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

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47

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.

71

72

73

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 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¶

242

243 **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

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

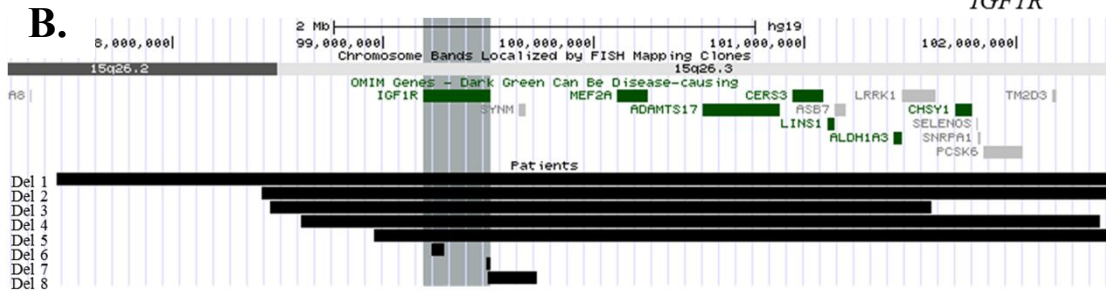
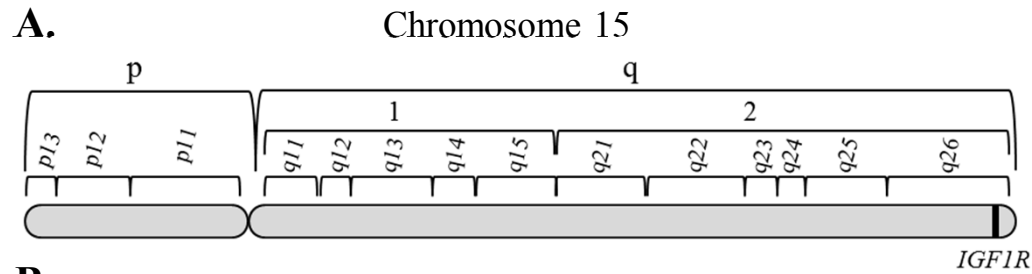
523 Competing interests: The authors have nothing to disclose.

