Case report

Infantile epilepsy associated with mosaic 2q24 duplication including SCN2A and SCN3A

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ABSTRACT

Epilepsies can be caused by specific genetic anomalies or by non-genetic factors, but in many cases the underlying cause is unknown.

Mutations in the SCN1A and SCN2A genes are reported in childhood epilepsies; in particular SCN1A was found mutated in patients with Dravet syndrome and with generalized epilepsy with febrile seizures plus (GEFS+).

In this paper we report a patient presenting with an atypical epileptic syndrome whose phenotype partially overlaps both Dravet syndrome and benign familial neonatal-infantile seizures (BFNIS).

Array-CGH analysis suggested the presence of a mosaic duplication (about 12 Mb) at the level of chromosome 2q23.3q24.3 involving SCN2A and SCN3A genes. Additional analyses (radiolabeled RFLP and quantitative PCR) confirmed the mosaicism of the duplication.

We suggest that the array-CGH analysis is mandatory for children presenting with epilepsy and psycho-motor retardation even without dysmorphisms or other clinical features suggesting a specific genetic/epileptic syndrome. The analysis must nevertheless be performed taking into account the possibility of a mosaicism.

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1. Introduction

Epilepsies are a group of heterogeneous neurological disorders characterized by recurrent and unprovoked seizures due to an abnormal electrical activity in the central nervous system.

While they can be caused by specific genetic anomalies (cytogenetic abnormalities,[1] single gene mutations[2]) and by non-genetic factors (infections, stroke, head trauma and brain tumors), in most cases the underlying cause is unknown.

Isolated epilepsy has been recently associated with mutations in a number of genes, the majority of which encode ion channels or receptors, including voltage-gated sodium, potassium, calcium, and chloride channels and receptors for acetylcholine and Y-aminobutyric acid (GABA).[2–6]

Mutations in the sodium channel genes (SCN1A and SCN2A) have been identified in monogenic childhood epilepsies. SCN1A mutations have been found in patients with generalized epilepsy with febrile seizures plus (GEFS+) and with Dravet syndrome; the latter typically presents with prolonged febrile convulsions in infants at about 6 months of age and worsens during the second year of life causing drug resistant seizures and cognitive and motor skill deterioration. Mutations in SCN2A have been found in families with benign familial neonatal-infantile seizures (BFNIS), a self-limiting disorder generally presenting between 2 days and 7 months with a spontaneous remission of seizures at about 18 months and normal cognitive outcome. Shi et al.[7] screened 59 patients with Dravet syndrome for mutations of SCN1A, SCN2A and GABRG2 and found three missense SCN2A mutations. Mutations of sodium channel SCN3A were recently found in a patient with cryptogenic pediatric partial epilepsy.[3] Functional analysis of sodium channel mutations (SCN1A, SCN2A and SCN3A) revealed that they are implicated in neuronal excitability suggesting that they play a role in the pathogenesis of epilepsy.

We report a patient affected with infantile epilepsy associated with mosaicism for a 12.01 Mb duplication of the long arm of chromosome 2 including SCN2A and SCN3A genes.

2. Clinical report

The propositus is a Caucasian male who was referred to our institute for evaluation of developmental delay and epilepsy. He was born at 38 weeks of gestation via spontaneous vaginal delivery to unrelated healthy parents following an uncomplicated pregnancy.

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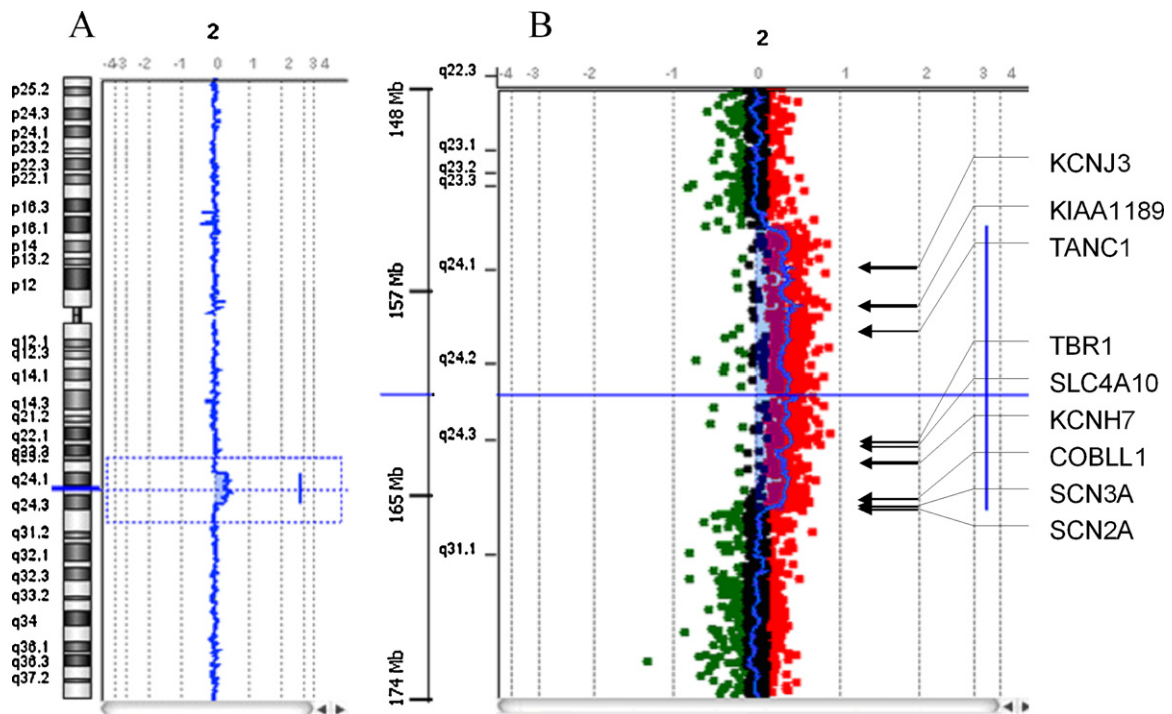[c] These authors contributed equally to this work.

