

SHORT REPORT

Intragenic deletions of the IGF1 receptor gene in five individuals with psychiatric phenotypes and developmental delay

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Haploinsufficiency of the gene encoding the insulin-like growth factor 1 receptor (*IGF1R*), either caused by telomeric 15q26 deletions, or by heterozygous point mutations in *IGF1R*, segregate with short stature and various other phenotypes, including microcephaly and dysmorphic facial features. Psychomotor retardation and behavioral anomalies have been seen in some cases. Here we report small, intragenic deletions of *IGF1R*, identified by chromosome microarray analysis in two unrelated families affected primarily with neuropsychiatric phenotypes including developmental delay, intellectual disability and aggressive/autoaggressive behaviors. The deletions are in frame, and both wild-type and mutant mRNAs are expressed as measured by quantitative real-time PCR. While short stature is considered a phenotypic hallmark of *IGF1R* haploinsufficiency, the present report suggests that in frame exon deletions of *IGF1R* present predominantly with cognitive and neuropsychiatric phenotypes. *European Journal of Human Genetics* (2013) 21, 1304–1307; doi:10.1038/ejhg.2013.42; published online 13 March 2013

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INTRODUCTION

The gene encoding the insulin-like growth factor 1 receptor (*IGF1R*), located on the long arm of chromosome 15, is involved in somatic development and glucose metabolism. Numerous telomeric deletion cases of 15q, resulting in the loss of *IGF1R* as well as rare cases of heterozygous missense and nonsense mutations of *IGF1R* have been reported. They were associated with various constellations of clinical findings, including growth deficit, microcephaly, developmental delay, mild facial dysmorphisms and skeletal deformations in affected individuals.^{1–11}

Usually intrauterine or postnatal growth deficits were moderate to severe in these patients. Notably, cognitive dysfunction of the reported individuals was either moderate, mild or absent.^{2–4} Significant psychiatric comorbidities beyond cognitive impairment have not been reported to date.

Although most cases represent large telomeric deletions, involving many genes, a recent report presented a three-generation Dutch family with a small (0.095 Mb) deletion, affecting only exons 11–21 of *IGF1R* and one nearby gene. Seven family members carried the deletion, and all were affected with short stature (–2.5 to –4.4 SD below the mean). All affected individuals showed average to above-average scores on IQ testing. Clinical neurodevelopmental deficits, namely mild speech and motor delay during early childhood, were detected in the index patient only.¹⁰

Here, we report five individuals with intragenic exon deletions of *IGF1R* in two unrelated families, segregating predominantly with cognitive and neuropsychiatric phenotypes. None of the five individuals had short stature, defined as equal or greater than 2 SD

below the mean for age and gender. In both families, the microdeletions were inherited maternally.

MATERIALS AND METHODS

Human subjects

Both probands were referred to the Medical Genetics Laboratories, Baylor College of Medicine (BCM), Houston, TX, USA, for clinical array comparative genomic hybridization (aCGH) analysis. The study was approved by the Institutional Review Board of BCM, and all participants were recruited after informed consent was obtained.

Array comparative genomic hybridization

Patient DNA was analyzed using the Baylor College of Medicine clinical genomic microarray (version 8.2 in individual I-1, version 8.1 in individual II-1), described in Boone *et al.*¹² Given the small deletion size, microarray findings were confirmed by polymerase chain reaction (Supplementary Figure S1) in individual I-1.

All genomic coordinates refer to the February 2009 assembly of the reference genome (GRCh37/hg19). Exon numbering is based on RefSeq transcript NM_000875.3.

