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# Two Distinct *De novo* Pathogenic Sequence Variants in the *SCN2A* Gene in Children with Early Infantile Epileptic Encephalopathy/Ohtahara Syndrome

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#### Abstract

Early infantile epileptic encephalopathy/Ohtahara syndrome represents a group of genetically heterogeneous disorders affecting normal brain development and functioning. In this work, we present a case of two unrelated children diagnosed with early infantile epileptic encephalopathy and associated multiple congenital abnormalities. To obtain genetic diagnosis, these children were examined through multi-step diagnostic algorithm including G-banded karyotype analysis and whole-genomic screening using array-based comparative genomic hybridization (array-CGH) with negative results. Additionally, these children and their unaffected parents were enrolled for our pilot study of targeted next-generation sequencing technology (NGS) using commercial panel ClearSeq Inherited DiseaseXT (Agilent Technologies) and consequent validation by Sanger sequencing. Our analysis detected two distinct *de novo* pathogenic sequence variants in the *SCN2A* gene, resulting in p.Met1545Val and p.Ala263Val changes on the *SCN2A* protein level and explaining their pathological phenotypes.

In conclusion, our findings indicate that NGS can become the optimal approach to the genetics diagnostics of disorders of central nervous system (CNS) which can lead to the discovery of new candidate genes.

**Keywords:** Intellectual disability; Epileptic encephalopathy; Ohtahara syndrome; array-CGH; NGS; *SCN2A* gene

# Introduction

Epileptic encephalopathies (EE) represent a group of epileptic conditions characterized by an abnormal cerebral electrical activity which result in progressive cerebral dysfunction. Based on the age of onset eight EE syndromes are defined: early myoclonic encephalopathy and Ohtahara syndrome (neonatal period), West syndrome and Dravet syndrome (infancy), myoclonic status in nonprogressive encephalopathies, Lennox-Gastaut syndrome, Landau-Kleffner syndrome and epilepsy with continuous spike waves during slow wave sleep (childhood and adolescent period) [1,2]. The most severe forms of them are characterized by extreme abnormalities in electroencephalographic (EEG) pattern which cause global developmental delay and intellectual disabilities due to irreversible and fatal brain damage.

Early infantile epileptic encephalopathy (EIEE)/Ohtahara syndrome (OS) represents the earliest form of neonatal EEs, because symptoms appear within the three months after delivery, often within 10 days of life [2,3]. Infants acutely develop tonic spasms with variable progression, localization, frequency per day, and with independence of the sleep cycle. OS typically result in severe structural brain malformations and is often accompanied by variety of metabolic disorders which may modify clinical features and their severity. The broad phenotypic heterogeneity of epileptic encephalopathies reflects a broad genetic heterogeneity with monogenic and polygenic forms of EE and all types of inheritance (autosomal dominant, autosomal recessive and X-linked) [4]. Whole-genome and whole-exome sequencing techniques have led to the identification of pathogenic sequence variants in a large number of genes which are essential for normal brain development and functioning. These genes play an important role in the formation of functional synapses, signal generation and its transmission.

In our work, we present two cases of unrelated children with epileptic encephalopathies resulting in severe intellectual disability and multiple congenital abnormalities. They were examined by the multi-step genetic diagnostic algorithm including G-banded karyotype and-CGH, however, we did not identify any pathogenic copy-number variants (CNVs) explaining their pathological phenotype. Additionally, these children and their unaffected parents were involved in our pilot study using targeted NGS with a commercially available panel of 2742 gene known in pathogenesis of human inherited diseases. Here, we present the findings after applying this panel to our probands.

# Material and Methods

Two families with affected children were diagnosed at the Department of Medical Genetics (University Hospital Brno, Czech Republic) in years 2015 and 2016. Before the procedure of any genetic analyses, parents signed the informed consent. The informed consent (institutional number 4-288/15/2) was approved by the Ethical Committee, University Hospital Brno. The peripheral blood samples were collected in sterile heparinized tube for cytogenetic analyses. Genomic DNA samples were obtained from peripheral blood in EDTA according to the standard DNA isolation process using MagNaPure system (Roche s.r.o, Basel, Swiss). We evaluated their quality and quantity by DNA agarose electrophoresis with TrackIt<sup>™</sup> 1 Kb Plus DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA), NanoDrop<sup>®</sup> ND-1000 (Thermo Fisher Scientific) and Qubit<sup>®</sup> 1.0 (Thermo Fisher Scientific).