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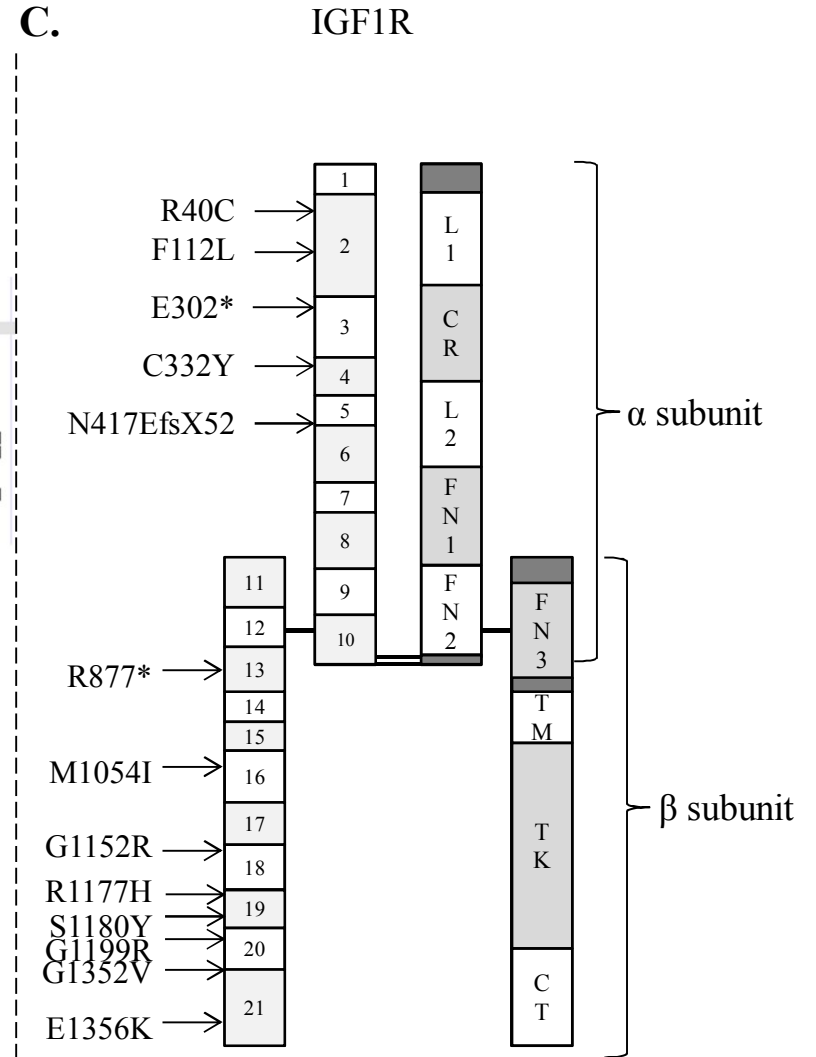
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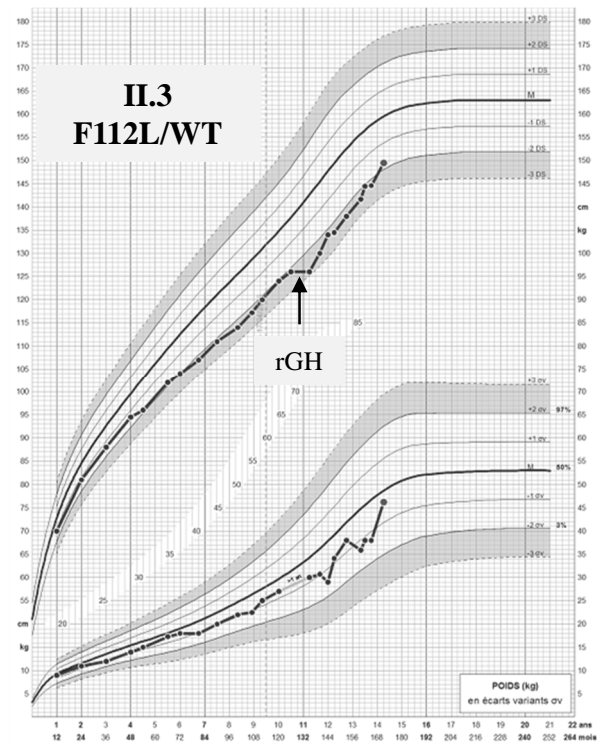
