Increasing knowledge in IGF1R defects: lessons from 35 new patients

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ABSTRACT

(248words)

Background: The IGF1R is a keystone of fetal growth regulation by mediating the effects of IGF-I and IGF-II. Recently, a cohort of patients carrying an *IGF1R* defect was described, from which a clinical score was established for diagnosis. We assessed this score in a large cohort of patients with identified *IGF1R* defects, as no external validation was available. Furthermore, we aimed to develop a functional test to allow the classification of variants of unknown significance (VUS) *in vitro.*

Methods: DNA was tested for either deletions or single nucleotide variant (SNV) and the phosphorylation of downstream pathways studied after stimulation with IGF-I by western blotting of fibroblast of nine patients.

Results: We detected 21 *IGF1R* defects in 35 patients, including eight deletions and 10 heterozygous, one homozygous, and one compound-heterozygous SNVs. The main clinical characteristics of these patients were being born small for gestational age (90.9%), short stature (88.2%), and microcephaly (74.1%). Feeding difficulties and varying degrees of developmental delay were highly prevalent (54.5%). There were no differences in phenotypes between patients with deletions and SNVs of *IGF1R.* Functional studies showed that the six missense SNVs tested were associated with decreased AKT phosphorylation.

Conclusion: We report eight new pathogenic variants of *IGF1R* and an original case with a homozygous SNV. We found the recently proposed clinical score to be accurate for the diagnosis of *IGF1R* defects with a sensitivity of 95.2%. We developed an efficient functional test to assess the pathogenicity of SNVs, which is useful, especially for VUS.

INTRODUCTION

Insulin-like growth factors IGF-I and IGF-II are major factors which stimulate fetal growth. Both bind to the type 1 IGF receptor (IGF1R). Binding of IGFs to this receptor leads to autophosphorylation of intracellular tyrosine residues, which in turn leads to activation of the phosphatidyl-inositide 3-kinase (PI3K)/AKT and mitogen-activated protein kinase (MAPK)/ERK signaling pathways, resulting in cellular proliferation and growth [1]. *IGF1R* is located at chromosome 15q26, contains 21 exons, and leads to the expression of a dimeric transmembrane tyrosine-kinase receptor (figure 1A. and 1C.) [2]. IGF1R and the insulin receptor (IR) share more than 50% homology and hybrid dimers can be generated, the function of which is still unclear [3].

The first description of the involvement of *IGF1R* defects in pre and post-natal growth failure was made by Pasquali *et al.* in the late seventies [4]. The authors described patients with intra-uterine growth retardation (IUGR), post-natal growth failure, and microcephaly due to a 15q26 terminal deletion that included *IGF1R* (OMIM #612626). Butler *et al.* then described a similar phenotype in patients with ring chromosome 15 with *IGF1R* deleted [5]. The phenotype-genotype correlation was unclear because of the difference in gene content of these large deletions. In 2013, Abuzzahab *et al.* reported a loss of function of the IGF1R in two children with either compound heterozygous pathogenic missense variants or a heterozygous pathogenic nonsense variant of *IGF1R* (OMIM #270450) [6]. Since then, many others have reported pathogenic variants in *IGF1R*, mainly in the heterozygous state and rarely in the compound heterozygous state (n = 3) [7 and references within]. Finally, in 2012, Gannagé-Yared *et al.* reported the first patient with a homozygous pathogenic variant [8]. Most of the reported cases were born small for gestational age (SGA) with no or poor catch-up growth, but the final heights are widely variable and can be in the normal range. Indeed variable phenotypic expression has already been reported, even in relatives carrying the same molecular defect, which makes the diagnosis of *IGF1R* defects difficult [9,10]. Furthermore, some authors have highlighted that the phenotype of patients with *IGF1R* defects overlaps with that of either Silver-Russell syndrome (SRS, OMIM #180860) or SHORT syndrome (OMIM

#269880), leading to the late diagnosis of *IGF1R* defects [11,12]. However, the presence of microcephaly appears to be highly specific for *IGF1R* defects. Thus, the recent international consensus about SRS mentioned *IGF1R* defects as a differential diagnosis of SRS in case of absence of relative macrocephaly, meaning that head circumference should be assessed in a patient born SGA with poor catch-up growth to distinguish between these etiologies [13]. From the first descriptive cohort of 25 patients with *IGF1R* defects, Walenkamp *et al.* proposed a clinical score to drive molecular investigations [14]. This score combines the following four items: birth length or weight < -1 standard deviation score (SDS), head circumference < -2 SDS at first presentation, height at first presentation < -2.5 SDS, and plasma IGF-I levels above the mean for age and gender. Molecular testing for *IGF1R* should be proposed if three or more items are present, with a sensitivity of 76% in their cohort [14]. Recently, Janchevska *et al.* identified two anomalies of *IGF1R* in a cohort of 64 patients born SGA with no catch-up growth, supporting the hypothesis that the prevalence of these defects is high enough to search for them in this particular group of patients [15]. Concerning the treatment of postnatal short stature, the efficiency of recombinant growth hormone (rGH) therapy in patients with *IGF1R* defects is still controversial and only isolated cases with variable age at onset, duration, and dose of treatment have been reported [7,14].

A few functional studies in either fibroblasts or cell lines have been reported, generally showing the inability of the mutated receptor to activate downstream pathways, especially phosphorylation of the receptor itself and/or AKT and rarely ERK [15–18]. In 2009, Fang *et al.* demonstrated *IGF1R* haploinsufficiency due to a mRNA decay phenomenon in a nonsense variant in exon 18 [10]. Most groups have not observed any effect on the expression of the transmembrane IGF1R in patients with missense variants [6,10,19,20].

We report here a large cohort of 21 *IGF1R* defects, including eight previously unreported pathogenic variants. Furthermore, we established phenotype-genotype correlations and assessed the efficiency of rGH therapy in these patients. Finally, we developed a reproductive functional test to assess the responsibility of variants of unknown significance (VUS) in the phenotype.

MATERIALS AND METHODS

Patients

Patients were either followed in our clinic or referred by other clinical centers for molecular analysis. A clinical file, including comprehensive clinical and biological data, growth charts, and treatment was completed for all patients. Each patient had been examined by a geneticist and/or a pediatric endocrinologist. Molecular diagnosis strategy following clinical presentation is depicted in supplementary figure 1. Written informed consent for participation was received either from the patients themselves or their parents, in accordance with French national ethics rules for patients recruited in France (Assistance Publique – Hôpitaux de Paris authorization n°681).

Auxologic methods

Length, weight, and head circumference at birth are expressed as SDS according to Usher and McLean charts [21]. Post-natal growth parameters are expressed as SDS according to Sempé charts [22]. The age of puberty onset (breast development for girls and testis enlargement (≥ 4mL) for boys) was considered to be normal from 8 to13 years for girls and 9 to 14 years for boys.

Molecular analysis

All molecular diagnosis of the *IGF1R* defects was performed in the same laboratory of molecular genetics. DNA was extracted from blood leukocytes using an in-house protocol after cell lysis by a salting out procedure, as previously described [23]. DNA was quantified using a NanoDrop ND-1000 Spectrophotometer (Invitrogen, France).

The main known molecular causes of SRS (loss of methylation at 11p15, maternal uniparental disomy of chromosome 7) or Temple syndrome (OMIM#616222) at the 14q32.2 locus were ruled out by methylation analysis, as detailed in a previous study [23].

IGF1R deletions were assessed by multiplex ligation-dependent probe amplification (MLPA) using the SALSA MLPA P217 IGF1R probe mix (MRC Holland, Amsterdam, Netherlands), following the

manufacturer's instructions. MLPA data were analyzed using the Novel Software Coffalyser.NET provided by MRC-Holland.

For single nucleotide polymorphism (SNP) microarray analysis, samples were processed using cytoSNP-12, or HumanOmniExpress-24 microarrays (Illumina, San Diego, CA, USA). Automated Illumina microarray experiments were performed according to the manufacturer's instructions. Images were acquired using an iScan System (Illumina). Image analysis and automated CNV calling were performed using GenomeStudio v.2011.1 and CNVPartition v.3.1.6. SNP profiles were analyzed by examination of signal intensity (Log R ratio, i.e. ln (sample copy number/reference copy number)) and allelic composition (BAF, i.e. B Allele Frequency).

For the detection of *IGF1R* SNV, DNA was amplified and sequenced by direct Sanger sequencing procedures, using the ABI PRISM Big Dye Terminator v3.0 Cycle Sequencing Kit and an ABI 3100 Genetic Analyzer (Life Technologies, Courtaboeuf, France). Sequences were then analyzed with SeqScape v2.6 (Life Technologies).

Variants are described in accordance with the recommendations of the Human Genome Variation Society. All the new variants were recorded in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar).

Variant interpretation was performed following the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) classification of variants [24]. Six main categories are evaluated according to these guidelines: population data (prevalence of the variant in control populations), computational in silico predictive data, functional characterization, segregation, de novo data and allelic data (e.g. variant detected in *trans* with a pathogenic variant for a recessive disorder). Depending on these data, variants are classified as benign or likely benign, pathogenic or likely pathogenic and some stay of uncertain significance.

