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The spectrum of intermediate SCN8A-related epilepsy

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Abstract:

Objective: Pathogenic variants in *SCN8A* have been associated with a wide spectrum of epilepsy phenotypes, ranging from benign familial infantile seizures (BFIS) to epileptic encephalopathies with variable severity. Furthermore, a few patients with intellectual disability (ID) or movement disorders without epilepsy have been reported. The vast majority of the published *SCN8A* patients suffers from severe developmental and epileptic encephalopathy (DEE). In this study, we aimed to provide further insight on the spectrum of milder *SCN8A*-related epilepsies. Methods: A cohort of 1095 patients was screened using a next-generation sequencing panel.

Further patients were ascertained from a network of epilepsy genetics clinics. Patients with severe DEE and BFIS were excluded from the study.

Results: We found 36 probands who presented with a *SCN8A*-related epilepsy and normal intellect (33%) or mild (61%) to moderate ID (6%). All patients presented with epilepsy between age 1.5 months and seven years (mean 13.6 months), and 58% of these became seizure free, 2/3 on monotherapy. Neurological disturbances included ataxia (28%) and hypotonia (19%) as the most prominent features. Interictal EEG was normal in 41%. Several recurrent variants were observed including Ile763Val, Val891Met, Gly1475Arg, Gly1483Lys, Phe1588Leu, Arg1617Gln, Ala1650Val/Thr, Arg1872Gln and Asn1877Ser.

Significance: With this study, we explore the electro-clinical features of an intermediate *SCN8A*-related epilepsy with mild cognitive impairment and for the majority a treatable epilepsy.

Key words: SCN8A, voltage-gated sodium channels, epilepsy, epilepsy genetics, intellectual disability

Introduction: SCN8A encodes the voltage-gated sodium channel Nav1.6, which is primarily expressed in excitatory neurons with high concentrations at the axon initial segment and the node of Ranvier. Pathogenic variants in SCN8A are associated with a spectrum of epilepsy phenotypes, ranging from rare families with benign familial infantile seizures (BFIS) to severe early onset developmental and epileptic encephalopathies (DEE) (EIEE13, OMIM #614558). Since the first case report published by Veeramah and colleagues in 2012¹, several reports have confirmed the role of SCN8A, primarily in patients with DEE²⁻⁹. The majority of patients with SCN8A DEE, described so far, have a severe phenotype characterized by early seizure onset, difficult to treat seizures, severe intellectual disability (ID), motor disorders and a relatively high mortality²⁻¹⁰. Functional studies of selected variants causing DEE, have revealed gain-of-function as the main pathogenic mechanism^{1; 6; 11; 12}. This gain-of-function comes from hyperactivity of the ion channel, due to elevated persistent sodium currents, hyperpolarizing shifts in the voltage dependence of activation or impaired channel current inactivation^{12; 13}. This mechanism is the opposite of the one that has been demonstrated in Dravet syndrome, which is characterized by loss-of-function variants in *SCN1A*¹⁴. *SCN1A* encodes the voltage gated sodium channel Nav1.1, which is primarily expressed in inhibitory interneurons. Variants in SCN2A, encoding a third voltage-gated sodium

channel in the human CNS, can lead to both gain- and loss-of-function, complicating things even more¹⁵. Awareness of these important differences in pathophysiology is necessary, as it might have therapeutic implications¹⁶.

We have recently detected a recurrent *SCN8A* variant, Glu1483Lys, in a very mild familial epilepsy phenotype¹⁷. We identified three unrelated families with a total of 16 family members who presented with BFIS and normal cognition. Five family members developed paroxysmal kinesigenic dyskinesia (PKD)¹⁷. Recently, Han et al. confirmed BFIS as part of the phenotypic spectrum in *SCN8A*-related epilepsies¹⁸. Beside these two extreme phenotypes, there is an increasing number of patients with a milder form of DEE. In this study, we describe the spectrum of the electroclinical features of this intermediate *SCN8A*- epilepsy phenotype, aiming to provide a clearer picture for clinicians, genetic counselors and affected families.

Methods and material:

We systematically screened all exons and exon-intron boundaries of *SCN8A* in a cohort of 1095 unselected patients with various forms of epilepsy using different next-generation sequencing (NGS) panels¹⁹. The panels included from 45 to 500+ genes related to epilepsy, intellectual disability or autism. Variants were assumed to be pathogenic if they arose *de novo*, or were inherited from an affected parent or affected/unaffected mosaic parent, and if they were nonsynonymous, splice-site altering or frameshift causing, and not present in controls in the gnomAD browser (see web resources). Sanger sequencing confirmed variants and segregation. Furthermore, detected variants were tested (PolyPhen-2, SIFT and MutationTaster) for predicted pathogenicity. ACMG and MPC scores are noted in table 1. All variants, except for one, were either pathogenic or likely pathogenic according to the ACMG criteria²⁰.

Four different metrics were used for in silico variant pathogenicity prediction:

1) The 'Missense badness, PolyPhen-2, and Constraint score' (MPC), which demonstrates a 5.8 times increased variant enrichment in cases compared to individuals from the general population for MPC scores $>2^{21}$

2) The 'paralog conservation score' (parazscore), which quantifies the amino-acid positions conservation across human proteins of the same gene family. A significant enrichment of disease-associated missense variants was observed at paralog-conserved sites²²;

3) The Grantham score, which accesses the effect of the amino acid substitution based on the properties of the amino acid exchange. It ranks amino acid substitutions from similar amino acids substitutions (0) to substitution which differs in their chemical properties ones (215)²³.
4) The allele frequency analysis based on the alleleFrequencyApp (see web resources), which calculates a maximum credible number of possible pathogenic alleles observed in gnomAD. The allele count is estimated based on the disease prevalence, the allelic and genetic heterogeneity and the variant penetrance. For *SCN8A* we specified the disease prevalence as one in 300 the allelic heterogeneity as 0.01, the genetic heterogeneity as 0.1 and the penetrance as 50% resulting in a maximum number of a single allele in gnomAD. We compared the corresponding value with the allele frequency of variants present in gnomAD.