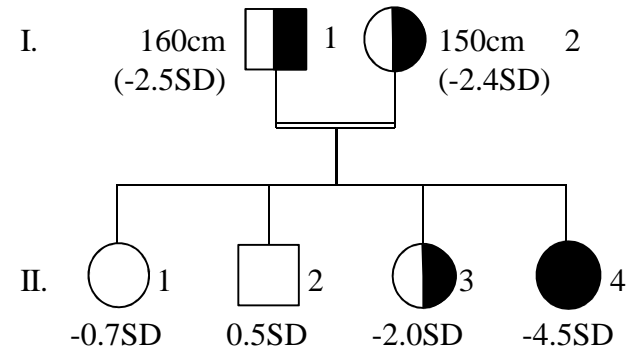
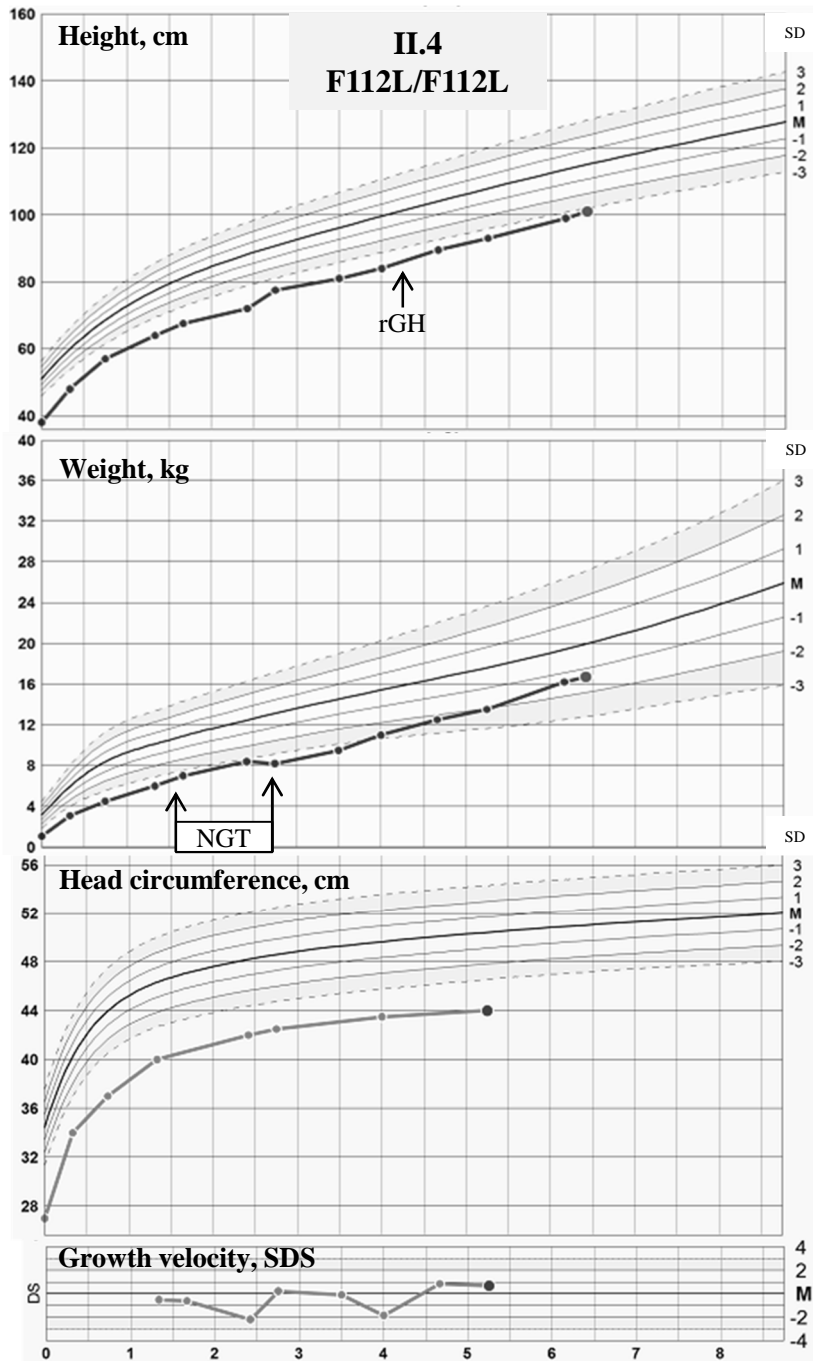
548 the data and revised the manuscript. Frédéric Brioude: analyzed the data, wrote and revised the
549 manuscript.

