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Disorders of IGFs and IGF-1R signaling pathways

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ABSTRACT

The insulin-like growth factor (IGF) system comprises two ligands, IGF-I and IGF-II, that regulate multiple physiological processes, including mammalian development, metabolism and growth, through the type 1 IGF receptor (IGF-1R). The growth hormone (GH)-IGF-I axis is the major regulator of longitudinal growth. IGF-II is expressed in many tissues, notably the placenta, to regulate human pre- and post-natal growth and development. This review provides a brief introduction to the IGF system and summarizes findings from reports arising from recent larger genomic sequencing studies of human genetic mutations in *IGF1* and *IGF2* and genes of proteins regulating IGF action, namely the IGF-1R, IGF-1R signaling pathway components and the IGF binding proteins (IGFBPs). A perspective on the effect of homozygous mutations on structure and function of the IGFs and IGF-1R is also given and this is related to the effects on growth.

1. Introduction

1.1. The system: regulators of IGFs, IGFBPs, IGF-1R, post receptor signaling

IGF-I and IGF-II act via the type 1 IGF receptor (IGF-1R), which is expressed ubiquitously both pre- and postnatally. The IGF-1R has a similar structure to the insulin receptor and is a dimeric (heterotetrameric) transmembrane glycoprotein encoded by *IGF1R* (De Meyts, 2015). The extracellular region of the mature protein is involved in ligand binding and comprises the α subunit and elements of the β subunit, whereas the intracellular region encompasses the tyrosine kinase domain within the β subunit (Adams et al., 2000; De Meyts, 2015). At physiological ligand concentrations the IGF-1R binds a single ligand. Upon ligand binding the IGF-1R undergoes significant structural rearrangement, with IGF contacting the IGF-1R at two separate sites within an IGF-1R binding pocket; i) the high affinity binding site 1 comprising residues from the so-called L1 and α CT domains and ii) a low affinity binding site 2 involving the FnIII-1 domain (Fig. 1a) (Ward et al., 2008).

Activation of the receptor upon IGF binding is regulated by a family of six high affinity IGF binding proteins (IGFBPs 1–6) (Bach, 2018; Forbes et al., 2012). Binding to IGFBPs precludes IGF interaction with the IGF-1R. IGFBPs act to transport IGFs in the circulation and to deliver IGFs to target tissues. The majority of IGFs are found in the circulation in a trimeric complex with IGFBP-3 or IGFBP-5 and the acid labile subunit

(ALS) (Boisclair et al., 2001). This complex prolongs the circulating half-life of IGFs (Guler et al., 1989). All IGFBPs bind both IGF-I and IGF-II with similar affinities, except IGFBP-6 which is essentially IGF-II specific (Bach, 2016). The tissue specific expression of the different IGFBPs is believed to provide tissue specificity to IGF action. The bioavailability of IGFs is further regulated by binding of IGFBPs to the extracellular matrix and by cleavage by extracellular IGFBP proteases (Bach, 2018; Forbes et al., 2012). Both of these processes result in a lowered affinity of the IGFBPs for IGFs and their subsequent release to bind to the IGF-1R. IGF-II concentrations are further regulated by the type 2 IGF receptor (IGF2R, also known as the cation-independent mannose 6 phosphate receptor) that targets IGF-II for lysosomal degradation (Brown et al., 2009).

Binding of IGF to the IGF-1R leads to activation of two main signaling cascades ((Taguchi and White, 2008): the insulin receptor substrate (IRS)-initiated phosphatidylinositol 3-kinase (PI3K)-AKT/mammalian target of rapamycin (mTOR) pathway, which predominantly leads to metabolic outcomes, and the SHC-initiated Ras-mitogen-activated protein kinase (MAPK) pathway, which controls mitogenic outcomes, such as cell growth and differentiation. These signaling cascades are complex, with multiple signaling molecules and adaptor proteins coordinating to result in the specific biological outcomes. They are also shared by the related insulin:insulin receptor signaling pathways leading to cross talk (Taniguchi et al., 2006).

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1.2. Knockout mice for *Igf1*, *Igf2*, *Igf1r*, *Igfbps*

The function of mammalian IGF ligands, their receptors and IGFbps has been investigated through knockout and overexpression studies in mice (Gicquel and Le Bouc, 2006; Yakar et al., 2018), which have been particularly informative as rodents and humans have similar system components and in general share similar regulatory and post receptor signaling mechanisms. Global *Igf1* knockout (*Igf1*^{−/−}) results in severe growth retardation and an adult body size 30% of wild type mice (Lupu et al., 2001). Essentially all tissues are smaller, and in particular bone growth is severely retarded. Tissue specific *Igf1* knockout models demonstrate the role of circulating IGF-I in regulation of body size and tissue growth and reveal autocrine actions in specific tissues such as

bone (recently reviewed by Yakar et al., 2018).

Similarly, *Igf2* knockout has significant impact on growth, with prenatal growth being particularly affected. At birth *Igf2*^{−/−} mice are 70% of wild type size (DeChiara et al., 1990). Recently it was shown that IGF-II is essential for longitudinal and appositional murine postnatal bone development, which involves proper timing of chondrocyte maturation and perichondrial cell differentiation and survival (Uchi-mura et al., 2017). Instead of a simple delay of growth, an uncoordinated growth plate development was noticed in these experiments. Furthermore, biochemical and two-photon imaging analyses identified elevated and imbalanced glucose metabolism in the *Igf2* null mouse. Global *Igf2* knockdown also highlighted the importance of IGF-II in young adult mice in maintaining both neuronal and intestinal stem cell niches

