CASE REPORT

Homozygous mutation of the IGF1 receptor gene in a patient with severe pre- and postnatal growth failure and congenital malformations

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Abstract

Background: Heterozygous mutations in the IGF1 receptor (*IGF1R*) gene lead to partial resistance to IGF1 and contribute to intrauterine growth retardation (IUGR) with postnatal growth failure. To date, homozygous mutations of this receptor have not been described.

Subject: A 13.5-year-old girl born from healthy first-cousin parents presented with severe IUGR and persistent short stature. Mild intellectual impairment, dysmorphic features, acanthosis nigricans, and cardiac malformations were also present.

Methods: Auxological and endocrinological profiles were measured. All coding regions of the *IGF1R* gene including intron boundaries were amplified and directly sequenced. Functional characterization was performed by immunoblotting using patient's fibroblasts.

Results: IGF1 level was elevated at 950 ng/ml (+7 s.d.). Fasting glucose level was normal associated with high insulin levels at baseline and during an oral glucose tolerance test. Fasting triglyceride levels were elevated. Sequencing of the *IGF1R* gene led to the identification of a homozygous variation in exon 2: c.119G>T (p.Arg10Leu). As a consequence, IGF1-dependent receptor autophosphorylation and downstream signaling were reduced in patient's fibroblasts. Both parents were heterozygous for the mutation.

Conclusion: The homozygous mutation of the *IGF1R* is associated with severe IUGR, dysmorphic features, and insulin resistance, while both parents were asymptomatic heterozygous carriers of the same mutation.

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Introduction

Intrauterine growth retardation (IUGR) is defined as a birth weight and/or length more than 2 s.d. below the mean corrected for gestational age (1). This finding reflects pathological processes that inhibit growth *in utero*. However, only 10% of these children are at risk for short stature as adults and the cause of this postnatal defect often remains unclear (2).

Insulin-like growth factor 1 (*IGF1*) and IGF1 receptor (*IGF1R*) gene defects have been identified in a few patients with IUGR. Severe IGF1 deficiency due to a homozygous mutation in the *IGF1* gene results in intrauterine and postnatal growth failure, microcephaly, intellectual disability, and deafness (3, 4, 5, 6). Mutations in the *IGF1R* gene in the heterozygous state have been recently described as a cause of IUGR (7) and

lead to partial resistance to IGF1 and contribute to IUGR with postnatal growth failure, microcephaly, and normal or increased levels of serum IGF1 and IGF binding protein 3 (IGFBP3), sometimes associated with modestly impaired intellectual development (8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21). Skeletal and cardiac abnormalities might also be mainly present in patients with terminal deletion of chromosome 15q including the *IGF1R* locus (22), but these conditions are possibly caused by deletion of other genes. So far, only two patients were described with a compound heterozygous mutation in the *IGF1R* gene (8, 20) while mutations in the homozygous state have never been reported. Here, we report for the first time the case of a 13.5-year-old girl harboring a homozygous mutation of the *IGF1R* gene presenting with severe IUGR and postnatal growth failure.

Materials and methods

Subject

The patient is the only child of healthy, first-cousin Lebanese parents. A written consent was obtained from both parents before performing all the analyses. Height and weight were compared with normative French references.

Biochemical analysis

IGF1, 25(OH)vitamin D (25(OH)D), and parathormone (PTH) were measured using commercial chemiluminescent assays on the automated Diasorin automate (Stillwater, MN, USA). 1,25-Dihydroxyvitamin D3 (1,25(OH)₂D) was measured using the Diasorin RIA. IGFBP3, GH, FSH, LH, estradiol (E₂), free thyroxine (T₄), TSH, and insulin were measured by commercial chemiluminescent assays (Immulite 2000, Llanberis, Gwynedd, UK). The homeostatic model assessment for insulin resistance index ($\text{HOMA-IR} = \text{fasting glucose (mmol/l)} \times \text{fasting insulin (mIU/ml)} / 22.5$) was calculated to assess insulin action (23). Biochemical parameters (serum electrolytes, blood glucose levels, calcium, phosphorus, alkaline phosphatase levels, liver, and renal function tests) were measured using a Kodak automated dry chemistry machine.

