increased joint spaces of the hand joints in long-term controlled acromegaly patients characterize an aspect of the late manifestations of well-controlled acromegaly and indicate persistent, possibly irreversible, cartilage hypertrophy.

INTRODUCTION

Acromegaly is a rare disease caused by overproduction of growth hormone (GH) from a pituitary adenoma[1]. This syndrome is characterized by a variety of complaints, increased co-morbidity and mortality. On radiographs, active acromegaly is characterized by increased joint space width, amongst other features[2]. However, the effects of cure or biochemical control of the disease on joint space width has not been fully elucidated. Nonetheless, patients with acromegaly have a high prevalence of joint pain and arthropathy even after long-term successful biochemical cure[3]. Although the changes in joints of patients with controlled acromegaly match the criteria of both radiological and clinical osteoarthritis (OA)[4], radiological characteristics differ between controlled acromegaly and primary OA: patients with acromegaly have more severe osteophytosis, but less joint space narrowing[4]. In addition, despite long-term control of acromegaly, the joint spaces appear to remain abnormally wide, reflecting irreversible cartilage hypertrophy. There are no quantitative data available on the magnitude of joint space width in patients with acromegaly compared with healthy controls.

The aim of the present study is to quantify joint spaces in hand radiographs of patients with long-term controlled acromegaly in comparison to age and gender related controls by means of semi-automated image analysis software[5]. The second aim was to explore potential factors that are associated with the size of the joint space in acromegaly patients, such as age at diagnosis, markers of severity, duration of acromegaly, and duration of remission. The third aim was to explore the relation between joint space width and specific joint complaints in these patients with controlled acromegaly.

PATIENT AND METHODS

Patients

In this case-control study we compared joint space width in hand radiographs between patients with long-term controlled acromegaly and controls derived from two epidemiological studies performed in our institution.

Acromegalic patients

All patients with long-term remission of acromegaly (i.e. more than 2 yr), according to a normal IGF-I concentration for age, were selected from our database. The Leiden Acromegaly Database includes baseline and follow-up results of all patients that have been treated in the Leiden University Medical Center, a tertial referral center with a dedicated pituitary surgeon, from 1977 onwards. They were invited to participate in a cross-sectional study aimed at evaluating the extent of joint disease in long-term controlled acromegaly patients[4]. We invited 126 patients. Eighty-nine patients (71%) agreed to participate.

The majority of patients was treated by primary transsphenoidal surgery, if necessary followed by additional treatment. Control of GH oversecretion was achieved by curative transsphenoidal surgery (n=49), surgery and radiotherapy (n=15), primary surgery followed by somatostatin analog treatment (n=15), all three modes of treatment (n=3), and primary somatostatin analog treatment (n=7). At the study visit, all patients were in biochemical remission for a mean of 14 years. Biochemical remission was defined as normal glucose suppressed serum GH concentrations below 0.38 mcg/L (in patients not treated with somatostatin analogs), serum GH concentrations below 1.9 μg/liter and normal IGF-I levels for age at yearly follow-up visits[6].

Controls

To compare the hand joint space width in acromegaly patients with normative data from control subjects, we composed an age- and gender matched control group from the database of The Leiden Early Arthritis Clinic (EAC, n=167)[7] and patients from a follow-up visit of the so called KART study (*vide infra*) (n=304). None of these controls had hand-symptoms. The EAC is a prospective study started in 1993 and includes patients with early arthritis with symptoms for less than 2 years[7]. The goal is to detect inflammatory disorders early in the disease state and to treat these accordingly. All parameters such as medical history and physical-diagnostics examination were obtained by trained staff consisting of rheumatologists and research nurses. Conventional radiographs of hands and feet and affected joints were obtained in all patients at baseline. For the purpose of the present study, we made a selection of patients without hand symptoms and used the hand radiographs of their inclusion visit. The KART study is an epidemiological study, which includes patients with traumatic or non-traumatic knee complaints. At a baseline and follow-up visit 10 years later, routine radiographs of the hands were obtained

in all patients. Since patients were not included in the KART study on the basis of hand joint pathology, we assumed that their hand joints are a valid sample of the general population.

All study protocols were approved by the Medical Ethics Committee. Written informed consent was given by all patients and controls who participated in the studies.

Diagnosis of osteoarthritis

In all subjects clinical OA was scored according to the criteria proposed by the American College of Rheumatology (ACR)[8].

Study parameters

Conventional radiographs of the hands

From all subjects with acromegaly and all KART controls, standardized digital hand radiographs were taken by the same radiological technician with standardized focus-detector distances. Of the EAC controls, 133 radiographs were analog and 39 were digital. To analyze the analog radiographs, they were digitized (VXR-12, VIDAR System Corporation, Herndon, VA). Hand joints were evaluated for presence of radiological OA using the Kellgren and Lawrence classification system [9;10]. This is a 5-point ranking scale (0 = normal, 4= severe OA). A score of 2 or higher is considered (mild) OA.

Image processing for determination of joint space width

Recently, at our institute, van 't Klooster *et al.* developed a semi-automatic quantitative method to measure joint space width in hand radiographs with a good correlation with semi-quantitative scoring of joint space narrowing[5;8]. Therefore, joint space width of the hands in the current study were measured using this semi-automatic image analysis program. First, this program loads the digital/digitized radiographs and the user separates the left and the right hand.

Subsequently, in each hand, 12 joints, being four distal interphalangeal (DIP), four proximal interphalangeal (PIP) and second to fifth metacarpo-phalangeal (MCP) joints, were analyzed semi-automatically in four steps: 1) identification of the joint locations; 2) detection of the proximal and distal margins of each joint; 3) measurement of the width of each proximal phalange, as a reference for the joint space width measurement interval; and 4) calculation of the width of the joint space within the determined measurement interval.

The automatic results of each step were reviewed by an expert (S.H.M) and corrected

if needed. For each subject, 24 measured joint spaces were reported, as well as the thickness of the proximal phalanges. The joints of the thumb were omitted from the analysis since they were not perpendicular to the imaging plane and could not be assessed reliably. The individual values and the mean results of áll joints, the MCP, PIP and DIP joints were used for analysis. The intra-individual variation between repeat readings (n=25) was low (< 5%), reflected by a kappa of >0.9. Within the EAC cohort we compared digitized analog and digital radiographs. There was no significant difference between both techniques in any of the mean joint spaces (P=0.30).

Pain

Self-reported painful joints were recorded on a standard diagram including all 24 hand joints. Pain at structural physical examination was recorded systematically for all 24 joints.

Disease characteristics in acromegaly patients

In order to estimate the duration of exposure to GH excess, the estimated disease duration prior to diagnosis and disease duration prior to remission were carefully assessed. Estimated onset of disease was based on the start of signs and symptoms and changes on photographs. The date of remission was the date of normalization of GH and IGF-I concentrations. The duration of follow-up was the period between diagnosis and study visit. The duration of remission was the period between normalization of GH/ IGF-I concentrations and study visit. During the study visit actual GH and IGF-I concentrations were measured. Pretreatment IGF-I / GH concentrations were recorded from the database. Due to the unavailability of IGF-I assays in our center prior to 1986, pretreatment IGF-I levels were available in 67 of 89 patients. The product of the duration of disease and the severity reflected by the logarithmically transformed GH/ IGF-I concentrations were used as a marker of exposure to GH excess.

Assays

At the study visit, and after 1993, serum GH level was measured with a sensitive immunofluorometric assay (Wallac, Turku, Finland), specific for the 22 kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit 0.3 μg/L; intra-assay coefficient of variation (CV) 1.6-8.4% of 0.1-15.4μg/L). Before 1992, GH was measured by RIA (Biolab,Serona, Coissins, Switzerland) calibrated against

WHO IRP 66/21(detection limit: 0.5 mU/l, interassay CV: < 5%; for the conversion of mg/l to mU/l, multiply by 2). From1986 to 2005, serum IGF-I concentrations were determined by RIA (Incstar, Stillwater, MN) with a detection limit of 1.5 nmol/liter and an interassay CV less than 11%. IGF-I is expressed as SD scores for age- and gender-related normal levels determined in the same laboratory [11]. From 2005, serum IGF-I concentration (ng/ml) was measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA). The intra-assay variation was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/l, respectively. IGF-I levels were expressed as age and gender dependent standard deviation (SD) score, using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals[12;13].

Statistical analysis

For data analysis, PASW 17 for Windows was used (SPSS Inc. Chicago IL). We reported the mean ± SD unless specified otherwise. For the comparison of the mean joint space width between the patients and the controls we used ANOVA analysis with adjustments for age and gender. For the correlation between the joint space width and the disease characteristics of acromegaly we used Pearson's correlation and linear regression analysis. Tertiles of serum GH and IGF-I concentrations and sex were used as fixed factors, age was used as a covariate. (Un)-standardized beta's (ß) were reported with 95% confidence intervals (95%CI). Mean joints spaces were dichotomized to normal (< 95th percentile of female or male controls per joint site) or increased (>95th percentile of female or male controls per joint site).

In addition we studied self-reported pain in relation to the joint space of the individual joints. Within acromegaly patients, individual joint space width was re-coded in tentiles per joint (n=12), separately for males and females. The tentile-score was used as a covariate in a general estimated equations model to take into account the intra-patient effect.

Results

Baseline characteristics *(Table 1)*

The mean age of the 89 included acromegaly patients was 58.2 ± 11.5 years and there was an equal gender distribution (49% females). At diagnosis, the mean IGF-I SD score was 7.4 ± 4.7

(n=67) and mean GH concentrations were 44.5 ± 63 mcg/L. The estimated mean duration of acromegaly prior to diagnosis was 8.8 ± 7.4 years. All patients were in biochemical remission of acromegaly and the mean duration of remission was 14 ± 6 years. The majority of the patients with acromegaly (84%) reported complaints of pain and stiffness of the hand at the time of the current evaluation. The prevalence of clinical OA of the hand in acromegaly according to the ACR criteria was 40%. The mean age of the 470 controls was 46 ± 12 years and 42% were females.

Table 1. Baseline characteristics

	Acromegaly	Controls
N	89	470
Age (yr)	58 ± 12	46 ± 12*
Gender (% female)	49	42
BMI (kg/m²)	28.5 ± 4.7	NA
Pre-treatment IGF-I SD score	7.4 ± 4.7	NA
Disease duration (yr)	8.8 ± 7.4	NA
Duration of remission (yr)	14.1 ± 6.2	NA
Age at diagnosis (yr)	41 ± 12	NA
Clinical hand osteoarthritis(%)	40	NA

Data are shown as mean ± SD, NA: not assessed, yr: years, *p<0.01

Hand joint space width in acromegaly and controls *(Table 2, Figure 1)*

In patients and in controls, males had wider joint spaces and larger proximal phalange widths than females (P<0.001). There was a negative correlation between joint space width and age for the MCP (R -0.31, P<0.003) and DIP joints in acromegalic patients, (R 0.22, P<0.04).

The mean joint space width of all 24 joints measured in the hands of acromegaly patients was increased compared with controls (mean difference adjusted for age and gender 0.26 mm (95% CI 0.22-0.30), P<0.001). Mean MCP joint spaces were increased by 24%, mean PIP joint spaces by 21%, and mean DIP joint spaces by 20% in patients compared with controls *(Table 2)*. The mean width of the proximal phalanx was also increased in acromegaly patients, by 7%, p <0.001).

Mean joint spaces above the 95th percentile of controls were observed in 53% of the MCP, 45% of the PIP, 27% of the DIP joint sites and in 64% (all 24 measured joints) of acromegalic patients. Only 9% of the acromegalic patients had mean joint space width (all joints) below the 50th percentile of controls.

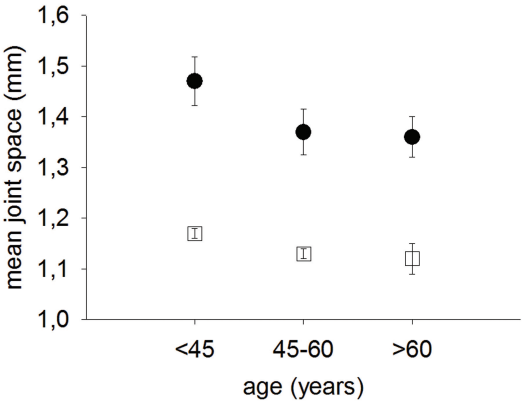


Figure 1. Increased mean joint space width in MCP joints in patients with acromegaly in comparison with controls. Dots are patients, squares are controls. Error bars represent means of 3 age groups.

Table 2. Difference in mean joint space width between acromegaly patients and controls.

Mean joint space width (mm)	Acromegaly patients N=89	Controls N=470	Mean difference	Adjusted mean difference*	P-value ** (2-tailed)
All joints	1.38 ± 0.25	1.15 ± 0.17	0.23 (0.19-0.27)	0.26 (0.22-0.30)	< 0.001
MCP	1.95 ± 0.34	1.61 ± 0.23	0.34 (0.28-0.40)	0.38 (0.33-0.44)	< 0.001
PIP	1.15 ± 0.23	0.95 ± 0.15	0.20 (0.16-0.24)	0.20 (0.17-0.25)	< 0.001
DIP	1.04 ± 0.30	0.89 ± 1.78	0.14 (0.10-0.19)	0.18 (0.14-0.23)	< 0.001
Mean width proximal phalanx (mm)	10.19 ± 1.26	9.40 ± 1.04	0.79 (0.54-1.0)	0.72 (0.55-0.90)	< 0.001

All measurements are expressed in millimeters ± SD. JSW= joint space width, MCP= metacarpophalangeal joint, PIP= proximal interphalangeal joint, DIP=distal interphalangeal joint. *Adjustments were made for age and gender. **All mean and adjusted mean differences were significant at the level P <0.001.

Influence of (previous) activity of acromegaly on joint space *(Table 3)*

For both MCP and PIP joints, there was a positive correlation between joint space width and pre-treatment serum GH and IGF-I concentrations (both R = 0.35, P=0.001). In contrast with the other hand joints, DIP joints were not significantly associated with parameters of acromegalic disease severity. A negative correlation was present between joint space width and age

at diagnosis (R – 0.28, P=0.009).

Using linear regression analysis, tertiles of pre-treatment GH and IGF-I SD concentrations were significantly associated with joint space width (mean of MCP joints and mean of all joints) with and without adjustments for age and gender (*Figure 2*).

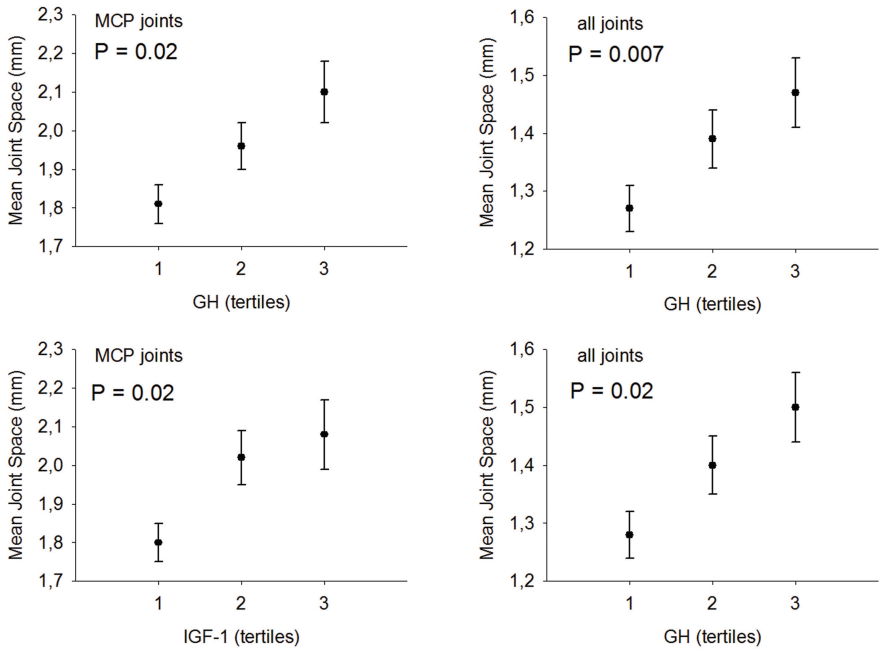


Figure 2. Mean joint space (± 95% CI) increases with increasing pre-treatment GH and IGF-I concentrations (left: mean joint space of MCP joints, right: mean joint space of all 24 measured hand joints, upper panels: tertiles of GH concentrations; lower panels: tertiles of IGF-I SD scores,)

A younger age at diagnosis of acromegaly was associated with larger joint spaces also after adjustments for the duration of follow-up. The mean joint space was not associated with different treatment modalities in this long-term well-controlled cohort of acromegaly patients. Joint space width was not associated with current biochemical markers of disease activity in this biochemically controlled population, neither was it associated with duration of remission or duration of follow-up.

Table 3. Linear regression analysis of factors influencing joint space width in MCP joints and all hand joints in acromegaly patients.

	MCP joints (N=8)	All joints (N=24)
Tertiles of pre-treatment GH	0.09 (0.03-0.184)	0.07(0.00 – 0.14)
Tertiles of pre-treatment IGF-I SD	0.14 (0.05-0.23)	0.09 (0.027-0.15)
Pre-treatment IGF-I SD score	0.018(0.02-0.035)	0.012(0.001-0.023)
Age at diagnosis (yr)	NS	NS

Data reported are unstandardized B (95% confidence interval) with adjustments for sex and present age. GH and IGF-I is categorized in groups according to three percentiles (GH 1st <12.5 mcg/L, 2nd 12.5-40 mcg/L, 3rd >40 mcg/L and IGF-I SD 1st <5.5, 2nd 5.5-9, 3rd >9). PIP and DIP joints were not influenced by markers of disease severity. NS = not significant.

Joint space width and relation with complaints

In general, there was no gender difference between the number of patients with self-reported pain in any joint of the hands. At individual joint level, as assessed with the structured diagram of 24 joints, pain was reported significantly more frequently by females in 16% of DIP joints, 21% of PIP joints and 10% of MCP joints in contrast with male patients who reported pain in 6.2% of DIP and PIP joints and 11.8% of MCP joints.

Joint pain was reported more frequently in joints with larger joint space width, when considering joint space width in tentiles per joint separately for gender within acromegaly patients (β for male patient 0.15 (0.05-0.26) P = 0.004 and for female patients β 0.04 (-0.02 – 0.16) p = ns) *(Figure 3)*.

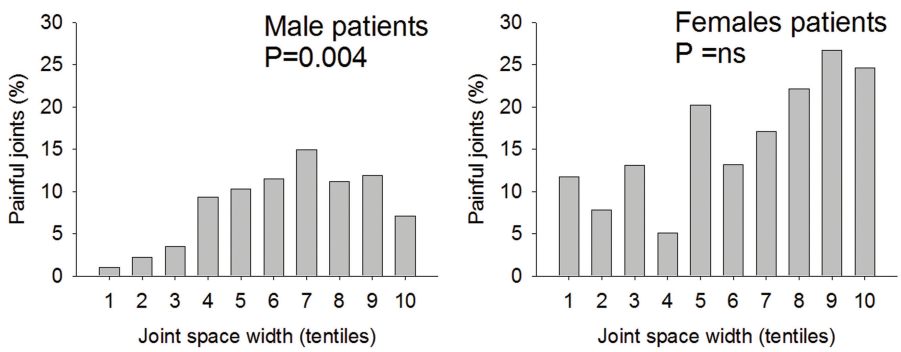


Figure 3. Distribution of self-reported pain in patients with increasing joint space width (in tentiles).

DISCUSSION

The aim of the present study was to quantify joint space width of hand joints in patients with long-term controlled acromegaly compared with age and gender matched controls. The data indicate that acromegaly patients have increased mean joint spaces in their hand joints, indicating persistent cartilage hypertrophy despite long-term control of the disease. In addition, joint space width was related to GH and IGF-I concentrations at the time of initial diagnosis, but not to current concentrations of GH and IGF-I, disease duration, or duration of follow-up. Finally, hand joints in which patients reported pain, were wider than asymptomatic hand joints. These observations characterize the late manifestations of well-controlled acromegaly in hand joints and indicate persistent, possibly irreversible, cartilage hypertrophy in these hand joints.

Arthropathy is an invalidating complication of active and well-controlled acromegaly[14]. However, little is known about the clinical characteristics and progression or regression of acromegalic joint disease after induction of biochemical remission. We recently reported an increased prevalence of clinical and radiological osteoarthritis in patients with long-term controlled acromegaly[4]. A remarkable finding of that study was the combination of severe osteophytosis with normal or even widened joint spaces. In the present study, we document that the joint space width of the hand joints in acromegaly, especially the PIP and MCP joints, were clearly wider than those of controls. The increased joint spaces of the small hand joints, which we measured in the present study, are in accordance with the appearance of wide joint spaces on hip and knee radiographs of long-term cured acromegalic patients[4]. Therefore, joint space widening appears to be a persistent feature of long-term controlled acromegaly in both small and large, weight- and non-weight bearing joints.

GH and IGF-I act both as growth factors for chondrocytes[14]. In accordance with this notion, joint space width correlated positively with pre-treatment GH and IGF-I concentrations. Age at diagnosis was negatively related to joint space width, compatible with biochemically more active acromegaly at young age or alternatively, with cartilage that is more responsive to GH induced cartilage hypertrophy in younger subjects. Joint spaces width was related to markers of disease severity at the time of initial diagnosis, but not to the duration of remission. This may suggest that cartilage hypertrophy, induced during the active phase of the disease, is maintained in the long-term after cure of acromegaly. Apparently, the transient period of active

acromegaly has resulted in a higher persistent set point of joint cartilage volume. Some short-term studies have suggested that cartilage hypertrophy may improve after successful treatment of acromegaly[15;16]. However, in our study mean joint space width was increased despite long-term control of acromegaly. Follow-up studies are required to determine whether this cartilage hypertrophy is stable or whether it progresses or regresses during controlled disease.

We used a new, semi-automated technique to quantify joint space width[5]. This method is accurate and efficient and can be used to determine joint space narrowing in patients with OA and has a good agreement with a semi-quantitative scoring system of joint space [17]. This method was able to detect quantitative differences in large joint space width in acromegaly patients compared with controls. Moreover, within the group of acromegalic patients, this method was sensitive enough to relate joint space width to markers of disease severity of acromegaly. The large number of abnormally wide joint spaces, which we defined as higher than the 95th percentile of controls, may even be underestimated because of the lower age of the controls. Joints with increased joint space width were more frequently painful than joints within the range of normal, especially in male patients. In this patients group, cartilage loss is probably not the main cause of pain and therefore, the reverse, i.e. cartilage hypertrophy, should be recognized as a risk factor for pain. Further study is needed to assess which factors determine pain, such as osteophytosis, or instability of joints.

In conclusion, long-term biochemical control of acromegaly is associated with wider joint spaces of the hand joints. The increased joint spaces characterize an aspect of the late manifestations of well-controlled acromegaly and indicate persistent, possibly irreversible, cartilage hypertrophy.

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Chapter 7.

CLINICAL OSTEOARTHRITIS PREDICTS PHYSICAL AND PSYCHOLOGICAL QUALITY OF LIFE IN ACROMEGALY PATIENTS.

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ABSTRACT

Objective: Quality of life (QoL) is decreased in patients with long term control of acromegaly. In addition, these patients suffer from irreversible osteoarthritis. The aim of this study was to assess the impact of joint-specific complaints, clinical and radiological signs of arthropathy on different aspects of quality of life in patients with acromegaly after long-term disease control.

Design: Cross-sectional study

Methods: We studied 58 patients (31 males), mean age 60 years (range 32-81 years), with strict biochemical control of acromegaly for a mean duration of 15 years. QoL was assessed by four health-related QoL questionnaires (HADS, MFI-20, NHP, SF-36) and one disease specific QoL questionnaire (ACROQOL). The outcomes of these questionnaires were compared with joint-specific self-reported complaints of pain/stiffness, clinical osteoarthritis based on American College of Rheumatology (ACR) criteria and radiological osteoarthritis based on the Kellgren-Lawrence (KL) scoring method.

Results: Long-term cured acromegaly patients had high pain scores of the spine, knee, and hip which limited physical functioning (mean difference -27.0, 95%-CI -9.5,-41.0) and psychological well-being (mean difference -44.4, 95%-CI -26.1,-60.9) (SF-36). Clinical osteoarthritis of the spine was associated mostly with impaired QoL scores, on physical, social, and emotional functioning, and on anxiety and depression. Remarkably, radiological osteoarthritis was not associated with impaired QoL.

Conclusion: These findings accentuate the importance of recognition of the clinical manifestations of arthropathy in patients with acromegaly despite long-term disease control.

INTRODUCTION

In acromegaly, excessive growth hormone (GH) and insulin-like growth factor type I (IGF-I) concentrations cause gradual changes in facial and acral appearances as well as in many internal tissues. After successful treatment of the GH excess, soft-tissue swelling diminishes and symptoms related to GH excess, such as perspiration and paresthesias, decrease. However, other acromegalic features, for instance changes in bone and cartilage, persist due to irreversible changes resulting in mild to severe degenerative joint disease [1,2] .

Quality of life (QoL) in acromegaly is impaired according to several cross-sectional studies in patients with active and controlled acromegaly[3-7]. Moreover, after long-term control, QoL scores remain impaired in comparison with healthy controls[8] ,without improvement in QoL scores after 4 years of follow-up with ongoing disease control[9]. Several factors affect QoL in acromegaly including activity of the disease, treatment modalities, and joint complaints. Several cross-sectional studies reported better QoL scores in patients with disease control in comparison with active disease[3-6]. In addition, longitudinal studies reported a beneficial effect of medical treatment on QoL[10-11]. The mode of treatment has also been associated with impaired QoL, i.e. irradiated patients had a greater and more progressive impairment in QoL[4,8,9], whereas after SMS analog treatment an impaired QoL was observed only in a single study[3]. Another factor that predicted impaired QoL on both physical and mental items in controlled acromegaly was the presence of self-reported joint-related complaints[1]. However, the scoring of these subjective joint complaints was crude and not joint-site specific. Recently, we demonstrated a high prevalence of clinical and radiological osteoarthritis in patients with long-term controlled acromegaly, when compared with controls, with relative risks up to 12[12]. The impact of acromegalic arthropathy, assessed by objective evaluation, on QoL is currently unknown. However, in general, musculoskeletal disorders and osteoarthritis caused by non-traumatic hip and knee disorders have a substantial negative impact on QoL, especially on the subscales with physical components, as demonstrated by a systematic review[13].

The aim of this study was to explain at least part of the variation in impaired QoL among long-term cured acromegaly patients by variations in osteoarthritis. Therefore, we explored the impact of subjective joint specific complaints, and objective clinical and radiological osteoarthritis on the different aspects of quality of life assessed by validated Qol questionnaires

in patients with long-term control of acromegaly. For this purpose, we carefully characterized the clinical aspects of joint disease according to the definitions of clinical and radiological osteoarthritis.

PATIENTS AND METHODS

Patients

All patients with acromegaly who were in long-term follow-up in our centre were invited to participate in a cross-sectional study on arthropathy[12]. The inclusion criterion for the present analysis, which aimed to address the relation between arthropathy and QoL, was a stable state of disease control for more than 2 years after successful treatment for acromegaly and a QoL assessment within 3 months prior to the current structured evaluation of clinical and radiological evaluation of arthropathy. Therefore, in this study we analyzed a subset of patients of two previously published but unrelated studies, which focused in a longitudinal design on the effects of long-term follow-up on QoL[9], and in a cross-sectional design, three months later, on the effects of acromegaly on arthropathy[12]. The patients were asked to participate in the structured joint assessment study after the QoL assessment study had been completed. Therefore, we had both extensive information on QoL scores prior to, and unrelated to, the arthropathy study and structured joint assessment.

Of the 126 patients invited for the arthropathy study, 37 patients (29%) preferred not to participate in this study for various reasons including co-morbidities, travel distance to the outpatients' clinic, and lack of time. Another 31 patients (25%) declined to participate in or were not selected for the longitudinal QoL assessment, for diverse reasons including lack of a previous QoL measurement, unwillingness to be confronted with psychological complaints, and lack of time. The studied patients (n=58) were not different from the non-included patients (n=68) in age, gender, body mass index (BMI), duration of disease, pre-treatment GH/IGF-I, type of primary treatment, duration of follow-up, and self-reported joint complaints based on an earlier study[1].

The first treatment option was transsphenoidal surgery by the same neurosurgeon, complemented when required by radiotherapy (prior to 1985), or SMS analogs (from 1985 onwards) in most patients. Primary medical treatment was given in a minority of patients in

the form of depot formulations of long-acting SMS analogs from 1998 onwards. Since 2003, Pegvisomant was considered as treatment for therapy-resistant acromegaly. After establishment of disease control, biochemical analyses for follow-up were performed on a yearly basis by oral glucose tolerance tests (except in medically treated patients), measurement of serum GH and IGF-I concentrations, and evaluation of other pituitary functions.

Control of acromegaly was defined as a normal glucose-suppressed serum GH of less than 0.38 μg/liter, random fasting serum GH levels less than 1.9 μg/liter, and normal IGF-I levels for age[8]. Patients not meeting these criteria were offered additional treatment (see above). Hypopituitarism was supplemented with thyroxine, hydrocortisone, testosterone or estrogens (in pre-menopausal women) according to the following definitions. Estrogen deficiency in women was present in case of LH/FSH deficiency in premenopausal women with prolonged amenorrea >1 year without adequate replacement therapy and all postmenopausal women. In men, LH/FSH deficiency was defined as testosterone level below the reference range (8.0 nmol/l). Thyroid stimulating hormone (TSH) deficiency was defined as a free T4 level below the reference range. Adrenocorticotrophic hormone (ACTH) deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55 μmol/l) after a corticotrophin releasing hormone test or insulin tolerance test. GH deficiency was not routinely assessed.

The study protocol was approved by the Medical Ethics Committee and all subjects gave written consent for their participation.

Protocol

For the longitudinal QoL study, QoL questionnaires were sent to the patients homes with prepaid envelopes in March/April 2007[9]. Patients who did not return the questionnaires were contacted by telephone. Subsequently, the patients were invited for a study visit in July/August 2007[12]. Standardized questionnaires for joint-complaints were completed by the patients (see below) and, thereafter, physical examination was performed by a single physician (MW) trained in structured joint assessment. Blood samples were taken in the post absorptive state to assess actual GH and IGF-I concentrations. Other relevant details of treatment and patient characteristics were derived from the patient records. Conventional radiographs were obtained in all patients, according to a standardized protocol (*vide infra)*.

Study parameters

Questionnaires

1. HADS (Hospital Anxiety and Depression Scale) The 14 items of the HADS pertain to anxiety and depression. Each item is measured on a 4-point scale. Scores for the anxiety and depression subscale range from 0 to 21 and for the total score from 0 to 42. A high score points to more severe anxiety and depression[14].

2. MFI-20 (Multi Dimensional Fatigue Index) The MFI-20 contains 20 statements to assess fatigue[15]. Every statement is measured on a five-point scale; scores vary from 0 to 20. Higher scores indicate higher experienced fatigue.

3. NHP (Nottingham Health Profile) The NHP consists of 38 yes/no questions, which are subdivided in 6 scales assessing impairments[16,17]. The total score is the mean of the 6 subscales. A high score is related to a worse QoL, values are between 0 and 100.

4. SF-36 (Short-Form 36) The 36 items of the SF-36 record general well-being during the previous 30 days[18,19]. Scores are expressed on a 0 to 100 scale. Higher scores are associated with better QoL.

5. ACROQOL (Acromegaly-Quality of Life) The ACROQOL is a disease specific questionnaire. Responses are given as frequency of occurrence or degree of agreement on a five-point scale. Parameters are expressed as percentage, from 0 to 100. Higher scores are associated with a better QoL[20].

Parameters of acromegalic disease

Disease duration was calculated from the estimated date of onset, using start of signs and symptoms, and facial changes on photographs to the date of normalization of serum IGF-I concentration after treatment. Duration of disease control was calculated from the date of normalization of serum IGF-I concentration after treatment until the study visit. Both surgically and/or irradiation cured patients and patients with controlled disease during SMS analog treatment were collectively referred to as having 'controlled disease'.

Biochemical parameters

Serum GH was measured with a sensitive immunofluorometric assay (IFMA) (Wallac, Turku, Finland), specific for the 22 kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit 0.01 μg/l; inter-

assay coefficient of variation (CV) 2.0-9.0% of 0.1-15.4 μg/l) from 1992 onwards, and, prior to 1992 with the radioimmunoassay (RIA) (Biolab/Serono, Coinsins, Switzerland) calibrated against WHO-IRP 66/21, with an inter-assay CV below 5% and a detection limit of 0.19 μg/l. Pretreatment GH concentrations were available from all patients.

From 1986 to 2005, serum IGF-I concentrations were determined by a RIA (Incstar; Stillwater, MN, USA) with a detection limit of 1.5 nmol/l and an inter-assay CV below 11%. IGF-I is expressed as standard deviation scores (SD score) for age- and gender-related normal levels determined in the same laboratory.[21] Therefore, baseline IGF-I concentrations prior to treatment of the disease were all measured with the same assay. From 2005 onwards, serum IGF-I concentration (ng/ml) was measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay CV was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/l, respectively. IGF-I levels were expressed as SD score, using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals[22,23] .

Radiological investigation and radiological scoring

Conventional radiographs of the knees (posterior-anterior (PA), weight-bearing, fixed-flexion[24] and lateral), hands (dorso-volar), hips (PA), and lumbar (PA and lateral) and cervical spine (Anterior-Posterior (AP) overview, AP transbuccal and lateral) were obtained from all participating patients following a standardized manner with a fixed film-focus distance and fixed joint position acquired by the same experienced radiographer and scored by a single experienced skeletal radiologist using the Kellgren-Lawrence (KL) scoring method[12,25].

The diagnosis of osteoarthritis

We used the following definitions for osteoarthritis:

1. Radiological osteoarthritis was defined as a KL score of ≥2 in at least 1 joint of the intervertebral discs, of the femorotibial joints of the knee, of the distal interphalangeal joints (DIPJs), proximal interphalangeal joints (PIPJs), first interphalangeal joints (1st IPJs), and first carpometacarpal joints (1st CMCJs) of the hand and of the hip

2. Clinical osteoarthritis was defined as pain and stiffness on most days of the preceding month and a KL score of ≥2 in at least one intervertebral disc,[26] in at least 2 hand joints, in the femorotibial joint[27] and in the hip[26].

3. Pain/stiffness or osteoarthritis of a knee, hip, or hand joint was considered as minimal unilateral at that particular joint-site. Pain/stiffness or osteoarthritis of the spine was considered as the presence or absence or complaints or minimal one intervertebral disc with a KL score ≥2, respectively.

For all definitions a joint prosthesis in the knees (n=1) and/or hips (n=2) as a result of end stage of osteoarthritis was excluded from that joint-specific analysis

Statistical analysis

SPSS for windows version 16.0 (SPSS inc., Chicago, IL, USA) was used for data analysis. Data was presented as mean (SEM), unless mentioned otherwise. Analysis of variance with standard adjustments for participant's age, gender, and radiotherapy was used when appropriate. Results of the analyses were expressed as denotations for p-values when decrease in quality of life was significant. To compare differences in QoL in patients with and without complaints of pain and stiffness of the different joint sites binary logistic regression analysis was used with the following parameters: age, gender, and radiotherapy.

RESULTS

Clinical characteristics

We studied 58 patients, 31 male and 27 female patients, with a mean age of 59.6±1.5 years. As shown in *Table 1*, all patients had (medically) controlled acromegaly for a mean of 14.8 years after (multimodality) treatment, and at least for 2 years. At the time of diagnosis of acromegaly mean serum GH concentrations were 41.2±3.5 μg/l, mean IGF-I SD scores were 7.5±0.8, and the mean estimated duration of active disease prior to disease control was 9.1±1.0 yrs (range 1 to 43 yrs).

At the time of the current evaluation, mean GH level was 0.7±0.1 μg/l, and mean IGF-I SD score was 0.57±0.2. There were no gender differences in BMI, age, duration of active disease or duration of disease control, serum GH concentrations, or in IGF-I SD-scores.

ACTH and TSH deficiency were present in 19% and 28% of patients, respectively. More female than male patients were considered hypogonadal (estrogen deficient) due to natural menopause. Sex hormone substitution therapy was given to 11 patients (23% of men

required testosterone and 15% of women used estrogens). There were no gender differences for the other pituitary hormone deficiencies.

Table 1 Clinical characteristics of the patients.

	Patients (n=58)
Gender (n(%))	
Males	31 (53 %)
Females	27 (47 %)
Age (yr)	59.6 (1.5)
BMI (kg/m²)	28.3 (0.6)
Treatment: (n(%))	
Surgery	31 (53 %)
Surgery + RT	15 (26 %)
Surgery + SMS	6 (10 %)
Surgery + RT + SMS	1 (2 %)
Primary SMS	5 (9 %)
Age at diagnosis	41.6 (1.7)
Disease duration (yrs)	9.1 (1.0)
Duration of controlled disease (yrs)	14.8 (0.9)
GH (μg/l)	
Pre-treatment	41.2 (3.5)
Current	0.7 (0.1)
IGF-I SD scores	
Pre-treatment	7.5 (0.8)
Current	0.57 (0.2)
Hypopituitarism (n(%))	19 (33 %)
Hypothyroidism	16 (28 %)
Hypogonadism	29 (50 %)
Hypocortisolism	11 (19 %)

Data are shown as mean and standard error of the mean (SEM), unless mentioned otherwise. N: number, BMI: body mass index, GH: growth hormone, IGF-I: insulin-like growth factor I, SD: standard deviation, RT: radiotherapy (pituitary irradiation), SMS: somatostatin analogs.

Prevalence of joint-specific complaints and osteoarthritis

Joint related complaints of pain and stiffness were reported by 94% of the patients. The hand was the most affected joint-site (84%), followed by the cervical and lumbar spine in 65% and

61% of patients, respectively (*Table 2*). Radiological osteoarthritis was mostly present in the spine (cervical spine 91% and lumbar spine 82%), followed by the hand in 68% of patients. Clinical osteoarthritis, the combination between complaints and radiological abnormalities, was also most prevalent in the spine (65%) and hand (46%). The hip and knee were the joint-sites least affected by clinical osteoarthritis, in 20% and 30% of patients, respectively.

Table 2. Prevalence of joint-specific complaints of pain and stiffness, clinical osteoarthritis, and radiological osteoarthritis in acromegaly patients with long-term disease control (N=58).