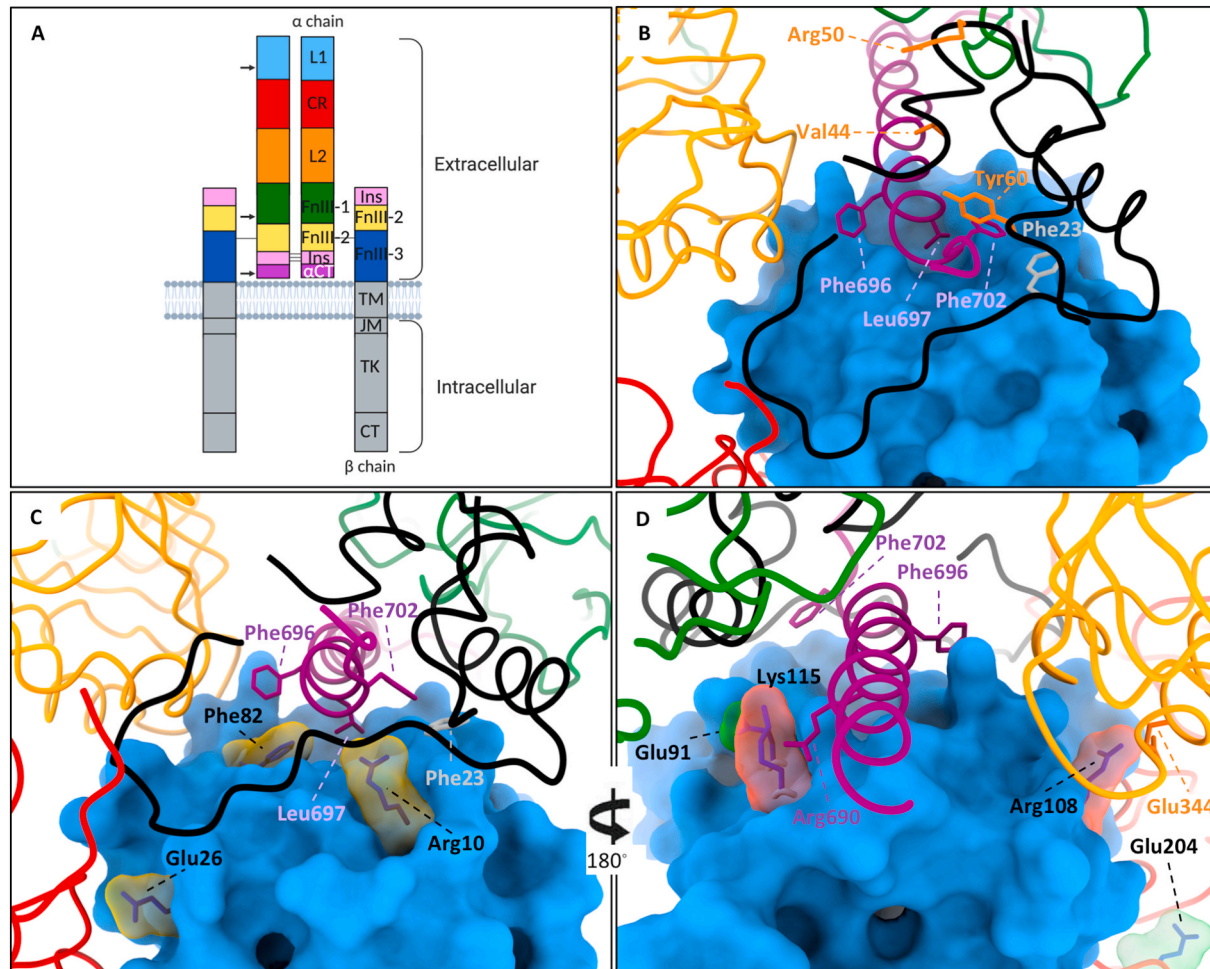


Fig. 1. A. Schematic of the IGF-1R highlighting the domain structure as follows: L1 (first leucine-rich repeat domain), CR (cysteine-rich domain), L2 (the second leucine-rich repeat domain), FnIII-1, FnIII-2 and FnIII-3 (the 1st, 2nd and 3rd fibronectin type III domains, respectively), Ins (insert domain, that incorporates the a C-terminal peptide (α CT)), TM (trans-membrane domain), the intracellular JM (juxtamembrane domain), TK (tyrosine kinase domain) and CT (C-terminal Tail). Arrows indicate domains involved in IGF binding. Colouring of domains is used in subsequent panels.

B. Residues of mature IGF-I (Val44, Arg50, Tyr60) that are affected by homozygous mutations are highlighted (orange) on the ribbon diagram of IGF-I (black) bound to the IGF-1R binding site 1, involving the L1 domain (space-filled blue) and the α CT (magenta). Tyr60 contacts Phe702 of the α CT peptide and its mutation to His would disrupt this hydrophobic interaction. The side chain of residue Arg36 contacts the L2 domain (orange ribbon) but is not resolved in this IGF-I:IGF-1R structure (6PYH.pdb, Li et al., 2019). IGF-I Phe23 (grey) lies within a hydrophobic pocket and anchors IGF-I on binding site 1.

C. Residues of mature IGF-1R (Arg10, Glu26 and Phe82) that are affected by homozygous mutations are highlighted (yellow space filled, all in L1 domain). Arg10 lies within binding site 1 and the hydrophobic pocket accommodating IGF-I Phe23 (grey). Arg10 mutation would disrupt ligand binding. Phe82 makes contact with the α CT peptide Phe696 (magenta) and mutation likely disrupts the binding of the α CT to the L1 domain, which would in turn affect ligand binding. Glu26 contacts Arg242 (CR domain) and mutation of Glu26 most likely affects IGF-1R stability. The additional residues encoded by the homozygous mutation Pro733-Arg734ins are not shown as they are not part of the existing structure.

D. IGF-1R residues (Arg108/Lys115 (orange space-filled) and Glu91/Glu204 (green space-filled)) affected by heterozygous mutations lie outside the binding site 1 and are seen upon rotation of the IGF-I:IGF-1R by 180°. Lys115 is in close proximity to Arg690 of the α CT.

Note: numbering in all panels refers to mature peptides. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Ziegler et al., 2019). Interestingly, *Igf2* $-/-$ mice catch up in weight to wild type after 3 months postnatal growth, whereas mutation in the human *IGF2* affects both pre- and post-natal growth (Yakar et al., 2018). The differences in IGF-II action in mice and humans may arise due to differences in postnatal expression, with *Igf2* expression declining in most mouse tissues in the postnatal period, whereas in humans the IGF-II serum and tissue levels remain high. Global overexpression of *Igf2* in transgenic mice does lead to an increase in overall size (Eggenschwiler et al., 1997; Sun et al., 1997). This also applies to humans with increased IGF-II serum levels (Beckwith-Wiedemann syndrome) who show both prenatal and postnatal overgrowth (discussed in detail below).

Further insight into the tissue specific roles of IGF-II has been provided from tissue specific knockout and overexpression studies. For example, placental specific *Igf2* knockout studies revealed the importance of IGF-II in placental function (Constancia et al., 2002) and overexpression in pancreatic beta cells and smooth muscle cells led to type 2 diabetes and cardiovascular defects, respectively (Devedjian et al., 2000; Zaina et al., 2003).

Mice with IGF-1R gene knockout are not viable and have severe *in utero* growth retardation (~45% of normal) (Liu et al., 1993). Haploinsufficient *Igf1r* mice (*Igf1r* $+/-$) have reduced circulating IGF-I levels and are growth retarded. Similarly, reduced circulating IGF-I levels in *Igfals* knockout mice result in reduced body weight and overall size (Ueki et al., 2000). This arises due to instability of the trimeric IGFBP-3/-5:ALS complex, rapid degradation of IGFBP-3 and clearance of IGF-I. Interestingly knockout of individual IGFBPs results in little or no change in phenotype as seen by the analysis of double-, triple-, or quadruple IGFBP knockout mice, presumably as IGFBPs have redundant functions (Bach, 2018). However, the key role played by IGFBP proteases in regulating IGF bioavailability was elegantly demonstrated in knockout studies of the pregnancy-associated plasma protein A (PAPP-A). PAPP-A knockout mice are 40% smaller than wild-type littermates. Double knockout of PAPP-A and IGFBP-4 lead to an increase in size over mice with PAPP-A knockout alone, demonstrating that cleavage of IGFBP-4 by PAPP-A promotes IGF release during development to promote growth (Conover et al., 2004; Ning et al., 2008).

In summary, knockout studies in mice provide an invaluable insight into the function of IGFs and IGF system components in regulating growth and development. However, mouse studies do not provide a complete picture as there are some key differences in the IGF system biology between mice and humans.

2. Human growth disorders associated with the IGF system

A recent explosion in the number of reported patients with mutations in IGF system components has been enabled by readily available and affordable gene sequencing technology. "Insulin-like growth factor I deficiency" is listed in the Online Mendelian Inheritance in Man registry (MIM #608747) and in the International Classification of Pediatric Endocrine Diagnoses (ICPED) (code 1B.4e) (Quigley and Ranke, 2016). It is one of the causes of a spectrum of disorders called "GH Insensitivity Syndrome (GHIS)", which further includes loss-of-function mutations of genes encoding the GH receptor (*GHR*) (Laron syndrome), intracellular signal transducer and activator of transcription factor *STAT5B* (*STAT5B*) and Acid-Labile Subunit (*IGFALS*), and activating mutations of *STAT3*, as well as conditions characterized by increased serum IGF-I concentrations such as *IGF1R* and *PAPPA2* defects (Storr et al., 2019) and defects of IGF-I signaling. An overview of the data relating to mutations in genes encoding the IGFs, IGF-1R and known IGF-1R signaling components will be now discussed with reference to how these studies inform our understanding of the IGF system's role in growth and development.