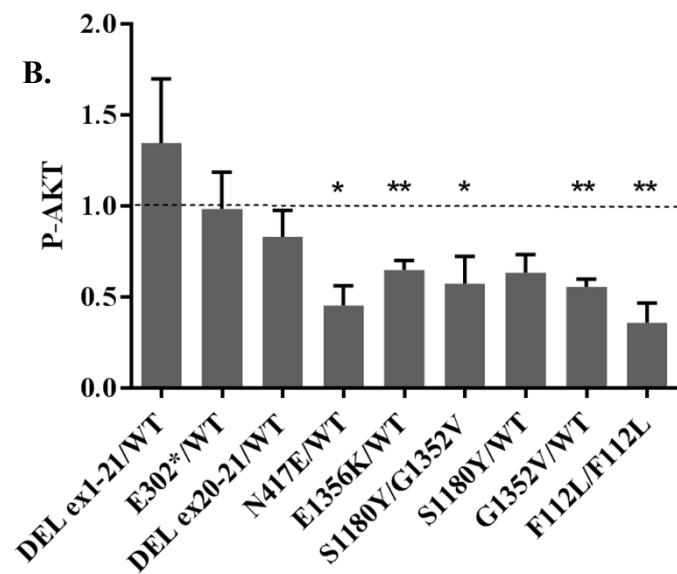
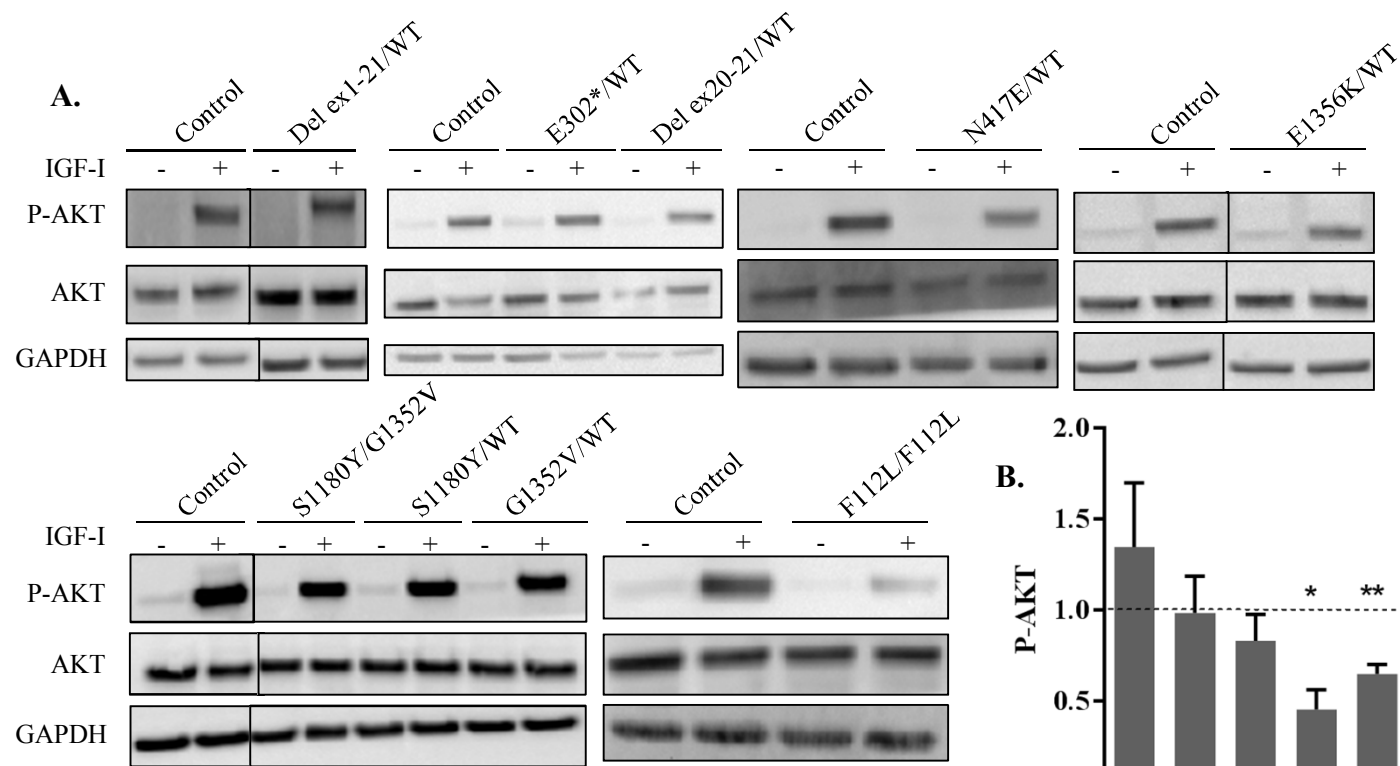
