# Clinical examples of disturbed insulin-like growth factor signaling: intrauterine and postnatal growth retardation because of mutations of the insulin-like growth factor 1 receptor gene

Raile K, Schneider A, Klammt J, Kratzsch J, Keller E, Seidel B, Garten A, Schmidt H, Pfäffle R and Kiess W

Leipzig and Munich, Germany

Insulin-like growth factors (IGF) are key players in regulating growth and development of pre- and postnatal tissues. In addition, insulin-like growth factor-1 (IGF-1) known to be a key stimulus of placental substrate uptake inhibits fetal placental catabolism and reduces placental lactate production. Insulin-like growth factor-1 receptor (IGF-1) deletions cause intrauterine failure to thrive. IGF-1R gene knockout experiments have revealed a mild pre- and postnatal growth deficit in heterozygous IGF-1R<sup>+/-</sup> mice. A gene dosage effect of the IGF-1R gene on embryonic and postnatal growth, and also on postnatal growth of tissues and organs has been investigated in clonal mice strains with a wide spectrum of IGF-1R deficiency. Approximately 10% of infants with intrauterine growth retardation (IUGR) remain small, but the causes remain unknown. Recently, monoallelic loss of chromosome 15q, mutations of the IGF-1 receptor gene, and loss of one copy of the IGF-1 receptor gene again owing to deletions of the distal long arm of chromosome 15 have been found in patients with intrauterine growth retardation and postnatal growth deficit. Binding of IGF-1 to erythrocytes in short children with IUGR has shown to be lower than in children with normal height. The number of IGF-1 receptor copies on human fibroblasts seems to be predictive of their proliferative response to IGF-1. Hemizygosity for IGF-1R can cause primary IGF-1

©2006, World J Pediatr. All rights reserved.

resistance despite normal or even elevated GH and/or IGF-1 serum concentrations. At present IGF-dependent growth in prenatal life seems to be largely independent of GH, except for a small effect just before birth, while IGFdependent growth after birth and particularly during puberty is strongly related to growth hormone (GH) action. In conclusion, mutations and deletions of the IGF-1 receptor gene lead to abnormalities in the function and/ or number of IGF-1 receptors. Alterations of the IGF-1 receptor signaling pathway seem to retard intrauterine and postnatal growth in humans. In the future, expression of such mutations in cells *in vitro* provides an opportunity to define the role of IGF-1 receptor in human growth and growth disorders.

World J Pediatr 2006;1:14-22

*Key words:* insulin-like growth factors; insulin-like growth factor 1 receptor; intrauterine growth retardation; short stature; mutation; heterozygous; chromosome 15

#### Introduction

Insulin-like growth factor-1 receptor (IGF-1R) as an important mediator of cell proliferation and longitudinal bone growth is a heterotetramer consisting of two alpha binding subunits and two beta subunits containing tyrosine kinase activity. The IGF-1R is closely related to the insulin receptor with a homology of 80%-95% in the tyrosine kinase domain.<sup>[1-3]</sup> Insulin-like growth factor 1 (IGF-1) binding to the IGF-1R causes the transmembrane activation of the tyrosine kinase activity of the IGF-1R.<sup>[4]</sup> In contrast to the insulin receptor, an impairment of

Author Affiliations: Hospital for Children and Adolescents (Raile K, Schneider A, Klammt J, Keller E, Seidel B, Garten A, Pfäffle R and Kiess W), Institute of Laboratory Medicine, Clinical Chemistry, and Molecular Diagnostics Medical Faculty (Kratzsch J), University of Leipzig; Hospital for Children and Adolescents, University of Munich (Schmidt H), Germany

**Corresponding Author:** Wieland Kiess, MD, Hospital for Children and Adolescents, University of Leipzig, Oststr. 21-25, D-04317 Leipzig, Germany (Tel: 49-341-9726000; Fax: 49-341-9726009; Email: kiw@ medizin.uni-leipzig.de)

the closely related IGF-1R has only been suggested on rare occasions under clinical circumstances. For instance, monoallelic loss of chromosome 15q, mutations of the IGF-1 receptor gene, and loss of one copy of the IGF-1 receptor gene again owing to deletions of the distal long arm of chromosome 15 have been found in patients with intrauterine growth retardation and postnatal growth deficit.<sup>[5]</sup> Binding of IGF-1 to erythrocytes in short children with IUGR has shown to be lower than in children with normal height.<sup>[6]</sup> The number of IGF-1 receptor copies on human fibroblasts seems to be predictive of their proliferative response to IGF-1. Hemizygosity for IGF-1R can cause primary IGF-1 resistance despite normal or even elevated growth hormone (GH) and/or IGF-1 serum concentrations. IGF-1R gene knockout experiments in mice have shown that mice carrying null mutations of both alleles exhibit severe and those of one allele, moderate embryonic and postnatal growth deficiency.<sup>[7-9]</sup> Recently, a compound heterozygous mutation of the human IGF-1R gene and a nonsense mutation (Arg59stop) that reduced the number of IGF-1 receptors on fibroblasts from the affected child have been observed in children with intrauterine growth retardation and highly elevated IGF-1 levels.<sup>[10]</sup> Since our first report, additional families with members presenting with intrauterine growth retardation, postnatal growth failure and carrying IGF-1R mutations have been identified by a number of investigators around the world. The phenotype of patients with IGF-1 receptor mutations and/or reduction of IGF-1 receptor numbers known to date are being presented. Their relevance to clinical medicine and a hypothesized potential for future research into growth and growth disorders is being discussed.

