Intrauterine twin discordancy followed by partial postnatal catch-up growth in a girl with a pathogenic IGF1R mutation

Short title: IGF1R mutation: Intrauterine twin discordancy

Paula Ocaranza1, Monique Losekoot2, Marie JE Walenkamp3, Christiaan de Bruin4, Jan M Wit4, Veronica Mericq1

1Institute of Maternal and Child Research, School of Medicine, University of Chile, Chile
2Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands
3Emma Children’s Hospital, Amsterdam UMC, Vrije Universiteit Amsterdam, Pediatric Endocrinology, Amsterdam, The Netherlands
4Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands

What is already known on this topic?
IGF1R mutations cause prenatal and postnatal decrease on linear growth. This mutation (p.Glu1050Lys) has been tested in vitro in fibroblasts showing decreases in phosphorylation of STAT5, a protein which upon activation acts as a transcription factor in the nucleus.

What this study adds?
The effect of this mutation in an intrauterine growth is tested for first time in discordant twins. The affected girl’s weight was decreased by 36% and 12% of length. This case highlights that intrauterine twin discordancy can be produced in some patients carrying IGF1R mutations.

Abstract
Background: IGF-1 is essential for normal human growth in utero and postnatally. It mediates its effects through the IGF-1 receptor (IGF1R), a widely expressed cell surface tyrosine kinase receptor.
Objective: The aim of the study was to analyze pre- and postnatal growth, clinical features and laboratory findings of discordant twins: one small for gestational age (SGA) girl and one appropriate for gestational age (AGA) brother in whom discordant postnatal growth persisted.
Patient and methods: A girl born with a low weight and length (-2.4 SDS) but borderline low head circumference (-1.6 SD) presented with a height of -1.7 SDS, in contrast to a normal height twin brother (0.0 SDS). Because of elevated serum IGF-1 levels, IGF-1 resistance was suspected.
Results: Sequencing revealed the presence of a previously described pathogenic heterozygous mutation (p.Glu1050Lys) in the SGA girl, not present in the parents nor the AGA twin brother.
Conclusions: The pathogenic IGF1R mutation in this girl led to intrauterine growth retardation followed by partial postnatal catch-up growth. Height in mid-childhood was in the lower half of the reference range, but still 1.7 SD shorter than her twin brother.

Keywords: Insulin-like growth factor type-1, Insulin-like growth factor type-1 receptor, small for gestational age, postnatal growth, intrauterine discordancy

Corresponding author: Dr. Veronica Mericq, Avenida Santa Rosa 1234, piso 2 IDIMI. Santiago-Chile
vmericq@med.uchile.cl

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Introduction
Insulin like growth factors (IGFs) are essential for intrauterine and postnatal growth and development [1]. The mitogenic effects of IGF-1 are mediated through the IGF-1 receptor (IGF1R), a cell surface tyrosine kinase receptor encoded by IGF1R (15q26.3) [2]. Synthesized as a single polypeptide precursor, the IGF1R undergoes proteolytic cleavage into α- and β-chains and forms a tetramer (α2β2), with the extracellular α2-subunits involved in ligand binding and the β2-subunits carrying intrinsic tyrosine kinase activities [2]. Ligand association leads to IGF1R autophosphorylation and activation of multiple downstream signaling pathways [3]. This signaling results in fetal somatic growth, whereas postnatal somatic growth is achieved through the synergistic interaction of growth hormone (GH) and IGFs, among other factors [4].
The role of IGFs and their receptors in growth and development was first studied in animal models in which the invalidation of the Igf1 and IGF1 genes in mice causes pre- and postnatal growth retardation [5]. Later, genetic studies in short children showed that absent or decreased expression of IGF1 leads to severe pre- and postnatal growth failure, and microcephaly [6-8], while heterozygous (or compound hypomorphic) mutations or deletions of IGF1R lead to a variable degree of pre- and postnatal growth failure and microcephaly [9-11].
Intrauterine growth retardation is a common condition in pregnancies and can lead to a small body size for gestational age (SGA) [12]. It can be caused by maternal, placental or fetal factors. Approximately 90% of SGA born children show catch-up growth in...
the first years of life [13, 14]. In these children no further diagnostic tests are carried out. In SGA born children with persistent short stature multiple genetic causes have been detected [15].

We report an SGA born girl with partial catch-up growth, but still 1.7 SD shorter than her appropriate for gestational age (AGA) born twin brother, with an unexpectedly elevated serum IGF-1, caused by a previously described pathogenic mutation in IGF1R (c.3148G>A, p.Glu1050Lys).