Functional test

Patient fibroblasts, obtained from skin biopsies after informed consent and control fibroblasts obtained from the Coriell Institute of Medical Research (Camden, N.J.), were cultured in Dulbecco's Modified Eagle Medium enriched with glutamate, sodium pyruvate, penicillin, streptomycin, and 10% fetal-calf serum at 37°C. After 24 h of serum-free culture in six-well plates, cells were stimulated with [50 ng/ml] IGF-I (Peprotech, US) for 10 min before lysis. We found these stimulation conditions to be the most accurate to assess both AKT and ERK phosphorylation in controls. For both non-stimulated and stimulated conditions, 4.2 to 12.1 µg of protein was deposited in a NuPAGE™ 4-12% Bis-Tris Gel (Thermo Fischer Scientific, US). Electrophoresis was performed on an XCell SureLock™ Mini-Cell Electrophoresis system (Thermo Fischer Scientific, US). Membranes were incubated with polyclonal antibodies against either phospho-AKT (Ser473, Cell Signaling, US, 1:2000), pan-AKT (Cell Signaling, 1:1000), phospho-ERK1/2 (Tyr204, Cliniscience, France, 1:800), ERK1/2 (Cell Signaling, 1:1000), or GAPDH for normalization (Cell Signaling, 1:2000). Then, membranes were incubated with an HRP-conjugated secondary antirabbit antibody (1:3,000), revealed with ChemiDoc™ XRS+ System (Bio-Rad, US), and analyzed with Quantity One v4.6.6 software. Immunoblot images were quantified using ImageJ 1.50 software (https://imagej.nih.gov).

mRNA quantification

Total mRNA was extracted from non-stimulated cells using NucleoSpin miRNA® (Macherey-Nagel) and cDNA obtained by reverse-transcriptase polymerase chain reaction (RT-PCR, Superscript II, Invitrogen, France). cDNA was then amplified and quantified on a QuantStudio 7 Flex Real-Time PCR system (Thermo Fischer) using primers localized in exons 7-8 by SYBR Green technology (Applied Biosystem, US).

Biological assays

IGF-I serum concentrations were determined by different techniques, as patients were followed in different centers. However, IGF-I levels were collected along with the normal values (NV) for most

patients. Thus, IGF-I levels were considered as high if >1 SDS according to the technique used. We express IGF-I levels as SDS according to age and gender from control matched references [25].

Statistical analysis

Characteristics of the population are described as percentages for qualitative variables or as SDS and mean (range) for continuous variables. For statistical analysis, Pearson's test was used for correlations, Fisher's test for dichotomous variables, and the t-test for continuous variables.

RESULTS

Genetic results

Between 2006 and 2018, 111 samples of DNA were tested for *IGF1R* mutations/deletions. We identified *IGF1R* defects in 35 patients from 20 different families. Aside from the 20 index cases, we identified *IGF1R* defects in 15 relatives, including three siblings, seven fathers, and five mothers. Among the 20 index cases, molecular analysis was prescribed for 13 patients for a clinical suspicion of an *IGF1R* defect and for seven for a clinical suspicion of SRS (Supplementary figure 1). The molecular diagnosis of the *IGF1R* defect was made at 9.2 years of age (0.8 to 18.1) for the index cases. Eight patients carried a heterozygous deletion (figure 1B.). Eleven carried a single nucleotide variant (SNV): 8 missense, two nonsense, and one insertion at the boundary of intron 5-exon 6. Sequencing of the cDNA of the latter variant obtained from lymphocytes confirmed that the inserted guanine was present in the cDNA, leading to a frameshift and a premature stop codon (N417Efs*52, Supplementary figure 2). One patient carried two missense SNVs. Among the 13 SNVs identified, 10 patients had a heterozygous SNV, one patient had compound heterozygous missense SNV, and one carried a homozygous missense SNV (figure 1). Parental DNA samples were available for 13 patients. Three inherited the anomaly from their mother, five from their father, two from both parents, and the anomaly arose *de novo* for three patients. Among the 13 variants, we identified eight new pathogenic or likely pathogenic variants (Table 1). Five deletions included the entire *IGF1R* gene, one interstitial and four terminal lengthening from 3.13 to 5.01Mb (figure

1B., Del1 to 5), whereas three included only part of *IGF1R* with length from 19kb to 234kb (figure 1B., Del6 to 8).

Clinical features

The intragenic deletions (exon 2, Del6, n = 3) and *IGF1R* terminal deletions (Del7, n = 2 and Del8, n = 1) did not include other disease-causing OMIM genes and were thus analyzed together with the SNVs for the clinical study. Clinical characteristics are shown in Table 2. There was no statistical difference in clinical presentation between patients with large deletions and pathogenic variants of *IGF1R*. We calculated the clinical score recently proposed by Walenkamp *et al.* for 21 patients for whom clinical data required for this scoring system were fully available (birth weight or length < -1 SDS, height at presentation < -2.5 SDS, head circumference at presentation < -2 SDS (microcephaly) and IGF-I level > 0 SDS) [14]. Twenty patients (95.2%) met at least three of the four criteria and 11 (52.4%) fulfilled all four. Among them, all had a birth weight or length < -1 SDS, 17 (81.0%) had a height at presentation below -2.5 SDS, and 19 (90.5%) had microcephaly. All 21 patients scored positive for elevated IGF-1 levels if considered at the different endpoints (including during rGH treatment). However, five patients (23.8%) would have not met this criterion if IGF-I levels were considered only prior to the initiation of rGH treatment. One patient (carrying Del6) did not achieve a positive clinical score, with only two items [being born with a height or weight < -1 SDS and high levels of IGF-I (during rGH treatment only)].

Given the clinical overlap between SRS and *IGF1R* defects, the Netchine-Harbison clinical scoring system (with a positive clinical diagnosis of SRS for a score of at least 4/6) was assessable for 10 patients and only one scored 4/6 [13,26] (lacking relative macrocephaly at birth and body asymmetry items). Nevertheless, most patients scored 3 out of 6, comprising the following items: being born SGA, post-natal growth retardation, and feeding difficulties.

					ACMP/AMP	
cDNA nomenclature NM_000875.4	**Reference**	**Amino-acid substitution** NP_000866.1	**ClinVar**	**GnomAD**	**Detailed staging**	**Variant classification#**
c.118C>T	This study	R40C	SCV000926288	Not reported	PM1 PM2 PP2 PP3	Likely pathogenic
c.384T>C	This study	F112L	SCV000926289	Not reported	PS4 PM1 PM2 PP1 PP2 PP3 **PS3**	Pathogenic
c.904G>T	This study	E302*	SCV000926290	Not reported	PVS1 PM2 PP3 PP4	Pathogenic
c.995G>A	This study	C332Y	SCV000926291	Not reported	PM1 PM2 PP2 PP3 BS4	Likely pathogenic
c.1247+1-1247+2insG	This study	N417Efs*52	SCV000926292	Not reported	**PS3** PM2 PM4 PP1 PP4	Pathogenic
c.3162G>A	This study	M1054I	SCV000926293	Not reported	PM1 PM2 PP2 PP3 PP4	Likely pathogenic
c.3454G>A	This study	G1152R	SCV000926294	Not reported	PS4 PM1 PM2 PP1 PP2 PP3 PP4	Pathogenic
c.3539C>A	This study	S1180Y	SCV000926295	Not reported	PS4 PM1 PM2 PP2 PP1 PP3	Pathogenic
c.2629C>T	[30]	R877*	rs150221450	AF: $3.977.10^{-6}$	PVS1 PM2 PP3 PP4	Pathogenic
c.3530G>A	[14]	R1177H	SCV000926296	Not reported§	PM1 PM2 PP2 PP3	Likely pathogenic
c.3595G>A	EGL Genetic Diagnostics	G1199R	rs886044448	Not reported	PS4 PM1 PM2 PP1 PP2 PP3 PP4	Pathogenic
c.4055G>T	dbSNP	G1352V	rs759808066	AF: $1.607.10^{-5}$	PM2 PP2 PP3 **PS3** BS4	Likely pathogenic¤
c.4066G>A	[14]	E1356K	rs746562843	AF: 642.10^{-5}	PM2 PP2 BS4 **PS3**	Likely pathogenic¤

Table 1

	All n = 35			Deletions n = 6			SNVs n = 29			p
	Mean	**Range**	**n (%)**	**Mean**	**Range**	**n (%)**	**Mean**	**Range**	**n (%)**	
Sex (Female/Male)			15/20			2/4			13/16	0.68
Birth parameters:										
Term (WA)	37.7	31.0;41.5	23	38.4	37.0;40.0	5	37.5	31.0;41.5	18	0.82
Preterm (< 37 WA)			5/23 (21.7)			0/5 (0)			5/18 (27.8)	0.55
Weight (SDS)	-2.5	-3.8;-1	23	-2.3	-3.2;-1.4	5	-2.5	-3.8;-1.0	18	0.58
SGA #(weight <- 2 SDS)			17/23 (73.9)			3/5 (60.0)			14/18 (77.8)	1
Length (SDS)	-3.3	-5.2;-1.4	22	-3.0	-4.2;-2	5	-3.4	-5.2;-1.4	17	0.46
SGA # (length < -2 SDS)			20/22 (90.9)			4/5 (80.0)			16/17 (94.1)	1
Head circumference (SDS)	-2.6	-3.9;-0.3	17	-2.4	-3.0;-1.6	5	-2.7	-3.9;-0.3	12	0.63
Microcephaly (HC< -2 SDS)			14/17 (82.4)			4/5 (80.0)			10/12 (83.3)	1
Relative macrocephaly#			3/17 (17.6)			1/5 (20.0)			2/12 (16.7)	1
Clinical features:										
Microcephaly			20/27 (74.1)			4/4 (100)			16/23 (69.6)	0.70
Feeding difficulties#			12/22 (54.5)			4/5 (80.0)			8/17 (47.1)	0.69
Anorexia/thinness			11/14 (78.5)			4/4 (100.0)			7/10 (70.0)	1.0
Enteral nutrition			5/14 (35.7)			2/4 (50.0)			3/10 (30.0)	1
Developmental delay			12/22 (54.5)			4/5 (80.0)			8/17 (47.1)	0.69
Language			6/22 (27.3)			3/5 (60.0)			3/17 (17.6)	0.31
Motor			6/22 (27.3)			1/5 (20.0)			5/17 (29.4)	1
Cognitive			12/22 (54.5)			4/5 (80.0)			8/17 (47.1)	0.69
Assistance in school			14/23 (60.9)			4/5 (80.0)			10/18 (55.6)	0.70
Clinical score ([14]):										
Birth weight or length < -1 SDS			21/21 (100)			4/4 (100)			17/17 (100)	
Microcephaly (HC< -2 SDS)			19/21(90.5)			4/4 (100)			15/17 (88.2)	
Height # (< -2.5 SDS)			17/21(81.0)			4/4 (100)			13/17 (76.5)	
IGF-I > 0 SDS			21/21 (100)			4/4 (100)			17/17 (100)	
Final height (SDS):	-2.5	-4.2;1.0	25	-2.9	-4.1;-2.1	5	-2.3	-4.2;1.0	20	0.30
Without rGH	-2.2	-4.2;1.0	13	-3.2	-4.1;-2.1	2	-2.0	-4.2;1.0	11	0.42
With rGH	-2.8	-4.2;-1.0	12	-2.8	-3.8;-2.1	3	-2.8	-4.2;-1.0	9	0.99
Short stature (< -2 SDS)			19/25 (76.0)			5/5 (100)			14/19 (73.7)	0.72

