# Acromegaly : irreversible clinical consequences

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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 50$^{th}$ 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 | d3-WT | P-value |
|---|---|---|---|
| | n=51 | n=35 | |
| **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 50$^{th}$ 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.

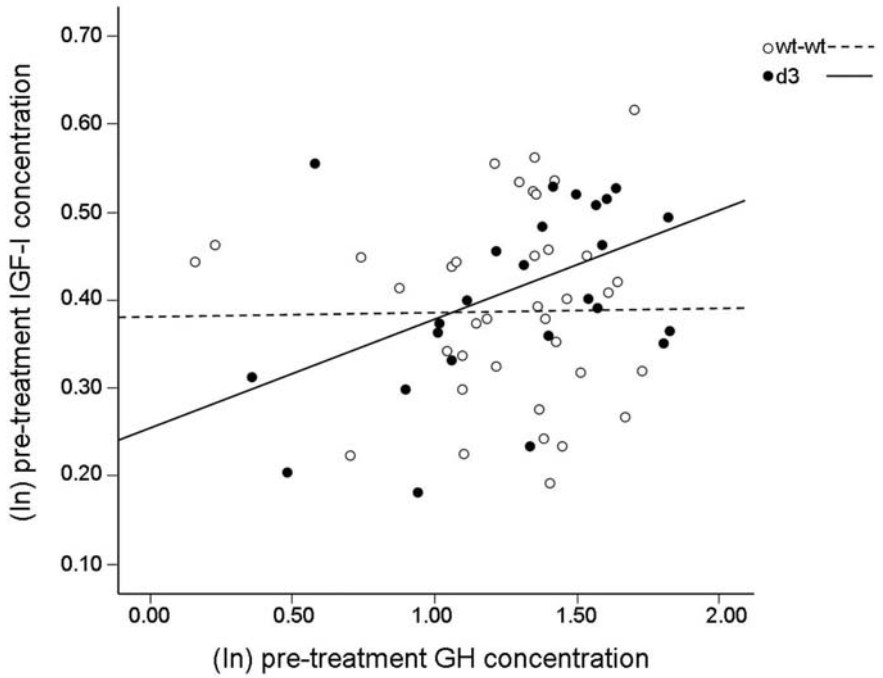


**Figure 1.** The relation between (ln) pre-treatment GH and IGF-I concentrations for GHR wt-wt and GHR d3. GHR wt-wt patients: unstandardized beta (B) 0.01, 95% CI -0.13-0.16 (p = 0.32) *vs.* GHR d3 patients B 0.28, 95% CI 0.19-0.37 (p = 0.01). The absolute arithmetic means for pre-treatment GH and 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<br>n=51 | d3<br>n=35 | p-value |
|---|---|---|---|
| **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, 1$^{st}$ CMC joints, and 1$^{st}$ IP joints). However, in the subgroup of patients with high pre-treatment GH concentrations (>50$^{th}$ 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.

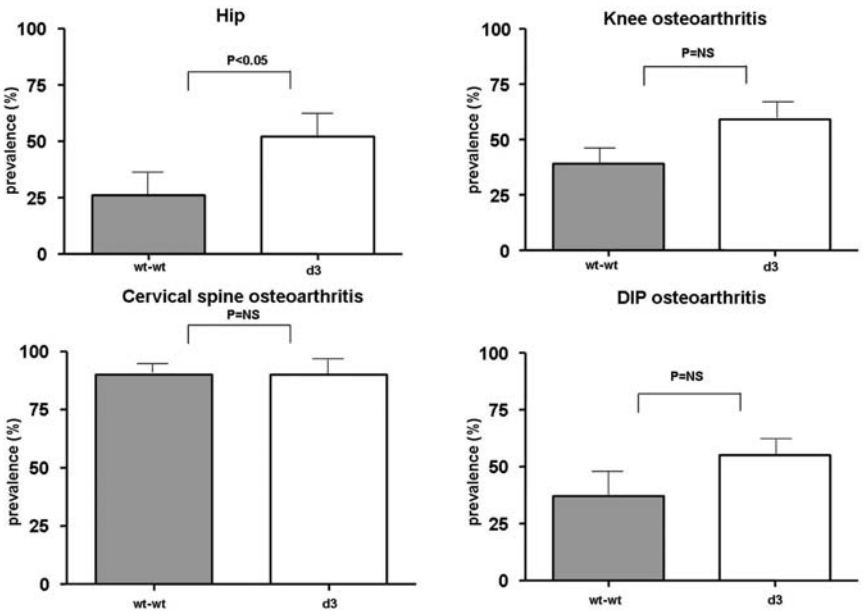


**Figure 2.** The prevalence of radiographic osteoarthritis of the hip, knee, cervical spine and, DIP joints of the hand for GHR wt-wt and GHR d3 in acromegalic patients with long-term cured disease. The same tendency was seen for the lumbar spine as for the cervical spine. Grey bars denote wt-wt (n=51) 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<br>n=51 | d3<br>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).

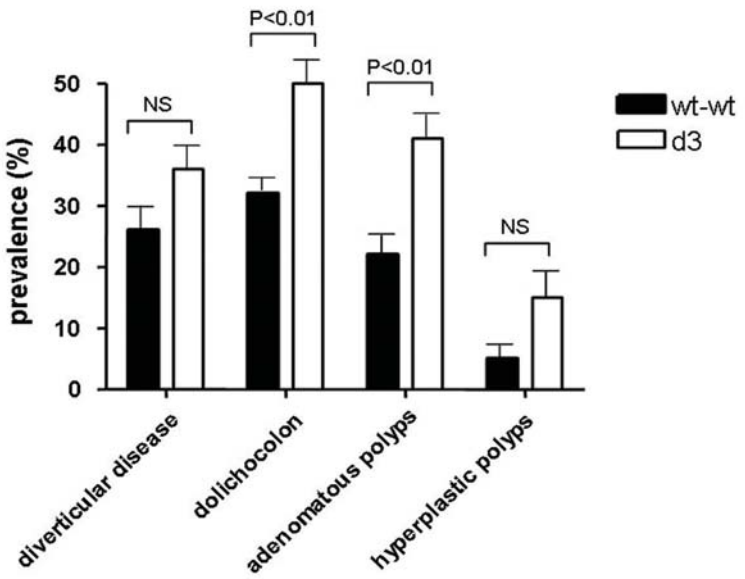


**Figure 3.** The prevalence (%) of colonic diverticular disease, dolichocolon, and colonic polyps in long-term cured acromegaly for the different d3GHR genotypes. Data were analyzed by binary logistic regression, with adjustments for age, gender, BMI, duration of active disease, pre-treatment levels 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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