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A Novel Mutation in Insulin-Like Growth Factor-1 Receptor (c.641-2A>G) Is Associated with Impaired Growth, Hypoglycemia, and Modified Immune Phenotypes

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Abstract

Introduction: Insulin-like growth factor 1 receptor (IGF1R) mutations lead to systemic disturbances in growth and glucose homeostasis due to widespread IGF1R expression throughout the body. IGF1R is expressed by innate and adaptive immune cells, facilitating their development and exerting immunomodulatory roles in the periphery.

Case Presentation: We report on a family presenting with a novel heterozygous *IGF1R* mutation with characterization of the mutation, IGF1R expression, and immune phenotyping. Twin probands presented clinically with short stature and hypoglycemia. Variable phenotypic expression was seen in two other family members carrying the *IGF1R* mutation. The probands

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Author Contributions

MRS researched and analyzed the data in figures 6–7 and wrote the manuscript; TPF researched and analyzed the data in figures 1 and 5 and wrote the manuscript; DJP and JAM researched the data in figure 7 and reviewed/edited the manuscript; AM provided samples described in Supplementary Tables 1–2 and reviewed/edited the manuscript; VH, RR, and AD researched and analyzed the data in figures 2–4 and reviewed/edited the manuscript; TMB and LMJ conceived the study and reviewed/edited the manuscript.

Statement of Ethics

All procedures were approved by local Institutional Review Boards and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from participants (or their legal guardian in the case of minors) prior to enrollment.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

were treated with exogenous growth hormone therapy and dietary cornstarch, improving linear growth and reducing hypoglycemic events. *IGF1R* c.641-2A>G caused abnormal mRNA splicing and premature protein termination. Flow cytometric immunophenotyping demonstrated lower IGF1R on peripheral blood mononuclear cells (PBMCs) from *IGF1R* c.641-2A>G subjects. This alteration was associated with reduced levels of T helper 17 (Th17) cells and a higher percentage of T helper 1 (Th1) cells compared to controls, suggesting decreased IGF1R expression may affect CD4⁺ Th cell lineage commitment.

Discussion: Collectively, these data suggest a novel loss-of-function mutation (c.641-2A>G) leads to aberrant mRNA splicing and IGF1R expression resulting in hypoglycemia, growth restriction, and altered immune phenotypes.

Keywords

growth; hypoglycemia; immunology; IGF1R

INTRODUCTION

Growth and glucose homeostasis are intricately regulated by hormonal insulin and insulin-like growth factor (IGF) signaling via tyrosine kinase receptors, including the IGF1 receptor (IGF1R) [1]. The *IGF1R* gene is found on chromosome 15q26.3 and consists of 21 exons, encoding for an ($\alpha\beta$)₂ homodimer with homology to the insulin receptor (IR) [2]. Mutations in *IGF1R* have been reported to affect ligand binding and/or decrease surface IGF1R expression [2]. Prior reports of *IGF1R* mutations are commonly heterozygous [3–15], with only two compound heterozygous [16, 17] and two hypomorphic homozygous mutations [14, 18] reported to date. Homozygous loss-of-function mutations are likely lethal, as observed in mouse studies [19].

IGF1R mutations are known to cause intrauterine growth retardation (IUGR), microcephaly, and failure to thrive postnatally [2, 20, 21]. The current therapy for rectifying growth defects associated with heterozygous *IGF1R* mutations is trial administration of recombinant human growth hormone (rhGH) intended to induce supraphysiological levels of IGF1, in complex with stabilizing IGF binding protein-3 (IGFBP-3), providing compensatory IGF1R signaling through the wild-type *IGF1R* allele [22]. In addition to growth defects, direct effects on glucose metabolism, including glucose intolerance, have been observed [13–15, 17, 23]. Hypoglycemia has been less frequently reported [24].

IGF1R is found to be ubiquitously expressed in most cells of the body including muscle, adipose, bone, as well as, innate and adaptive immune cells [25]. While IGF1R expression is known to be decreased on peripheral blood mononuclear cells (PBMCs) of subjects with *IGF1R* mutations [26, 27] and IGF1R signaling has been associated with modulation of immune function [25], there is limited understanding of the impacts of IGF1R on human innate and adaptive immunity *in vivo*. Although *IGF1R* mutations have not been associated with overt immune defects, we hypothesized that these mutations may be associated with sub-clinical immune phenotypic alterations. Information gained could potentially elucidate immune mechanisms in disorders with known IGF1 or IGF1R modulation. Here, we report a novel heterozygous *IGF1R* mutation, c.641-2A>G, leading to a splicing defect that impairs

IGF1R protein expression, altering the growth, glucose metabolism, and immune profile of affected subjects.

RESEARCH DESIGN AND METHODS

Subject Enrollment

All procedures were approved by local Institutional Review Boards and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from participants (or their legal guardian in the case of minors) prior to enrollment. Subjects affected by the *IGF1R* mutation were age- and sex-matched to one or two control subjects attending clinics at the University of Florida (UF; Gainesville, FL) and Emory University (Atlanta, GA). Peripheral blood samples were collected from non-fasted subjects by venipuncture in sodium heparin vacutainer tubes for IGF1R expression experiments and ethylenediaminetetraacetic acid (EDTA)-coated vacutainer tubes (BD Biosciences) for Immune Phenotyping experiments. Blood was rested overnight prior to processing to minimize variance due to transport times. Subject demographic information is presented in Supplementary Tables 1,2.

Glucose Metabolism, IGF, and IGFBP Quantification

Serum glucose was determined via the hexokinase method and hemoglobin A1c (HbA1c) by cation-exchange chromatography, performed by UF Health Pathology Laboratories. Serum IGF1 levels were measured by enzyme-labeled chemiluminescent immunometric assay, performed by either Quest Diagnostics or UF Health Pathology Laboratories. IGFBP-3 and IGFBP-1 levels were measured by quantitative chemiluminescent immunoassay and quantitative radioimmunoassay, respectively, both performed by ARUP Laboratories.

