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Eloïse Giabicani, Marjolaine Willems, Virginie Steunou, Sandra Chantot-Bastaraud, Nathalie Thibaud, Walid Abi Habib, Salah Azzi, Bich Lam, Laurence Bérard, Hélène Bony-Trifunovic, et al.

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1 **Increasing knowledge in *IGF1R* defects: lessons from 35 new patients.**

2 Eloïse Giabicani<sup>1</sup>, Marjolaine Willems<sup>2</sup>, Virginie Steunou<sup>1</sup>, Sandra Chantot-Bastaraud<sup>3</sup>, Nathalie Thibaud<sup>1</sup>,  
3 Walid Abi Habib<sup>1</sup>, Salah Azzi<sup>1</sup>, Bich Lam<sup>1</sup>, Laurence Bérard<sup>1</sup>, Hélène Bony-Trifunovic<sup>4</sup>, Cécile Brachet<sup>5</sup>,  
4 Elise Brischoux-Boucher<sup>6</sup>, Emmanuelle Caldagues<sup>7</sup>, Régis Coutant<sup>8</sup>, Marie-Laure Cuvelier<sup>9</sup>, Georges  
5 Gelwane<sup>10</sup>, Isabelle Guemas<sup>7</sup>, Muriel Houang<sup>1</sup>, Bertrand Isidor<sup>11</sup>, Claire Jeandel<sup>12</sup>, James Lespinasse<sup>13</sup>,  
6 Catherine Naud-Saudreau<sup>14</sup>, Monique Jesuran-Perelroizen<sup>15,16</sup>, Laurence Perrin-Sabourin<sup>17</sup>, Juliette Piard<sup>6</sup>  
7 Claire Sechter<sup>18</sup>, Pierre-François Souchon<sup>19</sup>, Caroline Storey<sup>10</sup>, Domitille Thomas<sup>1</sup>, Yves Le Bouc<sup>1</sup>, Sylvie  
8 Rossignol<sup>20,21</sup>, Irène Netchine<sup>1</sup>, and Frédéric Brioude<sup>1</sup>.

9

- 10 1. Sorbonne Université, INSERM, Centre de Recherche Saint Antoine, APHP, Hôpital Armand  
11 Trousseau, Explorations Fonctionnelles Endocriniennes, F-75012, Paris, France.
- 12 2. CHU Arnaud de Villeneuve, Département de Génétique Médicale, 34000 Montpellier, France.
- 13 3. APHP, Hôpital Armand Trousseau, Département de Génétique, UF de Génétique Chromosomique, F-  
14 75012, Paris, France.
- 15 4. CHU Amiens Picardie, Médecine Pédiatrique et Médecine de l'Adolescent, 80054 Amiens, France.
- 16 5. Hôpital Universitaire des Enfants Reine Fabiola, Université libre de Bruxelles, 1020 Bruxelles,  
17 Belgium.
- 18 6. Université de Franche-Comté, CHRU Saint Jacques, Centre de Génétique Humaine, 25030 Besançon,  
19 France.
- 20 7. CHU Nantes, Médecine Pédiatrique, 44000 Nantes, France.
- 21 8. CHU Angers, Endocrinologie et Diabétologie Pédiatriques, 49000 Angers, France.
- 22 9. CH Calais, Pédiatrie, 62100 Calais, France.
- 23 10. Université Paris Diderot, APHP, Hôpital Robert Debré, Endocrinologie et Diabétologie Pédiatriques,  
24 75019 Paris, France.
- 25 11. CHU Nantes, Service de Génétique Médicale, 44000 Nantes, France.

- 26 12. CHU Arnaud de Villeneuve, Pédiatrie Spécialisée Endocrinologie Gynécologie de l'Enfant et de  
27 l'Adolescent, 34000 Montpellier, France.
- 28 13. CH Métropole Savoie, UF de Génétique Chromosomique, 73000 Chambéry, France.
- 29 14. CH Bretagne Sud, Endocrinologie et Diabétologie Pédiatriques, 56100 Lorient, France.
- 30 15. Cabinet libéral d'endocrinologie-pédiatrique, 14 rue du Rempart Saint Etienne, 31000 Toulouse,  
31 France.
- 32 16. AFPEL, 59 800 Lille, France.
- 33 17. Université Paris Diderot, APHP, Hôpital Robert Debré, Unité de Génétique Clinique, 75019 Paris,  
34 France.
- 35 18. Université de Franche-Comté, CHU Jean Minjoz, Unité d'Endocrinologie et Diabétologie  
36 Pédiatriques, 25030 Besançon, France.
- 37 19. CHU Reims, American Memorial Hospital, Diabétologie et Endocrinologie Pédiatriques, 51100  
38 Reims, France.
- 39 20. Hôpitaux Universitaires de Strasbourg, Service de Pédiatrie, Strasbourg, France.
- 40 21. INSERM U1112, Laboratoire de Génétique Médicale, Institut de Génétique Médicale d'Alsace  
41 (IGMA), Faculté de Médecine de Strasbourg, Strasbourg, France.

42 **Corresponding author:**

43 Eloïse Giabicani, MD

44 Explorations Fonctionnelles Endocriniennes, Hôpital Armand Trousseau

45 26 avenue du Dr Arnold Netter, 75571 Paris cedex 12, France

46 Email : [eloise.giabicani@aphp.fr](mailto:eloise.giabicani@aphp.fr), phone : +33171738032, fax : +33144736127

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48 **Keywords:** IGF1R, IGF-I, AKT, fetal growth, intra-uterine growth retardation, small for gestational age,  
49 Silver-Russell syndrome, haploinsufficiency, homozygous variant.

