

De novo 8p23.1 deletion in a patient with absence epilepsy

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ABSTRACT – The 8p23.1 deletion syndrome is a rare multisystem disorder with high penetrance and a variable phenotypic spectrum that includes congenital heart disease (CHD), intellectual disability, behavioural problems, microcephalia, and sometimes epilepsy. Genomic copy number variations (CNVs) constitute an important genetic risk factor for common genetic generalised epilepsy syndromes (GGEs) and absence seizures. These variations, resulting either from copy loss (microdeletion) or copy gain (duplications), disrupt genes associated with neuronal development. Herein, we report an epilepsy patient who was affected by developmental delay, microcephalia, behavioural problems, CHD, and childhood-onset absence seizures. The patient had a 4-Mb *de novo* microdeletion at 8p23.1. Some of the genes in this region, particularly *XKR6* and *MIR597*, may be involved in the pathogenesis of absence seizures, suggesting that epilepsy may possibly be part of the phenotypic spectrum of the syndrome rather than a comorbid disorder. Thus, CNV screening for GGE plus patients may have important implications in clinical practice with regards to diagnostic classification, clinical management of the syndromic multisystem disorders, and, potentially, genetic counselling.

Key words: 8p23.1 deletion syndrome, *XKR6*, *MIR597*, absence seizure, genomic copy number variation

Typical absence seizures are a sudden, brief impairment of consciousness, lasting only 3-25 seconds, without a concomitant postictal phase, and occur multiple times per day. Seizures are aggravated by hyperventilation, and EEG shows a typical generalized, synchronous, bilateral 3-4 Hz spike and slow-wave discharges. Absence seizures are a type of idiopathic generalized

epilepsy and are mostly seen in childhood absence epilepsy (CAE). CAE is the most common childhood epilepsy that generally begins between 3 and 9 years of age (Loiseau *et al.*, 1995; Shinnar *et al.*, 2015). Children with CAE have a normal neurological examination and normal intelligence level, however, frequent seizures may affect mental performance (Loiseau *et al.*, 1995;

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Glauser and Loddenkemper, 2013). Cognitive changes, as well as linguistic and behavioural problems, may rarely be seen in patients with CAE (Shinnar *et al.*, 2015). The idiopathic generalized epilepsies have a predominantly genetic aetiology (Hughes, 2009). Mutations and susceptibility alleles have been identified in calcium channel, chloride, and GABA_A receptor genes, and the functional analysis of the variants of these ion channels has revealed increased excitatory activity in the cortex or thalamocortical regions (Maljevic *et al.*, 2006; Urak *et al.*, 2006; Hughes, 2009). To date, *GABRG2*, *GABRA1*, *GABRB3*, *CLCN2*, and *CACNA1H* genes and the 8q24 region have been reported as susceptibility loci in the development of CAE (Sugimoto *et al.*, 2000; Maljevic *et al.*, 2006; Urak *et al.*, 2006; Hughes, 2009). Genomic copy number variations (CNVs) constitute an important genetic risk factor for common genetic generalised epilepsy syndromes (Lal *et al.*, 2015) and absence epilepsies (Addis *et al.*, 2016). The recurrent hotspot microdeletions disrupt genes associated with neuronal development and function, suggesting fundamental neurodevelopmental pathways that may be important in epileptogenesis of absence seizures. The 8p23.1 microdeletion is a rare syndrome with the phenotypic characteristics of congenital heart disease (CHD), intellectual disability (ID), behavioural problems, and microcephaly due to loss of multiple neighbouring genes (Ballarati *et al.*, 2011). Microcephaly, physical dysmorphic anomalies, CHD, and behavioural problems may be mild or absent in this syndrome (Ballarati *et al.*, 2011). The severity of ID

and the occurrence of microcephaly appear to be related to the extent of the deletion (Claeys *et al.*, 1997). To the best of our knowledge, there are two reports of 8p23.1 microdeletion patients in the literature; one on a patient with EEG anomalies and another on two patients with absence epilepsy (Fagan *et al.*, 1988; Claeys *et al.*, 1997), however, a critical region or gene that causes epilepsy has not been identified yet. Besides having typical absence seizures and EEG anomalies, our patient also suffered from serious behavioural problems, microcephaly, and minor dysmorphic features, which is why we decided to pursue genetic analysis.