Genetic analysis

EDTA blood samples from the index case and both parents were collected for genetic studies. DNA was extracted from leucocytes by standard salt precipitation methods (24). DNA fragmentation, labeling, hybridization, and scanning were performed according to the manufacturer's protocols (<http://www.affymetrix.com>) using an Affymetrix Fluidics Station 450, Affymetrix Hybridization Oven 645 and Affymetrix GeneChip Scanner 3000 7G (Affymetrix, Inc., Santa Clara, CA, USA).

The coding sequences of the *IGF1R* gene (GenBank accession number: NM_000875) and IGF1 (IGF: NM_000618) were sequenced after DNA amplification by PCR. Primers were designed using Primer 3 (<http://frodo.wi.mit.edu>) and OLIGOS v.9.3 and checked for specificity using BLAST (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi>). DNA sequences were obtained from UCSC or GenBank databases. PCR were performed using Taq DNA polymerase (Invitrogen Life Technologies). Nucleotide numbering reflects the open reading frame of the cDNA. Amino acid numbering for IGF1R represents the mature IGF1R protein without the 30 amino acids signal peptide (UniProtKB accession number: P08069). Effects of the sequence variation on the protein were predicted using PolyPhen (<http://genetics.bwh.harvard.edu/pph/>).

Wild-type and mutant IGF1R were modeled using the freely available version of the Discovery Studio 3

Software (Accelrys, Inc., San Diego, CA, USA). The crystal structure of the first three domains of the IGF1R protein was obtained from the Protein Data Bank (PDB ID: 1IGR (25)).

Cell culture and immunoblotting

Patient's and control dermal fibroblasts were cultured in DMEM/F12 supplemented with 10% FBS under standard conditions. After an overnight starvation in serum-free medium containing 0.2% BSA, cells were incubated for 15 min with the indicated concentrations of recombinant human IGF1 (rhIGF1; Biozol, Eching, Germany). Lysates were prepared as described previously (16) followed by SDS-PAGE and immunoblotting to determine *IGF1R* expression, activation, and signal transduction. Primary antibodies were obtained from New England Biolabs (Frankfurt/Main, Germany; anti-phospho-IGF1R (Tyr1135/6)/IR(Tyr1150/1) (19H7); anti-phospho-Akt (Thr308) (244F9); anti-IGF1R β (111A9); anti-Akt (9272)) and from Sigma-Aldrich (anti- β -actin). HRP-conjugated secondary antibodies were from Thermo Fisher Scientific (Bonn, Germany).

Results

Clinical findings

The patient was born at 41 weeks of gestation after an unremarkable pregnancy and vaginal delivery, from healthy first-cousin Lebanese parents. At birth, her weight was 1650 g (-4.7 s.d.), her length 39 cm (-4.5 s.d.), and her head circumference 30 cm (-3.3 s.d.). A significant systolic murmur was heard. An echocardiogram evidenced an opened foramen ovale (FO), a small perimembranous ventricular septal defect (VSD), two narrowed pulmonary branch arteries with increased Doppler gradient (60 mm Hg), and a continuation of the inferior vena cava to the azygos vein, all of which were treated conservatively. She was able to sit at the age of 8 months and to walk alone at 1 year (Fig. 1).

At the age of 4.5 years, her general appearance was that of a thin, very small, non-cyanotic girl. Her weight was 9 kg (-3.8 s.d.) and height 87 cm (-4.5 s.d.). Upon examination, the child presented with reduced subcutaneous fat, triangular small face, up-slanting palpebral fissures, slight convergent strabismus, arched eyebrows, low-set ears, high-arched palate, brittle and eroded teeth, microretrognathism, pterygium colli, clinodactyly without flexion crease of the fifth fingers and gap between first and second toes. External genitalia and ophthalmological examination were unremarkable. An echocardiography at that time showed a closed FO, restrictive VSD with bulging of tissue of the tricuspid valve, and normalized left

Figure 1 Photographs of the patient at the age of (A) 2 months, (B) 9 years, (C) 13.5 years, and (D) X-ray of patient's bone age at 13.5 years. Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-12-0701>.

pulmonary artery diameter while the right one was still below normal values. The gradient at that level was about 50 mm Hg. Bone age was delayed by 6 months.