Genetic Analysis

Genomic DNA extracted from whole blood was used for whole exome sequencing and Sanger dideoxy-sequencing. Whole exome sequencing for Twin B was performed by Baylor Genetics, as previously described [28]. VCRome 2.1 in-solution exome probes [29] and additional probes for genes involved in over 3600 Mendelian diseases were used for exome enrichment. For targeted *IGF1R* sequencing, coding exon 3, including intron-exon junctions, were PCR amplified and sequenced as previously described [5]. The intronic primers for amplifying exon 3 were: forward, IGF1Rx3f (intron 2), 5'-GCAGTGAATGACCCAGAAGGATGC-3'; reverse, IGF1Rx3r (intron 3): 5'-CCGACAGAGTCTACCTTTGTGTGCTA-3'. Total RNA was extracted from PBMCs that were ficoll-purified from whole blood, and subjected to RT-PCR (reverse transcriptase-PCR) amplification to assess aberrant splicing of exon 3. The primers for amplifying cDNA sequences between exon 2 and exon 4 were: forward, (exon 2) 5'-GAGAGCCTCGGAGACCTCTTCC-3'; and reverse (exon 4), 5'-CTTCGGGCAAGGACCTTCACAA-3'. Microarray cytogenetic testing was performed via oligonucleotide-single nucleotide polymorphism (SNP) assays by Agilent Sureprint G3 human CGH+SNP for twin A and Affymetrix Cytoscan HD for twin B.

Flow Cytometric IGF1R Quantification

200 μ L of whole blood was stained with anti-human CD221-PE (1H7, Biolegend) for 30 minutes at 23°C in the dark. Red blood cells were lysed for five minutes at 23°C with 1-step Fix/Lyse Solution (eBioscience), followed by three washes with staining buffer (PBS + 2% FBS + 0.05% NaN₃). Events were acquired on an LSRFortessa (BD Biosciences) and analyzed with FlowJo software (v10.6.1; Tree Star).

Whole Blood Immune Phenotyping

200 μ L of whole blood was stained with six different panels of fluorescently-labeled anti-human antibodies, adapted from a standardized immunophenotyping panel published by Maecker et al. [30], for 30 minutes at room temperature (RT) in the dark. Antibody information is presented in Supplementary Table 3. Red blood cells were lysed for five minutes at 23°C with 1-step Fix/Lyse Solution (eBioscience), followed by three washes with staining buffer. Events were acquired on an LSRFortessa (BD Biosciences) and analyzed with FlowJo software (v10.6.1; Tree Star).

Statistical Analyses

Data were analyzed using GraphPad Prism software version 7.0. Data are presented as mean $\pm$ standard deviation (SD) unless otherwise specified. IGF1R expression was compared between *IGF1R* c.641-2A>G heterozygous subjects (IGF1R^{Het}) and unrelated control subjects via Mann-Whitney U test. Frequencies of broad immunophenotypes were compared using multiple t tests without correction for multiple comparisons, to accommodate the small number of IGF1R^{Het} subjects available for analysis. Frequencies of selected immunophenotypes were compared using Mann-Whitney U test in secondary analyses. P-values < 0.05 were considered significant.

RESULTS

Clinical Presentation of Family Members Carrying the *IGF1R* c.641-2A>G Mutation

Twin monozygotic female probands were born at 34 weeks gestation via caesarean section due to preterm premature rupture of membranes. Twin A had a birth weight of 1.53 kg (−1.51 standard deviation score (SDS) Fenton preterm growth chart), birth length of 43.2 cm (−0.31 SDS Fenton), and head circumference of 29 cm (−1.10 SDS Fenton). Twin B had a birth weight of 1.53 kg (−1.51 SDS Fenton preterm growth chart), birth length of 42 cm (−0.75 SDS Fenton), and head circumference of 29.5 cm (−0.77 SDS Fenton). The twins had hypotonia in infancy with failure to attain catch-up growth (in weight and length) in the first two years of life. Head circumferences were more than 2 SDS below the mean from 6 months to 2 years. Twin B also had congenital scoliosis with multiple fused hemivertebrae, tethered cord, and Chiari malformation. The twins had microarray-based comparative genomic hybridization testing which showed a 7q31.1 deletion (242–317 kb) of unknown clinical significance. The only coding gene in the deleted region is *IMMP2L*, which has not been linked with any phenotypes in the Online Mendelian Inheritance in Man (OMIM) database. While *Immp2l* knockout mice have been reported to show decreased body weight

prior to adulthood, there is evidence of haplosufficiency [34], suggesting that the 7q31.1 deletion observed in the twins is unlikely to be the cause of their failure to thrive.

Both were evaluated by a pediatric endocrinologist at 3 years 4 months of age for short stature. At that time, twin A weighed 11.6 kg (SDS -2.08) with a height of 87.7 cm (SDS -2.21). Twin B weighed 11.4 kg (SDS -2.4) with a height of 86 cm (SDS -2.65). Twin A also experienced hospital admission at age three years for decreased responsiveness and hypoglycemia (46 mg/dL) in the setting of acute otitis media and 16 hours of fasting. During the ensuing two years of follow up, they experienced intermittent episodes of mild hypoglycemia (55–70 mg/dL) on home glucometer, mostly during prolonged fasting.

