LETTER TO JMG

A novel mutation in a patient with insulin-like growth factor 1 (IGF1) deficiency

G Bonapace, D Concolino, S Formicola, P Strisciuglio


The insulin-like growth factors (IGFs; somatomedins) comprise a family of peptides that play important roles in mammalian growth and development. The principal members of this family are IGF1 and IGF2. IGF1 (somatomedin C), a 70 residue basic polypeptide, mediates many of the growth promoting actions of growth hormone (GH) and has metabolic and mitogenic effects. The major source of circulating IGF1 is the liver, but it is also produced in a wide variety of tissues and has endocrine and paracrine modes of action. The mature IGF1 peptide has A, B, C, and D domains with homology to insulin, and is highly conserved. It is produced as an inactive precursor, pre-pro-IGF1, with an additional carboxyterminal E region that plays an important role in the maturation of normal IGF1 peptide. This regulatory region is obtained by alternative splicing of the last two exons. IGF1 resides on the long arm of chromosome 12 (12q 22–24.1), and several molecular studies have demonstrated that the structure of this gene is very complex. The gene contains six exons, which extend over more than 85 kb on chromosomal DNA. For human IGF1, two potential primary translation products exist: IGF1A and IGF1B, with sizes of 153 and 195 amino acids respectively. The two precursors are synthesised from distinct messenger RNAs produced by alternative splicing of the primary transcript. IGF1A mRNA contains exons 1, 2, 3, 4, and 6 of the human IGF1 gene while IGF1B is encoded by exons 1, 2, 3, 4, and 5. It has been speculated that IGF1B plays the major role during intrauterine growth, while the same function during postnatal growth is taken over by IGF1A.

Recent studies have focused attention on the genetic causes of growth alterations. Mutations involving the molecular structure of GH or the function of the GH receptor have been described. Recently, a partial deletion of the gene for IGF1, resulting in intrauterine growth failure plus severe post-natal growth retardation, sensorineural deafness, and mental retardation has been found. In this study, we describe a new case of IGF1 deficiency associated with sensorineural deafness, severe pre- and post-natal growth failure, and delayed psychomotor development produced by a novel transversion T→A, which disrupts the normal consensus sequence for the polyadenylation site in the 3′ untranslated region of exon 6 of IGF1, leading to altered mRNA processing, which could account for the extremely low IGF1 circulating levels and for the clinical findings.

METHODS

Case report

The patient was born at 39 weeks’ gestation by caesarean section because of poor fetal growth. The pregnancy had until then been uneventful. The birth weight was 1480 kg (−4SD), length was 41 cm (−6.5 SD), and the head circumference was 26.5 cm (<5th percentile). Hypoglycaemia and icterus were not reported. His parents were second cousins and the family history showed several miscarriages. His mother was 26.5 cm (5th percentile). Hypoglycaemia and icterus were not reported. His parents were second cousins and the family history showed several miscarriages. His mother was 153 cm tall (5th percentile) and his father was 163 cm (5th percentile). His healthy sister, at the age of 9 years, was 120 cm tall (−1SD). The analysis of familial pedigree did not show the presence of a short stature condition segregating in the family. The clinical evaluation of the patient at the age of 19 months showed a weight of 4750 kg (−6.2 SD), a length of 64 cm (−6.2 SD), a head circumference of 40.5 cm (<5th percentile), a body mass index of 11.7 (>3rd percentile) and normal size genitalia. Delayed psychomotor development and poor responses to sound were noted.