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#### G-banded karyotype analysis and array-CGH technique

Cytogenetic analysis of karyotype was accomplished using standard G-banding procedure and twenty mitoses per patient were evaluated. After G-banded karyotype analyses with negative results (46,XY in patient 1 and 46,XX in patient 2, respectively) we performed wholegenomic screening chromosomal rearrangements by array-CGH using a SurePrint G3 CGH Microarray 4x180K (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's recommendations. Samples were matched with Human Genomic DNA reference (Promega, Madison, WI, USA). Array-CGH slides were scanned with Agilent Microarray Scanner. Data obtained using Agilent Feature Extraction software 12.0.2.2 and Agilent Cytogenomics 4.0, respectively, and visualized using Agilent Genomic Workbench Software 7.0.4.0. CNVs were detected using ADM-2 algorithm with filters of at least five neighbouring probes in region, minimal size of 100 kb in region and minimal absolute average log ratio of 0.25 as cut-off. All genomic positions were estimated on the human reference sequence GRCh37/hg19.

Some additional analyses and characterization of detected CNVs in patients and their parents were required to confirm their origin and localization and to specify their impact on the patient's phenotype. For these purposes we used targeted method of relative quantitative PCR (qPCR).

#### **Relative quantitative PCR**

Relative qPCR was designed and performed with two pairs of DNA primers (targeted to CNVs region and out of the CNVs region, respectively). The amount of 10 ng diluted genomic DNA samples (7.5 μl), specific DNA primers (5 μl of forward and reverse 5μM primers) and 12.5 µl reaction mix Power SYBR Green® PCR Master mix (Thermo Fisher Scientific) were prepared to the final reaction volume of 25 µl. Relative quantitation of target DNA regions (performed in duplicates) were run on StepOne using StepOne Software v2.3 (Thermo Fisher Scientific) following a recommended thermal protocol (95°C for 10 min followed by 40 cycles with 95°C for 15 sec, 60°C for 60 sec, and including final melt curve analysis up to 95°C (mode Step and Hold). Ct values were subtracted for the ERH gene (housekeeping gene) and for each tested DNA region to calculate  $2^{-\Delta\Delta CT}$  [5,6]. After the  $2^{-\Delta\Delta CT}$ calculation to get R-values we were able to confirm or to exclude the loss or gain of genetic material in the targeted DNA regions relatively to the ERH gene in patient's and parental DNA samples. DNA primer sequences are mentioned in Table 1 in supplementary material.

# Targeted NGS

High quality genomic DNA from peripheral blood of children's patients was used for the library preparation. A total of 200 ng DNA per sample were sheared separately by Covaris E-Series (Covaris, Woburn, MA, USA) and the size distribution of fragment was checked by Agilent 2200 Tape Station. Individual samples of DNA fragments were processed to obtain DNA libraries following SureSelect<sup>XT</sup> Target Enrichment System according to manufacturer's recommendation (Agilent Technologies). Prepared DNA libraries were captured using ClearSeq Inherited Disease<sup>XT</sup> Capture libraries (Agilent Technologies). This design includes 2742 genes directly implicated in the pathogenesis of inherited diseases and was developed in the collaboration with Medical Genetics, Charite Berlin. Captured DNA libraries were quality-controlled and quantified using Agilent 2200 TapeStation and Qubit\* 1.0 (Thermo Fisher Scientific). Then they were pooled to 4 nM, denatured and diluted to final concentration of 6pM and sequenced on Illumina MiSeq benchtop sequencer following manufacturer's recommendation (Illumina, Inc., San Diego, CA, USA).

#### **Bioinformatic NGS data processing**

Quality of raw sequencing files was checked using FastQC (v0.11.5) [7]. Presence of adapters was scanned using minion and swan (Kraken package, v15-065) [8]. Preprocessing of the raw sequencing files was performed using cutadapt (v1.11) [9] in following steps: I) Very low-quality ends were trimmed (Phred<5), II) Adapters from both reads of a pair were removed with minimal overlap of 3 bp and maximum of 10% mismatches in a matched sequence (readapters: R1- AGATCGGAAGAGCACACGTCTmoved GAACTCCAGTCAC, R2 - AGATCGGAAGAGCGTCGT-GTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTAT-CATT), III). Very short (<35 bp) and unpaired reads after the trimming were discarded. Preprocessed sequencing reads were mapped to a reference genome (hg19, UCSC Genome FTP http://hgdownload. cse.ucsc.edu/goldenPath/hg19/chromosomes/) [10,11] by BWA aln/ sampe (v0.7.15) [12]. Alignments were further processed with Stampy (v1.0.29) [13]. Aligned bam position and mate information sorted files was corrected using Same tools (v1.3) [14]. Since each library was sequenced in two separate sequencing runs, bam files were merged into one using Picard (v2.1.0) [15] for each sample. PCR duplicates were marked and removed using Picard. Duplicates clean bam files were indel realigned using Genome Analysis TK (v3.6) [16]. Base Quality Score Recalibration (BQSR) was performed in two steps using Genome AnalysisTK and dbSNP (v147) [17] variants were used as a set of known variants. Coverage of targeted regions was explored using bed tools (v2.23.0) [18]. Additional quality checks and statistics were obtained using Picard.