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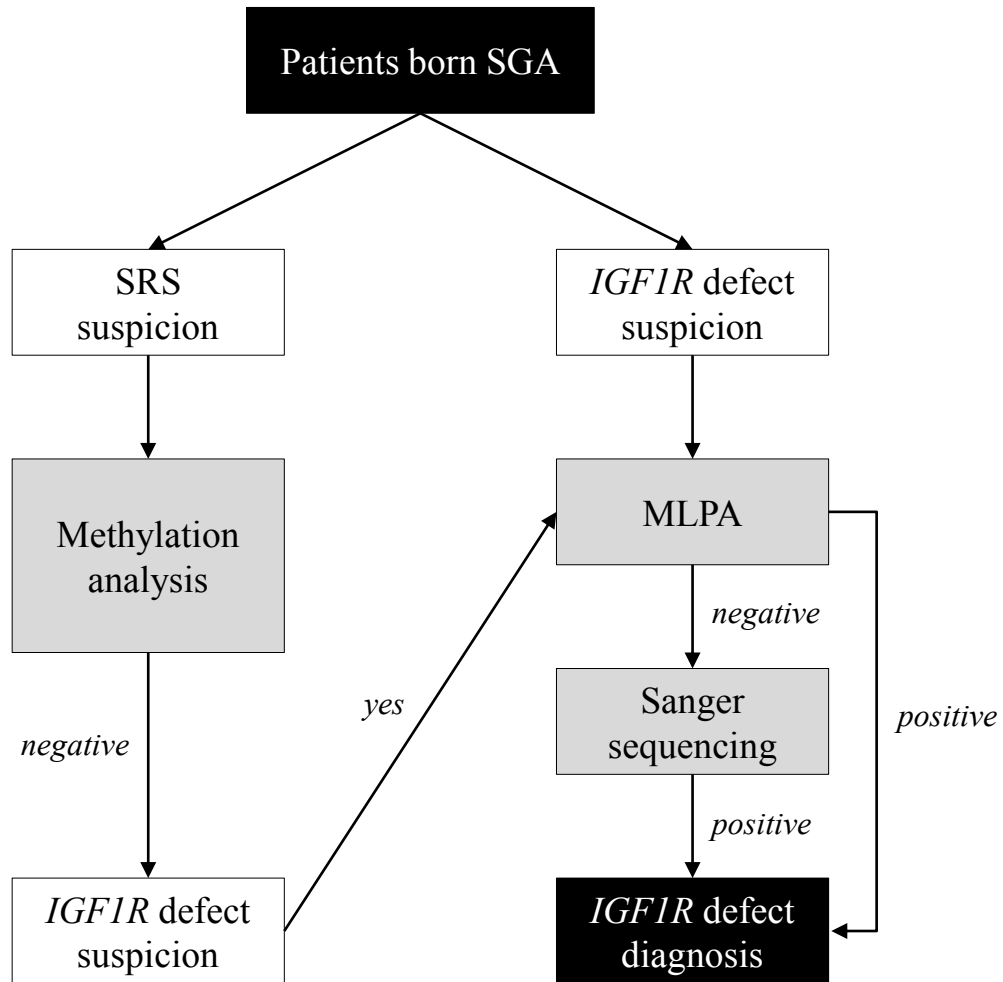