Quantitative real-time PCR

RNA was extracted from fresh, whole blood using the PAXgene Blood RNA Kit (PreAnalytiX, Hombrechtikon, Switzerland). RNA was treated with DNase using DNA-free kit (Ambion, Grand Island, NY, USA), and reverse-transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Grand Island, NY, USA). Real-time PCR amplification of *IGF1R* wild-type copy (exons 2–3 boundary) was done using Power SYBR Green Kit (Applied Biosystems) and primers IGF1R_F2 (5′-CGATGTGTGAGAAGACCACC-3′,

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mapping within exon 2) and *IGF1R_R* (5'-TGGCAGCACTCATTGTTCTC-3', mapping within exon 3 deleted in all patients; qPrimerDepot at <http://primerdepot.nci.nih.gov/>). Primers to amplify internal control *GAPDH* gene were *GAPDH_F1* (5'-AAGGTGAAGGTCGGAGTCAA-3') and *GAPDH_R1* (5'-AATGAAGGGGTCATTGATGG-3'; qPrimerDepot). Quantification of combined expression of mutant and wt alleles was done using TagMan assays specific for *IGF1R* exons 10–11 boundary and *GAPDH* as a control (Applied Biosystems). The relative expression of mutant allele of *IGF1R* was deduced by subtracting wild-type allele expression from combined mutant and wild-type allele expression. qPCR conditions included 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All experiments were done in triplicate.

RESULTS

Clinical cases

From June 2009 through November 2011, 15 632 individuals were referred to the Medical Genetics Laboratory of Baylor College of Medicine for clinical chromosome microarray testing. Among these, 10 probands were found to have heterozygous deletions encompassing sequence of the *IGF1R* gene (0.064% of all cases). In 7 of the 10 cases, the entire *IGF1R* gene was deleted, similar to previously described cases of *IGF1R* haploinsufficiency.^{6–9,11} One case deleted only the last exon of *IGF1R* and three exons of a neighboring gene, *PGPEP1L*. Two cases appeared particularly interesting, as they harbored intragenic deletions of one and three *IGF1R* exons, respectively. These two were subsequently studied in more detail, and clinical phenotypic information was obtained.

Case 1. Individual I-1, a 7-year-old boy of Indian-European descent, presented to the pediatric genetics clinic for evaluation of global developmental delay and abnormal behaviors. Psychiatric symptoms included hyperactivity, extreme temper and mood, aggressive and autoaggressive behavior. He had had two previous hospitalizations to psychiatric inpatient units for violent behaviors. At the time of his visit, he was on risperidone, guanfacine and dextroamphetamine/amphetamine. He had previously been evaluated by a developmental pediatrician, and developmental quotients were between 40 (for problem-solving) and 50 (for language and gross motor skills).

Body height was in the lower normal range on the 10th percentile. The remainder of the physical examination revealed borderline microcephaly (on the second percentile for age and gender) and subtle dysmorphic facial features, including mild synophrys, mild epicanthal folds and widely spaced teeth.

His mother, individual I-2, was a 31-year-old female with a history of bipolar and anxiety disorder, diagnosed at the age of 11 years, with one suicide attempt at age 26 years. She graduated from high school and never required special education. Information on early development was not available for this individual.

Case 2. Patient II-1, a 21-month-old boy, presented to the clinical geneticist for evaluation of abnormal behavior, speech delay, microcephaly and poor weight gain, as well as a family history of learning and growth problems. Body height was in the lower normal range (on the ninth percentile; Table 1).

The proband's brother (II-2) had global developmental delay, autoaggressive and aggressive/oppositional behavior toward others. Their mother (II-3) had a history of autoaggressive behaviors as a child, and cognitive delays. Body stature was low in the brother (3rd percentile), but within the normal range in the mother (30th percentile). No dysmorphic facial features or skeletal anomalies were present in individuals II-1, II-2 and II-3. At the time of referral to the clinical geneticist, none of the family members had been seen by a psychiatrist.

Molecular analysis

In proband I-1, aCGH revealed a heterozygous deletion of 2 kb (minimum interval) to 16 kb (maximum interval) in size (Figures 1a and c), deleting the entire exon 3 of *IGF1R* on chromosome 15q26.3 (Figure 1c). The same microdeletion was found in the proband's mother (I-2). In individual I-1, laboratory testing revealed IGF-1 and IGF-binding protein (IGF-BP) levels in serum within the normal range (IGF-1: 100 ng/ml; IGF-BP: 4.3 mg/l).

