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SHORT REPORT

Partial deletion of *GLRB* and *GRIA2* in a patient with intellectual disability

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We report about the partial *de novo* loss of *GLRB* and *GRIA2* in an individual with intellectual disability (ID). No additional mutations were found in either gene. *GLRB* itself does not seem to be a good candidate as it causes autosomal recessive hyperekplexia and no symptoms were found in the patient. Mutations of *GRIA2* have not been described as cause of ID to date. Nonetheless, it is a very attractive candidate because it encodes a subunit of a glutamate receptor, which is highly expressed in postsynaptic structures and has an important role in signal transduction across synapses. Although we were able to isolate a fragment of a fusion transcript of both genes from the patient's blood, we were not able to isolate a transcript with an open reading frame throughout the entire length. The reading frame could be restored by differential splicing, which might take place in brain tissue but not in blood. We assume that either haploinsufficiency of *GRIA2* or a *GLRB*/*GRIA2* fusion gene leading to a protein with dominant-negative properties is causing the phenotype of the patient.
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INTRODUCTION

Glutamate receptors can be found in the dendritic part of synapses and aberrations in genes of glutamate receptor subunits have been identified as cause of intellectual disability (ID), that is, *GRIN2A* and *GRIN2B*,[1] *GRIA3*[2,3] and *GRIK2*.[4] *GRIA2* encodes the GLUR2 subunit of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and is mainly expressed in brain.[5] GLUR2 controls important biophysical properties of AMPA receptors and is involved in learning, memory and synaptic plasticity.[6,7]

MATERIALS AND METHODS

See Supplement.

RESULTS

Clinical presentation of the patient

The 3-year-old boy presented with severe speech delay, gait abnormalities (tip toeing) and abnormal behavior (hyperactivity, attention deficit and aggressive behavior). The family history is unremarkable. No structural malformations could be identified. The body measurements at birth and at the age of 3 years were normal. Some minor anomalies were seen but with the strong resemblance to both unaffected parents (detailed clinical information and facial view in Figure 1 Supplementary Information).

Molecular karyotyping

Array-CGH revealed, beside frequently observed variations, an unknown deletion on the long arm of chromosome 4 with a size of 102 ± 9 kb (arr 4q32.1 (158 267 339 × 2, 158 272 983-158 375 402 × 1, 158 378 650 × 2)dn) (NCBI36/hg18).

Fluorescence *in-situ* hybridization (FISH)

FISH with fosmid G248P82551H7 (WI2-1059P14) (see Figure 1 for genomic position) confirmed the deletion in the patient. As the same probe gave regular signals on both chromosomes 4 of the parents (Figure 1), we conclude that the patient's deletion occurred *de novo*.

Sequencing

Sequencing of *GRIA2* uncovered two homozygous SNPs in the patient's DNA: rs4302506 and rs4475186. Both SNPs have a minor allele frequency (MAF) of $T = 0.362/456$ and are considered to be polymorphisms. Sequencing of *GLRB* uncovered three SNPs: rs12507409 was found in hemizygous state and results in a synonymous amino-acid exchange (MAF of $T = 0.03/38$); rs41280499 (MAF of $T = 0.452/568$) and rs3775724 (MAF of $T = 0.375/472$) were found in heterozygous state. No SNPs were found that might reveal the parental origin of the chromosome carrying the deletion.

Expression of *GLRB*, *GRIA2* and a fusion transcript in blood

Amplification of commercial cDNA samples derived from normal brain (Clontech, Mountain View, CA, USA) with exon-based, *GRIA2*-specific primers (Supplementary Table S1) resulted in several products of the *GRIA2* transcript (data not shown). None of these amplifications were successful when tested on blood derived cDNAs obtained from control persons. *GLRB* could also easily be amplified from normal brain cDNA. In contrast to *GRIA2*, we found *GLRB* expression in blood. To see if a fusion transcript is being produced we used a forward primer in exon 4 of *GLRB* (and a reverse primer in exon 6 of *GRIA2*. A specific product could be amplified from the patient's blood cDNA, but not from the parents' blood cDNA

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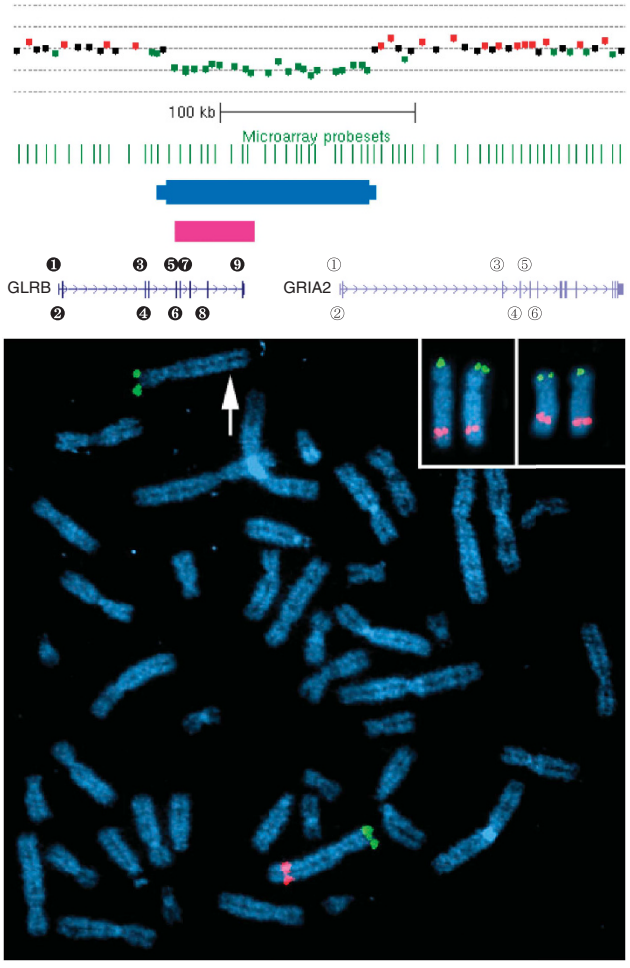