Table2

No deafness was reported in our cohort. One girl (carrying a missense SNV) had a slightly delayed onset of puberty (onset at 13.3 years), whereas the onset of puberty of the other 16 patients (10 boys) occurred at the normal age. Three patients were treated with GnRH analogs together with rGH at the onset of puberty to preserve the duration of growth due to a low predicted final height, despite the onset of puberty at a normal age. Four patients carrying a missense SNV had attention deficit hyperactivity disorder (ADHD), which required medication. Three patients developed obesity in childhood with metabolic syndrome for one as a young adult. One patient (father of two affected children) had early type 2 diabetes and one patient had episodes of hypoglycemia in infancy. Noticeably, four patients (two with *IGF1R* deletion, two with a missense SNV) had cardiac defects, including one case of transient inter-auricular communication (IAC), one of IAC and rhythmic troubles, one of patent foramen ovale, and one of severe cardiac insufficiency, which led to heart transplantation (carrying a missense SNV).

Only two cases of homozygous pathogenic variants have yet been reported [8,12]. Thus, the pedigree and growth curves of the girl with the F112L homozygous pathogenic variant are shown in figure 2. Although the girl with the homozygous pathogenic variant (II.4) showed severe growth retardation of approximately -4 SDS with tremendously elevated IGF-I [621 ng/mL (NV 20-300) at 1.6 years] and IGFBP-3 [5605 ng/mL (NV 800-3700)], both parents (I.1 and I.2) and one older sister (II.3) with the heterozygous pathogenic variant showed impaired postnatal growth of approximately -2 SDS, with a final height in the lower range of normal curves. The unaffected younger siblings (II.1 and II.2) showed normal growth around the mean. Furthermore, the homozygous carrier (II.4) had a patent foramen ovale, severe oeso-gastric reflux, anorexia requiring enteral support for one year (naso-gastric tube), and psychomotor delay with learning disability, whereas no other member of the family presented with such clinical features.

All clinical data are available for each patient in Supplementary Table 1.

rGH therapy

Eighteen patients received rGH treatment, starting at an age of 7.5 years (1.5;15.3) under the SGA European Medicines Agency (EMA) indication, with a mean height at the start of therapy of -3.8 SDS (-5.6; -1.6). The starting dose was 46.6 µg/kg/day (35.0;85.5) and was significantly increased for only five of the 15 patients for whom data on the dose evolution was available. For most patients (60.0%), the dose of rGH was not raised because of high serum levels of IGF-I. Among the 12 patients that completed rGH treatment and reached their final height, the mean height gain was 1.0 SDS (0.2;2.5), which positively correlated with the duration of treatment ($\rho = 0.76$, $p = 0.004$) and negatively correlated with the age at the start of rGH ($\rho = -0.68$, $p = 0.01$). IGF-I serum levels were high (over 1 SDS) for 11 (47.8%) patients before any treatment, with a mean of 1.9 SDS (-2.0;7.1), which rose to 3.3 SDS (0.3;9.5) under rGH therapy.

IGF1R functional test

We performed functional analysis on fibroblasts for seven index cases, two affected parents and four controls (figure 3). The six missense SNVs all showed a decrease in phosphorylated AKT, although the results for the S1180Y variant did not reach statistical significance ($p = 0.065$). Both G1352V and E1356K were predicted as VUS after *in silico* analysis and showed a significant decrease in AKT phosphorylation *in vitro* ($p = 0.009$ and $p = 0.002$, respectively), suggesting that these variants are likely pathogenic. There were no alterations of AKT phosphorylation for two patients carrying either a nonsense SNV or a chromosome 15q26.6 deletion that included the entire *IGF1R* gene. The results concerning ERK phosphorylation were highly variable and we observed no significant modifications in this pathway (Supplementary figure 3). All but one patient showed normal *IGF1R* expression. This patient, who carries a 15q26.6 heterozygous deletion, including *IGF1R*, showed expression of 37.7% of controls (Supplementary figure 4).

DISCUSSION

We report a large cohort of patients carrying various *IGF1R* defects and describe eight new pathogenic variants. Furthermore, we developed an *in vitro* functional test to assess the pathogenic impact of VUS.

As previously described, *IGF1R* defects are mainly present in the heterozygous state. Nevertheless, we found two patients with missense SNVs on both alleles, including one patient with compound heterozygous pathogenic variants and one with a homozygous one. The patient carrying the compound heterozygous SNVs did not phenotypically differ from the other patients although both variants were pathogenic. On the other hand, the homozygous pathogenic variant was associated with a more severe phenotype in terms of growth, microcephaly, and mental retardation relative to that of her relatives who carry the same variant in the heterozygous state.

With the advent of next-generation sequencing (including exome sequencing or a gene panel of growth disorders, microcephaly, or cognitive impairment, which can include *IGF1R*), the identification of SNVs will increase in the future. Thus, the description and registration of new SNVs with a precise phenotypic description is necessary to distinguish between those that are benign and those that are pathogenic. Furthermore, we demonstrated that functional characterization of such SNVs is sometimes necessary. In our cohort, such experiments were helpful for the classification of two SNVs reported as SNPs with a very low allele frequency and classified as VUS based on the ACMG/AMP recommendations because of incomplete penetrance (E1356K and G1352V) [24]. However, the definition of "unaffected" carrier was only based on the reported final heights of the two fathers who carried the variants, as other criteria were not available (birth parameters, head circumference and IGF-I levels).Those two variants were finally classified as likely to be pathogenic after demonstration of their functional consequences.

The *in vitro* studies showed impairment in the ability to activate downstream pathways for the receptors affected by missense SNVs, especially the AKT pathway. We were unable to demonstrate any significant functional consequences of deletions or nonsense SNV, unlike previous studies [10,27,28]. It is possible that discrepancies between our results on deletions and those of previous studies may be due to different

IGF-I concentrations used for stimulation. Indeed, Choi *et al.* showed a progressive increase in AKT phosphorylation in fibroblasts from a patient with an *IGF1R* deletion in response to increasing IGF-I concentrations from 1 to 400 ng/mL [28]. Ester *et al.* reported the same pattern with lower concentrations (5 to 20 ng/mL) [18]. Thus, it is possible that the IGF-I concentration we used (50 ng/mL) did not allow proper discrimination of AKT phosphorylation between deletions and controls [29]. Nevertheless, the aim of this functional study was to assess pathogenic impact of SNVs of unknown significance, and we found IGF-I concentration of 50 ng/mL to be effective. Unlike missense pathogenic variants, which may lead to a dominant-negative effect, deletions or nonsense variants may lead to haploinsufficiency. Although we could not quantify membrane IGF1R, we demonstrated that *IGF1R* mRNA levels were low in fibroblasts from one patient with a deletion, favoring haploinsufficiency [10,28,30].

Very recently, a scoring system has been proposed for a clinical suspicion of an *IGF1R* defect [14]. This clinical score showed 95.2% sensitivity for our cohort. All patients were born with weight or length < -1 SDS and microcephaly was almost always present. However, post-natal short stature (with a threshold set at -2.5 SDS) was inconstantly observed in our cohort. However, height at first evaluation was usually below -2 SDS. The clinical scoring system could be adapted for this item, so as not to miss patients with *IGF1R* defects for whom height is not severely affected but this will result in an increased number of patients that should be tested and thus, to a reduced specificity. Elevated circulating IGF-I levels were absent prior to rGH therapy for 23.8% of the patients in our cohort but IGF-I levels rose markedly after initiating rGH treatment. The absence of high IGF-I levels prior to rGH therapy can be explained by the previously described feeding difficulties of some patients with *IGF1R* defects, which can lead to nutritional deficiency and low basal levels of IGF-I [31]. This pattern of low IGF-I levels which increase rapidly after initiating rGH therapy, should alert clinicians to the possibility of an *IGF1R* defect in a child born SGA, especially with the presence of microcephaly. However, the high sensitivity of this clinical score favors its use in routine diagnosis to drive genetic tests. The specificity of this clinical score should be assessed in large cohorts of SGA patients with the help of molecular studies, as well as in patients with

idiopathic short stature since fetal growth restriction, although highly prevalent, is not constant in patients carrying *IGF1R* defect.