50 **Word count:** 4577

51 **ABSTRACT**

52 (248words)

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

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

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

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

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## 74 INTRODUCTION

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

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

99 #269880), leading to the late diagnosis of *IGF1R* defects [11,12]. However, the presence of microcephaly  
100 appears to be highly specific for *IGF1R* defects. Thus, the recent international consensus about SRS  
101 mentioned *IGF1R* defects as a differential diagnosis of SRS in case of absence of relative macrocephaly,  
102 meaning that head circumference should be assessed in a patient born SGA with poor catch-up growth to  
103 distinguish between these etiologies [13]. From the first descriptive cohort of 25 patients with *IGF1R*  
104 defects, Walenkamp *et al.* proposed a clinical score to drive molecular investigations [14]. This score  
105 combines the following four items: birth length or weight < -1 standard deviation score (SDS), head  
106 circumference < -2 SDS at first presentation, height at first presentation < -2.5 SDS, and plasma IGF-I  
107 levels above the mean for age and gender. Molecular testing for *IGF1R* should be proposed if three or  
108 more items are present, with a sensitivity of 76% in their cohort [14]. Recently, Janchevska *et al.*  
109 identified two anomalies of *IGF1R* in a cohort of 64 patients born SGA with no catch-up growth,  
110 supporting the hypothesis that the prevalence of these defects is high enough to search for them in this  
111 particular group of patients [15]. Concerning the treatment of postnatal short stature, the efficiency of  
112 recombinant growth hormone (rGH) therapy in patients with *IGF1R* defects is still controversial and only  
113 isolated cases with variable age at onset, duration, and dose of treatment have been reported [7,14].

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

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

## 124 **MATERIALS AND METHODS**

### 125 **Patients**

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

### 133 **Auxologic methods**

134 Length, weight, and head circumference at birth are expressed as SDS according to Usher and McLean  
135 charts [21]. Post-natal growth parameters are expressed as SDS according to Sempé charts [22]. The age  
136 of puberty onset (breast development for girls and testis enlargement ( $\geq 4\text{mL}$ ) for boys) was considered to  
137 be normal from 8 to 13 years for girls and 9 to 14 years for boys.

### 138 **Molecular analysis**

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

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

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

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

150 For single nucleotide polymorphism (SNP) microarray analysis, samples were processed using cytoSNP-  
151 12, or HumanOmniExpress-24 microarrays (Illumina, San Diego, CA, USA). Automated Illumina  
152 microarray experiments were performed according to the manufacturer's instructions. Images were  
153 acquired using an iScan System (Illumina). Image analysis and automated CNV calling were performed  
154 using GenomeStudio v.2011.1 and CNVPartition v.3.1.6. SNP profiles were analyzed by examination of  
155 signal intensity (Log R ratio, i.e.  $\ln(\text{sample copy number}/\text{reference copy number})$ ) and allelic composition  
156 (BAF, i.e. B Allele Frequency).

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

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

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



170 **Functional test**

171 Patient fibroblasts, obtained from skin biopsies after informed consent and control fibroblasts obtained  
172 from the Coriell Institute of Medical Research (Camden, N.J.), were cultured in Dulbecco's Modified  
173 Eagle Medium enriched with glutamate, sodium pyruvate, penicillin, streptomycin, and 10% fetal-calf  
174 serum at 37°C. After 24 h of serum-free culture in six-well plates, cells were stimulated with [50 ng/ml]  
175 IGF-I (Peprotech, US) for 10 min before lysis. We found these stimulation conditions to be the most  
176 accurate to assess both AKT and ERK phosphorylation in controls. For both non-stimulated and  
177 stimulated conditions, 4.2 to 12.1 µg of protein was deposited in a NuPAGE™ 4-12% Bis-Tris Gel  
178 (Thermo Fischer Scientific, US). Electrophoresis was performed on an XCell SureLock™ Mini-Cell  
179 Electrophoresis system (Thermo Fischer Scientific, US). Membranes were incubated with polyclonal  
180 antibodies against either phospho-AKT (Ser473, Cell Signaling, US, 1:2000), pan-AKT (Cell Signaling,  
181 1:1000), phospho-ERK1/2 (Tyr204, Cliniscience, France, 1:800), ERK1/2 (Cell Signaling, 1:1000), or  
182 GAPDH for normalization (Cell Signaling, 1:2000). Then, membranes were incubated with an HRP-  
183 conjugated secondary antirabbit antibody (1:3,000), revealed with ChemiDoc™ XRS+ System (Bio-Rad,  
184 US), and analyzed with Quantity One v4.6.6 software. Immunoblot images were quantified using ImageJ  
185 1.50 software (<https://imagej.nih.gov>).

186  
187 **mRNA quantification**

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

193 **Biological assays**

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

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

## 198 **Statistical analysis**

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

## 202 **RESULTS**

### 203 **Genetic results**

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

220 1B., Del1 to 5), whereas three included only part of *IGF1R* with length from 19kb to 234kb (figure 1B.,  
221 Del6 to 8).

## 222 **Clinical features**

223 The intragenic deletions (exon 2, Del6, n = 3) and *IGF1R* terminal deletions (Del7, n = 2 and Del8, n = 1)  
224 did not include other disease-causing OMIM genes and were thus analyzed together with the SNVs for the  
225 clinical study. Clinical characteristics are shown in Table 2. There was no statistical difference in clinical  
226 presentation between patients with large deletions and pathogenic variants of *IGF1R*. We calculated the  
227 clinical score recently proposed by Walenkamp *et al.* for 21 patients for whom clinical data required for  
228 this scoring system were fully available (birth weight or length < -1 SDS, height at presentation < -2.5  
229 SDS, head circumference at presentation < -2 SDS (microcephaly) and IGF-I level > 0 SDS) [14]. Twenty  
230 patients (95.2%) met at least three of the four criteria and 11 (52.4%) fulfilled all four. Among them, all  
231 had a birth weight or length < -1 SDS, 17 (81.0%) had a height at presentation below -2.5 SDS, and 19  
232 (90.5%) had microcephaly. All 21 patients scored positive for elevated IGF-1 levels if considered at the  
233 different endpoints (including during rGH treatment). However, five patients (23.8%) would have not met  
234 this criterion if IGF-I levels were considered only prior to the initiation of rGH treatment. One patient  
235 (carrying Del6) did not achieve a positive clinical score, with only two items [being born with a height or  
236 weight < -1 SDS and high levels of IGF-I (during rGH treatment only)].