SCN8A positive patients underwent a detailed clinical evaluation and patients with severe DEE and BFIS were excluded from the study. The criteria for severe DEE were defined as severe, pharmacoresistant epilepsy and developmental impairment, as previously reported ²⁴. BFIS is a self-limiting epilepsy syndrome characterized by afebrile seizures typically in clusters, with onset between four and eight months. Neurological examination, psychomotor development and the interictal EEG are normal and the children become seizure free within the first years of life and sustain seizure freedom without the aid of antiepileptic drugs (AEDs)²⁵.

Additional probands were collected from an international network of epilepsy genetics clinics. Data on clinical phenotype, genetics, neuroimaging and EEG were requested for all patients (and if possible relatives), who were included in the study. Seizures were classified according to the International League Against Epilepsy (ILAE)²⁶. All probands, and in case of minors, legal parents, provided written informed consent. The study was approved by the local ethical committees. Sodium channel blockers (SCBs) were defined as AEDs that target sodium channels and included carbamazepine (CBZ), oxcarbazepine(OXC), lamotrigine(LTG) and phenytoin (PHT). Data sharing: anonymized data will be shared by request from any qualified investigator.

Results:

By the use of targeted NGS screening of *SCN8A* in a cohort of 1095 patients, we identified 12 (1.2%) probands with a predicted pathogenic variant in *SCN8A*. Six of the patients fulfilled the

criteria for a severe DEE and thus excluded from this study. Of the 1095 patients screened, approximately 326 fulfilled a DEE diagnosis (this might be an underestimate, as referrals to our center are often lacking clinical information). In addition to the remaining six patients, we ascertained 30 additional probands/families with predicted pathogenic SCN8A variants through collaborating diagnostic and research laboratories. The Asn1877Ser variant was seen in three controls in gnomAD, but as the phenotype resembles a benign familial epilepsy, with several family members affected, and was seen in three probands in this study, it was included as being pathogenic. All variants, except #10 p.Tyr1241Cys, were classified as pathogenic or likely pathogenic according to the ACMG criteria²⁰. Not all missense variants in SCN8A are pathogenic and at 384 amino acid positions variants have been reported in the general population²⁷. Distinguishing disease-causing variants from benign is still a challenge in clinical genetics. Rarity of an allele is widely recognized as a necessary (though not sufficient) criterion for variant pathogenicity²⁰ (ACMG guidelines, but the key question "how common is too common?" remains poorly answered for many diseases.) Estimating the genetic and allelic heterogeneity offers an opportunity to identify variant cut-off frequency filters. Genetic heterogeneity is the maximum proportion of disease that is attributable to variation in a single gene, and allelic heterogeneity is the maximum proportion of variation within a gene that is attributable to a single allele. The patient variant 10 (c.3722A>G, p.Tyr1241Cys, NM_014191.3) was found once in the gnomAD²⁷ and discovEHR²⁸ database. The presence of a variant in databases represents not a pathogenicity exclusion criterion based on our allele frequency analysis for mild forms of epilepsy. The amino acid residue position is relatively conserved across voltage-gated sodium channels (positive parazscore of 0.23^{22}). In addition, the MPC score (MPC = 2.48) supports variant pathogenicity and the Grantham score of 194 indicates a likely pathogenic variant.

One of the probands (#30) has previously been mentioned briefly²⁹, but was included in this study since additional data had been collected.

In total, 36 probands/families were included. The detected *SCN8A* variants were mainly missense variants (33/36) scattered throughout the gene. In addition, two truncating variants (one frameshift (#4) and one stop (#30)), and one variant causing a nucleotide change eight basepairs upstream of the start codon (#1) were detected. Fourteen variants were located in the transmembrane domains and two in the poreforming domain, of the cytoplasmic variants, we

found one in the D1/D2 domain. The variant Asn544fs* was a frameshift variant with a clinical picture of absence epilepsy, inherited from an affected parent, and classified pathogenic according to the ACMG classification guidelines.

The variants either occurred *de novo* or segregated within the family in a dominant fashion (#2, #4, #7, #14, #15, #33 and #34, see figure 1 for selected pedigrees). In the family of proband #33 the variant was inherited from an affected mosaic parent. Mosaicism was also suspected in #15, but has not yet been confirmed. All variants were located at highly conserved residues and predicted possibly damaging according to computational prediction software (see methods and web resources). Mining the available literature and databases, eight variants were found to be recurrent either within this study or overall; Ile763Val³⁰, Val891Met³¹, Gly1475Arg^{10; 32}, Gly1483Lys¹⁷, Phe1588Leu, Arg1617Gln^{5; 7; 8; 33; 34}, Ala1650Val^{5; 7; 35} (different amino acid substitution, see discussion section), Arg1872Gln^{7; 12; 36} and Asn1877 Ser^{30; 35; 37}, with five of them (amino acid positions 763, 1475, 1617, 1650, 1872) seen in severe DEE phenotypes as well. Clinical data on all 36 probands and families are presented in table 1. Age at inclusion varied from nine months to 35 years, with a mean of 7.9 years. Seizure onset was between six weeks and seven years, with a mean of 13.6 months (SD ±17 m). Seizure semiology was very diverse, and included generalized tonic-clonic seizures (GTCs) (21), focal (14), tonic (8), myoclonic (6) and atonic seizures (8), as well as epileptic spams (2) and atypical / typical absences (12). Seizure severity did not progress over time and seizure triggers were not found. Three probands experienced convulsive status epilepticus (#3, #14, #33).