	Pain and stiffness (%)	Radiological osteoarthritis (%)	Clinical osteoarthritis (%)
Cervical spine	65	91	65
Lumbar spine	61	82	61
Hip	54	30	20
Knee	52	46	30
Hand	84	68	46
DIP		51	
PIP		47	
IP		32	
CMC		47	

Data represent percentage joint-specific complaints, radiographic, and clinical osteoarthritis. Complaints of osteoarthritis of a knee, hip, or hand joint was considered as minimal unilateral osteoarthritis at that particular joint. Osteoarthritis of the spine was considered as osteoarthritis at minimal one intervertebral disc. DIP: distal interphalangeal joints, PIP: proximal interphalangeal joints, IP: interphalangeal joints, CMC: carpometacarpal joints.

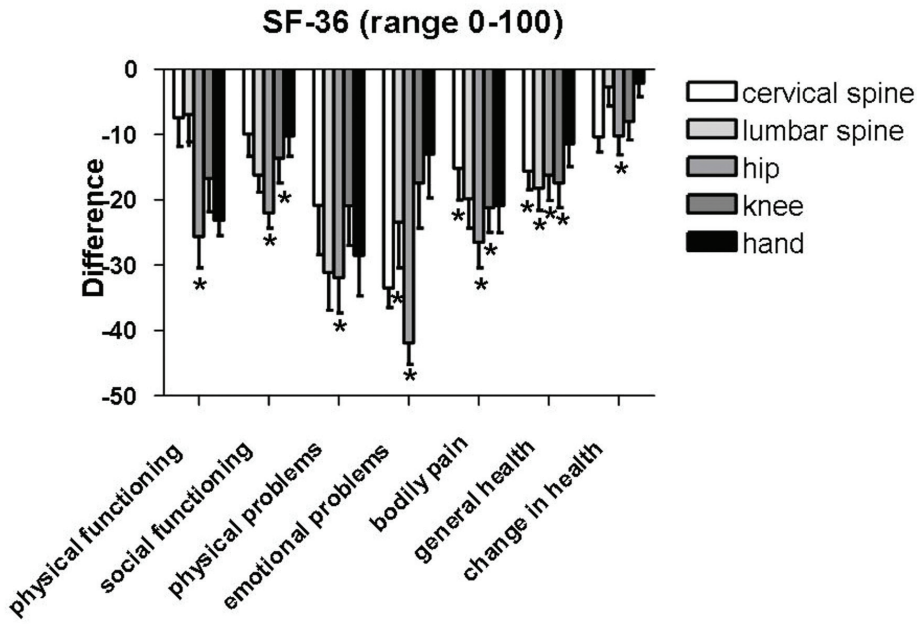
Quality of life

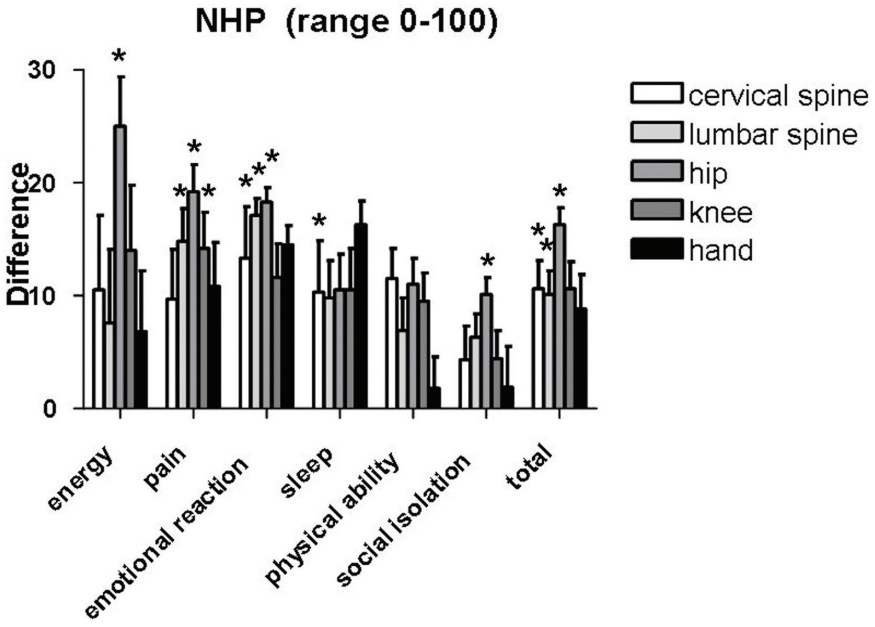
Quality of life, measured by 5 validated questionnaires (27 subscales), was not affected by gender, disease duration, duration of disease control, levels of pre-treatment or actual GH/IGF-I, or any degree of hypopituitarism. Age affected only few items, i.e. a higher age was associated with worse physical functioning (SF-36) (regression coefficient (R)= -0.296, p=0.002), but a better score on the pain subscale of the SF-36 (R=0.252, p=0.004). Radiotherapy (n=16, 28%) was associated with decreased scores on physical performance (ACROQOL), increased scores on energy, pain, physical ability, social isolation (NHP), and increased general fatigue (MVI-20), even when adjusted for duration of disease control, pre-treatment IGF-I, hypopituitarism, age, gender, and BMI (data not shown). There was no difference in QoL between surgery or primary somatostatin analog treatment.

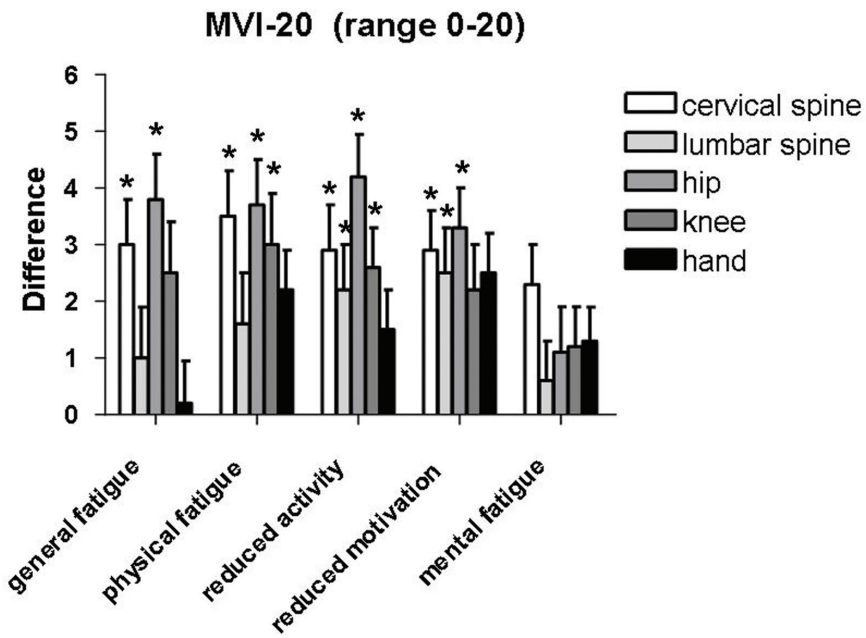
Impact of joint-specific complaints, radiological, and clinical osteoarthritis on QoL

Complaints of pain and stiffness

Figures 1A-E show the mean differences in QoL subscale scores between patients with and without pain and stiffness of the different joint sites. On all 5 questionnaires, patients with joint-complaints scored worse than patients without joint-complaints. Complaints of pain and stiffness of the hip were associated with the largest decrease in QoL, in 21 of the 27 assessed subscales, but these complaints did not influence the subscales sleep (NHP), mental fatigue (MFI-20), psychological, appearance, and personal relations (ACROQOL). Complaints of the spine, hip, and knee mostly affected physical and general subscales, however anxiety and depression (HADS) were also affected by complaints of the spine and hip. In addition, complaints of the knee affected the subscales psychological and appearance (ACROQOL). Complaints of the hand did not affect any subscale.




NHP (range 0-100)
Difference
30
20
10
0
cervical spine
lumbar spine
hip
knee
hand
energy
pain
emotional reaction
sleep
physical ability
social isolation
total


MVI-20 (range 0-20)
Difference
6
5
4
3
2
1
0
cervical spine
lumbar spine
hip
knee
hand
general fatigue
physical fatigue
reduced activity
reduced motivation
mental fatigue

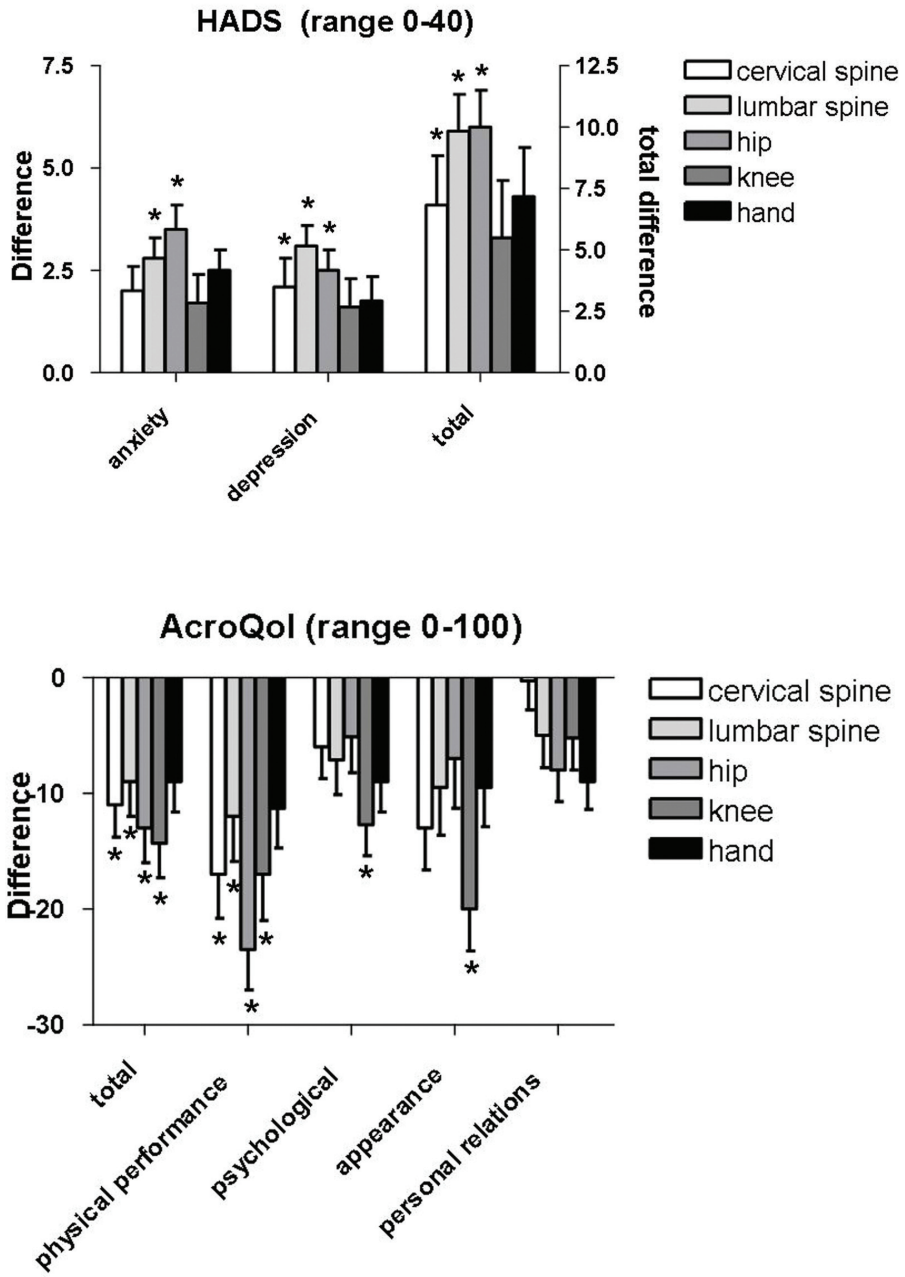


Figure 1 A-E. *p<0.05. Data were analyzed by binary logistic regression analysis with the following parameters: age, gender, and radiotherapy. Bars represent mean difference (SEM) in QoL-scores between patients with and without complaints of pain and stiffness (Difference = QoL-score of patients with complaints minus QoL-score of patients without complaints).

Radiological osteoarthritis

There was no difference in QoL scores between patients with and without radiological osteoarthritis at the different joint sites, not even when the degree of osteoarthritis was taken into account.

Binary logistic regression analysis with adjustments for age, gender, and previous RT was performed to assess the effect of radiological osteoarthritis on QoL. Except for radiological osteoarthritis of the hip negatively affecting bodily pain (SF-36) (adjusted ß= -0.05, p=0.01), radiological abnormalities of none of the other joint-sites significantly affected any QoL subscale.

Clinical osteoarthritis

Tables 3A&B show the QoL scores of patients with and without clinical osteoarthritis of the spine, hip, knee, and hand, respectively. Primary adjustments were made for factors known to influence QoL like age, gender, and radiotherapy. In addition, per analysis secondary adjustments were made for osteoarthritis at other joint-sites than the investigated joint-site. Patients with clinical osteoarthritis of the spine, hip, and knee had worse QoL scores on all subscales than patients without osteoarthritis of these joint-sites. Clinical osteoarthritis of the spine was additionally associated with impaired emotional (SF-36 and NHP) and physical functioning (SF-36, NHP, MFI-20, and ACROQOL), and anxiety and depression (HADS). Clinical osteoarthritis of the hip was associated with impaired physical functioning and reduced energy (SF-36, NHP, and MFI-20). Clinical osteoarthritis of the knee was associated with reduced activity (MFI-20) and pain (NHP). Described differences remained significant when adjusted for the presence of osteoarthritis at the other joint-sites. In patients with clinical hand osteoarthritis, only the subscale: sleep (NHP) was impaired when compared with patients without osteoarthritis of the hand, only when adjusted for the presence of osteoarthritis at the spine, hip, and knee.

Table 3A. Mean (SEM) QoL subscale scores for acromegalic patients with and without osteoarthritis, in the spine (lumbar and cervical spine), and hip, respectively

Table 3A	Clinical spine osteoarthritis		Clinical hip osteoarthritis	
	No OA N=17	OA N=41	No OA N=38	OA N=20
SF-36 (0-100)				
Physical functioning	84.7 (4.3)	72.6 (23.0)	**80.4 (3.3)**	**62.7 (8.4)***
Social functioning	93.3 (2.7)	77.2 (4.3)	83.9 (3.5)	77.8 (8.0)
Physical problems	90.0 (5.9)	58.9 (7.7)	71.3 (6.5)	63.8 (13.8)
Emotional problems	**95.5 (7.0)**	**62.0 (7.8)***	74.3 (6.7)	70.4 (12.9)
Bodily pain	**86.9 (17.5)**	**67.1 (25.8)***	**77.1 (4.0)**	**56.2 (7.9)***
General health	**72.7 (13.5)**	**54.4 (21.4)****	62.2 (3.4)	56.7 (9.1)
Change in health	**55.0 (16.4)**	**15.7 (15.7)***	**51.7 (2.6)**	**36.1 (4.4)****
NHP (0-100)				
Energy	20.2 (6.9)	30.7 (6.6)	**21.9 (4.7)**	**43.3 (13.8)***
Pain	**9.9 (4.4)**	**19.2 (4.7)****	13.0 (3.5)	27.0 (9.0)
Emotional reaction	**5.8 (2.6)**	**19.1 (5.6)****	12.6 (3.1)	21.7 (9.5)
Sleep	11.3 (4.6)	21.6 (5.1)	15.5 (3.8)	22.9 (9.4)
Physical ability	**5.1 (2.7)**	**16.6 (3.5)****	10.7 (2.7)	17.0 (5.9)
Social isolation	4.1 (3.2)	8.4 (3.0)	5.8 (2.4)	10.8 (5.6)
Total	**8.8 (2.5)**	**19.4 (3.7)***	15.3 (3.3)	24.7 (6.8)
MFI-20 (0-20)				
General fatigue	**10.0 (1.2)**	**13.0 (0.8)***	11.3 (0.7)	13.9 (1.6)
Physical fatigue	**9.4 (1.0)**	**13.0 (0.8)****	11.1 (0.7)	13.1 (1.4)
Reduced activity	**8.2 (0.9)**	**11.1 (0.8)***	**9.5 (0.7)**	**12.2 (1.3)***
Reduced motivation	**7.6 (0.8)**	**10.5 (0.7)****	9.4 (0.7)	10.0 (1.2)
Mental fatigue	**8.2 (0.9)**	**10.5 (0.7)***	9.8 (0.7)	8.3 (1.2)
HADS				
Anxiety (0-21)	**3.8 (0.9)**	**6.6 (0.7)****	5.5 (0.6)	6.0 (1.5)
Depression (0-21)	**2.9 (0.8)**	**6.0 (0.7)****	4.5 (0.6)	6.2 (1.6)
Total (0-42)	**6.7 (1.5)**	**12.6 (1.2)****	9.7 (1.0)	12.2 (2.8)
ACROQOL (0-100)				
Total	**71.2 (3.6)**	**60.9 (17.3)***	65.6 (2.7)	63.7 (5.3)
Physical performance	**74.1 (4.5)**	**57.0 (3.8)****	65.2 (3.4)	53.6 (6.7)
Psychological	69.6 (3.8)	63.1 (2.8)	64.1 (2.6)	69.5 (5.0)
Appearance	65.5 (5.1)	52.7 (3.7)	56.5 (3.3)	62.1 (7.2)
Personal relations	73.8 (3.7)	73.5 (15.4)	71.4 (2.9)	76.9 (4.1)

Table 3B. Mean (SEM) QoL subscale scores for acromegalic patients with and without osteoarthritis, in the knee and hand, respectively

Table 3B	Clinical knee osteoarthritis		Clinical hand osteoarthritis	
	No OA N=28	OA N=30	No OA N=29	OA N=29
SF-36 (0-100)				
Physical functioning	79.2 (3.6)	70.4 (7.1)	77.3 (4.6)	76.4 (4.7)
Social functioning	83.2 (4.0)	81.3 (6.3)	80.7 (5.0)	84.7 (4.1)
Physical problems	71.9 (6.4)	63.4 (13.6)	60.2 (9.1)	79.8 (6.8)
Emotional problems	72.9 (7.2)	71.6 (10.2)	62.1 (9.4)	84.8 (6.5)
Bodily pain	75.3 (4.2)	66.3 (8.0)	71.0 (4.7)	74.7 (5.9)
General health	62.8 (3.7)	55.9 (6.7)	61.4 (4.3)	60.5 (5.0)
Change in health	51.1 (2.9)	41.7 (3.6)	50.4 (3.5)	46.6 (3.4)
NHP (0-100)				
Energy	23.4 (5.2)	35.4 (11.1)	26.0 (6.9)	27.4 (7.0)
Pain	**11.1 (3.1)**	**28.2 (8.5)***	13.4 (4.0)	11.6 (5.5)
Emotional reaction	13.7 (3.5)	16.6 (7.2)	14.8 (4.5)	14.2 (4.5)
Sleep	14.7 (3.4)	24.2 (9.9)	11.7 (4.0)	22.5 (5.9)
Physical ability	9.4 (2.4)	20.2 (6.3)	10.6 (3.2)	13.7 (3.8)
Social isolation	5.7 (2.4)	10.4 (5.2)	10.9 (17.9)	2.9 (2.8)
Total	15.3 (3.1)	22.7 (7.2)	17.9 (4.2)	16.5 (4.2)
MFI-20 (0-20)				
General fatigue	11.5 (0.8)	12.9 (1.2)	12.4 (1.0)	11.4 (1.0)
Physical fatigue	11.0 (0.7)	12.9 (1.3)	11.6 (0.9)	11.5 (0.9)
Reduced activity	**9.5 (0.7)**	**11.7 (1.0)***	10.8 (0.9)	9.4 (0.9)
Reduced motivation	9.0 (0.7)	10.6 (1.1)	9.3 (0.8)	9.5 (0.9)
Mental fatigue	9.5 (0.7)	9.5 (1.2)	8.7 (0.8)	10.2 (0.9)
HADS				
Anxiety (0-21)	5.5 (0.7)	5.9 (0.9)	6.1 (0.8)	5.1 (0.7)
Depression (0-21)	4.9 (0.7)	4.8 (1.1)	5.2 (0.8)	4.5 (0.8)
Total (0-42)	10.1 (1.9)	10.8 (1.8)	11.0 (1.5)	9.5 (1.3)
ACROQOL (0-100)				
Total	66.3 (2.9)	62.2 (3.9)	64.0 (3.4)	66.3 (3.3)
Physical performance	64.5 (3.8)	57.8 (5.4)	60.2 (4.7)	64.9 (4.1)
Psychological	65.5 (2.9)	64.7 (3.7)	63.4 (3.2)	67.2 (3.3)
Appearance	58.6 (3.8)	55.6 (4.8)	55.7 (4.2)	59.7 (4.4)
Personal relations	72.2 (3.1)	73.9 (3.8)	70.7 (3.8)	74.6 (3.1)

Legend Tables 3A &B: Analysis of variance was performed with standard adjustments for age, gender, and radiotherapy. In addition, adjustments were made for osteoarthritis in three of the four joint-sites (spine, hip, knee, or hand) not defined as the dependent variable at that particular analysis, however, these adjustments were not of influence and therefore not mentioned. Age, gender, and radiotherapy were no independent parameters of osteoarthritis at the different joint-sites. *p<0.05, **p<0.01, OA: osteoarthritis. SF-36 and ACRO-QoL: higher scores, better performance. HADS, NHP, and MFI-20 higher scores: more impairment.

DISCUSSION

This is the first study in patients with acromegaly reporting the impact of joint-site specific joint complaints and osteoarthritis on QoL after long-term control of GH excess. Both self-reported joint complaints and clinical osteoarthritis were associated with impaired QoL. In contrast, radiological osteoarthritis was not associated with QoL scores. Interestingly, there appeared to be joint-site specific effects on QoL scores.

Clinical osteoarthritis of the spine seemed to have the greatest impact, not only on physical and general well being, but also on psychological well-being. We further demonstrated a relatively mild effect of clinical osteoarthritis of the hip and knee on QoL when compared with clinical osteoarthritis of the spine, whereas clinical hand osteoarthritis does only affect sleep.

Osteoarthritis in acromegaly is caused by degeneration of previously hypertrophied cartilage due to the anabolic effect of elevated GH and IGF-I levels during the active phase of the disease. Co-morbidity is a well-known determinant of health related-QoL. In previous studies we reported decreased QoL in patients with controlled acromegaly, especially in those patients with self-reported joint-related complaints[1,8]. Recently, we evaluated the characteristics of these joint complaints in cured patients. We observed a 4 to12 fold increased prevalence of clinical and radiological osteoarthritis in comparison to controls, using structured joint-assessment and radiographs. In contrast to joint-space narrowing observed in primary osteoarthritis, the widening of joint spaces, well known from active acromegaly, persisted also after long-term disease control[12].

Physical symptoms, QoL, and psychological well-being are closely related. We demonstrated an effect of spinal complaints and osteoarthritis on anxiety, depression and emotional problems. It has been reported that chronic pain of the lower back has clear functional and emotional impact since loss of vitality, persistent discomfort, sleeping difficulties, side effects of

drugs, economic and social concerns weighs heavily and cumulatively on affected individuals[28]. Furthermore, emotional disturbances (such as depression, increased somatic awareness, and anxiety reactions) were reactions to the presence of chronic pain. These psychological profiles and their reflection on social and occupational functioning might predispose to developing chronic low back pain[11]. Therefore, a potential limitation of this study might be the inability to show a causative effect of osteoarthritis on QoL or *vice versa*.

Osteoarthritis is the most common form of arthritis in persons over 50 years of age. A recent report from the World Health Organization (WHO) emphasized the impact of osteoarthritis on patients and society[29]. Almost all studies on the impact of osteoarthritis on QoL were solely investigated using the SF-36 questionnaire[30] and reported a large negative impact of osteoarthritis on all aspects of health when compared with normal controls[13].

Remarkably, we were unable to demonstrate an effect of clinical osteoarthritis of the hand on QoL, despite a high prevalence of complaints of pain/stiffness. In contrast, in primary osteoarthritis, there is a large impact of hand osteoarthritis on QoL[30]. Osteoarthritis in primary affected individuals consists of osteophytosis in combination with severe joint-space narrowing[31]. Osteoarthritis in long-term controlled acromegaly is predominantly caused by osteophytosis since the characteristical joint-space widening, known from active acromegaly persists[32], despite long-term disease control[12]. This severe osteophytosis might cause pain and stiffness as evidenced by the self-reported complaints, but might not interfere with functional abilities as much as when osteophytosis was combined with joint-space narrowing. In addition, the spine, hip, and knee are strained during every form of physical activity and osteoarthritis in these joint sites might therefore demonstrate a larger impact on QoL than osteoarthritis of the hand. Indicative for this notion is the fact that patients experience sleep difficulties from osteoarthritis of the hand, and not from osteoarthritis at the other joint sites. The above mentioned explanation might explain the discrepancy between the prevalence of radiological osteoarthritis on the one hand and clinical osteoarthritis on the other hand. In addition, radiological osteoarthritis can be present without causing complaints of pain and stiffness (yet), possibly due to an early stage of osteophytosis or relatively preserved joint-spaces[33].

In a previous study in 2004, we demonstrated that acromegaly patients experienced worse QoL than age- and sex matched controls, although there was considerable variation in outcome parameters among the acromegaly patients, we also demonstrated that patients with joint pains had even lower QoL scores[1]. Millar *et al*. confirmed these findings[34]. They

performed rheumatological examination in 58 patients with mostly short-term controlled acromegaly and related the outcome to their QoL scores based on the SF-36, AcroQol, and artritis impact measument scales 2 (AIMS2). Patients with joint complaints had significantly lower QoL scores than patients without joint complaints. In addition to our earlier study and to Millars study, we wanted to explain, at least part, of the individual variation in impaired QoL among long-term cured acromegaly patients. We therefore related joint side specific complaints achieved by patient records, questionnaires, physical (rheumatological), and radiological examination. Our data indicate a new finding: high pain scores of the spine had the greatest impact on not just physical functioning, but also general and psychological well-being. This Qol assessment was not biased for the awareness of joint abnormalities, since the patients were unaware that structured joint assessment would follow this QoL assessment.

In conclusion, long-term controlled acromegalic patients with an established diagnosis of osteoarthritis have high pain scores especially of the spine, knee, and hip, which impairs physical functioning, as well as general and psychological well-being. These findings underscore, in addition to the assumptions of Millar *et al*., the great importance of recognition of the complaints, pain management, and coping with pain in this patient group. Interventions directed towards reduction of pain and improvement of functional capacity and QoL should be developed and evaluated.

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Part III.

ACROMEGALY; LONG-TERM DISEASE OUTCOME, NOT JOINT RELATED.

Chapter 8.

HIGH PREVALENCE OF VERTEBRAL FRACTURES DESPITE NORMAL BONE MINERAL DENSITY IN PATIENTS WITH LOING-TERM CONTROLLED ACROMEGALY.

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Article submitted

ABSTRACT

Objective: To establish the prevalence of osteoporosis, vertebral, and non-vertebral fractures in acromegaly patients with long-term controlled disease and factors potentially influencing fracture risk.

Design: We conducted a case-control study

Patients and measurements: Eighty-nine patients (46% male, mean age: 58 years) were included. We studied vertebral and non-vertebral fractures, bone mineral density (BMD), and markers of bone turnover. In 48 patients BMD assessment was also obtained 7 years prior to the current study. To compare vertebral fracture prevalence, data from a sample of the Dutch population (n=3469) was used.

Results: Vertebral fracture prevalence was 59% (men 64%, women 54%), significantly increased when compared with controls (odds ratio up to 6.5), and independent of the duration of disease control, BMD, markers of bone turnover, and acromegalic disease characteristics. Mean number of vertebral fractures per patient was 3.4 ± 0.3 (range 1-8). There was no relationship between the number and severity of fractures, parameters of bone-turnover, and follow-up BMD measurements. BMD did not change during prolongation of follow-up by 7 years of controlled acromegaly.

Conclusion: There is a very high prevalence of vertebral fractures in acromegaly patients with long-term controlled disease, independently of BMD. In view of the significant morbidity and mortality associated with vertebral fractures in general and the inability of BMD to predict fracture risk in acromegalic patients, we propose to include vertebral fracture assessment, for example by lateral conventional radiographs of the spine in the screening of patients with acromegaly, both at diagnosis and during follow-up after establishment of disease control.

INTRODUCTION

Growth hormone (GH) and insulin-like growth factor I (IGF-I) act as anabolic hormones on bone by stimulating bone turnover, especially bone formation. It has also been suggested that the anabolic effects of GH on BMD may be sustained after cure of acromegaly[1-5], but duration of follow-up was relatively short in the majority of studies. The effects of GH excess on bone mineral density (BMD) appear to be less consistent. In active acromegaly, high circulating concentrations of GH and IGF-I are associated with increased cortical BMD[6-10]. The reported effects of GH/IGF-I excess on trabecular BMD are more variable, with studies reporting either increased or decreased BMD at trabecular sites possibly due to the variable presence of hypogonadism[1;3-5;11-13].

It remains unclear whether the overall increase in BMD observed in patients with acromegaly is associated with a decrease in fracture risk. Data on fracture risk are conflicting in patients with active as well as controlled acromegaly. Vestergaard *et al.* suggested that risk for non-vertebral fractures is decreased in patients with active acromegaly compared with controls, that these patients sustain fractures at a later age, and that the fracture risk is independent of gonadal status [14]. In contrast, Bonadonna *et al*. found that in post-menopausal women with active acromegaly, the risk for vertebral fractures was high even in the presence of normal BMD, but that vertebral fractures were always associated with low BMD in controlled disease[15]. The same group also demonstrated an increase in vertebral fracture risk in men with acromegaly, especially in those with hypogonadism, irrespective of BMD[16]. To date, the prevalence of fractures in relation with BMD has not been assessed in acromegalic patients after long-term biochemical control.

The main objective of our study was to establish the prevalence of vertebral and non-vertebral fractures in both men and women with controlled acromegaly for a mean of 14 years, and to study factors potentially modulating fracture risk in the controlled state of the disease.

PATIENTS AND METHODS

Patient population

We invited 126 patients with sustained disease control of acromegaly for more than 2 years to participate in this study. All patients had an established diagnosis of acromegaly and were followed at the Department of Endocrinology of the Leiden University Medical Center after successful treatment and control of disease activity[17]. Thirty-seven patients were unwilling or unable to take part in the study. They declined for various reasons such as illness, travel distance to the outpatients' clinic, lack of time, or psychological reasons. A total of 89 of the 126 invited patients (71%) were included. The 37 non-participating patients did not differ from the participating patients in age, gender, body mass index (BMI), duration of active disease, pre-treatment GH/IGF-I, type of primary treatment, duration of follow-up, and self-reported cervical, thoracic, and lumbar spine complaints (based on an earlier study[18]).

The first treatment option had always been transsphenoidal surgery performed by a single neurosurgeon and complemented when required by radiotherapy prior to 1985, or somatostatin (SMS) analogs from 1985 onwards. In a minority of patients primary treatment was given in the form of depot formulations of SMS analogs from 1998 onwards. Since the availability in 2003 of Pegvisomant in the Netherlands, this drug was also used as treatment for therapy-resistant acromegaly. After establishment of cure, disease activity was assessed on a yearly basis by measurement of serum GH and IGF-I concentrations, by oral glucose tolerance tests (except in medically treated patients), and by evaluation of other pituitary functions. The Medical Ethics Committee approved the study protocol and informed consent was obtained from all patients.

Study parameters

Disease activity

Activity of acromegaly was evaluated by measuring fasting serum GH and IGF-I concentrations, and by an oral glucose tolerance test. Control of acromegaly was defined by random fasting serum GH levels below 1.9 μg/L (~5 mU/L), normal IGF-I levels for age, and by normal glucose suppressed serum GH below 0.38 μg/L (~1 mU/L)[17]. Duration of disease control was based on time since normalization of IGF-I. Disease duration was estimated using the reported date of onset of symptoms and signs, including facial changes on photographs, to the date of

normalization of serum IGF-I concentration after treatment by transsphenoidal surgery, radiotherapy, and/or medical therapy.

Assessment of pituitary and gonadal function

For evaluation of pituitary function thyroid stimulating hormone (TSH) deficiency was defined as a free thyroxine (fT4) level below the normal laboratory reference range (absolute value <10 pmol/L). Adrenocorticotropic hormone (ACTH) deficiency was defined as an inappropriate increase in cortisol levels (absolute value <0.55 umol/L) after stimulation by corticotrophin releasing hormone, or insulin tolerance test.

The history of each individual patient was carefully reviewed with respect to their gonadal function from the time of diagnosis to the present evaluation[18-19]. Patients with adequately treated hypogonadism throughout follow-up, which was defined as gonadal hormone replacement therapy (HRT) started within one year after the onset of hypogonadism, were not considered hypogonadal. Thus, male patients with normal testosterone levels throughout follow-up (in relation to sex hormone binding globulin concentration) or short-term hypogonadism which had been adequately suppleted within one year after onset of hypogonadism were considered eugonadal. Male patients with a total testosterone concentration below 8 nmol/L, present for > 1 yr in follow-up or prior to diagnosis, were considered hypogonadal. Female patients with normal spontaneous menstrual cycle, estrogen or contraceptive use or with short-term amenorrhea subsequently treated with estrogen within 1 year, were considered eugonadal. Female patients with prolonged untreated amenorrhea in the presence of low serum oestradiol concentration of less than 70 nmol/L (and low LH/ FSH in postmenopausal women) or natural menopause, were considered hypogonadal.

All patients with hypopituitarism were appropriately treated with levothyroxine, hydrocortisone, testosterone, or estrogen substitution (in pre-menopausal women).

Biochemical assays

Serum GH was measured with a sensitive immunofluorometric assay (IFMA) (Wallac, Turku, Finland), specific for the 22 kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit 0.03 mU/L; interassay coefficient of variation (CV) 2.0-9.0% of 0.25-40 mU/L) from 1992 onwards, and previously with the radioimmunoassay (RIA) assay (Biolab/Serono, Coinsins, Switzerland) calibrated

against WHO-IRP 66/21, with an interassay CV below 5% and a detection limit of 0.5 mU/L.

Until 2005, serum IGF-I concentrations were determined by a RIA (Incstar; Stillwater, MN, USA) with a detection limit of 1.5 nmol/L and an inter-assay CV below 11%. IGF-I is expressed as standard deviation scores (SD) for age- and gender-related normal levels determined in the same laboratory[20]. From 2005 onwards serum IGF-I concentration (ng/ml) was measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay CV was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/L, respectively. IGF-I levels were expressed as SD score, using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals[21-22].

The markers of bone-turnover, beta-crosslaps (bone-resorption), and procollagen type 1 amino-terminal propeptide (P1NP) (bone-formation) were measured by an electrochemoluminescent immunoassay with a Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). Vitamin D25OH was measured by RIA (Incstar/DiaSorin, Stillwater, MN, USA).

Bone mineral density (BMD) measurements

Bone mineral density was measured at the lumbar spine (L1 to L4) and total hip using dual energy X-ray absorptiometry (DXA, Hologic QDR 4500, Hologic Inc., Waltham, MA, USA) equipped with reference values based on the National Health and Nutrition Examination Survey (NHANES III). The same apparatus was used at baseline and follow-up in patients in whom a baseline DXA was available. In these patients baseline T- and Z-scores were recalculated using the new NHANES III reference data.

World Health Organization (WHO) criteria were used to define osteopenia (T-score between -1.0 and -2.5) and osteoporosis (T-score of $\leq$-2.5).

Forty-eight of the 89 patients included in the study had a baseline BMD measurement 7 years prior to the current study when their acromegaly had already been controlled for a mean of 10 years. These data have already been published[2]. The current study assessed the effect of another 7 years (totally 17 years) of disease control on BMD.

Vertebral and non-vertebral fractures

Conventional lateral radiography of the thoracic and lumbar spine was performed in all

patients. Radiographs were obtained at a standard fixed film focus distance by a single, experienced radiology technician. The radiographs were blindly evaluated by a specialized radiologist with considerable experience in skeletal radiology (HK), as well as by two of the other authors (MW/NB), using the semi quantitative method proposed by Genant *et al.* for assessment of vertebral deformities and fractures examining vertebrae T4-L5[23]. The intra-observer variability was 1% for the lumbar spine and 3% for the thoracic spine. Inter-observer variability was 3% for the lumbar spine and 5% for the thoracic spine. The prevalence of non-vertebral fractures sustained after inappropriate trauma was evaluated by a structured self-reported questionnaire.

To enable comparison of the vertebral fracture prevalence with controls, we used the radiological data from the Rotterdam Study (n=3469), a prospective population based cohort study of individuals aged 55 years and over. The study was designed to investigate the incidence and determinants of chronic disabling diseases. Rationale and design have been described previously[23;25]. The Medical Ethics Committee of Erasmus University Medical School has approved the Rotterdam Study and written informed consent was obtained from each subject. At a follow-up visit, between 1997-1999, thoraco-lumbar radiographs of the spine were obtained. The follow-up radiographs were available for 3241 individuals, who survived an average of 7.4 years after baseline centre visit and who were still able to come to our research centre. All follow-up radiographs were scored for the presence of vertebral fracture as described earlier[25].

Statistics

SPSS for windows version 16.0 (SPSS inc., Chicago, IL, USA) was used for data analysis. Data are presented as mean (SEM), unless otherwise stated. BMD, T- and Z-scores of the lumbar spine at baseline and follow-up in patients with and without vertebral fractures, were analyzed by analysis of covariance with adjustments for age, gender, and BMI. Delta was calculated by: (follow-up value-baseline value) / baseline value. The prevalence of vertebral fractures in acromegaly was compared with a Dutch epidemiological control cohort by binary logistic regression analysis. Controls were the reference category. Factors potentially affecting vertebral fracture risk were identified by binary logistic regression analysis with adjustments for age, gender, BMI, parameters of disease activity, and hypopituitarism, when appropriate.

Comparisons of the prevalence of vertebral fractures between male and female acromegalic patients, grouped according to gonadal status, were performed by binary logistic regression analysis with adjustments for age, gender, and BMI.

RESULTS

Patient characteristics *(Table 1)*

Eighty nine patients, 46 male and 43 female patients, with controlled acromegaly for at least 2 years, were included. Mean age was 58.3 ± 10.9 years and male patients were significantly younger than their female counterparts (55.9 ± 10.7 *vs*. 60.8 ± 11.9 years, p = 0.04). The mean estimated duration of active disease prior to remission was 8.9 ± 7.3 years (range 1 to 45 yrs). All patients had controlled disease for a mean of 14 years (range 2 to 28 years). In 50 patients, disease control was obtained after surgery only. In case of inadequate disease control, surgery was followed by additional radiotherapy in 16 patients, treatment with SMS analogs in 14 patients, and both postoperative radiotherapy and SMS analog treatment in 3 patients. Six other patients had SMS analogs as primary medical treatment. As per inclusion criteria, biochemical control was maintained since remission in the majority of patients for >14 years (n=71), and in 18 patients for 2-14 years. Twenty patients (23%) were still using SMS analogs at the time of the current evaluation.