In proband II-1, aCGH revealed a heterozygous intragenic loss of 40 kb (minimum interval) to 58 kb (maximum interval) in size,

Table 1 Clinical and molecular characteristics of five individuals with *IGF1R* exon deletions

	I-1 (Proband)	I-2 (Mother)	II-1 (Proband)	II-2 (Brother)	II-3 (Mother)
Age at diagnosis	7 years	31 years	21 months	4 years	26 years
Sex	Male	Female	Male	Male	Female
Ethnicity	Indian-European	Indian-European	European	European	European
Cognitive function	Moderate ID	Normal	Global developmental delay	Global developmental delay	Impaired. No formal testing.
Body height	10th percentile (118.2 cm) Z-score: -1.3	5th percentile (152.5 cm) Z-score: -1.7	9th percentile (82.5 cm) Z-score: -1.3	3rd percentile (95 cm) Z-score: -1.9	30th percentile (160 cm) Z-score: -0.5
Psychiatric phenotype	Hyperactivity, aggressive and autoaggressive behavior	Bipolar and anxiety disorders	Autoaggressive behavior	Aggressive and autoaggressive behavior	Autoaggressive behavior during childhood
Molecular phenotype	<i>IGF1R</i> microdeletion, exon 3, 2 kb	<i>IGF1R</i> microdeletion, exon 3, 2 kb	<i>IGF1R</i> microdeletion, exons 3–5, 40 kb	<i>IGF1R</i> microdeletion, exons 3–5, 40 kb	<i>IGF1R</i> microdeletion, exons 3–5, 40 kb
Minimal interval	Chr15:99 434 081–99 435 876	Chr15:99 434 081–99 435 876	Chr15:99 403 439–99 443 615	Chr15:99 403 439–99 443 615	Chr15:99 403 439–99 443 615
Maximal interval	Chr15:99 424 326–99 439 855	Chr15:99 424 326–99 439 855	Chr15:99 393 779–99 451 587	Chr15:99 393 779–99 451 587	Chr15:99 393 779–99 451 587
Inheritance	Mat	Unknown	Mat	Mat	Unknown
IGF1 level	Normal	Unknown	Normal	Unknown	Unknown
IGF-BP	Normal	Unknown	Normal	Unknown	Unknown
Other chromosomal abnormalities	None	None	None	None	Dup 21q22.3. (18 kb)

Abbreviations: ID, intellectual disability; IGF1, insulin-like growth factor 1; IGF-BP, insulin-like growth factor-binding protein; Mat, maternal.

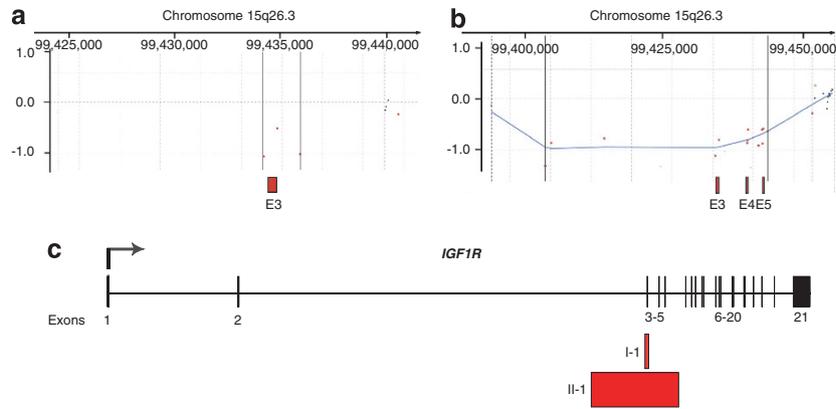


Figure 1 Intragenic deletions in *IGF1R* identified by exon-targeted aCGH. (a, b) Microarray plots of index patient I-1 (a) and II-1 (b). Deleted exons represented as red boxes. (c) Schematic representation of intragenic deletions of *IGF1R* in probands I-1 and II-1 affecting exons 3 and 3–5, respectively. Minimal intervals displayed (2 kb and 40 kb). Plotting is based on UCSC Genome Build hg19 and the minimum interval detected to be deleted by aCGH.