The interictal EEG was available in 33/36 patients and showed focal epileptiform abnormalities in 15 patients (45%), predominant in the posterior quadrants in (ten patients) or in the central/ centro-parietal / fronto-central regions (five patients), with or without bilateral spreading. Four patients (12%) had only generalized abnormalities (#6, #14, #22, #26). In 14 patients (39%) the interictal EEG was normal (#9, #11, #12, #13, #16, #17, #20, #23, #29, #34, #35) or normalized at follow up (#15). Ictal EEG was available in four patients and showed a focal discharge in two (#6, #11) and generalized spike or irregular spike and waves discharges in the other two (#19, #28). Cognitively, these patients fare well. Before seizure onset 26/36 (72%) had normal cognitive development, two/36 (6%) had a mild ID, one/36 had moderate ID (3%) and four/36 (11%) had developmental delay, not classified due to the young age of the patient. At follow-up, a

deterioration from normal intellect/developmental delay to mild ID or from mild ID/developmental delay to moderate ID was seen in seven (19%) patients, whereas 81% did not experience deterioration at seizure onset. After seizure onset (and at the time of follow-up) 22/36 (61%) had mild ID and two/36 (6%) had moderate ID.

Additional features included ataxia (10/36), hypotonia (7/36), language delay (5/36), autism/autistic features (4/36), movement disorders (3/36) including paroxysmal dyskinesia (3/36), gait disturbances (2/36), sleep disturbances (1/36), learning difficulties (2/36) and ADHD (1/36). See table 1 for details.

All probands were evaluated for their treatment response. Twenty-one/36 (58%) probands became seizure free. Monotherapy was sufficient in 12/21 (57%) probands, and included lamotrigine (LTG) (2), carbamazepine (CBZ) (1), phenytoin (PHT) (1), valproate (VPA) (1), vigabatrin (VGB) (1), ethosuximide (ETX) (1), phenobarbital (PB) (1) and levetiracetam (LEV) (1). In total 13/36 (36%) probands became seizure free with therapy that included a SCB either in monotherapy or in combination with other antiepileptic drugs (AEDs). One became seizure free with a small dose of cannabidiol. However, 7/36 (20%) probands became seizure free without the use of SCBs. Seizure offset was only available for six patients, and ranged between four and 10 years, mean age at seizure offset was 7.7 years.

Six probands had affected family members, segregating with the variants. For proband #5 a similar phenotype with absences and learning/language difficulties was seen in the sister, as well as the mother. For proband #7, the mother also had unspecified epilepsy and carried the variant; there was also an affected maternal grandfather, who did not have genetic analysis done. Likewise, proband #14 had an affected father, who carried the variant, and an affected paternal grandfather, who was not tested genetically. Proband #15 had a sister with a similar epilepsy phenotype, the variant was suspected to have been inherited from a mosaic parents, but this was not confirmed at the time of preparation of this paper. Proband #33 had an affected sister with a similar phenotype, and genetic investigations showed that the variant was inherited from an affected mosaic (28%) father. Proband #34 had an affected sister and mother, in whom the variant segregated.

Discussion:

Pathogenic variants in *SCN8A* have so far been described in patients with different epilepsy phenotypes, including rare families with BFIS and in >100 patients with mild to severe DEE. In this study, we describe the electro-clinical phenotype of 36 patients with intermediate epilepsies due to pathogenic variants in *SCN8A*. Patients with *SCN8A*-related severe DEE²¹ and BFIS¹⁸ were excluded from the study.

The variant at position c.-8A>G was assumed to be likely pathogenic because it occurred de novo and the clinical features of the patient resembled the phenotype seen in other SCN8A patients. The variant is located outside of the Kozak consensus sequence, but may lead to increased RNA stability or translational initiation or result in altered splicing pattern. These theories can only be confirmed by functional testing of the variant, which unfortunately was not possible in this study. Until then, the variant may need to be classified as a variant of unknown significance. All 36 patients presented with seizures in early childhood (mean 13.6 months). Before seizure onset, 72% probands had normal cognitive development. More than half of the probands became seizure free, 57% of these with monotherapy. Compared to the BFIS families, described by Gardella et al¹⁷, Anand et al³⁷ and Han et al¹⁸, the majority of the probands in this cohort have cognitive impairment, with 6% suffering from moderate ID, and 61% from mild ID. Furthermore, only 58% became seizure free compared to almost 100% of the BFIS patients; seizure freedom is exceptional in the severe DEE phenotype. The patients herein described also appeared to have additional neurological disturbances including primarily ataxia (in 28%) and hypotonia (in 19%). In the severe DEE cohort, the incidence of ataxia is around 11%, compared to the 28% of this cohort. However, it is important to notice that many of the patients suffering from severe SCN8A DEE are unable to walk autonomously. Furthermore, the patients in this intermediate cohort do not suffer from the spasticity and paraplegia or the extra-pyramidal/cerebellar symptoms that up to 50% of the severe DEE patients do²⁴. A few patients in this cohort (8%) had dyskinesia, which is also seen in both severe DEEs and BFIS families. Growth impairment (microcephaly or reduced growth) observed in severe DEE, was not seen in this cohort. Other prominent phenotypic features included language delay/difficulties in 14% and movement disorders not further specified in 8%. In mouse models, it has been shown that SCN8A is widely expressed, both in the motor neurons of the brain stem, as well as in many types of neurons in the cerebellum, where functional deficits in Purkinje cells have been found³⁸⁻⁴⁰, confirming the importance of *SCN8A* in motor function. This

could explain the involvement of the motor system, and why cerebellar atrophy and ataxia⁴¹, associated with intellectual impairment, appeared to be major features in subjects harboring *SCN8A* variants. The cerebellum does, however, also play an important role in language and grammar processing, verbal working memory and speech motor planning⁴², and a large proportion of *SCN8A* patients, including those reported in the present cohort, show an impairment of these functions as well.