Thirty-eight male patients were eugonadal (30 with preserved gonadal function and 8 with hypogonadism and adequate replacement treatment with androgens), whereas 8 males were hypogonadal (no or inadequate replacement treatment during follow-up). The mean testosterone level at the study visit was 14.2 ± 3.2 nmol/L. Five female patients were eugonadal (4 were pre-menopausal with normal gonadal function and 1 had adequate gonadal steroid replacement therapy for hypogonadism), whereas 38 women were post-menopausal. The mean duration of hypogonadism was comparable in males and females. All, but two patients were vitamin D replete as evidenced by $25(OH)_2$ D levels >75 nmol/L. Eight patients received calcium and vitamin D supplements and 3 patients had been treated with bisphosphonates for up to 4.5 years, but these drugs had been discontinued at least 2.5 years prior to the present study. Three patients were currently treated with bisphosphonates and had received these agents for a mean of 6.5 years (range: 3.5-9.5 years).

There were no differences in duration of active disease, duration of remission, serum GH levels and IGF-I SD-scores at diagnosis or at study evaluation, and the prevalence of pituitary hormone deficiencies, including luteinizing hormone (LH)/ follicle stimulating hormone (FSH) deficiency between both genders.

Table 1. Demographic and Clinical characteristics of acromegalic patients, grouped according to gender.

	Males N=46	Females N=43	P-value
Age (yrs)	55.9 (10.7)	60.8 (11.9)	0.04
BMI (kg/m²)	28.7 (4.5)	28.3 (4.9)	0.77
Treatment: (n(%))			
Surgery	31 (68 %)	19 (44 %)	0.12
Surgery + radiotherapy	7 (15 %)	9 (21 %)	0.86
Surgery + SMS	6 (13 %)	8 (19 %)	0.81
Surgery + radiotherapy + SMS	2 (4 %)	1 (2 %)	0.59
SMS	0 (-)	6 (14 %)	-
Disease duration (yrs)	8.1 (5.1)	9.7 (9.1)	0.81
Duration of disease control (yrs)	14.5 (6.5)	13.7 (6.0)	0.40
GH (µg/L)			
Pre-treatment	40.27 (49.65)	32.65 (45.81)	0.75
Current	0.87 (1.77)	0.95 (0.96)	0.75
IGF-I SD scores			
Pre-treatment	7.9 (4.7)	6.7 (4.7)	0.28
Current	0.6 (1.6)	0.5 (1.9)	0.75
Vitamine D 25 (OH) (nmol/L)	79.6 (4.6)	79.9 (3.8)	0.82
Beta-crosslaps (ng/ml)	0.29 (0.1)	0.41 (0.1)	<0.01
P1NP (ng/ml)	30.2 (2.9)	45.5 (4.2)	<0.01
Hypopituitarism (n(%))	13 (28 %)	16 (37 %)	0.41
Hypogonadal/ Natural menopause (n(%))	8 (17 %)	38 (88 %)	<0.01

Data are shown as mean (SD), unless mentioned otherwise. A p-value <0.05 was considered significant. Yr: years, kg/m²: kilograms per square meter, µg/L: micrograms per liter, SD: standard deviation, SMS: somatostatin analog, BMI: body mass index, IGF-I:insulin like growth factor, CI: confidence interval.

Markers of bone turnover were within the normal laboratory reference ranges in all patients. Estrogen-depleted women had higher rates of bone turnover than estrogen-replete women, hypogonadal males, or eugonadal males as evidenced by significantly greater P1NP and beta-crosslaps concentrations (data not shown).

Bone mineral density measurements

Mean BMD at the lumbar spine was 1.01 ± 0.02 g/cm², mean T-score was -0.51 ± 0.18, and mean Z-score was +0.45 ± 0.20. Mean BMD at the total hip was 0.88 ± 0.02 g/cm², mean

T-score was -0.47 ± 0.12, and mean Z-score was +0.46 ± 0.10. BMD. T- and Z-scores of the lumbar spine did not differ between patients with and without vertebral fractures, nor after adjustment for age, gender, BMI, and gonadal status (*Table 2*).

Five patients had osteoporosis and 14 patients had osteopenia at one or more sites.

Table 2. BMD, T- and Z-scores of the lumbar spine at baseline and follow-up in patients with and without vertebral fractures (VF).

	Vertebral fractures (VF)		Difference (95% -CI)	P-value
	No VF	VF		
	n=15 (34%)	n=27 (66%)		
BMD lumbar spine				
Baseline	1.06 (0.04)	1.00 (0.03)	-0.06 (-0.18 – 0.06)	0.29
Follow-up	1.01 (0.04)	1.00 (0.03)	0.01 (-0.10 – 0.11)	0.90
Delta	-0.01 (0.02)	0.00 (0.01)	0.02 (-0.02 – 0.06)	0.26
T-score lumbar spine				
Baseline	-0.19 (0.35)	-0.53 (0.27)	-0.23 (-1.23 – 0.82)	0.66
Follow-up	-0.50 (0.35)	-0.69 (0.26)	0.32 (-0.94 – 1.00)	0.95
Delta	-0.06 (0.25)	-0.10 (0.17)	-0.37 (-1.02 – 0.27)	0.25
Z-score lumbar spine				
Baseline	0.74 (0.42)	0.12 (0.27)	-0.49 (-1.50 – 0.53)	0.34
Follow-up	0.56 (0.41)	0.40 (0.26)	-0.01 (-0.95 – 0.93)	0.96
Delta	0.04 (0.16)	0.24 (0.96)	-0.46 (-3.39 – 2.47)	0.75

Data shown as mean (SEM) unless mentioned otherwise. BMD was measured in (g/cm^2). Data were analyzed by analysis of covariance with adjustments for age, gender, and BMI. Delta was calculated by: (follow-up value-baseline value) / baseline value. CI: confidence interval, n: number, BMD: bone mineral density, BMI: body mass index.

Vertebral and non-vertebral fractures

Prevalence of vertebral fractures

The prevalence of vertebral fractures was 59%, and was not different between patients with controlled disease for 2-14 years (57%) and those with controlled disease for longer than 14 years (59%). There was a gender difference in the prevalence of vertebral fractures with more men than women with one or more documented vertebral fracture (56 *vs.* 44%; p=0.02). Hypogonadal men had a significantly higher prevalence of vertebral fractures (86%) than eugonadal (19%) or hypogonadal (49%) women (p<0.05) (*Figure 1*).

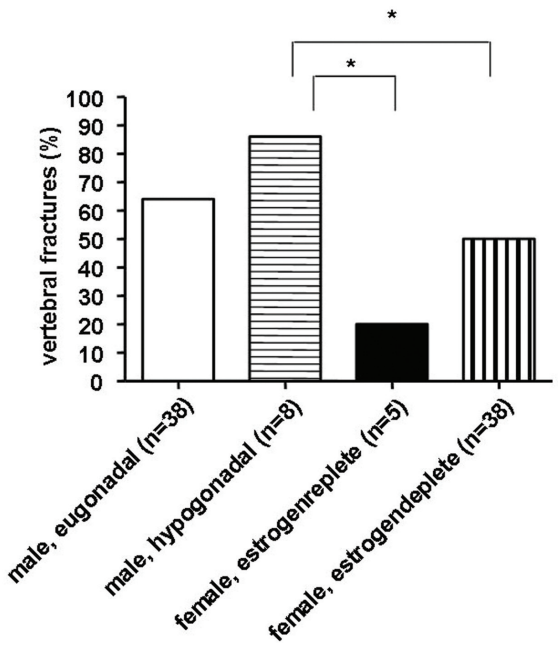


Figure 1. Prevalence of vertebral fractures in male and female cured acromegalic patients, grouped according to gonadal status. *p <0.05. Data were analyzed by binary logistic regression analysis with adjustments for age, gender, and BMI.

Fifty-five percent of patients had one or more fractures at the level of the thoracic spine and 18% had one or more fracture at the levels of the lumbar spine. Mean number of vertebral fractures was 3.4 ± 0.3 (range 1-8 fractures) per patient. The most common fractures were anterior wedge fractures (73%), followed by biconcave fractures (18%), and crush fractures (15%), ($p<0.01$). The grade of the vertebral fractures varied from mild (69%) to intermediate (20%) to severe (11%).

Patients demonstrated significantly more vertebral fractures than controls in all age groups. Odds ratio's varied from 6.5 (95% CI 3.4-12.4) in patients 61-65 year to 2.3 (95% CI 1.3-3.6) in patients >76 years when compared with controls (*Table 3*).

Prevalence of non-vertebral fractures

Thirty-one patients (35%) sustained one or more non-vertebral fractures during follow-up since establishment of biochemical control of acromegaly. The prevalence was 37% in men and 33% in women. Fractures of the feet, tibia, humerus, radius, wrist and hand were variably reported. The most common fracture sites were wrist (29%) and tibia (26%).

Table 3. prevalence of vertebral fractures by age category in acromegaly patients compared with a Dutch epidemiological control cohort

Age	Subjects		Vertebral fracture cases				
	Acromegaly	Controls	Acromegaly	Controls	Odds	95% CI	P-value
	n=89	n=3469	N (%)	N (%)	ratio		
<55	19	-	11 (59 %)	-	-	-	-
56-60	25	826	13 (53 %)	25 (3 %)	3.7	1.3-10.9	0.01
61-65	18	955	8 (44 %)	48 (5 %)	6.5	3.4-12.4	<0.01
66-70	15	796	10 (67 %)	72 (9 %)	4.4	2.4-8.2	<0.01
71-75	8	560	7 (88 %)	73 (13 %)	2.4	1.3-4.3	<0.01
>76	4	332	3 (75 %)	37 (11 %)	2.3	1.3-3.6	<0.01

Data were analyzed by binary logistic regression analysis. Controls were the reference category. Age in years.

Changes in BMD in patients with sustained disease remission

The subgroup of 48 patients who had a baseline DXA 7 years prior to the present study were in sustained remission for 17.4 ± 6.3 years. Seventeen of these patients (41%) had hypopituitarism with hormone substitution. Six patients had been treated with oral bisphosphonates, of whom 3 patients for up to 4.5 years but they had discontinued these drugs for at least 2 years before evaluation, and 3 patients were still receiving these agents for a mean of 7 years. These 6 patients were excluded from analysis of sequential changes in BMD.

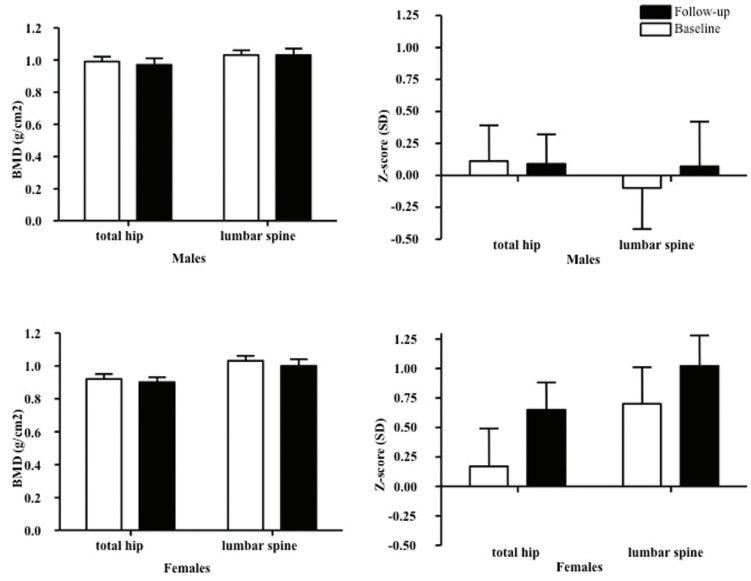


Figure 2. BMD and Z-scores in male and female cured acromegalic patients at baseline and follow-up. (n=42). In order to eliminate the effect of age and gender we used Z-scores in addition to BMD.

At baseline, mean BMD was 1.02 ± 0.03 g/cm^2 at the lumbar spine and 0.96 ± 0.03 g/cm^2 at the total hip. Four patients had osteoporosis and 11 patients had osteopenia at one or more sites. After a mean follow-up period of 7 years, mean BMD was 1.01 ± 0.03 g/cm^2 at the lumbar spine and 0.83 ± 0.02 g/cm^2 at the total hip. Five patients had osteoporosis and 18 patients had osteopenia at one or more sites. Overall, there was no significant change in BMD over time at either the lumbar spine or total hip sites. There was no significant difference in BMD changes between men and women (*Figure 2*).

Table 4. Factors potentially affecting vertebral fracture (VF) risk in acromegaly patients with long-term disease control.

	Vertebral fractures (VF)		
	No VF n=37	**VF n=52**	**P-value**
Gender (n(%))			0.02
Males	17 (46 %)	29 (56 %)	
Females	20 (54 %)	23 (44 %)	
Age (yrs)	56.5 (1.9)	59.5 (1.6)	0.23
BMI (kg/m^2)	27.9 (0.8)	28.9 (0.7)	0.89
Length (cm)	175.9 (1.8)	175.6 (1.4)	0.45
Weight (kg)	86.6 (2.9)	86.9 (2.6)	0.31
Age at diagnosis (yrs)	41.2 (2.2)	40.3 (2.1)	0.96
Disease duration (yrs)	8.7 (1.0)	8.9 (1.3)	0.52
Duration since diagnosis (yrs)	16.9 (1.5)	20.1 (1.4)	0.85
Duration of remission (yrs)	12.5 (1.0)	15.2 (1.0)	0.07
Pre-treatment fasting GH (ug/l)	36.4 (7.6)	37.0 (7.3)	0.77
Pre-treatment IGF-I SD scores	7.8 (0.9)	6.7 (0.8)	0.11
Hypogonadism (n(%))	18 (47 %)	27 (52 %)	0.85
P1NP (ng/ml)	38.3 (4.0)	37.2 (3.2)	0.66
Beta-crosslaps (ng/ml)	0.37 (0.04)	0.35 (0.03)	0.73
BMD lumbar spine (follow-up) (g/cm^2)	1.02 (0.04)	1.01 (0.02)	0.52
T-score lumbar spine	-0.40 (0.34)	-0.53 (0.21)	0.62
Z-score lumbar spine	0.63 (0.32)	0.57 (0.25)	0.58
BMD Total Hip (follow-up) (g/cm^2)	0.89 (0.03)	0.87 (0.02)	0.36
T-score total hip	-0.43 (0.24)	-0.50 (0.14)	0.38
Z-score total hip	0.55 (0.18)	0.41 (0.13)	0.51

Data are depicted as mean (SEM), unless mentioned otherwise. Data were analyzed by binairy logistic regression analysis, with adjustments for age, gender, BMI, parameters of disease activity, and hypopituitarism, when appropriate. SEM: standard error of the mean, n: number, yrs: years, kg: kilogram, m: meter, cm: centimeter, GH: growth hormone, IGF-I: insulin-like growth factor type I, SD: standard deviation, P1NP: procollagen type 1 amino-terminal propeptide, BMD: bone mineral density.

There was also no significant difference in baseline, delta BMD, or current BMD at the lumbar spine or at the total hip between patients with, and without documented vertebral fractures (*Table 2*). Changes in BMD from baseline were not affected by gender, current GH and IGF-I concentrations, duration of active acromegaly, duration of follow-up, gonadal status, or the presence of hypopituitarism. Pituitary irradiation was associated with a significant negative effect on BMD (p=0.05) and Z-scores (p=0.03) of the total hip, which persisted after adjusting for age and gonadal status (p=0.01).

Analysis of potential risk factors for increased vertebral fracture risk

Gender represented a significant risk factor for vertebral fractures, with men being significantly more at risk. There was no significant relationship between the prevalence of vertebral fractures and age, BMI, age at diagnosis, duration of active disease, pre-treatment or controlled levels of GH / IGF-I, type of treatment, duration of controlled disease, the presence of hypopituitarism, markers of bone turnover, treatment with bisphosphonates, and for current or delta BMD in patients with and without vertebral fractures (*Table 4*).

DISCUSSION

This study indicates a high prevalence of vertebral fractures in acromegalic patients with sustained controlled disease for a mean of 14 years. Approximately 60% of these patients had suffered at least one vertebral fracture. The prevalence of these vertebral fractures was considerably increased when compared with a large Dutch cohort of the general population[25]. These fractures in acromegaly patients occurred independently of age, severity or duration of disease activity, type of treatment, presence of hypopituitarism, or BMD, and despite normal vitamin D concentrations. There was a significantly increased prevalence of fractures in men, particularly in the presence of hypogonadism. Apparently, fractures are another feature of the irreversible changes in the skeleton present in acromegalic patients with long-term biochemical control[26] (see *Figure 3* for an image of the fractures)

There was no significant decrease in BMD at any site measured in the subgroup of patients in which BMD was measured with an interval of 7 years, despite increasing age of the population studied. We have previously demonstrated a sustained maintenance of BMD par-



Figure 3. This radiograph of the lumbar spine shows grade 1 and 2 morphometric vertebral deformities, some endsplate sclerosis, and osteophytosis. Apparent in this radiograph is the calcification of the aorta. These changes in a patient 18.5 years after control of disease activity illustrate how the irreversible, structural modifications of the spine caused by GH excess during the active period of acromegaly, may lead to an overestimation of lumbar spine BMD. Note the relatively wide intervertebral discs and mild posterior scalloping of the vertebral bodies, compatible with the underlying disorder.

ticularly at trabecular sites after >10 years of correction of GH excess[2]. In the present study, we extend these observations to >17 years of follow-up, suggesting that the beneficial effect of GH excess on BMD is long-lasting, although apparently this does not protect from risk of fractures, as an increase in BMD may not necessarily translate in improved bone quality[27].

Control data on vertebral fractures were derived from a large Dutch epidemiological study, that did not include subjects younger than 55, since the occurrence of vertebral fractures is very unlikely in that age group[25]. The prevalence of vertebral fractures in acromegalic patients largely exceeded the prevalence in the control population, in all age groups[25]. In addition, large European population-based cohort studies also report a much lower prevalence of vertebral

fractures in both men and women[28-32], comparable to the findings in the Dutch reference cohort. Our findings, based on the scoring of two independent scorers and confirmed by an experienced radiologist, demonstrate that the prevalence of vertebral fractures in acromegalic women, but even more so in acromegalic men, exceeds the reported prevalence of vertebral fractures in the general population, including the Dutch population.

The results of this study are in keeping with data from another group that studied the prevalence of vertebral fractures in 36 post-menopausal females and 40 male patients with controlled (~60%) or active acromegaly[15-16]. Those studies reported a comparable high prevalence of vertebral fractures of 53% in females, and 57% in male patients. However, some differences between our study and the previous studies have to be highlighted. First, the duration of disease control was much longer in our patients and there are no data available on vertebral fractures in acromegaly with controlled disease for more than 14 years. Second, all our patients were vitamin D replete and received hormonal substitution, when applicable. Logistic regression analysis demonstrated that, after correction for all potentially influencing factors, hypogonadism, particularly in men, was associated with vertebral fracture risk. Unfortunately, it was not possible to determine when the vertebral fractures had occurred, as most of these fractures were asymptomatic. As a consequence, we were unable to discriminate fractures that had occurred at the time of active acromegaly from those that occurred during the long period of disease control. Irrespective of this issue, these fractures may have significant clinical implications regarding morbidity and mortality since vertebral fractures are associated with decreased quality of life, increased morbidity and mortality, and increased risk of new (non)vertebral fractures[33-38]. Longitudinal studies are required to address these topics and to assess the contribution of other risk factors for these fractures, including vitamin D, (replacement for) hypopituitarism, and age.

GH and IGF-I are important anabolic hormones for bone. In fact, most of the effects of GH are mediated by systemic and/or local IGF-I, which enhances the differentiated function of osteoblasts and bone formation, although GH may also act directly on bone cells[39]. On the other hand, chronic GH and IGF-I excess, such as present in active acromegaly, may impair bone quality, independently of any change in BMD, and apparently increase the risk of fractures, the most important consequence of a decrease in bone quality.

The high prevalence of vertebral fractures in the presence of normal BMD may be due to an overestimation of lumbar spine BMD due to irreversible, structural modifications of the spine, such as degenerative changes and vertebral deformities as a consequence of a prolonged

exposure of the skeleton to GH excess in the active stage of acromegaly. BMD of the hip is less affected by structural changes in bone. However, there was no association between low BMD of the hip and vertebral fractures, in accordance with the results recently published by Mazziotti *et al.* [16]. Six patients were (in the past) treated with bisphosphonates, and these patients were excluded from analysis of sequential changes in BMD. The exclusion of these patients did not affect our conclusions.

In conclusion, our findings indicate a high prevalence of vertebral fractures in acromegalic patients during very long follow-up of controlled disease, independently of BMD. Our data also suggest that BMD is maintained during prolongation of follow-up of patients with biochemical control of acromegaly. In view of the significant morbidity associated with vertebral fractures, we advocate the inclusion of lateral conventional radiographs of the thoracic and lumbar spine in the screening of all patients with acromegaly both at diagnosis and during follow-up after establishment of disease control.

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Chapter 9.

ACROMEGALY IS ASSOCIATED WITH AN INCREASED PREVALENCE OF COLONIC DIVERTICULA: A CASE-CONTROL STUDY

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ABSTRACT

Objective: In acromegaly, overproduction of GH and IGF-I causes abnormal extracellular matrix regulation. We hypothesized that this may predispose to the development of colonic diverticula. Since the relation between acromegaly and colonic diverticula is unknown, the study aim was to assess the prevalence of colonic diverticula in patients with cured acromegaly.

Design: We conducted a case-control study.

Methods: We screened reports of colonoscopies performed for the purpose of screening for polyps in 107 patients with cured or biochemically controlled acromegaly and in 214 age- and sex-matched controls for the presence of diverticula, dolichocolon, and polyps. In patients, the findings were related to GH/IGF-I concentrations at the time of diagnosis of acromegaly and to the duration of GH/IGF-I excess.

Results: In acromegaly, colonic diverticula were present in 37% of patients, dolichocolon in 34%, and adenomatous polyps in 34%, which was increased compared with controls (OR 3.6, 95% CI: 1.4, 5.7; 12.4, 95% CI: 6.8, 18.0; 4.1, 95% CI: 1.9, 6.4, respectively). The presence of colonic diverticula was associated with both GH and IGF-I concentrations at the time of diagnosis of acromegaly, when adjusted for the duration of active disease. The presence of dolichocolon and adenomatous polyps was associated with higher IGF-I concentrations at diagnosis.

Conclusions: Acromegaly is associated with an increased prevalence of colonic diverticula. In addition to the known irreversible effect of GH excess on collagen of joints and cardiac valves, this observation indicates an irreversible effect of GH and/or IGF-I on the collagen in the colon.

INTRODUCTION

Diverticular disease is common in Western and industrialized societies [1;2]. Diverticula are most often found in the sigmoid and descending colon[1;3]. Although the pathophysiology of colonic diverticula is still incompletely understood, it is generally assumed that aging and low dietary fibre are the main pathogenic factors involved in this disease. Aging might be associated with altered motility and weakening of the colonic wall and low fibre diet leads to a small amount of stool, resulting in colonic segmentation. The intraluminal pressure tends to be high in the segmented colon and causes mucosal and submucosal herniation through the weakened muscle layer, and, consequently, colonic diverticula develop[4;5].

Acromegaly is a chronic, slowly developing disease caused by a growth hormone-secreting pituitary adenoma leading to increased circulating growth hormone (GH) levels and increased insulin-like growth factor-I (IGF-I) secretion by peripheral tissues, especially the liver. Well known gastro-intestinal manifestations associated with acromegaly are colon carcinoma, adenomatous polyps, and dolichocolon[6-8].

GH is involved in matrix regulation, since GH increases gene expression of the matrix metalloproteinases (MMPs), that are capable of altering the composition of the extracellular matrix[9]. Recently, changes have been found in heart valves and aortic root in patients with acromegaly, comparable to those observed in patients with Marfan's syndrome, which is a connective tissue disease[10;11]. Marfan's syndrome is also associated with colonic diverticula[12-14]. Therefore, we hypothesized, that in acromegaly abnormal extracellular matrix regulation caused by overproduction of GH and IGF-I may also predispose to the development of diverticula.

The prevalence, localization, and severity of colonic diverticula in acromegaly is currently unknown. Therefore, the aim of our study was to quantify the presence of colonic diverticula in a well-characterized cohort of cured acromegalic patients, and in age- and sex-matched controls. In addition, we related the prevalence of colonic diverticula, cecal intubation rate, increased bowel length referred to as dolichocolon, and adenomatous polyps to parameters of initial disease activity of acromegaly, in order to gain more insight into the pathophysiological processes involved in the colonic complications of acromegaly, including colonic diverticula.

PATIENTS AND METHODS

Patients

We screened the medical records of all acromegaly patients currently in our database for eligibility in the current analysis. Since 1992 all acromegaly patients were advised to undergo a colonoscopy to screen for colonic polyps[15]. Patients were eligible when a colonoscopy had been performed in our center (n=110) and after biochemical control or cure of acromegaly had been achieved (n=107).The included patients did not differ from the excluded patients in age at diagnosis, gender, BMI, GH and IGF-I concentrations at diagnosis, or type of treatment.

The initial diagnosis of acromegaly was based on the characteristic clinical signs and symptoms and confirmed by insufficient suppression of GH during a glucose tolerance test (normal response: GH nadir <0.38 μg/L), elevated age- and gender-adjusted IGF-I levels, and the presence of a pituitary adenoma on radiological imaging. The first treatment option in the majority of patients was transsphenoidal surgery (TPS) performed by a single specialized neurosurgeon. If necessary, adjuvant treatment was given by radiotherapy (prior to 1985) or somatostatin (SMS) analogs (from 1985 onwards). This treatment approach resulted in early postoperative control in 66% and late control in 90% of patients[16]. From 1998, in some patients primary treatment was given in the form of depot formulations of SMS analogs. From 2003, patients with inadequate control by surgery and/or long-acting SMS analogs were treated with pegvisomant.

Disease activity was assessed yearly by oral glucose tolerance tests (except in patients treated with SMS analogs or pegvisomant), measurement of fasting serum GH and IGF-I concentrations, and evaluation of other pituitary functions. Remission of acromegaly was defined as a normal glucose suppressed serum GH less than 1.25 (RIA assay until 1992) or 0.38 μg/L (IFMA assay from 1992 onwards), serum GH levels less than 1.9 μg/L (all years) and normal IGF-I levels for age (from 1986 onwards) at yearly follow-up visits[16-18]. Hypopituitarism was treated promptly with thyroxine, hydrocortisone, testosterone or estrogens (in pre-menopausal women) when deficiencies were documented using appropriate basal hormone and dynamic tests[19].

Disease duration was calculated from the estimated date of onset, using start of signs and symptoms, facial changes on photographs and, in case of disease cure or remission, to the

date of normalization of serum IGF-I concentration after transsphenoidal surgery or additional treatment. GH and IGF-I concentrations at diagnosis and at colonoscopy were used for analyses. The GH concentration at diagnosis was calculated as the mean of several random fasting GH concentrations prior to diagnosis. Since IGF-I measurements were performed since 1986, pre-treatment IGF-I concentrations were available in 69 of the 107 patients. We combined the duration and severity of exposure to the high circulating concentrations of GH and IGF-I SD by multiplying the duration of active disease (in years) with the natural logarithm of the pre-treatment GH and IGF-I SD concentrations These products of pre-treatment exposure to GH or IGF-I will be referred to as: duration*lnGH or duration*lnIGF-I SD.

Controls

We included an age- and sex-matched control population consisting of 214 patients who had colonoscopic evaluation with the primary aim to screen for colorectal polyps. In these controls colonoscopy was also performed with the primary aim to screen for colorectal polyps, because of rectal blood loss, a positive family history of colon cancer, or aspecific abdominal complaints. Each acromegalic patient was matched to 2 controls. Patients and controls were compared on the presence of diverticula, cecal intubation rate, dolichocolon, and on colorectal polyps. All colonoscopies were performed at the Leiden University Medical Center using a strict protocol, under the same conditions as the patients, and from 1995 onward. All controls were derived from the same geographical area as the patients. The medical ethical committee approved the review the medical records and did not require informed consent for this retrospective analysis.

Colonoscopic evaluation

Colonoscopy was performed routinely in all patients with an established diagnosis of acromegaly. The records of colonoscopies performed in the Leiden University Medical Center between 1992 and 2008 were used for the present analysis. Structured reports of the colonoscopy were retrospectively analyzed and scored for the presence of diverticula, cecal intubation rate, dolichocolon and colorectal polyps. In case of biopsies, the histopathology was recorded. In case of more than one colonoscopy, the most recent colonoscopy was used for analysis, although polyps taken by biopsies in previous colonoscopies were also taken into account.

All patients and controls were prepared with four liters of hypertonic polyethylene glycol solution (Kleanprep; Helix Bio-pharma Corp., Aurora, Ontario, Canada). The proce-

dures were performed under conscious sedation using midazolam and/or fentanyl. Cecal intubation was confirmed by identification of the orifice of the appendix and the ileocecal valve.

Hormone assays

Serum GH levels were measured with a sensitive immunofluorometric assay (IFMA) (Wallac, Turku, Finland), specific for the 22 kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit 0.01 μg/l; intra-assay coefficient of variation (CV) 1.6-8.4% of 0.01-15.38 μg/l) from 1992 onwards. For conversion of μg/l to mU/l, multiply by 2.6. Before 1992, GH was measured by RIA (Biolab, Serona, Coissins, Switzerland) calibrated against WHO IRP 66/21(detection limit 0.5 mU/L, interassay coefficient of variation <5%, for conversion from μg/l to mU/l, multiply by 2).

From 1986 up till 2005, serum IGF-I concentrations were determined by a radioimmunoassay (RIA) (Incstar; Stillwater, MN, USA) with a detection limit of 1.5 nmol/l and an inter-assay CV below 11%. IGF-I is expressed as SD scores (SD score) for age- and gender-related normal levels determined in the same laboratory[20]. From 2005 onwards serum IGF-I concentration (ng/ml) was measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay CV was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/l, resp. IGF-I levels were expressed as SD score, using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals[21;22].

Statistical analysis

SPSS for Windows version 16.0 (SPSS inc., Chicago, IL) was used for data analysis. Data are presented as mean (SEM). We dichotomized the data according to the presence or absence of colonic diverticula, dolichocolon, cecal intubation, or polyps. First, we performed comparisons between acromegalic patients and controls by binary logistic regression analysis, with adjustments for age at colonoscopy and gender, to estimate odds-ratios (OR) shown with corresponding 95% confidence intervals (CIs), see *Table 2*. Second, within patients with acromegaly we performed binary logistic regression analysis with presence of diverticula as dependent variable adjusted for several factors and covariates. OR's reflected in page 9 and 10 are the result of these analysis. Third, Multinomial logistic regression analysis was used, with disease duration in tertiles as dependent variable, to estimate OR's with corresponding 95% CIs. The lowest tertile

was the reference category, see *Table 4*. Colonic diverticula and gender were entered in the model as factors and the covariates were lnpre-treatment GH concentrations, lnpre-treatment IGF-I SD scores, age at colonoscopy, and BMI. The OR's and 95% CIs are not displayed, but were subsequently transformed to risk ratios (RR) and corresponding 95% CI using the approximation formulae described by Zhang *et al*.[23]. This approach was chosen because the OR's for common outcomes in a closed cohort is no good approximation of the RR.

RESULTS

Patient and treatment characteristics

We studied 107 patients, 60 men (56%) and 47 women (44%), with long term cure/biochemical control of acromegaly. At the time of diagnosis of acromegaly the mean age was 46.8 ± 1.2 yr, and at time of the most recent colonoscopy mean age was 56.4 ± 1.2 yr. The treatment characteristics of acromegaly are provided in *Table 1*.

Table 1: Clinical characteristics of patients with biochemical control of acromegaly

	Males N=60 (56 %)	**Females N=47 (44 %)**	**P-value**
Age at diagnosis (yr)	45.1 (1.6)	49.9 (1.7)	0.02
Age at colonoscopy (yr)	54.7 (1.6)	58.5 (1.8)	0.17
BMI at diagnosis (kg/m^2)	27.2 (0.6)	26.4 (0.7)	0.20
Pre-treatment GH (ug/l)	31.6 (4.4)	24.9 (5.3)	0.33
Pre-treatment IGF-I SD score (n=69)	7.6 (0.74)	7.0 (0.8)	0.62
Treatment for acromegaly			
Surgery	38 (63 %)	22 (47 %)	0.09
Surgery + RT	9 (15 %)	7 (15 %)	0.89
Surgery + RT + SMS	3 (5 %)	2 (4 %)	0.82
Surgery + SMS	7 (12 %)	8 (17 %)	0.42
Primary SMS	3 (5 %)	6 (13 %)	0.38
Primary SMS + pegvisomant	-	2 (4 %)	-
Duration of active disease (yr)	10.2 (1.3)	9.0 (1.3)	0.80
Duration of disease remission (yr)	9.9 (1.3)	10.2 (1.7)	0.43

Data are shown as mean (SEM), unless mentioned otherwise. P<0.05 was considered significant. Pre-treatment IGF-I SD score was only available in 69 patients. Abbreviations: SEM: standard error of the mean, SD: standard deviation, yr: year, BMI: body mass index, RT: radiotherapy, SMS: somatostatin (analogs), kg: kilogram, m: meter. Bold P-values reflect significant difference.

The mean estimated duration of active disease prior to diagnosis was 9.7 ± 1.0 yr and the mean duration of remission was 9.8 ± 0.9 yr. At the time of diagnosis of acromegaly, mean serum GH concentration was 29.1 ± 2.7 μg/l and mean IGF-I SD score was 7.4 ± 0.5 SD. At the time of colonoscopy, mean serum GH concentration was 1.01 ± 0.2 μg/l and mean IGF-I SD score was 0.58 ± 0.2 SD.

Patients were compared with 214 age- and sex-matched non-acromegalic controls, 120 men (56%) and 94 women (44%). In controls, the mean age at colonoscopy was 57.0 ± 1.8 yr, which was comparable to that of the patients. The percentage Caucasians in patients and controls was comparable (95% *vs.* 94%, p=0.91).

Prevalence of diverticular disease

As demonstrated in *Table 2*, diverticula were reported in 40 patients (37%), left-sided in 38 patients and right-sided in 4 patients.

Table 2. Increased prevalence of diverticular disease and other colon abnormalities in patients with acromegaly compared with controls.

	Acromegalic patients n=107	**Controls n=214**	**OR (95% CI)**
Diverticula	40 (37 %)	41 (19 %)	3.6 (1.4, 5.7)
Left-sited	38 (36 %)	39 (18 %)	3.6 (1.4, 5.6)
Right-sited	4 (4 %)	11 (5 %)	1.3 (0.4, 4.0)
Dolichocolon	36 (34 %)	2 (1 %)	12.4 (6.8, 18.0)
Cecal intubation rate	83 (78 %)	205 (96 %)	0.14 (0.1, 0.3)
Colonic polyps	51 (48 %)	47 (22 %)	3.6 (2.1, 5.2)
Hyperplastic	15 (14 %)	19 (9 %)	1.8 (0.9, 2.8)
Adenomatous	36 (34 %)	28 (13 %)	4.1 (1.9, 6.4)
Low grade	30 (28 %)	21 (10 %)	
Intermediate grade	3 (3 %)	4 (2 %)	
High grade	3 (3 %)	3 (1 %)	
Colonic malignancy	3 (3 %)	9 (4 %)	0.91 (0.1, 1.9)

Data are shown as number (%). Data were compared by binary logistic regression analysis, with adjustments for age at colonoscopy and gender. Results are demonstrated as odds ratio (OR) with 95% confidence interval (CI). Controls were the reference category. Bold OR's reflect significant difference.

This prevalence in acromegalic patients was higher compared with the prevalence in controls (19%) (p=0.002). In acromegaly, diverticula were identified at a younger age than in controls (58.0 ± 1.7 yr *vs.* 72.3 ± 1.8 yr, p<0.001). Thirty-five percent of controls had colonoscopy for symptomatic complaints. Exclusion of these controls from analysis did not affect our outcome.

Factors influencing diverticular disease in acromegaly patients

Age, gender, and BMI.

There was a positive correlation between female gender and the risk for diverticula (OR 3.1, 95% CI: 1.3,7.4). However, after adjustment for age at colonoscopy and BMI, this positive association was not statistically significant (OR 2.2, 95% CI: 0.8,6.1). Age at colonoscopy was positively associated with diverticula, even when adjusted for gender and BMI (OR 1.09, 95% CI 1.0,1.2). Age at diagnosis was not associated with diverticula when adjusted for gender and BMI.

Table 3. Differences in clinical characteristics and biochemical finding between acromegaly patients with and without diverticular disease.

	Diverticular disease		
	No n=68	**Yes n=39**	**P-value**
Gender (% males)	58 %	42 %	0.03
Age at diagnosis (yr)	45 (1)	51 (2)	0.01
Age at colonoscopy (yr)	54 (2)	63 (2)	0.01
BMI at diagnosis (kg/m^2)	27 (1)	27 (1)	0.91
Pre-treatment GH (ug/l)	29 (4)	29 (4)	0.92
Pre-treatment IGF-I SD score	7 (2)	7 (1)	0.86
Duration of active disease (yr)	9 (1)	14 (3)	0.02
Duration of disease remission (yr)	10 (1)	11 (2)	0.65

Data are shown as mean (SEM), unless mentioned otherwise. P<0.05 was considered significant. Abbreviations: SEM: standard error of the mean, SD: standard deviation, yr: year, BMI: body mass index, kg: kilogram, m: meter. Bold P-values reflect significant difference

GH/IGF-I concentrations at diagnosis and duration of active disease

Duration of active disease was almost twice as long in patients with diverticula (14.3 ± 3.1 yr) when compared with patients without diverticula (8.7 ± 0.9 yr) (p<0.001) and when adjusted for age, gender, and BMI logistic regression analysis demonstrated a significant positive relation between disease duration and diverticular disease (OR 1.09, 95% CI 1.0, 1.2). Pre-treatment

GH concentrations and IGF-I SD scores were not different between patients with and without diverticula (*Table 3*). However, when entered in the regression model together with duration of active disease, the relation between disease duration and diverticular disease became even stronger (OR 1.11, 95% CI: 1.1-1.2). As shown in *Table 4,* patients with the longest disease duration had increased relative risks (RR) for diverticula (highest tertile: >11 years).

The prevalence of hypopituitarism, initial treatment modalities, current use of SMS analogs or pegvisomant, and duration of remission were not associated with diverticula.

Table 4. Risk of presence of diverticular disease of patients with acromegaly in relation to tertiles of duration of acromegaly.