Raw variant calls were performed using VarScan2 (v2.4.2) [19] with default settings except: I) Minimal variant frequency 0.2, II) VarScan2 p-value 0.05. dbSNP ID was added if a match between dbSNP and raw variants was found using snpSift (v4.2) [20]. Filtering of the raw variants was done using VarScan2 with default settings except minimal p-value which was set to 0.05. SNPs in very close proximity to indels were removed from the calls. Effect of the variants, ClinVar (downloaded

Variables	Genomic analyses		Clinical data
	G-banded karyotype array-CGH and relative qPCR	Targeted NGS Sanger sequencing	Chinical data
Patient 1	46,XY arr[GRCh37] 18q21.32(57291428_57830158) x3 <i>de novo</i> origin	SCN2A gene NC_000002.11: g.166166923C>T (p.Ala263Val) <i>de</i> <i>novo</i> origin	Early myoclonic epileptic encephalopathy, severe psychomotoric retardation, spasticity, no apparent facial stigmatization, no speech, slightly abnormal dermatoglyphic pattern of upper limbs
Patient 2	46,XX arr[GRCh37] 1p36.32(2694731_3202657)x3 maternally inherited	SCN2A gene NC_000002.11: g.166243337A>G (p.Met1545Val) <i>de</i> <i>novo</i> origin	Early infantile myoclonic encephalopathy, severe psychomotoric retardation, epilepsy, axial hypotonia, limb spasticity, facial stigmatization, feeding difficulties, abnormal dermatoglyphic pattern of left palm

Table 1: The overview of clinical data and genomic analyses in patient 1 and patient 2. The patients were examined through multi-step diagnostic algorithm including clinical genetic evaluation and genetic counselling.

Variables		R-values		
		Duplication 18q21.32	Control region 18q12.1	
	Patient 1	1.46	1.13	
Family 1	Mother	0.88	1.08	
	Father	1.00	1.16	
		Duplication 1p36.32	Control region 1p31.1	
	Patient 2	1.48	0.92	
Family 2	Mother	1.60	1.05	
	Father	1.05	1.03	

Table 2: The verification of CNVs using R-values calculation based on relativeqPCR results. R-values were calculated for both the regions of CNVs and controlregions in families 1 and 2. Threshold R-values: <0.7 means loss of genetic</td>material in targeted DNA region, >1.3 means gain of genetic material in targetedDNA region.

on 10/18/2016) [21] and dbSNP annotation was added to the filtered variant calls using snpEff (v4.2). Final variants were extracted from the filtered variants by targeted regions and by association with genes of interest by vcftools (v0.1.15) [22] and bcftools (v1.3) [23]. Additional analyses and annotations were done using R (v3.3.1) [24] with libraries data [25] and Variant Annotation [26].

#### Sanger sequencing and data analysis

We validated every pathogenic or likely pathogenic single-nucleotide variant (SNV) and its de novo status in patients and their parents by targeted Sanger sequencing. DNA primers were designed using Primer3, Primer Blast, UCSC in-silico PCR and Oligo Analyzer tools. Then standard PCR were performed according to the manufacturer's recommendations. (Promega, Madison, WI, USA). PCR products were purified using Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase following manufactory recommendation (Thermo Fisher Scientific). Primers were synthetized by Integrated DNA Technologies (Coralville, IA, USA). Consequently, we performed the termination reaction to obtain ssDNA fragment library for Sanger sequencing (BigDye\* Terminator v3.1 Cycle Sequencing Kit following manufactory recommendation, Thermo Fisher Scientific). The overview of primer pairs for standard PCR and Sanger sequencing is presented in Table 2 in Supplementary material. Sequencing reactions were performed in the capillary electrophoresis DNA sequencer ABI 3130 (Thermo Fisher Scientific). Chromatograms were analysed using the software Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, MI, USA) and Chromas Lite v2.1 (Technelysioum Pty Ltd, South Brisbane, Australia).