237 Given the clinical overlap between SRS and *IGF1R* defects, the Netchine-Harbison clinical scoring  
238 system (with a positive clinical diagnosis of SRS for a score of at least 4/6) was assessable for 10 patients  
239 and only one scored 4/6 [13,26] (lacking relative macrocephaly at birth and body asymmetry items).  
240 Nevertheless, most patients scored 3 out of 6, comprising the following items: being born SGA, post-natal  
241 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 <b>PS3</b>	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	<b>PS3</b> 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 <sup>-6</sup>	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 <sup>-5</sup>	PM2 PP2 PP3 <b>PS3</b> BS4	Likely pathogenic¶
c.4066G>A	[14]	E1356K	rs746562843	AF: 642.10 <sup>-5</sup>	PM2 PP2 BS4 <b>PS3</b>	Likely pathogenic¶

242

243 **Table 1**

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

244 **Table2**

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

256 Only two cases of homozygous pathogenic variants have yet been reported [8,12]. Thus, the pedigree and  
257 growth curves of the girl with the F112L homozygous pathogenic variant are shown in figure 2. Although  
258 the girl with the homozygous pathogenic variant (II.4) showed severe growth retardation of approximately  
259 -4 SDS with tremendously elevated IGF-I [621 ng/mL (NV 20-300) at 1.6 years] and IGFBP-3 [5605  
260 ng/mL (NV 800-3700)], both parents (I.1 and I.2) and one older sister (II.3) with the heterozygous  
261 pathogenic variant showed impaired postnatal growth of approximately -2 SDS, with a final height in the  
262 lower range of normal curves. The unaffected younger siblings (II.1 and II.2) showed normal growth  
263 around the mean. Furthermore, the homozygous carrier (II.4) had a patent foramen ovale, severe oeso-  
264 gastric reflux, anorexia requiring enteral support for one year (nasogastric tube), and psychomotor delay  
265 with learning disability, whereas no other member of the family presented with such clinical features.

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

267 **rGH therapy**

268 Eighteen patients received rGH treatment, starting at an age of 7.5 years (1.5;15.3) under the SGA  
269 European Medicines Agency (EMA) indication, with a mean height at the start of therapy of -3.8 SDS (-  
270 5.6; -1.6). The starting dose was 46.6 µg/kg/day (35.0;85.5) and was significantly increased for only five  
271 of the 15 patients for whom data on the dose evolution was available. For most patients (60.0%), the dose  
272 of rGH was not raised because of high serum levels of IGF-I. Among the 12 patients that completed rGH  
273 treatment and reached their final height, the mean height gain was 1.0 SDS (0.2;2.5), which positively  
274 correlated with the duration of treatment ( $\rho = 0.76$ ,  $p = 0.004$ ) and negatively correlated with the age at the  
275 start of rGH ( $\rho = -0.68$ ,  $p = 0.01$ ). IGF-I serum levels were high (over 1 SDS) for 11 (47.8%) patients  
276 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  
277 therapy.

278 **IGF1R functional test**

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

290 **DISCUSSION**

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

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

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

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



315 IGF-I concentrations used for stimulation. Indeed, Choi *et al.* showed a progressive increase in AKT  
316 phosphorylation in fibroblasts from a patient with an *IGF1R* deletion in response to increasing IGF-I  
317 concentrations from 1 to 400 ng/mL [28]. Ester *et al.* reported the same pattern with lower concentrations  
318 (5 to 20 ng/mL) [18]. Thus, it is possible that the IGF-I concentration we used (50 ng/mL) did not allow  
319 proper discrimination of AKT phosphorylation between deletions and controls [29]. Nevertheless, the aim  
320 of this functional study was to assess pathogenic impact of SNVs of unknown significance, and we found  
321 IGF-I concentration of 50 ng/mL to be effective. Unlike missense pathogenic variants, which may lead to  
322 a dominant-negative effect, deletions or nonsense variants may lead to haploinsufficiency. Although we  
323 could not quantify membrane IGF1R, we demonstrated that *IGF1R* mRNA levels were low in fibroblasts  
324 from one patient with a deletion, favoring haploinsufficiency [10,28,30].

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

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

342 This cohort allowed us to better characterize the phenotype of patients with an *IGF1R* defect. As  
343 previously described, fetal and post-natal growth retardation, microcephaly, and elevated IGF-I serum  
344 levels were highly prevalent in our cohort [7,14]. As in previously reported cases, we identified several  
345 cardiac anomalies in these patients, mostly benign. However, one patient underwent heart transplantation  
346 because of severe cardiac failure. These findings are in accordance with previous observations and argue  
347 in favor of a systematic cardiac ultrasound evaluation when an *IGF1R* defect is identified [8,12,19,32].  
348 Another interesting feature of our cohort is the presence of ADHD in several patients, which was only  
349 been previously reported for one case [29]. Furthermore, as reported in the Dutch cohort, we found a high  
350 prevalence of feeding difficulties, sometimes requiring nutritional support, [9,11,14]. This latter feature  
351 may have misled some clinicians to consider a clinical diagnosis of SRS at first evaluation. Indeed patients  
352 with SRS or *IGF1R* defects share several symptoms, including being born SGA, post-natal growth  
353 retardation, and high circulating levels of IGF-I [33,34]. However patients with *IGF1R* defects usually  
354 present with microcephaly, which distinguishes them from SRS patients, for whom head circumference is  
355 relatively preserved at birth [13,26]. The recent international consensus on the diagnosis and management  
356 of SRS stated that *IGF1R* defects represents a differential diagnosis and may be considered easily after the  
357 major molecular defects of SRS are ruled out, especially for those patients with no relative macrocephaly  
358 [13].

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

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

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

377

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495

## 496 TABLES

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

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

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

## 507 FIGURES

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

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

516 **Figure 3. A.** Western blot showing phosphorylated-AKT (P-AKT), total AKT, and GAPDH for patients  
517 and controls. **B.** Quantification of AKT phosphorylation calculated as:  $R$  after IGF-I stimulation /  $R$  not  
518 stimulated, with  $R = \frac{[(P-AKT_{patient}/AKT_{patient})/GAPDH_{patient}]}{[(P-$   
519  $AKT_{control}/AKT_{control})/GAPDH_{control}]}$ . \* $p < 0.05$ ; \*\* $p < 0.01$ ; WT: wild type allele. Experiments  
520 were repeated from 3 to 6 times for each individual. Error bars represent the standard error of the mean.  
521 Del ex1-21 corresponds to Del4 in figure 1 and Del ex20-21 to Del7.