	Tertiles	Patients with diverticula (%)	Adjusted RR (95% CI)	p-value
Disease duration	**<5.0**	**18**	**1**	
(years)	5.0 – 11.0	23	2.01 (0.8, 3.6)	0.08
	>11.0	29	4.21 (2.4, 5.4)	<0.01

N=69. Data were analyzed by multinomial logistic regression analysis with tertiles of disease duration as dependent variable and the lowest tertile as reference category. Colonic diverticula and gender were entered in the model as factors and the covariates were ln pre-treatment GH concentrations, ln pre-treatment IGF-I SD scores, age at colonoscopy and BMI. Abbreviations: RR: relative risk, CI: confidence interval.

Cecal intubation rate and dolichocolon

The cecal intubation rate was significantly lower in patients (78%), compared with controls (96%) (p<0.001). Dolichocolon was present in 43 patients (34%), but only in 3 controls (1%) (p<0.001). Cecal intubation rate was significantly lower in patients with dolichocolon (64%) when compared with patients with normal colon length (89%, p<0.001).

Factors influencing dolichocolon

The presence of dolichocolon was not associated with age at colonoscopy, age at diagnosis of acromegaly, gender, or BMI. LnIGF-I SD scores at the time of diagnosis were associated with dolichocolon (OR 1.3, 95% CI 1.1-1.4) also when adjusted for the duration of pre-treatment exposure (OR 1.3, 95% CI: 1.1-1.5). LnGH at diagnosis was not associated with dolichocolon, nor when adjusted for disease duration.

There was no influence of hypopituitarism, type of treatment for acromegaly, or duration of remission on dolichocolon. Dolichocolon was more frequently present in patients with

diverticula than in patients without diverticula (odds 1.4, 95% CI: 1.2-1.7).

Colorectal polyps

In 51 patients (48%) polyps were found and biopsy or polypectomy was performed, with an equal distribution in men (46%) and women (49%). Histopathological examination showed hyperplastic polyps in 14% of patients and adenomatous polyps in 34% of patients (82% low grade, 4% intermediate grade, and 4% high grade dysplasia).

Patients had a higher prevalence of adenomatous polyps compared with controls (28% vs. 13%, $p<0.001$), whereas there were no differences between both groups with respects to the grades of dysplasia. Thirty-five percent of controls had colonoscopy for symptomatic complaints. Exclusion of these controls from the analysis did not affect the difference in the prevalence of polyps between patients and controls (28% vs. 12%, $p<0.001$).

Factors influencing colorectal polyps

Age at colonoscopy, age at diagnosis of acromegaly, gender, or BMI were not associated with adenomatous polyps. LnIGF-I SD concentration at diagnosis was associated with adenomatous polyps (OR 1.1, 95% CI: 1.01-1.14), even when entered in the regression model together with disease duration (OR 1.1, 95% CI: 1.03-1.12). LnGH concentrations at diagnosis were not associated with adenomatous polyps. There was no influence of hypopituitarism, type of treatment for acromegaly, or duration of remission on adenomatous polyps. There was no difference in the presence of diverticula and dolichocolon between patients with and without adenomatous polyps.

DISCUSSION

This case-control study in patients with cured/controlled acromegaly is the first to report that acromegaly is associated with colonic diverticula. In addition, we observed an association between colonic diverticula and duration/severity of previous exposure to increased circulating GH and IGF-I concentrations. The highest tertile of disease duration, adjusted for pre-treatment concentrations of GH and IGF-I SD scores, was associated with a more than four-fold increased risk for diverticula. This observation adds diverticular disease to the currently recog-

nized, irreversible effects of acromegaly.

Dolichocolon was also increased in the patient group and associated with IGF-I concentrations at diagnosis, when adjusted for the duration of active acromegaly. Dolichocolon is a well known feature of acromegaly[24]. However, we are the first to systematically evaluate the prevalence of this manifestation in a large population of patients with controlled acromegaly. Therefore, these observations stress that the severity of acromegaly is a determinant of the various manifestations of acromegaly with respect to colonic diseases, which also appears to include diverticular disease.

Diverticula are left-sided out-pouchings of colonic mucosa and submucosa that emerge through the muscularis propria[1;5]. Although risk-factors for diverticular disease including physical inactivity, constipation, obesity, smoking, and non-steroidal anti-inflammatory drugs (NSAIDs) have been described, the development of diverticula is mainly related to three essential precipitating factors: low fibre diet, altered motility, and colonic wall resistance. On a microscopic level colons with diverticulosis may have increased elastin in the taenia[25], and structural changes in the collagen that mimic those seen with aging[26]. This concerns not so much the total amount of collagen, but the cross-linking and an increase in type 3 fibres[27;28], which may decrease the tensile strength of the circular muscle, facilitating the formation of diverticula. Support for a pathophysiological role of alterations in connective tissue characteristics in diverticula are derived from observations in patients with connective tissue diseases like Marfan's and Ehlers-Danlos syndromes, who develop diverticula throughout the colon at a precocious age[12-14;28].

Diverticula in Marfan's syndrome are caused by an abnormally weak bowel wall, due to a mutation in the fibrillin gene, leading to disturbed connective tissue[13]. This same mechanism also leads to degeneration of cardiac valves and aortic root in these patients[10]. GH is also involved in matrix regulation. GH increases gene expression of the matrix metalloproteinases (MMPs), that are capable of altering the composition of the extracellular matrix[9]. In acromegaly, this altered matrix is thought to be responsible for changes found in heart valves and the aortic root[11], a mechanism known from Marfan's syndrome[10]. A crucial role of GH and IGF-I in the regulation of matrix regulation is further strengthened by the observations that both cardiac valve pathology and the presence of diverticula are strongly associated with the duration of exposure to abnormal GH and IGF-I concentrations[29].

In addition, in this study in patients with long-term follow-up of acromegaly, colonic

adenomatous polyps were associated with IGF-I SD concentrations at diagnosis of acromegaly, even when adjusted for the estimated duration of active acromegaly. In accordance with previous studies, documenting a high prevalence of adenomatous polyps in patients with acromegaly[30;31], we observed a higher prevalence of adenomatous polyps in comparison with our control group, even though our controls underwent colonoscopy for a medical reason, i.e. because of rectal blood loss, a positive family history of colon cancer, or aspecific abdominal complaints. The adenomatous polyp frequency in the controls was comparable with the frequency observed in general Caucasian population[32].

Some potential limitations of this study have to be addressed. Since the indication of colonoscopy in both patients and controls was screening for colorectal polyps, diverticular disease may have been underreported, despite the use of a structured protocol for colonoscopy reports. However, this may have underestimated, rather than overestimated diverticula and dolichocolon, and therefore does not affect our conclusions[33;34]. Moreover, it is unlikely this effect will be different between patients and controls. Thus, the control group is appropriate for comparison of prevalences of diverticular disease and dolichocolon. Second, although all patients and controls underwent colonoscopy for the purpose of polyp screening, the *a priori* chance for the presence of polyps and colorectal cancer will be different between patients and controls and also within the control group, dependent on the indication for colonoscopy, i.e. rectal bleeding, (symptomatic) or family history of colorectal cancer (asymptomatic). However, the prevalence of polyps in the controls is comparable to literature reports. Moreover, exclusion of symptomatic controls from the analysis did not affect our conclusions on the frequency of polyps or diverticula. Moreover, a potentially higher polyp prevalence in the controls due to the indication of rectal bleeding or familiar cancer risk, would decrease, rather than increase, the difference between patients and controls. Third, the fact that in 20% of colonoscopies in acromegaly cecal intubation failed, may potentially have underestimated the prevalence of diverticula and polyps in acromegaly. However, this would result in an underestimation of the prevalence of diverticular disease, which does not affect our conclusions with respect to the increased prevalence of colonic diverticula in acromegaly.

In conclusion, colonic diverticular disease is increased in patients with acromegaly compared with controls and diverticula were present at a significantly younger age. Diverticulosis in acromegaly was primarily associated with the duration of active disease, which became even stronger when adjusted for the extend of previous GH and IGF-I excess. In addition,

dolichocolon and adenomatous polyps were increased in patients when compared with controls, which were both associated with IGF-I concentrations at the time of diagnosis of acromegaly. We hypothesize that in patients with acromegaly diverticula develop due to disturbed matrix regulation caused by previous excess of GH and/or IGF-I.

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Part IV.

DIFFERENT EFFECTS OF THE EXON-3 DELETED GROWTH HORMONE RECEPTOR POLYMORPHISM

Chapter 10.

IMPACT OF THE EXON 3-DELETED GROWTH HORMONE RECEPTOR POLYMORPHISM ON SPONTANEOUS GROWTH AND THE GROWTH RESPONSE TO RECOMBINANT HUMAN GROWTH HORMONE THERAPY IN GROWTH HORMONE DEFICIENT (GHD) AND NON-GHD CHILDREN WITH SHORT STATURE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Context: The exon-3 deleted growth hormone receptor (GHR_{d3}) polymorphism is associated with an increased growth response to recombinant human GH (rhGH) therapy in some, but not all, studies in growth hormone deficient (GHD) and non-GHD children with short stature.

Objective: To assess the effects of GHR_{d3} on baseline height and the 1st year's growth response to rhGH treatment in pre-pubertal GHD and non-GHD children with short stature.

Design: Systematic review and meta-analysis

Methods: Fifteen studies reporting the effect of GHR_{d3} on growth parameters were included. Principal outcomes were baseline height SDS, and the weighted average of change in growth velocity (delta cm/yr) and height gain (delta height SDS) after 1 year of rhGH.

Results: In GHD, not in non-GHD, baseline height SDS was 0.159 SD higher (95%CI 0.020,0.298) in GHR_{d3} compared with $GHR_{wt\text{-}wt.}$ In GHR_{d3} rhGH therapy resulted in a higher increase in growth velocity (0.521 cm/yr, 95%CI 0.196,1.015) and height gain (0.075 SD; 95%CI 0.007, 0.143) compared with $GHR_{wt\text{-}wt}$. Meta-regression demonstrated a larger difference between GHR_{d3} and $GHR_{wt\text{-}wt}$ in studies using lower rhGH doses and carried out at a higher age, independently of the cause of short stature.

Conclusions: This meta-analysis in prepubertal children with short stature indicates that GHR_{d3} is associated with increased baseline height in GHD, but not in non-GHD. Furthermore, GHR_{d3} stimulates growth velocity by an additional effect of ~0.5 cm during the first year of rhGH treatment and this effect is more pronounced at lower doses of rhGH and higher age.

INTRODUCTION

The aim of treatment of short children with recombinant human growth hormone (rhGH) is to increase growth in childhood and adolescence, and to increase adult height[1]. Treatment with rhGH has been approved for the treatment of children with short stature caused by growth hormone deficiency (GHD), and, more recently, for short children with Turner syndrome, chronic renal failure, Prader-Willi-Labhart syndrome, SHOX-haploinsufficiency, and short children born small for gestational age (SGA). In addition, in the USA rhGH is approved for treatment of children with idiopathic short stature (ISS) (if height standard deviation score (SDS) <-2.25). The first year's growth response, as well as the adult height gain attained as a result of rhGH treatment, is influenced by several factors, including age and height deficit at the start of treatment, the underlying cause of short stature, the severity and duration of GHD, and the dose, injection frequency, and duration of rhGH therapy[2;3]. A possible predictor of adult height gain is the height velocity during the first year of rhGH treatment[4]. Recent reports have suggested that also genetic variations in the GH-IGF-I axis might affect the response to rhGH treatment.

GH acts at the target cell through the growth hormone receptor (GHR)[5]. After binding to the GHR, GH induces activation of the JAK-STAT pathway, ultimately leading to increased expression of IGF-I and other GH dependent genes. One of the genetic factors considered responsible for the variation in response to rhGH treatment is a common polymorphism in the GHR gene, leading to deletion of exon 3 (d3), which encodes a 22-aminoacid residue sequence in the extracellular domain[6;7]. Dos Santos *et al.* described in 2004 that this loss of exon 3 stimulates receptor expression and function, specifically by affecting the binding of rhGH, receptor processing, transport, stability, binding to other ligands, dimerization of GHR monomers, and signal transduction[8].

Since 2004 several studies have addressed the possible influence of the GHR_{d3} polymorphism on the growth response to rhGH treatment in children with diverse clinical conditions, including GHD[9-14], SGA[8;15-19], ISS[8;20;21], and Turner syndrome[22]. However, the results of these studies are remarkably inconsistent, because 6 reports [11;13;19;21;22] confirmed the findings of Dos Santos *et al.* [8], whereas another 8 reports[9;10;12;14;15;17;18;20] could not demonstrate a significant effect of the exon-3 deleted genotype (GHR_{d3}) on the growth response to rhGH. We hypo-

thesized that the discrepancies between these studies might be explained, at least in part, by the relatively small numbers of patients included in these studies, precluding sufficient statistical power.

Theoretically, variations in GH sensitivity due to genetic differences in the GHR can be compensated by reverse changes in endogenous pituitary GH secretion, which might mask the effect of the GHR polymorphism on spontaneous growth. Therefore, we also hypothesized that in patients with GHD this compensatory effect within the GH-IGF-I axis is disturbed. Consequently, one might expect that only in children with short stature due to GHD, but not in children with short stature due to other causes, this GHR_{d3} genotype is associated with a less severe growth failure. However, so far none of the studies in GHD children have been able to demonstrate such an effect on baseline height [4-9], which may be due to confounding factors and insufficient numbers of study subjects.

In view of these contradictory results on the effects of GHR_{d3} on growth parameters in response to rhGH treatment and since the effect on this polymorphism has never been demonstrated on baseline height SDS, we conducted a meta-analysis. The aims of the study were 1) to assess the effect of the GHR_{d3} on baseline height SDS in pre-pubertal GHD and non-GHD children, and 2) to assess the impact of the GHR_{d3} on height gain and change in growth velocity in response to 1 year rhGH treatment.

MATERIALS AND METHODS

Eligibility criteria

Two principal measures of outcome were used: 1) baseline height SDS in GHD and non-GHD children and 2) height gain and/or growth velocity in response to 1 year of rhGH treatment. Studies reporting one of these outcomes in prepubertal children and stratified according to the exon 3-deleted GHR polymorphism, i.e. $GHR_{wt\text{-}wt,}$ $GHR_{wt\text{-}d3,}$ $GHR_{d3\text{-}d3,}$ and/or GHR_{d3} (GHR_{d3} is the combination of $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ genotypes) were eligible for inclusion in the present study. A large proportion of studies reported outcomes for $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ separately. For the purpose of the present meta-analysis, we re-calculated the weighted mean of the outcome parameter of interest (baseline height and first year's growth velocity and height gain) as well as the combined standard error of the $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ group. Studies reporting

incomplete data or studies providing the effects of the separate genotypes on growth only in a figure could not be included in this meta-analysis.

Search strategy

We searched Medline, Embase, Web of Science, and the Cochrane Library for studies reporting the effect of d3GHR on growth parameters in response to rhGH treatment. Searches were performed using the following search strategy: d3GHR OR d3-GHR OR d3-growth hormone (GH) receptor OR (("Exon 3-deleted" OR "exon 3 deletion") AND Growth Hormone Receptor) OR (("exon 3" OR d3) AND (growth hormone receptor OR GHR OR gh receptor OR gh receptors OR growth hormone receptors) AND (polymorphism OR polymorphisms OR genotype OR genotypes OR isoform OR isoforms)). Searches were performed on the 4th of December 2008. In addition, the references of relevant articles were checked for additional articles. Abstracts of meetings and unpublished results were not included in the analysis.

Data review and data analysis

Data extraction and eligibility was assessed by two independent investigators (M.W. and N.B.). Inconsistencies in data extraction were resolved by consensus. We compared the effect of rhGH treatment and baseline height between subjects with at least one copy of the exon-3 deleted GHR, i.e. GHR_{d3}, and subjects without exon-3 deleted GHR, i.e. $GHR_{wt\text{-}wt}$. $GHR_{wt\text{-}wt}$ was used as the reference group. For studies assessing the effect of rhGH, the weighted mean difference in height gain (delta SDS) and growth velocity (cm/year) in the first year of treatment was calculated. The effect of the GHR_{d3} on baseline height, expressed as SDS, was assessed separately in GHD and non-GHD. A meta-analysis was performed for baseline height, height gain and growth velocity by using both a fixed effects model and a random effects model. To assess to which extent the effect measured by the set of single studies was hetero- or homogeneous, the I^2 index was assessed. The test seeks to determine whether there are genuine differences underlying the results of the studies (heterogeneity), or whether the variation in findings is compatible with chance alone (homogeneity), 0% is very homogeneous, 100% very heterogeneous. In addition, a meta-regression was performed to assess the effects of age, dose, and diagnosis on the effect of the different d3GHR genotypes on height gain and growth velocity. Statistical analyses were done in Comprehensive Meta-Analysis (version 2·0, Biostat, Englewood, New Jersey, USA) and in STATA for Windows (version 10.1, StataCorp, Texas, USA).

RESULTS

Literature search

We identified 136 studies by search in Medline, Embase, Web of Science, and the Cochrane Library (*Figure 1*).

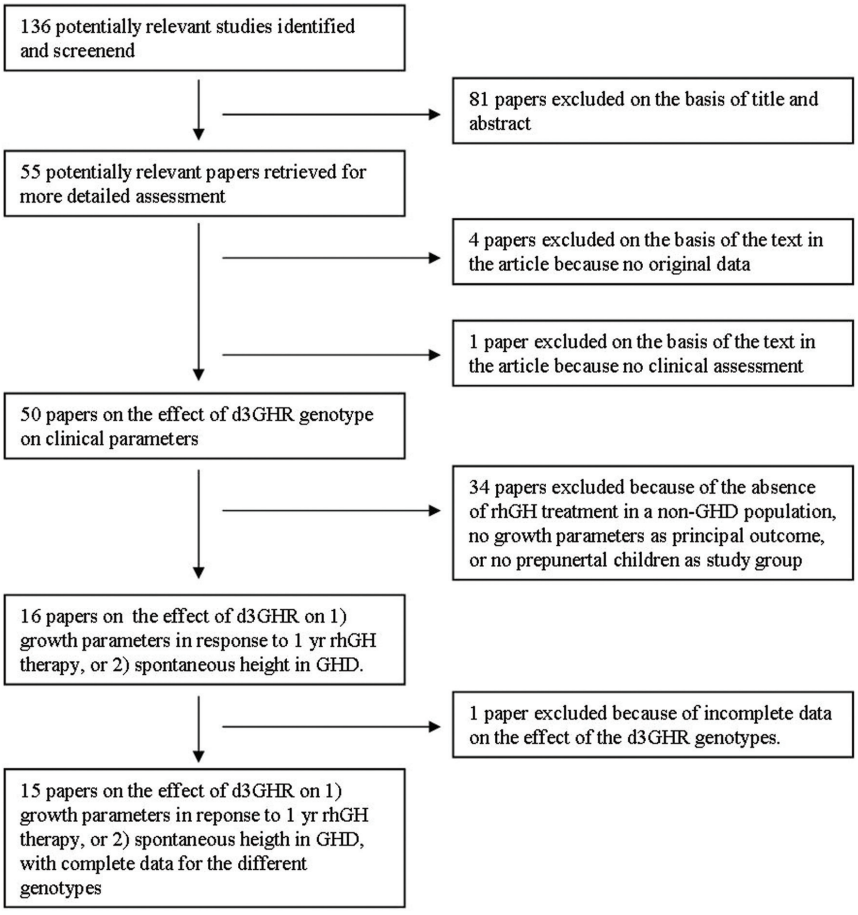


Figure 1. Summary of study assessment and exclusion stages.

We excluded 81 papers on the basis of title and abstract. Four of the 55 potentially relevant papers retrieved for more detailed assessment, were excluded from further analysis, because these studies did not report original data and one more could be excluded because the

effect of d3GHR on clinical parameters was not assessed. Additionally, 34 of the 50 papers with data on the effect of d3GHR on clinical parameters were excluded because of the absence of rhGH treatment in GHD or non-GHD populations, of reporting on growth parameters, or of pre-pubertal children as study group. The study from Pilotta *et al.*[23] did not provide precise data on genotypic differences and was therefore excluded from the present analysis. Consequently, a total of 15 studies were included in this meta-analysis (*Figure 1*).

These 15 studies provided data on the effect of rhGH therapy on height gain (n=1)[10], growth velocity (n=4)[8;12;13;21], or both (n=9) [9;11;14;15;17-20;22]. From 5 studies (one publication reported on 2 study populations[10]) [9-11;13] in GHD and from 11 studies (two publications reported on 2 different study populations [6;20] in non-GHD [13-19] data on baseline height could be extracted.

Study characteristics

Details of the 15 included studies are summarized in *Table 1*. Studies on the effect of d3GHR on baseline height or the growth response to rhGH were published between 2004 and 2008, and the number of included patients ranged from 28 to 240. Dos Santos *et al.*[8], Binder *et al.* [22] and de Graaff *et al.* [10] reported on 2 different study populations in the same paper, and for the purpose of the present analysis these populations were described and analyzed separately. Dos Santos *et al.* studied 2 separate populations of both SSA and ISS pre-pubertal children [8]. Binder *et al.* studied SGA and ISS children separately in the same paper[22], and De Graaff *et al.* [10] studied isolated GHD and multiple GHD pre-pubertal children in the same paper.

Indications for rhGH treatment were GHD (isolated GHD or in combination with multiple endocrine deficiencies) [9-14], small for gestational age (SGA) [8;15;17-19;22], idiopathic short stature (ISS) [8;20;21], and Turner syndrome [16;22]. The dosages of rhGH applied in the 15 included studies ranged from 26 μg/kg/day to 66 μg/kg/day and mean age ranged from 7 to 9 years. The genotypic distribution showed some inter-study variation, but in all included studies the distribution was according to the Hardy Weinberg Equilibrium (HWE). The distribution remained in HWE when all 1680 patients of the 15 studies were analyzed together. The genotypes included in this meta-analysis were: wt-wt=851 (51%), wt-d3=631 (37%), and d3-d3= 198 (12%).

Table 1. Overview of publications on the pharmacogenetic effects of rhGH in relation to d3GHR genotype on 1) baseline height in GHD and 2) growth velocity and/or height SDS gain in response to 1 year of rhGH treatment, arranged according to clinical condition

First author	N	Male	Age	GHR genotye (%)			Outcome	Significant effect of d3GHR polymorphism vs. wildtype		
		(%)	(yrs)	wt-wt	wt-d3	d3-d3		Effect of WT-D3	Effect of D3-D3	Effect of D3
GH deficiency in children										
Blum, 2006 (9)	107	68	7.0	59 (55%)	45 (42%)	3 (3%)	1. baseline height	NR	NR	No effect
							2. growth velocity and height gain	NR	NR	No effect
Jorge, 2006 (11)	58	62	8.9	28 (48%)	23 (40%)	7 (12%)	1. baseline height	NR	NR	↑ growth velocity
							2. growth velocity and height gain	NR	NR	No effect
Wan, 2007 (14)	154	70	7.8	79 (51%)	55 (36%)	20 (13%)	growth velocity and height gain	No effect	No effect	NR
De Graaff I,	40	NG	5.5	17 (43%)	19 (47%)	4 (9%)	1. baseline height	NR	NR	No effect
2008 (10)							2. height gain	NR	NR	No effect
De Graaff II, 2008 (10)	45	NG	4.8	19 (43%)	21 (47%)	5 (9%)	1. baseline height	NR	NR	No effect
							2. height gain	NR	NR	No effect
Marchisotti,	28	57	11.2	14 (50%)	11 (39%)	3 (11%)	1. growth velocity	NR	NR	No effect
2008 (12)										
Räz, 2008 (13)	181	54	6.6	90 (50%)	71 (39%)	20 (11%)	1. baseline height	No effect	↑ growth velocity	↑ growth velocity
							2. growth velocity	No effect	No effect	No effect
Small for gestational age										
Dos Santos I ,	76	61	6.6	36 (47%)	24 (32%)	16 (21%)	growth velocity	NR	NR	↑ growth velocity
2004 (8)										
Binder I, 2006 (22)	60	63	7.1	29 (48%)	23 (38%)	8 (14%)	growth velocity and height gain	↑ growth velocity	No effect	↑ growth velocity
Carrascosa, 2006 (17)	68	49	± 7.1	30 (44%)	32 (47%)	6 (9%)	growth velocity and height gain	No effect	No effect	NR
Tauber, 2007 (19)	240	58	6.6	144 (60%)	65 (27%)	31 (13%)	growth velocity and height gain	No effect	No effect	↑ growth velocity
Audi, 2008 (15)	219	50	9.1	99 (45%)	96 (44%)	24 (11%)	growth velocity and height gain	No effect	No effect	NR
Carrascosa,	49	72	7.7	23 (47%)	21 (43%)	5 (10%)	growth velocity and height gain	No effect	No effect	NR
2008 (18)										
Idiopathic short stature										
Dos Santos II, 2004 (8)	96	64	7.7	50 (52%)	38 (40%)	8 (8%)	growth velocity	NR	NR	↑ growth velocity
Carrascosa,	106	55	7.8	46 (43%)	42 (40%)	18 (17%)	growth velocity and height gain	No effect	No effect	NR
2008 (20)										
Ko, 2008 (21)	52	51	8.0	37 (71%)	13 (25%)	2 (4%)	growth velocity	NR	NR	↑ growth velocity
Turner syndrome										
Binder II, 2006 (22)	53	0	9	27 (51%)	15 (28%)	11 (21%)	growth velocity and height gain	No effect	↑ growth velocity compared with wt-wt and wt-d3	↑ growth velocity
Binder, 2008 (16)	48	0	9.1 - 16.0	24 (50%)	17 (36%)	7 (14%)	growth velocity	No effect	↑ period of high growth velocity	NR

Legend Table 1. Effect means: p<0.05, no effect: p>0.05. NR: not reported, yrs: years, GHR: growth hormone receptor, rhGH: recombinant human growth hormone (treatment). D3 = $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ *vs* $GHR_{wt\text{-}wt}$. The use of 2 different study groups in 1 paper is referred to as either I or II of those authors.

Meta-analysis

Baseline height in GHD pre-pubertal children (Figure 2A)

The effect of d3GHR on baseline height (SDS) in GHD children without previous rhGH treatment was assessed in five studies [9-11;13]. In 4 of these 5 studies baseline height (SDS) was higher in the GHR_{d3} than in the $GHR_{wt\text{-}wt}$ groups, which reached significance in only one study[11]. The mean difference in baseline height (SDS) between GHR_{d3} and $GHR_{wt\text{-}wt}$ in the combined data from these 5 studies was -0.159 (95% CI: -0.298, -0.020), which reflects a small positive effect in GHR_{d3} when compared with $GHR_{wt\text{-}wt}$ in the absence of rhGH treatment (I^2 0%). This was significant, even when with for age (p=0.04).

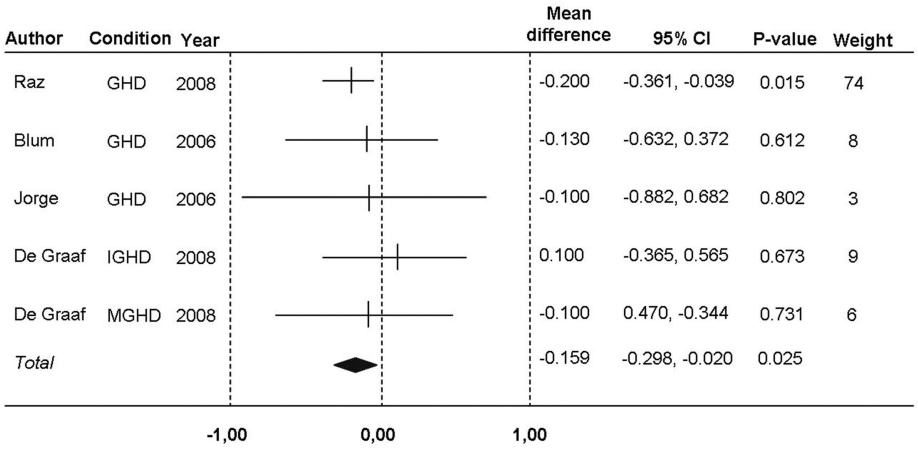


Figure 2A. Meta analysis of the effect of $GHR_{wt\text{-}wt}$ *vs.* GHR_{d3} genotypes on baseline height (SDS) in children with short stature caused by GHD. 'Total' represents fixed effects. A negative score points towards lower baseline height in $GHR_{wt\text{-}wt}$ subjects. De Graaff *et al.* studied isolated GHD (IGHD) and multiple GHD (MGHD) in the same paper[10]. CI: confidence interval. GHD: growth hormone deficiency, SGA: small for gestational age, ISS: idiopathic short stature.

Baseline height in non-GHD pre-pubertal children (Figure 2B)

In non-GHD children baseline height (SDS) was cross-sectionally assessed in 9 of 15 studies, and 2 of these 9 articles reported on 2 study populations[6;20]. None of these studies demonstrated a significant difference in baseline height (SDS) between GHR_{d3} and $GHR_{wt\text{-}wt}$. The mean difference in baseline height between GHR_{d3} and $GHR_{wt\text{-}wt}$ was -0.05 SDS (95% CI: -0.180,

0.080), which indicates the absence of a significant difference between GHR_{d3} and $GHR_{wt\text{-}wt}$ children (I^2 0%).

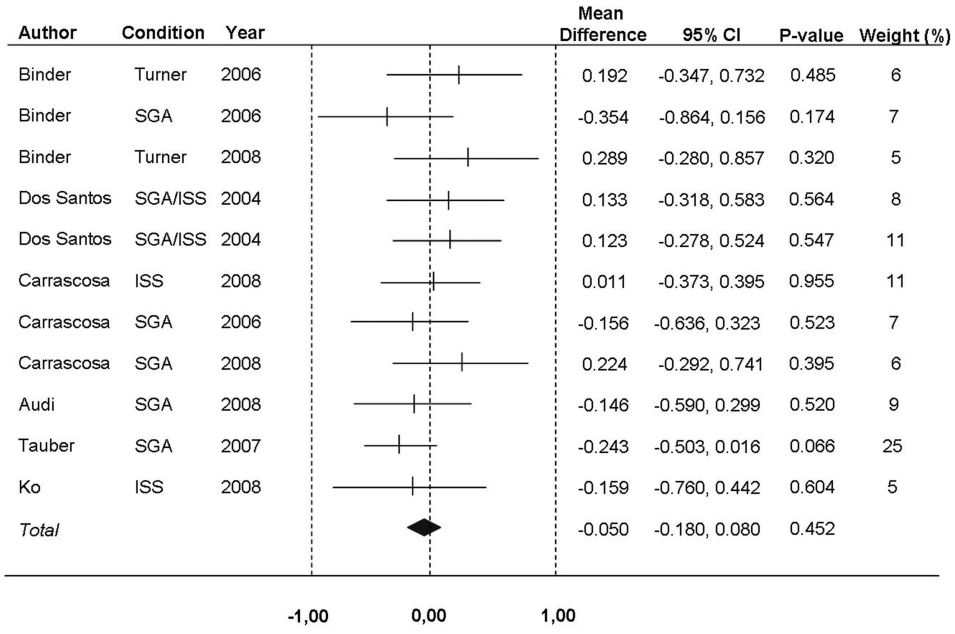


Figure 2B. Meta analysis of the effect of $GHR_{wt\text{-}wt}$ *vs.* GHR_{d3} genotypes on baseline height (SDS) in children with non-GHD short stature. 'Total' represents fixed effects. A negative score points towards lower baseline height in $GHR_{wt\text{-}wt}$ subjects. Binder *et al.* studied Turner syndrome and SGA, in the same paper[22]. Dos Santos *et al.* studied two populations of SGA/ISS in the same paper[8]. CI: confidence interval. GHD: growth hormone deficiency, SGA: small for gestational age, ISS: idiopathic short stature.

Height gain in response to 1 year rhGH treatment (Figure 3)

Height gain was measured as change in height SDS after 1 year of rhGH treatment. As demonstrated in *Figure 3*, for 8 of the 11 studies assessing the effect of height gain in GHD and non-GHD small children, the mean difference in height gain SDS after one year of rhGH treatment was lower for $GHR_{wt\text{-}wt}$ when compared with GHR_{d3} (mean difference -0.075, 95% C.I. - 0.143, -,007), which reflects 0.075 SD more increase in height in GHR_{d3} when compared with $GHR_{wt\text{-}wt}$ after 1 year of rhGH treatment. This pharmacogenetic effect was not statistically significant in the majority of the original studies. The I^2 for this comparison was 46%, indicating moderate heterogeneity [24].

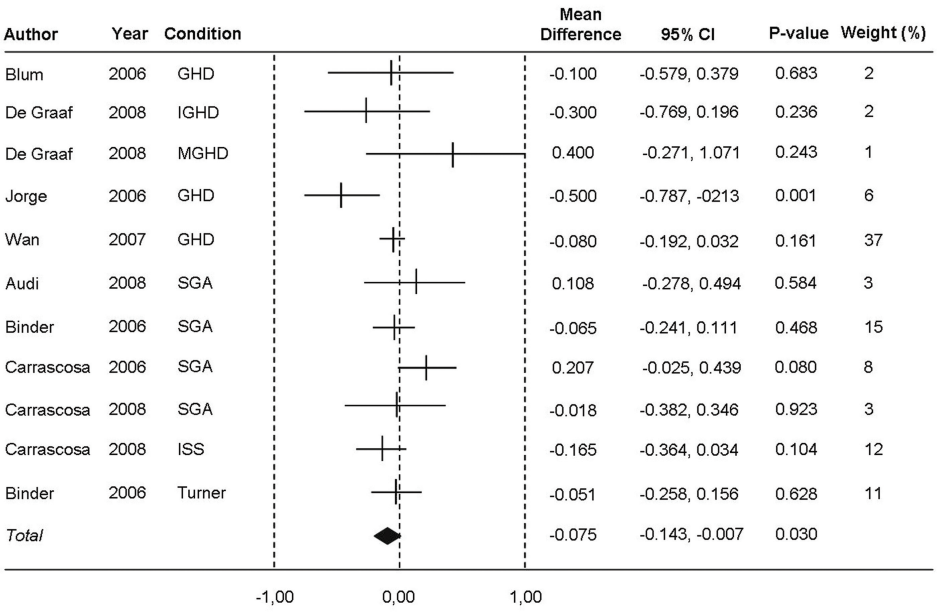


Figure 3. Meta-analysis of the effect of GHR_{wt-wt} *vs.* GHR_{d3} genotypes on height gain (SD) in children with short stature after 1 year of rhGH treatment. 'Total' represents fixed effects. A negative score points towards less height gain in GHR_{wt-wt} subjects. Binder *et al.* studied Turner syndrome and SGA, in the same paper[22]. De Graaff *et al.* studied isolated GHD (IGHD) and multiple GHD (MGHD) in the same paper[10]. GHD: growth hormone deficiency, SGA: small for gestational age, ISS: idiopathic short stature. CI: confidence interval.

First year's growth velocity in response to rhGH treatment (Figure 4)

For 16 studies the effects of d3GHR polymorphism on the first year's growth velocity, measured in cm/year, during rhGH treatment could be compared between GHR_{wt} and GHR_{d3} children. In 6 of these 16 studies there was a significant difference in growth velocity during rhGH treatment between GHR_{d3} and GHR_{wt-wt}, favoring $GHR_{d3.}$ After meta-analysis, mean growth velocity was lower for GHR_{wt-wt} compared with GHR_{d3} (mean difference -0.521 cm/yr, 95% CI: -0.709,-0.333), which reflects an additional increase in height of 0.521 cm in GHR_{d3} compared with GHR_{wt-wt} after 1 year of rhGH treatment. The I^2 was 67%, but the use of a random effects model did not influence the point estimate nor the statistical significance.

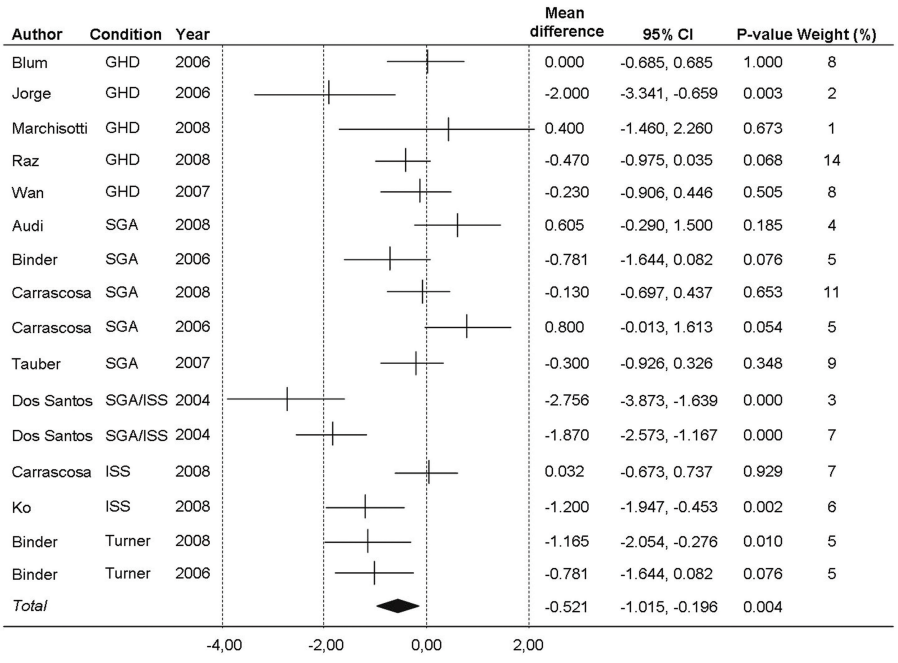
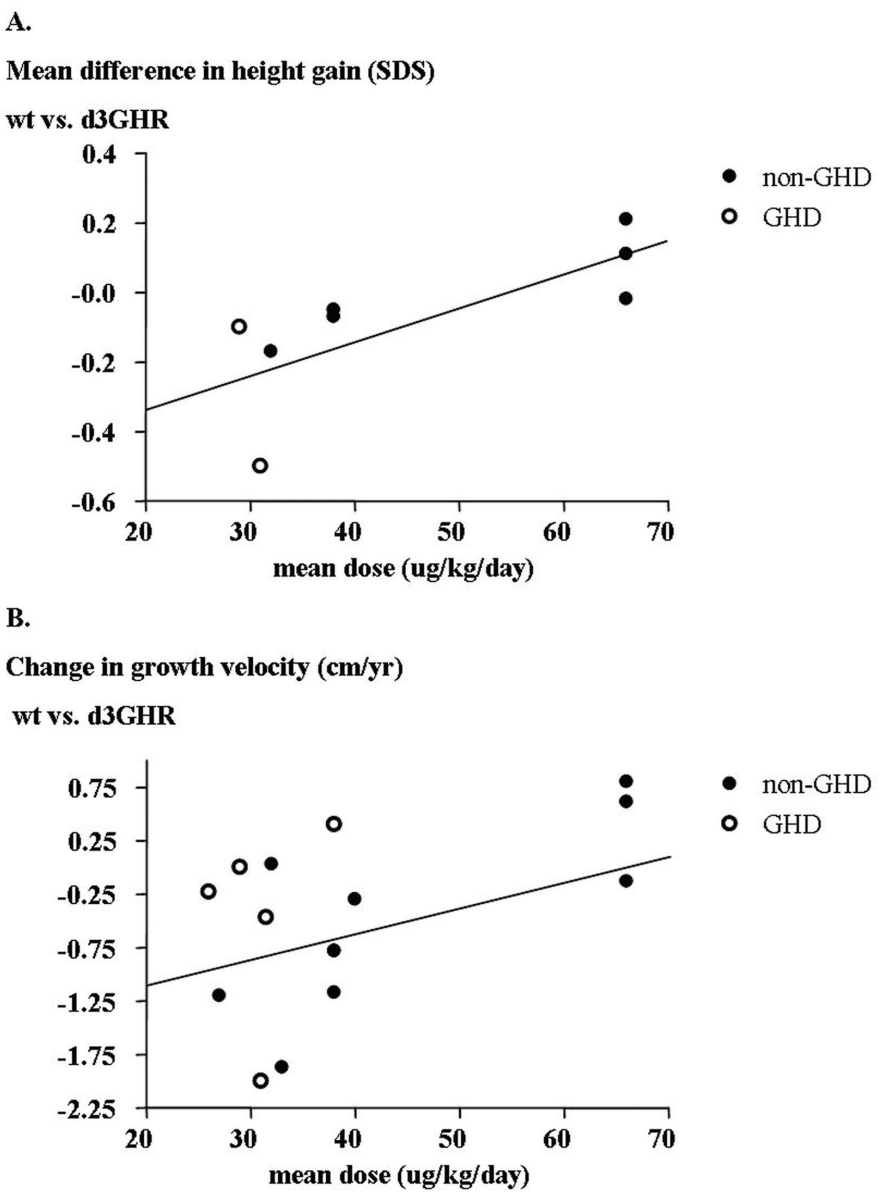


Figure 4. Meta-analysis of the effect of $GHR_{wt\text{-}wt}$ *vs.* GHR_{d3} genotypes on growth velocity (cm/yr) in children with short stature after 1 year rhGH treatment. 'Total' represents fixed effects. A negative score points towards lower growth velocity in $GHR_{wt\text{-}wt}$ subjects. Binder *et al.* studied Turner syndrome and SGA, in the same paper[22]. De Graaff *et al.* studied isolated GHD (IGHD) and multiple GHD (MGHD) in the same paper [10]. Dos Santos *et al.* studied two populations of SGA/ISS in the same paper[8]. CI: confidence interval. GHD: growth hormone deficiency, SGA: small for gestational age, ISS: idiopathic short stature.