Case study

A 7-year-old male patient, who was admitted to the Department of Child Neurology, Istanbul Faculty of Medicine, presented with sudden impairment of consciousness, as well as five-second periods of unresponsiveness, occurring at least 10 to 30 times per day for about six months. The antenatal and natal history was uneventful. He had undergone surgery for an ostium primum atrial septal defect, mitral valve cleft, and pulmonary artery stenosis when he was 6 months old. The patient had mental and motor developmental delay. He had mild ID and behavioural problems such as hyperactivity, outbursts of aggressiveness, and impulsiveness. He had a head trauma at the age of 5 due to a fall from a h8. Born to non-consanguineous

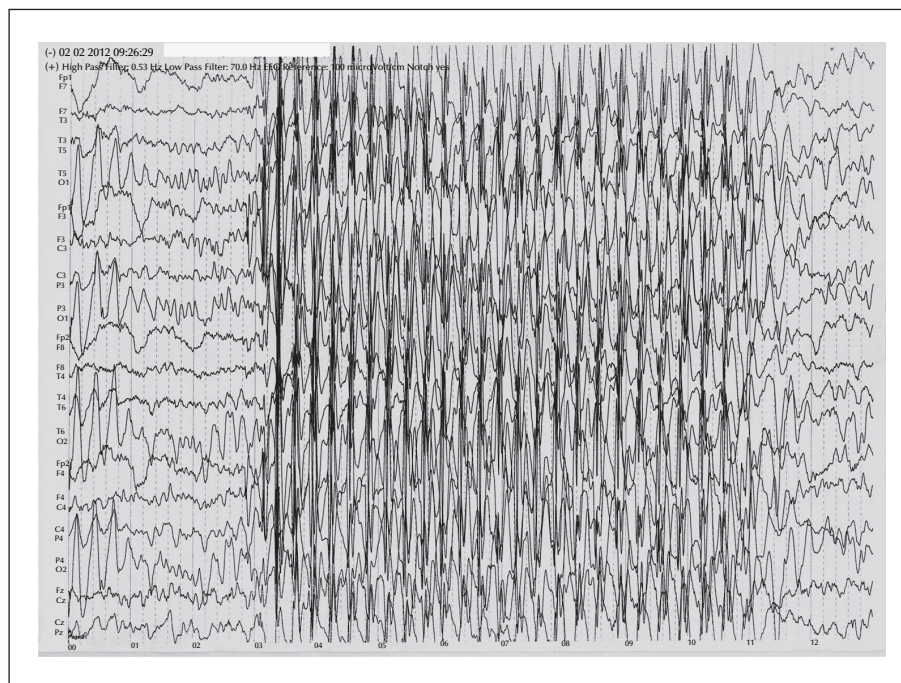


Figure 1. EEG recorded at age 9 years showing bilateral generalized 3-Hz spike and slow-wave discharges.