This cohort allowed us to better characterize the phenotype of patients with an *IGF1R* defect. As previously described, fetal and post-natal growth retardation, microcephaly, and elevated IGF-I serum levels were highly prevalent in our cohort [7,14]. As in previously reported cases, we identified several cardiac anomalies in these patients, mostly benign. However, one patient underwent heart transplantation because of severe cardiac failure. These findings are in accordance with previous observations and argue in favor of a systematic cardiac ultrasound evaluation when an *IGF1R* defect is identified [8,12,19,32]. Another interesting feature of our cohort is the presence of ADHD in several patients, which was only been previously reported for one case [29]. Furthermore, as reported in the Dutch cohort, we found a high prevalence of feeding difficulties, sometimes requiring nutritional support, [9,11,14]. This latter feature may have misled some clinicians to consider a clinical diagnosis of SRS at first evaluation. Indeed patients with SRS or *IGF1R* defects share several symptoms, including being born SGA, post-natal growth retardation, and high circulating levels of IGF-I [33,34]. However patients with *IGF1R* defects usually present with microcephaly, which distinguishes them from SRS patients, for whom head circumference is relatively preserved at birth [13,26]. The recent international consensus on the diagnosis and management of SRS stated that *IGF1R* defects represents a differential diagnosis and may be considered easily after the major molecular defects of SRS are ruled out, especially for those patients with no relative macrocephaly [13].

The efficiency of rGH therapy in this cohort is difficult to ascertain, as this was a retrospective and multicentric analysis with varying management in terms of the age at onset, the initial dose, dose adaptation, and discontinuation of treatment. Since the duration and age at the start of treatment significantly correlate with height gain, rGH treatment should be considered for patients with no catch-up growth at four years of age, under the EMA SGA indication. However, both clinicians and patients (or parents) should be aware of this unpredictable response to rGH therapy. Our *in vitro* experiments,

accounting for the functional consequences of the variants, were unable to distinguish between those patients who responded well or poorly to rGH therapy. It would be of interest however to set up such a prognostic tool. The high baseline IGF-I levels do not reflect the biological effect of IGF-I since the IGF1R signalization is impaired. These high IGF-I levels raised concern on potentially negative long term effects. Nevertheless, the pathophysiological comprehension of such elevated circulating IGF-I levels allows us to consider these levels only as a consequence of IGF-I resistance. Thus, IGF-I levels should not be interpreted and used in patients with *IGF1R* defects in the same manner as in unaffected patients.

In conclusion, we provide extensive clinical data on a large cohort of patients carrying *IGF1R* defects. We identified eight new pathological variants, including one homozygous pathogenic variant. We validated the clinical scoring system that has been recently proposed for patients with *IGF1R* defects. Finally, we developed a functional test to assess IGF1R activity *in vitro* that is useful for sorting VUS, which is of particular importance, especially for accurate genetic counseling.

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TABLES

Table 1. Description of the identified single nucleotide variants in the cohort and predictions of the pathological consequences. ACMP/AMP: American College of Medical Genetics and Genomics and the Association for Molecular Pathology classification of variants [24]. **PS3** corresponds to a pathogenic

effect in functional test. AF: allele frequency. # Classification performed using the InterVar classification system; § First reported in Walenkamp *et al.* [14]. ¤ Classified as "variant of unknown significance" before the functional test results.

Table 2. Clinical features of the patients with *IGF1R* defects. SNV: Single nucleotide variant. WA: weeks of amenorrhea; SDS: standard deviation score; SGA: born small for gestational age; HC: head circumference; rGH: recombinant growth hormone. # Items included in the Netchine-Harbison clinical scoring system for Silver-Russell syndrome diagnosis.

FIGURES

Figure 1. A. Schematic representation of the position of *IGF1R* on chromosome 15. **B.** Representation of the eight identified deletions using the UCSC (University of California Santa Cruz) software. **C.** Representation of the identified single nucleotide variants (SNV) from exons 1 to 21. Arrows indicate the SNVs identified in the cohort. The corresponding functional domains of the protein are shown to the right. L1 and 2: leucine-rich repeat domains; CR: cysteine-rich region; FN1 to 3: fibronectine type III domains; TM: trans-membrane region; TK: tyrosine kinase domain; CT: C-terminal segment.

Figure 2. Growth curves and pedigree of the family of the patient carrying the homozygous variant F112L/F112L. SDS: standard score deviation; NGT: naso-gastric tube.

Figure 3. A. Western blot showing phosphorylated-AKT (P-AKT), total AKT, and GAPDH for patients and controls. **B.** Quantification of AKT phosphorylation calculated as: *R* after IGF-I stimulation / *R* not stimulated, with *R* = [(P-AKTpatient/AKTpatient)/GAPDHpatient]/ [(P-AKTcontrol/AKTcontrol)/GAPDHcontrol]. *p < 0.05; **p < 0.01; WT: wild type allele. Experiments were repeated from 3 to 6 times for each individual. Error bars represent the standard error of the mean. Del ex1-21 corresponds to Del4 in figure 1 and Del ex20-21 to Del7.

STATEMENTS

Competing interests: The authors have nothing to disclose.

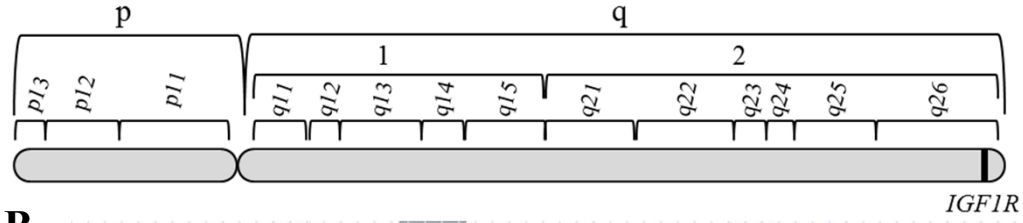
Contribution of the authors:

Eloïse Giabicani: wrote the manuscript, performed the experiments, collected and analyzed the data and revised the manuscript. Marjolaine Willems: collected and analyzed the data and revised the manuscript. Virginie Steunou: performed the experiments. Sandra Chantot-Bastaraud: performed the experiments, analyzed the data and helped in revising the manuscript. Nathalie Thibaud: performed the experiments Walid Abi Habib: analyzed the data and helped in revising the manuscript. Salah Azzi : performed the experiments, analyzed the data and helped in revising the manuscript. Bich Lam: performed the experiments, analyzed the data and helped in revising the manuscript. Laurence Bérard: collected data and helped to revise the manuscript. Hélène Bony-Trifunovic: collected data and helped to revise the manuscript. Cécile Brachet: collected data and helped to revise the manuscript. Elise Brischoux-Boucher: collected data and helped to revise the manuscript. Emmanuelle Caldagues: collected data and helped to revise the manuscript. Régis Coutant: collected data and helped to revise the manuscript. Marie-Laure Cuvelier: collected data and helped to revise the manuscript. Georges Gelwane: collected data and helped to revise the manuscript. Isabelle Guemas: collected data and helped to revise the manuscript. Muriel Houang: collected data and helped to revise the manuscript. Bertrand Isidor: collected data and helped to revise the manuscript. Claire Jeandel: collected data and helped to revise the manuscript. James Lespinasse: collected data and helped to revise the manuscript. Catherine Naud-Saudreau: collected data and helped to revise the manuscript. Monique Jesuran-Perelroizen: collected data and helped to revise the manuscript. Laurence Perrin-Sabourin: collected data and helped to revise the manuscript. Juliette Piard: collected data and helped to revise the manuscript. Claire Sechter: collected data and helped to revise the manuscript. Pierre-François Souchon: collected data and helped to revise the manuscript. Caroline Storey: collected data and helped to revise the manuscript. Domitille Thomas: performed the experiments, collected data and helped to revise the manuscript. Yves Le Bouc: collected data and helped to revise the manuscript. Sylvie Rossignol: collected data and helped to revise the manuscript. Irène Netchine: analyzed

548 the data and revised the manuscript. Frédéric Brioude: analyzed the data, wrote and revised the
549 manuscript.