IGF-1 is a pleiotropic hormone of the insulin family that exerts its biological functions in almost any tissue through autocrine, paracrine, and endocrine signaling mechanisms. Both IGF-1 and insulin mediate biologic effects through their specific cell surface receptors: IGF-1R and insulin receptor (IR). The IGF-1R and the IR are tyrosine kinase receptors and are composed of two extracellular *B*-subunits containing the ligand binding site and two intracellular β-subunits harbouring tyrosine kinase activity within their cytoplasmatic domain.<sup>[1]</sup> Each prereceptor peptide forms one set of  $\alpha/\beta$  subunits and both receptor proteins can form IGF-1R or IR homodimers as well as hybrid receptor dimmers of IGF-1R  $\alpha/\beta$  and IR  $\alpha/\beta$ hemi-receptors. Widely expressed in many mammalian tissues and cell types,<sup>[2,3]</sup> the hybrid receptors bind IGF-1 with an affinity similar to that of IGF-1R and insulin with an affinity lower than IR.<sup>[4-6]</sup> Distinctly, biological functions of the receptor types are still not clear and protein expression of the hybrid receptors might contribute to changes in insulin and IGF-1 sensitivity in target tissues.<sup>[7]</sup>

Recently we have reported heterozygous IGF-1R mutation in a mother and her two sons. Common phenotypes included pre- and postnatal growth retardation, somewhat impaired motor and mental development, primary microcephaly, but normal or even increased IGF-1 and IGFBP-3 serum levels.<sup>[10]</sup> The IGF-1R mutation found was in exon 2 of one IGF-1R allele and led to an early termination of transcription after 59 amino acids [Arg59Ter]. A putative and truncated receptor protein consisted only of a short c-terminal end of the IGF-1R  $\alpha$ -chain, unable to bind IGF-1 or to anchor into cell membrane. In fibroblasts and blood leucocytes, biallelic expression of the mutant and the wild type allele was demonstrated. We assumed that the family members with the [Arg59Ter] mutation represent the human phenotype of IGF-1R haploinsufficiency.<sup>[10]</sup> Recently, a new heterozygous IGF-1R mutation [Arg709Gln] at the cleavage site of the IGF-1 pre-receptor has been identified. The two affected family members, a mother and her daughter presented with pre- and postnatal growth deficit, microcephaly and mental retardation. Mutant prereceptor protein was found to be less well processed to mature  $\alpha/\beta$  IGF-1 receptor and overall IGF-1R surface protein, and IGF-1 receptor phosphorylation was reduced (Raile et al in press). Therefore, there is increasing evidence that reduced IGF-1R expression and signaling affects pre- and postnatal growth as well as brain growth and function.

IGF-1R gene knockout experiments in mice have shown a mild pre- and postnatal growth deficit in heterocygous IGF-1R<sup>+/-</sup> mice.<sup>[7]</sup> A gene dosage effect of the IGF-1R gene on embryonic and postnatal growth, as well as on growth of several tissues and organs was investigated in clonal mice strains with a wide spectrum of IGF-1R deficiency. The effect of reduced IGF-1R gene expression had the strongest impact not only on total fat tissue weight, but also on muscle, brain, lung, and spleen weight. Moreover, this disproportionate postnatal retardation of organ growth was stronger in males.<sup>[7,11,12]</sup>

In children born small for gestational age (SGA), binding of IGF-1 to erythrocytes was lower than in children of normal height and weight<sup>[6]</sup> and therefore, some degree of reduced IGF-1R expression was claimed to contribute to reduced growth in these children. In recent years, SGA children have been treated with recombinant human growth hormone (rhGH) and a number of studies have now demonstrated improvement of growth as long as GH treatment is continued.<sup>[13-15]</sup> However, the growth response is not as strong as usually in children with GH deficiency.

We investigated whether aspects of a gene dosage effect for the human IGF-1R gene is detected in subjects with the heterozygous IGF-1R mutation Arg59Ter that leads to inactivation of one IGF-1R allele. Then skin fibroblasts of one of the affected family members were examined for their IGF-1 and/or insulin signaling capability mediated either via IGF-1R, IR or IR/IGF-1R hybrid receptors. In contrast to patients with gene deletions of chromosome 15 resulting in a loss of one IGF-1R allele, no other genes or parts of the IGF-1R promoter region were deleted in [Arg59Ter] subjects.

# Insulin-like growth factors and the regulation of fetal growth

Growth during human fetal life represents the most rapid phase of human growth.<sup>[16]</sup> Fetal growth depends upon substrate and oxygen supply, vascularization of placental and fetal tissues, and a complex endocrine modulation of cellular proliferation, tissue expansion, inhibition of apoptosis, and tissue remodeling. IGF-1 and IGF-2 have both been used as important regulators of human fetal growth. IGF-1 is produced by the fetal liver.<sup>[12,17,18]</sup> This production is growth hormone independent but directly stimulated by insulin and fetal glucose uptake.<sup>[19]</sup> IGF-1 known to be a key stimulus of placental substrate uptake inhibits fetal placental catabolism and reduces placental lactate production. Targeted disruption of the mouse IGF-2 gene leads to a 40% reduction of fetal but normal postnatal growth. Disruption of the IGF-1 gene leads to both pre- and post-natal growth failure.<sup>[7,11]</sup> Mice with deletion of the IGF-1 receptor gene are of the most severe phenotype with only 45% of normal birth weights. Mice with an IGF-1 receptor knockout genotype usually die shortly after birth. Muscular hypotrophy leads to respiratory insufficiency in these animals pointing to the key role of the IGF-1 receptor for the development and expansion of skeletal muscle.