Fig. 1. Array-CGH; graphical output of mosaic duplication in 2q23.3q24.3. (A) Chromosome view. (B) Gene view (the genes potentially involved in the described phenotype are highlighted).

uncut bands (T and G, respectively), that stay for the fraction representing the two different alleles in each single amplification (G + T = 1.00), were performed using a densitometric test. In a normal heterozygous sample we observed the expected values of about 50% (0.50) for each band. In the proband, the uncut band (G) was about 70% (0.70) and the cut band (T) was about 30% (0.30) of the total amplification. These data indicate that the G-variant allele was duplicated in about 40% (0.40) of the cells, and the formula used to calculate it was:

$$\text{G allele}: \ 2a \times 0.5 + (1 - a) \times 0.5 = G$$

$$\text{T allele}: \ 1 - [2a \times 0.5 + (1 - a) \times 0.5] = T$$

where a is the fraction of cells with duplication.

These formulas describe the fraction of each single allele in a heterozygous state. In this case, since the G fraction was 0.70, the a value was 0.40 or 40% of the duplicate cells (in a normal heterozygous state, the a value should be 0 and the G value should be 0.50). The duplicate allele was found to be paternal, since the patient's father was homozygous for the G variant, while the mother was heterozygous (G/T).

The result of Real-Time quantitative PCR was 1.222 ± 0.118. A sample with no duplication produces a value of 100% (1.00 or 2/2), while an increased value of 50% (1.50 or 3/2) means that duplication involves all the cells. The increment of 22.2% according to our data indicates that 44% of the cells were involved in duplication (1.222 or 2.444/2).

5. Discussion

We reported the case of a child with atypical epileptic syndrome: while the type of seizure, the drug response, and the time of onset are similar to the findings observed in BFNIS, our proband presented seizure recurrence at the age of two years with intellectual disability and motor impairment, characteristic of the Dravet syndrome.

Array-CGH analysis suggested the presence of a mosaic 2q23.3q24.3 duplication (about 12 Mb). Additional analyses (radiolabeled RFLP and quantitative PCR) confirmed the mosaicism of the duplication, defined its value, and determined from which allele the alteration arose. These techniques, which are not routinely used for the characterization of genomic anomalies identified by array-CGH analysis, detected the duplication in the fraction between 40 and 51% of blood cells.

The duplication identified in our patient involves SCN2A and SCN3A genes.

SCN1A, SCN2A and SCN3A, a cluster of genes localized at 2q24.3, encode the alpha subunits of three voltage gated sodium channels (Na$_v$1.1, Na$_v$1.2, Na$_v$1.3) expressed in the central nervous system. Voltage gated sodium channels are also fundamental to the generation and propagation of action potentials in the brain and muscle cells. Mutations of these three genes have been described in patients with seizure disorders including generalized epilepsy with febrile seizures plus (GEFS+, OMIM no. 604233), severe myoclonic epilepsy in infancy (SMEI or Dravet syndrome; OMIM no. 607208), borderline SMEI (SMEB) and benign familial neonatal-infantile seizures (BFNIS).

Duplications involving SCN genes on 2q24.3 chromosome have been observed in only a few cases until now. Marini et al.[10] reported on a family with classical Dravet syndrome and an intragenic duplication of SCN1A. A duplication involving SCN2A, SCN3A and the last exon of SCN1A was recently identified in a patient with "familial neonatal seizures and intellectual disability".[11] Raymond et al.[12] have, moreover, described a female patient with neonatal-infantile epilepsy and a 2 Mb duplication at 2q24.3 including SCN1A, SCN2A and SCN3A.

In this report we described the first case of an epileptic patient with a genomic duplication involving SCN2A and SCN3A and excluding SCN1A.

As missense, gain of function mutations of SCN2A and SCN3A, leading to increased sodium current, have been previously reported in patients with epilepsy,[3,4,13] duplication of these two genes could potentially result in an increased expression of their

transcript with subsequent increased sodium current and neuronal excitability.

The other genes included in the duplication with a possible role in the pathogenesis of neurological anomalies are KCNJ3, KIAA1189, TANC1, TBR1, SLC4A10, KCNH7 and COBLL1. All these genes have been shown to be expressed in the brains of humans and experimental animals. In particular, SLC4A10 is a sodium bicarbonate transporter gene and was recently found to be disrupted in a balanced reciprocal translocation t(2;13)(q24;q31) or deleted in patients with seizures and mental impairment.[14,15]

In conclusion, the array-CGH analysis is mandatory for children presenting with epilepsy and psychomotor retardation even without dysmorphisms or other clinical features suggesting a specific genetic/epileptic syndrome. The analysis must nevertheless be performed taking into account the possibility of a mosaicism (i.e. analyzing DNA extracted from several tissues and confirming the obtained results with different techniques).

Moreover, molecular analysis of SCN2A and SCN3A genes should be performed in those children with no features of any specific epileptic syndrome described in the literature.

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