## 522 STATEMENTS

523 Competing interests: The authors have nothing to disclose.

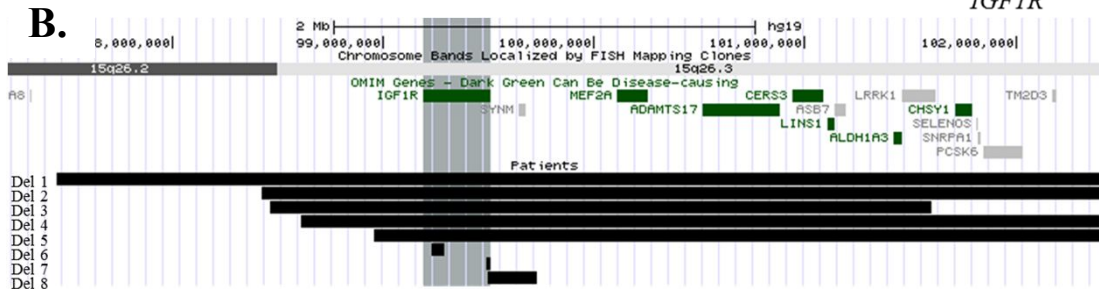
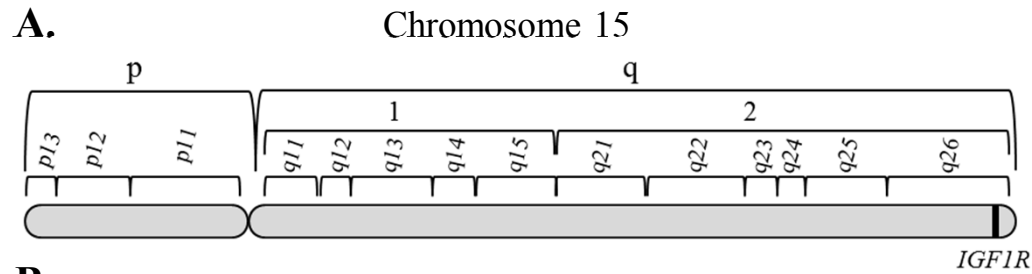
524 Contribution of the authors:

525 Eloïse Giabicani: wrote the manuscript, performed the experiments, collected and analyzed the data and  
526 revised the manuscript. Marjolaine Willems: collected and analyzed the data and revised the manuscript  
527 Virginie Steunou: performed the experiments. Sandra Chantot-Bastaraud: performed the experiments,  
528 analyzed the data and helped in revising the manuscript. Nathalie Thibaud: performed the experiments  
529 Walid Abi Habib: analyzed the data and helped in revising the manuscript. Salah Azzi : performed the  
530 experiments, analyzed the data and helped in revising the manuscript. Bich Lam: performed the  
531 experiments, analyzed the data and helped in revising the manuscript. Laurence Bérard: collected data and  
532 helped to revise the manuscript. Hélène Bony-Trifunovic: collected data and helped to revise the  
533 manuscript. Cécile Brachet: collected data and helped to revise the manuscript. Elise Brischoux-Boucher:  
534 collected data and helped to revise the manuscript. Emmanuelle Caldagues: collected data and helped to  
535 revise the manuscript. Régis Coutant: collected data and helped to revise the manuscript. Marie-Laure  
536 Cuvelier: collected data and helped to revise the manuscript. Georges Gelwane: collected data and helped  
537 to revise the manuscript. Isabelle Guemas: collected data and helped to revise the manuscript. Muriel  
538 Houang: collected data and helped to revise the manuscript. Bertrand Isidor: collected data and helped to  
539 revise the manuscript. Claire Jeandel: collected data and helped to revise the manuscript. James  
540 Lespinasse: collected data and helped to revise the manuscript. Catherine Naud-Saudreau: collected data  
541 and helped to revise the manuscript. Monique Jesuran-Perelroizen: collected data and helped to revise the  
542 manuscript. Laurence Perrin-Sabourin: collected data and helped to revise the manuscript. Juliette Piard:  
543 collected data and helped to revise the manuscript. Claire Sechter: collected data and helped to revise the  
544 manuscript. Pierre-François Souchon: collected data and helped to revise the manuscript. Caroline Storey:  
545 collected data and helped to revise the manuscript. Domitille Thomas: performed the experiments,  
546 collected data and helped to revise the manuscript. Yves Le Bouc: collected data and helped to revise the  
547 manuscript. Sylvie Rossignol: collected data and helped to revise the manuscript. Irène Netchine: analyzed