When comparing the present cohort to the severe *SCN8A* DEE population, in which the patients have earlier seizure onset, with more pronounced cognitive deficits as well as refractory epilepsy, we tried to identify possible predictive factors, that in newly diagnosed patients could help to detect those with a milder course as compared to those with a more severe evolution. First, within our cohort, the majority of probands have normal development prior to seizure onset (and 33% of them continue to develop normally) and maintain a normal EEG (41%). This is often not the case in probands who develop severe DEE, in which the majority usually are cognitively delayed from birth⁴³, or will present with cognitive difficulties early on and show changes in their EEGs. However, it is not an absolute feature, as we have observed several children with an extremely severe follow up, despite early normal development. Second, seizure onset occurred at a mean age of 13.6 months, compared to a mean age of 4 months in the DEE group⁴³. Anyway, it is worth to bear in mind that the age deviation in this cohort is quite large (SD±17 months), and thus these numbers should be interpreted carefully when counselling a family. Last, seizure freedom was obtained in 58% of the patients in this cohort and it was achieved rapidly and with monotherapy in 2/3. In contrast, only about 5% of patients in the severe DEE cohort achieve seizure freedom with monotherapy. This is important, albeit also a prerequisite in this study, where the more severe epilepsies were excluded.

Previously, it has been hypothesized, that seizures in patients with *SCN8A* variants should respond to SCBs ^{16; 17}. A beneficial effect of SCBs was observed in 36% of the patients reported in this study, either as monotherapy or in combination with other AEDs, and supportive of this, previous studies have shown a gain-of-function of the Arg1617Gln and Arg1872Leu¹² variants. Yet, 19% became seizure free without the use of SCBs, suggesting that seizure freedom should not be attributed solely to the use of SCBs, but also considered a phenotypic trait.

Interestingly, a partial benefit of LTG was also observed in the proband with a stop variant (#30). We can speculate that this unexpected finding may depend on genetic modifiers and differences in genetic background, or the fact that as the stop-codon is located at the c-terminal part of the protein, the transcript does not undergo nonsense-mediated decay.

Furthermore, we found three variants, i.e. one eight basepairs upstream of the start codon (#1), one frameshift variant (#4) and one stop variant (#30), all suspected to be loss-of-function, and all of them associated with an epilepsy phenotype. Previously, it has been hypothesized, that loss-of-function variants would not cause epilepsy⁴⁴, but rather present with ID with or without motor function abnormalities, such as ataxia^{2; 34; 41}. However, Blanchard et al. also describe a patient with epilepsy and ID, carrying a LOF variant¹¹, and two existing *Scn8a* knock-out mice models, supports the notion of epilepsy also being a feature of LOF of NaV1.6^{45; 46}. Other ion channel genes, including *SCN2A* have been shown to have a similar clinical picture, where both GOF and LOF variants may cause epilepsy. The underlying functional causes are yet to be fully elucidated. Variants were found throughout the gene (see figure 3), including the transmembrane segments, cytoplasmic loops and the inactivation gate, thus location in the gene is likely not predictable of functional or clinical effects of the variant, however, the figure does display the fact that the majority of the variants are found in domain three and four, the inactivation gate and the c-terminal, which may help guide variant interpretation.

Indeed, we identified several recurrent variants and observed a wide range of phenotypic variability for variant carriers. Ile763Val, Gly1475Arg, Arg1617Gln, Ala1650Val and Arg1872Gln were all seen in this study as well as in patients with DEE. Ile763Val has previously been described in a patient with intractable epilepsy and moderate ID³⁰, whereas we found it in two patients with focal epilepsy and mild ID (#5 and #6).

The Gly1475Arg variant is even more diverse and it has previously been identified in several patients, including a child with severe DEE, who died from probable SUDEP^{10; 32}. In this cohort, we found it in two patients with mild ID, and epilepsy controlled by SCBs. Both patients did suffer from hypotonia and ataxia.

Arg1617Gln has been seen in several DEE patients previously, including a girl with severe DEE who died after terminal progression of her disease^{2, 17}, whereas we found it in two patients with mild ID and treatable epilepsy (#24, #25). Still, they did display additional neurological disturbances

(language delay, dyskinesia) and their seizure onset was earlier (4 months), compared to the rest of this cohort. We also found the Arg1617GIn variant in one patient (#21), who displayed moderate ID as well as autism. Ala1650Val variant has not been described before (#26), but has been seen several times with a threonine (Ala1650Thr) substitution in patients with severe DEE^{5; 7; ³⁵. Arg1872GIn has also been seen several times in patients with severe DEE^{7; 36}, however we found it in a sib pair, with normal intellect and focal epilepsy (#34). The Gly1483Lys variant has previously been described in BFIS¹⁷; the patient in this study (#18) carrying this variant has indeed a very mild phenotype; with only speech delay, sporadic seizures and discrete focal EEG abnormalities. Why some variants show phenotypic heterogeneity and other variants do not, remains elusive. Of course, differences in the amino acid substitution might explain some of the difference, but this is true for just a few of the variants. In other cases, genetic modifiers and differences in the genetic background could underlie these observations. Further studies are needed to investigate this further.}

In conclusion, this study we provide further insight on the phenotypic spectrum of *SCN8A* epilepsy by focusing an intermediate phenotype characterized by treatable epilepsy with a later age of onset, mildly impaired cognitive development, as well as variable but in general mild neurological disturbances. A positive response to epilepsy treatment, especially with SCBs, was observed. Even if a wide range of phenotypes related to *SCN8A* variants can be expected (see illustration in figure 2), these findings highlight the presence of an increasing number of *SCN8A* patients with a phenotype of moderate severity.

