

## DATA REPORT

A 12p13 *GRIN2B* deletion is associated with developmental delay and macrocephalyNaoya Morisada<sup>1,2</sup>, Tomoaki Ioroi<sup>3</sup>, Mariko Taniguchi-Ikeda<sup>2</sup>, Ming Juan Ye<sup>2</sup>, Nobuhiko Okamoto<sup>4</sup>, Toshiyuki Yamamoto<sup>5</sup> and Kazumoto Iijima<sup>2</sup>

*N*-methyl D-aspartate receptor subtype 2B (GluN2B), encoded by *GRIN2B*, is one of the components of the *N*-methyl D-aspartate receptor protein. Aberrations in *GRIN2B* have been reported to be responsible for various types of neurodevelopmental disorders. We report a Japanese boy with an ~2 Mb interstitial deletion in 12p13 involving the entire *GRIN2B* gene, who presented with intellectual disability, motor developmental delay and marked macrocephaly.

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The *N*-methyl D-aspartate receptor (NMDAR) is one of the ionotropic glutamate receptors involved in intermediate excitatory neurotransmission in the mammalian central nervous system.<sup>1,2</sup> NMDARs are associated with higher cerebral function or cellular apoptosis after ischemic brain disease. They consist of three subunits, GluN1, GluN2 and GluN3, and the GluN2 subunit is further classified into GluN2A, GluN2B, GluN2C and GluN2D. *GRIN2B* is located on 12p13.1 and encodes GluN2B. Variants in *GRIN2B* are associated with various psychiatric disorders, including schizophrenia,<sup>3</sup> Parkinson's disease<sup>4</sup> and bipolar disorder.<sup>5</sup> *GRIN2B* mutations are also associated with an autosomal dominant form of intellectual disability (MRD6, OMIM#613970) and early infantile epileptic encephalopathy (EIEE27, OMIM#616139),<sup>6</sup> but these are rare diseases involving partial or entire *GRIN2B* deletions.

Here we report the case of a boy with mild intellectual disability and macrocephaly without epilepsy who has an ~2 Mb interstitial deletion in 12p13, including the entire *GRIN2B*. The patient is a 6-year-old Japanese boy who is the first child of non-consanguineous parents. His gestational age was 40 weeks and 0 days. His birth weight was 4,112 g (+2.91 s.d.), his length was 52 cm (+1.48 s.d.), and his head circumference was 36 cm (+2.2 s.d.). His parents and younger sister are healthy. He was referred to our hospital at an age of 7 months because of a motor developmental delay and intellectual disability. At that time, he was unable to hold his head up. He could not sit alone at the age of 1 year. At 2 years and 0 months, his height was 90.9 cm (+1.8 s.d.), his weight was 13.28 kg (+1.4 s.d.; Figure 1a), and his head circumference was 54.0 cm (+4.8 s.d.; Figure 1b). He showed macrocephaly, but his face was not disease-specific, and he had no epileptic convulsions. Cranial magnetic resonance imaging revealed no significant abnormality. His total developmental quotient, as assessed by the Kyoto Scale of Psychological Development 2001, was 60 (at 3 years and 6 months), 65 (at 5 years of age), and 67 (at 6 years and 1 month).<sup>7</sup> He was also diagnosed with autism spectrum disorder (ASD). Standard G-band karyotyping revealed a normal male karyotype (46, XY).

To identify the cause of his developmental delay, we performed chromosomal and genetic analysis after receiving written informed consent from his parents. All procedures were reviewed and approved by the Institutional Review Board of Kobe University School of Medicine and were performed in accordance with the ethical standards of the Declaration of Helsinki. We first performed fluorescence *in situ* hybridization and direct sequencing for *NSD1* (the nuclear receptor binding SET domain protein 1 gene on 5q35) because the patient's developmental delay and macrocephaly suggested that he had Sotos syndrome (SOTOS1; OMIM#117550). However, no aberrations were identified.

Next, we performed direct sequencing of *NFIX* (nuclear factor I/X gene on 19p13.3), whose mutation can cause Sotos syndrome-2 (SOTOS2; OMIM#614753).<sup>8</sup> We identified a heterozygous missense mutation: NM\_002501.2: c.830G>A, p.Arg277His. c.830G>A was not registered in the healthy control database in dbSNP (<http://www.ncbi.nlm.nih.gov/snp>), the Human Genetic Variation Database (<http://www.genome.med.kyoto-u.ac.jp/SnpDB/>) or ExAC (<http://exac.broadinstitute.org/>). Moreover, p.Arg277His was found to be a pathogenic amino-acid change through use of the functional prediction websites PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>), SIFT (<http://sift.jcvi.org/>) and Mutation Taster (<http://www.mutationtaster.org/>). However, the patient inherited this from one of his healthy parents; thus, we determined that c.830G>A was non-pathogenic.

