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Case study

Unusual association of SCN2A epileptic encephalopathy with severe cortical dysplasia detected by prenatal MRI

Silvia Bernardo [a,b,*], *Enrica Marchionni* [b,c], *Sabrina Prudente* [c], *Paola De Liso* [d], *Alberto Spalice* [d], *Antonella Giancotti* [e], *Lucia Manganaro* [a], *Antonio Pizzuti* [b,c]

[a] *Department of Radiological, Oncological and Pathological Sciences, Sapienza University of Rome, Policlinico Umberto I Hospital, Viale Regina Elena 324, Rome, Italy*
[b] *Department of Experimental Medicine, Sapienza University of Rome, Policlinico Umberto I Hospital, Viale Regina Elena 324, Rome, Italy*
[c] *IRCCS Casa Sollievo della Sofferenza, Mendel-laboratory, San Giovanni Rotondo, Italy*
[d] *Department of Pediatrics, Child Neurology and Psychiatry, Sapienza University of Rome, Policlinico Umberto I Hospital, Viale Regina Elena 324, Rome, Italy*
[e] *Department of Obstetrics, Gynecology and Urologic Sciences, Sapienza University of Rome, Policlinico Umberto I Hospital, Viale Regina Elena 324, Rome, Italy*

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ABSTRACT

We present an atypical association of SCN2A epileptic encephalopathy with severe cortical dysplasia. SCN2A mutations are associated with epileptic syndromes from benign to extremely severe in absence of such macroscopic brain findings. Prenatal MRI (Magnetic Resonance Imaging) in a 32 weeks fetus, with US (Ultrasonography) diagnosis of isolated ventriculomegaly showed CNS (Central Nervous System) dysplasia characterized by lack of differentiation between cortical and subcortical layers, pachygyria and corpus callosum dysgenesis. Postnatal MRI confirmed the prenatal findings. On day 6 the baby presented a focal status epilepticus, partially controlled by phenobarbital, phenytoin, and levetiracetam. After three weeks a moderate improvement in seizure control has been achieved with carbamazepine. Exome sequencing detected a *de novo* heterozygous mutation in the *SCN2A* gene, encoding the α_{II}-subunit of a sodium channel. The patient findings expand the phenotype spectrum of SCN2A mutations to epileptic encephalopathies with macroscopic brain developmental features.

* *Corresponding author*. Viale Regina Elena 324, Rome, Italy.
E-mail addresses: silviabernardo@live.it (S. Bernardo), enrica.marchionni@uniroma1.it (E. Marchionni), s.prudente@css-mendel.it (S. Prudente), paola.deliso@uniroma1.it (P. De Liso), a.spalice@tiscali.it (A. Spalice), antonella.giancotti@uniroma1.it (A. Giancotti), lucia.manganaro@uniroma1.it (L. Manganaro), antonio.pizzuti@uniroma1.it (A. Pizzuti).
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1. Introduction

Voltage-gated sodium channels (VGSCs) play an essential role in normal neurologic function and they are responsible for the initiation and propagation of action potentials. Structurally, they are integral membrane proteins consisting in an α subunit and one or more β subunits. Four main subtypes of VGSCs are present in human CNS: $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.6$, encoded by *SCN1A*, *SCN2A*, *SCN3A* and *SCN8A* genes, respectively. Mutations in sodium channel genes have been identified in multiple epilepsy syndromes.[1] In particular, *SCN2A* gene, encoding the α_{II}-subunit of the $Na_V1.2$, a brain expressed voltage gated sodium channel, has been associated with epileptic syndromes from benign to extremely severe,[2] without any structural counterpart.

We describe a premature baby with a *de novo* SCN2A missense mutation, affected by a severe drug-resistant epileptic encephalopathy and multiple brain malformations.