To investigate the growth failure, tests were performed for thyroid and sexual hormones, anti-gludin and anti-endomysium antibodies, and urine and plasma determination of amino acids and ammonia, in addition to karyotyping, radioimmunosorbent and radioallergosorbent testing, and the TORCH test for toxoplasma-rubella-cytomegalovirus-herpes and other viruses. All were found to be normal. At the age of 19 months, the bone age according to Greulich and Pyle was in the neonatal range. Audiograms showed profound bilateral sensorineural deafness. At the age of 2 years a magnetic resonance imaging of the brain and skeletal x ray were performed and found to be normal. The subsequent evaluation of the main growth regulator factors showed a basal GH serum concentration of 10 ng/ml with a peak after administration of arginine of 18 ng/ml (normal range 10–12 ng/ml), and a basal low serum IGFBP3 concentration of 1 ng/ml (normal range 3.7–152 ng/ml). An IGF1 generation test was performed according to the following

Key points

- We studied a new patient with short stature, sensorineural deafness, delayed psychomotor development, and IGF1 deficiency.
- By direct sequencing and expression analysis we found this clinical condition associated with a novel transversion T→A in the 3′ untranslated region of exon 6 of the IGF1 gene. The mutation in the homozygous state resides on the consensus sequence AATATA of the polyadenylation site and leads to a deregulated IGF1 mRNA maturation, altering the E domain of the IGF1 precursor.
- We conclude that this novel mutation, in changing the normal amino acid sequence for the E domain of the IGF1 precursor, accounts for the low circulating levels of IGF1 that causes the observed clinical condition.

Abbreviations: GH, growth hormone; IGFBP3, IGF binding protein 3; IGF, insulin-like growth factor; SSCP, single strand conformational polymorphism
protocol: 0.6 IU GH/day were administered intramuscularly to the patient for 7 days. Starting from the eighth day, the IGF1 and the IGF binding protein 3 (IGFBP3) serum concentrations were evaluated weekly as described for 3 consecutive weeks. The IGF1 serum levels did not change, whereas the serum basal IGFBP3 had been normal (3600 ng/ml) and did not change after the test (3800 ng/ml). The basal concentrations were evaluated weekly as described for 3 consecutive weeks. The IGF1 serum levels did not change, whereas the serum basal IGFBP3 had been normal (3600 ng/ml) and did not change after the test (3800 ng/ml). The basal IGFBP3 concentrations of the child’s parents were in the lower adult normal range (172 ng/ml and 159 ng/ml respectively, normal range 144–360 ng/ml).

Informed consent was obtained from the patient’s parents, and the study was approved by the ethics committee of the Faculty of Medicine of the University of Catanzaro.

**Molecular analysis**

DNA samples from normal controls, patient, and parents were extracted from whole blood by standard methods. Exons 1, 2, 3, 4, 5, and 6 of IGF1 were amplified by PCR using nucleotide primer pairs designed on the basis of the published IGF1 gene sequence (table 1). All of the primers were localised at 20 bp from the intron–exon boundary. To analyse the promoter region we used two specific primers, PRf and PRr, designed according to the published sequence (GenBank accession no. N_HSTGFAC-TXTO25.1). PCR amplification was performed using 100 ng of genomic DNA in a 30 μl volume with 250 μmol/l final concentration of dNTPs, a 1.5 mmol/l final concentration of MgCl2, 10pmol/l of each primer, and 1 U Taq DNA polymerase. After heating to 94°C for 3 minutes, 30 cycles were performed at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute before a final step of 72°C for 10 minutes. Each PCR product (15 μl) was electrophoresed on 1.8% agarose and visualised using ethidium bromide. The presence of point mutations in the IGF1 gene of patient and parents was studied by single strand conformational polymorphism analysis (SSCP) using a semi-automatic electrophoresis unit (Genephor; Amersham Pharmacia Biosystems, Piscataway, NJ, USA). The DNA samples from normal controls, patient, and parents were extracted from whole blood by standard methods.

**Table 1**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer sequence 5’-3’</th>
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<tr>
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<td>5F</td>
<td>AGTATCGGCCCCCAT</td>
</tr>
<tr>
<td>5R</td>
<td>ATGCTATAGAGGCTCTA</td>
</tr>
<tr>
<td>6F</td>
<td>TATAGGAATCATTTGCA</td>
</tr>
<tr>
<td>6R</td>
<td>CAGAGAGGAAATTTAGTC</td>
</tr>
</tbody>
</table>