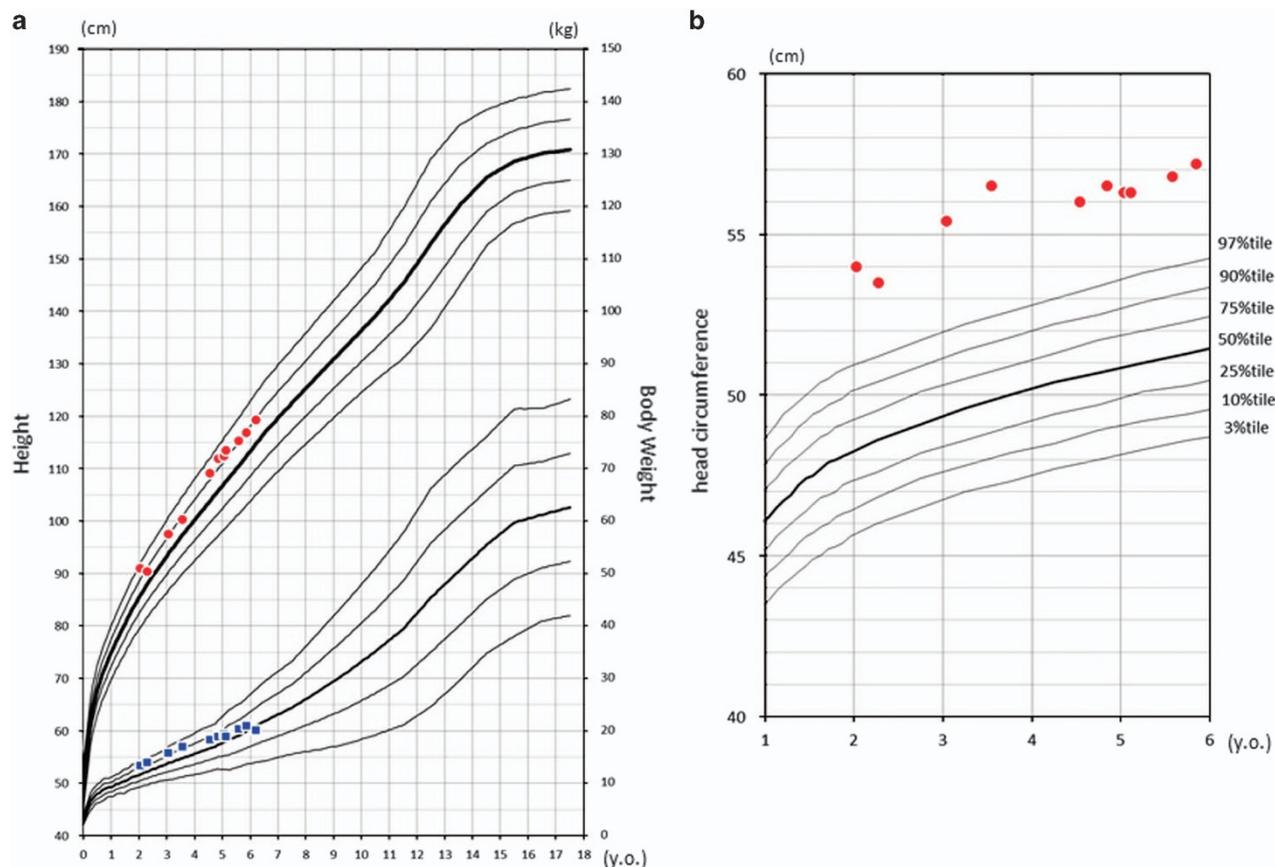
We then used the TruSight One Sequencing Panel on a MiSeq platform (Illumina, San Diego, CA) following the manufacturer's instructions to analyze 4,813 genes. However, no mutations were associated with the patient's neurological disorder. Array-based comparative genomic hybridization (array CGH, 180 k CGH+SNP, Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions identified an ~2 Mb heterozygous deletion in chromosome 12p (Figure 2a): arr[hg19]12p13.2p13.1 (12,482,956-14,436,199) x1. This region included 21 genes: *MANSC1*, *LOH12CR2*, *LOH12CR1*, *DUSP16*, *CREBL2*, *GPR19*, *CDKN1B*, *APOLD1*, *MIR613*, *DDX47*, *RPL13AP20*, *GPRC5A*, *MIR614*, *GPRC5D*, *HEBP1*, *HTR7P1*, *KIAA1467*, *GSG1*, *EMP1*, *C12orf36* and *GRIN2B*