Meta-regression

We performed a meta-regression analysis to assess whether the differences in response to rhGH between genotypes in growth velocity and height gain were dependent on the dose of rhGH, age, or cause of short stature (*i.e.* GHD versus non-GHD). Random effects meta-regression showed a significant interaction between rhGH dose and the difference in height gain between $GHR_{wt\text{-}wt}$ and GHR_{d3} (p=0.02) (*Figure 5A*) and suggested a trend for the interaction between rhGH dose and the difference in growth velocity between $GHR_{wt\text{-}wt}$ and GHR_{d3} (p=0.06) (*Figure 5B*) and This points towards smaller differences in outcome with increasing doses of growth hormone.

Figure 5AB



There was also an interaction between genotype and age for the treatment effect of rhGH: the differences in height gain between GHR_{wt-wt} and GHR_{d3} (p=0.02) (*Figure 5C*) and growth velocity (p=0.02) (*Figure 5D*) were more pronounced in studies with a higher mean age. Meta-regression demonstrated that age and dose were both significant predictors for the ef-

fect of the d3GHR genotypes (p=0.005 and p=0.002, respectively), irrespective of the cause of short stature (*i.e.* GHD *versus* non-GHD children). The cause of short stature did not significantly affect the difference between genotypes (p=0.069), although the trend observed

Figure 5CD

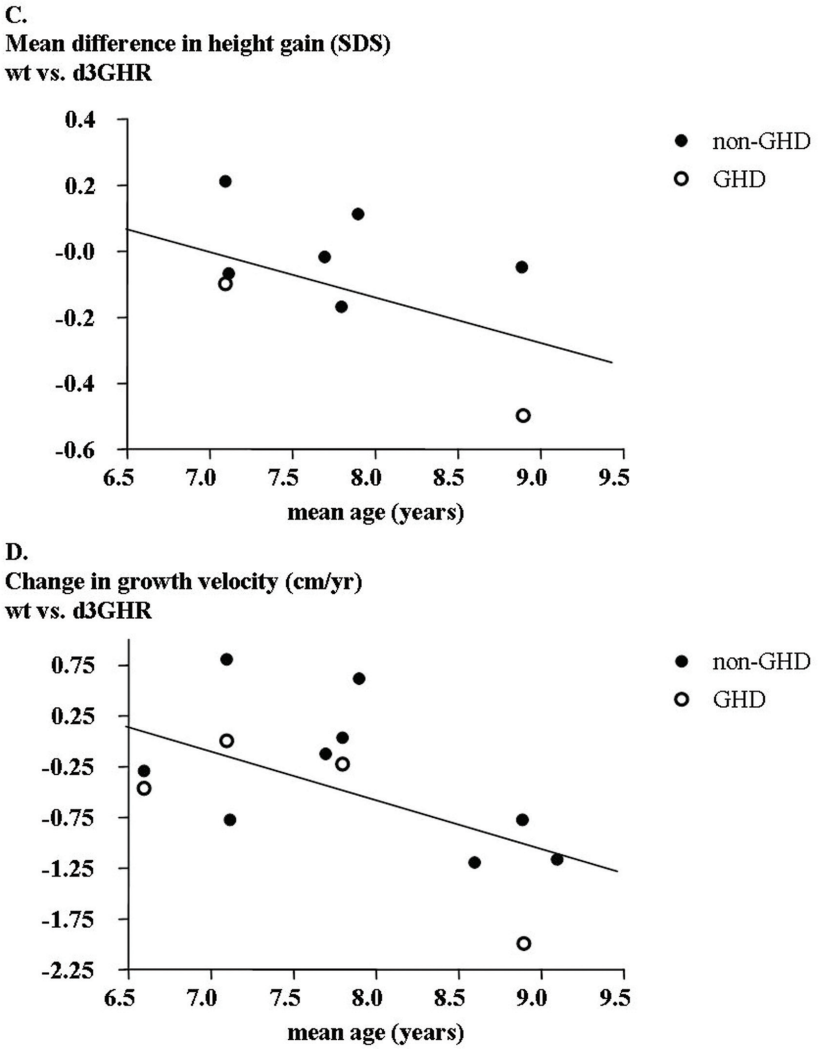


Figure 5 A-D. Association between the dose of rhGH / age and the difference between GHR_{wt-wt} and GHR_{d3} genotypes in change in growth velocity and height gain during 1 year of rhGH treatment. Regression coefficient (R) and 95% confidence interval (CI): Fig 5A: R 1.89, 95% CI -0.12, 3.17; Fig 5B: R 1.48, 95% CI 0.07, 0.88, Fig 5C: R -3.55, 95% CI -1.15, -7.25; Fig 5D:R -1.71, 95% CI -0.64, -2.99. GHD: growth hormone deficient.

in the interaction between genotype and diagnosis pointed towards a smaller effect in non-GHD children. When GHD and non-GHD patients were analyzed separately, this difference in height gain between $GHR_{wt\text{-}wt}$ and GHR_{d3} in non-GHD patients with short stature was not significant anymore (p=0.18), whereas it was significant in the GHD patients (p=0.01).

DISCUSSION

In the present meta-analysis, we show that the growth response during the first year of rhGH treatment, both expressed as height SDS gain and as growth velocity, is significantly increased in pre-pubertal short children with the GHR_{d3} genotype in comparison with the $GHR_{wt\text{-}wt}$ genotype. Moreover, this meta-analysis demonstrates an association between the stimulatory effect of the GHR_{d3} genotype and baseline height in children with GHD. Both findings are in line with the differences in activity between both GH receptor variants *in vitro*[8]. In addition, a lower rhGH dose and a higher age at onset of rhGH treatment were associated with a larger difference in growth response between $GHR_{wt\text{-}wt}$ and GHR_{d3} genotypes.

The studies included in the present meta-analysis differ with respect to the conditions causing short stature in children, the presence/severity of GHD, age (although all children were pre-pubertal with mean ages between 7 and 9 years), and the duration and/or dose of rhGH treatment. Nonetheless, the pharmacogenetic effects on the growth response to rhGH were remarkably constant across the various studies and the various indications for rhGH therapy. In the majority of studies on height SDS gain or first year's growth velocity, the effect of rhGH was larger in GHR_{d3} compared with $GHR_{wt\text{-}wt}$. Moreover, in 4 out of 5 included studies in GHD children baseline height (SDS) was higher in GHR_{d3} compared with $GHR_{wt\text{-}wt}$. The problem of statistical power in the individual studies is illustrated by the fact that the pharmacogenetic effects are largely consistent among the different studies, even though these effects were small and not always statistically significant. Unfortunately, the number of subjects in the studies included in the present meta-analysis was too small to reliably sub-analyze data for GHD and non-GHD children. In the meta-regression, the cause of short stature (GHD *versus* non-GHD) did not appear to be a significant predictor for first year's height gain and growth velocity in the total group. On the other hand, the difference in height gain between genotypes lost its statistical significance in a separate analysis of non-GHD children. Therefore, larger

studies are needed to enable firm conclusions on the (magnitude of the) effect of the GHR_{d3} on height gain in non-GHD children and to assess whether this effect is larger in GHD than in non-GHD children. In addition, we chose to analyze the pharmacogenetic effects in homozygous and heterozygous d3 deletions patients together, since the number of studies that reported separate results for GHR_{wt-d3} and GHR_{d3-d3} was too small. In analogy with the *in vitro* data by Dos Santos *et al.* which indicate increased bioactivity in GHR_{d3-d3} compared with GHR_{wt-d3}, additional studies are needed to establish whether there are comparable pharmacogenetic differences with respect to the growth response to rhGH between patients with the GHR_{wt-d3} and the GHR_{d3-d3} genotype[8].

The observation of a larger difference in first year's height gain and growth velocity between GHR_{wt-wt} and GHR_{d3} in patients treated with a lower rhGH dose in our meta-regression is in line with theoretical considerations that differences of any predictor will be uncovered more easily when a relatively low dose is given. With a high dose it would be more difficult to detect the subtle effects of variation in responsiveness. Our observation is in contrast to the suggestion that was raised in the recent review by Keni *et al*. that the GHR_{d3} genotype has a larger impact at higher doses. The discrepancy between our results and the analysis of Keni *et al.* may be caused by methodological differences, because Keni *et al.* did not perform a meta-analysis of the data and included only some of the studies included in our study [25]. The interaction between the pharmacogenetic effect with age can be explained by the relatively high GH responsiveness at a young age, which may overrule the subtle difference in responsiveness between the two genotypes. Since age and GH dose are independent predictors of the growth response to GH[4], it is uncertain what the relative effect of the GHR variant is in a prediction model including multiple parameters. We believe, however, that the small size of the effect estimated in the univariate analysis makes it unlikely that the assessment of GHR polymorphism will be a clinically useful predictor. In addition, in a large study in children with severe GHD no effect of the GHR polymorphism on height gain and final height was demonstrated[13]. Future studies should confirm this finding.

Interestingly, in 2 studies differences in growth parameters associated with the d3GHR genotypes were not reflected by different increases in IGF-I levels[16;22]. The opposite discrepancy was reported by Marchisotti *et al.,* who were unable to demonstrate an effect of the GHR_{d3} genotype on the growth response to rhGH in pre-pubertal GHD children, but did demonstrate an effect on IGF-I levels[12]. Therefore, one may speculate that IGF-I independent

effects of rhGH dose at the epiphyseal growth plates could also be involved in the pharmacogenetics of rhGH[26]. Alternatively, serum IGF-I levels may not be a good marker of the total IGF-I mediated effects at the epiphyseal growth plates, as autocrine and paracrine effects of locally secreted IGF-I (which are also influenced by rhGH therapy) are not reflected by circulating IGF-I concentrations[26].

The GHR_{d3} genotype is not expected to be associated with physiological variations in human growth or to be a primary cause of short-stature in humans, since potential variations in GH sensitivity due to genotypic differences in GHR activity can be compensated by alterations in endogenous pituitary GH secretion, which might mask the effect of the GHR polymorphism on spontaneous growth. In addition, the distribution of the d3GHR genotypes is in accordance to Hardy Weinberg equilibrium in all clinical conditions studied. Nonetheless, we hypothesized that in patients with GHD this compensatory effect within the GH-IGF-I axis does not function properly and, therefore, the GHR_{d3} genotype may be associated in GHD patients with increased baseline height. Individual studies in pre-pubertal GHD children did not report a stimulatory effect of this polymorphism on baseline height, but in this meta-analysis we were able to demonstrate a small effect, even when adjusted for age. As expected, this genotypic effect was not demonstrated on baseline height in non-GHD children. This finding is supported by observations of Lettre *et al*. who were unable to detect a role for common genetic variation in eight candidate genes of the GH/IGF-I axis in stature variation in the general population[27].

A possible limitation of this meta-analysis is the relatively small number of large studies on the effect of the d3GHR polymorphism on baseline or stimulated growth. In addition, in the various studies there were considerable differences in the degree of GH deficiency, which also may have influenced our findings. Despite the fact that both positive and negative results on the stimulatory effect on growth of the d3GHR polymorphism have been published, we can not exclude the presence of a publication bias in this field. However, this meta-analysis of 15 studies including a total of 1680 patient from the presently available peer-reviewed literature is useful to put available results into perspective.

In conclusion, this meta-analysis shows that the d3GHR genotype is significantly associated with a ~0.5cm/year higher growth response to 1 year of rhGH therapy in prepubertal children with short stature due to GHD and other causes (non-GHD). This pharmacogenetic effect is one of the many factors contributing to the growth response to rhGH therapy and

might be considered to be included in future prediction models[25]. These pharmacogenetic effects of GHR_{d3} on the growth response to rhGH treatment were stronger with lower rhGH dose and higher age. In addition, a d3GHR dependent effect was demonstrated on baseline height in GHD, but not in non-GHD children. Additional studies are required to establish to which extent these pharmacogenetic effects of the GHR_{d3} genotype on rhGH treatment translate into ultimate adult height gain.

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Chapter 11.

THE EXON 3-DELETED GROWTH HORMONE RECEPTOR POLYMORPHISM PREDISPOSES TO LONG-TERM COMPLICATIONS OF ACROMEGALY

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ABSTRACT

Objective: To evaluate the impact of the genomic deletion of exon 3 of the growth hormone receptor (d3GHR) on long-term clinical outcome of acromegaly in a well-characterized cohort of patients with long-term remission of acromegaly.

Design: We condected a cross-sectional study.

Methods: The presence of the d3GHR polymorphism was assessed in 86 acromegalic patients with long-term disease control and related to anthropometric parameters, cardiovascular risk factors, osteoarthritis, bone mineral density, colonic polyps and diverticulae, and dolichocolon.

Results: Fifty-one patients had two wild-type alleles (59%), whereas 29 patients (34%) had one allele and 6 patients (7%) had two alleles encoding for the d3GHR isoform. Carriers of the d3GHR isoform showed increased prevalence of osteoarthritis, especially of the hip (adjusted odds ratio (OR) 5.2, 95% CI 3.2-7.1), of adenomatous polyps (adjusted OR 4.1, 95% CI 2.4, 5.6) and dolichocolon (adjusted OR 3.2, 95% CI 1.8, 4.6). Anthropometric parameters, cardiovascular risk factors, bone mineral density and (non)vertebral fractures were not significantly different between patients with and without the d3GHR allele.

Conclusion: In patients with long-term cured acromegaly, the d3GHR polymorphism is associated with an increased prevalence of irreversible co-morbidities such as osteoarthritis, dolichocolon, and adenomatous colonic polyps, but not with other co-morbidities such as cardiovascular risk factors.

INTRODUCTION

Active acromegaly is associated with a wide variety of co-morbidities such as type 2 diabetes mellitus (DM), hypertension, obesity, arthropathy, colonic polyps, obstructive sleep apnea syndrome, and heart disease[1-6]. Some of these associated disorders persists despite long-term remission[7;8].

The clinical severity of acromegaly varies considerably between individuals. These differences may be mainly related to the severity and duration of active acromegaly. Recently, a polymorphism of the human growth hormone receptor (GHR), the genomic deletion of exon 3 (d3GHR), was associated with increased growth velocity in children with GH deficiency (GHD) during recombinant human GH (rhGH) replacement[8]. This polymorphism enhances GH signal transduction even though GH receptor binding is not altered[8-10]. In accordance, studies in untreated patients with acromegaly revealed that patients carrying a d3GHR allele had lower GH concentrations, but comparable serum IGF-I concentrations[11], a higher prevalence of type 2 DM, a trend towards more arthralgias, and they were less likely to achieve post-treatment normal IGF-I[13] than patients without this allele. This was not confirmed in another study[12]. However, to date, the ultimate clinical outcome of patients with acromegaly after successful biochemical treatment in relation to GHR isoforms has not been determined.

In accordance with the previously reported effects of the d3GHR in GHD and acromegaly, we hypothesized that the d3GHR polymorphism may affect the long-term susceptibility of acromegalic patients for the irreversible effects of GH. Therefore, the aim of this study was to assess the effects of the d3GHR polymorphism in a well-characterized cohort of acromegalic patients with long-term control of GH and IGF-I on long-term clinical outcome, including anthropometric parameters, cardiovascular risk factors, joint- and bone-related problems such as osteoarthritis, osteoporosis, vertebral fractures, and colonoscopic findings.

PATIENTS AND METHODS

Patients

We invited 126 consecutive patients in long-term biochemical remission for participation in the present study. Thirty-seven patients (29%) preferred not to participate for various reasons,

including co-morbidities, travel distance, lack of time, psychological reasons, or unwillingness to provide DNA. They were not different from the study population in disease characteristics or self-reported co-morbidities (data not shown).

From 1977 onwards, the first treatment option in the majority of patients was transsphenoidal surgery. If necessary, adjuvant treatment was given by radiotherapy (prior to 1985) or predominantly somatostatin analogs (from 1985 onwards). From 1998, some patients received primary somatostatin analog treatment. Disease activity was defined by insufficient suppression of serum GH levels during a 75 gram oral glucose tolerance test (normal response GH <0.38 μg/liter), supported by random fasting serum GH levels >1.9 μg/liter and increased IGF-I levels for age [97]. Pre-treatment IGF-I was available in 67 of the 86 patients. Upon treatment, disease activity was assessed yearly by glucose tolerance test, random GH and IGF-I levels. Patients cured by surgery or controlled during somatostatin analog treatment were collectively referred to as 'in remission'.

The Medical Ethics Committee approved the study protocol, and all subjects gave written consent for their participation.

Protocol

Patients were seen once at the outpatient clinic. Blood was drawn for fasting GH and IGF-I concentrations. Physical examination and structured joint assessment was performed. Conventional radiographs were obtained in a standardized manner[16] to assess the radiological aspects of acromegalic joints and the prevalence of vertebral fractures. Bone mineral density was measured using dual energy X-ray absorptiometry (DXA). Baseline and treatment characteristics were derived from patient records[15], including results of colonoscopies[6].

DNA collection and genetic analysis

DNA extraction was done 6-8 weeks after blood collection (8 ml). DNA concentrations and purity (OD260/280) were determined spectophotometrically using the nanodrop (Isogen, IJsselstein, the Netherlands). The exon 3 deletion in GHR gene was detected as described previously[17;33].

Based on the genotype patients could be divided into two groups: group 1: wild types (two wild type alleles; WT-WT), group 2: heterozygotes (one wild type allele and one d3GHR isoform; WT-d3) and homozygous deletion (two d3GHR isoforms; d3-d3). All patients with

minimal one d3GHR allele were referred to as d3 carriers. Expected allele frequencies were calculated by Hardy-Weinberg equilibrium.

Study parameters of long-term clinical outcome

Anthropometric measures and metabolic syndrome

Body weight, height, and waist- and hip circumference were measured. Body weight was measured to the nearest 0.1 kilogram, and body height was measured barefoot to the nearest 0.001 meter. BMI was calculated as weight (kg) divided by the square of height (m). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using the sphygmomanometric cuff method in sitting position.

The prevalence of the metabolic syndrome was assessed according to the NCEP-ATP III criteria[18], which is based on the presence of three or more of the following criteria: 1) fasting plasma glucose concentration ≥6.1 mmol/l (or treated); 2) triglycerides (TG) concentration ≥1.69 mmol/l; 3) high density lipoprotein (HDL) cholesterol concentration <1.04 mmol/l in men and <1.29 mmol/l in women (or treated); 4) blood pressure (BP) ≥130/85 mmHg (or treated); 5) waist circumference >102 cm in men and >88 cm in women. Patients treated with antihypertensive and lipid lowering medication when required, following standard patient care procedures were considered as either being hypertensive and/or having dyslipidemia.

Radiological osteoarthritis

Radiographs of the knees, hands, hips, and lumbar, thoracic, and cervical spine were made and scored by a single experienced musculoskeletal radiologist (HK) blinded for patient characteristics, according to the Kellgren-Lawrence atlas (KL)[19]. Reproducibility was good, as earlier described[16].

Radiological osteoarthritis was defined as a KL score of ≥2 in minimal one joint. The severity of osteoarthritis was defined as KL2-4, with KL4 being the most severe osteoarthritis. A joint prosthesis in the knees and/or hips as a result of end stage osteoarthritis was included as osteoarthritis in that particular joint.

Bone mineral density (BMD) measurements

Bone mineral density was measured at the lumbar spine (L1 to L4) and total hip using DXA (Hologic QDR 4500, Hologic Inc., Waltham, MA, USA) equipped with reference values based

on the National Health and Nutrition Examination Survey (NHANES III). World Health Organization (WHO) criteria were used to define osteopenia (T-score between -1.0 and -2.5) and osteoporosis (T-score of <-2.5).

Vertebral and non-vertebral fractures

Spinal radiographs were blindly evaluated by a specialized radiologist (HK), as well as by two of the other authors (MW/NB), using the semi quantative Genant's method for assessment of vertebral deformities and fractures examining vertebrae T4-L5[20]. The intra- and inter-observer variability was <5%. The prevalence of non-vertebral fractures sustained after inappropriate trauma was evaluated by a structured self-reported questionnaire.

Colonoscopic evaluation

The records of colonoscopies, performed routinely in all patients with acromegaly between 1992 and 2008, were used for the present analysis. Structured reports of the colonoscopy were retrospectively analyzed and scored for the presence of polyps, diverticula, cecal intubation rate, and dolichocolon. In case of polypectomy and/or biopsy, the histopathology was recorded. In case of more than one colonoscopy, the most recent colonoscopy was used for analysis, although polyps taken by biopsies and/or polypectomy during previous colonoscopies were also taken into account. Cecal intubation was confirmed by identification of the appendiceal orifice and ileocecal valve.

Assays

Serum GH was measured with a sensitive immunofluorometric assay (IFMA) (Wallac, Turku, Finland), specific for the 22 kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit 0.03 mU/l; interassay variation (CV) 2.0-9.0% of 0.25-40 mU/l) from 1992 onwards, and previously with the RIA assay (Biolab/Serono, Switzerland) calibrated against WHO-IRP 66/21, with an interassay CV below 5% and a detection limit of 0.5 mU/L.

From 1986 till 2005, serum IGF-I concentrations were determined by a radioimmunoassay (RIA) (Incstar; Stillwater, MN) with a detection limit of 1.5 nmol/l and an inter-assay CV below 11%. IGF-I is expressed as SD scores for age- and gender-related normal levels determined in the same laboratory[21]. From 2005 onwards serum IGF-I concentration (ng/ml) was

measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA). The intra-assay CV was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/l, respectively. IGF-I levels were expressed as SD score, using lambda-mu-sigma smoothed reference curves based on measurements in 906 healthy individuals[22;23].

A Hitachi modular P 800 autoanalyzer (Roche, Mannheim, Germany) was used to quantify serum concentrations of glucose and TG. HDL was measured with a homogeneous enzymatic assay (Hitachi 911, Roche, Mannheim, Germany).

Statistical analysis

SPSS for windows version 16.0 (SPSS inc., Chicago, IL) was used for data analysis. Data are presented as mean and SEM, unless specified otherwise. $P<0.05$ was considered significant.

Linear regression analyses, analyses of variance, and binary logistic regression analyses were used when appropriate. When applicable, adjustments were made for age, gender, BMI, duration of active disease, (log transformed) pre-treatment levels of GH and IGF-I, duration of remission, prevalence of pituitary insufficiencies, and treatment modalities.

The patients were divided into two groups (a low and a high pre-treatment GH group) on the basis of their pre-treatment GH concentrations below or above the 50th percentile (pre-treatment GH concentrations of 52.4 μg/L). All analyses were repeated separately for those patients with high and low pre-treatment GH concentrations.

RESULTS

Patient characteristics

We studied 86 patients (52% males) with a mean age of 58 years (range 31-83 years). Allele distribution was: 52 wt-wt (59%) and 35 d3 (wt-d3, 34% and d3-d3, 7%) and in Hardy Weinberg equilibrium ($P>0.05$).

All patients had well-controlled disease for a mean of 14 years (range 2-28). Wt-wt and d3 patients did not differ in applied treatments, estimated disease duration, duration of remission, and other patient characteristics (see *Table 1*).

The results are presented for wt-wt and d3 (d3-d3 and d3-wt) patients. When analyzed separately, the d3-d3 patients were younger at the time of diagnosis (d3-d3 34.0 ± 4.0

Table 1. Clinical Characteristics of the patients

	WT-WT n=51	d3-WT n=35	P-value
Gender (n males, (%))	28 (55%)	16 (46%)	0.91
Age (yr)	57.6 (1.5)	59.0 (1.5)	0.84
BMI (kg/m²)	28.7 (0.6)	28.5 (0.6)	0.85
Waist/hip ratio (m)	0.96 (0.0)	0.91 (0.1)	0.78
Treatment (n (%))			
Surgery	31 (61 %)	17 (48 %)	0.32
Surgery + RT	8 (16 %)	8 (22 %)	0.71
Surgery + SMS	9 (18 %)	4 (11 %)	0.82
Surgery + RT + SMS	1 (2 %)	2 (6 %)	0.68
SMS	2 (4 %)	5 (14 %)	0.61
Age at diagnosis (yr)	39.2 (1.7)	42.7 (2.6)	0.42
Disease duration (yr)	8.7 (0.9)	10.1 (1.7)	0.36
Duration since diagnosis (yr)	19.3 (1.2)	18.1 (2.9)	0.62
Duration of remission (yr)	15.1 (1.0)	13.0 (1.2)	0.41
GH (µg/l)			
Pre-treatment	87.5 (15.8)	108.5 (7.2)	0.12
Current	2.0 (0.3)	2.9 (0.6)	0.26
IGF-I SD			
Pre-treatment	7.5 (5.8)	7.2 (0.9)	0.71
Current	0.45 (0.3)	0.74 (0.1)	0.41
Hypopituitarism (n%)			
Any	13 (25 %)	16 (46 %)	0.36
TSH	8 (16 %)	10 (29 %)	0.41
LH/FSH	25 (49 %)	19 (54 %)	0.71
ACTH	10 (20 %)	12 (34 %)	0.32

Data are presented as mean (SEM) or as numbers (%). RT: radiotherapy, SMS: somatostatin analog.

vs wt-wt 39.2 ± 1.7, and wt-d3 44.6 ± 2.1 yr, P=0.01). In addition, these d3-d3 patients had a significantly lower BMI than the other genotypes (d3-d3 23.9 ± 0.9 kg/m² *vs* wt-wt 28.7 ± 0.6 g/cm², and wt-d3 29.4 ± 0.9 kg/m², P=0.03).

Biochemical parameters

Pre-treatment IGF-I concentrations were increased in all 67 patients (wt-wt: n=40; d3: n=27).

Pre-treatment GH and IGF-I concentrations and pre-treatment GH/IGF-I ratios were not different between genotypes (*Table 1*). However, d3 patients had a positive relation between (ln)GH levels and (ln)IGF-I SD scores, reflected by a unstandardized beta (B) of 0.28 (95% CI 0.19-0.37) whereas wt-wt patients did not show such a relationship (B 0.01, 95%CI -0.13-0.16) (*Figure 1)*. When patients with pre-treatment GH concentrations above the 50th percentile were analyzed separately, at any given (ln)pre-treatment IGF-I concentration, (ln)pre-treatment GH concentrations were lower in d3 patients when compared with wt-wt patients (P=0.03), even when adjusted for age and gender.

There were no differences in current GH and IGF-I concentrations or in the relation between current (ln)GH and (ln)IGF-I concentrations between the d3GHR genotypes.

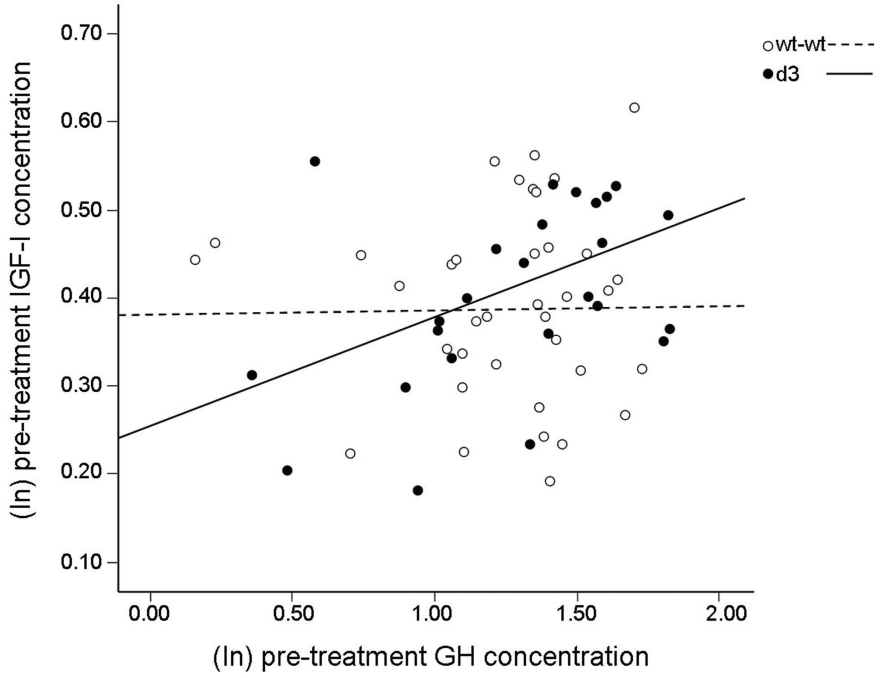


Figure 1. The relation between (ln) pre-treatment GH and IGF-I concentrations for GHR wt-wt and GHR d3. GHR wt-wt patients: unstandardized beta (B) 0.01, 95% CI -0.13-0.16 (p = 0.32) *vs.* GHR d3 patients B 0.28, 95% CI 0.19-0.37 (p = 0.01). The absolute arithmetic means for pre-treatment GH and IGF-I concentrations in GHRwt-wt patients were 87.5 ± 15.8 µg/L and 64.0 ± 4.7 nmol/L, resp, and for GHRd3 patients 108.5 ± 25.2 µg/L and 64.0 ± 4.9 nmol/L, resp.

Long-term complications

Cardiovascular risk factors and the metabolic syndrome

The prevalence of the metabolic syndrome in patients in long-term remission of acromegaly was similar in wt-wt and d3 patients (49% and 43%, resp.). As demonstrated in *Table 2,* there were no differences in prevalence of increased fasting glucose concentrations, decreased HDL concentrations, and increased abdominal fat in d3 patients in comparison with wt-wt patients. Lipid lowering medication was used by 18% of wt-wt patients and 22% of d3 patients (P=0.43).

Table 2. Components of the metabolic syndrome in acromegaly, for GHR wt-wt and d3 patients.

	wt-wt	d3	p-value
	n=51	n=35	
Increased fasting glucose	20 %	29 %	0.62
Increased blood pressure	26 %	18 %	0.73
Decreased HDL cholesterol	12 %	18 %	0.82
Increased TG levels	37 %	31 %	0.91
Increased waist circumference	61 %	63 %	0.89
Metabolic syndrome	49 %	43 %	0.84

Data are represented as n(%) unless mentioned otherwise. HDL: high density lipoprotein, TG: triglycerides. Data were analyzed with analyses of variance adjusted for age, gender, BMI, duration of active disease, pre-treatment levels of GH and IGF-I, duration of remission, prevalence of pituitary insufficiencies, and treatment modalities

There was no difference between genotypes in the prevalence of hypertension (P=0.41). Of 22% of patients with hypertension, 81% was already using antihypertensive medication and 19% was newly diagnosed during the study visit. Type 2 DM was prevalent in 14%(d3) *vs* 6% (wt-wt) of patients, which was not significantly different (OR 1.3, 95% CI 0.7, 2.0), nor after adjustment.

Radiological osteoarthritis

Osteoarthritis of the hip was more prevalent in patients with at least one d3 allele compared with wt-wt patients (51% *vs*. 26%, P=0.03), also when adjusted(*Figure 2*). This was not demonstrated for the other joints (knee, spine, DIP joints, PIP joints, 1st CMC joints, and 1st IP joints). However, in the subgroup of patients with high pre-treatment GH concentrations (>50th percentile) we also observed a significantly higher prevalence of osteoarthritis of the knee (58% *vs*. 37%, P=0.03) and DIP joints (80% *vs.* 50%, P=0.04) in d3 patients. In the lower pre-treatment GH group no difference in the prevalence of osteoarthritis was found between

genotypes.

The severity of osteoarthritis was not different between wt-wt and d3 genotypes, irrespective of the joints. The results were not influenced by additional adjustments for age or other risk factors for osteoarthritis such as BMI and occupational/exercise background.

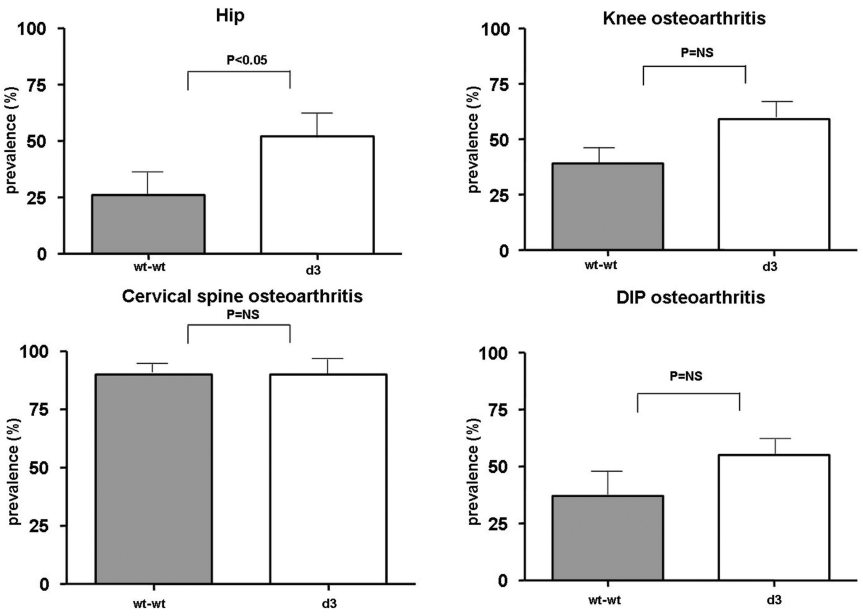


Figure 2. The prevalence of radiographic osteoarthritis of the hip, knee, cervical spine and, DIP joints of the hand for GHR wt-wt and GHR d3 in acromegalic patients with long-term cured disease. The same tendency was seen for the lumbar spine as for the cervical spine. Grey bars denote wt-wt (n=51) and white bars denote d3 (n=35) patients. DIP= distal interphalangeal joints. NS: not significant.

Bone mineral density

As shown in *Table 3,* BMD, T-scores, and Z-scores of the lumbar spine and hips were not different between wt-wt and d3 patients, even when adjusted for age, gender, and BMI. In addition, the prevalence of osteopenia and osteoporosis was not different between both genotypes.

Vertebral and non-vertebral fractures

There was no difference in the prevalence or total number of vertebral fractures in wt-wt and d3 patients (61 *vs.*57%,P=0.73), neither in relation to the localization of the fractures (thoracic or lumbar), type of fractures (wedge, crush, or biconcave), or in severity (data not shown). In

addition, the prevalence and localization of non-vertebral fractures was not different between both patient groups (16% *vs.* 17%,P=0.81).

Table 3. Bone mineral density of the lumbar spine and hip in acromegaly, for GHR wt-wt and GHR d3 patients.

	wt-wt n=51	d3 n=35	p-value
BMD lumbar spine	1.00 (0.03)	1.03 (0.03)	0.43
T-score lumbar spine	-0.64 (0.25)	-0.37 (0.28)	0.48
Z-score lumbar spine	0.44 (0.13)	0.53 (0.18)	0.26
Osteopenia lumbar spine n(%)	16 (39 %)	8 (26 %)	0.40
Osteoporosis lumbar spine n(%)	4 (10 %)	3 (10 %)	0.97
BMD hip	0.88 (0.02)	0.88 (0.03)	0.98
T-score hip	-0.47 (0.15)	-0.45 (0.23)	0.95
Z-score hip	0.25 (0.27)	0.71 (0.28)	0.65
Osteopenia lumbar spine n(%)	14 (35 %)	9 (31 %)	0.82
Osteoporosis lumbar spine n(%)	0 (-)	1 (3 %)	-

Data are represented as mean (SEM) unless mentioned otherwise. Data were analyzed by analysis of variance and binary logistic regression analysis, when appropriate and adjustments were made for age, gender, BMI, duration of active disease, pre-treatment levels of GH and IGF-I, duration of remission, prevalence of pituitary insufficiencies, and treatment modalities.

All analyses were adjusted and the results were not influenced by additional adjustments for occupational/exercise background.

Colonoscopic evaluation

In 52% of d3 patients and 39% of wt-wt patients colorectal polyps were found(P=0.02) (*Figure 3*). Histopathology demonstrated higher prevalence of adenomatous polyps in patients with at least one d3 allele (41%) compared with 22% in wt-wt patients (adjusted OR 3.3, 95% CI 2.0, 4.8). The grades of dysplasia were comparable (data not shown).