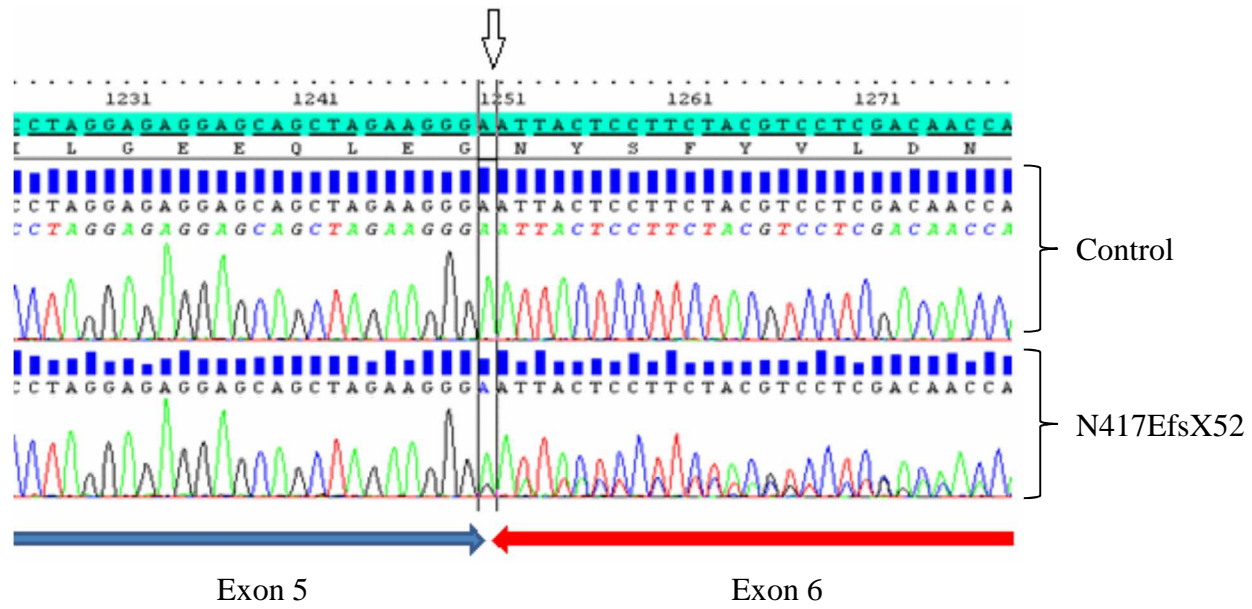
Name	Position [Hg19]	Size(Kb)
Del1	chr15 : 97450781-102461162	5 010.4
Del2	chr15 : 98426952-102461162	4 034.2
Del3	chr15 : 98464591-101599113	3 134.5
Del4	chr15 : 98610818-102397836	3 787.0
Del5	chr15 : 98954957-102461162	3 506.2
Del6	chr15 : 99229330-99290406	61.1
Del7	chr15 : 99492046-99511073	19.0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