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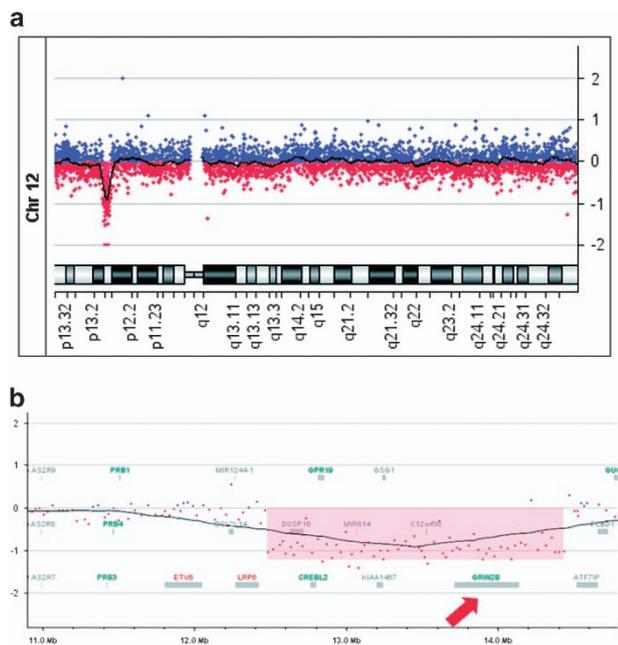
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**Figure 1.** Standard growth curve of the patient. He had a normal height and bodyweight (a) but a large head circumference (b). y.o. years-old.



**Figure 2.** An approximately 2 Mb deletion on the short arm of chromosome 12 was revealed by array CGH analysis (a). This region contains 21 genes, including *GRIN2B* (red arrow; b).

(Figure 2b). The deletion was not detected in either parent and was therefore considered to be *de novo*. Although one healthy control sample (nsv826262) in the database of genomic variants (<http://dgv.tcag.ca/dgv/app/home>)<sup>9</sup> carried a small deletion

including *GRIN2B* exon 4, we concluded that the *GRIN2B* mutations have a central role in the phenotype of this patient.

NMDARs are commonly assembled as two GluN1 subunits and two GluN2 subunits.<sup>10</sup> During the fetal and early postnatal period, GluN1/2B exists predominantly in the mammalian brain, and GluN1/2A is later dominant in the adult brain.<sup>11</sup> *GRIN2B* is essential for neural pattern formation, synaptic plasticity, short-term memory and language acquisition.<sup>12</sup> Moreover, disruptions or mutations of *GRIN2B* have been reported to be responsible for mild to severe intellectual disability<sup>6</sup> and autism.<sup>13</sup> Endele *et al.*<sup>6</sup> have previously reported two patients with chromosomal translocations of *GRIN2B* and 4 out of 468 patients with intellectual disability that had *GRIN2B* mutations. All patients showed behavioral anomalies including ASD, although none had epilepsy, facial dysmorphism or macrocephaly; patients with *GRIN2A* mutations frequently have epileptic seizures.<sup>6</sup> Disruption of *GRIN2A* is responsible for focal epilepsy and speech disorders, with or without intellectual disability (OMIM#245570).<sup>14</sup> *GRIN2C* and *GRIN2D* have not been shown to be associated with neurodevelopmental disorders.

Dimassi *et al.*<sup>12</sup> were the first to report patients with a 12p13.1 microdeletion including all or part of *GRIN2B*. The one female and two male patients affected presented with clinical phenotypes of moderate intellectual disability, delayed language development and mild facial defects, including an oval-shaped face, arched eyebrows and a high forehead.<sup>12</sup> Their growth was almost normal, including their head circumferences. Our patient showed macrocephaly but lacked the dolichocephaly and prominent head seen in Sotos syndrome. Because intellectual disability, language developmental delay and macrocephaly are common features of ASD patients, the 12p13 interstitial deletion should be considered as one responsible for non-specific ASD and intellectual disability.

In conclusion, array CGH is useful for disease diagnosis in non-specific neurodevelopmental patients. Our results should provide insight into the relationship between glutamate receptor expression and neurodevelopmental disease.

## HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at <http://dx.doi.org/10.6084/m9.figshare.hgv.845>.

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## COMPETING INTERESTS

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