**RESULTS**

Point mutations related to the clinical conditions in IGF1 were searched by PCR-SSCP and direct sequencing. After setting the more appropriate conditions in preliminary experiments on 30 unrelated healthy controls, 6 μl of PCR products from each of the six exons from normal controls, the parents, patient, and his healthy sister were mixed with an equal volume of formamide denaturing gel loading buffer. They were then heat denatured and analysed on a 12.5% non-denaturing acrylamide gel. The PCR products showing an anomalous SSCP pattern were sequenced. As illustrated in fig 1, SSCP analysis of exon 6 showed a different migration pattern from the wild type, characterised by a double band for both parents (lane 2 and 5) and a single anomalous shifted band for the patient (lane 3). The signal for the patient’s normal sister is shown in lane 4, and at the same position in lane 1 is the normal control. The promoter analysis of IGF1 was negative, as was the repetition of the SSCP on the negative exons, in less stringent conditions (data not shown). Fig 2 shows the results of direct sequencing of exon 6 in the father (first lane), mother (second lane), and proband (third lane). The wild type sequence of IGF1 gene from the databank is shown in the upper lane. The non-matching zones are indicated by “N”. On the terminus of the
polyadenylation signal, AATATA, is a T→A transversion, heterozygous in both parents and homozygous in the proband. To confirm that this DNA variant was the causative mutation and not a generic polymorphism, we first performed a direct sequencing of the entire IGF1 gene of the patient to exclude the presence of different molecular changes and then extended the sequencing analysis of exon 6 to the 100 unrelated healthy controls. No sequence alteration was found (data not shown). Notably, all members of the family lack the T nucleotide 2 bp upstream of the mutation.

This situation is probably due to a familial silent polymorphism, as we were able to find the same condition only in the proband’s healthy sister and not in other unrelated normal controls (data not shown). Finally, we performed molecular analysis on the GH receptor gene and were unable to find specific point mutations.

The effect of this novel mutation on the normal maturation of IGF1 mRNA was evaluated by RT-PCR and specific

Figure 1  Single strand conformational polymorphism analysis of IGF1 exon 6. Five ul of each PCR product from the various exon 6 IGF1 amplifications were added to 5 ul of 95% formaldehyde loading buffer, heat denatured, and run on a 12.5% acrylamide precast non-denaturing gel. A double band is present in lanes 2 and 5 for both parents of the proband. A single anomalous shifted band is evident in lane 3 (proband). The proband’s sister (lane 4) shows a normal pattern, the same as the control in lane 1.

Figure 2  Sequence analysis of IGF1 exon 6. Wild type IGF1 sequence from the databank is shown in the upper line (lower case). The non-matching bases are indicated by “N”. The poly-A consensus sequences are underlined. *Indicates the T→A transversion; heterozygous in both two parents (lanes 1 & 2) and homozygous in the proband (lane 3).

Figure 3  Expression analysis of exon 6 IGF1. After exon 6 cDNA specific amplification by RT-PCR, the products from the normal control (lane 2), father (lane 3), mother (lane 4), and patient (lane 5) were separated by electrophoresis on 2% agarose. Lane 1 contained 10 ul of phiX174 HindIII marker. The patient’s lane shows a shorter band than the control, approximately 340 bp. The same band is present together with the normal allele of 450 bp in both parents’ lanes.
amplification of exon 6 from cDNA. Fig 3 shows the RT-PCR products of exon 6 separated on a 2% agarose gel containing ethidium bromide and visualised by ultraviolet light. Lane 2 is the normal control, lane 3 is the father, lane 4 the mother, and lane 5 the patient. There are clear differences between the product sizes of the patient and his parents; both parent’s patterns show two bands of 450 bp and 340 bp, with the upper band corresponding to the normal size of exon 6, whereas in the patient only the shorter band at 340 bp is present. The anomalous 340 bp band found in the expression experiments was sequenced after cloning into TOP 10F vector system. Fifteen clones from four different plates were sequenced; fig 4 shows the resulting sequence. The mutant 5’ exon 6 IGF1 sequence diverges from that of the wild type, and the terminal region of this sequence continues onto the 3’ IGF1 exon 6 sequence. The Blast analysis of this anomalous intercalated sequence revealed a considerable homology to a clone KIAA0537 (Refseq accession NM 014840.1), localised downstream of IGF1 on chromosome 12.9 The RT-PCR primer position on the sequence is shown.