The combination of failure to thrive (low weight, short stature), hypotonia, and microcephaly was concerning for an underlying genetic etiology and whole exome sequencing was obtained for twin B revealing a likely pathogenic heterozygous mutation of *IGF1R* (c.641-2A>G). Additional heterozygous variants of unknown significance were detected in twin B (Supplementary Table 4, Supplementary Table 5), although, these were unlikely to be associated with the clinical observations. Of the heterozygous X-linked and autosomal dominant mutations identified, the novel c.3008C>T variant in *KMT2A* is the only mutation that could account for some, but not all, of their phenotype (Supplementary Table 4). Other mutations in this gene are associated with Wiedemann-Steiner syndrome, which presents with short stature and hypotonia [35]. However, the amino acid change from the novel mutation in Twin B is predicted to have a benign impact on *KMT2A* structure and function (Supplementary Table 4). A number of autosomal recessive variants of unknown clinical significance were also observed in twin B (Supplementary Table 5). Interestingly, two of the mutations were associated with microcephaly (Supplementary Table 5), however, as heterozygous recessive variants, these mutations would again be unlikely to be the underlying etiology for this observation in the twins. Overall, the whole exome sequencing results support the heterozygous *IGF1R* mutation (c.641-2A>G) as the causative mutation responsible for the phenotypes observed.

Growth factor testing revealed IGF1 levels were within normal reference ranges in both twins (twin A: 154 ng/mL, twin B: 115 ng/mL, reference range 55–248) (Figure 5A). Likewise, IGFBP-3 levels were within normal reference ranges for both twins (twin A: 4460 ng/mL, twin B: 3630 ng/mL, reference range 2169–4790) (Figure 5B). Random growth hormone levels were elevated or within normal reference ranges at 9.11 ng/mL and 2.59 ng/mL (reference range 0.1–6.2), for twin A and B, respectively. Their weight tracked along the 1st-5th percentile but with appropriate BMI and only mildly decreased prealbumin levels (14–16 mg/dL, reference range 20–40). They exhibited a mild bone age delay of 1 year 4 months at initial presentation and appropriate thyroid function. They were started on subcutaneous rhGH at 4 years 3 months of age (dosing 0.3 mg/kg/week) at a height of 93.2 cm (SDS -2.15) and 90.8 cm (SDS -2.74) for twin A and B, respectively. Height velocity improved with rhGH therapy (Figure 5C) by inducing supraphysiologic IGF1 and IGFBP-3 levels (Figure 5A,B), while HbA1c values did not rise significantly in either twin (5.2–5.4%, reference range <5.7). After one year of rhGH twin A's height SDS improved to -1.60 and twin B's improved to -2.12. Mild hypoglycemia persisted after initiation of rhGH therapy,

and intermittent supplementation with uncooked cornstarch (complex carbohydrate that is slowly digested) and protein powder were utilized to stabilize blood glucose levels.

The probands' older sister was born at 41 weeks gestation. Her birth weight was 2.7 kg (SDS -1.31), birth length was 48.3 cm (SDS -0.48), and head circumference is unknown. She experienced hypoglycemia (48 mg/dL) at age 7 years. However, she did not have short stature with a height of 114 cm (SDS -1.55). Her weight, 17.5 kg (SDS -2.04), and BMI, 13.5 kg/m², (SDS -1.58) were slightly low and attributable to stimulant medication use for attention deficit hyperactivity disorder, but with an appropriate prealbumin level of 21 mg/dL. She was found to have the same *IGF1R* heterozygous mutation with initial IGF1 level in the mid-normal range (192 ng/mL; reference range 57–277) and elevated IGFBP-3 (5920 ng/mL; reference range 2188–4996). Her IGFBP-1 level was 8 ng/mL (reference range 15–95) during mild hypoglycemia at age 7 years with a serum glucose of 64 mg/dL (random venous draw). She was started on nightly cornstarch and protein supplementation and achieved normoglycemia.

Maternal and paternal adult heights were 157.5 cm (5'2") and 180.3 cm (5'11"), respectively. The probands' mother was born at 36 weeks gestation and weighed 2.44 kg (-0.37 SDS Fenton). She reported subjective symptoms of hypoglycemia, as did maternal grandmother and paternal grandfather (Figure 1). The *IGF1R* sequencing revealed the same heterozygous mutation in the probands' mother.

Novel *IGF1R* c.641-2A>G Heterozygous Variant Drives Early IGF1R Protein Termination

We obtained genomic DNA from the affected family and conducted targeted sequencing after one proband (twin B) was found to have a mutation in *IGF1R* on whole exome sequencing. A likely pathogenic heterozygous variant, c.641-2A>G, was present in twin affected female probands, their mother, and one of their sisters (Figure 1, Supplementary Figure 1). The father and maternal half-sister do not carry the variant. This mutation was not present in the Genome Aggregation Database (gnomAD) [31] or the Exome Sequencing Project (ESP) [32] databases at the time of this writing, and as such, is deemed novel. The mutation modified the 3' acceptor splice site consensus sequence of intron 2, which was predicted to lead to skipping of exon 3, inducing a frameshift (Figure 2). The predicted abnormal splicing event was confirmed by Sanger analysis of *IGF1R* cDNA from the probands (Figure 3). This frameshift was predicted to lead to premature protein termination (p.Met214Thrfs*67) prior to the transmembrane domain, likely to impact IGF1R surface expression (Figure 4). Additionally, the two ligand-binding sites for IGF1 in each $\alpha\beta$ dimer, the first being composed of parts of the L1 and α -CT domains from different subunits and the second site composed of parts of Fn1 and Fn2 domains from the same subunit [33], were both predicted to be disrupted (Figure 4).