The partial overlapping of genetic and early clinical features in *SCN8A*-related epilepsies makes it difficult to provide proper counselling in these children so far. Further investigations are warranted to clarify this issue, as well as explore possible prognostic factors.

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Conflicts of interest:

None of the other authors have any conflict of interest to disclose.

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Ethical publication statement:

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.



Web resources:

- The ExAC browser: http://exac.broadinstitute.org
- The GnomAD database: http://gnomad.broadinstitute.org/
- SIFT: http://sift.jcvi.org
- Poly-Phen2: http://genetics.bwh.harvard.edu/pph2/
- MutationTaster: http://www.mutationtaster.org
- The allele frequency app: https://www.cardiodb.org/allelefrequencyapp

Key Bullets:

- The phenotypic spectrum of SCN8A is wide, from BFIS to DEE
- The intermediate phenotype is characterized by a treatable epilepsy and mild cognitive delay
- Prognostic markers remain elusive



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Author Man

Table 1. Clinical characteristics of the probands

Proba	Age at	Variant,	Pathogenicity	Family history	Age at	Seizure	Interictal EEG /	Cognition before	Treatment	Succesfull	Other features
nd #	inclusio	Inheritance,	(ACMG		seizure	types	Ictal EEG	sz onset /	response	monotherapy	
	n –	location	guidelines,		onset			cognition after sz			
		5	MPC)					onset			
1	2 y 8 m	c8A>G	Pathogenic	None	11 m	GTC, AA	EDs on the P-	N/ MID	Sz		Hypotonia,
	U	de novo					temporal		reduction:		uncoordinated
	_	5'UTR					regions		VPA 50%		movements,
							bilaterally, with				gait disturbance
		_					rare diffuse				
	Π	K					spreading				
2	9 y	c.411C>G	Likely	Mother, at 10-	2 y 2 m	Staring/	Temporal-O	MID/MID	Sz		Hypotonia,
		p.lle137Met	pathogenic	11 y: episodes		hypotonia	spikes / SW		reduction:		Hypothyroidism
		maternal	(PM1, PM2,	of loss of		to GTC, A, F	(left side		LTG, VPA,		Enuresis,
	<u> </u>	transmembr	PP1, PP2, PP3)	balance, lower		(visual)	predominance)		ZNS		sleep disorders
	C	ane domain		limb					No effect:		
		D1S1	MPC=1.66	hypostenia and					LEV, RFN,		
		_		falls, gait					трм		
				disturbance							
		D		and hand							
				tremor							
3	9 y 4 m	c.1122C>G	Likely	maternal	7 y	Nocturnal	Multifocal SW,	N / MID	Sz free: PHT,		Gait disturbance
		p.Asn374Lys	pathogenic	cousin with sz		frontal, T,	paroxysmal		VPA		
		de novo	(PS2, PP2, PP3)			GTC, SE	beta activity		No effect:		
		poreforming					that lasts		OXC, LEV,		
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	domain	MPC=1.83				between 1-2		ZNS, CLB		
						minutes				
4	c.1630_163	Pathogenic	See fig 1.	2 y 9 m	Ab	Slow wave and	N / MID	Sz free: VPA		
	1del	(PVS1, PM2,				SW discharges		+ ESM+		
	p.Asn544fs*	PP1)						AMD		
	39							Sz		
	maternal							reduction:		
	cytoplasmic							VPA + ESM		
	domain									
	D1/D2									
5	13 y 2287A>G	Pathogenic	Cousin with	7 wk	GTC	NA	NA / MID	Sz free: CLB,		Hypotonia,
	p.lle763Val	(PS1, PS2, PM1,	febrile sz					ZNS, PHT,	i	ataxia,
	de novo	PM2, PP2)						LTG, CBZ		chronic constipation,
	transmembr							Adverse		premature adrenarche,
	ane domain	MPC=1.51						effects: LEV		periodic leg movement
	D2S1							(behavior),		
								CLZ and LZP		
	-							(sleepiness)		
6	20 y c.2287A>G	Pathogenic	None	3 m	S, F	EDs, both	NA / MID	No effect:		Ataxia
	p.lle763Val	(PS1, PS2, PM1,				hemispheres		VPA, CBZ,		
	de novo	PM2, PP2)				Ictal: right F-		LTG, CLB,		
	transmembr					temporal onset,		acetazolami		
	ane domain	MPC=1.51				then rhythmic		de		
	D2S1					slow activity				
						propagating				

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								into the right				
								parasaggital				
								regions				
7	11 y	6	c.2671G>A	Pathogenic	See fig. 1	3 у	GTC, AA	CP and midline	N / MID	Sz free: CBZ	CBZ	ADHD
			p.Val891Me	(PS1, PM1,				spikes				
			t	PM2, PP1, PP2)								
			maternal									
			transmembr	MPC=3.14								
			ane domain									
		_	D2S5									
8	10 y	C	c.2806G>A	Likely	Father with ID	5 у	F, AA, FS	SW left F, C,	NA / MD	Sz free: LEV	LEV	Autistic features,
			p.Glu936Lys	pathogenic	and ADHD			right P		Adverse		behavioral problems
		C	non-	(PM1, PM2,						effects: TPM		
			maternal	PP1, PP2)								
			poreforming									
			domain	MPC=3.33								
9	9 y 9 i	m	c.3601G>A	Likely	None	8 m	М	Initially normal	DD / MID	Sz		Hypotonia,
			p.Glu1201Ly	pathogenic				(1y - 3y4 m),		reduction:		ataxia,
	_		s	(PS2, PM1,				then BG		VPA, LEV,		paroxysmal dystonia
		-	de novo	PM2, PP2)				slowing (7-10y)		CLB		
			transmembr					Postictal:		No effect:		
			ane domain	MPC=2.37				abundant beta,		STP		
			D3S1					diffusely slow		Sz		
								BG		aggravation.		
										охс		