The patient was seen again when she was 13.5 years old (Fig. 1). She was pleasant and showed a joyful mood. Her voice was high pitched. She started regular school but she so far had to repeat 2 years in her schooling. Her general aspect was unchanged. Auxological parameters were still below the third centile for age (height 135 cm (-4.4 s.d.), weight 30 kg (-3.2 s.d.), and head circumference (-5.6 s.d.)). An axillary acanthosis nigricans was noted, as well as a mild scoliosis and a truncal obesity. Her pubertal staging was Tanner B2P2, and her bone age corresponded to a chronological age of 15 years (Fig. 1) as determined by the Greulich–Pyle method (26).

Within the family, no other subjects were reported to be born with intrauterine growth failure and/or showed postnatal growth retardation. Her father's height was 173 cm (-0.66 s.d.) and her mother was 161 cm (-0.4 s.d.) tall. The subject's midparental target height of 160.5 cm was calculated from the heights of her parents.

Laboratory results

At 13.5 years old, when last seen, a fasting blood sample was collected for all measurements. Levels of free T_4 and TSH were normal. IGF1 level was very high (950 ng/ml ($+7$ s.d.)), while IGFBP3 was at the upper limit of normal (8.9 mg/l (3.1–9.5)) and the basal GH was normal (0.86 ng/ml). Respective values of FSH, LH, and E_2 were 4.2, 2.9 IU/l, and 39.4 pg/ml confirming onset of puberty. The fasting glucose level was normal (4.7 mmol/l), but baseline insulin levels were high (38 mIU/l; normal value <25 mIU/ml) resulting in

a HOMA-IR of 8.0 and thus indicating insulin resistance. Triglyceride levels were elevated (2.41 mmol/l; normal values <1.70 mmol/l). A 50 g oral glucose tolerance test (OGTT) was performed; at 2 h, the glucose level was 7.4 mmol/l and the insulin level 277 mIU/l (respective normal values <7.7 mmol/l and <150 mIU/l). Testosterone level was normal (<20 ng/ml). A slightly elevated calcium level (2.62 mmol/l, normal range 2.10–2.55) was noted associated with a slightly high phosphorus level (1.63 mmol/l, normal range 0.81–1.45), a normal PTH value (20 pg/ml, normal range of 6–36), a normal 25(OH)D level (28.9 ng/ml), and a high-normal 1,25(OH)2D level (79 pg/ml, normal range 18–71). The IGF1 measurement in the patient's mother was 201 ng/ml ($+0.7$ s.d.).

Genetic results

Chromosome studies of lymphocytes with high-resolution G- and R-banding were normal (46,XX). No pathogenic copy number variation (CNV), neither gain nor loss, was detected using the Whole-Genome 2.7M Array.

Sanger sequencing of the *IGF1R* gene led to the identification of a homozygous variation in exon 2: c.119G>T (Fig. 2). Both parents were heterozygous carriers of the mutation. No aberrations or polymorphisms were observed in the *IGF1* gene.

At the protein level, the *IGF1R* c.119G>T mutation is deduced to be a missense mutation, leading to the replacement of an arginine by a leucine at residue 10 (p.Arg10Leu). The amino acid exchange was predicted to be damaging according to PolyPhen and was absent in 150 Lebanese control chromosomes, thus suggesting a pathogenic impact of the variation. It affects an amino acid residue that is highly conserved among vertebrates, located in the ligand binding L1 domain. Introduction of the p.Arg10Leu mutation into the crystal structure model of the *IGF1R* ectodomain demonstrates the loss of hydrogen bonds between Leu10 and Asp8 in the mutant *IGF1R* (Fig. 3).

Functional results

To assess the impact of the leucine for arginine substitution on the biochemical properties of the receptor, skin fibroblasts of the patient and two sex-(control 1) and age (control 1 and 2)-matched control individuals were stimulated with increasing amounts of rhIGF1. Immunoblotting against tyrosine residues within the *IGF1R* β -subunit activation loop that become phosphorylated in response to IGF binding revealed diminished but not abrogated autophosphorylation in patient's fibroblasts. Total expression of the mutant *IGF1R* was unaffected (Fig. 4A and B). The activated *IGF1R* initiates a signaling cascade by binding and tyrosine phosphorylation of downstream molecules that

Figure 2 Electropherograms of the patient and her parents showing the replacement of the guanine by a thymine at position 119 (arrow) in exon 2 of *IGF1R* (c.119G>T). Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-12-0701>.

ultimately leads to the activation of major signaling nodes such as the protein kinase B/AKT. In patient's fibroblasts, impaired IGF-dependent receptor activation was paralleled by a requirement for higher IGF1 doses to achieve similar levels of AKT phosphorylation compared with control cells (Fig. 4C and D).