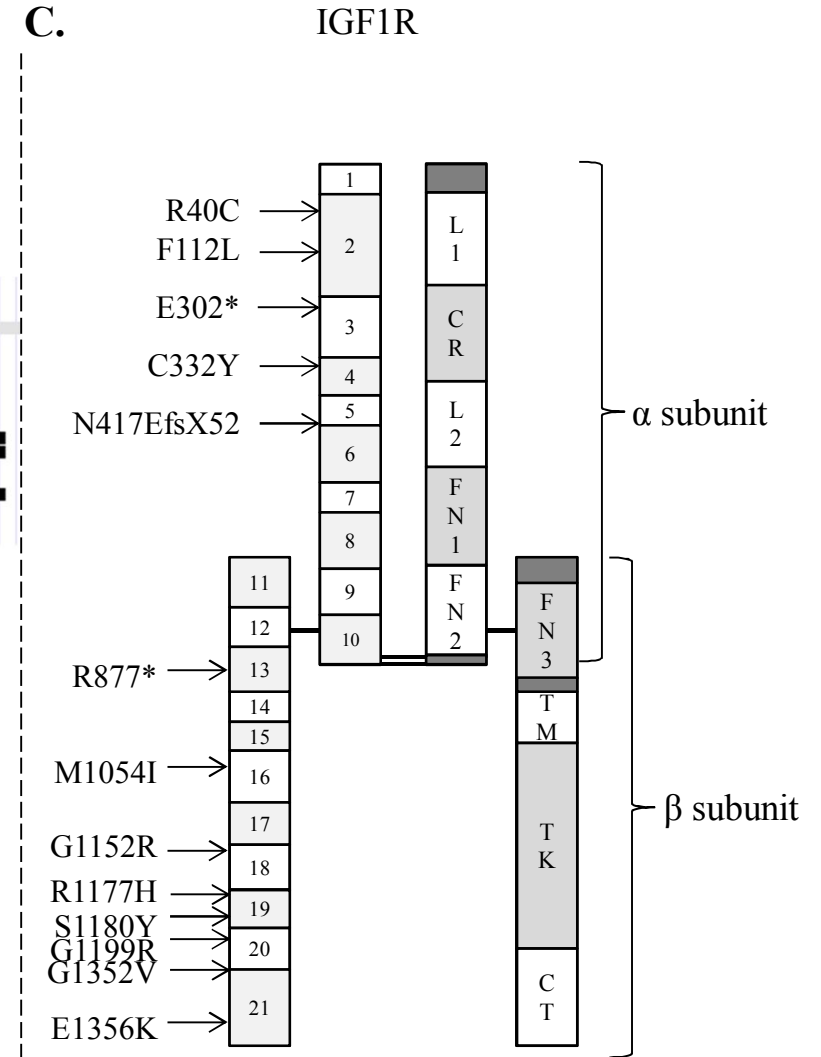


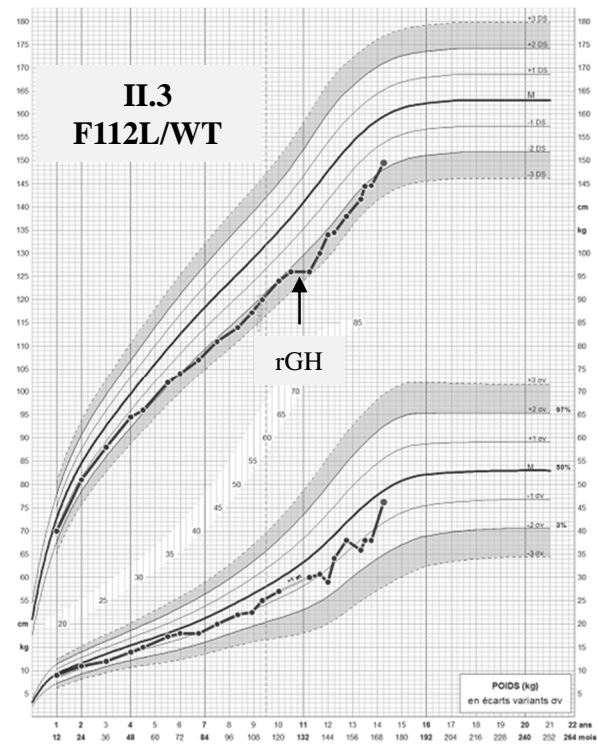
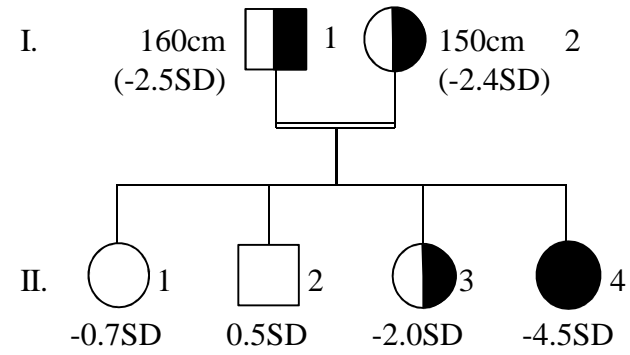
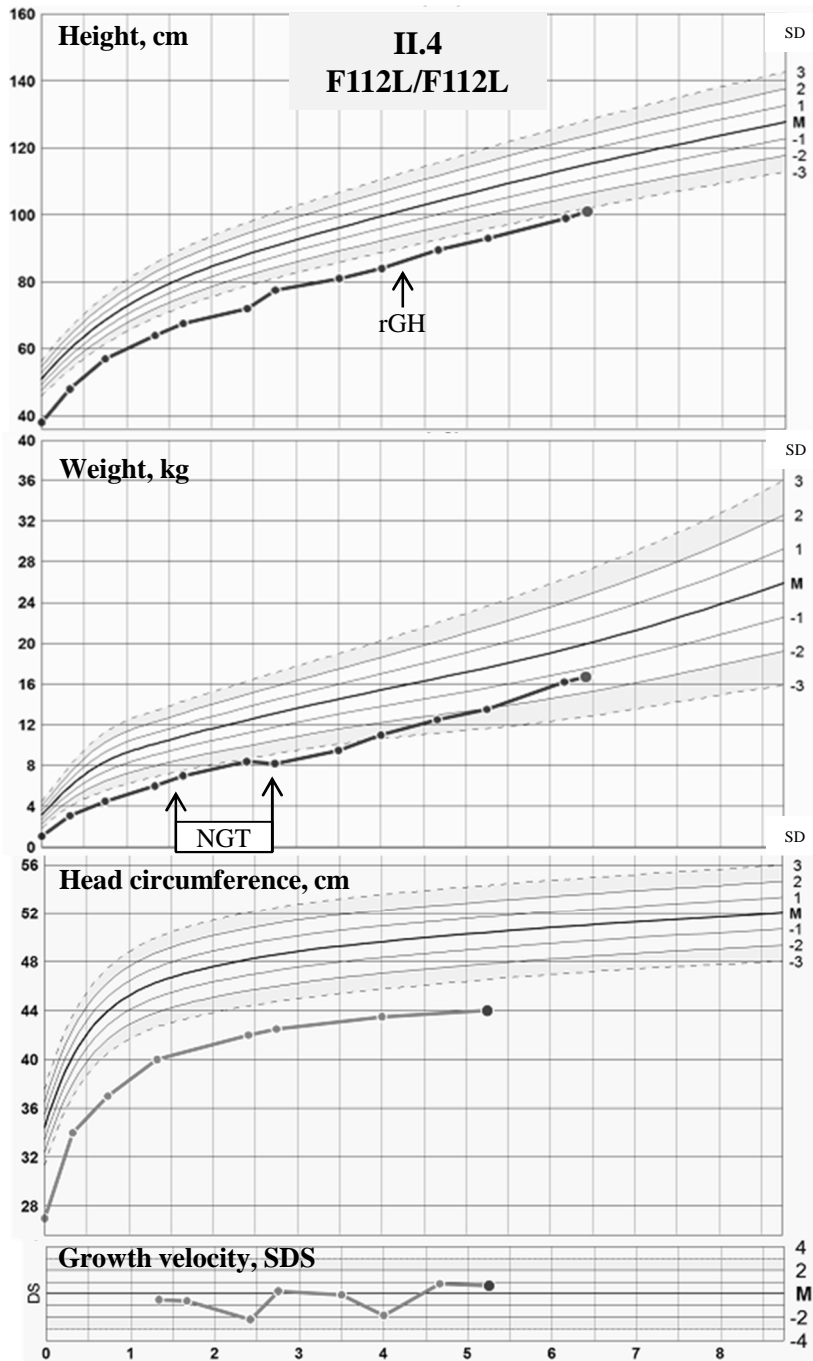
548 the data and revised the manuscript. Frédéric Brioude: analyzed the data, wrote and revised the  
549 manuscript.