2.1. Individuals with *IGF1* defects

Humans carrying genetic variants of *IGF1* have provided essential information on the biological role of IGF-I. Comparison of growth and

phenotype of such cases with individuals carrying pathogenic variants of genes encoding growth hormone (*GH1*) and its receptor (*GHR*) have elucidated the respective and partially overlapping roles in growth and development. Since the severe postnatal growth failure of children with complete absence of functional IGF-I is similar to that of children with complete GH deficiency or insensitivity, one may deduce that the effect of GH on postnatal growth is almost completely transmitted by IGF-I. In contrast, there are important differences between the clinical presentation of GH deficiency/insensitivity and IGF-I deficiency, particularly regarding intrauterine linear and cranial growth, development of the inner ear and other parts of the central nervous system, indicating GH-independent effects of IGF-I.

The reported cases with homozygous and heterozygous *IGF1* variants, though still limited, present a rather consistent picture of the associated clinical features, showing a spectrum of severity of the phenotype depending on gene dose (homozygous versus heterozygous) and biological activity of the *IGF1* variant (Table 1). There are three well-documented patients known with an apparent complete loss of IGF-I action. The first reported human *IGF1* variant was found in a 15.8 year old boy carrying a homozygous deletion of exons 4 and 5 (Woods et al., 1996). The second patient was a 55 year old male staying in an institution for the handicapped, who came under the attention of the pediatric endocrinologist following a request of his healthy brother to obtain genetic advice. He was homozygous for a non-functional variant (p. Val92Met, numbering including the 48 amino acid signal sequence = Val44Met in the mature peptide) (Denley et al., 2005a,b; Walenkamp et al., 2005). Recently, a third patient was described (Keselman et al., 2019) who proved homozygous for another missense variant (p. Tyr108His = Tyr60His in the mature peptide).

There is a still enigmatic case of a boy with an equally severe phenotype in which the genetic cause is still unsolved. In the initial report, the homozygous transversion T > A in the 3' untranslated region of exon 6 of *IGF1* was considered causative (Bonapace et al., 2003). This variant resides on the consensus sequence AATATA of the polyadenylation site and was shown *in vitro* to lead to a deregulated *IGF1* mRNA maturation, altering the E domain of the *IGF1* precursor. However, some years later the same allelic variant was also observed in homozygous and heterozygous state in normal height controls, corresponding to 4% of studied alleles (Coutinho et al., 2007). The three most frequently identified allelic variants of *IGF1* 3' UTR showed no effect on height SDS of adult controls nor on birth characteristics in small for gestational age (SGA) children (Coutinho et al., 2007). This patient may carry another homozygous variant in one of the non-screened regions of the gene, but there are no further reports to confirm this.

The main clinical features of a severe homozygous *IGF1* defect include extreme pre- and post-natal growth failure, poor feeding, severe microcephaly, retrognathia, sensorineural deafness and severe global developmental delay (Table 1) (Walenkamp and Wit, 2019). The finding of sensorineural deafness is consistent with the observations in postnatal IGF-I deficient mice, which show delayed inner ear maturation and neuronal loss (Camarero et al., 2001; Varela-Nieto et al., 2013). Birth length SDS varies between -4.3 and -6.5, much shorter than in patients with complete GH deficiency or insensitivity. Height SDS tends to decrease by age (-6.2, -6.9 and -8.5 at 3, 16 and 55 years, respectively) and a similar decrease may be seen in head circumference SDS (-6.1, -4.9 and -8.0). The deceased brother of the patient described by our group (Walenkamp et al., 2005) had a similar phenotype as the index case. The clinical features of this condition have confirmed the findings in *Igf1* knockout mice (Lupu et al., 2001; Yakar and Adamo, 2012) that IGF-I is a key factor in intrauterine growth and brain and inner ear development, and provided the insight that IGF-I secretion is independent of GH *in utero*.

The evolution of the clinical phenotype with advancing age is only known for the 55 year old patient carrying the Val92Met mutation. In this individual, additional observations included increased abdominal fat mass, facial dysmorphic features (including deep-set eyes, flat

Table 1Clinical, biochemical and genetic features of patients carrying *IGF1* variants.

Genetics ^a	Sex, Age (yrs)	BL SDS	BW SDS	BHC SDS	Height SDS	HC SDS	Hearing	Development	IGF-I SDS	Comment	Reference
Homozygous loss-of function variants											
Del exons 4, 5	M (15.8)	−5.4	−3.9	−4.9	−6.9	−5.3	Deaf	Delay	undetect	Anorexia, micrognathia, ptosis	Woods et al. (1996)
p.Val92Met	M (55) ^b	−4.3	−3.9		−8.5	−8.0	Deaf	Delay, IQ < 40	+7.3	Micrognathia	Walenkamp et al. (2005)
p.Tyr108His	M (3.2)	−6.3	−3.1		−6.2	−6.1	Deaf	Delay, ADHD	−1.1 to 3.0	Facial dysm	Keselman et al. (2019)
Unknown**	M (1.5)	−6.5	−4.0	−7.5	−6.2	−7.5	Deaf	Delay	low		Bonapace et al. (2003)
Homozygous variants with decreased function											
p.Arg84Gln	M (2.8)	−3.7	−2.4	−2.5	−4.9	−4.0	N	IQ 70-75	Variable	Anorexia	Netchine et al. (2009)
p.Arg98Trp	F (2.8)		−3.5		−3.2	−4.7	N	N			Shaheen et al. (2014)
	M (5.0)				−4.3	−5.4	N	N			
Heterozygous variants											
p.Ser83GlnfsX13	F (8.2)	−3.8	−2.9		−4.1	−2.4	N	Mild delay	−2.3	Anorexia, facial dysm	van Duyvenvoorde et al. (2010)
	M (6.2)	−1.0	−1.2		−4.6	−1.6	N		−2.6	XXX, facial dysm	
	F (35)	−1.6	−1.6		−3.5	−1.7	N		−1.8	Poor feeding	
	M (65)				−1.4	−1.8	N		−2.0		
c.207G > A, c.402+1G > C	M (8.7)	−0.6	−1.5		−4.0			ADHD	−2.2		Fuqua et al. (2012)
	F, mother				−4.6						
	F, grandm				−6.4						
Deletion	M (8.4)	−1.2	−1.5		−2.7	−3.4			Low nl	Micrognathia	Batey et al. (2014)
	Father				−2.0						
Del exons 4, 5	Father				−1.8				≈−2.1		Woods et al. (1996)
	Mother				−1.4				≈−2.2		
	F (10.0)				−1.0				≈−0.5		
p.Val92Met	N = 9 vs 44		3048 vs 3358 g		−1.0 vs −0.4	−1.0 vs 0.5			0.6 vs −0.3		Walenkamp et al. (2005)

Abbreviations: ADHD, attention deficit hyperactivity disorder; BL, birth length; BHC, birth had circumference; BW, birth weight; dysm, dysmorphism; F, female; grandm, grandmother; HC, head circumference; M, male; nl, normal; undetect, undetectable; vs, versus; yrs, years.

^a Residue numbers include the 48 amino acid leader sequence.

^b His deceased brother had an identical phenotype; **the genetic finding (T > A in untranslated region of exon 6), which would lead to altered E domain of the IGF-1 precursor, was later proven to be a polymorphism without functional importance (Coutinho et al., 2007).

occiput, a columella extending beyond the alae nasi), broad end phalanges, convex nails, hypermobility of the interphalangeal joints, restricted mobility of both elbows, hypertension, small testicular volume, micropenis, cataracts of both eyes and osteoporosis (Walenkamp et al., 2005; Walenkamp and Wit, 2019).