# Results

Two children patients and their unaffected parents were enrolled in our pilot study using NGS with commercially available targeted design ClearSeq Inherited Disease. We describe their cases in detail. The overview of genomic analyses and basic clinical data is presented in Table 1.

# **Clinical data**

Patient 1 is a boy (\*2007) born from the 1<sup>st</sup> gravidity to unrelated parents (both at the age of 30). His mother was suffering from viral infection in 4<sup>th</sup> week of gravidity, since 25<sup>th</sup> week on a diabetic diet with insulin therapy since 32<sup>nd</sup> week. Patient 1 was born on 39<sup>th</sup> week of gestation (3680 g/50 cm). His father declared no relevant health complications. Since delivery our patient has been suffering from seizures with opistotonus. After the diagnosis of pathological EEG he was medicated by anti-epileptic therapy with no response longterm. He has been seizure-free since 6<sup>th</sup> month of age. He is currently medicated by antiepileptic drugs phenytoinum (Epilan<sup>®</sup> D-Gerot) and vigabatrinum (Sabril<sup>®</sup>) and benzodiazepine (Diazepam<sup>®</sup>). Suspected mitochondrial and neurotransmission disorders, MERRF, NAPR and MELAS syndromes were excluded and CDKL5 gene analyses was negative (DNA sequence analyses and MLPA). He was diagnosed early myoclonic epileptic encephalopathy (EMEE) and severe psychomotoric retardation of unclear etiology, with spasticity. He exhibits no apparent facial stigmatization, no speech and slightly abnormal dermatoglyphic pattern of upper limbs. To reduce the negative symptoms and outcome, he performs Vojta and Bobath rehabilitation method combined with hippotherapy, He is currently under the supervision of psychologist and speech therapeutics.

Patient 2 is a girl (\*2014) born from the 1st gravidity to unrelated parents. She was delivered 5 days post-term, 3400 g/50 cm. After the delivery she suffered from tachycardia, hypotonia and myoclonia. After magnetic resonance imaging and EEG, she was diagnosed with early infantile myoclonic encephalopathy with poor response to antiepileptic therapy. Suspected metabolic disorders, myotonic dystrophy type 1 and TORCH syndrome were excluded. She was diagnosed severe psychomotor retardation with epilepsy, axial hypotonia and limb spasticity. She has apparent facial stigmatization (narrow forehead, long palpebral fissures, low-set ears, carp mouth) and abnormal dermatoglyphic pattern of left palm. She is fed through percutaneous endoscopic gastrostomy (PEG) feeding tube.

#### Whole-genomic analyses and their verification

Both patients showed a normal karyotype by G-banding. Consequently array-CGH on oligonucleotide DNA microarrays was performed with CNVs findings in both patients. 539-kb duplication of 18q21.32 chromosome: arr[GRCh37] 18q21.32(57291428\_57830158)  $x^3$  in patient 1 and 508-kb duplication of 1p36.32 chromosome: arr[GRCh37] 1p36.32(2694731\_3202657) × 3 in patient 2 were detected using 4X180K CGH Microarray. The CNVs findings were verified in both patients and their parents using relative quantitative PCR analysis. The presence of 18q21.32 duplication was detected only in patient 1 and it was shown to have *de novo* origin. We evaluated this CNV as a variant of undetermined significance. The 1p36.32 duplication in patient 2 was shown to be maternally inherited. Therefore, we evaluate it as a likely benign variant. The R-values for both the CNVs regions and regions outside the CNVs in our patients and their parents are presented in Table 2.

Due to the unexplained severe pathological phenotypes which were highly suspected to have genetic causes our patients were involved to the pilot project using targeted NGS approach with capture design ClearSeq Inherited disease. We preferentially focused on exonic variants and we filtered only non-synonymous exonic variants (SNVs and insertions/deletions) unique for the given patient. These variants were analysed in detail including the search in ClinVar [21], OMIM [27,28], dbSNP [17] and UniProtKB/Swiss-Prot [29] variant pages. Finally, our findings were correlated to patient's phenotype and were verified by independent analysis of Sanger sequencing in patients and their unaffected parents.