550

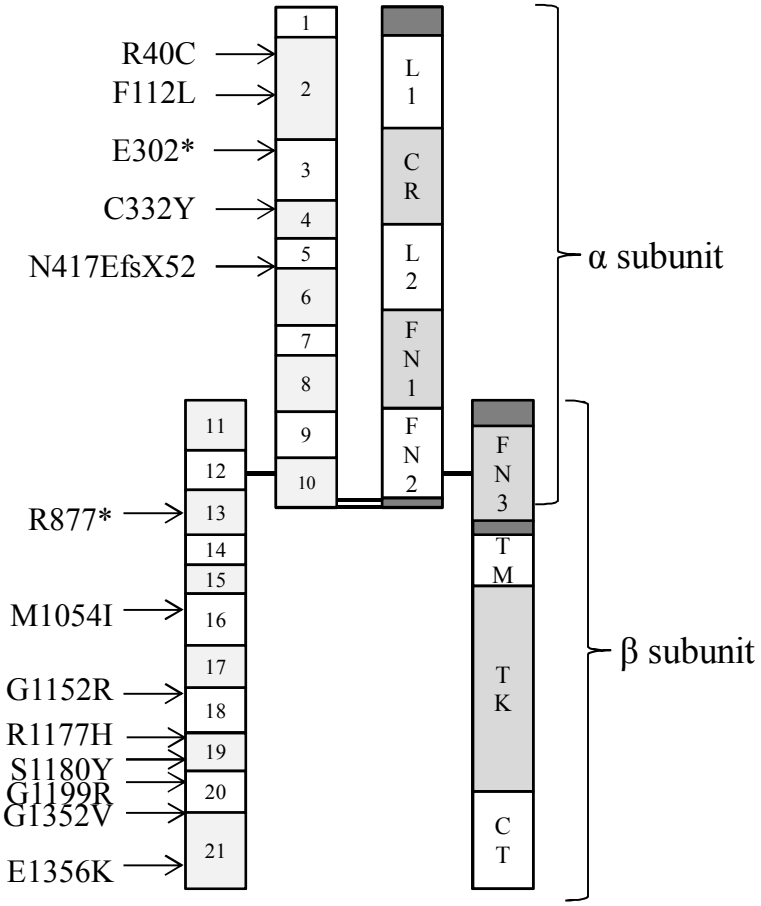
A. Chromosome 15

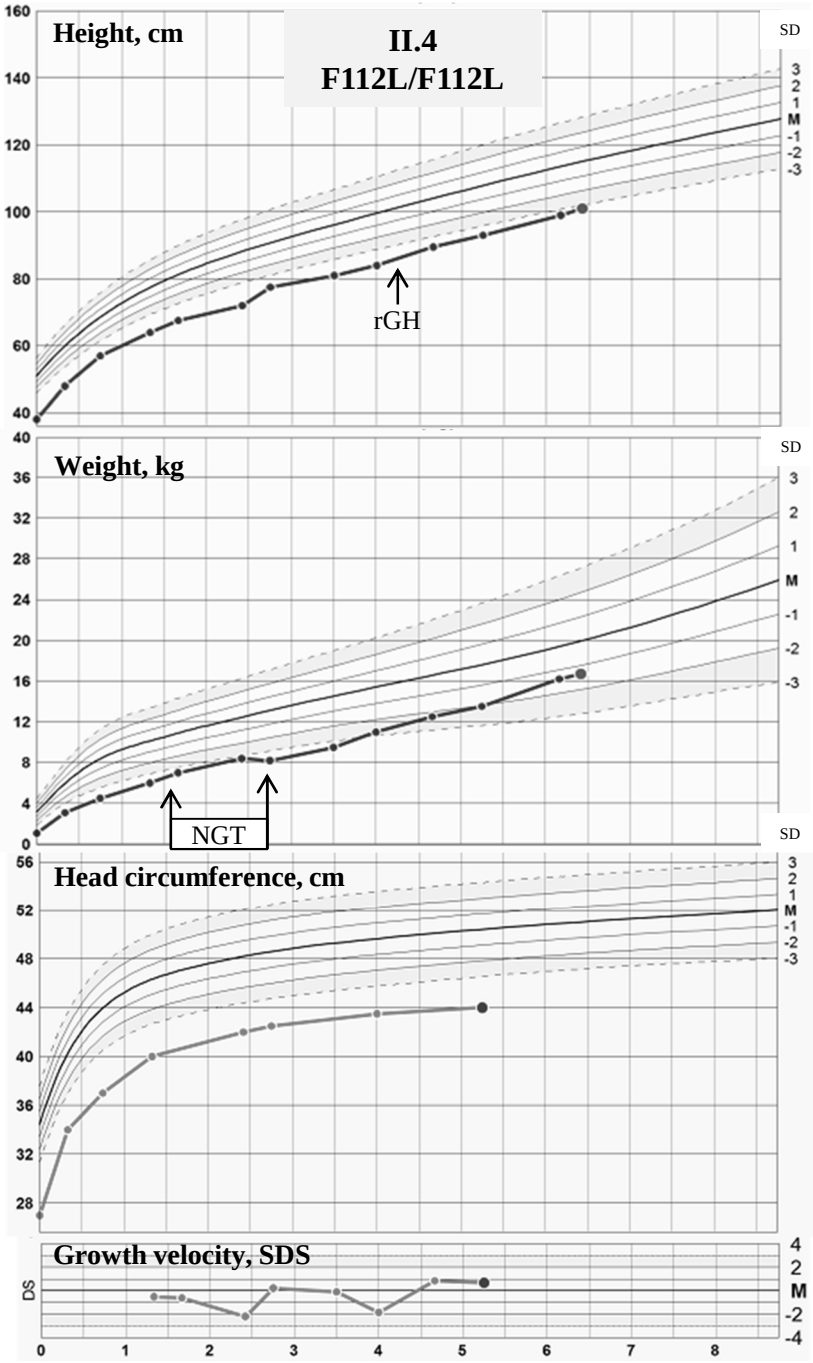


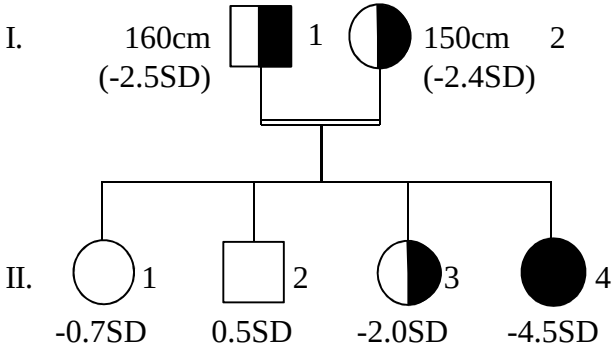
B.

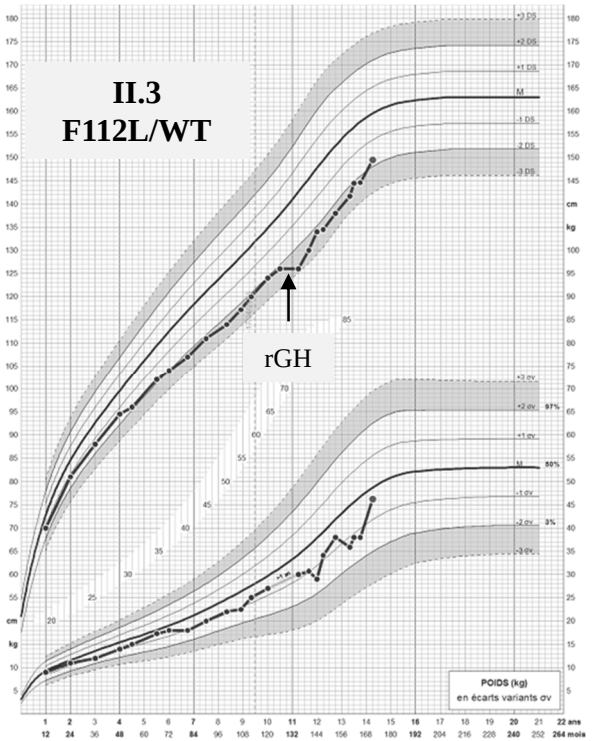
Name	**Position** [Hg19]	**Size(Kb)**
Del1	chr15 : 97450781-102461162	5 010.4
Del2	chr15 : 98426952-102461162	4 034.2
Del3	chr15 : 98464591-101599113	3 134.5
Del4	chr15 : 98610818-102397836	3 787.0
Del5	chr15 : 98954957-102461162	3 506.2
Del6	chr15 : 99229330-99290406	61.1
Del7	chr15 : 99492046-99511073	19.0
Del8	chr15 : 99496341-99730813	234.5