10	2 y	c.3722A>G	VUS	None	8 m	F, A	Focal posterior	NA / MID	Sz free: VGB	VGB	Paroxysmal dyskinesia
		p.Tyr1241Cy	(PM1, PP2)				bilateral EDs				
		S									
	Ċ	pending	MPC=2.48								
		transmembr									
		ane domain									
	C	D3S2									
11	2 y 1 m	c.3953A>G	Likely	None	3 m	F, GTC	Normal	N / N	Sz		Ataxia
		p.Asn1318S	pathogenic				Ictal: EDs on		reduction:		
	_	er	(PS2, PM2, PP2)				the P regions		CBZ, PER		
	С	de novo					bilaterally		No effect:		
		cytoplasmic	MPC=2.04						РВ, ОХС,		
		linker							CLB, ZNS		
		D3S4/D3S5							Adverse		
									effects: TPM		
									(hyperther		
									mia)		
12	2 y 3 m	c.3956C>A	Likely	None	2 m	C, T, GTC	Normal	N / MID	Sz		Ataxia,
		p.Ala1319As	pathogenic				Ictal EEG:		reduction:		Tremor, language delay
	+	р	(PS2, PM2, PP2)				parasagittal		РНТ		
	_	de novo					EDs, F rhythmic		No effect:		
		cytoplasmic	MPC=2.69				delta		VPA, PB		
		linker									
		D3S4/D3S5									

13	7 y 11 m	c.3967G>A p.Ala1323Th r, de novo cytoplasmic domain D3S4/D3S5	Likely pathogenic (PS2, PM2, PP2) MPC=2.22	None	5 m	FS, GTC	Normal	N / N	Sz free: VPA	VPA	
14	0	c.4391T>C	Likely	See fig. 1	7 m	A, GTC, SE	Diffuse	NA / MID	Sz		
		p.lle1464Th	pathogenic				abnormal		reduction:		
		r	(PM1, PM2,						TPM, VPA		
		paternal	PP1, PP2)								
	0	inactivation									
		gate	MPC=2.65								
15	10 y 6 m	c.4423G>A	Likely	See fig. 1	9 m	F to GTC, AA	Slowing over	NA / MID	Sz free: LTG	LTG	Hypotonia,
		p.Gly1475Ar	pathogenic				the temporal		Sz		ataxia,
	2	g,	(PS1, PM2, PP1,				region (3y),		reduction:		autistic features
		paternal,	PP2)				then normal		OXC, CBZ		
		inactivation					(9y)		No effect:		
	+	gate	MPC=1.54						LEV		
16	9 m	c.4423G>A	Pathogenic	None	4 m	F, T	Ictal: Slowing	NA / MID	Sz free: PHT	РНТ	Hypotonia,
		p.Gly1475Ar	(PS1, PS2, PP2)				and EDs on		No effect:		ataxia
		g					both		LEV		
		de novo,	MPC=1.54				hemispheres,				
		inactivation					mainly				

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		gate					temporal				
							regions				
17	9 y 9 m	c.4423G>A	Pathogenic	None	11 m	GTC, F	Normal	N / N	Sz free: CBZ	CBZ	
	C	p.Gly1475Ar	(PS1, PS2, PP2)						Sz		
		g							reduction:		
	<u> </u>	de novo	MPC=1.54						VPA		
	C	Blood									
	C	mosaicism									
		10%									
	_	inactivation									
		gate									
18	8 y	c.4447 G>A,	Pathogenic	None	11 m	F, GTC	Normal	N / N	SZ free:		Clumsy, shy, speech delay
		p.Glu1483Ly	(PS1, PS2, PS3,				background,		VPA, CBZ,		
		s	PM2, PP2)				small Spikes /		currently no		
		de novo					theta pointu in		AED		
		inactivation	MPC=2.13				the central				
		gate					regions				
19	2 y	c.4585A>G	Likely	None	4 m	GTC	Right posterior	N / N	No effect:		
		p.Met1529V	pathogenic				slow activity		LEV, PB,		
	- +-	al	(PS2, PM1,				during sleep		VPA		
		de novo	PM2, PP2)				Ictal:				
		transmembr					generalized				
		ane D4S1	MPC=1.2				spike				
							discharges				

20	11 y	c.4764C>G	Pathogenic	None	3.5 m	GTC, AA, T,	Normal	DD / MID	Sz free: CBZ	CBZ	Autism,
		p.Phe1588L	(PS1, PS2, PM1,			F					language delay
		eu	PM2, PP2)								
	-	de novo									
		transmembr	MPC=2.4								
		ane D4S3									
21	13 y	c.4764C>A	Pathogenic	None	5 m	F, GTC	NA	N / MID	Sz		Obesity
		p.Phe1588L	(PS1, PS2, PM1,						reduction:		
		eu	PM2, PP2)						PB, CBZ		
	_	de novo,									
	С	transmembr	MPC=2.4								
		ane D4S3									
22	2 y 8 m	c.4840A>G	Likely	None	4.5 m	AA, GTC, M,	Generalized SW	N / MID	Sz		
		p.Thr1614Al	pathogenic			A			reduction:		
		а	(PS2, PM2, PP2)						VPA, OXC		
		de novo									
		extracellular	MPC=2.17								
	C	domain									
		D4S3/D4S4									
23	7 y 5 m	c.4850G>C	Pathogenic	None	5 m	GTC	Normal	DD / MD	Sz free: LTG	LTG	Autism
		p.Arg1617Gl	(PS1, PS2, PM1,								
		n	PM2, PP2)								
		de novo									
		transmembr	MPC=2.4								
		ane D4S4									