The prevalence of diverticula was not statistically different, despite a slight tendency towards a higher prevalence in d3 patients (37%) when compared with wt-wt patients (26%). The cecal intubation rate was significantly lower in d3 patients (68%) compared with wt-wt (83%) (adjusted OR 0.2, 95% CI 0.1, 0.3). Dolichocolon was present in 17 d3 patients (49%) and in 16 wt-wt patients (32%) (adjusted OR 2.5, 95% CI 1.4, 3.6).

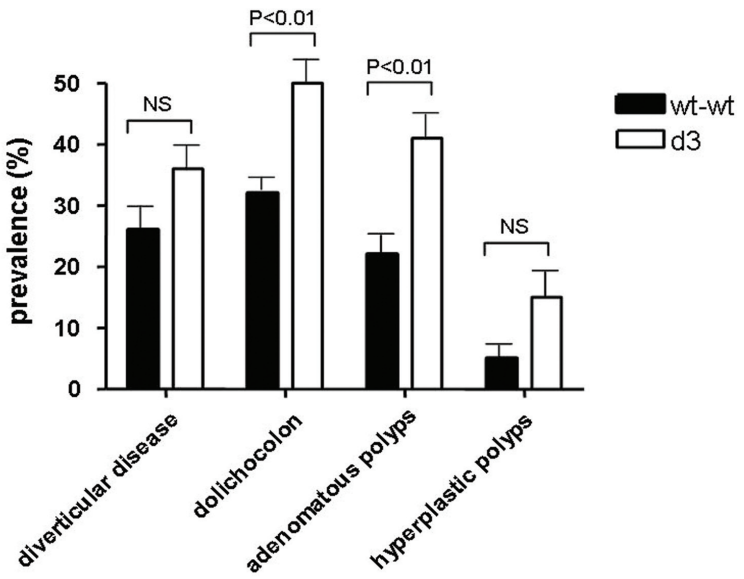


Figure 3. The prevalence (%) of colonic diverticular disease, dolichocolon, and colonic polyps in long-term cured acromegaly for the different d3GHR genotypes. Data were analyzed by binary logistic regression, with adjustments for age, gender, BMI, duration of active disease, pre-treatment levels of GH and IGF-I, duration of remission, prevalence of pituitary insufficiencies, and treatment modalities. NS: not significant

In addition, when the patients in the subgroup with high pre-treatment GH concentrations were analyzed separately, increased OR's for both adenomatous polyps (adjusted OR 4.1, 95% CI 2.4, 5.6) and dolichocolon (adjusted OR 3.2, 95% CI 1.8, 4.6) were demonstrated for d3 patients compared with wt-wt patients. The OR's were not different in the subgroup of patients with low pre-treatment GH concentrations.

DISCUSSION

In this study we evaluated whether the presence of the common functional GH receptor polymorphism, the deletion of exon 3, was related to an adverse late clinical outcome in a well-characterized cohort of patients with long-term remission of acromegaly. We demonstrated that the d3GHR was associated with a higher prevalence of colonic polyps and dolichocolon and

osteoarthritis, especially of the hip, knee, and DIP joints of the hand. Finally, the prevalence of other co-morbidities associated with acromegaly, such as metabolic syndrome and vertebral or non-vertebral fractures, did not differ between genotypes.

Patients with at least one d3 allele had a higher prevalence of osteoarthritis, mostly pronounced at the hip joint. In accordance, knee and DIP osteoarthritis was more prevalent in the d3 genotype in the subgroup with high pre-treatment GH concentrations. It is of note that the hip is the joint-site mostly affected by genetic or systemic changes[26]. Since almost all patients had osteoarthritis of the spine, the lack of difference between the d3GHR genotypes may also be caused by this high prevalence.

The d3 genotype was also associated with more colorectal adenomatous polyps and dolichocolon, another irreversible long-term complication of acromegaly. It has frequently been assumed that the prevalence of colonic polyps and dolichocolon in acromegaly was correlated to IGF-I concentrations[6;27-29]. A more active signal transduction in d3 patients is concordant with the finding of an increased prevalence of both adenomatous polyps and dolichocolon.

Co-morbidities including joint-complaints, osteoarthritis, hypertension, and colonic abnormalities are highly prevalent in patients with acromegaly despite long-term cured disease[5;7;15-16;27]. In contrast, type 2 DM is frequently observed in active disease, but is potentially reversible since disturbances in glucose metabolism normalize after cure and the prevalence of type 2 DM is not increased in patients with (long-term) disease remission[7;25]. We could demonstrate an effect of the d3GHR polymorphism on persisting co-morbidities such as osteoarthritis and colonic abnormalities, but not on type 2 DM. This is in contrast to the study of Mercado *et al*., which demonstrated an increased prevalence of type 2 DM in active acromegaly patients with the d3 genotype[13]. However, this can be explained by the fact that type 2 DM is less frequently observed in long-term cured acromegaly than in active acromegaly[36] and the prevalence of type 2 DM in our cohort is not increased in comparison with reference data[7]. The lack of effect of polymorphism on long-term outcome of type 2 DM or components of the metabolic syndrome, is in accordance with the fact that those co-morbidities appear to be reversible complications of acromegaly after biochemical control[7;15]. However, a power effect as reflected in the unadjusted and adjusted OR's might underlie the inability to demonstrate an effect of the d3GHR polymorphism on type 2 DM. Although hypertension does improve upon successful treatment of acromegaly, it can also be considered as a chronic complication of the disease since it persists in patients in remission. There was no significant effect of the d3GHR genotype on

the prevalence of hypertension.

We were also unable to demonstrate an effect of the d3GHR polymorphism on BMD and (non)vertebral fractures in our patients with long-term cure of acromegaly. Acromegaly is associated with increased BMD in combination with a high prevalence of vertebral fractures, especially with coincident hypogonadism[30]. Up to now, a relation between pre-treatment GH or IGF-I concentrations and these co-morbidities has not been reported, which is consistent with the lack of difference between the d3GHR genotype [31;32].

In acromegaly, four studies have addressed the relation between GH and IGF-I between the d3GHR genotypes [12;13;33;34]. In the first study, in 44 active acromegaly patients, GH levels were lower despite similar IGF-I concentrations in carriers of d3 genotype compared with the wt-wt genotype[11]. A second study in 148 active acromegaly patients found a subtle correlation between GH and IGF-I concentrations in d3 patients, but not in wt-wt patients[13]. Another recent study confirmed this subtle correlation between post-treatment, mainly elevated, GH and IGF-I concentrations in d3 patients[36]. The fourth study did not find a difference in IGF-I concentrations between d3GHR genotypes[12]. In our analysis mean pre-treatment GH and IGF-I concentrations did not differ between genotypes. However, subtle genetic differences in the GH receptor leading to a more active GH signal, may not be evident in GH-IGF-I relationships, based on single measurements of plasma concentrations. The observed difference in the correlation between GH and IGF-I levels between GHRwt and GHRd3 patients is too small to make firm conclusions. Moreover, GH and IGF-I concentrations are not only dependent on GH production and GH receptor activity, but also on many other factors, including age, gender and the use of estrogens. In addition, GH and IGF-I concentrations fluctuate according to diurnal (IGF-I) and/or episodic (GH) variations in secretion. These factors might explain the lack of a measured difference in single GH and IGF-I concentrations between the GH receptor genotypes in our and other studies. In addition, IGF-I plateaus at high GH levels, which may affect the relation between IGF-I and GH levels, especially in active acromegaly. Nonetheless, the observation of higher prevalences of GH related long-term complications is in accordance with the observation *in vitro* that the D3 polymorphism increases the GH signal[9].

We presented our data grouping together GHRwt-d3 and GHRd3-d3 patients. However, we did analyze some baseline aspects separately in the d3-d3 group and these patients appeared to be younger at the time of diagnosis of acromegaly and they also had a lower BMI than the other 2 patients groups. Kamenicky *et al*. have also recently described this very interesting

finding. It is tempting to speculate that the GHRd3-d3 genotype can promote the acromegalic phenotype at a younger age. However, the number of GHRd3-d3 patients in our study is too small to draw any reliable conclusion. Moreover, we can not exclude that the lower BMI may also be related to the lower age of these patients.

Since the design of the study was cross-sectional, we were unable to conclude whether the increased prevalence of osteoarthritis of the hip, knee, and DIP joints and of colonic polyps and dolichocolon was the result of enhanced activity of GH excess during uncontrolled acromegaly or whether it was due to the impact of the d3GHR polymorphism after remission of the disease. Another potential limitation is the presence of a survival bias, since we were only able to include patients alive during long-term follow-up.

Our findings indicate that the d3GHR influences the long-term, *i.e.* irreversible, complications of acromegaly. However, it is uncertain whether screening of all patients prior to treatment will be clinically relevant, since current treatment modalities enable biochemical control in almost all patients, irrespective of GHR genotypes. If acromegaly is appropriately controlled by treatment, the effects of the genotypes on the irreversible consequences of acromegaly will predominantly be the result of the activity and duration of the disease prior to appropriate treatment.

In conclusion, the d3GHR polymorphism is associated with more severe complications of acromegaly, reflected in an increased prevalence of osteoarthritis, especially of the hip, knee, and DIP joints of the hand, and of colonic polyps and dolichocolon. However, other co-morbidities such as metabolic syndrome, type 2 DM, changes in BMD, or (non)vertebral fractures are unaffected by the d3GHR polymorphism. Apparently, the ultimate impact of the d3GHR polymorphism on long-term complications of acromegaly is evident only on the irreversible effects of previous GH excess.

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Chapter 12.

THE EFFECT OF THE EXON-3 DELETED GROWTH HORMONE RECEPTOR POLYMORPHISM IN VARIOUS CLINICAL CONDITIONS: A SYSTEMATIC REVIEW.

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Article submitted

ABSTRACT

The GHR is the first key molecule mediating growth hormone (GH) action. In 2004 Dos Santos *et al.* were the first to link a common GH receptor (GHR) polymorphism, the exon 3 deleted GHR (d3GHR), to **increased responsiveness to recombinant human GH (rhGH) treatment** in children with short stature. Since then, this polymorphism has been under extensive investigation regarding its influence to the response on rhGH therapy in different clinical populations. This review focuses on studies evaluating the effect of the polymorphism on respectively growth, GH-insulin-like growth factor I (IGF-I) axis, metabolism, cardiovascular disease risk, and survival.

There are discrepant results on the effects of the d3GHR on the GH-IGF-I axis between studies in GHD and acromegaly. However, some of the few studies investigating the effect of this polymorphism on the GH-IGF-I axis indicate a positive effect of the GHR_{d3} genotype ($GHR_{d3\text{-}d3}$ or $GHR_{wt\text{-}d3}$) on IGF-I concentrations. The GHR_{d3} genotype seems to affect metabolism, since in patients with type 1 and 2 diabetes mellitus (DM) and in patients with impaired glucose tolerance (IGT) the frequency of the GHR_{d3} genotype is significantly lower than in the normal population. In addition, in a cohort of healthy Caucasian children and adolescents, higher insulin secretion in the presence of the d3GHR was demonstrated. These findings suggest that increased bioactivity of this allele confers a protection function against type 2 DM. The $GHR_{d3\text{-}d3}$ isoform is more frequently present in patients suffering from starvation, indicating that both the elevated GH gene expression and increased GHR-mediated GH responsiveness may constitute adaptive responses to the effects of persistent malnutrition, since increased circulating GH appears to form part of a physiological response to nutrition deprivation. The prevalence of the GHR_{d3} isoform in well-fed populations is currently unknown. But in patients with type 2 DM and IGT, the GHR_{d3} isoform is associated with more severe obesity than in patients with the other isoforms. In 2 cohorts studied, there was no association between cardiovascular risk factors like hypertension and stroke and the d3GHR polymorphism.

We conclude that the effects of the d3GHR polymorphism are not limited to subtle variations in growth parameters in (non)GHD children treated with rhGH, but includes many pathophysiological processes in which the GH-IGF-I axis is involved. The implications of these genotype-phenotype relationships of the GHR for routine clinical practice seem to be limited.

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      2. Type 2 Diabetes Mellitus
   C. Impaired glucose tolerance
   D. Conclusion

VI. Effect of d3GHR on cardiovascular diseases
   A. Pathogenesis
   B. Hypertension
   C. Stroke
   D. Conclusion

VII. Effect of d3GHR on survival
   A. Pathogenesis
   B. Obesity and starvation
   C. Conclusion

VIII. Summary and conclusions

I. Introduction

Human growth hormone (GH) promotes post-natal growth of the skeleton and soft-tissue as well as acts as an important regulator of bone turnover, muscle mass, and immune function. In addition, the GH-insulin-like growth factor type I (IGF-I) axis is important for metabolic control[1]. GH has several effects via the growth hormone receptor (GHR) on metabolism. Some of these effects are IGF-I-dependent, such as on glucose uptake and protein metabolism, whereas other effects are IGF-I-independent, including stimulation of insulin secretion, lipolysis, and gluconeogenesis[1].

The biological actions of GH are mediated by the activation of its cell-surface receptor, the GHR. The composition of this dimeric GHR depends on the assortments of coding polymorphisms of the GHR gene. Three variants of the GHR, that differ in the presence or absence of exon-3 (GHR_{wt-wt}, GHR_{wt-d3}, and GHR_{d3-d3}), are commonly seen[2]. In mammals, the variation causing the isoforms may occur at either the genomic[3] or mRNA splicing level[2]. The function of exon 3 is unknown, although this exon, and thus the deletion, is in close proximity to the GH binding site[4]. The loss of exon 3 appears to have little effect on the receptor, as GHR_{wt-d3} and GHR_{d3-d3} are stable and functional receptors without apparent differences in binding activity or internationalization compared with $GHR_{wt-wt.}$ While either allele alone is sufficient for normal growth[3], the presence of at least one deleted allele is thought to confer an increased growth response to GH therapy[5].

The presently known functional consequences of the exon 3 deleted GHR polymorphism in various clinical conditions and physiological processes are the subjects of this review.

I A. The GHR gene structure

The GHR is a member of the class I hematopoietic cytokine receptor superfamily, which currently includes more than 40 members[6]. The GHR gene is located on the short arm of chromosome 5 (p13.1-p12) and has 9 exons that encode the receptor and several additional exons in the 5-prime untranslated region. The coding exons span at least 87kb. The GHR consists of an extracellular domain of 246 amino acids (aa), a single transmembrane domain of 24 aa, and a cytoplasmic domain of 350 aa[7]. Exon 2 encodes an 18-amino-acid signal sequence. Exons 3 to

7 encode the extracellular domain. Exon 8 encodes the transmembrane domain, and exon 9 and 10 encode the cytoplasmic, signal-transduction domain and the 3-prime untranslated region. The active form of the GHR is a receptor homodimer[8;9]. The first step in GHR activation is the binding of GH to the GHR. GH binding to preformed dimers of GHRs located at the cell membrane surface induces rotation of the intracytoplasmatic subunits of the dimerized receptors, resulting in the activation of associated kinases like the Janus tyrosine kinase (JAK) 2, the mitogen-activated protein (MAP) kinase, and the phosphatidylinositol-3 (PI-3) kinase. The pathways involving these three kinases are assumed to account for the most signaling events triggered by the GHR[10] and are also referred to as the JAK/STAT (signal transducers and activators of transcription) pathway. Detailed description of the features of the receptor is beyond the scope of this review article, but numerous reviews have been published on this subject[10-16].

I B. Impaired growth due to mutations of the GHR

In a small number of humans, growth defects result from rare mutations of the GHR. Mutations have been found to alter various intrinsic features of the GHR that are required for proper activation of the receptor itself, or of some components of the GHR signaling cascades. A clinical consequence of inactivating mutations of the GHR is the GH insensitivity syndrome, first described by Laron in 1966. Laron syndrome is an autosomal, fully penetrant recessive disease resulting from GH resistance. Phenotypic characteristics are severe growth retardation, acromicria, small gonads and genitalia, abdominal adiposity, and high GH concentrations in combination with very low circulating IGF-I concentrations. The pathogenesis of this syndrome is explained by various molecular defects mainly affecting the GHR, including exon deletion and mutations (nonsence, frameshift, missense)[17]. Most of these mutations affect the extracellular domain of the receptor, which results in the absence of circulating GH binding protein (GHBP). Normal or high GHBP levels in GH resistance syndromes denote that the mutation resides within transmembrane or cytoplasmic domains of the GHR or affects post-receptor components. At present three types of mutations of the GHR have been described, resulting in different severities of the phenotypic consequences, i.e. expression failure[18], activation failure[19-21], and signaling failure[22-24] .

I C. GH resistance syndromes caused by post-GHR defects

Recently, two mutations have been described in STAT5B. First, a homozygous mutation in STAT5B was associated with GH insensitivity[25]. The mutated Stat5 was inactive and blocked all cellular events downstream in the JAK2-STAT5 cascade, including IGF-I deficiency. Second, a mutation was described leading to the total absence of detectable mature protein due to early termination. This mutation combines severe GH insensitivity with immunodeficiency[26;27]. In addition, mutations of IGF-I[28] or of the IGF-I receptor[29] leading to intrauterine and postnatal growth retardation have also been documented as causes of GH resistance syndromes. Both the post-growth hormone receptor mutations as the IGF-I (receptor) mutations are not the focus of this review.

I D. Polymorphisms of the GHR

Most of the GHR polymorphisms are single nucleotide polymorphisms or microdeletions. The functional role of most of these polymorphisms at the molecular level and the way they can modify GHR function at the physiologic level have not been elucidated. Polymorphisms of the GHR have been described in exon 3, 6 and 10[30]. One of these polymorphisms, the deletion of exon 3, has been studied more extensively because of its relatively high prevalence. The expression of the different d3GHR isoforms is consistent with simple mendalian inheritance of 2 different alleles in which exon 3 is spliced in an 'all or none' fashion. The alternative splicing of exon 3 in GHR transcripts is the result of an unusual polymorphism that significantly alters splicing of the transcript. The relatively high prevalence (~ 40% and ~10% for heterozygosity and homozygosity in the population, respectively) for the allele producing transcripts lacking exon 3 suggests the possibility that it may play a role in polygenically determent events, i.e. may have selective advantage under certain circumstances. A genetic polymorphism resulting in deletion of an entire exon from mRNA without compromising structure or function of the resultant protein is very unusual[31]. It was initially thought that the isoform lacking exon 3 was tissue dependent[32-34]. However, it became apparent that the exon 3 deletion was not tissue dependent but individual dependent[35].

The effect of the deletion of exon 3 has been investigated in several clinical conditions, some studies focusing on the single or double deletion of exon 3 as one genotype (GHR_{wt} *vs.* GHR_{d3}), and other studies regarding the single or double deletion of exon 3 as two different

genotypes ($GHR_{wt\text{-}wt}$ *vs.* $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$).

II. Effects of d3GHR on GH/IGF-I axis

II A. Pathogenesis

Biologically active GH binds its transmembrane receptor (GHR), which dimerizes to activate an intracellular signal transduction pathway, resulting in complex effects including synthesis and secretion of IGF-I. IGF-I is responsible for most anabolic and mitogenic actions of GH, including growth promotion and development of organs and tissues during all growth stages[36]. IGF binding protein 3 (IGFBP3) is the main of several proteins which increases IGF-I half-life, transports it to the target cells and guides its interaction with membrane receptors. Serum IGF-I and IGFBP3 are usually reduced in patients with GHD.

The prevalence of the different isoforms of the GHR is comparable between GHD, acromegaly, and healthy subjects, which excludes a role of GH in regulating GHR isoform expression patterns including the d3GHR. The question is whether the different d3GHR isoforms explain some of the individual differences in GH and IGF-I concentrations in patients with disturbances in the GH-IGF-I axis. For the purpose of this review we separately analyze the effects of the d3GHR on diseases of the GH-IGF-I axis, i.e. in children with GHD, adults with GHD, patients with active and controlled acromegaly.

II B. Growth hormone deficiency

GHD is a clinical condition in children, in whom GH production is insufficient for normal height gain and development of bone and muscle. GHD is a clinical condition in adults, in whom GH production is inadequate for the maintaining of muscle- and bone mass and other GH dependent body functions. There are several causes for GHD including congenital and acquired pituitary lesions.

II B1. *Growth hormone deficiency in children*

GHD in children is responsible for impaired height gain, bone and muscle development. The effect of the GHD will only develop some months after birth and, if left unadjusted, is responsible for late puberty. Clinical signs are impaired growth, immature face, and (a slightly) obese

stature.

Five studies in GHD children have investigated the effects of d3GHR genotype on the GH-IGF-I axis[37-41]. The effect of d3GHR genotype on baseline IGF-I concentrations in GHD was studied by Blum *et al*., who reported lower IGF-I concentrations in GHR_{wt-wt}[37]. Another study demonstrated genotype specific differences in IGF-I concentrations in response to (short-term) rhGH treatment, with the lowest response in GHR_{wt-wt} despite equal GH and IGFBP3 concentrations in the different genotypes[39]. This is in accordance with the theory proposed by Dos Santos[5], of enhanced growth in GHR_{d3} in children who are small for gestational age (SGA) or have idiopathic short stature (ISS). In fact, they provided evidence for increased bioactivity of GHR_{d3} *in vitro* in response to various GH concentrations compared with GHR_{wt-wt}[5]. In addition, a slightly higher GH sensitivity regarding short-term IGF-I generation during rhGH therapy was seen in ISS patients with the GHR_{d3} genotype[42]. The three remaining studies in GHD children were unable to demonstrate any effect on IGF-I[38;40;41]. Studies in Turner's syndrome of Cushing's disease did not observe any effect on IGF-I either[43;44].

In conclusion, two of the seven studies providing data on the effect of the d3GHR genotype on the GH-IGF-I axis in GHD demonstrated a genotype specific difference. This effect was uniform in both studies, indicating higher IGF-I concentrations in the GHR_{d3} genotype, compared with GHR_{wt-wt}[37-41]. There were no genotype-fenotype relationships with respect to GH or IGFBP3 concentrations. Additional studies are required to establish whether the subtle clinical effect of the GHR_{d3} genotype is reflected in circulating IGF-I concentrations.

II B2. *Growth hormone deficiency in adults*

The syndrome of GHD is different in adults compared with children. Although GH requirements decrease after puberty, the percentage of prepubertal GHD subjects who have insufficient GH reserve during retesting in adulthood, varies considerably among the different studies with a frequency of persistence of GHD between 20 and 87%[45]. In adulthood, the metabolic actions of the GH/IGF-I axis take place at plasma levels which are markedly lower than those needed to promote longitudinal growth. IGF-I concentrations in adults are positively related to GH secretion patterns and are able to detect part of the adult patients with suspected GHD. Although many adult patients with GHD have normal IGF-I levels, there is agreement that IGF-I is the best parameter during follow-up of rhGH replacement therapy[46].

Van der Klaauw *et al*. showed that the GHR_{d3} genotype was associated with differen-

ces in effects of rhGH during short-term, but not during long-term, individually adjusted rhGH replacement therapy in adults with GHD[47]. In accordance to children with GHD[37;39], a higher increase in IGF-I levels in GHR_{d3} patients compared with $GHR_{w\text{-}wt}$ patients was demonstrated after a comparable dose of rhGH[47]. Meyer *et al.* demonstrated in a German population of adult GHD patients on 1 year of rhGH therapy that GHR_{d3} patients required 25% less GH for the same IGF-I levels compared with $GHR_{wt\text{-}wt}$ patients[48]. Finally, Moyes *et al.* studied a large cohort of, at baseline comparable, adults with GHD on an identical dose of rhGH for 1 year. After 1 year of rhGH treatment, patients with $GHR_{d3\text{-}d3}$ demonstrated a significantly larger increase in IGF-I concentrations compared with patients with $GHR_{wt\text{-}wt}$. However, when $GHR_{d3\text{-}d3}$ and $GHR_{wt\text{-}d3}$ patients were analyzed together this difference with $GHR_{wt\text{-}wt}$ was not present[49]. In contrast to these 3 studies, a Swedish study in GHD adults treated for 1 year with individually titrated rhGH dose did not demonstrate any effect of the GHR_{d3} on IGF-I levels[96].

In conclusion, four studies focused on the effect of d3GHR on the GH-IGF-I axis in response to rhGH in GHD adults, with contrasting short-term results. However, the differences between the genotypes in effect on the GH-IGF-I axis were subtle, or only demonstrable in $GHR_{d3\text{-}d3}$ *vs.* $GHR_{wt\text{-}wt.}$ When patients with $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ are analyzed together, the effect vanished. Moreover, most of the above mentioned studies included relatively low numbers of patients, resulting in low statistical power. Only prospective and large studies with large numbers of all genotypes will have the required statistical power to identify a potential, additional contribution of d3GHR polymorphism to the GH-IGF-I axis in response to GH therapy.

II C. Active and controlled acromegaly

Acromegaly is a chronic, slowly progressive disease caused by a growth hormone-secreting pituitary adenoma leading to increased circulating GH levels and increased IGF-I secretion by peripheral tissues, especially the liver. The GHR_{d3} genotype has been associated with increased IGF-I levels[37;39;47]. Several studies assessed the effects of the d3GHR polymorphism on the disease course in acromegaly. An underlying concept was that the d3GHR polymorphism may have caused a stimulation of the GH-IGF-I axis and therefore rendered acromegalic patients with the GHR_{d3} genotype more susceptible for the effects of GH.

Only a few studies have focused on the effect on biochemical parameters of the d3GHR genotype in active acromegaly patients. One study in patients with newly diagnosed

active acromegaly revealed that patients carrying a GHR_{d3} allele had lower GH concentrations, but comparable serum IGF-I concentrations, than patients without this genotype, in accordance with the notion of a more active GH signal[50]. However, Kamenicky *et al.* was not able to show an effect of the GHR_{d3} genotype on GH-IGF-I relationships in 105 patients with active acromegaly[51]. Another study, studied 152 patients at baseline and 1 to 5 years post-treatment. Although these authors observed no effects on GH and IGF-I concentrations at baseline in GHR_{d3} patients, these patients were less likely to achieve normal IGF-I concentrations during treatment for acomegaly[10]. However, the pathophysiological mechanism behind these unconfirmed observations is not completely understood.

Circulating lymphoid cells express GHR variants, and, therefore, peripheral lymphocytes may be useful for evaluating GHR physiology. Ochao *et al.* compared expression patterns of the GHR isoforms in lymphocytes between healthy individuals and patients with active acromegaly[52]. In healthy subjects a predominance of the GHR_{wt-wt} isoform was demonstrated. This is in accordance with the theory that the absence of exon 3 does not impair GHR physiology[53]. In addition, GHR_{wt-wt} was negatively correlated with IGF-I levels in healthy individuals. In contrast, acromegalic patients demonstrated similar amounts of the GHR_{wt-wt} and GHR_{d3} isoforms, and these isoforms neither correlated with circulating GH levels nor with IGF-I levels. This suggests that the interaction between GH and its receptor may be altered in acromegaly.

In conclusion, there are large discrepancies between the results of the few studies in acromegaly on the effect of the d3GHR isoforms on the GH-IGF-I axis. However, since the main objectives of these three studies were different, additional larger studies are required in both active and (long-term) controlled acromegalic patients to clarify the effects of the d3GHR genotypes on the GH-IGF-I axis in acromegaly and to confirm the interesting observation that the d3GHR polymorphism may be associated with an adverse biochemical treatment result.

II D. Conclusion

There are discrepancies between the studies in both GHD and acromegaly on the effect of the d3GHR on the GH-IGF-I axis. Nonetheless, the majority of these studies indicate a positive effect of the GHR_{d3} genotype on IGF-I concentrations[37;39;47;52]. However, considering the relatively small number of studies, which in general include relatively low numbers of subjects, it is hard to draw firm conclusions at present.

III. Effect of d3GHR on growth

III A. Pathogenesis

Under normal physiological circumstances, linear growth is a variable trait, individually different and dependent on numerous factors, both genetical and environmental. Just as spontaneous linear growth, stimulated growth in response to rhGH treatment varies extensively amongst individuals, also resulting from both genetic and non-genetic effects. One of the genetic factors considered responsible for this variation in stimulated growth is the expression of exon 3 of the GHR. The loss or retention of exon 3 is thought to affect receptor expression or function, specifically by affecting the binding of human growth hormone, receptor processing, transport, stability, binding to other ligands, dimerization of GHR monomers, or signal transduction as described by Dos Santos *et al*. in 2004[5]. They demonstrated that GHRd3 induced a higher transcriptional activity of the reporter construct in response to various GH concentrations than $GHR_{wt\text{-}wt}$[5]. Potential physiological variations in GH sensitivity due to GHR differences in short children with an otherwise normal GH-IGF-I axis can be compensated by adaptations of endogenous pituitary GH secretion, which might mask the effect of the GHR polymorphism on basal growth rate. In addition, there is no evidence that the d3GHR influences normal adult height variation[54]. However, in patients with GHD this compensatory effect of the GH-IGF-I axis is deficient.

III B. Children with short stature due to growth hormone deficiency

GHD is a condition of inadequate production of GH. Deficiency of GH produces significantly different problems at various ages. GHD can be congenital or acquired in childhood or adult life. It can be partial or complete. It is usually permanent, but sometimes transient. It may be an isolated deficiency or occur in association with deficiencies of other pituitary hormones. In newborn infants the primary manifestations may be hypoglycemia. In later infancy and childhood, growth failure may be the major effect.

GH deficiency is treated by rhGH. A fixed rhGH dose, calculated according to weight and body surface area, accelerates growth from a pre-treatment rate of 3-4 cm/year to 6-12 cm/year in the first year of therapy in children[55]. This therapeutic effect can be further improved by earlier treatment, larger rhGH doses, individualized doses based on IGF-I concentrations[56], and

more frequent administrations of rhGH[55]. Since this therapeutic efficacy of rhGH shows high inter-individual variation, there is possibly a role for the complex cascade of numerous genes by which GH regulates growth velocity, including the d3GHR polymorphism.

Several studies have been published on the effect of the exon 3 deleted GHR polymorphism with some reporting positive and others negative results with respect to the influence of this polymorphism on growth parameters in GHD children. It is important to note that those studies were performed in different populations, harboring variations in their responses to GH therapy. In addition, there are differences between studies in growth parameters, the dosages of rhGH, and durations of follow-up. These studies are summarized below in *Table 1*. Jorge *et al.* were the first to extend the findings, described by Dos Santos *et al.*, of altered GHR function by the deletion of exon 3 in GHD children[5;57]. Both increased growth velocity in the first year of treatment with rhGH and increased final height were achieved in children with non-isolated GHD with the GHR_{d3} genotype. In addition, Räz *et al.* also demonstrated an increased first and second year growth velocity for both the $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ genotypes in comparison with the $GHR_{wt\text{-}wt}$ in children with isolated GHD, but they were unable to replicate the finding of Jorge *et al.* on increased final height[41;54;57].

In contrast, other studies reported negative results on these pharmacogenetic effects of rhGH. Blum *et al.* were unable to confirm a stimulating effect of the GHR_{d3} genotype on growth parameters in 107 children with severe GH deficiency after 1 year of treatment with rhGH in a study in which $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ patients were pooled (GHR_{d3})[37]. This was in keeping with de Graaff *et al*. and Pilotta *et al*., although the latter published not enough data to be represented in *Table 1*[38;40]. Higher IGF-I levels have been attributed to the GHR_{d3} genotype, although this did not result in increased height gain or growth velocity[37;39].

Most studies focussed on the short-term results of rhGH treatment, i.e. growth velocity or height gain in the first 1 or 2 years of treatment. To date, only 2 studies reported on the effect of d3GHR on final height after treatment with rhGH. Jorge *et al.* demonstrated a stimulating effect for both the GHR_{d3} isoform as for the $GHR_{d3\text{-}d3}$ isoform, which could not be explained by pre-treatment characteristics or differences in rhGH dose[57]. Raz *et al.* demonstrated a stimulating effect of the GHR_{d3} isoform in the short-term after rhGH treatment in a large cohort of GHD children, but were unable to demonstrate this effect on final height[41]. Of all studies on the effect of the d3GHR on growth in GHD children, Raz *et al*. were able to report a difference in basal growth rates prior to treatment as reflected by a difference in

baseline height between the different genotypes[41]. However, in a recent meta-analysis of studies reporting baseline height, we showed that there is indeed a subtle difference between GHR_{d3} and $GHR_{wt\text{-}wt}$ genotypes in children with GHD[58].The discrepancies in the effect of the d3GHR polymorphism on the response to (all individualized) doses of rhGH treatment seen in studies on GHD in children can possibly be explained by variations in underlying conditions, individual conditions of the patients studied, severity of GHD, stringency on the criteria used for diagnosis of GHD, duration of rhGH treatment, and the relatively small numbers of subjects included in the studies. In addition, Herishanu *et al*. were unable to demonstrate differences in growth delay in children with Cushing's disease according to the different variants of the d3GHR[43].

In conclusion, in most individual studies and in a recent meta-analysis, the growth response during the first year of rhGH treatment, both expressed as height SDS gain and as growth velocity, is slightly, but significantly increased in pre-pubertal short children with the GHR_{d3} genotype, albeit with a small increase of approximately 0.5cm/yr, in comparison with the $GHR_{wt\text{-}wt}$ genotype[58]. This polymorphism therefore explains only a small part of the relatively large inter-individual differences in linear growth response. Therefore, there may be other, up till now unknown, genetic factors including the GHR c.1319 G>T polymorphism[59], that influence growth velocity in GHD patients after GH therapy.

III C. Children with short stature not due to growth hormone deficiency

The cause of growth failure in the majority of short children who do not have GHD is unknown. These otherwise normal children with idiopathic short stature (ISS) or born small for gestational age (SGA) secrete normal amounts of GH in response to pharmacologic stimulation[30]. The importance of the GHR in modulating the growth promoting action of GH is demonstrated by the abnormal growth of children with complete insensitivity to GH due to inactivating mutations in the gene for the GHR (Laron dwarfism)[36]. These non-GHD short children may secrete GH normally and yet have defects in the ability of target cells to respond to GH. Such defects could occur either at the GHR or the intracellular mediators of GH signaling. In addition, serum concentrations of the GHBP in children with ISS have shown to be low, suggestive for abnormalities in the GHR gene[60;61].

Since 2001 and 2003, the US Food and drug Administration (US FDA) and the European regulatory agency, respectively, allowed the treatment of short children born SGA

with rhGH. In 2003, rhGH was approved by the US FDA to stimulate growth in children with ISS, and soon after it was also approved in Europe. Children with a height of 2.25-3.00 SD below the mean for their ages are now eligible for rhGH prescription. Although most children with SGA/ISS respond to treatment with rhGH with increases in their linear growth rates, their responses are more limited than those of children with GHD who are treated similarly. In accordance to the observations in GHD, in non-GHD children there are relatively large intra-individual variations in response to rhGH treatment. Indicators for a good response on rhGH treatment in this non-GHD patient group include young age, low baseline height, low baseline IGF-I levels, and high rhGH dose. The GHR_{d3} genotype is thought to influence the growth response in non-GHD children, since Dos Santos *et al.* reported on increased growth velocity in SGA and ISS children with the GHR_{d3} genotype and treated with rhGH[5]. They further demonstrated that the GHR_{d3} genotype was not primary related to the genesis of short stature and it had no effect on growth rates or hormonal parameters before start of rhGH therapy[5]. In addition, GHR isoforms are not involved in the etiology of short stature[62]. For the purpose of this review, SGA and ISS are discussed separately.

III C1. *Small for gestational age*

Children SGA have a birth weight below the 10th percentile for that gestational age, usually due to intrauterine growth restriction (IUGR). The status of being born SGA can be a consequence of fetal, maternal, or environmental factors, but the exact (multifactorial) etiology remains usually unknown. The majority of infants born SGA (85%) experience spontaneous catch-up growth in the first few months and up to 2 years, followed by a normal pattern of development. Those 15% failing to experience catch-up growth do not reach their target height and remain short throughout life[63;64]. The mechanisms involved in catch-up growth remain unclear. The finding of higher basal GH levels suggest GH hypersecretion as a factor for early catch-up growth[65]. On the other hand, birth weight, birth length, gestational age, and mid-parental height have also been identified as factors influencing catch-up growth[63;65]. Although the majority of these children is non-GHD, rhGH treatment improves average linear growth in these children[5]. This effect is largest during the first 2 years of treatment and diminishes after 4-6 years of continuation of rhGH[66]. When rhGH is discontinued catch-down in growth velocity may occur[66].

Dos Santos *et al.* treated pre-pubertal patients born SGA with normal GH secre-

tion with supraphysiological rhGH replacement doses and demonstrated a strong relationship between GHR_{d3} genotype and growth velocity. Shortly after, these findings were confirmed by two other groups[67;68] despite the fact that SGA children are heterogeneous regarding the origin of the short stature[69]. In accordance, increased spontaneous post-natal growth velocity in the GHR_{d3} genotype compared with the $GHR_{wt\text{-}wt}$ genotype was reported by Jensen *et al.*[70]. In addition, de Graaff *et al.* demonstrated a higher frequency of GHR_{d3} in children with GHD born SGA compared with GHD born appropriate for gestational age[38].

Conflicting findings were reported by four Spanish studies of the same group who were unable to demonstrate an effect of the GHR_{d3} genotype on pre- or post-pubertal growth after rhGH treatment in varying doses[71;72] or without rhGH treatment[73]. These contrasting observations can be caused by the relatively heterogeneous age distribution of the cohorts studied (large proportions post-pubertal children), the genotype assignment techniques used, and/or the exclusion of patients with a minor increase in height (<0.5 SDS)[74].

A single unconfirmed study studied the effect of the GHR_{d3} genotype in pre-natal growth in SGA and showed decreased fetal growth velocity in this genotype[70]. However, fetal growth is generally considered independent of fetal GH levels, whereas fetal IGF-I levels are associated with fetal size[75-79].

Only one study assessed the long-term effect of d3GHR polymorphism on near final height (adolescence), demonstrating increased height in patients with the GHR_{d3} isoform[41].

III C2. *Idiopathic short stature*

ISS refers to extreme short stature (<3 SD below the mean), despite minimal 2 of the following: normal size for gestational age at birth, normal body proportion, no evidence for endocrine dysfunction, no chronic organic or psychiatric disease, no emotional disturbances, and normal food intake. In ISS the short stature is due to decreased growth rate without a diagnostic explanation after an ordinary growth evaluation[5]. Only 5-9% of ISS children reach an adult height lower than 2 SD below the mean[80] and rhGH treatment is recommended to increase height in these non-GHD children.