There are two patients known with a homozygous variant of *IGF1* with a less severe clinical picture (Table 1). A mild pre- and postnatal growth failure, mild intellectual deficit and normal hearing was observed in a French patient carrying a homozygous *IGF1* mutation (p.Arg84Gln = Arg36Gln in mature peptide) which is transcribed into a mutated IGF-I protein with some remaining function (Netchine et al., 2009). A patient from Saudi Arabia carrying a homozygous variant (p.Arg98Trp = Arg50Trp in mature peptide), recently classified as a variant of unknown significance (VUS according to ACMG guidelines) (Keselman et al., 2019; Richards et al., 2015), showed a similarly relatively mild phenotype (Shaheen et al., 2014). The lack of studies on segregation and *in vitro* functionality makes it impossible to be sure about the pathogenicity of this variant (Walenkamp and Wit, 2019).

The serum IGF-I concentration is of little help for establishing the diagnosis. Serum IGF-I was undetectable in the patient with the deletion of exons 4 and 5 (Woods et al., 1996), but the case with the p.Val92Met mutation showed an extremely high IGF-I concentration, as measured by radioimmunoassay (Walenkamp et al., 2005). In the French patient with partial IGF-I deficiency serum IGF-I concentrations varied between assays (Netchine et al., 2009), while in the Argentinian case IGF-I concentrations showed unexplained variations using the same assay (Keselman et al., 2019; Walenkamp and Wit, 2019).

GH secretion as assessed by baseline GH concentration and GH peak

in a stimulation test appears to be increased in childhood (Keselman et al., 2019; Walenkamp et al., 2005; Woods et al., 1996) and had normalized in the 55 year old patient (Walenkamp et al., 2005). Severe insulin resistance was observed in the first reported patient (Woods et al., 1996), but the adult with the Val92Met mutation only had a mild glucose intolerance, though functional studies showed that binding or activation of both insulin receptor isoforms was not detectable even at micromolar concentrations (Denley et al., 2005a,b). The patients with Arg84Gln and Tyr108His mutations (Keselman et al., 2019; Netchine et al., 2009) did not show insulin resistance.

Heterozygosity for a pathogenic mutation can cause short stature (Batey et al., 2014; Fuqua et al., 2012; van Duyvenvoorde et al., 2010) with a wide height SDS range (Table 1). In the two Dutch siblings no dominant negative effect of the truncated protein was shown (van Duyvenvoorde et al., 2011). An effect of heterozygosity for a pathogenic *IGF1* variant on growth and adult stature was also shown in studies in relatives of patients with a homozygous *IGF1* variant (Keselman et al., 2019; Woods et al., 1996). In the large pedigree of the Dutch family with the Val92Met variant, heterozygous carriers had a significantly lower birth weight, height, head circumference, and a higher serum IGF-I concentration than carriers of the wild-type gene (Walenkamp et al., 2005). These observations suggest that haploinsufficiency of *IGF1* may be one of the genetic causes of children presenting with moderate short stature who may be initially registered as SGA with failure to catch-up or as idiopathic short stature (ISS). Such gene-dose effect has also been documented for *IGFALS* and *STAT5B* variants (Domene et al., 2004; Scalco et al., 2015; van Duyvenvoorde et al., 2008) and several genes associated with skeletal dysplasias, e.g. *SHOX* and *NPR2* (Baron et al.,

2015).

In the general population polymorphisms in the *IGF1* promoter region and serum IGF-I concentration may be one of the many factors influencing intra-uterine and postnatal growth (Arends et al., 2002; Ester et al., 2009; Fujihara et al., 2012; Kim et al., 2010; Murphy et al., 2006; Okada et al., 2010; Vaessen et al., 2002; Yang et al., 2008). Furthermore, methylation of *IGF1* CpG-137 in the P2 promoter was shown to be associated with birth length (Le Stunff et al., 2018). In general, however, genes associated with the GH-IGF axis represent a small minority of the more than 400 genes associated with height in genome-wide association studies (Lettre et al., 2007; Wood et al., 2014).

So far, activating *IGF1* variants or duplications have not been discovered, but one would expect tall stature in individuals with such variants, in line with the observations of overgrowth in patients with duplications or activating variants of *IGF1R* (Kant et al., 2007; Lin et al., 2018; Okubo et al., 2003).

To date, there is little information on therapeutic options for patients with IGF-I deficiency. The homozygous carrier of the exon 4 and 5 deletion was treated with recombinant human IGF-I (rhIGF-I) for one year, which increased height velocity, bone size and improved body composition and insulin sensitivity (Woods et al., 2000). The Italian boy with the undetermined genetic defect was also treated with rhIGF-I with an acceptable growth response (Concolino et al., 2010). The patient with partial IGF-I deficiency was treated with recombinant human GH (rhGH) resulting in an insufficient growth response (Netchine et al., 2009). The growth response to rhGH treatment in the two siblings carrying a pathogenic heterozygous *IGF1* mutation (van Duyvenvoorde et al., 2010) was considered sufficient to continue treatment up to adult height.

2.2. Individuals with *IGF2* defects

The paternally expressed gene encoding Insulin-like Growth Factor 2 (*IGF2*) on chromosome 11p15.5 plays a critical role in placental and body growth (MIM # 147470) (Gicquel and Le Bouc, 2006). As mentioned above, already in 1990 the *Igf2* knockout studies in mice showed that IGF-II is important for fetal growth (DeChiara et al., 1990). The discovery of an epimutation of the telomeric imprinting center region on chromosome 11p15.5 in Silver-Russell syndrome (SRS) (Gicquel et al., 2005), as well as copy number variants involving the 11p15.5 region (Begemann et al., 2012; Demars and Gicquel, 2012; Heide et al., 2018; Tumer et al., 2018; Wakeling et al., 2017), showed that a functional defect of IGF-II might be one of the main causes of poor growth in SRS. In fact, approximately 50% of cases with SRS are caused by a loss of methylation of the telomeric domain in the 11p15.5 region, resulting in downregulation of paternal *IGF2* expression (Azzi et al., 2014; Gicquel et al., 2005; Netchine et al., 2007). Later, other disruptions in the HMGA2-PLAG1-*IGF2* pathway were shown to cause an SRS phenotype (Abi Habib et al., 2018). At the other end of the spectrum of growth, biallelic *IGF2* expression leads to fetal overgrowth and an increased risk for childhood cancer (Demars and Gicquel, 2012).

Additional indirect proof of the effect of IGF-II deficiency in humans was provided by a case report on a short woman (height −3 SDS) born with a severely decreased birth size (birth weight −5.4 SDS) and presenting with an atypical early diabetes mellitus (associated with insulin resistance), altered fat distribution and lactation failure. She carried a balanced chromosomal translocation t(1;11)(p36.22;p15.5) and further studies showed that a break point 184 kb upstream of the paternally derived *IGF2*, separating it from some telomere enhancers, resulted in reduced expression in some mesoderm-derived adult tissues (Murphy et al., 2008).

The first direct evidence of the effect of IGF-II deficiency was provided by a patient with a paternally transmitted form of SRS caused by an *IGF2* loss-of-function variant [p.Ser64Ter] (Begemann et al., 2015). Later, several other patients were detected with paternally transmitted *IGF2* variants, the majority of which perturbed expression (Abi Habib

et al., 2018; Grosse et al., 2017; Liu et al., 2017; Poulton et al., 2018; Rockstroh et al., 2019; Tumer et al., 2018; Yamoto et al., 2017). The phenotype is typical for SRS, including low birth size (SGA), relative macrocephaly and short stature at preschool age, and patients had varying degrees of feeding problems and neuropsychomotor developmental delay. Other findings included cardiac and male genital anomalies, facial dimorphism and finger deformities (Rockstroh et al., 2019; Vasques et al., 2019). These observations showed that IGF-II deficiency not only affects prenatal growth, but also postnatal growth. Serum IGF-II appears to have a limited diagnostic value for the differential diagnosis of children born SGA (Binder et al., 2017).