IGF1R Protein Expression is Decreased in *IGF1R* c.641-2A>G Het Subject PBMCs

We assessed levels of IGF1R protein expression in affected family members compared to age- and sex-matched subjects (Supplementary Table 1). IGF1R expression has previously been observed at lower levels in bulk PBMCs of subjects with *IGF1R* mutations using flow cytometry [26, 27]. The *IGF1R* c.641-2A>G heterozygous (*IGF1R*^{Het}) subjects, including

the rhGH-treated twins, showed significantly lower IGF1R expression on PBMCs, at $50 \pm 8\%$ of control levels (Figure 6), suggestive of haploinsufficiency. Here, we also used forward and side-scatter characteristics to gate on granulocytes, monocytes, and lymphocytes in order to understand which PBMC populations showed reduced IGF1R expression in the *IGF1R*^{Het} subjects. IGF1R expression was significantly reduced in the *IGF1R*^{Het} subjects as compared to controls, throughout all PBMC sub-populations analyzed, to the following proportions of levels observed in controls: $40 \pm 4\%$ in granulocytes, $55 \pm 7\%$ in monocytes, and $69 \pm 15\%$ in lymphocytes (Figure 6), indicating a global defect in IGF1R expression.

***IGF1R* c.641-2A>G Het Subjects Show Altered CD4⁺ T Cell Skewing**

We postulated that *IGF1R* haploinsufficiency, evident in PBMCs, might impact immune function [25] in the *IGF1R*^{Het} subjects. Flow cytometric immunophenotyping was performed on peripheral blood to measure major subsets of CD4⁺ and CD8⁺ T cells, effector CD4⁺ T cells (Teff), CD4⁺ regulatory T cells (Treg), follicular helper T cells (Tfh), B cells, natural killer (NK) cells, dendritic cells (DC), and monocytes. The immune profiles of the *IGF1R*^{Het} subjects were quite similar to those of controls, with the exception of Teff populations (Figure 7A, Supplementary Table 2, Supplementary Table 6). Here, *IGF1R*^{Het} subjects showed significantly lower percentages of CD4⁺CD45RO⁺CD183⁻CD196⁺ T helper 17 (Th17) cells and significantly higher percentages of CD4⁺CD45RO⁺CD183⁺CD196⁻ T helper 1 (Th1) cells than age- and sex-matched controls (Figure 7B,C), suggesting a shift in effector CD4⁺ T cell phenotype. Despite the rhGH-induced IGF1 upregulation in the twin probands (Figure 5A), Teff phenotypes were similar between all *IGF1R*^{Het} subjects (Figure 7B,C).

DISCUSSION

We report a novel variant, *IGF1R* c.641-2A>G, which leads to premature protein termination and reduced IGF1R protein surface expression. Haploinsufficiency resulted in variable expressivity in this family affected by a splice site mutation in the *IGF1R* gene. Associated hypoglycemia was found even in family members less affected by short stature and poor growth. In the twin probands most affected, their growth responded to supratherapeutic exogenous rhGH administration, and all three affected children had improvement in hypoglycemia with dietary cornstarch administration.

The risk of hypoglycemia in these patients with the *IGF1R* c.641-2A>G mutation is likely multifactorial. IGF1 levels were in the mid to upper half of the normal reference range for age for all three affected children at baseline screening, and hypoglycemia occurred before and after rhGH-induced supraphysiologic increases in IGF1 levels in the twin probands, similar to the pattern of hypoglycemic events observed in another heterozygous *IGF1R* mutation [24]. It is known that a 50% loss of IGF1R expression increases insulin sensitivity, as supported by elevated insulin-induced AKT activation in a previously characterized *IGF1R* mutation [8]. Increased hybrid IGF1R and IR heterodimers may also contribute to hypoglycemia due to increased blood glucose uptake from IGF1 binding as compared to IGF1R homodimers. Future studies should address whether similar mechanisms occur in those affected by *IGF1R* c.641-2A>G to promote hypoglycemia. IGF1-induced glucose

uptake is reported to be no more than 6% of insulin's capacity [36], but IGF1 can be found at concentrations 100x higher than insulin in plasma concentration. These effects may be exacerbated if the bioavailability of IGF1 is increased, as would be expected in the case of suppression of IGF1 levels in the setting of mild hypoglycemia for the older sibling of the twin probands. IGF1 also suppresses GH and hepatic glucose production [37, 38], leading to decreased counter-regulatory compensation for hypoglycemia during times of stress or low glycogen [24]. Administration of rhGH did not alleviate the hypoglycemia, and thus GH deficiency is not a contributor to their hypoglycemia. However, we speculate that supraphysiologic IGF1 levels from rhGH could bind to IGF1R-IR heterodimers or cross-bind with the IR (though at lower affinity than insulin binding). While glucose intolerance, rather than hypoglycemia [24], has been more frequently observed in subjects with other *IGF1R* mutations [13–15, 17, 23], it has been suggested that hypoglycemia in early life could evolve into glucose intolerance in later life as a consequence of β cell apoptosis with the loss of pro-survival IGF1R signaling [39].

Decreased IGF1R protein expression has been previously reported in subjects with other heterozygous mutations in the *IGF1R* gene [3, 5, 7, 8, 12, 14, 24]. While most of these studies utilized fibroblasts to assess IGF1R expression, we confirmed that PBMCs may serve as an accessible source for the means of monitoring IGF1R expression in patients with short stature and suspected *IGF1R* mutations [26, 27]. We also extended beyond these previous studies [26, 27] to show that defective IGF1R expression could be observed throughout lymphocyte, monocyte, and granulocyte sub-populations.

Fibroblasts from subjects carrying *IGF1R* haploinsufficiency mutations have previously been shown to possess defects in IGF1-induced IGF1R signaling [3–5, 7–11, 14, 15, 17, 24], suggesting that decreased IGF1R protein expression directly correlated with reduced IGF1R signaling. However, in PBMCs, the role of IGF1R may be more complex as signaling appears to require simultaneous stimulation of the PI3K/Akt pathway through additional surface receptors, including but not limited to Toll-like receptors (TLR) or the T cell receptor (TCR) [40–42]. This confounded our ability to directly measure and compare IGF1R signaling between genetically diverse human subjects with variable responses to these secondary immune stimuli.