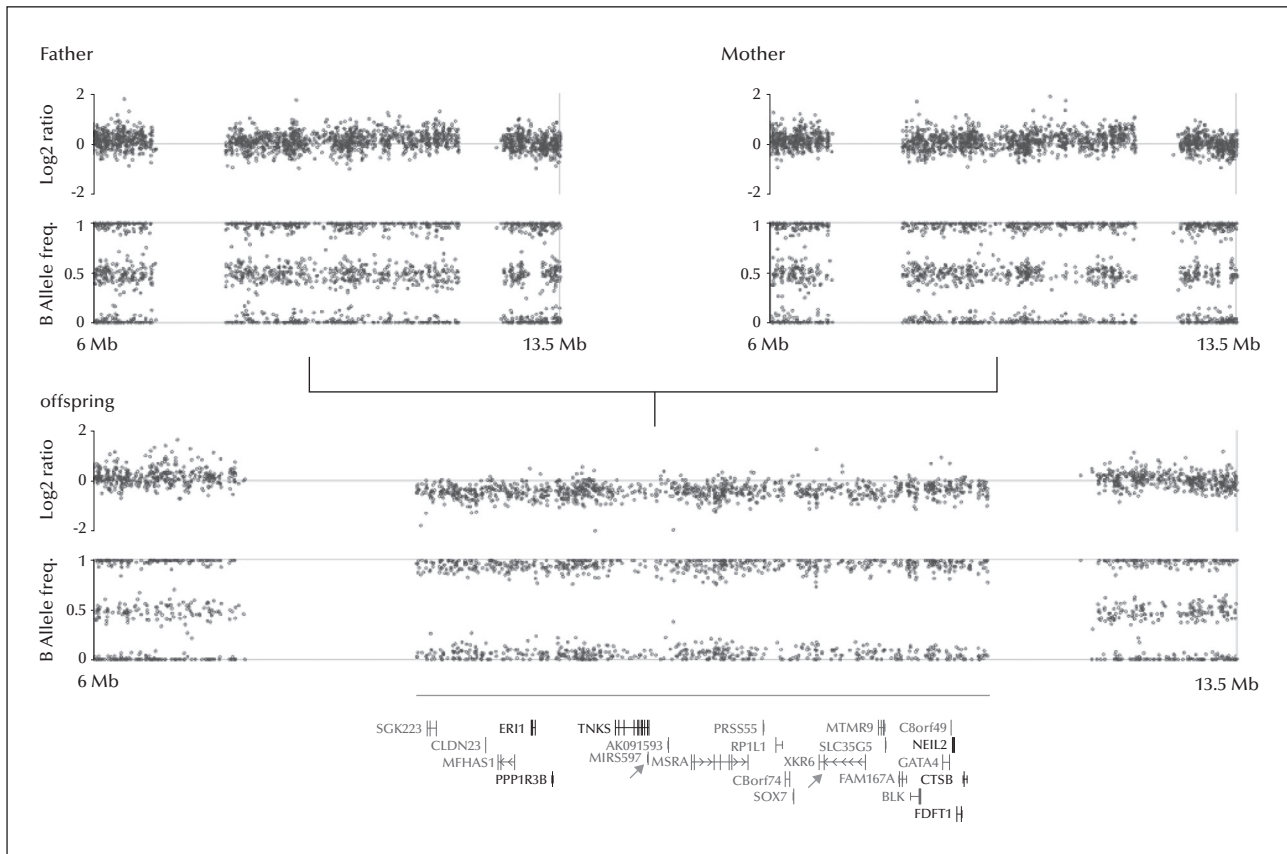


Figure 2. SNP signal intensity log₂ ratios and B allele frequencies (BAF) of the father (top left), the mother (top right), and the offspring (centre). The 8p23.1 deletion in the offspring shows a segment of 1,100 markers with decreased log₂ ratios and complete loss of heterozygosity. The red line shows the minimal critical region of the deletion. Genomic organization of UCSC genes are indicated below. Small arrows point to *MIR597* and *XKR6*.

parents, he had two healthy sisters and a brother. There was no family history of neurological or hereditary diseases or febrile convulsions.

The patient's general physical examination revealed microcephalia, high-arched palate, small ears, and swan neck deformity of the fingers. His neurological examination was normal. His IQ score was 65. Standard EEGs showed bilateral temporal-parietal-occipital and centro-temporal unconnected multiple epileptogenic foci and generalized 3-Hz spike and slow-wave discharges (*figure 1*). There were no structural lesions except for a post-traumatic, right frontal cortico-subcortical gliotic lesion on brain MRI which was approximately 1 mm wide. During 11 years of follow-up, he was treated with valproic acid and did not develop any other types of seizure. The seizures were almost fully controlled during this period of time. After obtaining written informed consent, the patient, mother, and father all underwent genotyping based on Affymetrix AXIOM genotyping array. The Axiom CNV summary tools (Affymetrix, Santa Clara, CA, USA) were used for calculating log₂ ratios of signal intensities and B allele frequencies obtained from genotyping

data. CNV calling was carried out using the Nexus Copy Number package version 7.5 (BioDiscovery Inc., Hawthorne, CA, USA). CNV screening revealed a 4-Mb *de novo* deletion of the chromosomal region 8p23.1 (8,109,255-12,166,733 bp [GRCh37/hg19]) in the offspring (*figure 2*). There were no other CNVs observed in the patient and the screening of exons of the *GABRG2* gene by PCR and Sanger sequencing gave negative results.

Discussion

Careful clinical investigation suggested that the index patient was affected by a multisystem disorder consistent with the 8p23.1 microdeletion syndrome. The 8p23.1 microdeletion is a contiguous gene syndrome which shows multiple and unrelated clinical features due to loss of multiple neighbouring genes (Ballarati *et al.*, 2011). Each of the genes gives rise to a distinct phenotype when deleted. In our patient, the deleted 4-Mb region included about 100 genes, most of which encode for non-coding RNAs. Microcephaly, physical

dysmorphic anomalies, CHD, and behavioral problems may be mild or absent in this syndrome (Ballarati *et al.*, 2011). The severity of ID and the occurrence of microcephaly appear to relate to the extent of the deletion (Claeys *et al.*, 1997). The *GATA4* and *SOX7* genes are expressed in the heart and appear to function in the same pathway, contributing to heart anomalies (Claeys *et al.*, 1997). Regarding the other genes in this region, a specific phenotype-genotype association has not yet been described.