In an interesting patient carrying both an 11p paternal duplication (resulting in the double expression of *IGF2* as reported in Beckwith-Wiedemann syndrome) and a 15q terminal deletion, including the *IGF1R* deletion, resulting in *IGF1R* haploinsufficiency (Giabicani et al., 2019) birth size was normal, but the patient experienced growth retardation in early childhood. The authors speculated that IGF-II plays a key role in fetal growth, independently of IGF-1R signaling, and that its role is less important in postnatal growth.

IGF-II variants are not only associated with poor growth but may also have an effect on the risk of diabetes mellitus type 2 (T2D) (Mercader et al., 2017). In individuals with and without T2D of Latino descent, a novel genetic variant in *IGF2* was associated with ~20% reduced risk for T2D. This variant prevents splicing between *IGF2* exons 1 and 2 and is associated with a specific, allele-dosage-dependent reduction in the expression of *IGF2* isoform 2.

The pathophysiology of some other forms of syndromic short stature may be associated with *IGF2* silencing. Convincing proof for such phenomenon was offered by experiments on fibroblasts from four children with 3-M syndrome (caused by mutations in *CUL7* (n = 1), *OBSL1* (n = 2) and *CCDC8* (n = 1)) compared with controls (Murray et al., 2013). The authors concluded that 3-M syndrome is associated with a gene expression profile of reduced *IGF2* expression and increased *H19* expression similar to that found in SRS. They speculated that loss of autocrine IGF-II in the growth plate may be associated with the short stature seen in children with 3-M syndrome.

At the other end of the spectrum, the tall stature of individuals with Sotos syndrome (MIM #117550) may be associated with overexpression of *IGF2*, via hypomethylation of one of the differentially methylated regions (DMRs), i.e. a loss of the establishment or maintenance of *IGF2*-DMRO methylation during the postimplantation period (Watanabe et al., 2020). These observations are in line with a study by Visser et al. (2012) showing deregulation of the MAPK/ERK pathway in Sotos syndrome, which results in altered hypertrophic differentiation of *NSD1* expressing chondrocytes and may be a determining factor in overgrowth and accelerated skeletal maturation in Sotos syndrome (Visser et al., 2012).

2.3. Individuals with homozygous and heterozygous variants of *IGF1R*

The pleiotropic hormones IGF-I and IGF-II act through binding to the IGF-IR. *IGF1R* is located on the distal part of the long arm of chromosome 15 (15q26.3). Since the 1970s, several terminal deletions of the distal long arm of chromosome 15, resulting in allelic loss of *IGF1R* and thus partial IGF-I resistance, have been described (Butler et al., 1988; Giabicani et al., 2020; Pasquali et al., 1973; Roback et al., 1991; Walenkamp et al., 2019). Patients with these heterozygous deletions show pre- and post-natal growth retardation (Choi et al., 2011) and a variable degree of other clinical manifestations (Giabicani et al., 2020; Walenkamp et al., 2019). Based on these case reports it was generally assumed that the growth failure in these patients was caused by haploinsufficiency of *IGF1R*, but this could obviously not be considered direct evidence.

The paper of Abuzzahab et al., in 2003 was the first to show that a heterozygous or compound heterozygous pathogenic variant of *IGF1R* is associated with pre- and postnatal growth retardation, as well as

microcephaly and a high or relatively high serum IGF-I concentration (Abuzzahab et al., 2003). In the following years this was confirmed by a large number of case reports. Walenkamp et al. calculated that up to 2019 there had been described 36 different (likely) *IGF1R* pathogenic variants, as well as a number of variants of uncertain significance (Walenkamp et al., 2019). A total of 13 additional novel pathogenic variants were reported in two subsequent papers describing French and German cohorts (Giabicani et al., 2020; Gopel et al., 2020).

The large majority of individuals carrying *IGF1R* variants are heterozygous for a single nucleotide variant (SNV) (usually missense) and the second largest group consists of individuals carrying deletions encompassing *IGF1R* (28, 23 and 11% in the large Dutch, French and German cohorts, respectively) (Giabicani et al., 2020; Gopel et al., 2020; Walenkamp et al., 2019). Biallelic variants are quite rare; so far, only five patients (from four families) with homozygous *IGF1R* variants have been reported (Gannage-Yared et al., 2013; Giabicani et al., 2020; Gopel et al., 2020; Prontera et al., 2015), and four patients (from three families) with compound heterozygous *IGF1R* variants (Abuzzahab et al., 2003; Fang et al., 2012; Giabicani et al., 2020). Selected clinical, biochemical and genetic features of these cases are shown in Table 2.

With regard to the clinical presentation of individuals carrying heterozygous SNVs or deletions, the number of case reports have grown too large to refer to all of them. Instead, here we present the average data

from the two recent large case series (Giabicani et al., 2020; Walenkamp et al., 2019) (Table 3). In general, individuals carrying monoallelic *IGF1R* variants present with a less severe phenotype than patients carrying a biallelic variant. However, there is a clear overlap with regard to clinical features, such as birth size, height, head circumference between individuals carrying biallelic and mono-allelic variants.

When assessing the clinical features of the reported cases, one must consider that all reports have suffered from referral bias. The phenotype of the first reported patients with *IGF1R* variants was rather extreme, so that thereafter clinicians have been looking for *IGF1R* variants in patients with similar clinical features. Thus, genetic analysis of *IGF1R* was usually requested if a patient showed the “classical” clinical features (prenatal and postnatal growth failure, microcephaly and a serum IGF-I concentration in the upper half of the normal range or above). A more representative picture of the phenotypic range can be obtained when one focuses on the clinical characteristics of affected relatives, in whom it is not rare that at least one of the classical features is absent. Only if data become available on large sets of individuals tested for *IGF1R* variants independently of their clinical presentation, will the real scope of the phenotypic range be assessed.

Since most short children with a pathogenic *IGF1R* variant were born SGA, many have been treated with rhGH according to this accepted indication. The general picture arising from multiple case reports has

Table 2

Clinical, biochemical and genetic features of patients carrying homozygous or compound heterozygous *IGF1R* variants (according to *IGF1R* NM_000875.4; HGVS nomenclature).

Genetics	Sex, age (yrs)	BL SDS	BW SDS	BHC SDS	Height SDS	HC SDS	IGF-1 (ref, ng/mL or SDS)	Development	Phenotype	HSDS Fa, Mo	Reference
Homozygous loss-of-function variants											
c.119G>T (p. Arg40Leu)	F (13.5)	-4.5	-4.7	-3.3	-4.4	-5.6	7.0 SDS	Delay	Facial dysm, pterygium colli, heart malformation, reduced s.c. fat, clinodactyly, high-pitched voice, axillary acanthosis, scoliosis, truncal obesity	Fa -0.7 Mo -0.4	(Gannage-Yared et al., 2013)
c.2201G>T (p. Pro733 Arg734ins25)	F (0.75)	-3.2	-4.1	-5.5	-3.2	-5.3	285, 352 (18-146)	Delay	Facial dysm, heart malformation, bilateral sensorineural hearing loss, atrophic left iris SHORT syndrome	Fa -2.2 Mo -2.3	(Prontera et al., 2015)
c.334T>C (p. Phe112Leu)	F (5.2)	-3.8	-4.0	-3.3	-4.6	-4.9	4.1 SDS	Delay	Heart malformation, severe oesophago-gastric reflux, anorexia	Fa -2.5 Mo -2.4	(Giabicani et al., 2019)
c.168G>T (p. Glu56Asp)	F (5.0)	-3.4	-2.6	-2.45	-4.1	-3.9	335 (2.4yrs), 479 on GH, max 719	Mild verbal delay	Round doll-like face, big eyes, dense black eyebrows and scalp, low hairline in the neck		(Gopel et al., 2019)
	F (4.3)	-4.0	-2.3		-4.6	-2.3	161 (10 mo), 335 on GH, max 452		Same features plus mild flow acceleration in the pulmonary trunk		
Compound heterozygous loss-of-function variants											
c.413G>A (p. Arg138Gln)/c.435A>C (p. Lys145Asn)	F (14.0)		-3.5		-4.8 (AH)		1130 ng/mL (ref 191-462)	Non-verbal learning disorder	Obsessive tendencies, excessive fantasy role playing, social phobias. Poor response to GH	Fa -2.8 Mo -1.6	(Abuzzahab et al., 2003)
c.361G>A (p. GluE121Lys)/c.700G>A (p. Glu234Lys)	M (3.4) F (4.4)	-3.5 -0.8	-2.8 -3.2	-3.2	-7.3 -5.9 -3.4	-5.1	253 ng/mL (ref 54-161) 410 ng/mL (19.8-141)	Mild delay Gross motor delay	Facial dysm, antibody-negative diabetes mellitus from 14.5 yrs. Central adiposity. Facial dysm, strabismus, clinodactyly, multiple café-au-lait lesions, epidermal cyst, Burkitt lymphoma	Fa -1.4 Mo -1.2	(Fang et al., 2012)
c.3539C>A (p. Ser1180Tyr)/c.4055G>T (p. Gly1352Val)	M (6.6)	-2.7	-2.2	-3.0	-2.5	-4.3	4.2 SDS	Delay	pyelic dilatation, mild cognitive delay (mainly language)	Fa -0.7 Mo -0.4	(Giabicani et al., 2019)