24	9 y	c.4850G>A	Pathogenic	Paternal first	5 m	GTC, M, A	NA	DD / MID		Language delay,
		p.Arg1617Gl	(PS1, PS2, PM1,	cousin with						paroxysmal dyskinesia
		n	PM2, PP2)	microcephaly						
		de novo								
		transmembr	MPC=2.4							
		ane D4S4								
25	24 y	c.4850G>A	Pathogenic	None	NA	GTC, F	NA	MID / MID	Sz free: CBZ,	Extra-pyramidal signs
		p.Arg1617Gl	(PS1, PS2, PM1,						VPA	
		n	PM2, PP2)							
		de novo								
		transmembr	MPC=2.4							
		ane D4S4								
26	7 y	c.4949 C>T	Pathogenic	None	11 m	M, A, T, S,	Generalized	NA / MID	Sz free: CLB,	Hypotonia,
		p.Ala1650Va	(PS1, PS2, PM2,			АА	EDs		RUF, KDSz	supraventricular
			PP2)						adverse	tachycardia
		de novo							effects: LEV	
		cytoplasmic	MPC=2.26						(increased	
		D4S4/D4S5							frequency),	
	_								ZNS (new sz	
		+							type), VPA	
									(neurodevel	
									opmental	
									regression)	
27	9 y	c.4961T>A	Likely	None	2 y 8 m	Ab	Generalized 3	NA / MID	No effect:	Ataxia

			p.lle1654As	pathogenic				Hz SW. Spikes		VPA		
			n	(PM1, PM2,				occipital right				
		-	unknown	PP2)				and left				
			transmembr									
	Ι.		ane domain	MPC=2.92								
			D4S5									
28	4 y		c.5273T>C	Likely	None	2 y	Ab, FS	Mild BG	N / N	Sz free: ETX	ETX	
			p.Val1758Al	pathogenic				slowing,		No effect:		
			a	(PS2, PM1,				irregular		LEV		
		_	de novo	PM2, PP2)				generalized				
			transmembr					EDs, OIRDA				
			ane D4S6	MPC=2.46				Ictal: irregularly				
		()						generalized,				
			_					3Hz SW				
	1							followed by				
								rhythmic				
								diffuse delta				
		C						activity				
		С						(clinically:				
		+	5					arrest of				
		_	5					ongoing				
								activity)				
29	9 y I		c.5311G>A	Likely	None	6 m	GTC, T	Normal	N / N	Sz		
			p.Val1771lle	pathogenic						reduction:		
			de novo	(PS2, PM2, PP2)						CBZ		

30 6 y 6 S458C>T Pathogenic None 3-4 m M in hands Trains of SW, ight CP. NA / MID Sz reduction: Ianguage delay 30 6 y 6 y 6 y 6 y 6 y Pathogenic None 3-4 m M in hands Trains of SW, ight CP. NA / MID Sz reduction: Ianguage delay 2 de novo PM2, PP2) PM2, PP2) None 6 m T EDS over the posterior lobe N / N Sz free: PB PB Reflux 31 15 y C5497C>C Likely None 6 m T EDS over the posterior lobe N / N Sz free: PB PB Reflux 32 7 minknown P22 MPC=2.44 None 9 m F, A, AA EDS, mid CP N / Mild learning disability Sz free: CBD CBD EDS N o effect: LEV EDS No effect: LEV EDS No effect: EDS No effect: EDS No effect: EDS EDS No effect: EDS EDS No effect: EDS EDS No effect: EDS EDS No effect:<				C-terminal									
30 6 y c.5458C>T Pathogenic None 3-4 m M in hands Trains of SW, right CP. NA / MID Sz Ataxia, Ianguage delay 31 15 y c.5497G>C Likely None 6 m T EDs over the posterior lobe N / N Sz free: PB PB Reflux 31 15 y c.5497G>C Likely None 6 m T EDs over the posterior lobe N / N Sz free: PB PB Reflux 32 3 y c.5597G>A, Likely None 9 m F, A, AA EDs, mid CP N / Mild learning disability Sz free: CBD CBD 32 1 y c.5597G>A, Likely None 9 m F, A, AA EDs, mid CP N / Mild learning disability Sz free: CBD CBD 32 1 y c.5597G>A, Likely None 9 m F, A, AA EDs, mid CP N / Mild learning disability Sz free: CBD CBD 12 1 y c.5597G>A, Likely None 9 m F, A, AA EDs, mid CP N / Mild learning disability Sz free: CBD CBD 14 c.5597G>A, N <td< td=""><td></td><td></td><td></td><td></td><td>MPC=2.02</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>					MPC=2.02								
b.Arg1820* (PVS1, PS1, brazenia kinetowia powerina kinetowia p	30	6у	Ť	c.5458C>T	Pathogenic	None	3-4 m	M in hands	Trains of SW,	NA / MID	Sz		Ataxia,
<i>ie novo</i> PM2, PP2) PM2, PP2) Image: PP2 imag				p.Arg1820*	(PVS1, PS1,			and fingers	right CP.		reduction:		language delay
31 5 y c,5497G>C Likely None 6 m Loss EDs over the posterior lobe N / N Sz free: PB PB Reflux 31 15 y c,5497G>C Likely None 6 m F F DS over the posterior lobe N / N Sz free: PB PB Reflux 10 m/nown P2) MPC=2.44 None P				de novo	PM2, PP2)				Irregular delta		LTG		
Image: sector				C-terminal					activity on the				
 31 15 y c.5497G>C Likely p.Asp1833H pathogenic (PM1, PM2, UNC, UNC, CTerminal PP2) 32 3 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 4 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI pathogenic (PS2, PM2, PP2) 5 y c.5597G>A, Likely p.Arg1866GI p.Arg1866GI			C						left side				
p.Asp1833H pathogenic p.Asp1833H pathogenic posterior lobe No effect: LEV is nknown P2,0 nP2,0	31	15 y		c.5497G>C	Likely	None	6 m	т	EDs over the	N / N	Sz free: PB	РВ	Reflux
is unknown C-terminal(PM1, PM2, P2) 				p.Asp1833H	pathogenic				posterior lobe		No effect:		
unknown C-terminalPP2) MPC=2.44<				is	(PM1, PM2,						LEV		
C-terminal MPC=2.44 <			С	unknown	PP2)								
32 3 y c.5597G>A, Likely None 9 m F, A, AA EDs, mid CP N / Mild learning disability Sz free: CBD CBD, CBD, CBZ, CLB, CBZ, CBZ, CBZ, CBZ, CBZ, CBZ, CBZ, CB				C-terminal									
32 3 y c.5597G>A, Likely None 9 m F, A, AA EDs, mid CP N / Mild learning Sz free: CBD CBD p.Arg1866GI pathogenic n, (PS2, PM2, PP2) V V V V V V V V V V V V V V V V V V V			()	MPC=2.44								
p.Arg1866Gl pathogenic disability No effect: n, (PS2, PM2, PP2) Does the second s	32	3 у	Ι	c.5597G>A,	Likely	None	9 m	F, A, AA	EDs, mid CP	N / Mild learning	Sz free: CBD	CBD	
n, (PS2, PM2, PP2) CBZ, CLB,				p.Arg1866Gl	pathogenic					disability	No effect:		
				n,	(PS2, PM2, PP2)						CBZ, CLB,		
de novo LEV, TPM,				de novo							LEV, TPM,		
C-terminal MPC=2.39 VGB, VPA,				C-terminal	MPC=2.39						VGB, VPA,		
ST			С								ST		
Adverse			t	5							Adverse		
effects: CBZ			-	5							effects: CBZ		
(drowsiness											(drowsiness		
and severe											and severe		
cognitive											cognitive		
disturbance)											disturbance)		