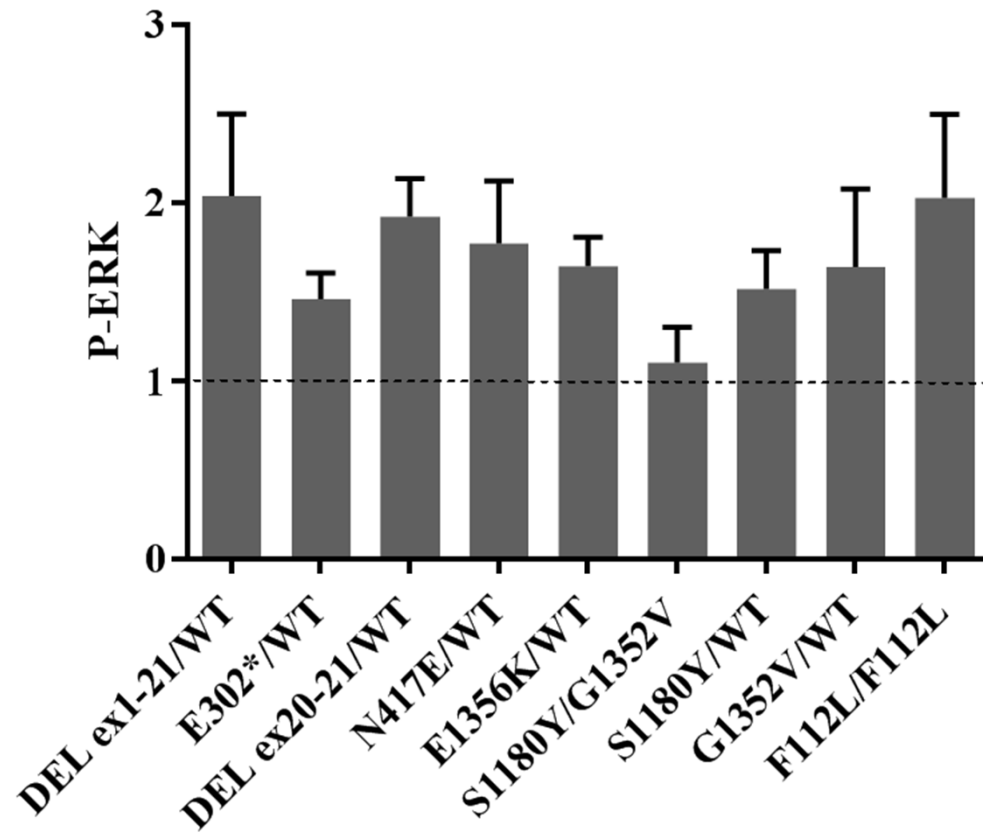


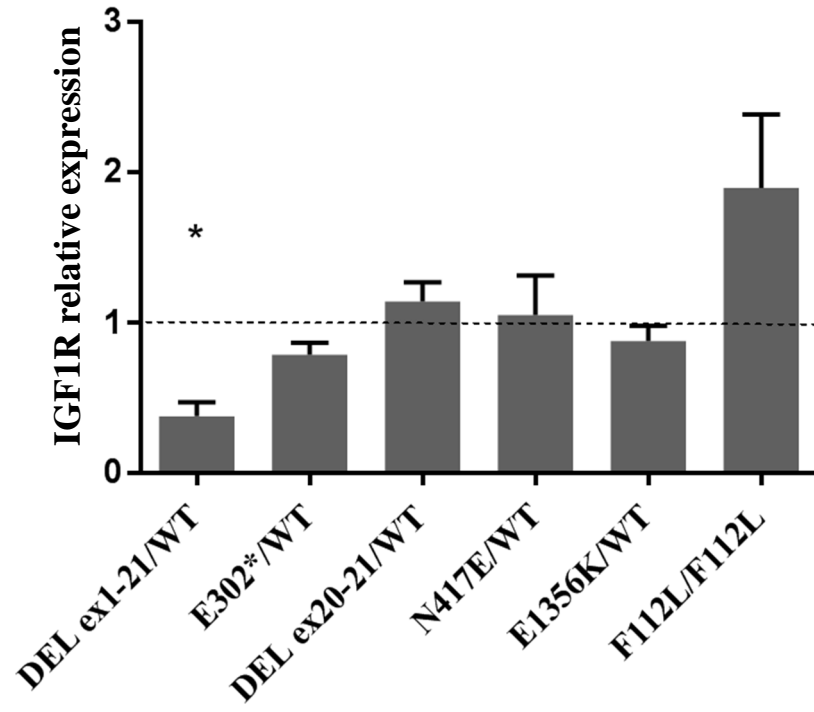












	PATIENTS		GENETICS				FAMILY			TERM (WA)
	NAME	SEX	NOMENCLATURE	PROTEIN	ALLELIC STATUS	SEGREGATION	MOTHER HEIGHT (SDS)	FATHER HEIGHT (SDS)	TARGET HEIGHT (SDS)	
ABBREVIATIONS										
ADHD: attention deficit hyperactivity disorder	patient1	M	15q26.3(98954957_102461162)x1	NA	HTZ	mother	-4,1	-0,5	-2,1	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.