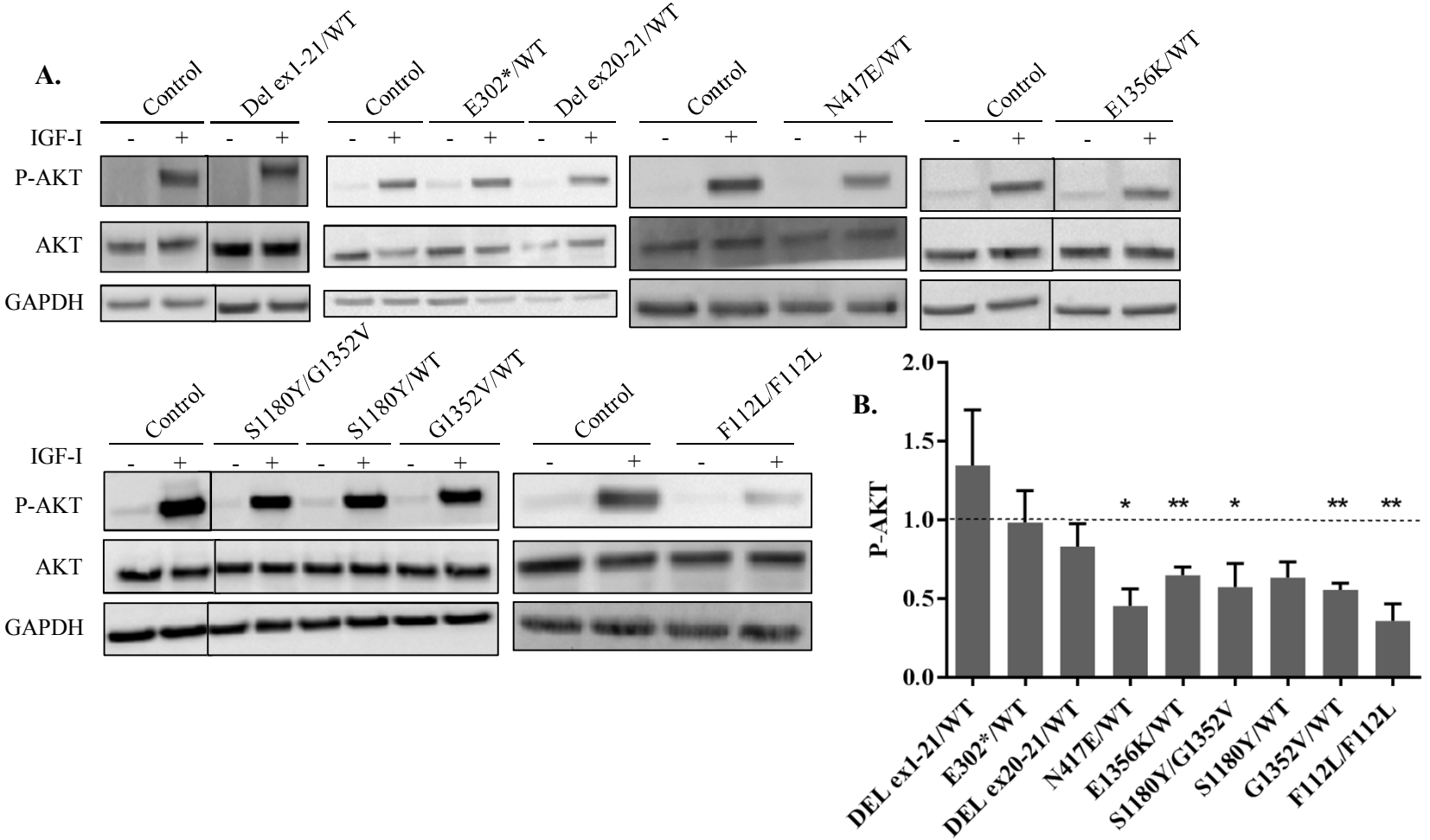
C. IGF1R




Height, cm
II.4
F112L/F112L
SD
rGH
Weight, kg
NGT
Head circumference, cm
Growth velocity, SDS
DS


I.
160cm
(-2.5SD)
1
150cm
(-2.4SD)
2
II.
1
2
3
4
-0.7SD
0.5SD
-2.0SD
-4.5SD


II.3
F112L/WT
rGH
POIDS (kg)
en écarts variants σ


A.
Control
Del ex1-21/WT
Control
E302*/WT
Del ex20-21/WT
Control
N417E/WT
Control
E1356K/WT
IGF-I
- + - + - + - + - + - + - + - + - +
P-AKT
AKT
GAPDH
Control
S1180Y/G1352V
S1180Y/WT
G1352V/WT
Control
F112L/F112L
IGF-I
- + - + - + - + - + - +
P-AKT
AKT
GAPDH
B.
P-AKT
2.0
1.5
1.0
0.5
0.0
*
**
*
**
**
DEL ex1-21/WT
E302*/WT
DEL ex20-21/WT
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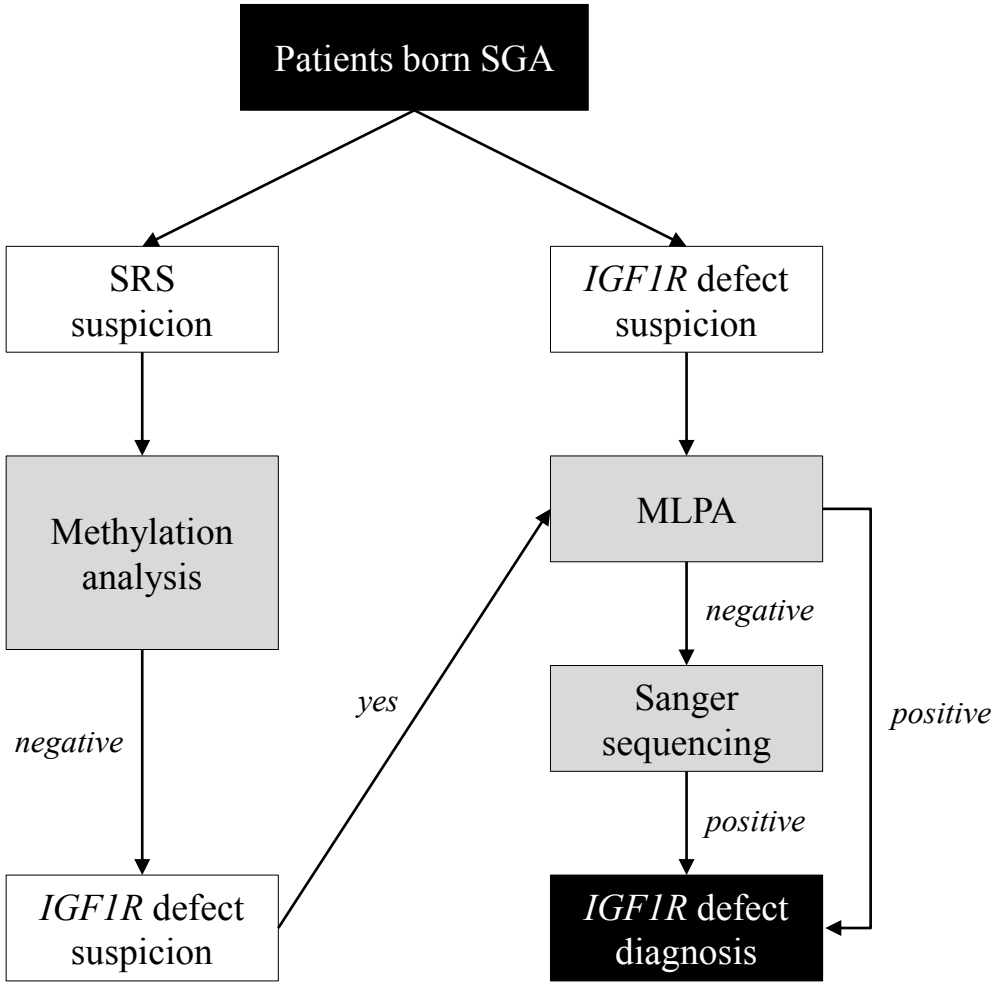
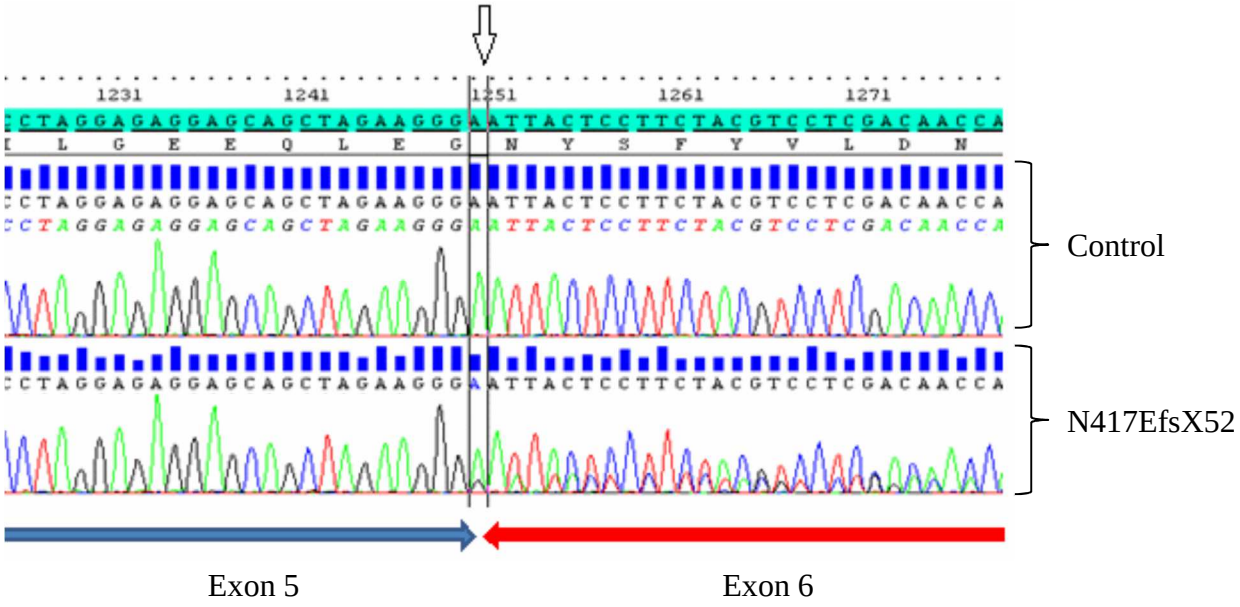
G1352V/WT
F112L/F112L


Patients born SGA
SRS suspicion
IGF1R defect suspicion
Methylation analysis
MLPA
negative
Sanger sequencing
positive
negative
yes
positive
IGF1R defect suspicion
IGF1R defect diagnosis