#### Genetics and biochemistry of the IGF-1 receptor

In humans, the IGF-1 receptor gene is located on the distal long arm of chromosome 15 (15q26.3). It is synthesized as a large precursor protein that undergoes extensive post-translational modifications including cleavage and glycosylation.<sup>[1]</sup> The receptor belongs

to the family of the insulin receptor and the insulin receptor-related receptor.<sup>[2]</sup> The mature and functional IGF-1 receptor is a heterotetramer consisting of two alpha and two beta-subunits. The alpha subunits form the extracellular domain for ligand binding. The IGF-1 receptor binds IGF-1 with high affinity and IGF-2 and insulin with lower affinity. In contrast, the insulin receptor is activated by low concentrations of insulin, but higher doses of IGFs are required for activation of the insulin receptor. No ligand has been found for the insulin receptor-related receptor as vet.<sup>[2,20,21]</sup> The beta-subunits of the receptors containing intracellular tyrosine kinase domains are responsible for transphosphorylation of the receptors. Phosphorylation of the IGF-1 receptor leads to interaction with a number of signaling molecules, phosphorylation of insulin-receptor substrates, activation of PI3-kinase (phosphatidyl-inositol-3) and MAP kinase.<sup>[1,2]</sup>

# Monoallelic loss of chromosome 15, ring chromosomes and deletions of the distal arm of chromosome 15

Monoallelic loss of chromosome 15q, and loss of one copy of the IGF-1 receptor gene again because of deletions of the distal long arm of chromosome 15 have been observed in patients with intrauterine growth retardation and postnatal growth deficit.<sup>[22,23]</sup> Binding of IGF-1 to erythrocytes in short children with IUGR has shown to be lower than in children with normal height.<sup>[6]</sup> The number of IGF-1 receptor copies on human fibroblasts seems to be predictive of their proliferative response to IGF-1.<sup>[24]</sup> Fibroblasts from patients with monoallelic loss of the IGF-1 receptor gene show a reduced ligand binding. In a tall child with three copies of the IGF-1 receptor gene, however, accelerated growth has been ascribed to the overactivation of the receptor kinase resulting from the increased binding of the ligand.<sup>[25,26]</sup> It is concluded that hemizygosity for IGF-1R can cause primary IGF-1 resistance despite normal or even elevated GH and/or IGF-1 serum concentrations. Patients with loss of material from the distal arm of chromosome 15 show intrauterine growth retardation, postnatal growth deficits, occasional craniofacial and skeletal abnormalities, and mild to moderate mental retardation [27-31]

# Phenotype of a boy with a mutation of the human IGF-1 receptor gene

We reported a male infant who was born after an uneventful pregnancy at term.<sup>[10]</sup> At birth, the baby had symmetric growth retardation with a weight of 2000 g (-3.5 SDS), a length of 40 cm (-5.8 SDS), and

microcephaly (head circumference 31.0; -4.6 SDS). The mother's height was 148 cm (-2.6 SDS) and the father's height 178 cm (+0.1 SDS), the calculated target heigh for boys was 169.5 cm (-1.1 SDS). Postnatally, the infant failed to catch up growth and remained below the 3rd percentile for normal boys. The baby was referred to our unit at the age of 14 months with extreme short stature (-3.8 SDS). On clinical examination, we noticed microcephaly and a mild delay of motor development, and delay of speech development. Phenotype description at the age of 5 vears was as follows: receded frontal hair border, bushy eyebrows, broad nasal bridge, broad and rounded nasal tip, long and smooth philtrum, slit-like mouth, thin upper lip, broad, everted and fleshy lower lip, short fingers especially the thumbs, clinodactyly, wide set nipples, and pectus excavatum.<sup>[10]</sup> Followup radiographs revealed a retarded bone age of 1-1.5 vears. Most importantly, IGF-1 levels were elevated within a range of +1.1 to +2.3 SD, while IGFBP-3 levels initially were normal but dropped to negative SD-values at the age of 4.5 years.<sup>[32]</sup> Because of short stature and reduced growth rate we assessed stimulated (arginine, insulin, GHRH) as well as spontaneous nocturnal growth hormone secretion. Surprisingly, all GH stimulation tests that are thought to function via hypothalamic stimulation as well as spontaneous nocturnal GH secretion gave the result of partial growth hormone deficiency, inspite of elevated IGF-1 levels. Only stimulation with GHRH resulted in normal GH secretion. Thus, hypophyseal function per se was normal but hypothalamic-hypophyseal communication appeared to be suppressed. Secretion of TSH, ACTH, prolactin, and LH/FSH was normal. The results of chromosome analysis, blood counts, electrolytes, liver and kidney function tests were also normal.

# Heterozygous mutation of the IGF-1 receptor gene in one family

The patient described above, his mother and brother were found to be heterozygous for the point mutation CGA $\rightarrow$ TGA (Arg59stop) in exon 2 of the IGF-1 receptor gene. Sequencing of all exons encoding the IGF-1 receptor showed no additional mutation, thus a compound heterozygous mutation on both alleles was excluded. Autosomal dominant inheritance was suggested and the grandfather (I/1) presenting short stature would be a candidate to carry the mutation, unfortunately he was lost to follow-up. The mutation creates a novel DdeI site, resulting in two additional fragments of 97 and 155 bp to the 252 bp PCR product.<sup>[10]</sup> Equal amounts of mutant and normal allele of cDNA were seen in the patient and his mother, indicating that both alleles are expressed in peripheral cells.

# Expression of IGF-1 receptor protein in skin fibroblasts

Skin fibroblasts of our patient and of 3 control cell lines were analysed for expression levels of IGF-1 receptor protein.<sup>[10]</sup> The cells that were specifically labelled with a PE-stained, monoclonal anti human IGF-1 receptor antibody were quantified by flow cytometric analysis. Compared to skin fibroblasts from control donors, fibroblasts from our patient were stained significantly less in number of specifically labelled cells and with less median intensity. We conclude that less IGF-1 receptor protein was present on fibroblasts of our patient. Despite this fact, we failed to see differences in IGF stimulated thymidine uptake or proliferation (WST-1 assay, cell counts) between these cell lines.

## In vitro aspects of Arg59Ter IGF-1R mutation

We found reduced IGF-1R protein expression, reduced IGF-1 dependent IGF-1R phosphorylation, and reduced phosphorylation of Akt, a downstream target of IGF-1R signaling in Arg59Ter fibroblasts (*Raile et al in press*). Therefore, phosphorylated Akt reflects fewer phosphorylated IGF-1R on the cell surface of Arg59Ter fibroblasts, suggesting a dose dependent effect of IGF-1R expression on IGF-1 signaling.

