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Genetic causes of growth hormone insensitivity beyond *GHR*

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Abstract

Growth hormone insensitivity (GHI) syndrome, first described in 1966, is classically associated with monogenic defects in the GH receptor (*GHR*) gene which result in severe post-natal growth failure as consequences of insulin-like growth factor I (IGF-I) deficiency. Over the years, recognition of other monogenic defects downstream of GHR has greatly expanded understanding of primary causes of GHI and growth retardation, with either IGF-I deficiency or IGF-I insensitivity as clinical outcomes. Mutations in *IGF1* and signaling component *STAT5B* disrupt IGF-I production, while defects in *IGFALS* and *PAPPA2*, disrupt transport and release of circulating IGF-I, respectively, affecting bioavailability of the growth-promoting IGF-I. Defects in *IGF1R*, cognate cell-surface receptor for IGF-I, disrupt not only IGF-I actions, but actions of the related IGF-II peptides. The importance of IGF-II for normal developmental growth is emphasized with recent identification of defects in the maternally imprinted *IGF2* gene. Current application of next-generation genomic sequencing has expedited the pace of identifying new molecular defects in known genes or in new genes, thereby expanding the spectrum of GH and IGF insensitivity. This review discusses insights gained and future directions from patient-based molecular and functional studies.

Keywords GH insensitivity · IGF-I deficiency · IGF-I insensitivity · GH-IGF-I axis

1 Growth hormone insensitivity, GHI: Historical perspective

The development of human pituitary growth hormone (hGH), followed by the manufacture of recombinant-DNA-derived GH, revolutionized therapy for children with GH deficiency (GHD). To date, tens of thousands of children worldwide have been treated with GH.

The diagnosis and management of GHD has been hampered significantly by recognized limitations in our ability to establish a firm diagnosis in many cases. Major problems include: 1) non-physiologic nature of GH stimulation tests; 2) lack of a satisfactory methodology for resolving conflicting data from two or more stimulation tests; 3) arbitrary definitions of what constitutes a subnormal response to provocative testing; 4) insufficient attention to effects of age, pubertal status, sex steroid priming; and 5) reproducibility of GH assays. With the recognition of the pivotal role of insulin-like growth factors (IGFs) in the regulation of skeletal growth, it has been proposed that a more appropriate diagnostic categorization would be the classification of disorders resulting in IGF deficiency [1].

Such a classification, first proposed in 1996 [1], is shown in Table 1. At the time of the writing, almost twenty-five years ago, everything shown in bold italics was theoretical. In subsequent years, patients with these defects have been reported.

The initial examples of GH insensitivity were reported by Laron and colleagues, who described "three siblings with hypoglycemia and other clinical and laboratory signs of growth hormone deficiency, but with abnormally high concentrations of immunoreactive serum growth hormone" [2]. To date, several hundred such cases have been identified worldwide, the majority from the Mediterranean region or from Ecuador, in presumed descendants of Spanish "conversos" (Jews who converted to Christianity during the Inquisitions of the 15th and 16th centuries) [3]. As more molecular defects of the *GHR*

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Table 1 Classification of IGF deficiency (1996)

(I) Hypothalamic dysfunction
(II) Pituitary GHD
(III) GH Insensitivity
  (A) Primary GHI
    (1) GH receptor deficiency
      (a) Mutations/deletions of the extracellular domain of the GH receptor
      (b) Mutations/deletions of the transmembrane domain of the GH receptor
      (c) Mutations/deletions of the intracellular domain of the GH receptor
    (2) Abnormal GH signal transduction due to defects distal to the GH receptor
  (B) Secondary GHI
    (1) Malnutrition
    (2) Liver disease
    (3) Chronic disease
(IV) Primary defects of IGF synthesis
(V) Primary defects of IGF transport/clearance
(VI) IGF resistance

From: Rosenfeld RG: Biochemical diagnostic strategies in the evaluation of short stature: the diagnosis of insulin-like growth factor deficiency. *Horm Res* 46:170–173, 1996

Everything shown in ***bold italics*** was theoretical at the time of the writing (1996)

Table 2 Proposed classification of growth hormone insensitivity

Primary GH insensitivity (Hereditary defects)
1. GH receptor defect (may be positive or negative for GH binding protein
  a. Extracellular mutation
  b. Cytoplasmic mutation
  c. Intracellular mutation
2. GH signal transduction defects (distal to cytoplasmic domain of GH receptor)
  a. STAT5B mutations
3. IGF-I defects
  a. IGF-I gene deletion or mutation
  b. IGF-II gene deletion or mutation
  c. IGF-I transport defects
    (1) ALS mutations
    (2) PAPPA-2 mutations
  d. IGF-I receptor defects
4. Bioinactive GH molecule (responds to exogenous GH)

Secondary GH insensitivity (Acquired defects)
1. Circulating antibodies to GH that inhibit GH action
2. Antibodies to the GH receptor
3. GH insensitivity caused by malnutrition, liver disease, catabolic states, diabetes mellitus
4. Other conditions that cause GH insensitivity

Derived from Backeljauw PF, Dattani MT, Cohen P, Rosenfeld RG: Disorders of Growth Hormone/Insulin-like Growth Factor Secretion and Action. *In:* Sperling MA (ed) Pediatric Endocrinology, Elsevier, Philadelphia 2014

gene have been identified, however, it has become apparent that this condition exists worldwide.

As additional molecular defects resulting in GH and IGF insensitivity have been uncovered, the diagnostic classification of GHI has evolved (Table 2). Correlations between genotype and GHI phenotype, however, remain imperfect, but there is increased appreciation for the wide phenotypic spectrum of GHI, even within the *GHR* gene classically associated with the severest form of GHI [4]. In this review, we will summarize new genetic defects downstream of the GHR and highlight how they contribute to the spectrum of GHI.

2 Molecular defects associated with IGF-I deficiency

The linear growth promoting effects of GH are mediated by regulating hepatic and peripheral IGF-I production. Secreted hepatic IGF-I circulates in complex with IGF binding proteins (IGFBP), particularly IGFBP-3, and acid labile subunit (ALS, aka IGFALS). Circulating IGFBP-3 and ALS are also targets of human GH (hGH) regulation and produced predominantly from the liver. Measurements of serum IGF-I, IGFBP-3 and ALS, have thus served as diagnostic markers of *in vivo* responsiveness to GH, both pituitary-derived hGH and exogenous recombinant hGH (rhGH). Remarkably, our understanding of how GH regulates hepatic IGF-I, IGFBP-3 and ALS, in humans has remained limited, despite insights gained from mammalian and non-mammalian models. Identifying molecular etiologies of IGF-I deficiency has expanded our understanding beyond classical *GHR* deficiencies [5–7], aided by the advent of next-generation genomic sequencing.

The binding of GH to cell surface homodimeric GHR, a type I cytokine receptor lacking intrinsic tyrosine kinase activities, initiates Janus kinase 2 (JAK2)-dependent [8] signal transduction, in which JAK2 phosphorylates the 7 tyrosines within the human GHR intracellular domain [9], and phosphorylates signaling components recruited to the activated GHR (Fig. 1). Although genetic defects have been identified in many of components involved in GHR-JAK2 signaling, only monogenic defects within the signaling component *STAT5B* and *IGF1*, a known gene target of STAT5B, have proven to be causal of severe primary IGF-I deficiency. The genetic defects in these two genes are reviewed below. It is of note that an estimated ~60% of children diagnosed with GHI, IGF-I deficiency and short stature, do not carry mutations in *GHR*, *STAT5B*, or *IGF1* [10], suggesting molecular understanding of IGF-I deficiency and GHI remain to be more fully defined.