1231
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1251
1261
1271
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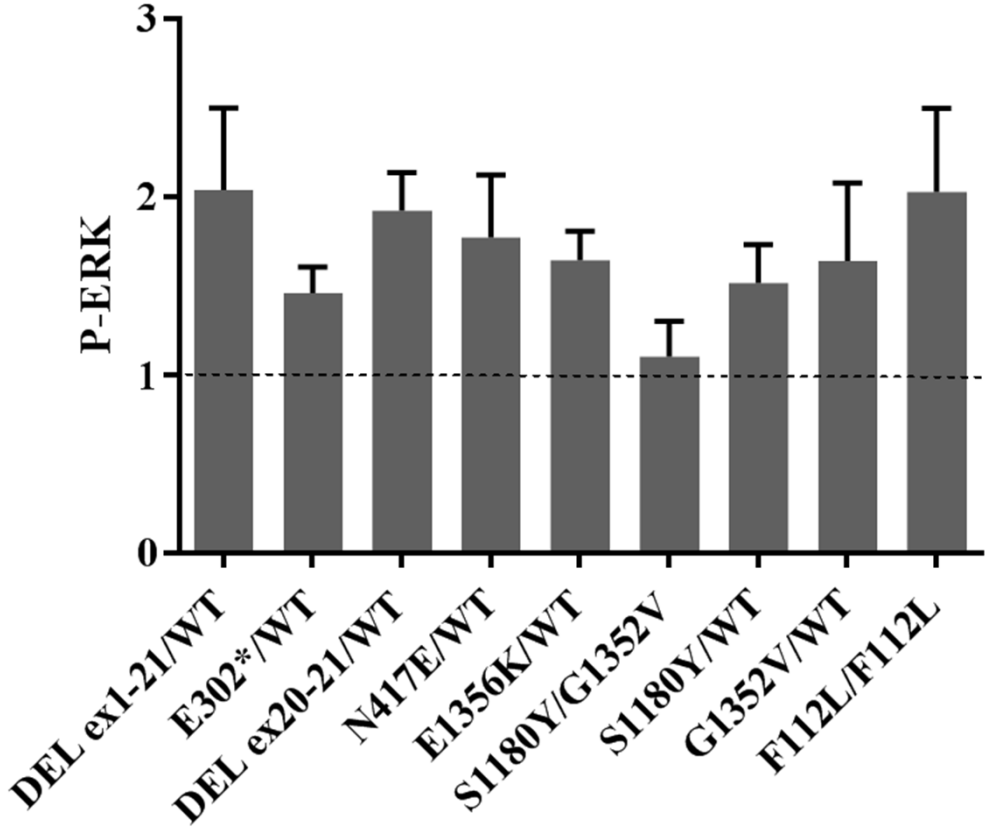
Control
N417EfsX52
Exon 5
Exon 6


P-ERK
3
2
1
0
DEL ex1-21/WT
E302*/WT
DEL ex20-21/WT
N417E/WT
E1356K/WT
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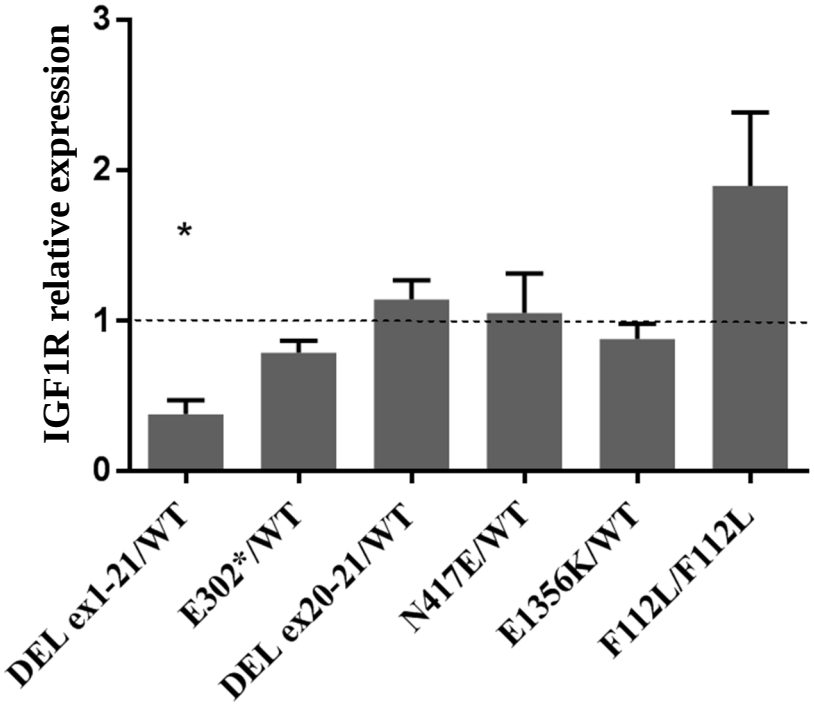
S1180Y/WT
G1352V/WT
F112L/F112L


IGF1R relative expression
3
2
1
0
*
DEL ex1-21/WT
E302*/WT
DEL ex20-21/WT
N417E/WT
E1356K/WT
F112L/F112L

ABBREVIATIONS

ADHD: attention deficit hyperactivity disorder
BL: birth length
BW: birth weight
DVT: developmental
GnRH: gonadotropin
HC: head circumference
HTZ cp: compound HTZ
HTZ: heterozygous
IAC: interatrial communication
min: minimum
NA: not available
POF: patent foramen ovale
rGH: recombinant growth hormone
SDS: standard deviation score
SRS: Silver-Russell syndrome
WA: weeks of amenorrhea

PATIENTS		GENETICS				FAMILY			
NAME	SEX	NOMENCLATURE	PROTEIN	ALLELIC STATUS	SEGREGATION	MOTHER HEIGHT (SDS)	FATHER HEIGHT (SDS)	TARGET HEIGHT (SDS)	TERM (WA)
patient1	M	15q26.3(98954957_102461162)x1	NA	HTZ	mother	-4,1	-0,5	-2,1	40
relative1	F	15q26.3(98954957_102461162)x1	NA	HTZ	unknown	-0,6	-1,7	-1,3	NA
patient2	M	15q26.3(98610818_102397836)x1	NA	HTZ	de novo	1,9	0,8	1,4	39
patient3	M	15q26.2q26.3(98426952_102461162)x 1	NA	HTZ	unknown	-2,4	1,4	-0,3	37
patient4	M	15q26.2q26.3(97450781_102461162)x1	NA	HTZ	unknown	0	2,4	1,3	39
patient5	F	15q26.2q26.3(98464591_101599113)x1	NA	HTZ	de novo	-1,6	0,8	-0,5	37
patient6	M	15q26.3(99492046_99511073)x1	NA	HTZ	unknown	NA	NA	NA	NA
relative6	M	15q26.3(99492046_99511073)x1	NA	HTZ	father	-1,6	-3,5	-2,8	38,3
patient7	F	15q26.3(99496341_99730813)x1	NA	HTZ	de novo	-1,5	-1,7	-1,7	35
patient8	F	15q26.3(99229330_99290406)x1	NA	HTZ	father	-1,5	-1,2	-1,5	32
relative8a	M	15q26.3(99229330_99290406)x1	NA	HTZ	father	-1,5	-1,2	-1,2	31
relative8b	M	15q26.3(99229330_99290406)x1	NA	HTZ	unknown	-3,8	0	-1,7	41
patient9	M	c.118C>T	R40C	HTZ	unknown	-0,9	-3,3	-2	41
patient10	F	c.384T>C	F112L	HMZ	father + mother	-2,4	-2,5	-2,6	34
relative10a	F	c.384T>C	F112L	HTZ	unknown	0,3	-1	-0,5	NA
relative10b	F	c.384T>C	F112L	HTZ	unknown	-2,4	-2,5	-2,6	NA
relative10c	M	c.384T>C	F112L	HTZ	unknown	-2,4	0	-1	NA
patient11	F	c.904G>T	E302*	HTZ	unknown	-2,2	-2,5	-2,5	34
patient12	F	c.995G>A	C332Y	HTZ	father	-1,8	-1,3	-1,7	40
relative12	M	c.995G>A	C332Y	HTZ	unknown	NA	NA	NA	NA
patient13	M	c.1247+1-1247+2insG	N417EfsX52	HTZ	mother	-2,8	-0,5	-1,4	41,5
relative13	F	c.1247+1-1247+2insG	N417EfsX52	HTZ	unknown	-2,9	-2	-2,2	NA
patient14	M	c.2629C>T	R877*	HTZ	unknown	-1,3	-1	-1	37
patient15	M	c.3162G>A	M1054I	HTZ	unknown	-0,6	-0,8	-0,6	38,5
patient16	F	c.3454G>A	G1152R	HTZ	father	-2,9	-4,1	-3,8	40
relative16a	M	c.3454G>A	G1152R	HTZ	unknown	-2,4	-1,7	-1,8	NA
relative16b	M	c.3454G>A	G1152R	HTZ	father	-2,9	-4,1	-3,3	39
patient17	M	c.3530G>A	R1177H	HTZ	unknown	-3,2	-2,2	-2,5	38
patient18	M	c.3539C>A/c.4055G>T	S1180Y/G1352V	HTZ comp	father + mother	-2,4	1	-0,7	40
relative18a	M	c.4055G>T	G1352V	HTZ	unknown	NA	NA	NA	NA
relative18b	F	c.3539C>A	S1180Y	HTZ	unknown	NA	NA	NA	NA
patient19	F	c.3595G>A	G1199R	HTZ	mother	-2,9	0	-1,6	38,5
relative19	F	c.3595G>A	G1199R	HTZ	unknown	NA	NA	NA	NA
patient20	F	c.4066G>A	E1356K	HTZ	father	-1,1	0,5	-0,4	37
relative20	M	c.4066G>A	E1356K	HTZ	unknown	NA	NA	NA	NA