DISCUSSION
We describe a new case of IGF1 deficiency, with severe pre- and post-natal growth failure, sensorineural deafness, and delayed psychomotor development. Our patient has a striking phenotypic similarity to the first IGF1 deficiency case described by Woods et al.7 The clinical and biochemical findings gave an absence of response to the IGF1 generation test in our patient prompted us to look for a possible specific defect in IGF1 production after the exclusion of both GH and GH receptor deficiency. By molecular analysis, we discovered the presence of a novel mutation (transversion T→A) on the polyadenylation signal AATATA in the 3’ untranslated region of IGF1 exon 6, homozygous in the patient and heterozygous in the parents. The healthy sister presented a normal IGF1. By cloning and sequencing of RT-PCR IGF1 exon 6 products, we demonstrated that the mutation disrupts the correct consensus sequence for the normal polyadenylation pathway and deregulates the splicing and the maturation of mRNA.

Several mechanisms have been proposed to control IGF1 peptide production and action. It has been demonstrated that IGF1 is bound to binding proteins while in circulation, and that this is the first step in the regulation of its action.10 IGF1 transcription may determine how and where IGF1 is active, and several start sites for transcription have been identified.11 Alternative splicing is another possible mechanism for the regulation of IGF1 expression as has been suggested for other genes, such as the calcitonin/calcitonin related genes, in which different combinations of exons produce different peptides in different tissues.12 This idea has been recently confirmed by the observation that the new splice variant of IGF1 present in human liver is obtained by the use of a cryptic 5’ donor splice site at the junction of exons 5 and 6, confirming that in vivo cryptic sites are used to regulate the IGF1 production.13 14 In our patient, there is no evidence of alteration in IGF1 binding protein production, very low IGF1 circulating levels are present, and the mutation resides on the IGF1 gene region important for transcription and normal precursor processing. Therefore, we focused our attention on mRNA production and maturation as a possible functional target for the mutation. By quantitative RT-PCR (data not shown) we first assessed that the mutation does not affect the transcription rate of IGF1. By gel analysis of the RT-PCR products we found that in our patient the exon 6 product amplified from cDNA was shorter than normal (340 bp v 450 bp). After cloning and direct sequencing of the RT PCR exon 6 product, we found that the initial part of the exon is fused to a sequence identified in the gene databank as clone KIAA0537, normally located downstream of IGF1,7 and
consequently, all the normal codifying sequence of the exon was skipped. This was a surprising result. As it has been demonstrated that the normal polyadenylation signal on the 3’ region is important for the correct splicing of the mRNA, particularly to mark the 3’ end of the terminal exon, it could be speculated that because the novel mutation disrupts the polyadenylation signal, it may deregulate the normal scanning mechanisms by the splicing machinery of the intron-exon junction, leading to an aberrant delayed splicing into the KIAA transcript. The most important functional consequence of the presence of the KIAA sequence into the 3’ terminal region of exon 6 is the complete alteration of the B domain of pre-pro-IGF1 encoded by this exon, which plays a pivotal role in the maturation of the IGF1 active peptide.

CONCLUSION

We believe our molecular data defining the first case of a homozygous defect in the polyadenylation signal of IGF1 associated with severe pre- and post-natal growth failure, sensorineural deafness, and delayed psychomotor development are consistent with the fundamental role played by IGF1 in normal growth and with the previously described pathways involved in the regulation of the active peptide. Specifically, we conclude that this novel mutation, disrupting the normal domain of the precursor required for the production of the active mature peptide, results in low circulating levels of IGF1, which causes the clinical condition observed in our patient, which also includes microcephaly and mental retardation. This is supported by several studies using transgenic mouse models, which showed effects on neuronal migration and oligodendrocyte differentiation, and have thus demonstrated the important role of IGF1 in brain development.

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