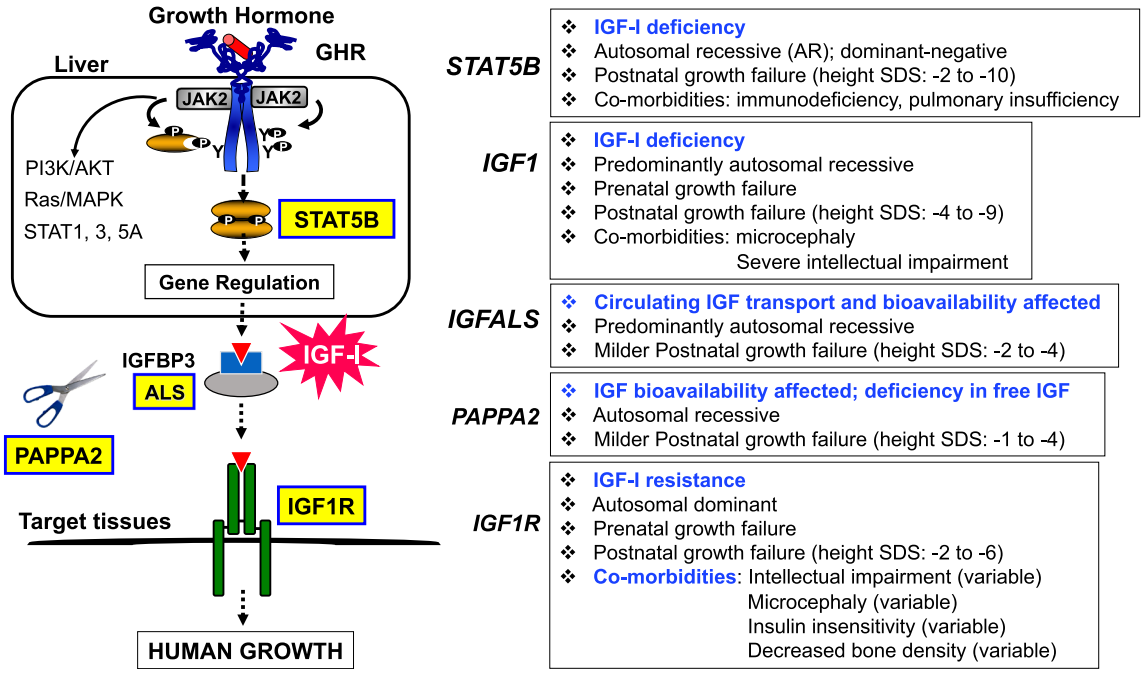


Fig. 1 Schematic of the human GH-IGF-I axis. Interaction of GH with cell surface dimeric GH receptors (GHR) leads to recruitment of Janus kinase 2 (JAK2), and subsequent activation of multiple signaling cascades. Recruited STAT5B is tyrosine phosphorylated by JAK2, homodimerizes, and translocate to the nucleus, where it binds DNA, regulating production of circulating IGF-I, IGFBP-3 and ALS. PAPPA2 proteolysis of circulating IGFBP-3 and -5, generates free IGF-I. Mutations in a number of components along this axis result in GHI and IGF deficiency (*STAT5B*, *IGF1*), IGF bioavailability (*IGFALS*, *PAPPA2*) or IGF resistance (*IGF1R*). Co-morbidities associated with mutations are indicated

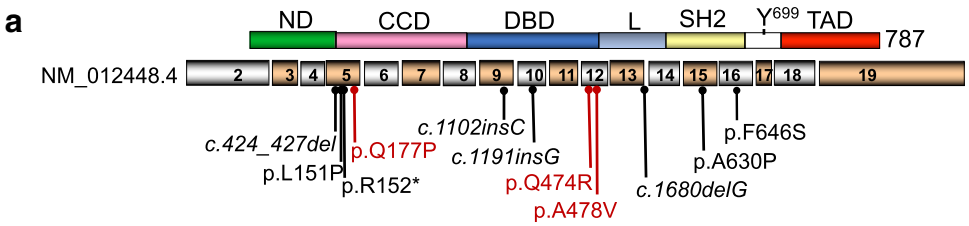
2.1 GH signal transduction defect: *STAT5B* mutations

The human signal transducers and activators of transcription (STATs) are a ubiquitously expressed family of 7 structurally similar cytosolic proteins that are activated by multiple growth factors and cytokines and participate in a plethora of biological functions [11]. To date, pathophysiological genetic defects, germline and somatic, have been identified in all STATs except STAT5A [12–16]. STAT5A is most closely related to STAT5B, sharing >90% amino acid identity. Distinct immune deficiencies are associated with each defective STAT, but only with germline STAT5B deficiency (MIM #245590) are severe growth impairments and impaired IGF-I production consistently observed.

Four of the 7 STATs (STAT1, 3, 5A, 5B) can be activated by the mammalian GH-GHR system. In humans, the pivotal role of STAT5B in transducing GH signals to the *IGF1* gene was confirmed upon identification of homozygous, autosomal recessive, *STAT5B* inactivating mutations associated with severe GHI, IGF deficiency, and post-natal growth failure [17–24]. The first homozygous inactivating *STAT5B* mutation was reported in a 16-yrs old female who had normal *GHR* but a clinical growth phenotype reminiscent of classical GHR deficient patients [17]. The identified missense STAT5B p.A630P mutation, located in the SH2 domain (Fig. 2a), resulted in domain and protein instability [25, 26], very poor immune detection and no activation in both primary cells [17, 27] and reconstituted systems [28]. This was the first indication that in humans, unlike in rodent *Stat5b*$^{-/-}$ knock-out models [29, 30], the closely related STAT5A, demonstrably functional in patient cells [28], could not compensate for loss of STAT5B.

Subsequent homozygous *STAT5B* mutations identified in STAT5B deficient patients, 4 males and 7 females, further supported the phenotypic resemblance to patients carrying autosomal recessive *GHR* mutations [4, 7, 31, 32], including mild facial dysmorphic features, such as a prominent forehead, depressed nasal bridge and high-pitched voice [17, 19, 22]. *STAT5B* mutations identified by targeted Sanger sequencing or whole exome sequencing (WES), are scattered throughout the *STAT5B* gene (Fig. 2a, b). Five out of the 8 mutations result in early protein termination (1 nonsense, 4 frame-shifts) and 3 are missense mutations of which only 1 (*c.452 T> C*, p.Leu151Pro) appeared to be expressed as well as wild-type STAT5B in EBV-transformed B cells [24]. The remaining 2 missense mutations, include the first described STAT5B mutation [17], are both located within the SH2 domain [23].

The growth phenotype for STAT5B deficiency can be summarized as follows [33]. Birth size was appropriate for gestation (8 out of 9 patients) and birth was generally without complications although for 6 out of the 9 cases, birth was before 37 weeks of gestation. Postnatal growth failure in all cases was observed, consistent with the degree of IGF deficiency, and indistinguishable from those with GHI syndrome



b

Affected individuals	Exon	cDNA	Protein	Reference
Homozygous				
2#	5	*c.424_427del*	p.Leu142Argfs*20	Pugliese-Pires PN, et al, 2010, Eur J Endocrinol 163:349-355
1	5	*c.452T>C*	p.Leu151Pro	Acres MJ, et al, 2019, J Allergy Clin Immuno 143:413-416 e4
2	5	*c.454C>T*	p.Arg152*	- Bernasconi A, et al, 2006, Pediatrics 118:e1584-e1592. - Boyanovsky A, et al, 2009, 8th Joint Meeting of the Lawson Wilkins Pediatric Endocrine Society/European Society for Paediatric Endocrinology, P01-067
1	9	*c.1102insC*	p.Gln368Profs*9	Vidarsdottir S, et al, 2006, J Clin Endocrinol & Metab 91:3482-3485
1	10	*c.1191insG*	p.Asn398Glufs*16	Hwa V, et al, 2005, J Clin Endocrinol & Metab 90:4260-4266
2#	13	*c.1680delG*	p.Glu561Argfs*17	Hwa V, et al, 2007, Horm Res 68:218-224
1	15	*c.1888G>C*	p.Ala630Pro	Kofoed EM, et al, 2003, N Engl J Med 349:1139-1147
1	16	*c.1937T>C*	p.Phe646Ser	Scaglia PA, et al, 2012, J Clin Endocrinol & Metab 97:E830-E839
Dominant-Negative				
2#	5	*c.530A>C*	p.Gln177Pro	Klammt J, et al, 2018, Nat Commun 9:2105
4$	12	*c.1421A>G*	p.Gln474Arg	Klammt J, et al, 2018, Nat Commun 9:2105
5$	12	*c.1433C>T*	p.Ala478Val	Klammt J, et al, 2018, Nat Commun 9:2105