BIRTH PARAMETERS			rGH THERAPY					GROWTH		DEVELOPMENT						
WEIGHT (SDS)	LENGTH (SDS)	HC (SDS)	rGH	AGE AT ONSET	HEIGHT AT ONSET (SDS)	DURATION	HEIGHT GAIN (SDS)	HEIGHT min (SDS)	FINAL HEIGHT (SDS)	ASSISTED SCHOOL	DVT	LANGUAGE DELAY	MOTOR DELAY	COGNITIVE DELAY	HC <-2 SDS	FEEDING DIFFICULTIES
-1,9	-2	-3	yes	4,1	-3,0	10,9	1,4	-3,3	-2,4	1	1	1	0	1	1	1
NA	NA	NA	no	-	-	-	-	NA	-4,1	NA	NA	NA	NA	NA	NA	NA
-1,4	-2,3	-1,6	no	-	-	-	-	-2,1	-2,2	1	1	1	0	1	NA	0
-2,3	-4,2	-2,33	yes	2,9	-5,6	on going	on going	-5,7	NA	1	1	1	1	1	1	1
-3,2	-3,1	-3	yes	1,5	-4,6	14,7	2,5	-4,6	-2,1	0	0	0	0	0	1	1
-2,6	-3,4	-2,3	yes	3,0	-5,1	3,5	1,5	-5,3	-3,8	1	1	0	0	1	1	1
NA	NA	NA	no	-	-	-	-	NA	NA	NA	NA	NA	NA	NA	NA	NA
-2,1	-3,1	NA	yes	6,4	-3,1	on going	on going	-3,2	-3,5	1	1	0	0	1	1	1
-2,5	-3,8	-2,8	no	-	-	-	-	-4,8	NA	0	0	0	0	1	1	0
-3,5	-3,8	-2,6	yes	2,9	-3,5	11,2	2,1	-3	-1,8	1	1	1	1	1	1	1
-2	-2,5	NA	yes	7,2	-1,6	7,2	0,7	-2,4	-1	0	1	0	1	0	0	0
-1,4	NA	NA	no	-	-	-	-	NA	-1,2	0	0	0	0	0	0	NA
-2,3	-2,5	NA	no	-	-	-	-	-2,7	-2,7	1	0	0	0	0	1	0
-3,8	-4	-3,3	yes	4,1	-4,6	on going	on going	-4,5	NA	1	1	1	1	1	1	1
NA	NA	NA	no	-	-	-	-	NA	-2,4	NA	NA	NA	NA	NA	0	NA
NA	NA	NA	yes	11,3	-2,8	on going	on going	-2,2	NA	1	NA	NA	NA	NA	0	NA
NA	NA	NA	no	-	-	-	-	NA	-2,5	NA	NA	NA	NA	NA	0	NA
-1	-1,4	-0,3	yes	12,8	-4,4	2,8	0,4	-4,4	-4,2	1	1	0	0	1	1	0
-3,2	-4,8	-3,9	yes	9,5	-2,7	on going	on going	-2,8	NA	1	1	0	1	1	1	1
NA	NA	NA	no	-	-	-	-	NA	-1,3	NA	NA	NA	NA	NA	NA	NA
-2,8	-3,9	NA	yes	10,4	-3,3	5,8	0,1	-3,3	-3,2	0	0	0	0	0	1	0
NA	NA	NA	no	-	-	-	-	NA	-2,8	NA	NA	NA	NA	NA	NA	NA
-1,8	-3,1	-2,8	no	-	-	-	-	-3	NA	NA	0	0	0	0	1	0
-2,5	-2,9	-0,3	yes	15,3	-2,7	0,3	0,2	-2,6	-2,5	1	0	0	0	0	1	0
-2,7	-3,3	NA	yes	4,2	-4,8	4,3	0,1	-4,7	NA	1	1	0	0	1	1	1
NA	NA	NA	no	-	-	-	-	NA	-4,2	0	NA	NA	NA	NA	NA	NA
-3,7	-5,2	-3,5	yes	11,8	-4,5	6,2	0,4	-4,6	-4,1	0	0	0	0	0	1	1
-2,6	-4,2	-2,9	yes	5,5	-3,4	10,1	1,7	-3,4	-3	0	0	0	0	0	1	0
-2,2	-2,7	-3	no	-	-	-	-	-2,5	NA	1	1	1	1	1	1	1
NA	NA	NA	no	-	-	-	-	NA	1	NA	NA	NA	NA	NA	0	NA
NA	NA	NA	yes	7,5	NA		NA	NA	-2,1	NA	NA	NA	NA	NA	1	NA
-2,1	-3,7	-3,7	yes	11,7	-4,3	4,1	1,4	-4,4	-2,9	0	0	0	0	0	1	0
NA	NA	NA	no	-	-	-	-	NA	-2,9	NA	NA	NA	NA	NA	NA	NA
-2,8	-2,6	-3,6	no	-	-	-	-	NA	NA	NA	NA	NA	NA	NA	0	1
NA	NA	NA	no	-	-	-	-	NA	0,5	NA	NA	NA	NA	NA	NA	NA

CLINICS	LAST EVALUATION			IGF-I BEFORE TREATMENT				IGF-I DURING TREATMENT				CLINICAL SCORE (Walenkamp et al. 2019)				
OTHERS	AGE	HEIGHT (SDS)	HC (SDS)	AGE	IGF1 (ng/mL)	IGF-I SDS	IGF1 >0 SDS	AGE	IGF1 (ng/mL)	IGF-I SDS	IGF1 >0 SDS	SCORE	BW/BL <-1 SDS	HEIGHT <-2,5 SDS	HC <-2 SDS	IGF1 >0 SDS
Hypoglycaemia	15,8	-1,6	-1,5	3,7	279	5,5	1	5,1	521	8,8	1	4	1	1	1	1
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA
Knees luxation	18,9	-2,2	NA	8,2	460	3,6	1	12,1	704	2,9	1	2/3	1	0	NA	1
IAC	12,8	-2,5	-2,8	2,9	95	0,6	1	4,2	135	1,1	1	4	1	1	1	1
Overweight	18,0	-2,1	NA	1,5	25	-2,0	0	2,5	169	2,9	1	4	1	1	1	1
GnRH analogs	15,5	-3,6	NA	2,6	138	1,1	1	4,2	306	3,7	1	4	1	1	1	1
	NA	-3,5	NA	56,2	224	1,5	1	NA	NA	NA	NA	NA	NA	NA	NA	1
ADHD	13,5	-3,1	NA	NA	NA	NA	NA	6,7	460	6,0	1	4	1	1	1	1
IAC	10,4	-4,5	-5,1	10,3	414	0,6	1	NA	NA	NA		4	1	1	1	1
	14,0	-1,4	NA	1,9	112	0,9	1	5,9	220	1,3	1	4	1	1	1	1
ADHD, cryptorchidism	19,1	-0,9	NA	7,2	142	-0,3	0	9,2	556	3,9	1	2	1	0	0	1
	49,5	-1,2	-1,6	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	0	NA
	17,0	-2,5	na	14,7	968	4,5	1	NA	NA	NA	NA	3	1	0	1	1
POF, hypothyroidism	5,2	-3,7	-4,9	4,0	314	4,3	1	5,2	710	9,8	1	4	1	1	1	1
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	NA
ADHD	13,7	-2,2	NA	11,3	269,3	-1,2	0	11,7	582	1,6	1	1/3	NA	0	0	1
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0	NA
GnRH analogs	15,3	-4	NA	12,8	947	4,1	1	13,8	1470	7,9	1	4	1	1	1	1
ADHD, GnRH analogs	11,3	-2,2	NA	9,3	353	0,5	1	10,3	510	1,4	1	4	1	1	1	1
	NA	-1,3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	16,6	-3,2	NA	10,0	252	-0,2	0	11,6	449	1,3	1	4	1	1	1	1
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA
	7,1	-2,2	NA	5,4	273	3,5	1	NA	NA	NA	NA	4	1	1	1	1
Obesity	21,5	-2,5	-2,7	15,2	1260	7,1	1	16,1	908	4,4	1	3	1	0	1	1
	12,6	-4,7	na	4,2	226	2,1	1	8,1	964	7,4	1	4	1	1	1	1
Type II diabetes	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA
	18,1	-4,1	-4,1	11,8	300	-0,1	0	15,7	412	0,2	1	4	1	1	1	1
	15,1	-1,7	NA	5,6	310	4,3	1	6,6	653	9,5	1	4	1	1	1	1
Pyelic dilatation	6,6	-1,6	-4,3	5,4	304	4,2	1	NA	NA	NA	NA	3	1	0	1	1
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	NA
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA
	20,8	-2,9	NA	12,9	383	-0,2	0	14,6	790	3,0	1	4	1	1	1	1
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA
Heart failure	6,2	-1,1	-1,8	0,7	151	2,0	1	NA	NA	NA	NA	3	1	1	0	1
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

SUPPLEMENTARY DATA

Table 1. General data of the whole cohort of patients carrying an *IGF1R* defect.

Figure 1. Molecular strategy adopted for *IGF1R* defects diagnosis in the laboratory. SGA: small for gestational age, SRS: Silver-Russell syndrome, MLPA: multiplex ligation-dependent probe amplification. *IGF1R* defect suspicion was based on the presence of microcephaly and/or elevated IGF-I levels. ,

Figure 2. cDNA sequencing for the patient carrying the NM_000875.4:c.1247+1_1247+2insG variant, responsible for a one-base-pair insertion, leading to a frameshift and premature stop codon (N417EfsX52).

Figure 3. Quantification of ERK phosphorylation calculated as:

[(P-ERKpatient/ERKpatient)/GAPDHpatient]/ [(P-ERKcontrol/ERKcontrol)/GAPDHcontrol]. WT: wildtype allele. Experiments were repeated from 3 to 6 times for each individual. Error bars represent the standard error of the mean. Del ex1-21 corresponds to Del4 in figure 1 and Del ex20-21 to Del7.

Figure 4: IGF1R cDNA expression in fibroblasts. Primers used:

Igf1R-219-F: ACAGGGATCTCATCAGCTTCAC and Igf1R-219-R: TCCACCATGTTCCAGCTGTT. The amplicon length was 109 bp, spanning exons 7 and 8. Del ex1-21 corresponds to Del4 in figure 1 and Del ex20-21 to Del7.