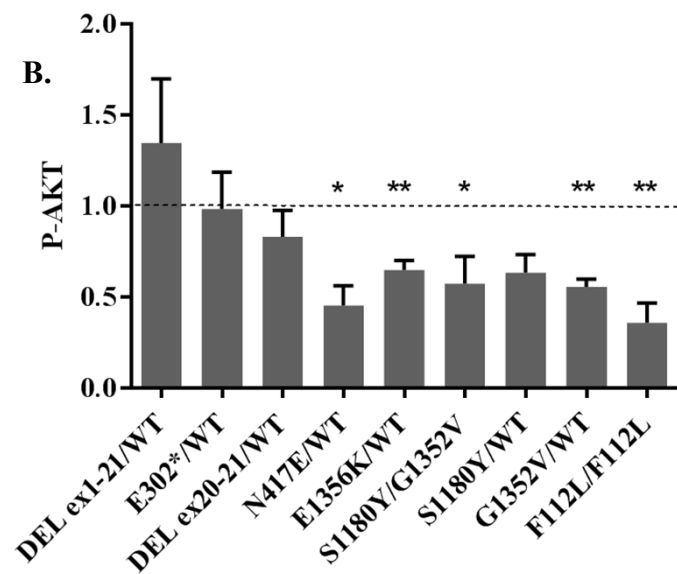
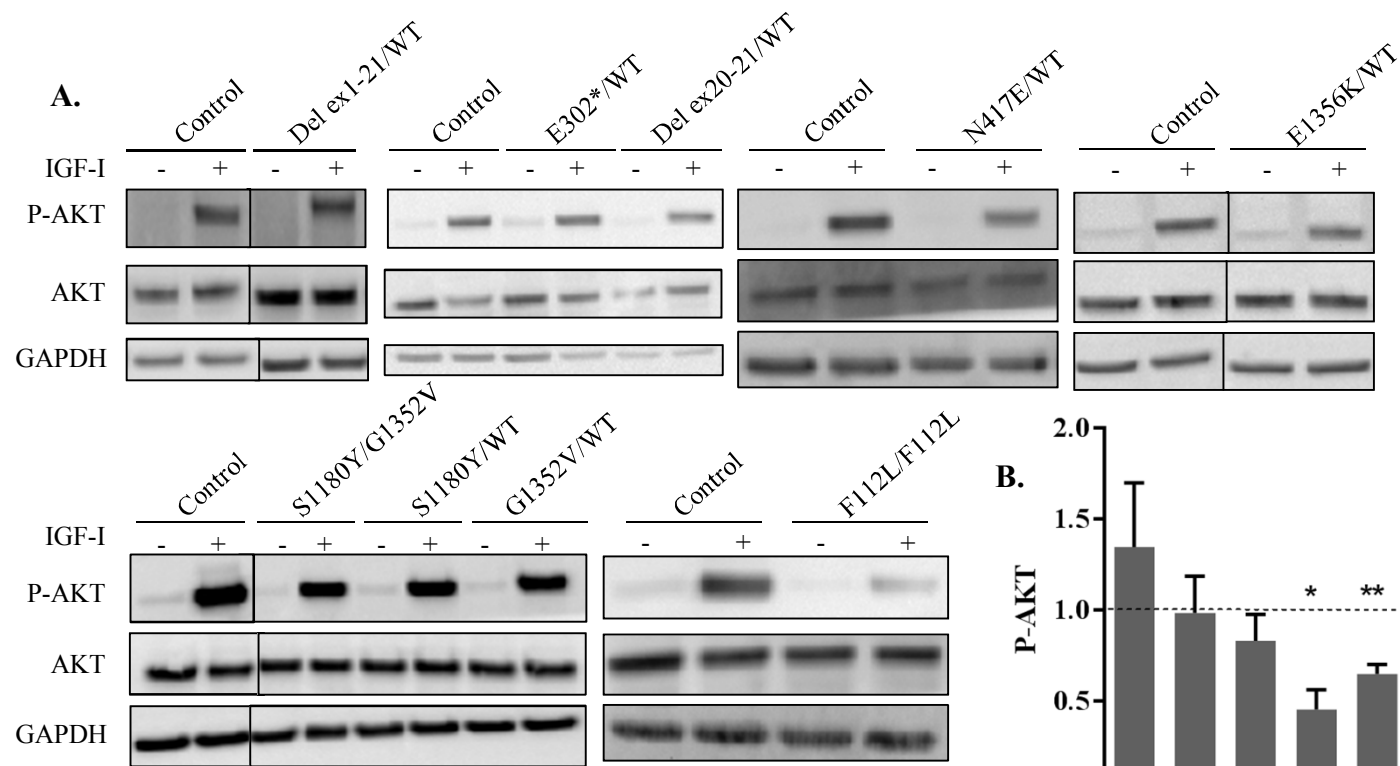
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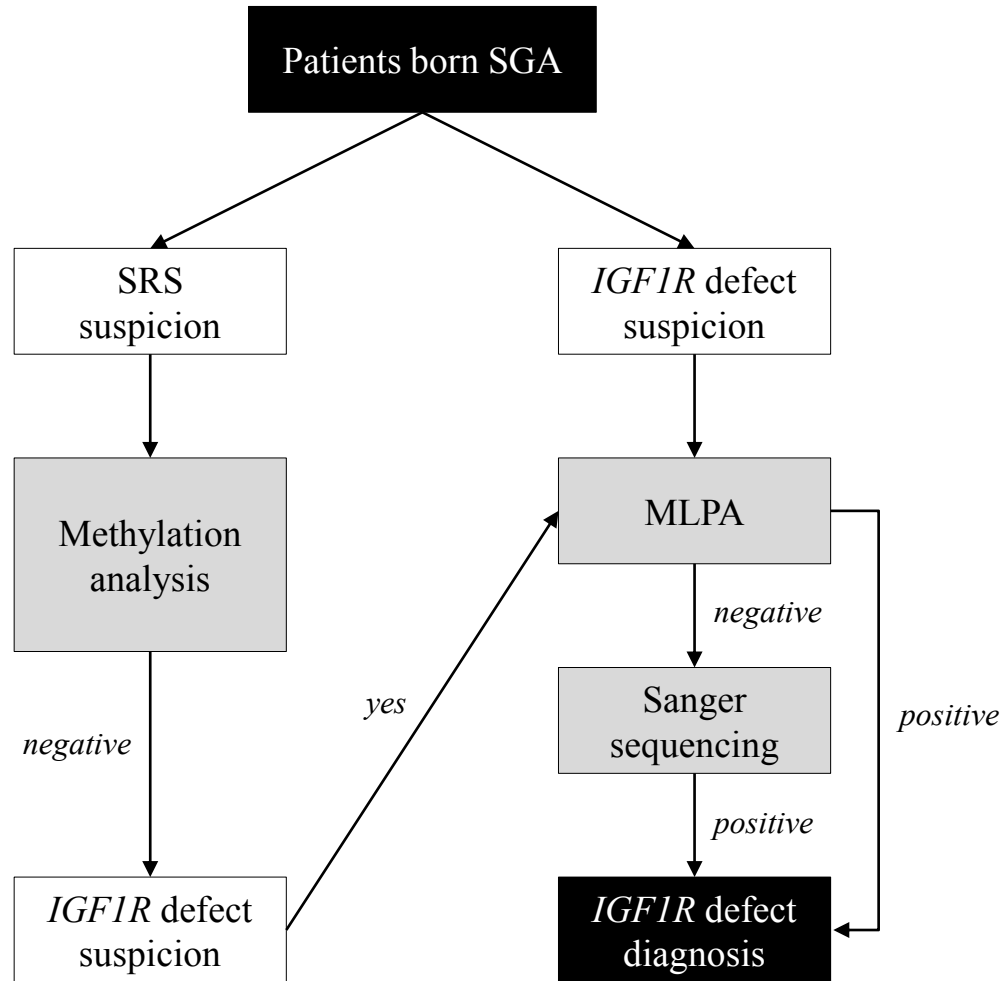