Surprisingly, we found enhanced insulin-dependent Akt phosphorylation and IR/IGF-1R autophosphorylation in IGF-1R depleted Arg59Ter fibroblasts compared to controls. Total expression of IR protein was not changed but IGF-1R protein was decreased as expected. This would reflect in vitro resistance to IGF-1 but increased sensitivity to insulin in Arg59Ter fibroblasts (Raile et al in press). Recent studies in fibroblasts from IGF-1R- knockout mice (R-) that overexpress IGF-1R, IR-isoform A, and IR-isoform B alone or in combination gave evidence that IR and IGF-1R homodimers bind their respective ligand with a high affinity. Hybrid receptors predominantly bind IGF-1, with slight differences in binding characteristics whether IR-A or IR-B forms these hybrid receptors.<sup>[33,34]</sup> Entingh-Pearsall et al investigated IGF-1 and insulinsignaling in selective IGF-1R knockout (IGF-1Rko) adipocytes that were transfected to overexpress both IGF-1R and IR.<sup>[35]</sup> IGF-1 was able to induce a weak phosphorylation of IR, whereas insulin could not activate IGF-1R. They suggested that IR/IGF-1R hybrids mediate this transactivation of IR by IGF-1.<sup>[35]</sup> In our studies, we found lower IR/IGF-1R hybrids in

| Mutation  | Frequency<br>(Cohort)                      | Serum IGF-1<br>(ng/ml) | Growth retardation                     | CNS-abnormalities   | References                 |
|---|--|------------------------|--|---|----------------------------|
| IGF-1R<br>IGF-1R  | 1/11                                       | 121-222                | prenat                                 | Microcephaly  | [8]                        |
| Arg59Ter<br>(heterozygous)                              | (IGF-1 resistence)<br>mother/two half sons | 355                    | postnat.                               | Delay in speech, social interaction, motor skills         | [0]                        |
| IGF-1R<br>Arg108Gln<br>Lys115Asn(compound heterozygous) | 1/42<br>(IUGR)                             | 63<br>1130<br>1770     | prenat.<br>postnat.                    | Microcephaly<br>Abnormal speech                           | [8]                        |
| IGF-1R<br>Arg709Gln<br>(heterozygous)                   | 1/24<br>(IUGR)<br>mother/daughter          | 208                    | prenat.<br>postnat.                    | Microcephaly<br>Low IQ (60)                               | [9]                        |
| IGF-1R<br>Glu1050Lys<br>(heterozygous)<br>IGF-1         | mother/daughter                            | 68                     | (prenat. mother)<br>(prenat. daughter) | Microcephaly,<br>Discrete developmental delay             | Van der Kamp,<br>Endo 2005 |
| IGF-1<br>D12S346<br>(homozygous)                        | Single case                                | Not detectable         | prenat.<br>postnat.                    | Developmental delay,<br>microcephaly,<br>central deafness | [33]                       |
| IGF-1<br>Polyadenylation Signal,T-A                     | Single case                                | 1                      | prenat.<br>postnat.                    | Developmental delay,<br>microcephaly,<br>central deafness | [31]                       |
| IGF-1<br>Val44Met<br>(Homozygous/heterozygous)          | Family study                               | Homozygous:<br>normal  | prenat.<br>postnat.                    | Developmental delay,<br>microcephaly,<br>central deafness | [32]                       |

Table. Overview of known IGF-1R mutations and comparison of IGF-1 mutations and their respective phenotype in terms of growth, CNS-abnormalities and IGF-1 levels

Arg59Ter fibroblasts than in control cell lines. Some investigators have reported increased IGF-1R/IR hybrid formation in muscle extracts of obese subjects with insulin resistance and hyperinsulinemia. A higher level of hybrid receptor versus IR homodimers was claimed to contribute to insulin resistance in obesity. High glucose concentration was found to up-regulate muscle hybrid receptors and this mechanism was thought to be of clinical relevance in insulin resistant and diabetic patients.<sup>[10,36,37]</sup> In contrast to these studies in insulin resistant patients, subjects with partial monosomy 15q26.1-->qter syndrome or one reported case with decreased IGF-1 surface binding were reported to have recurrent hypoglycemia.<sup>[5,25]</sup> This clinical finding would be in analogy with increased insulin sensitivity in IGF-1R depleted Arg59Ter fibroblasts, but we did not find hypoglycemia in our family and had normal insulin sensitivity in our index patient.

#### Phenotypic aspects of IGF-1R haploinsufficiency

A clinical syndrome of IGF-1 resistance (OMIM #270450) has been postulated in terms of end-organ insensitivity to IGF-1. Reported cases who were small at birth grew slow postnatally with normal or increased levels of GH and IGF-1.<sup>[38]</sup> IGF-1 resistance because of

reduced IGF-1R expression was postulated to explain the short stature phenotype of African eve Pygmies. No mutations within the IGF-1 receptor gene have been found so far but data were obtained from immortalized hybridoma cells, not from native tissues.<sup>[39]</sup>

IGF-1 resistance owing to reduced IGF-1 receptor number has been proposed to explain the growth restricted phenotype of patients with loss of the distal long arm of chromosome 15q26 resulting in haploinsufficiency for the IGF-1R gene.<sup>[22-25,40,41]</sup> Common phenotypic features of partial monosomy 15q26.1-->qter syndrome were intrauterine growth retardation, postnatal growth failure, microcephaly, facial abnormalities, including high arched palate, abnormal ears, hypertelorism, and skeletal abnormalities like clinodactyly, club feet and scoliosis. Some clinical abnormalities overlap with Russel-Silver syndrome, but as one fundamental difference, most patients with deletion of 15g ter have more or less delayed motor development and impaired psychosocial skills.<sup>[25,30]</sup> In vitro, fibroblasts from patients with partial trisomy 15g showed increased but those with monosomy 15q ter decreased IGF-1 ligand binding and IGF-1R tyrosine kinase activation.<sup>[25,42]</sup> These data suggest a gene dosage effect of the human IGF-1R gene and the clinical features of growth abnormalities

have been supported by *in vitro* studies. Up to the present, these cases of an euploidy of the IGF-1R gene are difficult to discriminate specific IGF-1R-dependent phenotype from other gene deletions, which might contribute to a more heterogenous phenotype.