In patient 1 we identified total of 17 012 DNA sequence variants, when 16 120 of them (94.76%) with known reference single-nucleotide polymorphism identifier (rsID). As a result of multi-step analytical process we identified a heterozygous NC\_000002.11:g.166166923C>T transition affecting the *SCN2A* gene resulting in a p.Ala263Val substitution (Figure 1). This variant has been previously detected in patients with neonatal seizures and EIEE/OS [30,31]. To support our assumption of its pathogenicity we performed confirmative analysis by targeted Sanger sequencing in patient 1 and his parents. We proved the heterozygosity for p.Ala263Val substitution in patient 1 and confirm its *de novo* origin as presented in the Figure 2.

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Figure 1: Pathogenic genetic variant NC\_00002.11:g.166166923C>T in the SCN2A gene leading to amino acid substitution p.Ala263Val. The variant is present in heterozygous state in patient 1 (NGS data visualization in IGV 2.3.28).



*de novo* origin. The part of chromatograms showing the substitution NC\_000002.11:g.166166923C>T resulting in p.Ala263Val in the SCN2A protein which was detected in the heterozygous state in patient 1.

In patient 2 we identified total of 18 277 DNA sequence variants and 17 436 of them (95.40%) were classified with known rsID. After NGS data analysis and variant filtering we detected a heterozygous NC\_000002.11:g.166243337A>G transition affecting the SCN2A gene resulting in a p.Met1545Val substitution (Figure 3). To our knowledge, less than ten cases of this likely pathogenic variant have been reported so far [32,33]. Consequently, we performed targeted Sanger sequencing in patient 2 and her parents. We confirmed de novo origin and heterozygous state for p.Met1545Val in patient 2, as shown in Figure 4. This result gives a strong evidence for its pathogenic or likely pathogenic effect, respectively.

# Discussion

We present two cases of children with epileptic encephalopathies. They have been examined through multi-step diagnostic procedure including G-banded karyotype and array-CGH with negative results. Consequently, they were examined by targeted NGS using commercial panel ClearSeq Inherited DiseaseXT including 2742 genes associated to human congenital diseases, followed by verification by Sanger sequencing in their parents. Here, we focused on a detail characterization and clinical consequences of two unique pathogenic sequence variants in the SCN2A gene, p. Ala263Val in patient 1 and p.Met1545Val in patient 2.

The SCN2A gene is located at 2q24.3 chromosome region and encodes a sodium voltage-gated channel alpha subnuit 2, which belongs to nine-member sodium channel alpha gene family [34]. Voltagegated sodium channels are transmembrane glycoprotein complexes which function in generation and propagation of action potentials in neurons and muscles. They comprise of one pore-forming alpha subunit which may be associated with either one or two beta subunits [35]. The SCN2A protein composes of four internal homology repeats, each of them contains eight potential transmembrane segments, and multiple glycosylation and phosphorylation sites [36]. The SCN2A gene is predominantly expressed in brain tissue, especially in neurons and glia. The encoded SCN2A protein is distributed along the surface of granule cells in cerebellum and it is suggested its primary involvement in the transmission of information to Purkinje cells [37]. It plays a fundamental role in controlling electrical excitability during CNS development and plasticity [38]. Recent studies highlighted its important role in psychiatric diagnoses and neurologic diseases through the identification of multiple SCN2A pathogenic sequence SNVs. Predominantly, they were identified and confirmed as one of

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Figure 3: Likely pathogenic genetic variant NC\_000002.11:g.166243337A>G in the SCN2A gene leading to amino acid substitution p.Met1545Val. The variant is present in heterozygous state in patient 2 (NGS data visualization in IGV 2.3.28).



important causes in the wide spectrum of epileptic encephalopathies exhibiting a highly variable clinical picture [39,40].

*SCN2A*-related disorders represent a group of clinically variable pathological phenotypes. Allelic variants in the *SCN2A* gene have been identified as pathogenic in many children with severe clinical condition defined as early infantile epileptic encephalopathy type (EIEE) and in children with less severe benign familiar neonatal infantile seizures (BFNIS) [41]. EIEE/OS belongs to a group of genetically and clinically distinct epileptic encephalopathies. Approximately 75% of EIEE may progress to West syndrome, a diagnosis with an adverse prognosis including the arrest of psychomotor development and abnormal EEG pattern negatively affecting brain functions [42]. Wolff et al. defined two groups of patients with distinct seizure onset (before or after three months of age). These patients exhibit variable additional phenotypic features related to epilepsy, different impact of pathogenic variants in the *SCN2A* gene to encoded protein resulting in different response to treatment by Na+ channel blockers [43].