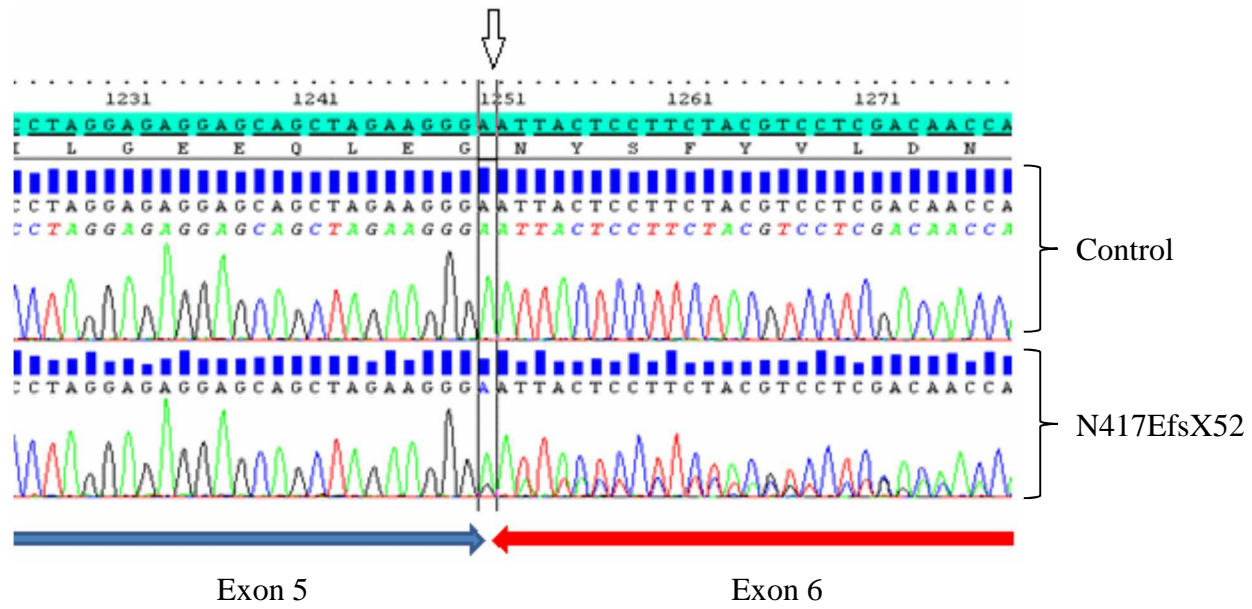
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Del2	chr15 : 98426952-102461162	4 034.2
Del3	chr15 : 98464591-101599113	3 134.5
Del4	chr15 : 98610818-102397836	3 787.0
Del5	chr15 : 98954957-102461162	3 506.2
Del6	chr15 : 99229330-99290406	61.1
Del7	chr15 : 99492046-99511073	19.0
Del8	chr15 : 99496341-99730813	234.5