Broad immunophenotyping efforts revealed that *IGF1R* c.641-2A>G Het subjects show largely normal proportions of innate and adaptive immune populations. However, a significant shift in CD4⁺ Th cell lineage commitment, with an increase in Th1 and a decrease in Th17 populations was observed. These data are in agreement with studies suggesting that IGF1R signaling modulates CD4⁺ T cell function by inhibiting Th1 skewing [43] and promoting Th17 skewing [44], both *in vitro* and *in vivo* [45]. Our interpretation of this immune phenotype resulting from impaired IGF1R signaling is potentially complicated by the supraphysiological IGF1 levels induced by the twins' rhGH treatment. Reassuringly, the mother and older sister were not treated with rhGH, suggesting that our observations in the family are likely reflective of the impact of the shared genetic mutation. Regardless, this immune phenotype should be assessed in subjects with other *IGF1R* haploinsufficiency mutations in order to verify that the phenotype is associated with hampered IGF1R expression.

While the classical role of Th1 cells is to drive immunity against intracellular pathogens and Th17 cells to protect against extracellular pathogens, these subsets play important roles in a wide variety of diseases including autoimmunity and cancer [46]. Although standardized normal reference ranges for Th1 and Th17 cells for age have yet to be developed [30], the modest shifts observed in CD4⁺ T cell skewing in the *IGF1R* c.641-2A>G Het subjects are likely consistent with their lack of overt immune defects. However, the potential for similar IGF signaling-related shifts in Th1/Th17 ratios contributing to disease [47] in populations with underlying genetic risk should still be considered. The data presented herein illustrate a previously unreported heterozygous *IGF1R* mutation with variable clinical phenotype and demonstrate the novel concept of *IGF1R* haploinsufficiency potentially affecting immune function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Clemmons DR: Metabolic actions of insulin-like growth factor-I in normal physiology and diabetes. *Endocrinol Metab Clin North Am* 2012;41:425–443, vii–viii. [PubMed: 22682639]
2. Klammt J, Kiess W, Pfäffle R: IGF1R mutations as cause of SGA. *Best Pract Res Clin Endocrinol Metab* 2011;25:191–206. [PubMed: 21396585]
3. Harmel EM, Binder G, Barnikol-Oettler A, Caliebe J, Kiess W, Losekoot M, Ranke MB, Rappold GA, Schlicke M, Stobbe H, Wit JM, Pfäffle R, Klammt J: Alu-mediated recombination defect in IGF1R: haploinsufficiency in a patient with short stature. *Horm Res Paediatr* 2013;80:431–442. [PubMed: 24296753]
4. Leal AC, Montenegro LR, Saito RF, Ribeiro TC, Coutinho DC, Mendonca BB, Arnhold JJ, Jorge AA: Analysis of the insulin-like growth factor 1 receptor gene in children born small for gestational age: in vitro characterization of a novel mutation (p.Arg511Trp). *Clin Endocrinol (Oxf)* 2013;78:558–563. [PubMed: 22998174]
5. Fang P, Schwartz ID, Johnson BD, Derr MA, Roberts CT, Hwa V, Rosenfeld RG: Familial short stature caused by haploinsufficiency of the insulin-like growth factor I receptor due to nonsense-mediated messenger ribonucleic acid decay. *J Clin Endocrinol Metab* 2009;94:1740–1747. [PubMed: 19240156]
6. Kawashima Y, Hakuno F, Okada S, Hotsubo T, Kinoshita T, Fujimoto M, Nishimura R, Fukushima T, Hanaki K, Takahashi S, Kanzaki S: Familial short stature is associated with a novel dominant-negative heterozygous insulin-like growth factor 1 receptor (IGF1R) mutation. *Clin Endocrinol (Oxf)* 2014;81:312–314. [PubMed: 24033502]
7. Wallborn T, Wüller S, Klammt J, Kruis T, Kratzsch J, Schmidt G, Schlicke M, Müller E, van de Leur HS, Kiess W, Pfäffle R: A heterozygous mutation of the insulin-like growth factor-I receptor causes retention of the nascent protein in the endoplasmic reticulum and results in intrauterine and postnatal growth retardation. *J Clin Endocrinol Metab* 2010;95:2316–2324. [PubMed: 20357178]