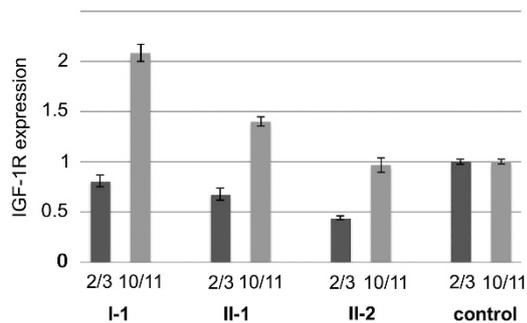


Figure 2 Quantitative real-time PCR analysis of *IGF1R* mRNA in peripheral blood. '2/3' Indicates the abundance of wild-type *IGF1R* mRNA, amplified by using primers flanking the exon 2/exon 3 boundary. '10/11' Indicates the abundance of total (wild-type and mutant) *IGF1R* mRNA, using primers flanking the exon 10/exon 11 boundary. The abundance of mutant *IGF1R* mRNA can be deduced by subtracting the wild-type (2/3) expression from the total (10/11) expression. Note the inter- and intra-familial variability of *IGF1R* mRNA expression. All expression levels are normalized to *GAPDH* and to a normal, age-matched control. All reactions were carried out in triplicate; error bars indicate SD.

equivalent to exons 3–5 of *IGF1R* (Figures 1b and c). The same microdeletion was found to be present in the proband's mother (II-3) and in one brother (II-2). The mother also carried a microduplication in 21q22.3 (minimal interval: chr22:47 848 308-47 866 257) containing one RefSeq gene, *PCNT*, biallelic loss-of-function mutations of which cause microcephalic osteodysplastic primordial dwarfism type II and Seckel syndrome. Duplications of *PCNT* have not been associated with human disease.

No other aCGH abnormalities were found in II-1 or II-2. In II-1 IGF-1 and IGF-BP plasma levels were within normal range (121 ng/ml; IGF-BP: 3.2 mg/l).

Quantitative real-time PCR analysis was conducted to determine the expression and stability of both wild-type and mutant alleles of *IGF1R* (Figure 2). While primers and probes flanking the exons 10–11 boundary of *IGF1R* were used to determine the total abundance of (wild-type and mutant) *IGF1R* mRNA, those flanking the exons 2–3 boundary detect wild-type *IGF1R* only, given that exon 3 is deleted in all patients included in this report. Expression levels were normalized to *GAPDH* and to a normal, age-matched control. The abundance of mutant *IGF1R* mRNA can be deduced by subtracting wild-type allele expression (exons 2–3 product) from total expression (exons 10–11 product). In all individuals tested, total expression (as determined by

the exons 10–11 product) was higher than wild-type expression (as determined by the exons 2–3 product), suggesting that both the wild-type and mutant alleles are stably expressed on an mRNA level, at least in peripheral blood. The ratio of mutant allele expression to wild-type allele expression was 1:1.58 (individual I-1), 1:1.07 (individual II-1) and 1:1.21 (individual II-2), respectively.

DISCUSSION

The IGF-1 receptor contributes to somatic growth and brain development by mediating cell proliferation, survival and differentiation.¹³ The IGF1 receptor consists of two extracellular alpha-subunits that provide a binding site for IGFs and two transmembrane beta-subunits associated with a tyrosine-kinase-dependent second messenger system. In the central nervous system, the subsequent signaling cascade mediates a neuroprotective effect by inhibiting apoptosis.^{14–16}

Previously reported cases of terminal 15q deletions entailing *IGF1R* haploinsufficiency had heterogeneous phenotypes, including intrauterine and postnatal growth delay, microcephaly, dysmorphic facial features, skeletal anomalies and speech and motor delay. Missense and nonsense mutations in *IGF1R* segregated with short stature, mild dysmorphic facial features, mostly mild developmental delay and mild psychiatric features in one reported case.^{1–5}

Although the variable phenotype of previously reported patients with 15q26.3 deletions may be attributable, at least in part, to genes other than *IGF1R*, it is notable that the smallest *IGF1R* deletion reported to date segregated predominantly with short stature and no or mild neuropsychiatric disorders.¹⁰ In that family described by Veenma *et al*, seven family members carried a deletion of exons 11–21 of the *IGF1R* gene and what at the time of publication used to be a hypothetical protein-locus (LOC145814), and is now annotated as a RefSeq gene, *PGPEPIL*, encoding pyroglutamyl-peptidase-I-like. Body heights in the seven probands were 4.04, 2.72, 4.42, 3.01, 2.5, 4.0 and 3.09 (mean: 3.4) SD below the age-specific reference groups.¹⁷ Mild motor and speech delay and behavioral abnormalities were present in only one of the seven cases.