Figure 1 Partial deletion of *GLRB* and *GRIA2*, confirmed by array-CGH and FISH. Top: array-CGH data revealed an unknown deletion of 102 ± 9 kb, supported by 21 probes with significantly reduced signal intensity (green boxes on lower dashed line). Middle: UCSC-browser (hg19) diagram of the *GLRB/GRIA2* region (chr4:157 970 647–158 289 501). The deletion (blue box, with thinner parts representing the breakpoint regions) removes exons 5–9 of *GLRB* (exon numbers in black circles) and exons 1–2 of *GRIA2* (white circles). The pink box represents the genomic position of fosmid G248P82551H7, which was used in FISH. Bottom: absence of the fosmid-specific FISH-signal (pink) on one of the patient's chromosomes 4 (white arrow) validates the array-CGH data. As both chromosomes 4 (marked by a green chr4-specific subtelomeric probe) of each of the parents display the pink fosmid-signal (integrated boxes, upper right), the patient's deletion probably occurred *de novo*. The green vertical lines below the array data represent the genomic position of the corresponding probes on the Agilent SurePrint G3 Human CGH Microarray (Agilent, Santa Clara, CA, USA; 2 × 400 K, AMADID 021850).

(data not shown). Sequencing revealed a fusion transcript in which exon 4 of *GLRB* was spliced to exon 3 of *GRIA2*. The predicted result of this transcript fusion is a truncated *GLRB* protein (p.Met99fs) with an appendix of 20 amino acids of unique sequence (SAPSFREESMLFLDFMTRSL*).

DISCUSSION

Mutations in *GLRB* are associated with hyperekplexia.[8,9] This disorder has been described as an autosomal recessive disorder and haploinsufficiency was excluded as possible cause.[8,9] We assume that the partial deletion of *GLRB* itself does not have a major impact on the patient's ID, because no other mutations were found in this gene and no ID is described in patients with mutations in one or both alleles of *GLRB*.

We hypothesize that *GRIA2* is involved in the patient's ID. It was previously suggested from mouse models that *GRIA2* might contribute to ID in Coffin–Lowry-syndrome.[10] Tzschach *et al*[11] reported on a patient with a balanced translocation t(2;5)(p21;q12.1) and *de novo* loss of roughly 9 Mb on chromosome 4, including *GLRB* and *GRIA2*. The clinical presentations of this patient and our patient do not show specific overlapping abnormalities. However, it cannot be excluded that our patient might develop severe obesity in the second or third decade of life as the patient reported by Tzschach *et al*.[11] They suggest haploinsufficiency of one or several genes as likely cause and *GRIA2, GLRB, NPY1R* and *NPY5R* as candidate genes that might cause the phenotype. No overlapping deletions were listed in the DECIPHER database by the end of October 2011. The ECARUCA database contains 26 cases with overlapping deletions.[12] All but one are microscopically visible. One patient (ECARUCA ID 4689) with multiple malformations, short stature, microcephaly and ID is listed with a submicroscopic deletion with a size of 5.76 Mb containing more than 30 genes.

The Database of Genomic Variants describes four copy number changes that are found in *GRIA2* (variation no. 2551, 3520, 80534 and 92490), which are different from the one found in our patient. Each CNV was found in only one individual of the original 270 Hapmap samples or the Yoruban subgroup, respectively.[13,14] None of these CNVs was reproduced faithfully even within the same work and the same samples. Instead two different duplications, one deletion and one inversion affecting *GRIA2* were reported presumably for the same sample. As nothing is known about the individual the only conclusion that can be drawn is that CNVs in *GRIA2* are rare.

Hamdan *et al*[15] sequenced 197 genes that encode glutamate receptors in each of 95 sporadic cases of nonsymdromic ID. The supplementary information contains a homozygous mutation in *GRIA2* (Asp781His) that was inherited from both parents and is predicted to be 'probably pathogenic' by Polyphen2 (http://genetics.bwh.harvard.edu/pph2/). There was no confirmation of or information on this result upon request. It remains open if the parents are affected or if this data has been validated.

Four genes that encode subunits of glutamate receptors have been described as a cause of nonsyndromic ID: *GRIK2, GRIN2A, GRIN2B* and *GRIA3*.[1–4] Autosomal recessive (*GRIK2*), autosomal dominant (*GRIN2A, GRIN2B*) and X-linked (*GRIA3*) traits have been observed for these genes.

As we did not find a mutation in all exons and flanking intron areas of *GRIA2* we have no evidence for an autosomal recessive mechanism in our patient. So haploinsufficiency of *GRIA2* might be the underlying mechanism. The fusion transcript derived from *GLRB* and *GRIA2* and detected only in the blood of our patient results in a frame shift mutation with a premature stop codon. An open reading frame might arise from various splice forms, for example, from exon 3 of *GLRB* spliced to exon 3 or 4 of *GRIA2*. We can neither prove nor exclude that this might happen in the patient's brain. A potential dominant-negative effect of the derivative fusion protein could explain the pathogenicity of this deletion. To our knowledge there is only one reported case of a fusion gene that causes ID.[16]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)