# Common phenotype of known human IGF-1R and IGF-1 mutations

To the present, four different human IGF-1R mutations have been reported; each seems to highlight distinct aspects of IGF-1R function. First, the mutation reported by us results in inactivation of one IGF-1R allele and in lower mRNA and protein expression of intact IGF-1R and represents a dose reduction of IGF-1R.<sup>[10]</sup> Second, the compound heterozygous IGF-1R mutations Arg108Gln/Lys115Asn that changes both the binding characteristics of the IGF-1 binding pocket within the  $\alpha$ -subunit and leads to a reduced IGF-1 binding affinity and receptor phosphorylation.<sup>[10]</sup> Third, the recently reported heterozygous mutation Arg709Gln changes the highly conserved cleavage site<sup>[43]</sup> and finally a new heterozygous mutation Glu1050Lys resides within the tyrosine kinase domain and leads to reduced autophosphorylation of IGF-1R and reduced postreceptor signaling [Van der Kamp et al., 2005 Abstract Endocrine Society Annual Meeting]. Thus mutations of key regions including ligand binding pocket, cleavage site and tyrosine kinase domain and corresponding phenotypes have been reported. In all known mutations, common clinical sequelae are intrauterine growth retardation and postnatal lack of catch up growth. The degree of psychomotor delay and microcephaly is more variable, but some developmental abnormalities have been observed in all individuals.

The phenotype of IGF-1 deficient patients with almost no detectable IGF-1 levels is more prominent in mental retardation, microcephaly and growth deficit before and after birth.<sup>[17,44,45]</sup> Moreover, severe insulin resistance is present if IGF-1 function is absent and insulin sensitivity is increased under treatment with rhIGF-1.<sup>[18]</sup>

Increased or normal IGF-1 serum levels have been detected in all individuals with IGF-1R mutations. At least, IGF-1 levels are inadequately normal for their reduced growth rate. The highest IGF-1 levels have been seen in a girl with heterozygous Arg108Gln/Lys115Arg mutation, resulting in a much lower IGF-1 binding affinity of the ligand binding pocket of the mutant IGF-1R protein.<sup>[10]</sup> In our study, IGF-1 levels were elevated in the older and normal in the younger brother. After growth hormone treatment, one of the patients showed high IGF-1 serum levels, even higher than those

observed in SGA patients treated with comparable doses of GH.<sup>[46]</sup> Possible explanations for increased IGF-1 levels are: (1) less IGF-1 dependent suppression of GH secretion by dysfunctional IGF-1 receptor protein, relative GH hypersecretion and high endogenous IGF-1 generation, and (2) clearance of serum IGF-1 by binding to its high affinity IGF-1 receptor, internalization of ligand receptor complexes, and finally intracellular degradation of IGF-1. Fewer surface receptor protein or less binding affinity of mutant IGF-1R protein might further increase IGF-1 serum levels.

### Growth hormone treatment in IGF-1 resistance

The rationale behind growth hormone treatment in patients with IGF-1R haploinsufficiency could be as follows: first, using direct growth promoting effects of GH via its own receptor, and second, increasing circulating and autocrine/paracrine IGF-1 levels to overcome IGF-1 resistance.<sup>[47]</sup>

Growth hormone treatment in cohorts of SGA children without known monogenetic cause of growth retardation has been reported widely. The reported height gain in a cohort of SGA children treated with rhGH (33  $\mu$ g • kg<sup>-1</sup> • d<sup>-1</sup>) improved from a baseline height at start from -3.0 to -2.0 SDS approximately +1.8 SD within the first three years of GH treatment, whereas our boy grew within the reported range and improved from -2.5 to -1.5 SD within the first 2 years.<sup>[46-48]</sup> IGF-1 levels achieved during GH therapy were higher (+1.8 -3.0 SD) than the reported after GH treatment at 33  $\mu$ g • kg<sup>-1</sup> • d<sup>-1</sup>.<sup>[46]</sup>

### Discussion

Insulin-like growth factors are involved in classical endocrine circuits, like growth hormone dependent longitudinal growth during childhood and adolescence, or paracrine and autocrine functions, affecting embryonal proliferation, differentiation, and regression of various organs and tissues.<sup>[3]</sup> The IGF-1 receptor mediates most effects of both IGF-1 and -2 on proliferation and inhibition of apoptosis.<sup>[2]</sup> Recently, our group together with Dr. Chernausek's group in Cincinnati, USA, has reported the first cases of perinatal growth retardation and postnatal lack of catch up growth despite sufficient IGF-1 levels. In addition, these growth abnormalities are accompanied by a distinct phenotype with microcephaly.<sup>[49]</sup>

Because of growth deficiency at high-normal IGF-1 levels, we suspected previously a mutation within the IGF-1 signaling cascade, which led us to detect a heterozygous mutation of the IGF-1 receptor, resulting in a stop codon of one allele and to reduce the number of IGF-1 receptor proteins on skin fibroblasts. Interestingly, we found an attenuated response of growth hormone secretion determined spontaneously during night and after insulin hypoglycemia and arginine stimulation.<sup>[16]</sup> In contrast, direct stimulation of the pituitary gland by GHRH resulted in a normal peak secretion of GH. These findings might indicate inhibitory feedback mechanisms of IGF-1 on GH secretion at the hypothalamic and/or pituitary level. In men, continuous infusion of IGF-1 decreased peak levels of GH secretion either spontaneously or after stimulation. We speculate that this mutation could lead to IGF-1 insensitivity despite high IGF-1 levels on (peripheral) target organs for longitudinal growth, and that inhibition of GH secretion might still be functional at the hypothalamic and pituitary level, resulting in decreased GH secretion and decreased IGFBP-3 synthesis.