Our findings suggest a wider phenotypic spectrum of *IGF1R* mutations, including significant neuropsychiatric ailments. We describe two small intragenic *IGF1R* deletions, including exon 3 and exons 3–5, with maternal inheritance in two unrelated families. These cases of intragenic exon deletions of *IGF1R* appear to be rare (2 in 15 632 in our cohort of samples submitted for clinical, exon-targeted oligonucleotide chromosome microarray analysis).

No validated intragenic exon deletions of *IGF1R* were listed in the database of genomic structural variation (dbVar), neither in the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources at the time of submission of the manuscript.

Phenotypes in our five patients included global developmental delay or intelligence deficit (4/5), and autoaggressive (4/5) and aggressive (2/5) behaviors during childhood. In addition, in one of our cases bipolar and anxiety disorders with one suicide attempt were reported. Our cases exhibited a mean body height of -1.34 SD below the mean for age and gender, using the 2000 CDC growth charts for the United States as a reference, consistent with borderline low or even normal stature (Table 1).

The phenotypic spectrum of our cases extends beyond the phenotypes resulting from *IGF1R* point mutations described previously. These were located in exons 2, 7, 11 and 16, respectively.^{1–5} Short stature was present in all cases, mild motor and/or speech developmental delay in 4/9 cases and mild intellectual disability – with a Wechsler intelligence quotient of 60 – in one case of a patient with a missense mutation in exon 11. This missense mutation was detected in a girl whose mother, a carrier of the same mutation, did not show any neuropsychiatric abnormalities and had normal intelligence.² Mild psychiatric anomalies, namely anxious affect, obsessive tendencies, psychomotor agitation, were present in one case.¹ Other neuropsychiatric symptoms, specifically aggressive or autoaggressive behaviors, were not described in these patients.

Interestingly, both cases reported herein delete segments in frame, as exon 3 has 312 bp, and exons 3–5 a total of 606 bp, both of which are multiples of three. Quantitative real-time PCR studies show that mRNA from both wild-type and mutant alleles is detectable in peripheral blood, suggesting stability of both products. It is conceivable that both might still be translated into functional proteins. Unfortunately, IGF1R protein is not expressed at sufficient levels in lymphocytes to be detectable by western blot analysis (data not shown). However, given the patients' phenotypes, with absence of significant short stature or skeletal anomalies, one could speculate that the resulting, mutated proteins work reasonably well, stimulating somatic growth, but still cause cognitive and psychiatric phenotypes, based on IGF1R's role in the central nervous system.

Postnatal impact of *IGF1* knockout in mice is strongest in brain regions that exhibit a high degree of plasticity into adult life, for example, hippocampal granule cells, reflecting a long-lasting IGF-dependency of cells in these brain areas.^{18,19} Although IGF1 interactions with its receptor are complex and influenced by IGF-1-binding proteins, postnatal IGF1R dysfunction would also be expected to functionally impact these brain areas. During embryonic development, however, IGF1 mRNA is transiently expressed in many brain regions, including areas containing long projection neurons.^{18,20,21}

Although IGF1R dysfunction in hippocampal neurons might be associated with the predominance of learning and memory deficits seen in many patients with *IGF1R* mutations, one could hypothesize that disturbances of cortical long-projection neurons might account for some of the neuropsychiatric symptoms seen in our patients.¹⁸

However, it remains elusive how such differential impact on distinct neuronal subgroups might be caused by different deletions and mutations affecting the *IGF1R* gene.

Discovery of further clinical cases is warranted in order to establish clear phenotype–genotype causality of heterozygous *IGF1R* dysfunction.

CONFLICT OF INTEREST

Drs Schaaf, Stankiewicz and Cheung are faculty members of the Department of Molecular and Human Genetics at Baylor College of Medicine, which derives revenue from the chromosomal microarray analysis offered in the Medical Genetics Laboratory. The remaining authors declare no conflict of interest.

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