Fig. 2 STAT5B mutations. **a** Schematic of human STAT5B protein and corresponding exons based on transcript NM_012448.4. Homozygous mutations (black) and dominant-negative mutations (red) are indicated. ND, N-terminal domain; CCD, coiled-coiled domain; DBD, DNA binding domain; L, linker; SH2, ν-src homology 2; TAD, transcriptional activation domain. Y^{699}, tyrosine 699 that is phosphorylated. **b** mutations tabulated. #, siblings; $, includes family members

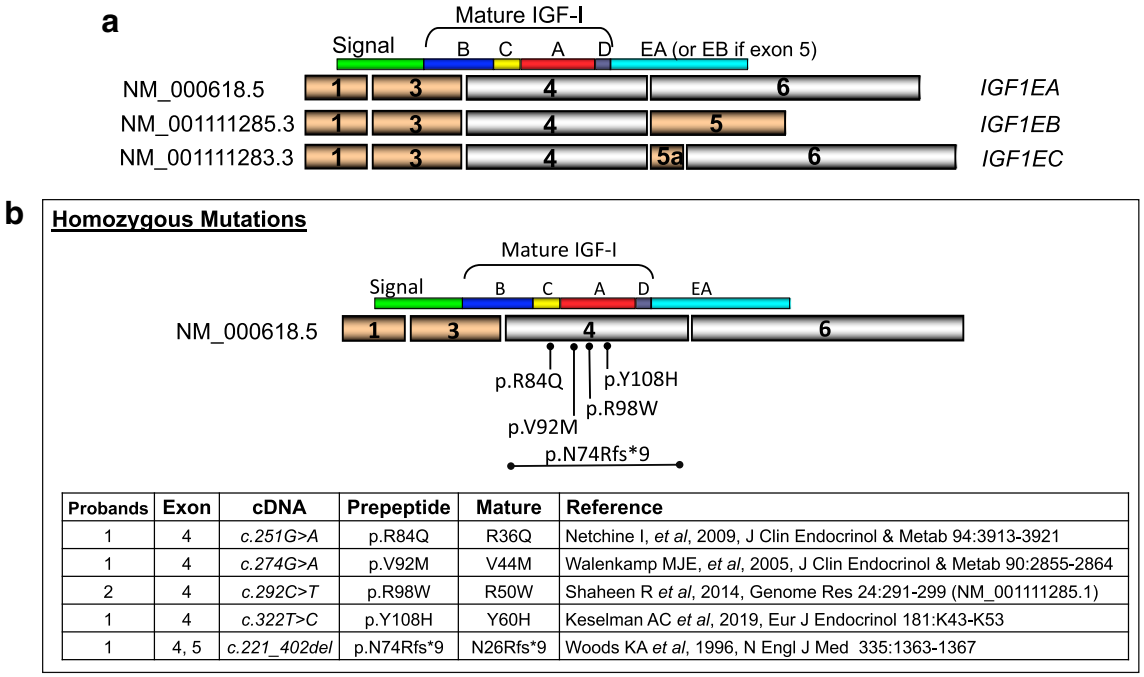
due to *GHR* mutations [34]. Height SDS at first report (ages 2 yrs. to 31 yrs) ranged from −3.0 SDS to −9.9 ($n = 11$). Bone age was delayed in 7 out of 7 patients [19, 22]. Puberty was also consistently delayed (7/8 patients), reflecting the low levels of circulating IGF-I. As expected, serum IGF-I was below normal in all 11 known STAT5B deficient patients, as was serum IGFBP-3 (10/10) and IGFALS (6/6) when analyzed. Unique to STAT5B deficiency, serum prolactin was markedly elevated in 6 of 8 patients, independent of gender.

In addition to homozygous mutations, dominant-negative inactivating STAT5B mutations (Fig. 2b) were recently reported in patients who exhibited GHI, IGF-I deficiency and post-natal growth deficits less severe than those characteristic of typical homozygous STAT5B deficiency [33] but significantly lower when compared to asymptomatic carriers of autosomal recessive STAT5B mutations. Strikingly, the mean height SDS of the 11 subjects carrying dominant-negative STAT5B mutations resembled those of children with dominant-negative *GHR* mutations [35–40] and children carrying *GHR* pseudo-exon 6 [41–43]. These clinical observations, together with severe height deficits comparable between autosomal recessive GHR deficiency and STAT5B deficiency, fully supported the importance of the GHR-STAT5B signaling for post-natal linear growth and IGF-I production in response to GH.

Additional co-morbidities of STAT5B deficiency absent in patients who are GHR deficient, are the significant immune dysregulation [27, 32, 44, 45] with potentially fatal pulmonary insufficiency. As documented for the first reported case, the patient carrying STAT5B p.A630P succumbed to pulmonary insufficiency by age 30 yrs. [23, 44]. Since multiple cytokines activate STAT5B and STAT5A, frequently referred together as STAT5, the clinical observation of immune deficiency in STAT5B deficient patients indicate the inability of functional STAT5A to compensate for loss of STAT5B. To date, only one STAT5B deficient patient appears to lack the typical severe immune and pulmonary problems associated with STAT5B deficiency, surviving to age 31 yrs. [46], while 2 younger patients presented with autoimmune and other immune irregularities but without pulmonary sequalae [23, 24, 44]. Dominant-negative *STAT5B* inactivating mutations only mildly impaired immunity, in contrast to distinct growth effects [33]. Of note, severe immune complications in a 33 yr old male was attributed to a dominant-negative STAT5B (p.Q206R in the CCD), although stature of the patient was not indicated [47] and it is unclear if symptoms could be due solely to the heterozygous missense variant reported. Collectively, these clinical observations suggest that STAT5B functions critical for growth and IGF-I production can be delineated from those critical for immune functions, with mechanisms still needing to be clarified.

2.2 Disruption of IGF-I production: *IGF1* mutations

The human *IGF1* gene, >84 kilobases (kb) on chromosome 12q23.2, consist of 6 exons, which, through alternative splicing from two promoters, generates multiple pre-propeptide transcripts [48]. Exons 3 and 4, common to all transcripts, encode for 4 structural peptide domains (B, C, A and D, Fig. 3a) of the mature peptide [49–51]. A homozygous



Probands	Exon	cDNA	Prepeptide	Mature	Reference
1	4	*c.251G>A*	p.R84Q	R36Q	Netchine I, *et al*, 2009, J Clin Endocrinol & Metab 94:3913-3921
1	4	*c.274G>A*	p.V92M	V44M	Walenkamp MJE, *et al*, 2005, J Clin Endocrinol & Metab 90:2855-2864
2	4	*c.292C>T*	p.R98W	R50W	Shaheen R *et al*, 2014, Genome Res 24:291-299 (NM_001111285.1)
1	4	*c.322T>C*	p.Y108H	Y60H	Keselman AC *et al*, 2019, Eur J Endocrinol 181:K43-K53
1	4, 5	*c.221_402del*	p.N74Rfs*9	N26Rfs*9	Woods KA *et al*, 1996, N Engl J Med 335:1363-1367

Fig. 3 Rare homozygous *IGF1* mutations. **a** Three major *IGF1* transcripts expressed by most tissues, are from promotor P1. Exons correlating to propeptides, consisting of signal peptide (residue 1–32) followed by common domains B, C, A and D encoded by exons 3 and 4, and variable domain E which is post-translationally removed; **b** Homozygous *IGF1* mutations are indicated and tabulated

deletion of exons 4 and 5 was the first identified human *IGF1* mutation, resulting in a predicted truncated peptide, p.Asn74Argfs*9, and undetectable serum IGF-I concentrations [52]. Four homozygous *IGF1* defects have since been reported, all missense (Fig. 3b), located in domains C or A, encoded by exon 4 [53–56]. Three of the four missense mutant IGF-I peptides were still detectable in sera of patients, but lost or demonstrated significantly reduced affinity, for the IGF-I receptor, IGF1R [53–57]. The pathogenicity of the fourth missense variant, p.Arg98Trp, was not investigated [55], although predicted to be damaging by *in vitro* programs such as PolyPhen-2, and, thus, remains a variant of unknown significance.