8. Raile K, Klammt J, Schneider A, Keller A, Laue S, Smith R, Pfäffle R, Kratzsch J, Keller E, Kiess W: Clinical and functional characteristics of the human Arg59Ter insulin-like growth factor I receptor (IGF1R) mutation: implications for a gene dosage effect of the human IGF1R. *J Clin Endocrinol Metab* 2006;91:2264–2271. [PubMed: 16569742]
9. Kruis T, Klammt J, Galli-Tsinopoulou A, Wallborn T, Schlicke M, Müller E, Kratzsch J, Körner A, Odeh R, Kiess W, Pfäffle R: Heterozygous mutation within a kinase-conserved motif of the insulin-like growth factor I receptor causes intrauterine and postnatal growth retardation. *J Clin Endocrinol Metab* 2010;95:1137–1142. [PubMed: 20103656]
10. Inagaki K, Tiulpakov A, Rubtsov P, Sverdlova P, Peterkova V, Yakar S, Terekhov S, LeRoith D: A familial insulin-like growth factor-I receptor mutant leads to short stature: clinical and biochemical characterization. *J Clin Endocrinol Metab* 2007;92:1542–1548. [PubMed: 17264177]
11. Labarta JJ, Barrio E, Audí L, Fernández-Cancio M, Andaluz P, de Arriba A, Puga B, Calvo MT, Mayayo E, Carrascosa A, Ferrández-Longás A: Familial short stature and intrauterine growth retardation associated with a novel mutation in the IGF-I receptor (IGF1R) gene. *Clin Endocrinol (Oxf)* 2013;78:255–262. [PubMed: 22738321]
12. Kawashima Y, Kanzaki S, Yang F, Kinoshita T, Hanaki K, Nagaishi J, Ohtsuka Y, Hisatome I, Ninomoya H, Nanba E, Fukushima T, Takahashi S: Mutation at cleavage site of insulin-like growth factor receptor in a short-stature child born with intrauterine growth retardation. *J Clin Endocrinol Metab* 2005;90:4679–4687. [PubMed: 15928254]
13. Burkhardt S, Gesing J, Kapellen TM, Kovacs P, Kratzsch J, Schlicke M, Stobbe H, Tönjes A, Klammt J, Pfäffle R: Novel heterozygous IGF1R mutation in two brothers with developing impaired glucose tolerance. *J Pediatr Endocrinol Metab* 2015;28:217–225. [PubMed: 25153223]
14. Prontera P, Micale L, Verrotti A, Napolioni V, Stangoni G, Merla G: A New Homozygous IGF1R Variant Defines a Clinically Recognizable Incomplete Dominant form of SHORT Syndrome. *Hum Mutat* 2015;36:1043–1047. [PubMed: 26252249]
15. Walenkamp MJ, van der Kamp HJ, Pereira AM, Kant SG, van Duyvenvoorde HA, Kruithof MF, Breuning MH, Romijn JA, Karperien M, Wit JM: A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. *J Clin Endocrinol Metab* 2006;91:3062–3070. [PubMed: 16757531]
16. Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, Kiess W, Klammt J, Kratzsch J, Osgood D, Pfäffle R, Raile K, Seidel B, Smith RJ, Chernauek SD, Group IGRIS: IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med* 2003;349:2211–2222. [PubMed: 14657428]
17. Fang P, Cho YH, Derr MA, Rosenfeld RG, Hwa V, Cowell CT: Severe short stature caused by novel compound heterozygous mutations of the insulin-like growth factor 1 receptor (IGF1R). *J Clin Endocrinol Metab* 2012;97:E243–247.
18. Gannagé-Yared MH, Klammt J, Chouery E, Corbani S, Mégarbané H, Abou Ghoch J, Choucair N, Pfäffle R, Mégarbané A: Homozygous mutation of the IGF1 receptor gene in a patient with severe pre- and postnatal growth failure and congenital malformations. *Eur J Endocrinol* 2013;168:K1–7. [PubMed: 23045302]
19. Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A: Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* 1993;75:59–72. [PubMed: 8402901]
20. Ester WA, van Duyvenvoorde HA, de Wit CC, Broekman AJ, Ruivenkamp CA, Govaerts LC, Wit JM, Hokken-Koelega AC, Losekoot M: Two short children born small for gestational age with insulin-like growth factor 1 receptor haploinsufficiency illustrate the heterogeneity of its phenotype. *J Clin Endocrinol Metab* 2009;94:4717–4727. [PubMed: 19864454]
21. David A, Hwa V, Metherell LA, Netchine I, Camacho-Hübner C, Clark AJ, Rosenfeld RG, Savage MO: Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. *Endocr Rev* 2011;32:472–497. [PubMed: 21525302]
22. Walenkamp MJE, Robers JML, Wit JM, Zandwijken GRJ, van Duyvenvoorde HA, Oostdijk W, Hokken-Koelega ACS, Kant SG, Losekoot M: Phenotypic Features and Response to GH Treatment of Patients With a Molecular Defect of the IGF-1 Receptor. *J Clin Endocrinol Metab* 2019;104:3157–3171. [PubMed: 30848790]