MicroRNA genes are small non-coding RNA molecules which are responsible for regulating gene expression. The consequence of unbalanced expression of calcium channel, chloride, and GABA_A receptor genes can cause absence epilepsies. *MIR597* (microRNA-597) targets *GABRB3*, which is known to be involved in epileptogenesis of absence epilepsies (Kan *et al.*, 2012). The deletion of this microRNA gene may therefore be involved in the pathogenesis of absence seizures in our patient, as well as in other patients with the 8p23.1 microdeletion syndrome with an epilepsy phenotype. In addition, the protein product of the *XKR6* gene, which was among the deleted genes, localizes with a plasma membrane protein of unknown function, and is expressed only in the frontal lobe (Suzuki *et al.*, 2014). This gene has not yet been associated with a disease, but its presence in this region is remarkable. The frontal cortex is particularly associated with absence seizures (Hughes, 2009). Various near-infrared spectroscopy studies have revealed increased oxygenation in the frontal areas, and EEG studies have shown activation in dorsolateral and orbital frontal areas (Hughes, 2009). The clinical changes during ictal absence seizures, including alteration of awareness and arrest of motor initiation, may reflect frontal lobe dysfunction. The absence seizures and pattern of behavioural problems observed in our patient may also reflect frontal lobe dysfunction.

This case report strengthens the evidence that epilepsy may be part of the phenotypic spectrum of 8p23.1 deletion syndrome, and/or haploinsufficiency of several genes with functional diversity. Until further experimental evidence to the contrary is provided, it is possible that the comorbidity of rare classic 8p23.1 deletion syndrome and typical absence seizures in the index case is merely coincidental. Nevertheless, it is highly recommendable to carry out CNV and/or whole-exome sequencing (WES) for GGE plus patients, since it may have important implications on clinical practice with regards to diagnostic classification, clinical management of the syndromic multisystem disorders, and, potentially, genetic counselling.

Supplementary data.

Summary didactic slides are available on the www.epilepticdisorders.com website.

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References

- Addis L, Rosch RE, Valentin A, *et al.* Analysis of rare copy number variation in absence epilepsies. *Neurol Genet* 2016; 2(2): e56.
- Ballarati L, Cereda A, Caselli R, *et al.* Genotype-phenotype correlations in a new case of 8p23.1 deletion and review of the literature. *Eur J Med Genet* 2011; 54: 55-9.
- Claeys I, Holvoet M, Eyskens B, *et al.* A recognizable behavioural phenotype associated with terminal deletions of the short arm of chromosome 8. *Am J Med Genet* 1997; 74: 515-20.
- Fagan K, Wilkinson I, Allen M, *et al.* The coagulation factor VII regulator is located on 8p23.1. *Hum Genet* 1988; 79: 365-7.
- Glauser TA, Loddenkemper T. Management of childhood epilepsy. *Continuum* 2013; 19(3): 656-81.
- Hughes JR. Absence seizures: a review of recent reports with new concepts. *Epilepsy Behav* 2009; 15: 404-12.
- Kan AA, Erp S, Derijck AAHA, *et al.* Genome-wide microRNA profiling of human temporal lobe epilepsy identifies modulators of the immune response. *Cell Mol Life Sci* 2012; 69: 3127-45.
- Lal D, Ruppert AK, Trucs H, *et al.* Burden analysis of rare microdeletions suggests a strong impact of neurodevelopmental genes in genetic generalised epilepsies. *PLoS Genet* 2015; 11(5): e1005226.
- Loiseau P, Duche B, Pedespan JM. Absence epilepsies. *Epilepsia* 1995; 36: 1182-6.
- Maljevic S, Krampfl K, Cobilanschi J, *et al.* A mutation in the GABA-A receptor alpha-1-subunit is associated with absence epilepsy. *Ann Neurol* 2006; 59: 983-7.
- Shinnar S, Cnaan A, Hu F, *et al.* Long-term outcomes of generalized tonic-clonic seizures in a childhood absence epilepsy trial. *Neurology* 2015; 85: 1108-14.
- Sugimoto Y, Morita R, Amano K, *et al.* Childhood absence epilepsy in 8q24: refinement of candidate region and construction of physical map. *Genomics* 2000; 68: 264-72.
- Suzuki J, Imanishi E, Nagata S. Exposure of phosphatidylserine by Xk-related protein family members during apoptosis. *J Biol Chem* 2014; 289(44): 30257-67.
- Urak L, Feucht M, Fathi N, *et al.* A *GABRB3* promoter haplotype associated with childhood absence epilepsy impairs transcriptional activity. *Hum Mol Genet* 2006; 15: 2533-41, Erratum: *Hum Mol Genet* 2006 ; 15 : 3272.

TEST YOURSELF



- (1) In which epilepsy syndrome are absence seizures mostly seen?
- (2) Which genes have been reported as susceptibility loci in the development of idiopathic generalised epilepsy or CAE?
- (3) What kind of clinical features are associated with 8p23.1 deletion syndrome?
- (4) What are the pathophysiological mechanisms known to be associated with absence epilepsy?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".