Homozygous *IGF1* mutations identified to date are inherited as autosomal recessive defects (MIM #608747). Consistent clinical features are intra-uterine growth retardation (IUGR), microcephaly, retarded intellectual development and severe postnatal growth failure, features which, interestingly, overlap with the phenotype of microcephalic primordial dwarfism [58]. Indeed, the variant of unknown significance, *IGF1* p.Arg98Trp, identified in siblings, was part of a cohort clinically diagnosed with primordial dwarfism disorder [55]. Additional features of severe IGF-I deficiency observed in 3 of the patients include facial dysmorphism, sensorineural deafness, and global developmental delay [52, 53, 56, 59], and insulin resistance was described in the first reported patient [52, 60]. Cranial circumferences of these 3 probands ranged from −8.0 to −4.9 SDS, with height SDS -9.0 to −6.2 [52, 53, 59]. The patient carrying the p.Arg84Gln mutation who, in contrast, did not have hearing loss and had a relatively milder clinical phenotype, with cranial circumference of −2.5 SDS, and height SDS, −4.5 [54]. *In vitro* evidence suggest the p.Arg84Gln mutation still retained residual IGF-I function compared to p.Val92Met [54], further suggesting that severity of phenotype may correlate with severity of mutant IGF-I dysfunction. It is noteworthy that co-morbidities observed in patients carrying *IGF1* mutations are absent in patients who carry *GHR* or *STAT5B* mutations, supporting IGF-I production independent of the GH-STAT5B pathways in the fetus and possibly in peripheral tissues.

The identification of autosomal recessive *IGF1* mutations suggests that one normal copy of the *IGF1* gene may be sufficient for relatively normal growth. A deletion of 262 kb on chromosome 12 which included the entire *IGF1* gene, however, was associated with microcephaly and short stature, suggesting *IGF1* haploinsufficiency could be pathogenic [61]. The deletion, inherited from the short statured father, was revealed by CNV (copy number variant) analysis in a male patient age 8 years and 5 months whose head circumference was −3.4 SDS and height SDS, −2.7. Serum IGF-I concentration of the young proband was low-normal. It remains unclear whether non-coding genomic regions outside the *IGF1* coding regions contributed to the clinical phenotype. Two other reported pathological heterozygous *IGF1* defects are a splicing mutation (*c.402 + 1G > C*) that led to excision of exon 4 [62], and a four nucleotide duplication in exon 4 (*c.243_246dupCAGC*) which created a frameshift and early protein termination [63]. For both, the mutations segregated

with short stature (height SDS -1.4 to −6.4) in the respective families, serum IGF-I concentrations were low-normal or modestly below the normal range, and serum IGFBP-3 and ALS levels were normal to elevated. Of note, the splicing *c.402 + 1G > C* variant is predicted to generate a truncated IGF-I peptide similar to the first described autosomal recessive *IGF1* mutation in which exons 4 and 5 were deleted [52]. While parents of the proband carrying exons 4–5 del were asymptomatic and of normal stature, albeit below the mean, carriers of *c.402 + 1G > C* were significantly short statured [62]. Altogether, it remains to be fully elucidated how heterozygous *IGF1* defects (haploinsufficient, severely truncated peptides) exerted their pathological effects on growth.

3 Molecular defects affecting IGF-I bioavailability

The bioavailability of IGF-I is dependent on its release from associated IGFBPs, which has a higher affinity ($k_d \sim 10^{-10}$ M) for IGF-I than does cell surface IGF1R ($k_d \sim 10^{-8}$-10^{-9} M). IGFBPs thus act both as carriers of IGFs, prolonging the half-life of the IGFs, and as modulators of IGF availability and activity [64, 65]. No human mutations in IGFBPs have been identified to date. In the circulation, the IGF-I-IGFBPs binary complexes are further protected by ALS. Here, we discuss defects in two genes which affect IGF-I bioavailability: *IGFALS*, encoding the carrier ALS protein, and *PAPPA2*, a IGFBP-3 and IGFBP-5 protease recently demonstrated to be clinically important for normal linear growth.

3.1 IGF-I transport defects: *IGFALS* mutations

The majority of post-natal circulating IGF-I is generated by the liver, with production regulated by pituitary-derived GH. IGF-I circulates bound to IGFBPs, mainly IGFBP-3 and IGFBP-5, ~85% of which form ternary complexes with ALS, a soluble protein of ~85 kDa. The ternary complex formation, a 1:1:1 ratio of IGF:IGFBP:ALS, significantly prolongs the half-life of circulating IGF-I-IGFBP binary complexes (and IGF-II-IGFBP complexes, see below) [66]. An updated structural rendition of ALS, a glycoprotein belonging to the leucine-rich repeat (LRR) superfamily, proposes 21 LRR motifs that are arranged in a highly structured 3-dimentional horseshoe-shape with positive charged patches on the outer surface and an extensively negatively charged inner concave surface which may permit interactions with IGFBP-3 and IGFBP-5 [67].

The *IGFALS* gene (16p13.3) encoding ALS, is a small gene spanning 3.3 kb and carrying only 2 exons (exon 1 encoding only 5 of the 605 amino acid residue ALS protein). The first homozygous mutation of *IGFALS* (MIM *601489, #615961), a frameshift defect, *c.103delG*, was described in 2004 in a GHI patient whose most striking feature was a mismatch between extreme deficiencies of circulating IGF-I, IGFBP-3 and ALS and a relatively mild growth failure of height SDS -2.1 [68]. To date, over 30 homozygous and compound heterozygous *IGFALS* mutations in more than 62 patients have been formally reported, inherited as recessive defects. Patients with ALS deficiency (ACLSD) were phenotypically similar to the first described case, presenting with height deficits ranging from −4.0 SDS to −2.0 at time of report, some of whom reached final heights within the normal range [7, 69–73]. Puberty delay and insulin insensitivity have been frequently reported. An absence of ALS leads to loss of ternary complex formation and serum IGF-I concentrations consistent with severe IGF-I deficiency. Pharmacokinetics evidence, however, support the hypothesis that production of serum IGF-I is normal but rapidly cleared [74]. The preservation of local production of IGF-I, notably at the growth plate, furthermore, likely contributes towards tempering growth failure, a feature which is significantly more impaired when IGF-I deficiency is global.

Partial ALS deficiency has recently been proposed to be associated with heterozygous *IGFALS* carriers. The clinical phenotype indicated that although stature was within normal ranges, these asymptomatic carriers were statistically shorter than relatives who were normal for *IGFALS* [69]. In two reports, heterozygosity for *IGFALS* mutations resulted in approximately 1.0 SDS height loss in comparison to non-carriers [75, 76], whereas homozygosity or compound heterozygosity gave a further loss of 1.0–1.5 SD [75]. It has been suggested that identified heterozygous *IGFALS* variants could be the etiology for a subset of children classified as idiopathic short stature (ISS) [77, 78].