Discussion

Here, we report the case of a 13.5-year-old girl with IUGR, postnatal growth failure, mild developmental delay, dysmorphic facial features, clinodactyly, heart malformations, and insulin resistance. *IGF1R* analysis revealed the presence of a novel missense mutation in exon 2 in the homozygous state.

Few patients with *IGF1R* mutations have been reported (8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21). In all of them except two (8, 20), mutations were in the heterozygous state. Patients were born small for gestational age with a birth weight ranging between -3.4 and -1.5 s.d. and birth length between -5.0 and -0.3 s.d. without postnatal catch-up growth. In addition, some of these patients presented slightly dysmorphic features such as triangular shape of the face, microcephaly, clinodactyly, small hands and feet, and proximally placed thumbs (8, 14, 17). Mild delay of mental and psychomotor development has been reported for some cases (8, 13, 16).

Compared with most heterozygous patients with *IGF1R* mutations, our patient has a more severe growth delay and thus resembles in her auxological characteristics the compound heterozygous patients reported so far (8, 20). She also shares with these patients several facial and developmental abnormalities including dental anomalies and mild developmental delay.

Additional findings that were observed in at least one of the compound heterozygous siblings reported by Fang *et al.* (20) and our homozygous patient were mild facial dysmorphisms, clinodactyly, and convergent strabismus. Moreover, our patient was born with a cardiac defect – a finding that is frequently observed in carriers of heterozygous 15q26 deletions (including the *IGF1R* locus) but has been ascribed to genes located proximally to the *IGF1R* gene (22). However, SNP array analysis in our patient did not show any pathogenic CNV and, thus, the cause of the heart defect remains elusive.

Interestingly, our patient presented with several metabolic abnormalities. Symptoms of insulin resistance such as high baseline and post-OGTT insulin levels, hypertriglyceridemia, loss of adipose tissue, and acanthosis nigricans were noted. The *IGF1R* protein shares high homology in structure with the insulin receptor (IR) and activates comparable downstream signal transduction pathways (27). It has also been demonstrated that the *IGF1R* can form hybrid receptors with the IR with crucial consequences for ligand specificity and signaling capabilities (28). In two previous studies, enhanced IR activation was observed in parallel with a reduction of the *IGF1R* protein dose (11, 12). However, in both patients, abnormalities in glucose metabolism have not been observed. In contrast, reports of other patients with an *IGF1R* defect revealed an HbA1c in the upper normal range in one subject (16), mild insulin resistance in another patient (11), or suggested an association between a heterozygous *IGF1R* nonsense mutation found in several members of an Italian family with variable alterations in carbohydrate metabolism (19). Most interestingly, the compound heterozygous *IGF1R* patient recently reported by Fang *et al.* (20) presented with symptomatic

Figure 3 Visualization of the three-dimensional structure of the human IGF1R ectodomain (PDB accession number: 1IGR). (A) The affected arginine residue at position 10 (arrow) resides at the surface of the L1-Cys-rich (CR)-L2 IGF binding pocket; molecule is shown as ribbon diagram superimposed by the solvent surface; atoms of arginine 10 are displayed as van der Waals spheres. (B and C) Replacement of Arg10 by leucine (arrow heads) results in the loss of hydrogen bonds (dashed lines) to the essential IGF-interacting aspartic acid residue at position 8 (32). Arg10Leu displayed as ball-and-stick, Asp8 as stick representation. Graphics generated using the free version of Accelrys' Discovery studio 3.0. Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-12-0701>.

diabetes mellitus; although in contrast to our patient, the basis for diabetes appeared to be primary insulin deficiency. Mice expressing a dominant-negative *Igf1r* in skeletal muscle develop hyperinsulinemia at an early age with normal glucose levels, which later on evolves

to hyperglycemia and overt diabetes (29). Furthermore, defects in the insulin secretion of pancreatic β -cells were observed in these mice, which is in line with findings in a mouse model of β -cell-specific deletion of the *Igf1r* gene (30). The distinct manifestations of disturbed glucose homeostasis observed in our patient and that reported by Fang might thus be a reflection of the multifaceted functions and complex interactions of the IGF1R as a cofactor in the etiology of type 2 diabetes in different tissues. Thus, although pieces of information are accumulating that point to a direct interrelationship between *IGF1R* mutations and a disturbed glucose homeostasis in affected patients, direct evidence is still lacking and more specific studies, particularly in typical insulin-responsive tissues, are necessary.