Abbreviations: AH, adult height; BL, birth length; BHC, birth had circumference; BW, birth weight; dysm, dysmorphism; F, female; Fa, father; HC, head circumference; M, male; Mo, mother; ref, reference range; s.c., subcutaneous; yrs, years.

Table 3

Clinical and biochemical and genetic features of heterozygous carriers of heterozygous *IGF1R* deletions or single nucleotide variants (SNVs) in Dutch and French cohorts (Giabicani et al., 2020; Walenkamp et al., 2019).

Cohort	N	Birth length SDS (mean, range)	Birth weight SDS (mean, range)	Birth head circumference SDS (mean, range)	Height SDS (mean, range)	Head circumference SDS (mean, range)	IGF-1 (mean, range)	Feeding difficulty	Developmental delay
Deletions									
Netherlands	7	−2.9 −4.5; −1.3	−2.2 −3.3; −1.1	−1.7 −3.0; −0.5	−3.3 −5.5; −2.1	−2.2 −4.1; −0.8	1.2 −1.3; 2.5	6/7	7/7
France	12	−3.1 −4.2; −2.0	−2.3 −3.5; −1.4	−2.5 −3.0; −1.6	−3.8 ^a −5.7; −2.1	−2.8 ^b −5.1; −1.5	1.3 ^c −2.0; 5.5	6/9	7/10
SNVs									
Netherlands	18	−2.5 −5.0; −1.0	−2.1 −3.7; −0.4	−1.6 −2.9; 0.0	−2.9 −4.0; −1.7	−2.6 −4.2; −0.5	1.2 −1.3; 3.2	9/12	5/13
France	23	−3.4 −5.2; −1.4	−2.6 −3.8; −1.0	−2.7 −3.9; −0.3	−3.5 −4.7; −2.2	−3.6 ^d −4.9; −1.8	2.5 −1.2; 7.1	6/13	5/12

^a Minimal height in the growth curves.

^b Last HC available, data for only 4 patients.

^c Before or without rhGH therapy.

^d Last HC available, data for only 5 patients.

recently been confirmed by the results of rhGH treatment in three large case series (Giabicani et al., 2020; Gopel et al., 2020; Walenkamp et al., 2019). Treatment with rhGH has a positive effect on height velocity, but this effect is less and more variable, than that observed in short children born SGA without an *IGF1R* variant. During treatment with a dose of 33 µg/kg body weight/day, serum IGF-I concentration often increases to levels well above the reference range, and so far most clinical investigators are of the opinion that this may be tolerated because of the partial IGF-I resistance (Giabicani et al., 2020; Walenkamp et al., 2019).

Besides severe loss-of-function variants with a substantial effect on skeletal and cranial growth, there are probably also rare *IGF1R* variants with a mild or moderate effect on stature in an apparently healthy population (de Graaff et al., 2013; Kansra et al., 2012), and *IGF1R* variants have also been associated with longevity (Bonafe et al., 2003).

At the other side of the spectrum of linear growth, a few cases have been reported with tall stature due to either *IGF1R* duplication (Kant et al., 2007; Okubo et al., 2003) or an activating variant (Lin et al., 2018).

2.4. Implications of human mutations for the understanding of the IGF – IGF-1R interaction

Mutations of the *IGF1*, *IGF2* and the *IGF-1R* can affect overall expression, protein stability or result in a change within the coding region leading to a poorly functioning protein. Current understanding of how specific mutations of the human IGF ligands and the IGF-1R affect ligand binding and activation of the IGF-1R has been greatly assisted by recent advances in structural biology, allowing the visualization of the activated receptor in complex with IGF-I and IGF-II (Li et al., 2019; Xu et al., 2020). Structures provide molecular detail of the primary IGF binding site 1 (involving the L1 domain and αCT helical peptide) and the low affinity binding site 2 (in the FnIII-1). With this knowledge and the understanding that IGFs act to control growth and development, the clinical features of several IGF-I mutations can be explained, and at the same time insight into the mechanism of ligand binding and receptor activation is gained.

For example, as originally predicted, residue Val92 of IGF-I (Val44 in mature IGF-I) is in a region involved in engagement with the IGF-1R (specifically with the αCT peptide in binding site 1) (see Fig. 1b). The conservative substitution of Val92 to Met in the p.Val92Met homozygous mutation is not tolerated, highlighting this residue is critical for IGF-1R engagement (Denley et al., 2005a,b; Walenkamp et al., 2005). Similarly, Tyr108 of IGF-I (Tyr60 in mature IGF-I) makes direct contact with the αCT peptide and interacts with part of the site 1 hydrophobic pocket in which the important IGF-I Phe71 (Phe23 in mature IGF-I) binds and acts as the main anchor residue (Fig. 1b). Substitution with

the smaller and basic histidine (Tyr108His) most likely disrupts this hydrophobic pocket and results in a poor binding affinity. The less severe clinical picture arising from the Arg84Gln mutation can be explained by the observation that Arg84 (Arg36 in mature IGF-I) of the C-domain contacts the IGF-1R L2 domain outside the site 1 hydrophobic pocket (Fig. 1b, side chain not resolved in this structure). This interaction contributes to the overall binding affinity but is not essential. *In vitro* mutagenesis studies show mutation of this residue results in a 4–5 fold reduction in affinity (Jansson et al., 1998; Netchine et al., 2009). Intriguingly, the mild phenotype of the individual with the Arg98Trp mutation cannot be as easily explained, as Arg98 (Arg50 in mature IGF-I) does not make close contact with the receptor in any of the structures determined to date. Mutation of Arg98 to Ala only reduces IGF-1R binding affinity by 1.5 fold (Denley et al., 2005a,b; Jansson et al., 1998).

If we consider the four known homozygous mutations of the IGF-1R extracellular domain (Table 2) in a similar manner it is also possible to understand the impact on IGF-1R activation and downstream signaling leading to patient phenotypes listed in Table 2. The c119G > T (pArg40Leu) mutation lies in the L1 domain directly within the primary IGF binding site 1 that comprises the L1 domain and αCT helical peptide (Fig. 1c). *In vitro* analysis of Arg40 (Arg10 in mature IGF-1R) mutation to Ala demonstrates the critical role this residue plays in ligand binding and IGF-1R activation (Li et al., 2019; Whittaker et al., 2001).