2. Materials and methods

2.1. *Targeted next-generation sequencing*

Genomic DNAs from the proband and her parents were extracted from whole blood by the Nucleospin Blood KIT (Macherey-Nagel, Düren, Germany), after having obtained informed consent. The proband and her unaffected parents were subjected to the exome sequencing of 4.813 clinically relevant genes by the TruSight One sequencing panel (Illumina, San Diego, CA, USA) according to manufacturer's instructions. The samples were paired-end sequenced on a MiSeq desktop sequencer (Illumina, San Diego, CA, USA) and mapped to the human genome reference sequence (GRCh37, hg19). The generated vcf files were loaded in the "Variant Studio" software (Illumina, San Diego, CA, USA). Filtering criteria included coverage >20 reads, variant frequency >20%, minor allele frequency <0.01 and variants selected for mutation type including nonsense, missense, frameshift and splice variants.

As from pedigree analysis this case was initially presumed with a recessive mode of inheritance or an autosomal dominant mode due to a *de novo* mutation, variants were investigated which were heterozygous in the parents and homozygous or compound heterozygous in the proband or *de novo* in the proband. All filtered variants were further investigated in Alamut visual version 2.6.0 (Interactive Biosoftware, Rouen, France). Finally, databases including dbSNP, OMIM, UniGene, ClinVar Exome Variant Server and ExAC were used to the evaluation of genes and mutation background information.

2.2. *Validation of the* de novo *mutation by Sanger sequencing*

For validation of the c.751G>A variant located in the exon 7 of the *SCN2A* gene (NM_001040142.1), primers were designed using Primer-BLAST, which amplified a 326-bp amplicon from genomic DNA.

Standard PCR conditions were used with the forward 5′-TGGCATTCTGCATGACATTT-3′ and reverse primer 5′-TACCATTCCCATCCAATGAA-3′ (Sigma–Aldrich, Milan, Italy).

Sanger sequencing of the amplicons followed using an ABI3130xl 16-capillary sequencer (Life Technologies, Carlsbad, CA, USA). The parents, proband and 50 controls were sequenced.

3. Case study

A US scan at 31 weeks gestational age in a 38-years-old woman revealed bilateral ventriculomegaly. Parents were healthy and non-consanguineous with an unremarkable familiar history. The woman was negative for recent infections and standard fetal karyotype was normal (46,XX). Their first son was healthy.

Fetal MRI performed at 32 weeks confirmed the severe ventriculomegaly and evidenced a completely altered parenchymal intensity suggesting a neuronal disorganization with lack of differentiation between cortex and subcortical layers associated with pachygyria and anomalies of cortical gyration. Corpus callosum was normal in length but extremely thin (Fig. 1A).

Cesarean section was performed at 34 weeks for fetal tachycardia. The baby was hypotonic and required intubation and ventilation support. Head circumference was 32.6 cm (75th–90th percentile). Post-natal imaging showed multiple recent ischemic areas in the fronto-parietal lobes and in the periventricular white matter, other findings confirmed the fetal MRI.

On day 6, she developed a focal status epilepticus with bradycardia and oxygen desaturation, partially controlled by phenobarbital, phenytoin and levetiracetam. Interictal EEG showed epileptiform multifocal abnormalities, evolved in pseudo periodic epileptiform diffuse pattern after few weeks. Since multiple daily seizures persisted, levetiracetam was replaced by carbamazepine at day 29 with a moderate improvement. After genetic counseling, array-CGH resulted negative.

Then, the proband and the unaffected parents' DNA was extracted from peripheral blood for diagnostic exome sequencing of 4813 clinically relevant genes (TruSight One sequencing panel – Illumina).

3.1. *NGS using the TruSight one panel and bioinformatics analysis*

The proband and her parents were sequenced in parallel by a MiSeq desktop sequencer (Illumina), each generating over 24 million aligned sequences and around 70,000 variants.

The first analysis filtered for a recessive mode of inheritance and yielded negative results with exclusion of all variants by filtering or not meeting analysis criteria.

Therefore, all variants in common between the proband and her parents were excluded, allowing for the detection of a heterozygous *de novo* missense mutation (c.751G>A, p.Val251Ile) in the *SCN2A* gene (NM_001040142.1).

The Val251Ile mutation affected a highly conserved amino acid and was predicted to be damaging by different