23. Mohn A, Marcovecchio ML, de Giorgis T, Pfaeffle R, Chiarelli F, Kiess W: An insulin-like growth factor-I receptor defect associated with short stature and impaired carbohydrate homeostasis in an Italian pedigree. *Horm Res Paediatr* 2011;76:136–143. [PubMed: 21811077]
24. Solomon-Zemler R, Basel-Vanagaite L, Steier D, Yakar S, Mel E, Phillip M, Bazak L, Bercovich D, Werner H, de Vries L: A novel heterozygous IGF-1 receptor mutation associated with hypoglycemia. *Endocr Connect* 2017;6:395–403. [PubMed: 28649085]
25. Smith TJ: Insulin-like growth factor-I regulation of immune function: a potential therapeutic target in autoimmune diseases? *Pharmacol Rev* 2010;62:199–236. [PubMed: 20392809]
26. Wang SR, Carmichael H, Andrew SF, Miller TC, Moon JE, Derr MA, Hwa V, Hirschhorn JN, Dauber A: Large-scale pooled next-generation sequencing of 1077 genes to identify genetic causes of short stature. *J Clin Endocrinol Metab* 2013;98:E1428–1437.
27. Ocaranza P, Golekoh MC, Andrew SF, Guo MH, Kaplowitz P, Saal H, Rosenfeld RG, Dauber A, Cassorla F, Backeljauw PF, Hwa V: Expanding Genetic and Functional Diagnoses of IGF1R Haploinsufficiencies. *Horm Res Paediatr* 2017;87:412–422. [PubMed: 28395282]
28. Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, Braxton A, Beuten J, Xia F, Niu Z, Hardison M, Person R, Bekheirnia MR, Leduc MS, Kirby A, Pham P, Scull J, Wang M, Ding Y, Plon SE, Lupski JR, Beaudet AL, Gibbs RA, Eng CM: Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med* 2013;369:1502–1511. [PubMed: 24088041]
29. Bainbridge MN, Wang M, Wu Y, Newsham I, Muzny DM, Jefferies JL, Albert TJ, Burgess DL, Gibbs RA: Targeted enrichment beyond the consensus coding DNA sequence exome reveals exons with higher variant densities. *Genome Biol* 2011;12:R68. [PubMed: 21787409]
30. Maecker HT, McCoy JP, Nussenblatt R: Standardizing immunophenotyping for the Human Immunology Project. *Nat Rev Immunol* 2012;12:191–200. [PubMed: 22343568]
31. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Consortium EA: Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285–291. [PubMed: 27535533]
32. Auer PL, Reiner AP, Wang G, Kang HM, Abecasis GR, Altshuler D, Bamshad MJ, Nickerson DA, Tracy RP, Rich SS, Leal SM, Project NGENS: Guidelines for Large-Scale Sequence-Based Complex Trait Association Studies: Lessons Learned from the NHLBI Exome Sequencing Project. *Am J Hum Genet* 2016;99:791–801. [PubMed: 27666372]
33. Kavran JM, McCabe JM, Byrne PO, Connacher MK, Wang Z, Ramek A, Sarabipour S, Shan Y, Shaw DE, Hristova K, Cole PA, Leahy DJ: How IGF-1 activates its receptor. *Elife* 2014;3
34. Lu B, Poirier C, Gaspar T, Gratzke C, Harrison W, Busija D, Matzuk MM, Andersson KE, Overbeek PA, Bishop CE: A mutation in the inner mitochondrial membrane peptidase 2-like gene (*Immp2l*) affects mitochondrial function and impairs fertility in mice. *Biol Reprod* 2008;78:601–610. [PubMed: 18094351]
35. Jones W, Dafou D, McEntagart M, Woollard W, Elmslie F, Holder-Espinasse M, Irving M, Saggart A, Smithson S, Trembath R, Deshpande C, Simpson M: De Novo Mutations in *MLL* Cause Wiedemann-Steiner Syndrome; *Am J Hum Genet*, 2012, vol 91, pp 358–364. [PubMed: 22795537]
36. Guler HP, Zapf J, Froesch ER: Short-term metabolic effects of recombinant human insulin-like growth factor I in healthy adults. *N Engl J Med* 1987;317:137–140. [PubMed: 3299085]
37. Moxley RT, Arner P, Moss A, Skottner A, Fox M, James D, Livingston JN: Acute effects of insulin-like growth factor I and insulin on glucose metabolism in vivo. *Am J Physiol* 1990;259:E561–567.

38. Pratipanawatr T, Pratipanawatr W, Rosen C, Berria R, Bajaj M, Cusi K, Mandarino L, Kashyap S, Belfort R, DeFronzo RA: Effect of IGF-I on FFA and glucose metabolism in control and type 2 diabetic subjects. *Am J Physiol Endocrinol Metab* 2002;282:E1360–1368.
39. Robertson K, Lu Y, De Jesus K, Li B, Su Q, Lund PK, Liu JL: A general and islet cell-enriched overexpression of IGF-I results in normal islet cell growth, hypoglycemia, and significant resistance to experimental diabetes. *Am J Physiol Endocrinol Metab* 2008;294:E928–938.
40. Mirdamadi Y, Bommhardt U, Goihl A, Guttek K, Zouboulis CC, Quist S, Gollnick H: Insulin and Insulin-like growth factor-1 can activate the phosphoinositide-3-kinase /Akt/FoxO1 pathway in T cells. *Dermatoendocrinol* 2017;9:e1356518.
41. Walsh PT, Smith LM, O'Connor R: Insulin-like growth factor-1 activates Akt and Jun N-terminal kinases (JNKs) in promoting the survival of T lymphocytes. *Immunology* 2002;107:461–471. [PubMed: 12460191]
42. Wolters TLC, Netea MG, Hermus ARMM, Smit JWA, Netea-Maier RT: IGF1 potentiates the pro-inflammatory response in human peripheral blood mononuclear cells via MAPK. *J Mol Endocrinol* 2017;59:129–139. [PubMed: 28611056]
43. Bernabei P, Bosticardo M, Losana G, Regis G, Di Paola F, De Angelis S, Giovarelli M, Novelli F: IGF-1 down-regulates IFN-gamma R2 chain surface expression and desensitizes IFN-gamma/STAT-1 signaling in human T lymphocytes. *Blood* 2003;102:2933–2939. [PubMed: 12842994]
44. Erlandsson MC, Töyrä Silfverswärd S, Nadali M, Turkila M, Svensson MND, Jonsson IM, Andersson KME, Bokarewa MI: IGF-1R signalling contributes to IL-6 production and T cell dependent inflammation in rheumatoid arthritis. *Biochim Biophys Acta Mol Basis Dis* 2017;1863:2158–2170. [PubMed: 28583713]
45. Villares R, Kakabadse D, Juarranz Y, Gomariz RP, Martínez-A C, Mellado M: Growth hormone prevents the development of autoimmune diabetes. *Proc Natl Acad Sci U S A* 2013;110:E4619–4627.
46. Kaiko GE, Horvat JC, Beagley KW, Hansbro PM: Immunological decision-making: how does the immune system decide to mount a helper T-cell response? *Immunology* 2008;123:326–338. [PubMed: 17983439]
47. Dardalhon V, Korn T, Kuchroo VK, Anderson AC: Role of Th1 and Th17 cells in organ-specific autoimmunity. *J Autoimmun* 2008;31:252–256. [PubMed: 18502610]

ESTABLISHED FACTS

- *IGF1R* mutations impact growth and glucose metabolism.
- IGF1R signaling modulates immune function, as demonstrated by *in vitro* human and *in vivo* mouse studies.