The variant p.Ala263Val has been already evaluated as pathogenic in a large amount of patients with EIEE/OS [44]. The amino acid alanine in the position 263 is located in the hydrophobic S5 segment of transmembrane domain I of the SCN2A protein. Functional studies on cell culture experiments have indicated that the alteration in ion channel causes gain-of-function neuronal hyperexcitability with a 3-fold increase in persistent Na+ current [30]. Schwarz et al. presented a cohort of patients with distinct SCN2A pathogenic sequence variants to observe common clinical characteristics [45]. The seizures recurred more frequently, and they persisted longer than in less severe BFNIS. There were presented also cases of patients with the same variant p.Ala263Val that was detected in our patient 1. The patients were diagnosed an apparent hypotonia and sleeping difficulties. They exhibited neonatal-onset complex episodic neurologic phenotype, including ataxia and myoclonia. The Na+ channel blocker treatment may reduce the negative symptoms of EIEE/OS and improve the prognosis of significant proportion of affected children. Wolff et al. (2017) reviewed a large cohort of 201 patients with SCN2A-related disorders including 71 patients not reported previously [43]. They reported six cases of recurrent p.Ala263Val variant. Three of them were diagnosed BFNIS with late onset episodic ataxia while three others exhibited more severe phenotypes (epileptic encephalopathy/Ohtahara syndrome) with adverse prognosis, as presented in our patient 1.

In contrast to p.Ala263Val variant, the p.Met1545Val variant in the *SCN2A* gene has been detected in less than ten patients with EIEE so far. The amino acid methionine in the position 1545 is located in the

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hydrophobic S1 segment of transmembrane domain IV of the SCN2A protein. However, its alterations and their functional impact have not been estimated so far. In 2016, Dilena et al. presented a case of newborn male with a manifestation of epileptic encephalopathy, characterized by abnormal EEG pattern and tonic-clonic migrating partial seizures [33]. The neurological examination showed hypotonia, poor movement and feeding difficulties as were noticed in our patient 2. The condition was poorly responding to a wide range of anti-epileptic drugs, but Na+ channel blocker carbamazepine therapy as prevention to seizure relapses had shown to be effective. Due the fact the developmental delay is notable; it probably indicates a significant contribution of epilepsies and seizures to abnormal brain functional development. Gokben et al. presented a study of early-onset epileptic encephalopathies using targeted NGS panel including sixteen genes [46]. They detected twelve pathogenic or likely pathogenic sequence variants and defined de novo heterozygous p.Met1545Val in the SCN2A gene as a novel pathogenic variant with a causative effect in patients with EIEE/OS.

As described in clinical data, both our patients share key features including epileptic encephalopathy with poor response on first-choice anti-epileptic therapy associated with severe psychomotor delay and hypotonia, however some apparent features are specific for each of them (the absence of apparent facial stigmatization and feeding difficulties in patient 1). Both the detected *SCN2A* pathogenic variants (p.Ala263Val in patient 1, p.Met1545Val in patient 2) change the structure of transmembrane domains in the protein, therefore we expect that some variants in other genes and non-coding sequence variants may modify the phenotypes and can explain the existing phenotypic variability between our patients and among patients with epileptic encephalopathies with causative variants in the *SCN2A* gene.

#### Conclusion

In our study we present two cases of children with epileptic encephalopathies, intellectual disabilities and multiple congenital abnormalities that were enrolled in our pilot study using targeted NGS as a novel step in diagnostic algorithm at the Department of Medical Genetics (University Hospital, Brno). We detected two *de novo* distinct pathogenic sequence variant in the *SCN2A* gene which change the structure of transmembrane domains in the *SCN2A* protein. Thus, we proved that *SCN2A* pathogenic sequence variants play an important role in the pathogenesis of EE with adverse prognosis.

In conclusion, our study confirmed that targeted NGS with a commercially available gene panel could represent a suitable alterative to whole-exome or whole-genome sequencing, respectively. Its advantage especially consists in more targeted analysis of disease-causing genes; therefore, we suggest targeted NGS as an additional step after whole-genome screening of CNVs using array-CGH. This combination of two sensitive and precise methods could significantly improve the quality of genetic counselling in affected families. Furthermore, recent findings show that targeted NGS may contribute to the discovery of rare pathogenic sequence variants in the molecular genetic diagnostics of CNS disorders.

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