Similarly, the clinical features arising from the Phe112Leu homozygous mutation can be explained by observing the location of Phe112 in the IGF-I:IGF-1R complex structure (Li et al., 2019). Phe112 (Phe 82 in mature IGF-1R) is found on the surface of the L1 domain. Upon ligand binding the αCT helical peptide shifts position on the surface of the L1 domain, which brings αCT into contact with Phe112 (Fig. 1c). This movement of the αCT helical peptide is absolutely required to form the primary IGF binding site 1. Substitution of Phe112 with the smaller Leu residue lacking an aromatic ring structure is likely to disrupt key hydrophobic interactions between Phe112 and the αCT helical peptide (Fig. 1c), thereby perturbing the high affinity site 1 interaction.

Another homozygous mutation c.2201G > T p.(Pro733Arg734ins25) results in an extension of 25 amino acids at the end of the αCT helical peptide. In order for IGF-I to bind to site 1 the end of the αCT helical peptide needs to thread through the IGF-I C-domain. An extension to the αCT peptide is likely to hinder its ability to thread through the C-domain which would affect the ability to bind with high affinity.

Of the four homozygous mutations in the IGF-1R extracellular domain Glu56 (Glu26 in mature IGF-1R) is not directly within binding site 1 but lies within the interface between the L1 and CR domains. We speculate the Glu56Asp mutation affects stability of the IGF-1R, although this is yet to be investigated. There have been no reports we

are aware of describing *in vitro* expression of a Glu56 IGF-1R mutant.

There are also two compound heterozygous variants within the IGF-1R extracellular domain, interestingly also both introducing changes close to but not directly within the IGF-I binding region (Fig. 1d). Each of the combined mutations (Arg138Gln/Lys145Asn and Glu121Lys/Glu234Lys) are likely to be affecting overall receptor stability as the residues involved make contacts with adjacent domains within the IGF-1R structure. Interestingly, both a Glu121Ala IGF-1R and a Lys115Ala IGF-1R can be expressed *in vitro* and the mutations have little effect on IGF-I binding affinity, suggesting it is the combination of both mutations in the heterozygous variants that leads to poor IGF-1R activity.

In this review we have limited our discussion to the homozygous mutations of the IGF-1R extracellular domain, but similar analyses could be made for the intracellular domain mutants and the existing heterozygous mutations. In general, the relative effect mutations have on expression, peptide stability or ability to bind IGF is responsible for the spectrum of severities of the related clinical features.

2.5. Individuals with genetic disorders affecting post-receptor signaling

As summarized above, binding of IGF-I and IGF-II to the IGF-1R initiates two main post-receptor signaling cascades. Following autophosphorylation the IGF-1R binds the adaptor proteins IRS-1 and IRS-2 which then recruit PI3K leading to activation of the Akt/mTOR pathway. A second adapter SHC recruits downstream signaling molecules resulting in the MAP kinase cascade, including the Erk1/2 kinases. Many other proteins including 14-3-3 adapter proteins and protein kinase C, regulate these signaling pathways. It is noteworthy that these signaling pathways are not restricted to the IGF-IR, but are also activated by insulin and other tyrosine kinase receptor ligands (Rabiee et al., 2018). While in theory *IRS1* and *IRS2* variants could affect linear growth, so far these have only been associated with an increased risk of non-insulin dependent diabetes mellitus (Esposito et al., 2003; Wang et al., 2001). There are no reports indicating that loss of function may inhibit linear growth (Rabiee et al., 2018).

2.5.1. YWHAE and CRK

Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation proteins (YWHA, otherwise known as 14-3-3 proteins) are adapter proteins that bind many components of signaling pathways, including the IGF-1R signaling molecules (e.g. IRS2). The YWHA epsilon polypeptide, also called “14-3-3 protein, epsilon isoform” is encoded by *YWHAE*. A potential role of this gene in the regulation of growth has been suggested by findings in patients with a heterozygous terminal deletion of chromosome 17p13.3 (Miller-Dieker syndrome, MIM # 247200). This syndrome is characterized by lissencephaly, distinctive facial appearance, intellectual disability, severe neurological dysfunctions (including seizures, eye coloboma, chiari malformation and white matter abnormalities), and short stature. Different lengths and positions of the deletions influence the phenotype. Loss of function variants of *PAFAH1B1* (encoding platelet-activating factor acetylhydrolase, isoform 1b, subunit 1, previously called *LIS1*) cause nonsyndromic lissencephaly (MIM # 601545), but if combined with *YWHAE* haploinsufficiency brain and neurological abnormalities are more severe (Noor et al., 2018). At present, it is believed that *YWYAE* haploinsufficiency is primarily associated with brain disorders, in particular abnormal cortical organization (Chiari malformation), learning disability and seizures (Enomoto et al., 2012; Mignon-Ravix et al., 2010; Noor et al., 2018). The growth retardation in this syndrome was initially suggested to be caused by haploinsufficiency of *CRK*, a gene located next to *YWHAE* (Bruno et al., 2010; Nagamani et al., 2009), but a recent case report describing a short individual (−2.8 SDS for Dutch references (Schonbeck et al., 2013)) with only a disruption of *YWHAE*, suggests that *YWHAE* haploinsufficiency does play a role, possibly in combination with *CRK* haploinsufficiency.

Additional information about the causative role of the various genes in the 17p13.3 region can be obtained from patients with 17p13.3

duplications, usually subcategorized into class I duplications involving *YWHAE* but not *PAFAH1B1* and class II duplication which extend to involve *PAFAH1B1* (Bruno et al., 2010). The majority of patients with class I duplications show overgrowth (Bruno et al., 2010; Curry et al., 2013; Henry et al., 2016), in one case associated with a pituitary adenoma (Henry et al., 2016).

Our interpretation of the currently available literature is that there appears to be a gene-dose effect of both *YWHAE* and *CRK* regarding linear growth. In that perspective it is noteworthy that not only *YWHAE* plays a role in IGF-1R signaling, but *CRK* as well. C-Crk and CrkII have been reported to interact with (and be phosphorylated by) the IGF-1R (Henry et al., 2016; Jin et al., 2000; Klammt et al., 2004).

2.5.2. PIK3R1

Heterozygous pathogenic phosphatidylinositol 3-kinase regulatory subunit alpha (*PIK3R1*) variants are associated with SHORT syndrome (Chudasama et al., 2013; Dymont et al., 2013; Thauvin-Robinet et al., 2013). The name of SHORT syndrome is based on an acronym: S for Short stature, H for Hyperextensibility of joints and/or inguinal hernia, O for ocular depression, R for Rieger abnormality and T for teething delay. Additional clinical features include intrauterine growth restriction, several facial dysmorphisms and wrinkled and thin skin (Avila et al., 2016). The *PIK3R1* mutations disrupt not only IGF-1R signaling but also insulin signaling, so that patients are predisposed to insulin resistance and diabetes. Downstream effects are mediated by lower levels of phosphorylation of proteins in the AKT and mTOR signaling pathway (Chudasama et al., 2013; Dymont et al., 2013; Thauvin-Robinet et al., 2013). SHORT syndrome bears similarities with IGF-I resistance syndrome (*IGF1R* variants) and Silver-Russell syndrome (presumably because of the important role of diminished IGF-II action in this syndrome). In fact, one of the children with a homozygous *IGF1R* variant presented clinically as SHORT syndrome, so that the authors suggested to call this presentation “SHORT syndrome 2” (Prontera et al., 2015).