These clinical and hormonal abnormalities are associated with a heterozygous mutation leading to a deletion of one IGF-1 receptor allele. Therefore our index case represents the human phenotype of a heterozygous IGF-1 receptor knockout. We found that the mutation is expressed at the mRNA level and that skin fibroblasts carrying this mutation express less IGF-1 receptor protein. In mice, a total knockout of both alleles of the IGF-1R leads to death perinatally<sup>[7]</sup> but inducible heterozygous IGF-1R knockout leads to reduced growth postnatally, which is also related to the degree of IGF-1 receptor reduction.<sup>[8]</sup> Furthermore, different combinations of mutant receptor alleles had dose-dependent effects on the number of IGF-1 receptors on the cell surface, suggesting that a deficiency of IGF-1R does not induce allelic regulation.<sup>[9]</sup> In our case, we identified the same mutation in the patient's mother and his newborn brother, suggesting autosomal dominant inheritance. So far, all family members with the IGF-1 receptor mutation presented reduced perinatal growth and beyond this, a lack of postnatal catch up growth with mild impairment of final or actual height. The finding of a heterozygous effect of the IGF-1 receptor gene mutation would indicate that this genotype could be prevalent in children with intrauterine growth retardation and lack of postnatal catch up growth.<sup>[6]</sup> This suggests heterozygous mutations of the GH receptor gene or the IGF-1 gene.<sup>[17,19]</sup>

For the human IGF-1 receptor, a gene dosage effect on somatic growth was suggested from patients with partial monosomy 15q26Ter, resulting in haploinsufficiency for their IGF-1R gene. Common clinical findings of monosomy 15q26Ter syndrome included mental retardation, microcephaly and growth retardation before and after birth. Furthermore, the mouse phenotype of a variable IGF-1R knockout suggested that there is a sex specific and organ specific impact of IGF-1R depletion on organ and body size.<sup>[8,9]</sup> We report two brothers and their mother, who carried the Arg59Ter mutation on one IGF-1R allele, resulting in early termination of IGF-1R protein transcription and approximately 50% reduction of IGF-1R protein on peripheral tissues, as demonstrated by skin fibroblasts and leukocytes.<sup>[10]</sup> Clinical findings in members of this family with the heterozygous IGF-1R mutation and *in vitro* studies using Arg59Ter skin fibroblasts, provide evidence for a gene dosage effect of the human IGF-1R (*Raile et al in press*).

In clinical follow up, both half brothers showed a common phenotype with pre- and postnatal growth retardation, primary microcephaly, retarded psychomotor development, minor dysmorphisms and normal or elevated IGF-1 serum levels. *In vitro*, we found reduced IGF-1R expression in skin fibroblasts carrying the [Arg59Ter] mutation, compared to fibroblasts from age and gender matched controls. Furthermore, both Arg59Ter mutant and wildtype IGF-1R alleles were expressed equally at the mRNA level.<sup>[10]</sup> Therefore, the human phenotype found in the family is related to that of heterozygous IGF-1R knockout mice, as reported by Holzenberger.<sup>[8,9]</sup>

### Conclusions

A gene dosage effect is hypothesized for the IGF-1R on the basis of partial monosomy 15q-ter syndrome, with loss of one IGF-1R copy and from IGF-1R gene deleted mice. We report clinical and *in vitro* functional aspects on example of a heterozygous inactivation of the human IGF-1R by the Arg59Ter IGF-1R mutation. *In vivo*, clinical findings include prenatal and postnatal growth retardation, microcephaly and mental retardation. *In vitro*, reduced IGF-1R protein expression is observed on the cell surface and in total cell lysates. Preliminary *in vitro* data suggest that signaling of IGF-1 and insulin are strongly interwoven at the receptor level for example by way of receptor hybrid formation and that alterations of each ligand/receptor system might have a strong impact on signal transduction of both IGF-1 and insulin.<sup>[50-53]</sup>

### Acknowledgements

We wish to thank Ursula Banning, Jutta Ullmann, and Antje Berthold, for their technical support, and to Dr. S. Chernausek and Dr. R. Smith for sharing ideas, data and a basic interest in science. **Funding:** This study was supported by grants from the German Endocrine Society (to A.S.), the German Diabetes Society (to K. R., B.S. and A. G.) and IZKF, Leipzig, Germany (projects B11, B15 and B18 to W.K.) through BMBF, Bonn, Germany.

**Ethical approval:** The study was approved by the ethical committee of the Medical Faculty of the University of Leipzig, Germany.

#### Competing interest: None.

**Contributors:** WK and SA proposed and designed the study, KR and WK wrote the first draft of the paper, all other authors contributed with lab experiments, patient recruitment and scientific discussions as well as in revising the manuscript.