In accordance to SGA children, Dos Santos *et al.* reproduced their finding of a strong relationship between GHR_{d3} genotype and growth velocity in pre-pubertal children with ISS, treated with supraphysiological rhGH replacement doses[5]. Toyoshima *et al.* demonstrated that children with ISS carrying the GHR_{d3} allele presented higher GH sensitivity regarding short-

term IGF-I generation during rhGH treatment[42]. This finding supports the rhGH pharmacogenetic findings in relation to GHR_{d3} genotype, described by Dos Santos *et al.*[5]. In addition, although the GHR_{d3} genotype was less frequent in Japanese (20%)[81] and Koreans (17-22%)[82] than in Caucasians (25-44%), in Korean pre-pubertal ISS children the GHR_{d3} genotype significantly affected growth velocity in short-term rhGH treatment. In contrast, the Spanish study by Carrascosa *et al*., like in SGA, was unable to demonstrate an effect of d3GHR genotypes on height gain or height velocity during 1 to 2 years of rhGH treatment in a large cohort of prepubertal Spanish children with ISS[74]. Again, these authors excluded patients with poor growth response (<0.5 SD in 2 years) to rule out the main genotype abnormalities of the GH-IGF-I axis[29;30]. This could have created a selection bias and possibly they were therefore not able to show a stimulating effect of GHR_{d3} genotype on growth velocity. At present, there are no data are available on the effect of the d3GHR polymorphism on final height in ISS.

In conclusion, 13 studies have been published on the effect of d3GHR on stimulated growth in non-GHD. Although the different studies are conflicting, a meta-analysis of the data in prepubertal children with short stature indicates that GHR_{d3} is associated with increased baseline height in GHD, but not in non-GHD[58]. Furthermore, GHR_{d3} stimulates growth velocity by an additional effect of ~0.5 cm during the first year of rhGH treatment and this effect is more pronounced at lower doses of rhGH and higher age[58].

III D. Turner syndrome

Turner syndrome is a chromosomal disorder affecting females in whom all or part of one of the X-chromosome is absent. Short stature, a prominent feature of patients with Turner's syndrome, occurs in 100% of patients with 45,X0 karyotype and in 95.2% of patients with non-45, X karyotype. The cause of growth failure in Turner's syndrome is currently unknown, but theories exist about a dysfunctional GH response resulting in partial GH insensitivity on the one hand and a primary bone defect on the other hand[83]. Treatment during childhood consists of early GH therapy in supraphysiological doses and estrogen replacement around the normal age of puberty. An average gain of 10 cm (range 3.9–24.8 cm) in final height may be achieved by the early introduction of growth-promoting therapies[83]. Inter-individual variations in response on rhGH treatment are possibly explained by differences in parental height, age at start of GH treatment, and dose of rhGH[83].

Turner syndrome is a more specific and homogenous disorder of linear growth failure

and of different severity and origin than short stature due to GHD, SGA, or ISS. Girls with Turner syndrome with the $GHR_{d3\text{-}d3}$ genotype on a high dose of rhGH for 1 year demonstrated a significantly higher growth velocity than their $GHR_{wt\text{-}wt}$ and $GHR_{wt\text{-}d3}$ counterparts and exceeded their growth prediction by far[29;67]. Accordingly, the $GHR_{wt\text{-}wt}$ Turner girls failed to reach their height prediction, and $GHR_{wt\text{-}d3}$ showed intermediate responsiveness to rhGH. The effect of the GHR_{d3} genotype was far more evident in Turner girls than in patients born SGA[67]. When the total period of (a low dose of) rhGH administered in Turner girls was taken into account, $GHR_{d3\text{-}d3}$ genotypes were associated with enhanced growth velocity for a longer time period and a height gain almost twice as great when compared with the other two genotypes, despite comparable levels of rhGH admitted and independent of an effect on serum levels IGF-I[84]. In a Korean population with a known relatively low allelic prevalence of the d3GHR, there was no effect of this polymorphism on linear growth in 115 prepubertal patients with Turner's syndrome[44].

In conclusion, only three studies have focused on the effect of the d3GHR polymorphism on stimulated growth in Turner syndrome, two of them (both of the same group) reporting increased height gain and growth velocity in the short term and increased final height in favor of GHR_{d3} when compared with $GHR_{wt\text{-}wt.}$ This clear effect of the GHR_{d3} genotype is possibly due to the homogeneity of the population studied.

III E. The effect of d3GHR genotypes on basal height

Data on the effect of the d3GHR polymorphism on spontaneous growth in non-GHD children are scarce, but conflicting. Only 2 studies were performed both in SGA[70;85]. Jensen *et al.* demonstrated a positive effect of the GHR_{d3} isoform, whereas Audi *et al.* could not find an effect of the GHR_{d3} isoform on spontaneous growth[70;85]. We meta-analyzed these findings and we were unable to demonstrate a difference in baseline growth between the different genotypes in non-GHD children[54].

III F. Conclusion

The different studies on growth in response to rhGH treatment in different clinical conditions demonstrate a variety of results regardless of the cause of shortness (GHD or non-GHD). In addition to the factors held responsible for intra-study variation (*vide supra*), it is likely that differences between studies are at least in part caused by small sample sizes and heterogeneous

characteristics of the patients studied. False-positive findings are more likely with small sample sizes and for quantitative trait loci phenotypical variations tend to be overestimated with small sample sizes[41]. A recent meta-analysis in prepubertal children with short stature supports this finding, indicating that GHR_{d3} is associated with increased baseline height in GHD, but not in non-GHD children.

The GHR_{d3} genotype is not expected to be associated with physiologic variations in human growth or to be a primary cause of short-stature in humans, since potential variations in growth hormone sensitivity due to the GHR differences in non-GHD short children can be compensated by endogenous pituitary growth hormone secretion, which masks the effects of GHR polymorphisms on basal growth rate[5]. When this compensation is impossible, however, like in GHD children, the d3GHR genotype affects the growth response (spontaneous or stimulated). We demonstrated this hypothesis in a recent meta-analysis, indicating that the baseline growth in GHD children with de GHR_{d3} isoform is significantly increased[58].

IV Effect of d3GHR on other endocrine disorders

IV A. Pathogenesis

Deficient or excessive GH secretion induces typical clinical syndromes, with changes in many tissues, organs and organ functions, and altered body composition. In addition to physical symptoms and co-morbid diseases, patients with GH diseases experience a large range of complaints, i.e. functional limitations, diminished work-related productivity, social isolation, fatigue, poor health-related quality of life, etcetera[85;91]. However, there are major individual differences in the expression and severity of (co-)morbidity. Even after (long-term) control of the altered GH status (in acromegaly) strong individual differences in clinical outcome are reported[86]. We discuss several studies suggesting a possible effect of the d3GHR polymorphism on disease presentation in patients with active or biochemically well-controlled GHD or GH excess, respectively. In these patients natural feedback regulation within the GH-IGF-I axis is absent, implying that a possible effect of the different d3GHR isoforms should be demonstrable.

IV B. Growth hormone deficiency in adults

In adult patients with GHD, three groups of GHD can be identified: 1) childhood onset GHD with GH replacement in childhood and with normal stature, 2) GHD without GH replacement in adulthood with short stature, and 3) patients with adult-onset GHD due to different causes but mainly related to (treatment for) pituitary adenomas. Other causes for adult onset GHD are traumatic brain injury, sub-arachnoidal hemorrhage, or subdural haematoma. Therefore, GHD in adults is a heterogeneous syndrome. In contrast to the clear cut efficacy parameters of rhGH treatment in children (height gain, growth velocity, and final height), the effects of rhGH treatment in GHD adults are more subtle, like improvement in quality of life, muscle strength, lipid metabolism, body composition, and bone mineral density[92;93]. Several studies have assessed whether the large inter-individual variability in response to rhGH replacement therapy in GHD adults might be partly attributed to the d3GHR polymorphism in addition to more obvious causes such as differences in cause and severity of GHD.

GH and IGF-I affect lipogenesis and HDL cholesterol expression[94;95]. Van der Klaauw *et al*. demonstrated that the GHR_{d3} genotype contributed to the beneficial changes induced by rhGH in lipid metabolism[47]. However, there was no significant effect of the polymorphism on clinical parameters including BMI, total cholesterol, LDL cholesterol, and blood pressure[47]. In accordance, a recent Swedish study did not find a pharmacogenetic difference in response to rhGH between the genotypes in body composition[96].

In conclusion, two studies focused on the effect of d3GHR on clinical outcome in GHD adults after rhGH treatment, with contrasting short-term results. The number of participating patients, age, rhGH dose, and distribution of d3GHR isoforms were comparable in both studies. In contrast to short children, in whom the primary efficacy of GH therapy is easily evaluated by growth velocity and final height, the end-points to define responsiveness to rhGH therapy in adults are more subtle and diverse. Larger prospective studies with appropriate statistical power are necessary to clarify the additional contribution of d3GHR in the responses to rhGH therapy.

IV C. Acromegaly

The clinical severity of acromegaly varies considerably between individuals both during active disease and after long-term remission of acromegaly, most likely related to the severity and duration of active disease. However, since the GHR_{d3} genotype has been reported to increase

growth velocity in children with GHD during rhGH therapy[5;57;97] and since in GHD the GHR_{d3} genotype has been associated with increased IGF-I levels[37;39;47], assumptions have been made regarding an effect of the d3GHR polymorphism on the disease course in acromegaly. It has been hard to prove the theory that the d3GHR polymorphism may have caused a change in the GH-IGF-I axis and therefore rendered acromegalic patients with the GHR_{d3} genotype more susceptible for the effects of GH, since only few studies on this topic have been conducted showing different results (*vide supra*). However, On the other hand, the limited pharmacogenetic effect of enhanced signal transduction in GHR_{d3}, patients can also be overruled by the excess of GH. We therefore discuss studies in active and controlled acromegaly focusing on the clinical consequences of the disease.

IV C1. *Active acromegaly*

Several studies focused on the effect of clinical parameters in patients with active acromegaly. Mercado *et al*. observed that GHR_{d3} patients with active acromegaly had an increased prevalence of type 2 DM (odds ratio 2.0). However, this was not confirmed by others[43;51;98;99]. In addition, Mercado *et al*. could not demonstrate an effect of the d3GHR polymorphism on gender, height, BMI, percentage of macroadenomas, and arterial hypertension between the GHR genotypes in active acromegaly, although patients with the GHR_{d3} genotype were younger at diagnosis[51]. The late clinical outcome of acromegalic patients after successful treatment in relation to these GHR isoforms needs to be confirmed. Montefusco *et al*. studied 76 patients with active acromegaly retrospectively and reported a significantly lower BMI in GHR_{d3} compared with $GHR_{wt\text{-}wt}$ and equal prevalences of type 2 DM and hypertension in all genotypes, although GHR_{d3} carriers had more frequently a normal glucose tolerance[99]. Thus, these data indicate that the GHRd3 genotype is associated with glucose metabolism and body weight in patients with active acromegaly.

IV C2. *Controlled acromegaly*

Recently, our group reported the late clinical outcome of patients with acromegaly in relation to the different GHR genotypes[100]. In 86 patients, the GHR_{d3} genotype was associated with adverse clinical outcome with respect to osteoarthritis, dolichocolon, and adenomatous colonic polyps but not with other co-morbidities such as cardiovascular risk factors. These observations await confirmation by other groups.

IV C3. *Response to treatment*

With respect to treatment response, Bianchi *et al.* recently demonstrated that in acromegalic patients with active disease after unsuccessful neurosurgery and somatostatin anolog treatment, GHR_{d3} patients required significantly lower doses of Pegvisomant for a shorter period of time to achieve normalized IGF-I levels, when compared with $GHR_{wt\text{-}wt}$ patients[101]. This is compatible with a higher sensitivity of the GHR_{d3} genotype. However, the exact mechanism underlying this observation is not yet understood. This might include more efficient binding of Pegvisomant to the receptor in GHR_{d3} patients, or increased affinity for pegvisomant of the GHR_{d3}. Bernabeu *et al.* confirmed these results showing that the GHR_{d3} genotype predicted an improved response to pegvisomant therapy in acromegaly[102].

In conclusion, in acromegalic patients the GHR_{d3} genotype has shown to positively affect glucose metabolism and body weight and negatively affect osteoarthritis, dolichocolon, and adenomatous polyps. The therapeutic response to somatostatin analogs or pervisomant was better in the GHR_{d3} variant. However, this is based on few studies with varying inclusion criteria and different study outcomes.

IV D. Conclusion

In the different studies on the effect of the d3GHR on the GH-IGF-I axis in both GHD and acromegalic subjects effects of the d3GHR were either absent or rather subtle. In prepubertal children with short stature the GHR_{d3} is associated with increased baseline height in GHD, but not in non-GHD. Furthermore, GHR_{d3} stimulates growth velocity by an additional effect of approximately 0.5 cm during the first year of rhGH treatment. In acromegaly, there may be an effect of the d3GHR on clinical outcome, i.e. osteoarthritis, dolichocolon, and adenomatous polyps. Moreover, the therapeutic responses to somatostatin analogs or pervisomant were better in the GHR_{d3} variant in the patients with active acromegaly. Up till now we can not simply explain these findings by the theory that GHR_{d3} patients are more susceptible to the effects of GH, based on the finding of Dos Santos *et al.* that there is an increased bioactivity of GHR_{d3} *in vitro* in response to various GH concentrations. Large, prospective studies are needed to confirm these differences in clinical outcome in GHR_{d3} patients compared with $GHR_{wt\text{-}wt}$ patients.

V. Effect of d3GHR on metabolism

V A. Pathogenesis

Blood glucose levels are closely regulated in health. The principal organ of glucose homeostasis is the liver, which absorbs and stores glucose (as glycogen) in the post-absorptive state and releases it into the circulation between meals to match the rate of glucose utilization by peripheral tissues.

The GH-IGF-I axis is important for metabolic control[1;39;47]. GH has many metabolic effects, including IGF-I dependent effects, such as glucose uptake and protein metabolism, and IGF-I independent effects such as the stimulation of insulin secretion and pancreatic beta cell proliferation[103] and the decrease of pheripheral insulin sensitivity through inhibition of glucose disposal in skeletal muscle[104]. The question rises whether the increased bioactivity of the GHR_{d3} genotype affects metabolism.

V B. Diabetes Mellitus

V B1. *Type 1diabetes mellitus*

Type 1 DM is a disease resulting from insulin deficiency. Diabetic nephropathy is one of the complications associated with DM. Recent evidence has suggested that the glomerular podocyte is a target for GH action, and, therefore, the GH-IGF-I axis may play a role in the development of diabetic nephropathy[105;106]. Another recent study has demonstrated that deficiency of GHR in mice causes a reduction in systolic blood pressure and plasma renine levels, as well as an increase in aortic endothelial NO synthase levels[107]. Gu *et al*. therefore investigated whether the d3GHR polymorphism was involved in the pathogenesis of diabetic nephropathy in patients with type 1 DM[108]. However, they were unable to associate the d3GHR genotypes and diabetic nephropathy in these patients, although the allele frequency of GHR_{d3} was low, and not in Hardy Weinberg equilibrium, and comparable to the frequency found in Swedish patients with type 2 DM (*vide infra*)[109].

In conclusion, in patients with type I DM, the allele frequency of the d3GHR is not in accordance to HWE. No effect of the d3GHR was demonstrable on diabetic nephropathy.

V B2. *Type 2 diabetes mellitus*

Type 2 DM is a multi-genetic disease and an increasing problem worldwide. The disease may be present in the sub-clinical form for years before diagnosis, demonstrable by impaired glucose tolerance (IGT) (*vide infra*). Since the GH-IGF-I axis is important for metabolic control[1], there might be an effect of the d3GHR polymorphism on metabolic diseases. It was recently reported that a polymorphism of the IGF-I promoter caused lower IGF-I levels and a increased risk of type 2 DM[110;111].

The question was raised whether the $GHR_{d3\text{-}d3}$ genotype with increased GHR sensitivity potentially leading to increased IGF-I levels would protect against type 2 DM. Strawbridge *et al.* demonstrated that the allele frequencies of the d3GHR in type 2 DM patients disagrees with the Hardy Weinberg equilibrium, due to a lower proportion of $GHR_{d3\text{-}d3}$. These findings suggest that increased bioactivity of this allele confers a protection function against type 2 DM[109] due to increased IGF-I levels as a result of increased GH sensitivity which promotes glucose uptake[109] and decreased insulin sensitivity. This effect has also been demonstrated in centenarians[112]. However, if other factors outweigh this protective effect of $GHR_{d3\text{-}d3}$ genotype, the resulting type 2 DM in subjects with $GHR_{d3\text{-}d3}$ genotype seems to have a more severe phenotype associated with increased age-adjusted IGF-I and BMI[109], which is possibly due to an increase in GHR_{d3} activity.

In conclusion, the only study focusing on the effect of the d3GHR genotype on type 2 DM shows a lower allele frequency of GHR_{d3} in patients with type 2 DM[109]. This was in accordance to the allele frequency demonstrated in type 1 DM[108]. However, in GHR_{d3} patients with type 2 DM, the disease seems to be more severe than in the other genotype.

V C. Impaired glucose tolerance

IGT is a pre-diabetic state, which is associated with insulin resistance and increased risk of cardiovascular pathology. IGT may preceed type 2 DM by many years. IGT is also a risk factor for mortality. Obesity and a lack of exercise make progression to overt DM more likely.

In accordance with the findings in patients with type 1 and 2 DM, the d3GHR genotype does not meet the Hardy Weinberg equilibrium in patients with IGT[109]. In addition, a recent study in 145 healthy Caucasian children and adolescents higher insulin secretion during an oral glucose tolerance test was found in children who carry at least one d3GHR allele, when adjusted for the degree of insulin sensitivity[113].

In conclusion, the $GHR_{d3\text{-}d3}$ genotype may positively influence pancreatic beta cell compensatory capacity and therefore protects against IGT. This effect is comparable to patients with type 2 DM.

V D. Conclusion

There are discrepancies between the several studies in GHD and acromegaly on the effect of the d3GHR on the GH-IGF-I axis. However, the majority of studies indicate a positive effect of the GHR_{d3} genotype on the IGF-I concentrations[37;39;47;52], but regarding the small number of studies it is hard to draw firm conclusions. Moreover, since GH and IGFBP3 concentrations are not different for the different genotypes, one might assume that an increase in IGF-I secretion is caused by enhanced receptor expression or function, like binding of GH, receptor processing, transport, stability, and signal transduction, resulting in an increase in IGF-I concentrations. Uncovering the mechanism will promote our understanding of the GH-IGF-I axis. Therefore prospective studies are needed.

VI. Effect of d3GHR on cardiovascular diseases

VI A. Pathogenesis

Genetic factors are known to play a major role in hypertension[114]. A combination of genome wide linkage scans and candidate gene analysis has identified a variety of genetic loci that contribute to blood pressure variation and risk of hypertension[115]. Arterial hypertension itself is an important risk factor for stroke, largely because hypertension itself is associated with the formation of atheromatous deposits, which can narrow or obstruct cerebral arteries. The etiology of cerebrovascular stoke is heterogenous, however, with a strong genetic component[116].

Short stature has long been known to be associated with an increased risk of arthero-sclerosis[117] and coronary heart disease[118]. Short stature is also associated with elevated pulse pressure, a surrogate marker of arterial stiffness[119]. Since the risk of both hypertension and stroke appears to be inversely correlated with adult height, individual variation in the genes encoding GH and its receptor (GHR) might be associated with hypertension and stroke. The question rises whether the d3GHR polymorphism is one of the genetical factors influencing the risk on hypertension and stroke.

VI B. Hypertension

Hypertension can be classified either essential (primary) or secondary. Essential hypertension indicates that no specific medical cause can be found to explain a patient's condition. Secondary hypertension indicates that the high blood pressure is a result of (*i.e.*, secondary to) another condition, such as kidney disease or tumours (pheochromocytoma and paraganglioma). Persistent hypertension is one of the risk factors for strokes, heart attacks, heart failure and arterial aneurysm, and is a leading cause of chronic renal failure. Even moderate elevation of arterial blood pressure leads to shortened life expectancy.

Only one study has been performed regarding the effect of d3GHR on the risk for development of hypertension. A borderline non-significant association between females with hypertension and the GHR_{d3} phenotype was demonstrated, however the population studied was small[120].

In conclusion, with regard to the current literature, no relationship exists between d3GHR genotype and the risk for development of hypertension. More research in larger populations is required.

VI C. Stroke

Stroke is the rapid loss of brain functions due to a disturbance in the blood vessels supplying the brain. This can be due to ischemia caused by thrombosis or embolism or due to a hemorrhage. As a result, the affected area of the brain is unable to function, leading to inability to move one or more limbs on one side of the body, inability to understand or formulate speech, or inability to see one side of the visual field. A stroke can cause permanent neurological damage, complications, and death. It is the number two cause of death worldwide and may soon become the leading cause of death worldwide. Risk factors for stroke include advanced age, hypertension (high blood pressure), previous stroke or transient ischemic attack (TIA), diabetes, high cholesterol, cigarette smoking, and atrial fibrillation. High blood pressure is the most important modifiable risk factor of stroke.

The same authors investigating the effect of d3GHR on developing hypertension, investigated the effect of d3GHR on the risk for stroke, but failed to demonstrate any effect[120].

In conclusion, no effect of the d3GHR genotype on the risk for stroke was demonstrable. However, more studies in larger populations are needed.

VI D. Conclusion

Hypertension and stroke are major causes of morbidity and mortality. The risk for both hypertension and stroke appears inversely related with adult height, but the inter-individual variation in the d3GHR is not associated with stroke and hypertension. The association between stature and hypertension and stroke could not be exerted solely via a genetic influence upon stature. In addition, final height in the normal population, in contrast to GHD of small patients treated with rhGH, is not determined by variation in the d3GHR, but by other genetic and environmental factors like adult height, nutrition, etc. The risk on hypertension and stroke seems not to be mediated by the d3GHR polymorphism. However, only 2 populations have been studied, so it is hard to draw firm conclusions. Studies in other genetic populations might add new information to this topic.

VII. Effect of d3GHR on survival

VII A. Pathogenesis

The deletion of exon 3 was shown to be a genomic event that occurred in the several steps during the evolutionary lineage from Old World monkeys genome to Pongina and Hominima genomes [120;121]. Following the long-lived integration of proviral sequences in the vicinity of exon 3 of the GHR gene, a homologues recombination seems to have occurred in humans, resulting in the excision of exon 3 in one of our ancestors. It is unknown how this polymorphism has become as frequent as up to 50% of Caucasian people. Because of its current frequency it is clear that the homologues recombination is a very ancient event. Is it thought that the exon 3 deletion had played a favorable role, fitting with the Darwinian evolution of our species and favoring its propagation in given human subgroups [122], but up till now, no functional role could have been identified that substantiates this speculation. Focusing on extreme populations might contribute to the identification of the role of the GHR_{d3} genotype.

VII B. Obesity and starvation

Obese patients are at risk of early death, mainly from diabetes, coronary heart disease and cerebrovascular disease. The greater the obesity; the higher the morbidity and mortality rates.

In contrast, in many areas of the world, people are on the verge of malnutrition. Al-

though the basic condition of protein-energy metabolism (PEM) is the same in all parts of the world from whatever cause, malnutrition resulting from long periods of near-total starvation produces unique clinical appearances in children virtually never seen in the West.

Only few studies have investigated the role of the d3GHR polymorphism in obesity or starvation. Strawbridge *et al*. demonstrated that in patients with type 2 DM and IGT the prevalence of the $GHR_{d3\text{-}d3}$ isoform was significantly lower in a non-diabetic control population[109]. In addition, in this small proportion of type 2 DM and IGT patients with the $GHR_{d3\text{-}d3}$ genotype the phenotype was associated more with severe obesity and increased IGF-I levels[109].

In contrast, in a West-African population with persistent under-nutrition a high prevalence (70%) of GHR_{d3} genotypes was reported[123]. Starvation increases GH pulse frequency and amplitude resulting in an increase in circulating GH levels[124]. Coupled with reduced insulin secretion, this leads to increased lipolysis and ketogenesis, insulin resistance and conservation of glucose and protein[125]. The maintenance of a high level of circulating GH in malnourished individuals may therefore have been of selective value. However, increased lipolysis gives rise to elevated levels of circulating free fatty acids which have been shown, at least *in vitro*, to decrease the expression of GHR by approximately 50%[126]. Decreased amounts of GHR on the cell surface would be expected to lead to reduced secretion of IGF-I. The presence of at least one GHR_{d3} allele, demonstrated in 70% of the West-African population, may serve to ameliorate these effects of decreased expression of the GHR by virtue of its increased responsiveness to GH, and IGF-I levels may consequently be near normal.

VII C. Conclusion

In conclusion, the $GHR_{d3\text{-}d3}$ isoform is more frequent in patients suffering from starvation, indicating that both the elevated GH gene expression and increased GHR-mediated GH responsiveness may constitute adaptive responses to the effects of persistent malnutrition, since increased circulating GH appears to form part of a physiological response to nutrition deprivation. The prevalence of the GHR_{d3} isoform in well-fed populations is currently unknown. But in patients with type II DM and IGT, with the scarcely prevalent GHR_{d3} isoform, this isoform is associated with more severe obesity than in patients with the other isoforms. Further research on this interesting topic is required.

VIII. Summary and Conclusions

This review focused on the presently known functional consequences of the exon 3 deleted GHR polymorphism in various clinical conditions and physiological processes. We conclude that the effects of the d3GHR polymorphism are not limited to subtle variations in growth parameters in (non)GHD children with short stature treated with rhGH and to phenotype-genotype correlations and pharmacogenetic responses to therapy in patients with acromegaly. The effects also seems to affect many pathophysiological processes in which the GH-IGF-I axis is involved.

Two major implications of this review emerge. First, most studies included relatively low numbers of subjects, resulting in a low statistical power and explaining at least in part conflicting results between studies. In addition, most studies did not included independent additional cohorts for confirmation of their results. Second, the implications of these genotype-phenotype relationships of the GHR for routine clinical practice seem to be limited.

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Part V.

DISCUSSION AND SUMMARY

Chapter 13.

GENERAL DISCUSSION, CONCLUSIONS, AND SUMMARY

CONTENTS

I. Introduction

II. Osteoarthritis and joint-related problems in acromegaly

III. Acromegaly; long-term disease outcome, not joint-related

IV. Different effects of the exon-3 deleted growth hormone receptor polymorphism

V. General discussion and concluding remarks

Part I. Introduction

In this thesis a number of observations have been described in acromegalic patients during long-term follow-up with cured or biochemically well-controlled disease. These observations focused on persistent consequences of the disease, which actually represent irreversible effects. In these studies we evaluated the acromegalic arthropathy, bone quality and fractures, colonic abnormalities, and quality of life. In addition, we studied genotype-phenotype relationships of the d3GHR polymorphism on long-term clinical outcome in acromegaly. Moreover, we have meta-analyzed the effect of this polymorphism on spontaneous and stimulated growth in (non)-GHD short children, in order to help us understand the ongoing effect of this polymorphism in acromegaly, even after long-term cured disease.

Part II. Osteoartritis and joint-related problems in acromegaly

The prevalence of arthropathy in acromegaly is striking, also after long-term cure of GH overproduction. The aim of the study described in **Chapter 2** was the structural, clinical, and radiological assessment of the large joint-sites in 89 acromegaly patients with disease remission for a mean of 14 years after multimodality treatment, in comparison with 67 age-matched controls and a rheumatologic Dutch reference group.

The most frequent manifestation in our study was osteoarthritis, preferentially affecting the spine and even at remarkably young ages. The prevalence of clinical and radiological osteoarthritis of the hip, hand, and knee reached up to 80%. The prevalence of osteoarthritis in

patients exceeded the prevalence in controls at all joint-sites. The characteristical radiological changes observed in these patients with cured acromegaly consist of the combination of wide joint spaces and severe osteophytosis, which had not been described before.

This high prevalence of arthropathy in combination with the preservation of the extremely wide joint spaces, indicative of thickened cartilage, was previously thought to be present only in the active phase of the disease[1-5]. However, this case-control study demonstrated that prior GH excess has irreversible, deleterious late effects on clinical and radiological aspects of joints in patients with long-term cure of acromegaly.

The assumption is that persistent exposure of the tissues to pathologically elevated GH and IGF-I levels induces progressive changes in the joints. Cartilage proliferation occurs unevenly and produces a thickened, but mechanically unstable, joint surface[6,7]. In combination with laxity of periarticular ligaments and muscle weakness this leads to joint instability[6]. On the weight-bearing surfaces of the joints cartilage ulceration occurs and the reparative process deposits excessive amounts of fibrocartilage over the damaged areas. This is followed by development of osteophytes, formation of subchondral cysts, and joint space narrowing[6]. Ultimately, these pathophysiological processes become irreversible and self-perpetuating, with more mechanical trauma causing additional joint deformity that, in turn, leads to yet more structural damage to the articular tissues and after this point independently of the actual levels of GH and IGF-I. Our results support the hypothesis that the second step in the pathogenesis of osteoarthritis in acromegaly is independent of GH and IGF-I. Therefore, attention should be directed towards the recognition of joint-related problems in (long-term) cured acromegaly.

In conclusion, prior GH excess has irreversible, deleterious late effects on clinical and radiological aspects of joints in patients with long-term cure of acromegaly.

The few previous studies provided conflicting data on the impact of serum GH and IGF-I levels on clinical and radiological arthropathy. Moreover, these were mainly based on patient cohorts with active acromegaly. The study described in **Chapter 3** was conducted to identify factors influencing the development of radiological osteoarthritis during long-term control of acromegaly, focusing on disease specific parameters, GH and IGF-I concentrations and duration of the active disease, adjusted for the well-known determinants of primary osteoarthritis in 67 patients with long-term cured acromegaly.

This study documents that the risk to develop osteoarthritis in these patients is pre-

dicted by IGF-I concentrations at the time of initial diagnosis. An almost five-fold increase in risk for radiological osteoarthritis of the hip and hand was observed in patients in the highest tertile of pre-treatment IGF-I SD scores compared with patients in the lowest tertile of pre-treatment IGF-I scores. In addition, the extensiveness of osteoarthritis in all joints was associated with pre-treatment IGF-I SD scores. There were no other disease specific predictors for osteoarthritis

IGF-I stimulates chondrocytes to synthesize extracellular matrix components in cartilage. The action of IGF-I is mediated through the type I IGF receptor[8;9]. IGF-I may also influence osteoarthritis by osteophyte formation[8;9]. In this context, it is interesting to note that Meulenbelt *et al.* observed that the IGF-I allele 3 is associated with radiological osteoarthritis in the general population at any joint site (knee, hip, hand, and spine), and that carriers of the IGF-I 3 allele were predisposed for radiological osteoarthritis at any possible joint[10]. This association was strongest in subjects with radiological osteoarthritis of the hip. In general, radiological osteoarthritis of the hip has a specific genetic and geographically prevalence pattern, which suggests the involvement of systemic factors[10;11]. Moreover, the hip is the joint, which is the least affected by occupational and physical causes of osteoarthritis[11]. Therefore, in long-term cured patients with acromegaly, the hip is also most prone to secondary osteoarthritis caused by systemic increase of levels of IGF-I during the active phase of the disease and, apparently, this mechanism influences the prevalence of osteoarthritis even after long-term cure.

Thus, the development and extensiveness of osteoarthritis in long-term cured acromegaly patients is predicted by IGF-I concentrations at the time of initial diagnosis.

Primary osteoarthritis preferentially affects post-menopausal women. However, acromegaly is equally distributed in men and women. In **Chapter 4** we evaluated whether female gender is also a risk factor for osteoarthritis in acromegaly.

We compared pain and stiffness, clinical parameters and radiographs of the knee, hand, and hip between 46 male and 43 female patients (mean age: 58 years) with controlled acromegaly for a mean of 14 years. In addition, the gender-specific prevalence of osteoarthritis was compared with age- and environment-matched controls and of radiological osteoarthritis in a Dutch population sample.

Radiological and symptomatic osteoarthritis of the knee, distal interphalangeal joint, and hip was equally prevalent in male and female patients. Clinical osteoarthritis was similar-

ly prevalent in both genders except for hand osteoarthritis, which was encountered more in women. In comparison with controls and the historical population sample, the prevalence of osteoarthritis was increased in women with acromegaly, and even more so in men with acromegaly.

In acromegaly, the prevalence of osteoarthritis at all joint sites is similar in male and female patients. The near equal gender distribution of osteoartrhitis in acromegaly contrasts with the gender difference that is observed in primary osteoarthritis in which post-menopausal women are more frequently affected than men[12], suggesting that the overproduction of GH and IGF-I overrules the potential role of risk factors, including hormonal factors, in primary osteoarthritis.

Estrogen deficiency has been implicated as a contributing factor in conditions such as osteoporosis, gout, and osteoarthritis[13]. Estrogens also have well-known effects on the somatotrope system, affecting both GH[14;15] and IGF-I secretion[16]. In the liver estrogens inhibit GH-dependent IGF-I production. Interestingly, in 1951, estrogens were proposed as the first medical treatment in women with acromegaly[17]. In acromegaly, both the protective effect of estrogens, which is also observed in primary osteoarthritis, and the inhibitory action of estrogens on IGF-I production resulting in less severe IGF-I exposure may relatively protect pre-menopausal women. However, the ultimate effects of acromegaly on joint pathophysiology appear to be so strong that the prevalence of osteoarthritis is highly increased in both male and female patients with acromegaly. This may explain the lack of gender difference in the prevalence of osteoarthritis.

We can conclude that in long-term cured acromegaly patients the prevalence of osteoarthritis is equally prevalent in male and female patients, which is in contrast to the prevalence pattern in primary osteoarthritis.

In **Chapter 5** we compared the distribution of osteophytes and joint space narrowing between patients with acromegaly and primary generalized osteoarthritis to gain insight into the pathophysiological process of GH and IGF-I mediated osteoarthritis.

Doubtful osteoarthritis (Kellgren and Lawrence score 1 or more) of hips and knees on radiographs were compared between 84 patients with controlled acromegaly for a mean of 14.0 years and 189 patients with primary generalized osteoarthritis.

The osteoarthritis phenotype in secondary osteoarthritis present in patients with

long-term cured acromegaly differs from that in patients with primary osteoarthritis with generalized nature, since hips and knees in patients with long-term cured acromegaly were characterized predominantly by osteophytes and these osteophytes were observed frequently without joint space narrowing indicating preservation of articular cartilage in these joints. Patients with acromegaly reported less functional disability of the hips and knees than patients with primary generalized osteoarthritis.

Arthropathy due to GH hypersecretion results in osteophytosis and, to a lesser extent, in joint space narrowing. This observation suggests that the GH-IGF-I system is mainly involved in bone formation resulting in osteophytosis but may protect against cartilage loss.

We assume that in well-controlled acromegalic patients, who had pathologically elevated levels of circulating GH and IGF-I in the past, and in whom predominantly osteophytosis without joint space narrowing is seen, GH and/or IGF-I are implicated in the initiation of osteophyte formation. Recently, the elevation of GH stimulated IGF-I expression in the synovial cells and peripheral cells adjacent to the articular cartilage seemed to lead to the initiation of osteophyte formation in mice[18]. This might suggest that other factors are involved in osteophyte formation rather than that osteophytes are merely a secondary feature following osteoarthritic changes[18]. In the active phase of acromegaly, arthropathy is associated with hypertrophy of cartilage and soft-tissue[19]. We are the first to demonstrate that in long-term cured acromegalic patients articular cartilage is maintained, reflected by the absence of joint space narrowing even despite the presence of severe osteophytes. In addition, these preserved joint spaces may protect against pain caused by osteophytes and, therefore, prevent acromegaly patients from a decrease in functional capability.

In conclusion, arthropathy caused by acromegaly results in osteophytosis and not in joint space narrowing.

Chapters 2-5 have shown that acromegalic arthropathy is an invalidating complication of acromegaly and although it shares features with primary osteoarthritis, joint space narrowing is infrequently seen in acromegalic patients[20].

Since the late effects of acromegaly on hand joints have not been characterized, the aims of **Chapter 6** were 1) to compare joint space width of hand joints between patients with long-term controlled acromegaly and age- and gender-matched controls, 2) to identify factors associated with increased joint space width, and 3) to assess the relation between joint space

abnormalities and complaints of joint pain in acromegalic patients.

In a cross-sectional study in 89 patients (age 58 ± 12 yr, 49% women) with long-term controlled acromegaly and 471 age- and gender matched controls without hand symptoms (age 46 ± 12, 42% women) radiological joint space width of hand joints were measured by automated image analysis[21].

Patients with long-term remission of acromegaly had 20-24% wider mean joint spaces at the MCP, PIP and DIP-joints. Mean joint space width of all hand joints exceeded the 95th percentile of controls in more than 60% of the patients. Only a small portion (9%) of the patients had mean joint spaces below the 50th percentile of controls. Higher GH and IGF-I concentrations at the time of diagnosis were associated with larger joint space width at the MCP-joints. However, age at diagnosis and duration of remission of acromegaly was not related to joint space. There was a gender difference since in males increased joint spaces were associated with more self-reported pain, which was not the case in females.

This new, semi-automated technique to quantify joint space width[21] to determine joint space narrowing in patients with osteoarthtritis was able to detect quantitative differences in joint space width in acromegaly patients compared with healthy controls. Moreover, within the group of acromegalic patients, this method was sensitive enough to relate joint space width to markers of disease severity of acromegaly. The large number of abnormally wide joint spaces, which we defined as higher than the 95th percentile of controls, may even be underestimated because of the lower age of the controls. Joints with increased joint space width were more frequently painful than joints within the range of normal, especially in male patients. In this patient group, cartilage loss is probably not the main cause of pain but, rather, the reverse, i.e. cartilage hypertrophy, should be recognized as a risk factor for pain. Additional studies are needed to assess which factors determine pain, such as osteophytosis, or instability of joints.

The increased joint spaces of the hand joints in long-term controlled acromegaly patients characterize an aspect of the late manifestations of well-controlled acromegaly and indicate persistent, possibly irreversible, cartilage hypertrophy.

Quality of life (QoL) is decreased in patients with long-term control of acromegaly[22]. In addition, these patients suffer from irreversible osteoarthritis. The aim **Chapter 7** was to assess the impact of joint-specific complaints, clinical and radiological signs of arthropathy on different aspects of QoL in patients with acromegaly after long-term disease control.

QoL was assessed in 58 long-term cured acromegaly patients by four health-related QoL questionnaires (HADS, MFI-20, NHP, SF-36) and one disease specific QoL questionnaire (ACROQOL). The outcomes of these questionnaires were compared with joint-specific self-reported complaints and clinical and radiological osteoarthritis.

The patients reported high pain scores of the spine, knee, and hip which limited physical functioning and psychological well-being. Clinical osteoarthritis of the spine was associated mostly with impaired QoL scores. In addition to impaired physical and social functioning, spinal osteoarthritis was associated with emotional functioning, anxiety, and depression. Remarkably, radiological osteoarthritis was not associated with impaired QoL.

Our findings underscore the great importance of recognition of the complaints, pain management, and coping with pain in this patient group. Multimodality interventions directed towards reduction of pain and improvement of functional capacity and QoL should be developed and evaluated.