Based on detailed phenotypes of 32 individuals with SHORT syndrome and *PIK3R1* mutation it was shown that the major features described in the SHORT acronym were not universally seen and only half had four or more of the classic features (Avila et al., 2016). The commonly observed clinical features of SHORT syndrome seen in the cohort included intrauterine growth restriction (IUGR, <10th percentile), postnatal growth restriction, lipotrophy and the characteristic facial gestalt. Anterior chamber defects and insulin resistance or diabetes were also observed but were not as prevalent. The less specific (minor) features of SHORT syndrome include teething delay, thin wrinkled skin, speech delay, sensorineural deafness, hyperextensibility of joints and inguinal hernia. The authors recommended regular monitoring of glucose metabolism, an echocardiogram, and ophthalmological and hearing assessments (Avila et al., 2016).

2.5.3. Protein Kinase Cε (PRKCE)

Recently, a 13-year-old girl with the clinical characteristics of SHORT syndrome was reported with normal results of *PIK3R1* sequencing, but a *de novo* mutation in *PRKCE* (Protein Kinase Cε) (Alcantara et al., 2017). The authors showed that the variant causes impaired AKT activation via compromised mTOR2 complex function.

2.6. Speculation on additional genetic abnormalities associated with the GH-IGF axis in the human

Experimental data and insights from laboratory studies suggest that additional genes associated with the GH-IGF-axis could have a role in the etiology of human growth disorders. We believe that the coming decades will see an uncovering of several human growth disorders associated with this axis which are yet unknown. Below we identify genes possibly involved in growth disorders, albeit currently speculative and only supported by indirect evidence. Of note, another potential cause of short or tall stature could be through the disturbance of

microRNAs that regulate the expression of *GH*, *GHR*, *IGFs*, *IGF1R*, and downstream signaling peptides in different animal species. This has recently been reviewed (Cirillo et al., 2020) and will not be further discussed here.

2.6.1. Genes involved in regulating expression, translation, trafficking and secretion of IGF-I

Production of IGF-I and IGF-II requires interaction with the chaperone Glucose Regulated Protein 94 (GRP94), a ubiquitously expressed endoplasmic reticulum (ER)-resident chaperone that is essential for growth and development of multicellular organisms (Marzec et al., 2016). The amount of secreted IGFs is proportional to the GRP94 activity (Ostrovsky et al., 2009). A non-common SNP (P300L) with frequencies of 1–4% in various populations was found first in a child with primary IGF deficiency. P300L supported only 58% of IGF secretion relative to wild type GRP94 and recombinant P300L showed impaired nucleotide binding activity. The authors concluded that mutations in *GRP94* that affect its IGF-chaperone activity may represent a novel causal genetic mechanism that limits IGF biosynthesis (Marzec et al., 2016), but this observation needs confirmation.

2.6.2. IGF binding proteins

The availability of IGF-I to body tissues is regulated by a set of IGF-binding proteins (IGFBPs) and proteases to liberate IGF-I from its complex with acid-labile subunit (ALS) and IGFBP-3 or -5. So far, variants have only been detected in the human gene encoding ALS (*IGFALS*) and the gene encoding the protein PAPP-A-2 (*PAPPA2*) (for further details see the respective papers in this special issue).

Most classical IGFBPs (1–6) have an inhibitory effect on IGF-I availability, according to mouse and *in vitro* experiments (Bach, 2018). IGFBPs have ~10 fold higher affinity for IGFs than the IGF-1R (Bach, 2018). Therefore, one could hypothesize that a duplication or activating mutation of the genes encoding these proteins might lead to short stature. Given the role of IGFBP-3 and IGFBP-5 in the formation of the complex with IGF-I and ALS, one could also speculate that a loss of function variant of IGFBP-3 may have a similar effect as ALS deficiency, and thus would lead to modest short stature. The effect of knockout of IGFBP-3 in mice is dependent on the model and has either no effect (Scully et al., 2016) or promotes growth (Yakar et al., 2009). Therefore, it is difficult to predict the phenotype of a loss of function variant of IGFBP-3 in humans.

IGFBP-6 has a high affinity for IGF-II, ~50 times higher than for IGF-I (Bach, 2018). IGFBP-6 transgenic mice in which human IGFBP-6 cDNA was expressed under the control of the glial fibrillary acidic protein promoter showed high levels of hIGFBP-6 and reduced plasma IGF-I, and a significantly reduced body weight (Bienvenu et al., 2004).

There are also indications that variants in genes encoding IGFBP-4 or the IGFBP-4-specific protease pregnancy-associated plasma protein A (PAPP-A) and its regulatory protein stanniocalcin 2 (STC2) may be associated with linear growth. Two rare coding variants in the *STC2* gene have been found to be associated with increased height (Marouli et al., 2017). STC2 binds and inhibits PAPP-A, and PAPP-A has a role in cleaving and reducing bioactive levels of IGFBP-4 (Jepsen et al., 2015). Thus, in theory, a loss-of function variant of *STC2* is expected to cause a lack of inhibition of PAPP-A, which would presumably lead to increased cleavage of IGFBP-4, resulting in elevations in free IGF-I and thus increased stature (Guo et al., 2018a,b). In contrast, decreased cleavage of IGFBP-4 (for example by a specific variant in *IGFBP4*, or by gain-of-function variants of *STC2*) would be expected to be associated with short stature. This is in line with the fact that both *PAPP-A* and *IGFBP-4* have been associated with height by GWASs (Wood et al., 2014) and that homozygous loss of function mutations in *PAPPA2* result in short stature (Dauber et al., 2016).

Besides the well-known IGFBPs 1–6, there is another class of IGF binding proteins that binds IGFs with low affinity, e.g. IGF binding protein-like 1, also called IGFBP-related protein 4 (IGFBPRP4), encoded

by *IGFBPL1*. A recent case report described a case with a duplication 9p24p12 involving a.o. *IGFBPL1* (Amasdl et al., 2016), who was short and with a low serum IGF-I concentration. Cases with such *de novo* duplication are known to show a clinical syndrome including developmental delay, short stature, microcephaly, facial dysmorphism, hand and toe anomalies, heart defects and/or ocular manifestations (Amasdl et al., 2016), and low serum IGF-I was previously observed in two other cases (Fujita et al., 1976; Stagi et al., 2014). Recently, indirect support for an interaction between IGF-I and IGFBPL1 was provided when IGFBPL1 was identified as a novel regulator for the growth of retinal ganglion cell axons (Guo et al., 2018a,b). IGFBPL1 bound to IGF-I and subsequently induced calcium signaling and mammalian target of rapamycin (mTOR) phosphorylation to stimulate axon elongation. Blockage of IGF-I signaling abolished IGFBPL1-mediated axon growth, and vice versa, IGF-I required the presence of IGFBPL1 to promote RGC axon growth (Guo et al., 2018a,b). IGFBPL1 is closely related to IGFBP-7, with 52% amino acid sequence homology and 43% amino acid identity, and shares a similar domain structure (Cai et al., 2005). IGFBP-7 acts as an IGF-I/-II antagonist which can block IGF-1R activation (Evdokimova et al., 2012). Biallelic loss of function of IGFBP7 causes retinal arterial macroaneurysm with supraaortic pulmonic stenosis (OMIM #614224).

3. Conclusion

The fundamental role of the IGF system in the regulation of growth in the human has been confirmed by clinical observations in individuals carrying pathogenic variants in one of the multiple components of the system summarized here, and by knockout mouse studies. There is no doubt that a loss of function of IGF-I, IGF-II, ALS, PAPP-A-2 and IGF-1R lead to growth failure in both species, with some common clinical features arising in all human cases. Given the complexity of the system and of the post-receptor pathways, we expect that in the coming decades more gene defects associated with this axis will be uncovered, which will lead to a further expansion of insight in the role of the system in growth and development.

Author contributions

BF and JMW wrote the manuscript, JMW prepared the tables and BF and AB designed the figures.

Declaration of competing interest

The authors have no competing interests for this paper.

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