All *IGFALS* defects are located in exon 2, encoding the mature ALS peptide. *IGFALS* defects include a spectrum of missense and nonsense mutations, duplications, in-frame duplication, frameshift insertions and deletions [7, 79]. Functional integrity of identified variants has not been systematically evaluated (e.g. serum ALS concentrations assessed by ELISA, immunoblot analysis, and ability to form ternary complexes). One missense variant functionally well characterized and of particular note is a homozygous p.D440N identified in a 12 year old male with height SDS of −2.9 and typical biochemistries consistent with ALS deficiency [80]. The p.D440N mutation, within LRR17 on the ALS inner concave surface of the updated ALS model [67], created a consensus motif for N-glycosylation [80]. Detailed *in vitro* functional analysis supported the mutation generating a hyperglycosylated form of ALS with impaired secretion and ternary complex formation [81]. For other ALS missense variants, the highly structured and charged characteristics of the ALS protein are unlikely to easily accommodate amino acid substitutions [67]. Truncated protein variants, if stably expressed, could destroy the ability to bind IGF-IGFBP complexes, although precise interactive sites between ALS and IGF-IGFBP have yet to be determined.

3.2 Disruption of IGF-I bioavailability: Inactivating *PAPPA2* mutations

The human pregnancy-associated plasma protease A2 gene, *PAPPA2*, on chromosome 1q23-q25, encodes for a large 1791 amino acid residue protein, >200 kDa in size (Fig. 4a). A circulating metalloproteinase member of the pappalysin family, PAPPA2 specifically cleaves IGFBP-3 and IGFBP-5.

Recessive mutations in the *PAPPA2* was recently reported in 2 unrelated families in which the five affected children had postnatal retardation with heights discordant from their mid-parental target height [82]. The degree of short stature was variable, with heights ranging from −1.0 to −3.8 SDS. Serum GH and IGF-I levels were markedly elevated in the patients and elevated levels of ALS, IGFBP-3 and IGFBP-5 were noted, leading to initial speculations that these patients were classic cases of IGF-I resistance. Target sequencing of *IGF1R*, a direct cause of IGF-I resistance (see below), however, were unrevealing.

Whole exome sequencing (WES) analysis identified, in the first family, a homozygous frameshift mutation in *PAPPA2* (*c.1927_1928insAT*, p.D643fs25*) which resulted in undetectable *PAPPA2* protein and elevated IGF-I-IGFBP-ALS ternary complex formation [82]. In the second family, an expressed homozygous *PAPPA2* missense mutation (*c.3098C > T*, p.A1033V), independently identified by WES, was functionally impaired and could not proteolyze either IGFBP-3 or IGFBP-5 in *in vitro* reconstitution assays [82]. For all 5 affected children, although total serum IGF-I were high, serum free IGF-I and bioactive IGF-I, both parameters not typically measured, were abnormally low. In addition to short stature, moderate microcephaly, thin long bones, low bone mineral density, and insulin resistance were variably reported [82–85]. Modest growth responses to recombinant human IGF-I therapy have been reported [83, 86], supporting the importance of free IGF-I for growth. Interestingly, mouse models, *Pappa2* knock-out [87] and, more recently, *Pappa2* knock-in of the human p.A1033V mutation [88], recapitulated the human phenotypes and biochemistries. Both the human studies and mouse models provided the first evidence that free IGF-I is more critical than total IGF-I for the negative modulation of GH secretion from the hypothalamic-pituitary axis (Fig. 4b) [82, 88, 89].

Altogether, inactivating mutations in *PAPPA2* suggest that the prolonged sequestration of IGFs by IGFBPs is clinically significant, presenting as "IGF-I resistance" because of high total serum IGF-I levels but proven as a new mechanism for modulating IGF-I bioavailability.

4 Molecular defects associated with IGF-I resistance

Clinically, resistance to IGF-I actions have been based in part on normal or elevated serum IGF-I incongruent with growth failure and significant short stature. However, this definition is very broad as there are many growth disorders, such as those associated with skeletal dysplasia, in which mutations are outside of the traditional GH-IGF axis pathway. Here, we will focus on *IGF1R*, a gene which directly impacts IGF-I actions. We will also discuss recent identification of defects in the *IGF2* gene (encoding the IGF2 peptide) associated with elevated IGF-I but with phenotypic features of Silver-Russell Syndrome.

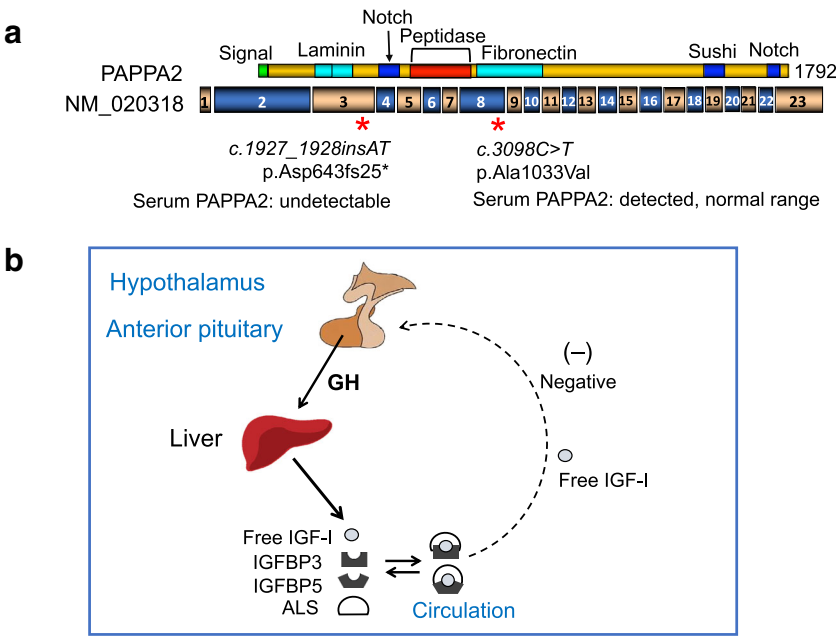


Fig. 4 Human *PAPPA2* mutations. **a** Schematic of protein and exons encoding the protein. The two described mutations are indicated. **b** A simplified model proposing that the negative feedback loop of circulating IGF-I for regulating GH secretion is dependent on free IGF-I availability

4.1 Disruption of IGF-I action: *IGF1R* mutations

IGF-I mediates its growth-promoting actions by binding to and activating IGF1R, a ubiquitously expressed cell-surface tyrosine kinase receptor, which also mediates the biological effects of IGF-II. The IGF1R protein is encoded within 21 exons of the 315 kilobase *IGF1R* gene on chromosome 15q26.3. Synthesized as a single polypeptide precursor including a secretory signal peptide of 30 amino acid residues, the IGF1R is proteolytically cleaved into α- and β chains and reassembled as a mature, functional, tetramer ($\alpha_2\beta_2$). IGF1R is structurally similar to the insulin receptor, with the extracellular dimeric α subunits involved in ligand binding (affinity for IGF-I ≥ IGF-II >> insulin), and intrinsic tyrosine kinase activity located in the β_2 subunits [90]. Ligand binding by IGF1R induces receptor autophosphorylation and subsequent activation of multiple downstream signaling pathways, including the PI3K/Akt and MAPK/Erk pathways important for cell survival and cell growth [91].

In 1991, chromosomal 15q26-qter deletions (MIM #612626) in an infant and other patients had led to speculation that the multitude of shared co-morbidities, including microcephaly, severe intrauterine growth retardation (IUGR) and post-natal growth deficiency, could be related to documented loss of 1 copy of the *IGF1R* gene [92]. Mouse knock-out models in 1993 subsequently indicated the importance of IGF-I and IGF1R for normal mammalian growth [93, 94]. In both knock-out models, IUGR was of note. Post-natal growth for *Igf1*$^{-/-}$ was significantly retarded by ~60% but the *Igf1r*$^{-/-}$ knock-out died perinatally, due to respiratory failure as consequences of muscle hypoplasia [93, 94]. It was not until 2003, however, that the first human *IGF1R* mutations (MIM *147370) was reported [95]. A heterozygous nonsense mutation (reported as R59* using amino acid numbering based on mature peptide lacking the 30 amino acid signal peptide) and compound heterozygous missense mutations (mature peptide nomenclature, R108Q/K115N), were reported in children presenting with IUGR, post-natal growth failure and variable co-morbidities [95]. Since this first report, the number of heterozygous mutations and cases reported have rapidly increased [7, 96], expedited by recent availability and application of genomic whole exome sequencing for genetic screening of short-statured patient cohorts. Between 2016 and 2020, for example, more than 40 potential pathological *IGF1R* variants have been identified [97–109], adding to the previous list of ~20 mutations.