### References

- 1 Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, et al. Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. EMBO J 1986;5:2503-2512.
- 2 Rosenfeld RG. Insulin-like growth factors and the basis of growth. N Engl J Med 2003;349:2184-2186.
- 3 LeRoith D, Werner H, Breitner-Johnson D, Roberts CT Jr. Molecular and cellular aspects of the IGF-1 receptor. Endocrine Rev 1995;16:143-163.
- 4 Butler AA, LeRoith D. Minireview: tissue-specific versus generalized gene targeting of the IGF-1 and IGF-1R genes and their roles in insulin-like growth factor physiology. Endocrinology 2001;142:1685-1688.
- 5 Bierich JR, Moeller H, Ranke MB, Rosenfeld RG. Pseudopituitary dwarfism due to resistance to somatomedin: a new syndrome. Eur J Pediatr 1986;142:186-188.
- 6 Ducos B, Cabrol S, Houang M, Perin L, Holzenberger M, Le Bouc Y. 2001 IGF type 1 receptor ligand binding characteristics are altered in a subgroup of children with intrauterine growth retardation. J Clin Endocrinol Metab 2001;86:5516-5524.
- 7 Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. Mice carrying null mutations of the genes encoding insulinlike growth factor (IGF-1) and type 1 IGF receptor (IGF-1R). Cell 1993;87:59-72.
- 8 Holzenberger M, Leneuve P, Hamard G, Ducos B, Perin L, Binoux M, et al. A targeted partial invalidation of the insulinlike growth factor I receptor gene in mice causes a postnatal growth deficit. Endocrinology 2000;141:2557-2566.
- 9 Holzenberger M, Hamard G, Zaoui R, Leneuve P, Ducos B, Beccavin C, et al. Experimental IGF-1 receptor deficiency generates a sexually dimorphic pattern of organ-specific growth deficits in mice, affecting fat tissue in particular. Endocrinology 2001;142:4469-4478.
- 10 Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, et al. IGF-1 receptor mutations resulting in intrauterine and postnatal growth retardation. N Engl J Med 2003;349:2211-2222.
- 11 DeChiara TM, Efstratiadis A, Robertson EJ. A growthdeficiency phenotype in heterozygous mice carrying an IGF-2 gene disrupted by targeting. Nature 1990;345:78-80.
- 12 Powell-Braxton L, Hollingshead P, Warburton C, Dowd M, Pitts-Meek S, Dalton D, et al. IGF-1 is required for normal embryonic growth in mice. Genes Dev 1993;7(12B): 2609-2617.

- 13 de Zegher F, Hokken-Koelega A. Growth hormone therapy for children born small for gestational age: height gain is less dose dependent over the long term than over the short term. Pediatrics 2005;115:e458-462.
- 14 de Zegher F. Endocrinology of small-for-gestational-age children: recent advances. Horm Res 2004;62(Suppl 3): 141-142.
- 15 Fjellestad-Paulsen A, Simon D, Czernichow P. Short children born small for gestational age and treated with growth hormone for three years have an important catch-down five years after discontinuation of treatment. J Clin Endocrinol Metab 2004;89:1234-1239.
- 16 Giustina A, Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 1998;19:717-797.
- 17 Woods KA, Camacho-Hubner C, Savage MO, Clark AJ. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med 1996;335:1363-1367.
- 18 Woods KA, Camacho-Hubner C, Bergman RN, Barter D, Clark AJ, Savage MO. Effects of insulin-like growth factor I (IGF-1) therapy on body composition and insulin resistance in IGF-1 gene deletion. J Clin Endocrinol Metab 2000;85:1407-1411.
- 19 Goddard AD, Covello R, Luoh SM, Clackson T, Attie KM, Gesundheit N, et al. Mutations of the growth hormone receptor in children with idiopathic short stature. N Engl J Med 1995;333:1093-1098.
- 20 Klammt J, Garten A, Barnikol-Oettler A, Beck-Sickinger AG, Kiess W. Comparative analysis of the signaling capabilities of the insulin receptor-related receptor. Biochem Biophys Res Commun 2005;327:557-564.
- 21 Klammt J, Barnikol-Oettler A, Kiess W. Mutational analysis of the interaction between insulin receptor and IGF-1 recdeptor with c-CRK and CRK-L in a yeast two hybrid system. Biochem Biophys Res Commun 2004;325:183-190.
- 22 Siebler T, Lopaczynski W, Terry CL, Casella SJ, Munson P, De Leon DD, et al. Insulin-like growth factor I receptor expression and function in fibroblasts from two patients with deletion of the distal long arm of chromosome 15. J Clin Endocrinol Metab 1995;80:3447-3457.
- 23 Roback EW, Barakat AJ, Dev VG, Mbikay M, Chretien M, Butler MG. An infant with deletion of the distal long arm of chromosome 15 (q26.1-qter) and loss of insulin-like growth factor 1 receptor gene. Am J Med Genet 1991;38:74-79.
- 24 Schmidt A, Chakravarty A, Brommer E, Fenne BD, Siebler T, De Meyts P, et al. Growth failure in a child showing characteristics of Seckel syndrome: possible effects of IGF-1 and endogenous IGFBP-3. Clin Endocrinol 2002;57:293-299.
- 25 Okubo Y, Siddle K, Firth H, O'Rahilly S, Wilson LC, Willatt L, et al. Cell proliferation activities on skin fibroblasts from a short child with absence of one copy of the type 1 IGF receptor gene and a tall child with three copies of the IGF-1 receptor gene. J Clin Endocrinol Metab 2003;88:5981-5988.
- 26 Nagai T, Shimokawa O, Harada N, Sakazume S, Ohashi H, Matsumoto N, et al. Postnatal overgrowth by 15q-trisomy and intrauterine growth retardation by 15q-monosomy due to familial translocation t(13;15): dosage effect of IGF-1R? Am J Med Gen 2002;113:173-177.
- 27 De Lacerda L, Carvalho JA, Stannard B, Werner H, Boguszewski MC, Sandrini R, et al. *In vitro* and *in vivo* responses to short-term recombinant human IGF-1 in a

severly growth-retared girl with ring chromosome 15 and deletion of a single allele for the type 1 IGF receptor gene. Clin Endocrinol 1999;51:541-550.