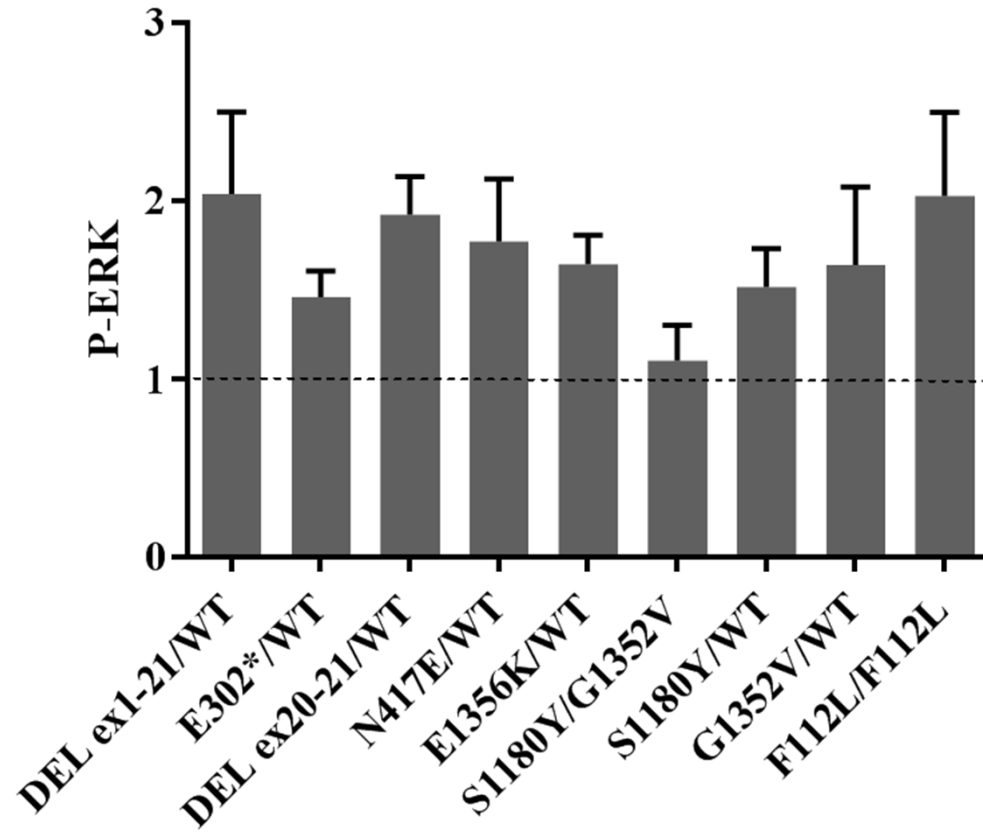


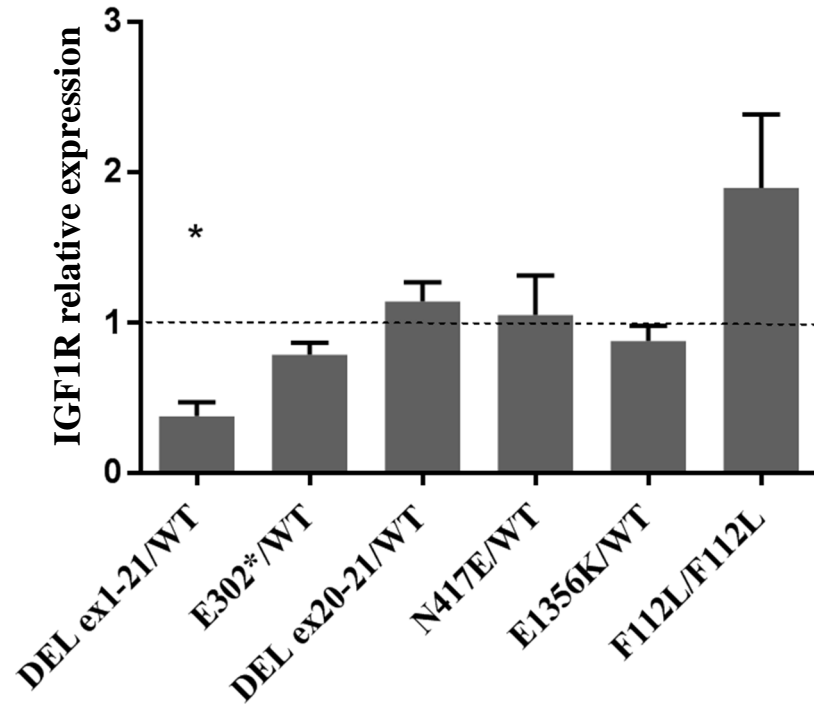














	PATIENTS		GENETICS				FAMILY			TERM (WA)
	NAME	SEX	NOMENCLATURE	PROTEIN	ALLELIC STATUS	SEGREGATION	MOTHER HEIGHT (SDS)	FATHER HEIGHT (SDS)	TARGET HEIGHT (SDS)	
<b>ABBREVIATIONS</b>										
ADHD: attention deficit hyperactivity disorder	patient1	M	15q26.3(98954957_102461162)x1	NA	HTZ	mother	-4,1	-0,5	-2,1	40
BL: birth length	relative1	F	15q26.3(98954957_102461162)x1	NA	HTZ	unknown	-0,6	-1,7	-1,3	NA
BW: birth weight	patient2	M	15q26.3(98610818_102397836)x1	NA	HTZ	de novo	1,9	0,8	1,4	39
DVT: developmental	patient3	M	15q26.2q26.3(98426952_102461162)x 1	NA	HTZ	unknown	-2,4	1,4	-0,3	37
GnRH: gonadotropin	patient4	M	15q26.2q26.3(97450781_102461162)x1	NA	HTZ	unknown	0	2,4	1,3	39
HC: head circumference	patient5	F	15q26.2q26.3(98464591_101599113)x1	NA	HTZ	de novo	-1,6	0,8	-0,5	37
HTZ cp: compound HTZ	patient6	M	15q26.3(99492046_99511073)x1	NA	HTZ	unknown	NA	NA	NA	NA
HTZ: heterozygous	relative6	M	15q26.3(99492046_99511073)x1	NA	HTZ	father	-1,6	-3,5	-2,8	38,3
IAC: interatrial communication	patient7	F	15q26.3(99496341_99730813)x1	NA	HTZ	de novo	-1,5	-1,7	-1,7	35
min: minimum	patient8	F	15q26.3(99229330_99290406)x1	NA	HTZ	father	-1,5	-1,2	-1,5	32
NA: not available	relative8a	M	15q26.3(99229330_99290406)x1	NA	HTZ	father	-1,5	-1,2	-1,2	31
POF: patent foramen ovale	relative8b	M	15q26.3(99229330_99290406)x1	NA	HTZ	unknown	-3,8	0	-1,7	41
rGH: recombinant growth hormone	patient9	M	c.118C>T	R40C	HTZ	unknown	-0,9	-3,3	-2	41
SDS: standard deviation score	patient10	F	c.384T>C	F112L	HMZ	father + mother	-2,4	-2,5	-2,6	34
SRS: Silver-Russell syndrome	relative10a	F	c.384T>C	F112L	HTZ	unknown	0,3	-1	-0,5	NA
WA: weeks of amenorrhea	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					FEEDING	
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 DELAY	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



## 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:

$$\frac{[(P-ERK_{patient}/ERK_{patient})/GAPDH_{patient}]}{[(P-ERK_{control}/ERK_{control})/GAPDH_{control}]} \quad WT:$$
 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.