The accumulated genetic data clearly support *IGF1R* gene dosage effects on growth, with mutations that lead to IGF1R haploinsufficiency able to impart distinct clinical growth impairment while three copies of *IGF1R* gene have been associated with tall stature [110, 111]. When *IGF1R* variants were functionally evaluated, either in cells derived from the patient and/or in *in vitro* reconstitution systems, expression and IGF-I-induced IGF1R signaling have been demonstrated to be reduced [112–115] in an I*GF1R* haploinsufficiency state, although binding of IGF-I may remain normal [115–117], stressing the necessity of bi-allelic expression of the *IGF1R* gene for full biological activity. Further, rare homozygous mutations, four reported to date [104, 109, 118, 119] (Fig. 5), all in the α-chain, appear to retain residual functions as was demonstrated for IGF1R p.R40L [118], IGF1R p.P733_R734ins25 (an in-frame insertion of intron 10 [119]), and p.F112L [109]. Of note, expression and function of a recently reported homozygous mutations, p.E56D [104], is unknown. Nevertheless, the implications are that complete ablation of *IGF1R* may be lethal for human as for rodent models.

Nucleotide changes with correlating amino acid alterations are found scattered throughout the IGF1R coding sequence (Fig. 5), consistent with a lack of "hot-spots" for inactivating *IGF1R* mutations. Evidence of pathogenicity has been demonstrated for many, but not all identified *IGF1R* variants. In particular, for the ~65% of IGF1R labeled as missense mutations (40 out of ~60 reported mutations), functional assessments can help determine if the variant is pathological (a mutation) or benign. Additional insights gained include mechanism of dysfunction such as dominant-negative effects [120], abnormal receptor trafficking [121], inactivated kinase activities [120, 122]. *In lieu* of functional analysis, in silico programs have been heavily utilized for prediction of potential protein damaging effects of variants. Walenkamp et al. [103], for example, utilized the most comprehensive, multi-level, set of criteria to inform pathogenicity of missense variants in absence of functional tests. The assessment involved InterVar (http://wintervar.wglab.org), a bioinformatics software tool for clinical interpretation of genetic variants following guidelines set by the American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) and co-segregation of variants in nuclear family, as the first criterion; the next set of criteria involved prediction algorithms (SIFT, Mutation Taster, PolyPhen-2) integrated with clinical characteristics in various combinations; and final expectation that all likely pathogenic variants should have a CADD score (https://cadd.gs.wshington.edu) of >24. From this analysis, 14 missense variants were deduced to be likely pathogenic and 6, likely benign [103].

The remaining 35% of all reported IGF1R mutations, consisting of nonsense and frameshifts as consequences of in/dels or splicing defect, lead to predicted early protein termination and are readily accepted as pathogenic without functional proof. The importance of validating pathogenicity even for deletion mutations, however, was emphasized in a recent case in which an in-frame deletion of 3 nucleotide, c.*80_82del*, encoding a single amino acid, pT28del, was identified in a subject selected for genetic analysis as part of a cohort of short statured children with high IGF-I [106]. The

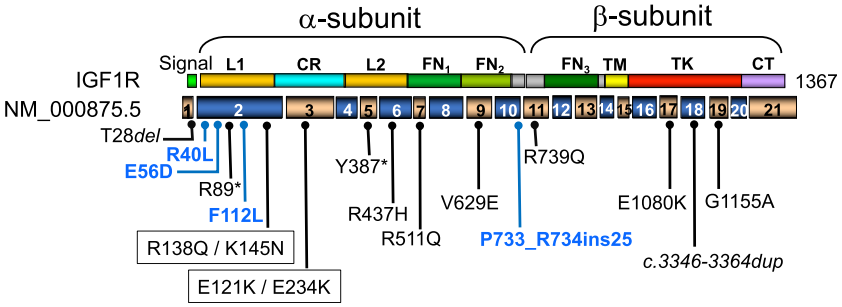


Fig. 5 Schematic of IGF1R protein and encoding exons. Sample of mutations from the ~60 reported, are shown, scattered along the gene. Homozygous, in blue; severest compound heterozygous mutations, boxed. Remaining mutations are heterozygous and autosomal dominant. L1 and L2, receptor L-domains; CR, Cysteine-rich region; FN, fibronectin type-III regions; TM, transmembrane domain, TK, tyrosine kinase domain; CT, C-terminal region

amino acid Thr28 lies within the secretory signal peptide of IGF1R (residues 1–30). Removal of the signal peptide is essential for appropriate localization of IGF1R to the cell surface and the loss of Thr28 was predicted to impact normal cleavage of the signal peptide (http://www.cbs.dtu.dk/services/SignalP/). In functional reconstitution studies, the mutant IGF1R did not accumulate intracellularly as might be expected if unable to traffic to the cell surface, but was, surprisingly, poorly expressed, suggesting that loss of a functional secretory signal may have re-directed the mutant peptide for degradation [106]. Although patient cells were not available for analysis, the authors concluded that this novel heterozygous mutation, the first to be described in the signal peptide of IGF1R, was causal of the growth deficiency in the young female patient (short stature, height SDS -3.1; microcephaly, head circumference of −3.6 SD; elevated serum IGF-I).

The most severe clinical presentations are from patients carrying homozygous *IGF1R* mutations [104, 109, 118, 119] and 2 cases of *IGF1R* compound heterozygous mutations [95, 123] (see Fig. 5). These children presented with IUGR, microcephaly, post-natal height SDS ranging from −3.75 to −5.9. Co-morbidities include global developmental delay, intellectual impairment, insulin resistance evident in teenage years, and sensorineuronal hearing loss. Interestingly, two recently reported compound heterozygous variants are associated with milder phenotypes [101, 109]. The functional impacts of p.R895Q/E769Q is unknown [101]. Each heterozygous variant, predicted to be deleterious by MutationTester, was inherited from a parent of normal stature, and the male child, born appropriate for gestational age, was notable for growth retardation with a height SDS of −2.6 by age 4 yrs., but no endocrine evaluations were performed [101]. The second inherited compound heterozygous mutation, p.S1180Y/G1352S, was identified in a boy who was born small for gestational age (birth weight, −2.2 SD; birth length, −2.7) and microcephalic (head circumference of −3 SD), but by age 6, exhibited improved growth, height SDS -1.6, although he remained microcephalic and serum IGF-I remained elevated above the normal range [109]. IGF-I-induced AKT signaling of primary dermal fibroblasts from the patient and parents demonstrated ~50% blunted responses [109]. Since the father, who carried p.G1352V was of normal stature, height SDS 1.0, and the mother who carried p.S1180Y, was short with height SDS -2.4, further investigations are needed to reconcile cellular studies with clinical phenotype.

Phenotype-genotype correlation remains limited. A simple clinical scoring system was recently proposed for indication of *IGF1R* genetic analysis [103]. Three clinical features (birth weight and/or birth length SDS, height SDS, and head circumference SDS at presentation) and IGF-I biochemistries (IGF-I SDS) were scored 1 each, with a positive score of 3 or more suggesting *IGF1R* is a strong candidate causal of clinical presentation and should be genetically analyzed. Sensitivity of this clinical score was calculated to be approximately 76% in biased cohorts of patients with short stature [103], and ~95% in an independent cohort evaluation of IGF1R defects [109].

It is likely that the number of pathological heterozygous *IGF1R* variants, either as a monogenic defect or in combination with other molecular defects [103, 124], will increase amongst patients born SGA (small for gestational age) without postnatal catch-up growth. A concurrent rapid functional screen could help expedite clinical management decisions. One intriguing functional method which deserves further exploration, is detection of cell surface IGF1R expression and signaling by flow cytometry (FACS, fluorescent-activated cell sorting), which can be performed on patient peripheral blood mononuclear cells, PBMCs, or primary fibroblasts if available, both of which has undergone successful FACS analyzed to assess variant IGF1R expression, cell-surface localization and IGF-I response [115].