In addition, so far, we do not have any plausible explanation for the accelerated bone age as the patient presented no signs of an accelerated puberty nor elevated endogenous GH. Hyperinsulinism may play a role as obese children have some bone advancement.

Lastly, the slightly elevated calcium despite a normal PTH level might suggest a role for the GH/IGF1 system in calcium metabolism. High calcium level with normal PTH has been described in two acromegalic patients in whom the hypercalcemia was 1,25(OH) $_2$ D dependent (31). Whether excess GH or IGF1 increases the production of 1,25(OH) $_2$ D in humans remains to be elucidated.

Receptor activation studies using patient's fibroblasts demonstrated an impaired capability of the mutant Arg10Leu receptor to tyrosine-phosphorylate itself and to activate the AKT downstream signaling pathway. However, the underlying mechanism remains speculative. By analogy to the hormone-receptor interface of insulin/IR, the highly conserved Arg10 of the IGF1R was initially suggested to be directly involved in ligand binding (25). However, alanine scanning experiments did not confirm such an assumption (32). Replacement

Figure 4 The *IGF1R* Arg10Leu mutation impairs IGF1-dependent IGF1R autophosphorylation and downstream signaling. Skin fibroblasts of the patient and two control subjects were stimulated with increasing amounts of IGF1 as described in the Subjects and methods section. Immunoblots were densitometrically quantified and normalized to total IGF1R (A) or AKT (C) expression. Control 2 stimulated with 20 nM rhIGF1 was set to 1; the mean of three independent experiments $\pm$ S.E.M. is shown. (B and D) Depict representative experiments showing diminished IGF1 dose-dependent autophosphorylation of the mutant IGF1R β -subunit (pY-IGF1R β) and reduced AKT phosphorylation at threonine residue 308 (pT-AKT) compared with wild-type controls with unaffected total IGF1R β and AKT expression respectively. Immunoblotting against β -actin was performed as loading control.

of Arg10 by the branched chain amino acid leucine instead of alanine, however, might have more detrimental consequences for IGF binding (Fig. 3). Other mechanisms, such as impaired plasma membrane trafficking, might also contribute to the reduced IGF1 response.

Reconstitution studies of previously reported heterozygous mutations have shown that most of them result in a complete loss of function. However, receptors expressed from the wild-type allele in these patients can be assumed to partly compensate for the aberrant allele. Moreover, due to the composition of the IGF1R of two (hemi)-receptor halves, a substantial proportion of receptors will be assembled out of two wild-type receptor species assuring residual receptor activity. To date, homozygous mutations have not been described in humans, but mice null mutants for the *Igf1r* gene invariably die at birth from respiratory failure (33). Thus, we suggest that only hypomorphic *IGF1R* mutations in a homozygous state as found in our patient are compatible with life, whereas loss-of-function mutations affecting both *IGF1R* alleles can be expected to be lethal.

Finally, we suggest that the asymptomatic phenotype of both parents, despite the presence of a heterozygous mutation, is the consequence of the hypomorphic nature of the Arg10Leu variant. Across all the family generations, there was no report of intrauterine or postnatal growth failure. From our results, it can be concluded that the great phenotypic variability observed in patients with IGF1 resistance is not only caused by the genetic background and/or environmental factors but also due to the type of mutations that influence the severity of the phenotypic appearance (10).

In conclusion, we describe for the first time a homozygous mutation of the *IGF1R* gene in a 13.5-year-old girl. This mutation is associated with severe IUGR, intellectual impairment, and an insulin-resistant state. Both parents were heterozygous carriers of the mutation without any clinical consequence, highlighting the possibility of asymptomatic carriers of heterozygous *IGF1R* mutations.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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