**Materials and Methods**

**Subjects**

Informed consent was obtained from the family to participate and provide samples (DNA, whole blood), in compliance with the Institutional Ethics Committee at San Borja-Arriarán’s Hospital (Santiago, Chile).

**Sample Procurement**

Genomic DNA was isolated from peripheral blood from the patient, sibling and both parents. The samples were sent to the Laboratory for Diagnostic Genome Analysis, Dept of Clinical Genetics at the Leiden University Medical Center (LUMC) for routine genetic testing of IGF1R. Targeted Sanger sequencing of the complete coding region exon 1-21 including intron/exon boundaries (NM_000875.3) was performed as previously reported [10, 16]. MLPA assay (MRC Holland kit P217-B2) containing probes for IGF1R exon 1-21 was performed for the detection of deletions or duplications [16].

**Statistical Analysis**

Comparisons between groups were not performed in this study.

**Results**

**Clinical presentation of the index patient**

The Chilean female index patient was part of a bichorial biamniotic twin, born after a pregnancy interrupted due to premature membrane rupture and metrorrhagia. The patient showed in utero growth discordancy at week 21 and was born SGA at 33 weeks of gestational age, with a birth weight of 1.48 kg (-2.4 SDS) [17], a birth length of 39 cm (-2.4 SDS) and head circumference of 29.5 cm (-1.6 SDS) (Figure 1a). During her first days of life, she was hospitalized for gastric distress. Several episodes of gastroesophageal reflux with and without cyanosis were reported after hospitalization.

The parents were not consanguineous. Paternal and maternal heights were 176.9 cm (-0.1 SDS) and 157.9 cm (-1.0 SDS), respectively, with a target height of -0.45 SDS [18]. The father reported normally timed puberty and the mother’s pubertal development was slightly delayed (menarche 14 years). Paternal grandfather and -mother had a height of 170 cm (-0.9 SDS) and 165 cm (0.4 SDS), and maternal grandparental heights were 162 cm (-2.1 SDS) and 157 cm (-1.0 SDS), respectively (Figure 2).

She consulted the Pediatric Endocrine Unit for evaluation of short stature at 1.25 yrs of age because of postnatal growth discordancy with her twin brother (Table 1). Height was 69.4 cm (-3.0 SDS), arm span 70.0 cm, weight 6.87 kg (-2.8 SDS for age), weight for height -2.2 SDS [19], and head circumference 44.8 cm (-1.3 SDS). Physical examination revealed normal body proportions and a small midface, mild frontal bossing, a thin upper lip, and mild hypertelorism. Bone age was delayed by 3 months. A normal female karyotype (46 XX) was found. Serum IGF-1 concentration was high (194 ng/mL; reference range (RR) < 131 ng/mL) and IGFBP-3 levels in the upper normal range (3.1 mg/L; RR = 1.1 – 3.6 mg/L). Independent walking was achieved at 1.25 yrs. Her appetite was poor and selective.

In the subsequent 8 years she visited the clinic several times (Table 1). Psychomotor development was normal. Height remained below -2 SDS up to 3 yrs and then increased (Figure 1b). Bone age at 3.75 yrs was delayed but identical to chronological age at 6.33 years. At age 8.92 yrs she was prepubertal and a small diffuse goiter was noted, confirmed by finding of a small thyroid cyst at ultrasound with normal thyroid function during follow-up. Over the years, her circulating IGF-1 levels and IGFBP-3 concentrations remained high (Table 1).

**Twin brother of the patient**

The male twin brother of the index patient was born at 33 weeks of gestational age with a weight of 2.0 kg and length of 44 cm. Growth data are shown in Table 1. At 1.75 years of age, his height was 83 cm and weight was 13.3 kg (Figure 1c). Thereafter his height SDS increased to close to the reference mean (Table 1) and slightly above conditional target height SDS, and remained stable afterwards (Figure 1d). He has no associated morbidities nor dysmorphic features (Figure 3).

**Genetic studies**

Since the clinical and biochemical characteristics of the index patient were consistent with IGF-1 resistance which could be caused by a deletion or an inactivating mutation in the gene encoding IGF1R, targeted sequencing and MLPA was performed for IGF1R on genomic DNA from whole blood from the index patient. Sequence analysis showed a heterozygous nucleotide substitution at position 3148 (c.3148G>A), changing glutamic acid to lysine at position 1050 of the mature IGF1R protein (p. Glu1050Lys). This heterozygous mutation was not encountered in the twin brother nor in both parents. It was confirmed by PP16 analysis that the index patient was the daughter of this couple.