Therapeutic options for patients with IGF1R insufficiency are currently limited to rhGH, as the majority of these patients are born SGA without catch-up growth, which is a clinical condition approved for rhGH therapy. Response, however, have been highly variable [103, 104]. Serum IGF-I concentrations during treatment should be carefully monitored as pretreatment IGF-I levels are typically already elevated.

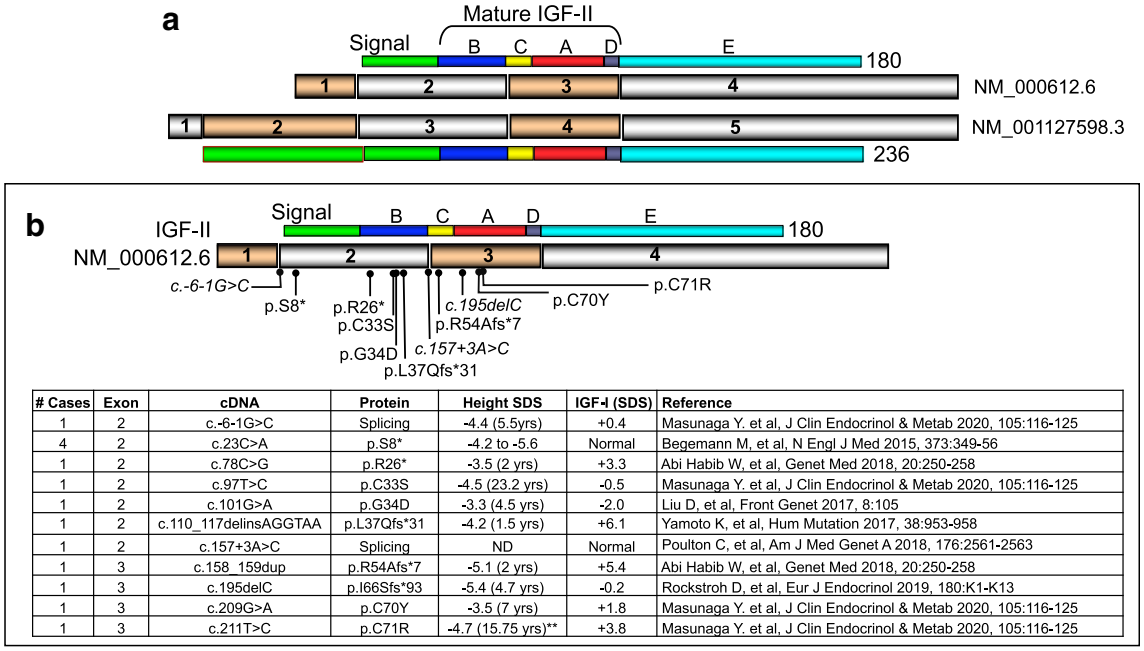
4.2 Inactivating mutations in imprinted *IGF2*

IGF-II is a member of the insulin and insulin-like family best known for its importance in pre-natal growth. Circulating IGF-II, like IGF-I, is produced predominantly by the liver, but unlike post-natal IGF-I, IGF-II production is not regulated by GH. Similar to IGF-I, IGF-II circulates in ternary complex, first forming preferential binary complexes with IGFBP-3. As expected, inactivating *IGFALS* mutations resulted in significantly reduced serum IGF-II detection [125], while elevated serum IGF-II were detected in patients carrying *PAPPA2* mutations [82].

IGF-II is encoded by *IGF2*, a maternally imprinted gene located on chromosome 11p15.5, a region which also contains several paternally imprinted genes including *H19* and *CDKN1C*. The insulin gene, *INS*, is adjacent to, and upstream of, *IGF2*, and is outside the control of the 11p15.5 imprinted locus. In most tissues *IGF2* is expressed from the paternal allele, except in the brain and liver where imprinting is lost and expression is from both the maternal and paternal alleles [126, 127]. The *IGF2* gene, ~20.5 kb in size, consists of 5 putative promoters and 9 exons which generates multiple transcripts that may be tissue and developmentally regulated. The final precursor protein of 20 kDa, encoded by exons 7, 8 and 9, undergoes post-translational processing to generate a mature protein of ~7.5 kDa. The 5 putative promoters located on non-coding exons 1–6, from which the multiple transcripts are generated, has led to confusion in cDNA nomenclature as well as the actual number of exons. Specifically, cDNA nomenclature of all identified *IGF2* mutations, except for one, are based on transcript NM_000612 which carries 4 exons designated 1 to 4. NM_000612 encodes for the canonical prepro-protein of 180 amino acids (Isoform 1) including a 24 amino acids signal peptide (Fig. 6a). For the one *IGF2* mutation not based on NM_000612 [128], the utilized NM_001127598.6 transcript carries 5 exons which encodes a larger prepro-protein of 236 amino acid. The only difference between the two transcripts is that NM_001127598.6 includes sequences for an in-frame 56 amino acids added to the N-terminus of the 180 amino acid prepro-protein found in NM_000612 (Fig. 6a).

Availability of IGF-II clearly cannot compensate for an IGF-I deficiency state despite robust IGF-II expression in utero compared to IGF-I, higher post-natal serum IGF-II concentrations, and comparable ability to bind and signal through IGF1R. A role for IGF-II in human growth, however, has been supported by observations in patients with Silver-Russell syndrome with molecular defects resulting in hypomethylation of the paternally derived *H19*-differentially methylated region and decreased *IGF2* expression [130]. Similar growth restriction has been observed in patients with mutations of *CDKN1C* on chromosome 11p15.5 [131].

The first documented human *IGF2* mutation (MIM *147470) was reported by Begemann and colleagues, in 2015 [128]. The paternally inherited nonsense *IGF2* mutation (NM_001127598.2: c.191C → A, p.Ser64Ter; equivalent to c.23C > A, p.S8*, NM_000612) was identified in a multigenerational family in whom four members had evidence of



# Cases	Exon	cDNA	Protein	Height SDS	IGF-I (SDS)	Reference
1	2	c.-6-1G>C	Splicing	-4.4 (5.5yrs)	+0.4	Masunaga Y. et al, J Clin Endocrinol & Metab 2020, 105:116-125
4	2	c.23C>A	p.S8*	-4.2 to -5.6	Normal	Begemann M, et al, N Engl J Med 2015, 373:349-56
1	2	c.78C>G	p.R26*	-3.5 (2 yrs)	+3.3	Abi Habib W, et al, Genet Med 2018, 20:250-258
1	2	c.97T>C	p.C33S	-4.5 (23.2 yrs)	-0.5	Masunaga Y. et al, J Clin Endocrinol & Metab 2020, 105:116-125
1	2	c.101G>A	p.G34D	-3.3 (4.5 yrs)	-2.0	Liu D, et al, Front Genet 2017, 8:105
1	2	c.110_117delinsAGGTAA	p.L37Qfs*31	-4.2 (1.5 yrs)	+6.1	Yamoto K, et al, Hum Mutation 2017, 38:953-958
1	2	c.157+3A>C	Splicing	ND	Normal	Poulton C, et al, Am J Med Genet A 2018, 176:2561-2563
1	3	c.158_159dup	p.R54Afs*7	-5.1 (2 yrs)	+5.4	Abi Habib W, et al, Genet Med 2018, 20:250-258
1	3	c.195delC	p.I66Sfs*93	-5.4 (4.7 yrs)	-0.2	Rockstroh D, et al, Eur J Endocrinol 2019, 180:K1-K13
1	3	c.209G>A	p.C70Y	-3.5 (7 yrs)	+1.8	Masunaga Y. et al, J Clin Endocrinol & Metab 2020, 105:116-125
1	3	c.211T>C	p.C71R	-4.7 (15.75 yrs)**	+3.8	Masunaga Y. et al, J Clin Endocrinol & Metab 2020, 105:116-125