- 28 Heath-Monning E, Wohltmann HJ, Mills-Dunlap B, Daughaday WH. Measurement of insulin-like growth factor I responsiveness of fibroblasts of children with short stature: identification of a patient with IGF-1 resistance. J Clin Endocrinol Metab 1987;64:501-507.
- 29 Rasmussen SK, Lautier C, Hansen L, Echwald SM, Hansen T, Ekstrom CT, et al. Studies of the variability of the genes encoding the insulin-like growth factor receptor and its ligand in relation to type 2 diabetes mellitus. J Clin Endocrinol Metab 2000;85:1606-1610.
- 30 Fukushima K, Komatsu H, Matsumoto M, Kobayashi H, Tsukimori K, Satoh S, et al. IGF-related proteins at birth in a case of antenatally diagnosed Silver-Russell syndrome. Pediatr Res 2002;51:323-327.
- 31 Tollefsen SE, Heath-Monning E, Cascieri MA, Bayne ML, Daughaday W. Endogenous insulin-like growth factor binding proteins cause IGF-1 resistance in cultured fibroblasts from a patient with short stature. J Clin Invest 1991;87:1241-1250.
- 32 Kratzsch J, Blum WF, Schenker E, Keller E. Regulation of growth hormone (GH), insulin-like growth factor (IGF)I, IGF binding proteins -1, -2, -3 and GH binding protein during progression of liver cirrhosis. Exp Clin Endocrinol Diabetes 1995;103:285-291.
- 33 Pandini G, Frasca F, Mineo R, Sciacca L, Vigneri R, Belfiore A. Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. J Biol Chem 2002; 277:39684-39695.
- 34 Spampinato D, Pandini G, Iuppa A, Trischitta V, Vigneri R, Frittitta L. Insulin/insulin-like growth factor I hybrid receptors overexpression is not an early defect in insulin-resistant subjects. J Clin Endocrinol Metab 2000;85:4219-4223.
- 35 Entingh-Pearsall A, Kahn CR. Differential roles of the insulin and insulin-like growth factor-I (IGF-1) receptors in response to insulin and IGF-1. J Biol Chem 2004;279:38016-38024.
- 36 Federici M, Porzio O, Lauro D, Borboni P, Giovannone B, Zucaro L, et al. Increased abundance of insulin/insulin-like growth factor-I hybrid receptors in skeletal muscle of obese subjects is correlated with *in vivo* insulin sensitivity. J Clin Endocrinol Metab 1998;83:2911-2915.
- 37 Federici M, Giaccari A, Hribal ML, Giovannone B, Lauro D, Morviducci L, et al. Evidence for glucose/hexosamine *in vivo* regulation of insulin/IGF-1 hybrid receptor assembly. Diabetes 1999;48:2277-2285.
- 38 Lanes R, Plotnick LP, Spencer EM, Daughaday WH, Kowarski AA. Dwarfism associated with normal serum growth hormone and increased bioassayable, receptorassayable, and immunoassayable somatomedin. J Clin Endocrinol Metab 1980;50:485-488.
- 39 Hattori Y, Vera JC, Rivas CI, Bersch N, Bailey RC, Geffner ME, et al. Decreased insulin-like growth factor I receptor expression and function in immortalized African Pygmy T cells. J Clin Endocrinol Metab 1996;81:2257-2263.
- 40 Tonnies H, Schulze I, Hennies H, Neumann LM, Keitzer R, Neitzel H. De novo terminal deletion of chromosome

15q26.1 characterised by comparative genomic hybridisation and FISH with locus specific probes. J Med Genet 2001; 38:617-621.

- 41 Tonnies H. Comments on "congenital Diaphragmatic Hernia: is 15q26.1-26.2 a candidate locus?" Am J Med Genet A 2004; 131:224.
- 42 Hammer E, Kutsche K, Haag F, Ullrich K, Sudbrak R, Willig RP, et al. Mono-allelic expression of the IGF-1 receptor does not affect IGF responses in human fibroblasts. Eur J Endocrinol 2004;151:521-529.
- 43 Kawashima Y, Kanzaki S, Yang F, Kinoshita T, Hanaki K, Nagaishi J, et al. Mutation at cleavage site of IGF receptor in a short stature child born with intrauterine growth retardation. J Clin Endocrinol Metab 2005;90:4679-4687.
- 44 Bonapace G, Concolino D, Formicola S, Strisciuglio P. A novel mutation in a patient with insulin-like growth factor 1 (IGF-1) deficiency. J Med Genet 2003;40:913-917.
- 45 Walenkamp MJ, Karperien M, Pereira AM, Hilhorst-Hofstee Y, van Doorn J, Chen JW, et al. Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. J Clin Endocrinol Metab 2005;90:2855-2864.
- 46 Arends NJ, Boonstra VH, Mulder PG, Odink RJ, Stokvis-Brantsma WH, Rongen-Westerlaken C, et al. GH treatment and its effect on bone mineral density, bone maturation and growth in short children born small for gestational age: 3-year results of a randomized, controlled GH trial. Clin Endocrinol (Oxf) 2003;59:779-787.
- 47 Arends NJ, Boonstra VH, Hokken-Koelega AC. Head circumference and body proportions before and during growth hormone treatment in short children who were born small for gestational age. Pediatrics 2004;114:683-690.
- 48 Edouard T, Trivin C, Lawson-Body E, Pinto G, Souberbielle JC, Brauner R. Extreme short stature after intrauterine growth retardation: factors associated with lack of catch-up growth. Horm Res 2004;61:33-40.
- 49 Kiess W, Kratzsch J, Keller E, Schneider A, Raile K, Klammt J, et al. Clinical examples of disturbed IGF signaling: mutations of the insulin-like growth factor I receptor (IGF-1R) gene. Rev Endocr Metab Dis 2005;6:183-187.
- 50 Bailyes EM, Nave BT, Soos MA, Orr SR, Hayward AC, Siddle K. Insulin receptor/IGF-1 receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting. Biochem J 1997;327(Pt 1):209-215.
- 51 Seely BL, Reichart DR, Takata Y, Yip C, Olefsky JM. A functional assessment of insulin/insulin-like growth factor-I hybrid receptors. Endocrinology 1995;136:1635-1641.
- 52 Soos MA, Whittaker J, Lammers R, Ullrich A, Siddle K. Receptors for insulin and insulin-like growth factor-I can form hybrid dimers. Characterization of hybrid receptors in transfected cells. Biochem J 1990;270:383-390.
- 53 Soos MA, Field CE, Siddle K. Purified hybrid insulin/insulinlike growth factor-I receptors bind insulin-like growth factor-1, but not insulin, with high affinity. Biochem J 1993; 290 (Pt 2):419-426.

Received September 8, 2005 Accepted after revision November 11, 2005