**Discussion**

In this study, we report a patient who presented with pre- and postnatal growth retardation resulting from a de novo heterozygous IGF1R mutation in exon 16 (c.3148G>A, p. Glu1050Lys). Substitution of this highly conserved amino acid residue, located in the intracellular tyrosine kinase domain, is associated with a change in charge of the amino acid and in silico analysis predicts inactivation of the IGF1R leading to a partial resistance to IGF-1. This mutation was not identified in the patient’s twin AGA born normal-statured brother nor in other family members.

Fetal growth and development are influenced by maternal, placental and fetal factors [1]. A variety of maternal and utero-placental factors may constrain the growth of the fetus. In this interesting experiment of nature the role of maternal and placental factors are well controlled and separated from the role of fetal factors. A series of elegant investigations in mice, complemented by case studies in humans, have convincingly demonstrated the critical role of the IGF system in pre- and postnatal [5]. Targeted
disruption of the gene encoding Igf-2 in mice resulted in a 40 percent reduction in fetal growth but otherwise normal postnatal growth, demonstrating the important role of IGF-2 in intrauterine growth. Disruption of the gene for Igf-1 led to a similar decrease in birth weight but was also characterized by persistent postnatal growth failure. Furthermore, deletion of the gene encoding Igf1r, which mediates the growth-promoting actions of both IGFs, resulted in birth weights that are only 45 percent of normal and these mice generally died within hours after birth from respiratory insufficiency resulting from muscular hypoplasia [5]. The relevance of these findings for human growth was supported by human reports. Homozygous mutations of IGF-1 were found in a few patients presenting with severe pre- and postnatal growth failure, microcephaly and deafness [6, 7]. Several reports have been published about patients with IGF-1 resistance due to molecular defects in the IGF1R who present with a variable degree of pre-and postnatal growth retardation [9].

Short stature is a common problem confronting pediatric endocrinologists. After exclusion of systemic or skeletal diseases or overt hormonal deficiencies, clinicians are often unable to provide a definitive diagnosis for the etiology of an individual patient’s short stature. An important clue for the cause of short stature is to register whether prenatal growth was either normal or reduced. Because of the persistent short stature in our patient and the high IGF-1 levels, we suspected a mutation within the IGF1R signaling cascade. Our hypothesis led us to the detection of a de novo heterozygous mutation of IGF1R in exon 16, resulting in the replacement of a Glu residue at position 1050 by a Lys residue. So far, mutations in IGF1R almost always result in intrauterine growth retardation (IUGR), and postnatal catch-up growth has not been documented. Aberrant IGF1R expression is described to lead to IGF1R haploinsufficiency [20, 21], disturbed processing of the proreceptor [22, 23], decreased ligand binding [24], abrogated IGF1R tyrosine kinase activity and reduced receptor autophosphorylation [10, 25, 26]. In line with the previously reported adult patient (with a birth weight and length of -2.1 and -0.3 SDS, respectively, and a height SDS of -3.3 at presentation, and an adult head circumference SDS of -3.0), the mutation led to a clinically significant prenatal and postnatal growth failure, though postnatal growth of our patient is less affected compared to almost all cases with IGF1R haploinsufficiency described to date. This mutation was also associated with microcephaly, but it did not affect intellectual development. Our patient reported feeding problems during the first year of life and poor appetite, which previously has been associated with the same and other IGF1R mutations [10]. This mutation was not present in her twin brother and parents, who all have normal stature. Our results provide strong evidence that this variant is likely to be the underlying cause of the intrauterine growth retardation and mild postnatal short stature observed in this patient.

Most of the IGF1R mutations have been described in children born SGA. The first human IGF1R defects were described by Abuzzahab et al in 2003 [9] and only a few compound heterozygous cases have been described thereafter [9, 27]. Most of the described cases are heterozygous carriers of IGF1R mutations [10, 20-23, 25, 26, 28-34]. To date only two single patients carrying a homozygous mutation have been described [35, 36]. The phenotype is variable, presumably depending on the impact of the mutation on the function of the IGF1R. The most common feature described in the reported patients included intrauterine growth retardation (IUGR) [11, 37], postnatal growth failure and microcephaly [11, 37, 38].