Fig. 6 Schematic of Human *IGF2* protein and encoding exons. **a** Of the two transcripts shown, NM_000612, encoding the canonical IGF-II protein, is the basis for cDNA and protein nomenclature in 11 of 12 mutations reported. The nomenclature of the first mutation was based on NM_001127598 [128]. IGF-II peptide structure is similarly organized as IGF-I; **b** The 11 germline, non-mosaic, mutations are schematically indicated, based on exons of NM_000612.6, and tabulated. The one reported mosaic mutation [129] is not shown

growth restriction. Noteworthy was that the mutation impacted both intrauterine and postnatal growth. Birth weight SDS ranged from −2.7 to −5.3, birth length SDS from −4.2 to −4.9, and latest height SDS from −1.6 to −4.0. In addition, relative macrocephaly was of note, and affected individuals had dysmorphic features similar to those associated with Silver-Russell syndrome, including triangular facies, frontal bossing, micrognathia or retrognathia, low-set ears and clinodactyly [132]. The nonsense mutation, within the signal sequence, would be predicted to abrogate synthesis of the mature IGF-II protein.

Since the first report, 11 other *IGF2* mutations have been identified [129, 133–137] (Fig. 6b). The spectrum of mutations include nonsense [128, 135], frameshifts [133, 135, 137], splicing [129, 136], and missense [129, 134]. Four of the missense mutations are cysteine residues involved in disulfide bonds which would be predicted to be disrupted by amino acid substitutions and likely affect integrity of the IGF-II structure [129]. One of the 4 cysteine substitution, p.Cys45Ser (*c.143G > C*), interestingly, was detected only in leukocytes and not in buccal cells, hair follicles or nails, consistent with somatic mosaicism [129]. The patient had fetal growth restriction, microcephaly, mild post-natal growth failure and high serum IGF-I but otherwise lacked many of the phenotypic features for Silver-Russell Syndrome.

Clinical features associated with the 11 germline *IGF2*, summarized [129], indicate 100% (14/14 patients) concordance with Netchine-Harbison scoring system features for Silver-Russell syndrome [132]. Birth length was −4.2 ± 0.9 SDS ($n = 14$), birth weight − 3.9 ± 0.8 (n = 14), birth head circumference − 1.6 ± 0.7 (n = 14). Postnatal deficits include height − 4.6 ± 0.9 ($n = 12$), weight − 3.8 ± 1.4 ($n = 11$), and head circumference − 2.2 ± 1.0 (n = 11). Triangular face was noted for all 14 patients. Other frequently observed features were clinodactyly in 12/14 (85.7%) patients, motor delay in 9/12 (75%) and speech delay 8/11 (72.7%).

Endocrine evaluations were not available for all 14 patients. As summarized [129], serum GH mg/L (peak value) were normal 16.1 ± 7.0 ($n = 6$), serum IGF-I were often elevated (1.5 ± 2.5 SDS, n = 12, Fig. 6b) and serum IGFBP-3 upper end of normal (0.5 ± 2.2, $n = 9$). Serum IGF-II were low normal (−1.6 ± 0.8, $n = 10$), consistent with biallelic expression of IGF-II from the liver. These observations suggest that normal-elevated IGF-I concentrations cannot fully compensate for the abnormal production of IGF-II and, further, that low normal circulating IGF-II by itself is also insufficient for normalizing growth and co-morbidities. Long-term rhGH treatment with dosage of 50 to 64 μg/kg/day ($n = 5$) appeared to have improved stature [128, 135] while lower dosages ($n = 2$) were ineffective [129]. It remains unclear whether the postnatal growth retardation reflects the persistent hypomorphic actions of IGF-II or is the consequence of abnormal programming of IGF-mediated growth in utero.

5 Perspective

The revelation of new molecular defects in known genes and the discovery of new genes causing a spectrum of GH and IGF insensitivity states have led to greater appreciation for non-typical cases of GHI that are frequently less easy to diagnose. Deeper insights into biological processes important for normal developmental growth are gained. The molecular causes of congenital IGF-I deficiency remain surprisingly limited. Beyond classical *GHR* defects, only monogenic defects in *STAT5B* and *IGF1* result in comparable global IGF-I deficiencies. For *IGFALS*, a target of GH-STAT5B signaling, the apparent IGF-I deficiencies associated with inactivating *IGFALS* mutations are consequences of rapid clearance of circulating IGFs and IGFBPs. In contrast, prolonged retention of circulating IGFs in ternary complex, as observed when *PAPPA2* is defective and total serum IGF-I was high but free IGF-I levels were low, results in growth failure comparable to *IGFALS* deficiency. The implications, not surprisingly, are that there are co-ordinated fine-tuned balances between prolonging half-life of circulating IGF-IGFBP complexes and timely release of IGF-I for normal biological actions. Moreover, in contrast to global IGF-I deficiencies, the relatively milder growth deficits of *IGFALS* and *PAPPA2* defects lend support to contributions of peripheral IGF-I in growth and development. Peripheral IGF-I production by itself, however, is insufficient to overcome circulating IGFs deficits.

The contribution of IGF-II to human growth came to the fore with the recent identification of mutations of this paternally expressed gene. A few key features have emerged, including developmental growth effects strongly correlating to Silver-Russell syndrome, detectable circulating IGF-II consistent with *IGF2* not imprinted in the liver, and presence of serum IGF-II is insufficient to counteract loss of *IGF2* expression in imprinted peripheral tissues. Further, presence of IGF-I cannot rescue *IGF2* defects and, conversely, normal *IGF2* expression similarly cannot compensate for loss of *IGF1* expression, suggesting IGF-I and IGF-II contribute independently to human growth.

IGF1R mediates the biological effects IGF-I and IGF-II. A gene dosage effect is evident with *IGF1R*, but not with *GHR*. The few rare homozygous *IGF1R* mutations and compound heterozygous mutations retained some residual signaling activities, consistent with accepted paradigm that total ablation of IGF1R may be lethal. The number of *IGF1R* mutations in particular has expanded rapidly in the past 4 years, in part because the phenotype of SGA without catch-up growth, microcephaly and elevated serum IGF-I concentrations can be indicative of potential *IGF1R* defects.

There is clearly a continuum of GHI beyond GHR, not only along the GH-IGF growth axis, but also within each gene. Growth and co-morbidity phenotypes ranged from severe to mild, associated with autosomal recessive mutations and

autosomal dominant defects, respectively, with milder GHI phenotypes likely to be more frequent. A greater appreciation for the spectrum of GHI, from classical to non-classical, correlating to molecular defects, should encourage investigation and more targeted treatments.

6 Future challenges

The application of next-generation genomic sequencing continues to expedite identification of new genetic defects associated with growth disorders. Recognition of significant defects from the numerous rare or private variants uncovered, remains a challenge, given that there is a lack of "hot spot" in known genes along the GH-IGF-IGF1R axis. Thus, more often than not, the variant is a variant of unknown significance (VUS), or in a gene not known to be part of the axis. The contributions of non-coding sequences have yet to be evaluated. A second challenge is that the ready availability of genetic analysis often generates long lists of VUS which has far outpaced ability to rapidly screen and efficiently validate functional pathogenicity, a concern not entirely mitigated by the numerous in silico programs freely available for predicting pathogenicity. This has contributed to poor genotype-phenotype correlations as mechanisms of pathogenicity are lacking. Further, poor genotype-phenotype correlations may be complicated by the fact that the growth disorder may be due not to monogenic defects but may reflect digenic or oligogenic effects, further challenging analysis of genomic data. The advantages of genomic advances, however, outweigh these concerns as greater knowledge of the molecular, cellular and mechanisms of growth disorders will be gained, towards improving management of patients.

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Data availability Yes

Compliance with ethical standards

Conflict of interest/competing interests V.H. has a patent for the use of PAPPA2 as a growth-promoting agent.

Code availability not applicable.

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