33		c.5615G>A	Likely	See fig. 1	6 wk	F, clusters	Normal	N / N	Sz free: CBZ	Learning difficulties
		p.Arg1872Gl	pathogenic			of GTC, SE			+ LEV	
	1 - t-	n	(PS1, PM2, PP1,							
	Ċ	paternal	PP2)							
		(mosaic)								
	<u> </u>	cytoplasmic	MPC=2.39							
	C	c-terminal								
34	14 y	c.5630A>G,	Likely	None	5 m	F clustering	Normal	N / N	Sz free: LTG LTG	Language delay
		p.Asn1877S	pathogenic							
	_	er,	(PS1, PM2, PP2)							
	C	pending								
		cytoplasmic	MPC=2.04							
		c-terminal								
35	4 y 4 m	c.5630A>G	Likely	See fig. 1	6 m		Normal	N / MID	Sz free: LTG,	
		Asn1877Ser	pathogenic						VPA	
		paternal	(PS1, PM2, PP1,							
		cytoplasmic	PP2)							
		c-terminal								
			MPC=2.04							
36	4 y 6 m	c.5630A>G	Likely	None	7 m	GTC, T, AA	Sporadic EDs,	N/ MID	Sz	Ataxia,
	_	p.Asn1877S	pathogenic				FC bilateral >		reduction:	clumsiness
		er	(PS1, PM2, PP2)				right		VPA, PB,	
		unknown							ТРМ	
		cytoplasmic	MPC=2.04							
		c-terminal								

30

<u>Abbreviations</u>: A: Atonic, AA: Atypical Absences, Ab: Absences, ADHD: Attention Deficit Hyperactivity Disorder, AMD: amantadine, BG: Background, CAE: Childhood Absence Epilepsy, CBZ: Carbamazepine, CLB: Clobazam, CLZ: Clonazepam, D:Domain, DD: Developmental Delay, ED: Epileptiform Discharges, FS: Febrile seizures, F: Focal, GTC: Generalized Tonic-Clonic, KD: Ketogenic diet, LAC: Lacosamide, LEV: Levetiracetam, LTG: Lamotrigine, , LZP: Lorazepam, MD: Moderate Intellectual Disability, MID: Mild Intellectual Disability, m: months, M: Myoclonic, NA: Not available, N: Normal, O: Occipital, OXC: Oxcarbazepine, P: Parietal, PER: Perampanel, PHT: Phenytoin, RFM: Rufinamide, S:SE: Status Epilepticus, ST: Stiripentol, S: Spasms, SW: Spike and Wave complexes, sz: Seizures, T: Tonic, TPM: Topiramate, VGB: Vigabatrin, VPA: Valproic Acid, y: years, ZNS: Zonisamide

Figure legends:

Figure 1. Pedigrees of SCN8A-related epilepsies showing segregation of the variant with the phenotype

Figure 2. The spectrum of SCN8A-related epilepsy

Figure 3. A partial display of published variants, with the recurrent DEE and known LOF variants, as well as those identified in this study

Author

Family of proband #4

Family of proband #7



Epilepsy Mild ID Behavioral issues Learning difficulties This article is protected by copyright. All rights reserved

epi_14705_f1.tiff

Mild phenotype, childhood seizures, normal cognition intermediate phenotype, treatable seizures, mild ID Moderate DEE, periods of scizure freedom Severe DEE. Central Vision Impairment, SUITEP

lanuscr Z utl

epi_14705_f2.tiff



- Intelletual disability or ataxia without epilepsy (Wagnon et al., 2017; Liu et al., 2019)
- Intermediate epilepsy (this study)
- DEE variants (previously published)
- Recurrent variants DEE and intermediate epilepsy

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Author **N**