Study Limitations
The affected Glu residue at position 1050, is located in the strongly conserved serine-threonine/tyrosine-protein kinase catalytic domain. A study limitation was the absence of functional studies, as fibroblasts from skin biopsies were not available. However, because of the persistent short stature in our patient and the high IGF-1 levels, we suspected a mutation within the IGF1R signaling cascade. Our hypothesis led us to the detection of a de novo heterozygous mutation of IGF1R in exon 16, resulting in the replacement of a Glu residue at position 1050 by a Lys residue. So far, mutations in IGF1R almost always result in intrauterine growth retardation (IUGR), and postnatal catch-up growth has not been documented. Aberrant IGF1R expression is described to lead to IGF1R haploinsufficiency [20, 21], disturbed processing of the proreceptor [22, 23], decreased ligand binding [24], abrogated IGF1R tyrosine kinase activity and reduced receptor autophosphorylation [10, 25, 26].

In conclusion, we describe a discordant pair of twins in whom the effect of this IGF1R mutation on the function of the IGF1R. The most common feature described in the reported patients included intrauterine growth retardation and mild postnatal short stature observed in this patient.

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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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References


Figure 1. A, B, C, D) Growth chart of the patient and her twin brother.  (A) and (B) Growth charts of the patient carrying the mutation . (C) and (D) Growth charts of the normal statured brother.
Length-for-age and Weight-for-age percentiles, Birth to 24 months

Figure 1B.
Figure 1C.
Figure 1D.
Stature-for-age and Weight-for-age percentiles, 2 to 20 years

Father
Mother
Target height

Published May 30, 2000 (modified 11/1/2005)
SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).
http://www.cdc.gov/growthcharts
PC PAL - Grow4OP 2.6.0.493
Figure 2. Pedigree of the index patient with the IGFIR mutation. Height SDS is indicated in brackets and persons who were checked for the IGFIR mutations are indicated (*).
Figure 3. Picture of the twins taken in July 2014.

Table 1: Clinical and biochemical characteristics of the index patient and her twin brother.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Height cm (SDS)</th>
<th>Weight kg (SDS)</th>
<th>BMI cm (SDS)</th>
<th>HC cm (SDS)</th>
<th>Bone Age</th>
<th>IGF-1 ng/mL (RR)</th>
<th>IGFBP-3 mg/L (RR)</th>
<th>Height cm (SDS)</th>
<th>Weight kg (SDS)</th>
<th>BMI (SDS)</th>
<th>HC cm (SDS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth data</td>
<td>39 (-2.5)</td>
<td>1.48 (-2.4)</td>
<td>29.5 (-1.6)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>44 (0.0)</td>
<td>2.0 (-0.6)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>1.25</td>
<td>69.4 (-3.0)</td>
<td>6.87 (-2.8)</td>
<td>14.3 (-1.3)</td>
<td>44.8 (-1.3)</td>
<td>1 yr</td>
<td>194 (&lt;131)</td>
<td>3.1 (1.1-3.6)</td>
<td>NA</td>
<td>10.7 (0.2)</td>
<td>NA</td>
<td>48 (0.9)</td>
</tr>
<tr>
<td>3.08</td>
<td>86.2 (-2.1)</td>
<td>10 (-3.5)</td>
<td>13.5 (-2.3)</td>
<td>NA</td>
<td>NA</td>
<td>269 (&lt;289)</td>
<td>4.3 (&lt;4.3)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>3.75</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>3 yr</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4.25</td>
<td>95.5 (-1.6)</td>
<td>13.1 (-1.9)</td>
<td>14.4 (-0.8)</td>
<td>NA</td>
<td>NA</td>
<td>330 (&lt;289)</td>
<td>4.5 (&lt;4.3)</td>
<td>104.8 (0.2)</td>
<td>20.9 (1.6)</td>
<td>19.0 (2.4)</td>
<td>NA</td>
</tr>
<tr>
<td>4.75</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>4 yr</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>6.33</td>
<td>109 (-1.6)</td>
<td>17 (-1.7)</td>
<td>14.3 (-0.7)</td>
<td>49 (-1.7)</td>
<td>6.5 yr</td>
<td>417 (&lt;286)</td>
<td>NA</td>
<td>118.3 (0.2)</td>
<td>22 (0.1)</td>
<td>15.7 (0.2)</td>
<td>53.5 (1.0)</td>
</tr>
<tr>
<td>8.92</td>
<td>122.0 (-1.8)</td>
<td>21 (-2.1)</td>
<td>14.1 (-1.3)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>132.2 (-0.1)</td>
<td>37.7 (1.4)</td>
<td>21.6 (1.7)</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; HC, head circumference; NA, not available; RR, reference range