NOVEL INSIGHTS

- The novel heterozygous *IGF1R* mutation, c.641-2A>G, results in abnormal mRNA splicing and premature IGF1R termination.
- *IGF1R* mutation carriers demonstrated varied phenotypic expression, with growth failure in twin probands and hypoglycemia, reduced Th17, and increased Th1 cell percentages in all affected family members.

Figure 1. Pedigree of family with *IGF1R* c.641-2A>G (het) variant.

The twin female probands (arrows) are III-3 (Twin A, P1) and III-4 (Twin B, P2). Family members who had *IGF1R* gene sequenced are noted with an asterisk (*), and those carrying the heterozygous mutation are noted with a plus sign (+). Family members with reported hypoglycemia are shaded gray and those with short stature are shaded black. Height standard deviation score (SDS) for age and sex based on CDC growth charts are found in parentheses (adult height SDS provided or most recent heights for children; twin proband height SDSs are pre-rhGH treatment).

Figure 2. *IGF1R* c.641-2A>G (het) variant induces early *IGF1R* protein termination. *IGF1R* c.641-2A>G (het) mutation (underlined) in the 3' consensus sequence for spliceosomal recognition (bolded DNA sequence) of intron 2 is predicted to induce skipping of exon 3, leading to premature protein termination. Gene diagram modified from ImmunoBase. NCBI reference sequence for gene: NG_009492.1; protein: NP_000866.1.

Figure 3. *IGF1R* c.641-2A>G (het) mutation induces skipping of exon 3.

Targeted Sanger sequencing analysis of genomic DNA shows *IGF1R* c.641-2A>G (het) mutation within intron 2, and sequencing of *IGF1R* cDNA from probands (P1, P2) and unaffected wild-type (WT) family members confirms that this causes an abnormal splicing event, leading to skipping of exon 3 in the probands.

Figure 4.

IGF1R c.641-2A>G (het) mutation induces premature stop codon disrupting ligand-binding and membrane attachment. (A) Linear depiction of domains of IGF1R ($\alpha\beta$)₂ homodimer with cell membrane shown as gray horizontal block. Location of IGF1R frameshift denoted by black horizontal dashed line. (B) Three-dimensional model of wild-type (WT) IGF1R complexed with IGF1. The two IGF1 binding sites per $\alpha\beta$ dimer include: 1) Parts of the L1 and α -CT domains from different subunits and 2) Parts of Fn1 and Fn2 domains from the same subunit. (C) Three-dimensional model of IGF1R p.Met214Thrfs*67. Dashed lines are indicative of domains past the predicted stop codon, which would not be translated from the *IGF1R* c.641-2A>G allele. The two IGF1 binding sites on each $\alpha\beta$ dimer are predicted to be disrupted. Figure modified from Kavan, et al. [33].

Figure 5. Clinical laboratory and growth data for probands (twin A and twin B). (A) Serum IGF1 and (B) IGFBP-3 levels with the vertical line indicating initiation of rhGH therapy and horizontal lines indicating the reference range at age 5 years (shaded area). (C) CDC age and gender-based height growth chart for probands. Arrow at initiation of rhGH therapy.

Figure 6. IGF1R protein expression is significantly reduced on PBMCs of *IGF1R*^{Het} subjects. Whole blood staining was performed to assess IGF1R levels via flow cytometry. (A) Representative histograms of CD221 (IGF1R) expression on bulk PBMC, granulocyte, monocyte, and lymphocyte populations based on size and granularity, of an age and sex-matched control (black) as compared to an *IGF1R*^{Het} subject (gray). (B) Geometric mean fluorescence intensity (gMFI) of CD221 is significantly reduced on *IGF1R*^{Het} PBMCs, granulocytes, monocytes, and lymphocytes. Twins (circles), older sister (triangles), and mother (squares) shown with their respective age-matched controls distinguished by shape. Mann-Whitney test: *, $p < 0.05$; **, $p < 0.01$.

Figure 7. Broad immunophenotyping reveals modulation of CD4⁺ T cell skewing in $IGF1R^{Het}$ subjects.

Whole blood was analyzed for percentages of sub-populations of naïve and memory CD4⁺ and CD8⁺ T cells (circles), effector CD4⁺ T cells (triangles), regulatory T cells (squares), follicular helper T cells (diamonds), naïve and memory B cells (inverted triangles), natural killer cells, dendritic cells, and monocytes (circles with crosses). For more detail on the populations analyzed, see Supplementary Table 2 and Supplementary Figures 2–4. (A) Volcano plot of immune phenotypes in control versus $IGF1R^{Het}$ subjects reveals that Th1 (CD3⁺CD4⁺CD45RO⁺CD183⁺CD196⁻) cells were significantly decreased and Th17 (CD3⁺CD4⁺CD45RO⁺CD183⁺CD196⁺) cells were significantly increased in whole blood of controls as compared to $IGF1R^{Het}$ subjects. Multiple t tests were performed with a significance threshold of $p = 0.05$ (horizontal dashed line). (B) Representative gating of CD196 and CD183 on CD3⁺CD4⁺CD45RO⁺ cells from a control and an $IGF1R^{Het}$ subject. (C) While the percentages of Th2 (CD183⁻CD196⁻) and Th1/17 (CD183⁺CD196⁺) were not altered, Th17 (CD183⁺CD196⁺) were significantly decreased and Th1 (CD183⁺CD196⁻) were significantly increased in $IGF1R^{Het}$ subjects. Twins (circles), older sister (triangles), and mother (squares) shown with their respective age-matched controls distinguished by shape. Mann-Whitney test: *, $p < 0.05$; **, $p < 0.01$.