Physical symptoms, QoL, and psychological well-being are closely related. We demonstrated a relation between spinal complaints and osteoarthritis on anxiety, depression, and emotional problems. It has been reported that chronic pain of the lower back has clear functional and emotional impact since loss of vitality, persistent discomfort, sleeping difficulties, side effects of drugs, and economic and social concerns weighs heavily and cumulatively on affected individuals[28]. Furthermore, emotional disturbances (such as depression, increased somatic awareness, and anxiety reactions) were reactions to the presence of chronic pain. In addition, radiological osteoarthritis can be present without causing complaints of pain and stiffness, possibly due to an early stage of osteophytosis or relatively preserved joint-spaces.

Considering the impact of clinical osteoarthritis of the spine on Qol, we emphasize the recognition of these complaints and a focus on pain management and coping with pain in this patient group.

Part III. Acromegaly; long-term disease outcome, not joint-related

Despite long-term disease remission or even cured disease, acromegaly patients suffer from a wide variety of co-morbidities. In addition to arthropathy, cardiovascular and metabolic abnormalities[23], hypertension[24;25], type 2 DM, and impaired glucose tolerance have been reported. Furthermore, active acromegaly is associated with increased prevalence of malignancies,

especially of the gastro-intestinal tract[26] and with altered bone mineral density and increased fracture risk. Chapters 7 and 8 focus on other complications of acromegaly, present even after long-term disease control.

The aim of the study described in **Chapter 8** was to establish the prevalence of osteoporosis, vertebral, and non-vertebral fractures in acromegaly patients with long-term controlled disease and factors potentially influencing the risk on fractures. Vertebral and non-vertebral fractures, bone mineral density (BMD), and markers of bone turnover were studied in 89 patients in comparison with data from a sample of the Dutch population.

The prevalence of vertebral fractures was almost 60% and was significantly increased when compared with controls. The prevalence of these fractures was independent of the duration of disease control, BMD, markers of bone turnover, and characteristics of acromegalic disease.

In this study, we demonstrated a very high prevalence of vertebral fractures in acromegalic women, and even more so in men, with long-term controlled disease. There was no relation between the vertebral fractures and BMD. In view of the significant morbidity and mortality associated with vertebral fractures in general and the apparent inability of BMD to predict fracture risk in these acromegalic patients, we propose to include vertebral fracture assessment in the screening of patients with acromegaly, both at diagnosis and during follow-up after establishment of disease control.

GH and IGF-I are important anabolic hormones for bone. In fact, most of the effects of GH are mediated by systemic and/or local IGF-I, which enhances the differentiated function of osteoblasts and bone formation, although GH may also act directly on cortical bone cells[27]. On the other hand, chronic GH and IGF-I excess, such as present in active acromegaly, may impair bone quality, independently of any change in BMD, and apparently increase the risk of fractures, the most important consequence of a decrease in bone quality. Since the clinical appearance of the fractures was asymptomatic, we were unable to discriminate fractures that had occurred at the time of active acromegaly from those which had occurred during the long period of disease control. Irrespective of this issue, these fractures may have significant clinical implications regarding morbidity and mortality, since vertebral fractures are associated with a decreased quality of life, an increased morbidity and mortality, and an increased risk of new (non)vertebral fractures[28-33]. At present, it is unclear what the optimal therapeutic strategy is to

decrease fracture risk in patients with acromegaly.

We conclude that there is a very high prevalence of vertebral fractures in acromegalic patients despite long-term controlled disease, which can cause increased morbidity and mortality.

In acromegaly, overproduction of GH and IGF-I cause abnormal extracellular matrix regulation. One of the pathophysiological changes in patients with Marfan's syndrome, a disease also caused by disturbed matrix regulation, is the development of colonic diverticula. In **Chapter 9** we hypothesize that abnormal matrix regulation in acromegaly may predispose to the development of colonic diverticula. Diverticula, dolichocolon, and colorectal polyps were assessed in 107 patients with cured and/or controlled acromegaly and in 214 age- and sex-matched controls. The findings were related to GH and IGF-I concentrations at the time of diagnosis of acromegaly.

The prevalence of diverticula, dolichocolon, and adenomatous polyps was increased in patients when compared with controls. Diverticula were associated with both GH and IGF-I concentrations at the time of diagnosis of acromegaly, when adjusted for the duration of active disease. The presence of dolichocolon and adenomatous polyps was associated with higher IGF-I concentrations at diagnosis.

Acromegaly is associated with an increased prevalence of colonic diverticula. In addition to the known irreversible effect of GH excess on collagen of joints and cardiac valves, this observation indicates an irreversible effect of GH and/or IGF-I on the collagen of the colon.

Support for a pathophysiological role of alterations in connective tissue characteristics in diverticula are derived from observations in patients with connective tissue diseases like Marfan's and Ehlers-Danlos syndromes, who develop diverticula throughout the colon at a precocious age[34-37]. Diverticula in Marfan's syndrome are caused by an abnormally weak bowel wall, due to a mutation in the fibrillin gene, leading to disturbed connective tissue[35]. This same mechanism also leads to degeneration of cardiac valves and aortic root in these patients[38]. GH is also involved in matrix regulation. GH increases gene expression of the matrix metalloproteinases (MMPs), which are capable of altering the composition of the extracellular matrix[39]. In acromegaly, this altered matrix is thought to be responsible for changes found in heart valves and the aortic root[40], a mechanism known from Marfan's syndrome[38]. A crucial role of GH and IGF-I in the regulation of matrix regulation is further strengthened by the observations that

both cardiac valve pathology and the presence of diverticula are strongly associated with the duration of exposure to abnormal GH and IGF-I concentrations in acromegaly[41].

Therefore, we conclude that there is an irreversible effect of excessive exposure to GH and/or IGF-I in acromegaly on collagen in the colon, resulting in the development of diverticula. Diverticula are another, hitherto unrecognized, feature of acromegaly.

Part IV. Different effects of the exon-3 deleted growth hormone receptor polymorphism

In 2004 Dos Santos *et al.* described an increased growth response in carriers of the d3GHR polymorphism (GHR_{d3} compared with GHR_{wt-wt}) in children born small for gestational age (SGA) and with idiopathic short stature (ISS) in response to rhGH treatment[42]. In addition, they documented increased receptor activity of GHR_{d3} compared GHR_{wt} in vitro[41]. Since 2004, several studies have addressed the possible influence of this common polymorphism of the GHR (d3GHR), present in 35% of the normal population, on the growth response to recombinant human GH (rhGH) treatment in children with different clinical conditions, including GHD[43-48], SGA[42;49-53], ISS[42;54;55], and Turner syndrome[56]. However, the results of these studies are remarkably inconsistent, because 6 reports[45;47;53;55;56] confirmed the findings of Dos Santos *et al.*[42], whereas another 8 reports[43;44;46;48;49;51;52;54] could not demonstrate a significant effect of the exon-3 deleted genotype (GHR_{d3}) on the growth response to rhGH.

In **Chapter 10** we hypothesized that the discrepancies between these studies might be explained, at least in part, by the relatively small numbers of patients included in these studies, precluding sufficient statistical power. Therefore, we conducted a structured meta-analysis on the 15 studies, which assessed the effect of GHR_{d3} on baseline height and the 1st year's growth response to rhGH treatment in pre-pubertal GHD and non-GHD children with short stature.

We demonstrate that the growth response during the first year of rhGH treatment is significantly increased in pre-pubertal short children with the GHR_{d3} genotype in comparison with the GHR_{wt-wt} genotype. Moreover, there is an association between the stimulatory effect of the GHR_{d3} genotype and baseline height in children with GHD. Both findings are in line with the differences in activity between both GH receptor variants *in vitro*[42]. In addition, a lower rhGH dose and a higher age at onset of rhGH treatment were associated with a larger diffe-

rence in growth response between GHR_{wt-wt} and GHR_{d3} genotypes.

This meta-analysis in prepubertal children with short stature indicates that GHR_{d3} is associated with increased baseline height in GHD, but not in non-GHD. Furthermore, GHR_{d3} stimulates growth velocity by an additional effect of ~0.5 cm during the first year of rhGH treatment and this effect is more pronounced at lower doses of rhGH and higher age.

The problem of insufficient statistical power in the individual studies is illustrated by the fact that the pharmacogenetic effects are largely consistent among the different studies, even though these effects were small and not always statistically significant. The GHR_{d3} genotype is not expected to be associated with physiological variations in human growth or to be a primary cause of short-stature in humans, since potential variations in GH sensitivity due to genotypic differences in GHR activity can be compensated by alterations in endogenous pituitary GH secretion, which might mask the effect of the GHR polymorphism on spontaneous growth. We hypothesized that in patients with GHD this compensatory effect within the GH-IGF-I axis did not function properly and, as we expected, the GHR_{d3} genotype was associated with increased baseline height in GHD patients. As expected, this genotypic effect was not demonstrated on baseline height in non-GHD children

In conclusion, the GHR_{d3} is associated with increased baseline height in GHD, but not in non-GHD children. Furthermore, GHR_{d3} stimulates growth velocity by an additional effect of ~0.5 cm during the first year of rhGH treatment.

In analogy with Chapter 10, we evaluated the impact of the genomic deletion of exon 3 of the growth hormone receptor on long-term clinical outcome of acromegaly in a well-characterized cohort of patients with long-term remission of acromegaly in **Chapter 11**.

The genetic distribution of the d3GHR was assessed in 86 acromegalic patients in long-term disease control and related to anthropometric parameters, cardiovascular risk factors, osteoarthritis, bone mineral density, colonic polyps and diverticula, and dolichocolon.

The d3GHR polymorphism is associated with more severe complications of acromegaly, reflected in an increased prevalence of osteoarthritis, colonic polyps, and dolichocolon. Other co-morbidities such as metabolic syndrome, type 2 DM, changes in BMD, or (non)vertebral fractures are unaffected by the d3GHR polymorphism. Apparently, the ultimate impact of the d3GHR polymorphism on long-term complications of acromegaly is evident only on the irreversible effects of previous GH excess.

It has frequently been assumed that the prevalence of colonic polyps and dolichocolon in acromegaly was correlated to IGF-I concentrations[57-60]. A more active signal transduction of GH in GHR_{d3} patients is concordant with the finding of an increased prevalence of both adenomatous polyps and dolichocolon. Acromegaly is associated with increased BMD in combination with a high prevalence of vertebral fractures, especially with coincident hypogonadism[61]. Up till this study, a relation between pre-treatment GH or IGF-I concentrations and these co-morbidities had not been reported, which is consistent with the lack of difference between the d3GHR genotypes[27;62]. This study shows that co-morbidities including joint-complaints, osteoarthritis, hypertension, and colonic abnormalities are highly prevalent in patients with acromegaly despite long-term cured disease[20;63-65]. The effect of the d3GHR polymorphism had no effect on DM. This is explained by the fact that type 2 DM is frequently observed in active disease, but is potentially reversible since disturbances in glucose metabolism normalize after cure and the prevalence of type 2 DM is not increased in patients with (long-term) disease remission[63-66].

In conclusion, the d3GHR polymorphism is associated with more severe outcome of acromegaly for the irreversible complications of GH excess, but not for the reversible complications.

Since the first publication on enhanced growth in children with the GHR_{d3} genotype in 2004, several studies were performed evaluating the effect of this polymorphism in various clinical conditions. We systematically reviewed these studies in **Chapter 12**, in order to establish the functional consequences of the exon 3 deleted GHR polymorphism.

Discrepancies exist between different studies on the effect of the d3GHR on the GH-IGF-I axis in GHD and acromegaly, and no evident effect of the GHR_{d3} genotype on the GH and/or IGF-I concentrations could be established evaluating these studies[43;46;67]. The presence of d3GHR had a small effect on clinical outcome; including increased growth velocity in (non)-GHD children treated with rhGH treatment, and increased basal height in GHD children. In acromegaly, the d3GHR was associated with a worse outcome of irreversible complications of acromegaly (more osteoarthritis, dolichocolon, and adenomatous polyps). In addition, the polymorphism was associated with a better treatment response to somatostatin analogs or Pegvisomant. The effects of the d3GHR on the response to rhGH treatment in GHD adults were less evident. However, it is more complicated to establish clear genotype-phenotype

relationships in adults, since the treatment endpoints of rhGH in adults are less clear than in children with GHD treated with rhGH.

The GHR_{d3} genotype seems to affect metabolism. In patients with DM type I and II and with IGT the frequency of the GHR_{d3} genotype is significantly lower than in the normal population. In addition, in a cohort of healthy Caucasian children and adolescents higher insulin secretion was demonstrated in the presence of the d3GHR[68]. These findings suggest that increased bioactivity associated with this allele confers a protection function against type II DM due to increased IGF-I levels as a result of increased GH sensitivity that promotes glucose uptake and decreased insulin sensitivity[68].

The $GHR_{d3\text{-}d3}$ isoform is more frequently present in patients suffering from starvation, indicating that both the elevated GH gene expression and increased GHR-mediated GH responsiveness may constitute adaptive responses to the effects of persistent malnutrition, since increased circulating GH appears to form part of a physiological response to nutrition deprivation. The prevalence of the GHR_{d3} isoform in well-fed populations is currently unknown. However, in patients with type II DM and IGT, the GHR_{d3} isoform is associated with more severe obesity than in patients with the other isoforms. Additional research on this interesting topic is required.

We conclude that the effect of the d3GHR polymorphism is not limited to subtle variations in growth parameters in (non)-GHD children with short stature treated with rhGH, but the effect affects many pathophysiological processes in which the GH-IGF-I axis is involved to some extent, despite the fact that the effect of the d3GHR on the GH-IGF-I axis itself is not yet completely elicidated. From a clinical perspective, the effects of the presence of the d3GHR are so small that it does not affect diagnostic or therapeutic approaches.

Part V. General discussion and concluding remarks

Acromegaly is a slowly progressive disease of exaggerated somatic growth and distorted proportion arising from hypersecretion of GH and IGF-I. The active phase of the disease causes enormous morbidity, including phenotypic changes, arthropathy, cardiovascular and pulmonary complications, and malignancies. Nowadays treatment options have been well established and almost all patients reach a state of controlled or cured disease. However, the studies described in this thesis document that there are severe long-term consequences of acromegaly, despite accu-

rate disease cure or control, which are, to a more or lesser extent, immobilizing for the patients.

Athropathy was the most reported long-term consequence by the patients and showed to have great impact on QoL. Arthropathy was the consequence of severe osteoarthritis at multiple joint-sites. Osteoarthritis was equally prevalent in males and females. The extend and severity of osteoarthritis was related to the plasma levels and duration of GH and IGF-I excess during the active phase of the disease. The characteristics of the osteoarthritis were very typical and consisted of severe osteophytosis in the absence of joint space narrowing, which contrasts with joint space narrowing in primary osteoarthritis. Joint space widening was already known to be present in the active phase of acromegalic disease, but we described that despite long-term disease control and severe osteophytosis, this joint space widening persists, reflecting persistent cartilage hypertrophy.

Colonic abnormalities, including diverticula, adenomatous polyps, and dolichocolon were highly prevalent in acromegaly patients and associated with the duration and amount of GH and IGF-I excess.

The prevalence of vertebral fractures was very high en increased, compared with controls. Vertebral fractures were not associated with any acromegalic disease characteristic and were independent of BMD and gonadal status.

Finally, we meta-analyzed differences in growth response to rhGH treatment in GHD and non-GHD short children. This variation is growth response was partly explained by a polymorphism in the d3GHR. In addition, we investigated the effect of this polymorphism on the long-term consequences of acromegaly. This polymorphism of the d3GHR was associated with more severe complications of acromegaly, reflected in an increased prevalence of irreversible complications of the disease, such as osteoarthritis, colonic polyps, and dolichocolon. The reversible complications, i.e. DM type II, metabolic syndrome and changes in BMD, were not affected by this polymorphism.

In view of the significant morbidity in acromegaly patients, both at diagnosis and during long-term follow-up after establishment of disease control, we propose that screening for osteoarthritis, and vertebral fractures should be included in the follow-up of acromegalic patients. In addition, clinicians should be aware of the increased prevalence of diverticula in the colon, which may be associated with increased complications and complaints.

It is essential to recognize these long-term consequences in order to establish appropriate follow-up and (multidisciplinary) care in these patients to limit the persisting complex

morbidity. Both doctors and patients should be aware of these persisting consequences to prevent inappropriate expectations with respect to the long-term results of treatment.

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Chapter 14.

NEDERLANDSE SAMENVATTING

Dit proefschrift beschrijft onderzoek naar de lange-termijn gevolgen van langdurig gecureerde of biochemisch gecontroleerde acromegalie. De studies beschreven in dit proefschrift richten zich op gewrichtsklachten, botdichtheid, vertebrale fracturen, darmafwijkingen en kwaliteit van leven. Daarnaast hebben wij ons gericht het verschil in genotype-fenotype relaties welke tot expressie kwamen bij de verschillende varianten van het groeihormoon receptor polymorfisme (d3GHR). Om het effect van het d3GHR polymorfisme op de lange-termijn gevolgen van acromegalie te begrijpen hebben wij een meta-analyse gedaan naar de effecten van dit polymorfisme op spontane en gestimuleerde groei bij al dan en niet GH deficiënte kinderen.

Alle patiënten die meededen aan deze studies waren langdurig genezen van hun acromegalie. Daarnaast werden al deze patiënten langdurig gevolgd in het Leids Universitair Medisch Centrum.

Acromegalie kan goed behandeld worden met (een combinatie van) chirurgie, radiotherapie en/of medicijnen. Dit resulteert in de meeste gevallen tot stabiele of genezen ziekte, kortweg 'genezen ziekte'[1;2]. Desondanks ervaart een groot deel van de patiënten een sterk verminderde kwaliteit van leven[3]. Dit proefschrift richt zich op de verschillende klinische aspecten van de ziekte na langdurige genezing.

Deel I van het proefschrift richt zich op gewrichtsproblemen. Acromegalie patiënten met langdurig genezen ziekte geven aan veel last te hebben van gewrichtspijnen[4]. Omdat de exacte prevalentie en de oorzaak van deze gewrichtspijnen onbekend was bij deze patiënten, hebben we dit in **Hoofdstuk 2** onderzocht. Door middel van vragenlijsten, klinisch en radiologisch onderzoek zijn de gewrichten in kaart gebracht bij 89 patiënten met langdurig genezen acromegalie.

Bij ongeveer 80% van de patiënten was artrose aanwezig in minimaal 2 grote gewrichten, met als voornaamste lokalisatie de wervelkolom, hand, knie en heup. Artrose in acromegalie werd gekenmerkt door veel en ernstige osteofyten in combinatie met wijde gewrichtsspleten. Dit laatste is een verschijnsel reeds bekend uit de periode van actieve acromegalie, het is echter nooit eerder beschreven bij langdurig genezen patiënten[1-6].

Deze studie toont aan dat gewrichtsspleet verbreding, als uiting van verdikt kraak-

been, zoals bekend van actieve acromegalie[5-9], blijft bestaan ondanks langdurig genezen ziekte. Daarnaast geeft de blootstelling aan langdurig hoge concentraties GH en IGF-I irreversibele schade aan de gewrichten van patiënten met genezen acromegalie, dit uit zich onder andere in de vorm van osteofyten, een vorm van secundaire artrose.

Er bestaat veel onduidelijkheid over de mate van invloed van hoge concentraties GH en IGF-I op het ontstaan van gewrichtspijn[4]. Gezien de in Hoofdstuk 2 bewezen hoge prevalentie van artrose bij genezen acromegalie patiënten is de studie beschreven in **Hoofdstuk 3** gericht op de identificatie van voorspellende, acromegalie specifieke, factoren van invloed op het ontstaan van artrose.

Bij 67 patiënten met genezen acromegalie werden ziektespecifieke kenmerken als hoogte van het GH en IGF-I ten tijde van de diagnose, de ziekte duur, leeftijd bij krijgen van de ziekte en remissieduur gecorreleerd aan de gegevens verkregen door vragenlijsten, klinisch en radiologisch gewrichtsonderzoek van de rug, hand, knie en heup.

Onze resultaten lieten zien dat het risico op het ontwikkelen van artrose en de uitgebreidheid van de artrose wordt bepaald door de hoogte van het IGF-I ten tijde van de diagnose van de acromegalie. Andere ziektespecifieke voorspellers op het ontstaan van artrose werden niet gevonden.

Artrose komt in de algemene populatie meer voor bij (post-menopauzale) vrouwen dan bij mannen[10]. Hoewel het niet helemaal duidelijk is hoe dit precies komt, wordt er een rol toebedeeld aan de beschermende werking van oestrogenen, welke wegvalt na de menopauze[10]. Acromegalie is een ziekte met een gelijke prevalentie onder vrouwen en mannen[2;11;12]. In **Hoofdstuk 4** onderzoeken wij of het vrouwelijke geslacht ook een risicofactor is voor artrose in acromegalie.

Gegevens verkregen uit vragenlijsten, klinisch en radiologisch gewrichtsonderzoek van de hand, heup en knie werden vergeleken tussen 46 mannen en 43 vrouwen met langdurig genezen acromegalie. Daarnaast werden deze gegevens vergeleken met geslachtsspecifieke gegevens van twee gezonde controle populaties[12].

Er was geen geslachtsverschil in de prevalentie van gewrichtsklachten en radiologische artrose van de hand, heup en knie. Klinische artrose was bij deze patiënten gelijk verdeeld voor de heup en knie, alhoewel meer vrouwen hadden last van handartrose dan mannen. Vergeleken

met de controle populaties was de prevalentie van artrose verhoogd bij vrouwen met acromegalie, en dit was zelfs nog evidenter voor mannelijke patiënten.

Uit deze bevindingen concluderen wij dat de overproductie van GH en IGF-I een potentiële beschermende rol van oestrogenen op artrose teniet doet.

Het karakteristieke kenmerk van radiologische artrose bij acromegalie, beschreven in hoofdstuk 1, bestaat uit veel osteofyten met relatief gespaarde gewrichtsspleten[14]. In **Hoofdstuk 5** hebben we de radiologie gegevens van onze acromegalie patiënten vergeleken met die van een cohort patiënten met primaire gegeneraliseerde artrose[15], dit om inzicht te krijgen in het pathofysiologisch proces dat ten grondslag ligt aan GH/IGF-I gemediëerde artrose.

De kenmerken van radiologische artrose van de heup en knie werden vergeleken tussen 84 acromegalie patiënten met secundaire artrose en 189 controles met gegeneraliseerde primaire artrose.

Het fenotypische kenmerk van artrose tussen deze 2 populaties verschilde aanzienlijk. Artrose werd bij acromegalie voornamelijk gekenmerkt door osteofyten zonder gewrichtsspleet vernauwing, iets dat niet of nauwelijks voorkwam bij primaire artrose. Opvallend was dat acromegalie patiënten veel minder klachten aangaven bij een zelfde uitgebreidheid van de osteofytose vergeleken met controles met primaire artrose.

Artrose ten gevolge van excessieve GH productie resulteert in osteofytose en nauwelijks tot gewrichtsspleet vernauwing. Het GH-IGF-I systeem is betrokken bij de botaanmaak dat resulteert in osteofytose[16]. Dit endocriene systeem lijkt tegelijkertijd te beschermen tegen gewrichtsspleet vernauwing.

Uit de hoofdstukken 2-5 blijkt dat gewrichtsklachten bij acromegalie patiënten een zeer invaliderend effect hebben[14]. Deze gewrichtsklachten hebben sommige kenmerken van primaire artrose, zoals osteofyten. Echter, een ander belangrijk kenmerk van primaire artrose: gewrichtsspleet vernauwing, wordt maar nauwelijks gezien bij acromegalie patiënten.

In **Hoofdstuk 6** brengen wij met de zogenaamde 'automated image analysis' methode de gewrichtspleten van de hand in kaart bij 89 langdurig genezen acromegalie patiënten en 471 gezonde controles, met een 3-ledig doel: 1) het vergelijken van de gewrichtsspleten tussen acromegalie patiënten en controles, 2) het identificeren van (ziektespecifieke) factoren die geassocieerd zijn met de wijdte van de gewrichtsspleten en 3) het beoordelen van de relatie tussen de

wijdte van de gewrichtsspleten en de gewrichtspijn.

Acromegalie patiënten hadden ongeveer 25% wijdere gewrichtsspleten dan controles, vooral in de MCP, PIP en DIP gewrichten. Meer dan 60% van de patiënten hadden gewrichtsspleten die wijder waren dan het 95e percentiel bij controles en maar 10% van de patiënten hadden een wijdte van de gewrichtsspleten, die zich onder het 50e percentiel van de controles bevond. De concentraties van GH en IGF-I ten tijde van de actieve acromegalie waren geassocieerd met wijdere gewrichtsspleten van de MCP gewrichten. Leeftijd ten tijde van diagnose of duur van de remissie was niet geassocieerd met de wijdte van de gewrichtsspleet. Bij mannelijke patiënten waren wijdere gewrichtsspleten meer geassocieerd met pijn dan bij vrouwelijke patiënten.

De wijde gewrichtsspleten bij langdurig genezen acromegalie patiënten zijn een uiting van irreversibele kraakbeenhypertrofie, ontstaan tijdens de actieve fase van de ziekte. Deze kraakbeen hypertrofie persisteert, ondanks langdurige biochemische controle van de acromegalie.

Acromegalie patiënten met langdurig genezen ziekte worden gekenmerkt door een verminderde kwaliteit van leven[17]. Daarnaast leiden deze patiënten aan irreversibele artrose, zoals in voorgaande hoofdstukken beschreven[14;18]. Het doel van **Hoofdstuk 7** was het in kaart brengen van de impact van gewrichtsspecifieke klachten, klinische en radiologische artrose op de verschillende aspecten van kwaliteit van leven.

Kwaliteit van leven werd geanalyseerd bij 58 patiënten met langdurig genezen acromegalie door middel van 4 gezondheid gerichte vragenlijsten (HADS, MFI-20, NHP en SF-36) en 1 ziekte specifieke vragenlijst (ACROQOL). De uitkomst van de vragenlijsten werd gecorreleerd aan gegevens verkregen via vragenlijst, klinisch en radiologisch gewrichtsonderzoek.

Gerapporteerde klachten van de rug, heup en knie hadden grote impact op de kwaliteit van leven. Klinische artrose van de rug had het grootste effect op de kwaliteit van leven, niet alleen op de fysieke en sociale subschalen, maar ook de psychologische subschalen werden sterk beïnvloedt. Radiologische artrose was niet gecorreleerd met kwaliteit van leven.

Bij patiënten met acromegalie is het ondanks de langdurige genezing van de ziekte van groot belang aandacht te besteden aan gerapporteerde pijnklachten, gezien de grote nadelige invloed hiervan op kwaliteit van leven. Daarnaast zal een multidisciplinaire aanpak van deze problematiek van grote waarde kunnen zijn in de follow-up van genezen acromegalie patiënten.

Deel II van dit proefschrift richt zich op lange-termijn gevolgen van genezen acromegalie, anders dan gewrichtsproblemen. Als co-morbiditeit van acromegalie wordt naast gewrichtspijn gerapporteerd over cardiovasculaire problematiek[19], metabole veranderingen, hypertensie en diabetes mellitus type II[20;21]. Daarnaast is actieve acromegalie geassocieerd met een verhoogde kans op maligniteit, vooral van het maag-darm systeem[22], met veranderingen van de botdichtheid en een verhoogde kans op (vertebrale) fracturen[23].

Recent is beschreven dat de overproductie van GH en IGF-I bij acromegalie leidt tot een abnormale regulatie van de extracellulaire matrix[24]. Eén van de pathofysiologische veranderingen bij Marfan's syndroom, een ziekte eveneens gekenmerkt door een verstoorde matrix regulatie, is de ontwikkeling van divertikels van het colon[25-28]. In **Hoofdstuk 8** stellen wij dat de abnormale matrix regulatie bij acromegalie patiënten leidt tot de ontwikkeling van divertikels van het colon.

Van 107 acromegalie patiënten werden restrospectief coloscopie verslagen bestudeerd en er werd gescoord op de aanwezigheid van divertikels, (adenomateuze) poliepen en dolichocolon. De bevindingen werden vergeleken met 214 controles van vergelijkbare leeftijd en geslacht. Bij patiënten werden de bevindingen gecorreleerd aan de hoogte van het GH en IGF-I ten tijde van de diagnose van de ziekte en aan de duur van de actieve ziekte.

De prevalentie van divertikels, poliepen en dolichocolon was verhoogd in patiënten vergeleken met controles. De prevalentie van divertikels was gerelateerd aan de hoogte van zowel het GH als het IGF-I ten tijde van de diagnose, indien gecorrigeerd werd voor de duur van de actieve ziekte. De prevalentie van dolichocolon en adenomateuze poliepen was gerelateerd aan de IGF-I concentratie ten tijde van diagnose van de acromegalie.

Concluderend is dit de eerste studie die een verhoogde prevalentie van colon divertikels bij acromegalie patienten beschrijft. We kunnen derhalve aan de bekende irreversibele effecten van GH en IGF-I op de gewrichten[18] en hartkleppen[24], nu het effect van GH en IGF-I op het collageen van het colon toevoegen.

GH en IGF-I hebben een anabole werking op bot en stimuleren voornamelijk de botaanmaak[29]. Deze anabole effecten blijven bestaan na de korte termijn (<10 jaar) genezing van acromegalie[30-33]. In **Hoofdstuk 9** hebben wij de prevalentie van osteoporose en (vertebrale) fracturen onderzocht bij 89 patiënten met gemiddeld 17 jaar genezen acromegalie. Tevens hebben wij onze bevindingen vergeleken met een gezonde controle populatie en getracht acromegalie

specifieke factoren te correleren aan de vertebrale fracturen.

De prevalentie van vertebrale fracturen bij patiënten met langdurig genezen acromegalie was bijna 60% en sterk verhoogd vergeleken met controles van dezelfde leeftijd en geslacht. Het voorkomen van vertebrale fracturen waren onafhankelijk van de duur van de remissie, botdichtheid, botstofwisseling, GH, IGF-I en gonadale status. De prevalentie was osteoporose was erg laag, zelfs na langdurige remissie van de acromegalie.

Bij patiënten met langdurig genezen acromegalie is de botdichtheid niet van invloed op het ontstaan van vertebrale fracturen. Gezien de morbiditeit en mortaliteit geassocieerd met vertebrale fracturen in de normale populatie raden wij aan de screening op vertebrale fracturen te implementeren in de follow-up van acromegalie.

Deel III van dit proefschrift richt zich op het groeihormoon receptor polymorfisme, de deletie van exon 3 (d3GHR). In 2004 werd in een cohort niet-GH deficiënte kleine kinderen voor het eerst beschreven dat dragers van het d3GHR polymorfisme een verhoogde groeirespons toonden in reactie op behandeling met exogeen GH[34]. In de jaren hierop volgend hebben verschillende groepen geprobeerd deze bevindingen te repliceren in diverse populaties[34-42], met tegenstrijdige resultaten.

In **Hoofstuk 10** beschrijven we een meta-analyse van de verschillende studies die sedert 2004 zijn gedaan naar de farmacogenetica van rhGH bij d3GHR, daar er veel tegenstrijdige studies zijn gepubliceerd sedert 2004. Onze hypothese was dat het verschil in uitkomst tussen de diverse studies ten minste deels verklaard kon worden door het relatief kleine aantal patiënten bestudeerd in sommige studies, met andere woorden, door een lage statistische power.

Wij hebben een meta-analyse verricht van 15 studies die het effect van het d3GHR polymorfisme op spontane groei en 1e-jaars groei na stimulatie met exogeen GH, in prepuberale kinderen met en zonder GH deficiëntie, gerapporteerd hebben.

Uit deze meta-analyse kunnen wij concluderen dat het d3GHR polymorfisme is geassocieerd met verhoogde spontane groei in GH deficiënte kinderen, maar niet in kleine kinderen zonder GH deficiëntie. Daarnaast zorgt dit polymorfisme bij kleine kinderen behandeld met exogeen GH voor een extra groeisnelheid van ongeveer 0.5 cm tijdens het eerste jaar van de behandeling.

In navolging op hoofdstuk 10 en 11 hebben wij in **Hoofdstuk 11** het effect van het d3GHR

polymorfisme op de lange termijn uitkomst van acromegalie onderzocht.

De genetische distributie van de d3GHR was bepaald in 86 patiënten met genezen acromegalie. De bevindingen werden gerelateerd aan gegevens over lichaamssamenstelling, cardiovasculaire risicofactoren, artrose, botdichtheid, vertebrale fracturen en darmafwijkingen.

Het d3GHR polymorfisme is geassocieerd met ernstigere lange termijn complicaties van acromegalie, zoals een hogere prevalentie van artrose, adenomateuze poliepen en dolichocolon. Andere co-morbiditeiten van acromegalie, zoals het metabool syndroom, type 2 diabetes mellitus en vertebrale fracturen waren niet geassocieerd met het d3GHR polymorfisme.

Het d3GHR polymorfisme is geassocieerd met een ernstigere uitkomst van acromegalie, maar dit is alleen aantoonbaar wanneer het irreversibele effecten van het eerdere GH overschot betreft.

Sinds de publicatie van Dos Santos *et al*. in 2004 over een stimulerend effect van het d3GHR polymorfisme op groei bij kleine kinderen behandeld met rhGH therapie, is een flink aantal andere onderzoekers in hun voetspoor getreden, echter in verschillende populaties en met wisselende resultaten. In **Hoofdstuk 12** doen wij een poging om op een systematische manier al deze studies de revue te laten passeren, om op deze manier het effect van dit polymorfisme goed in kaart te kunnen brengen. Wij richten ons op het effect van het d3GHR polymorfisme op de GH-IGF-I as, groei bij (te) kleine kinderen, klinische gevolgen van ziektes van de GH-IGF-I as, metabolisme, cardiovasculaire ziekten en overleving.

Er bestaat geen duidelijk effect van het d3GHR polymorfisme op de GH-IGF-I as. De verschillende studies in acromegalie, GHD bij volwassenen en GHD bij kinderen zijn erg heterogeen in opzet, populatie grootte en statistische analyses[43-45]. Ondanks het feit dat er geen duidelijk effect van het d3GHR polymorfisme op de GH-IGF-I as kan worden aangetoond zijn er wel degelijk (kleine) effecten te zien van dit polymorfisme op de kliniek. Bij (te) kleine kinderen met en zonder GHD met het GHR_{d3} genotype was de groeisnelheid als reactie op rhGH therapie verhoogd met ongeveer 0.5 cm/jaar. Patiënten met acromegalie en het GHR_{d3} genotype hadden een hogere prevalentie van de irreversibele co-morbiditeit van de ziekte, zoals artrose, dolichocolon en adenomateuze darmpoliepen. Het GHR_{d3} genotype had een gunstig effect op het glucose metabolisme, lichaamsgewicht en de respons op medicamenteuze behandeling van acromegalie.

Het GHR_{d3} genotype lijkt ook een effect te hebben op het metabolisme. De fre-

quentie van het GHR_{d3} genotype is significant lager bij patiënten met type 1 en 2 DM en met glucose intolerantie vergeleken met normale controles. Daarnaast is bij een cohort gezonde blanke kinderen en adolescenten met het GHR_{d3} genotype een hogere insuline secretie vastgesteld [46]. Deze resultaten duiden erop dat het d3GHR polymorfisme beschermt tegen DM. Het pathofysiologische concept hierachter is dat de verhoogde GH gevoeligheid hogere IGF-I concentraties geeft, welke een gunstig effect hebben op de glucose opname en insuline gevoeligheid[46]. Het GHR_{d3} genotype is in een hogere frequentie aanwezig bij mensen in gebieden waar chronisch voedseltekort is. Het idee is dat de verhoogde GH responsiviteit bijdraagt aan het adaptieve proces dat bestaat bij persisterende voedseldeprivatie. Er lijkt geen effect te zijn van het d3GHR polymorfisme op cardiovasculaire risico factoren, alhoewel er maar een beperkt aantal studies onderzoek naar dit effect hebben gedaan.

De effecten van het d3GHR polymorfisme blijven niet beperkt tot subtiele variaties in groei parameters bij al dan niet GHD kinderen met korte lengte behandeld met rhGH therapie, maar hebben in zekere mate effect op vele processen waarbij de GH-IGF-I as is betrokken. Vanuit een klinisch perspectief zijn de effecten van het GHR_{d3} zo beperkt, dat het niet van belang is voor diagnostische en therapeutische beslissingen.

Concluderende opmerkingen

Dit proefschrift beschrijft de lange termijn gevolgen van een eerder overschot aan GH en IGF-I bij patiënten gemiddeld 17 jaar genezen van acromegalie. Gezien de aanzienlijke morbiditeit van deze patiënten, zowel ten tijde van diagnose als ook na genezing van de ziekte, stellen wij voor om deze patiënten te screenen op artrose en vertebrale fracturen. Het is van uitermate groot belang om de lange termijn gevolgen van de ziekte te onderkennen, om op die manier adequate follow-up en goede (multidisciplinaire) zorg te kunnen bieden. Het doel hiervan is de persisterende complexe morbiditeit zo veel mogelijk te beperken en mogelijk een verdere achteruitgang van kwaliteit van leven te voorkomen. Zowel de behandelend arts als de patiënt moet zich bewust zijn van de lange termijn gevolgen van acromegalie, om op die manier irreële verwachtingen met betrekking tot herstel van bepaalde verschijnselen te voorkomen.

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CURRICULUM VITAE

Monica Johanna Elisabeth Wassenaar werd geboren op 24 december 1980 te Rotterdam. Als plattelandsmeisje groeide zij op in Strijen en volgde de middelbare school 'C.S.G. Willem van Oranje' te Oud-Beijerland waar zij in 1999 haar VWO diploma behaalde. Vanaf de 3e klas van de middelbare school wist zij reeds dat zij dokter wilde worden. Helaas werd zij tot twee maal toe uitgeloot. Die tijd werd ingevuld met een studie Klinische Neuropsychologie aan de Vrije Universiteit te Amsterdam, werk als fotomodel en het bewandelen van vele catwalks als mannequin. Om haar oorspronkelijke missie in het leven te volgen en haar bestaan iets meer diepgang te geven besloot zij in 2002 nog één maal mee te loten voor de opleiding Geneeskunde. Omdat drie maal scheepsrecht is werd zij ingeloot en geplaatst aan de Universiteit Leiden. In mei 2007 behaalde zij haar arts-examen. Vanaf juli 2007 was zij verbonden als arts-onderzoeker aan de afdeling Endocrinologie en Metabolisme van het Leids Universitair Medisch Centrum, onder leiding van Prof. Dr. J.A. Romijn, dr. A.M. Pereira, dr. N.R. Biermasz en in samenwerking met dr. M. Kloppenburg (reumatologie) en dr. H.M. Kroon (radiologie). Na een geslaagde zoektocht naar het meest uitdagende specialisme, heeft zij per begin april 2010 haar draai gevonden als arts-assistent psychiatrie bij Mentrum Kliniek 1e Constantijn Huygensstraat, te Amsterdam.

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