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1 Further delineation of phenotypic spectrum of SCN2A-related disorder.

2

3 **Running Title:** SCN2A-related disorder

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13

14 **Conflicts of interest**

15 None to declare for all authors
16

17 **Data Availability**

18 The data that support the findings of this study are available in the supplementary
19 material of this article.
20

21 **DDD statement**

22 The DDD study presents independent research commissioned by the Health
23 Innovation Challenge Fund [grant number HICF-1009-003], a parallel funding
24 partnership between Wellcome and the Department of Health, and the Wellcome
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6 the Wellcome.

7

8 **Abstract**

9 *SCN2A*- related disorders include intellectual disability, autism spectrum disorder,
10 seizures, episodic ataxia and schizophrenia. In this study, the phenotype-genotype
11 association in *SCN2A*-related disorders was further delineated by collecting detailed
12 clinical and molecular characteristics. Using previously proposed genotype-
13 phenotype hypotheses based on variant function and position, the potential of
14 phenotype prediction from the variants found was examined.

15 Patients were identified through the Deciphering Developmental Disorders study and
16 gene matching strategies. Phenotypic information and variant interpretation evidence
17 was collated. 17 previously unreported patients and 5 patients who had been
18 previously reported (but with minimal phenotypic and segregation data) were
19 included (10 males, 12 females; median age 10.5 years). All patients had
20 developmental delay and the majority had intellectual disability. Seizures were
21 reported in 15/22 (68.2%), 4/22 (18.2%) had autism spectrum disorder and no
22 patients were reported with episodic ataxia. The majority of variants were *de novo*.
23 One family had presumed gonadal mosaicism. The correlation of use of sodium
24 channel-blocking antiepileptic drugs with phenotype or genotype was variable.

1 These data suggest that variant type and position alone can provide some predictive
2 information about the phenotype in a proportion of cases, but more precise
3 assessment of variant function is needed for meaningful phenotype prediction.

4

5

6

7 **Keywords**

8 Intellectual disability; *SCN2A*; autism spectrum disorder, Developmental delay,
9 episodic ataxia

10

11

12

13 **Introduction**

14 Sodium voltage-gated channel α subunit 2 is a member of the sodium channel alpha
15 subunit gene family and encodes sodium channel $Na_v1.2$ (*SCN2A* OMIM *182390).

16 Voltage-gated sodium channel $Na_v1.2$ is one of the four major sodium channels in
17 the brain and consists of four domains, with each domain comprising of 6

18 transmembrane segments (see figure 1). The S3 segment acts as the voltage sensor
19 and the S5 and S6 segments form the ion selectivity pore (Hedrich 2019).

20 Pathogenic variants in *SCN2A* are associated with different neurological and
21 neurodevelopmental phenotypes including developmental and epileptic

22 encephalopathies (DEE), self-limiting neonatal-infantile epilepsy (SLNIE), episodic
23 ataxia (EA), developmental delay (DD), intellectual disability (ID), autism spectrum

24 disorder (ASD), and schizophrenia (Reynolds 2020). While many *SCN2A* variants

1 have been identified associated with these phenotypes, only a relatively small
2 number of these have been functionally characterised.

3 DEE is an umbrella term describing diseases characterised by both delayed
4 neurodevelopment and epilepsy. SLNIE is characterised by early onset seizures that
5 usually resolve in early childhood with normal cognitive development and a family
6 history suggestive of autosomal dominant inheritance (Scheffer 2017). Lauxman *et*
7 *al.* characterised variants in patients with both SLNIE and DEE using whole cell
8 patch-clamp recordings demonstrating evidence of gain of function (GoF) effects
9 from these variants (Lauxmann 2018). Ben-Shalom *et al* demonstrated that the
10 majority of ASD-related *SCN2A* variants resulted in a complete loss of Nav1.2
11 function (Ben-Shalom 2017). Therefore, it would be expected that all protein-
12 truncating variants (PTVs) (with assumed loss of function (LoF) effect) would result
13 in an ASD and/or ID phenotype and if seizures are present, it would be expected that
14 they are later onset (after infancy). This genotype-phenotype paradigm has been
15 corroborated by further studies (Begemann 2019) although not unsurprisingly,
16 occasional exceptions to this paradigm have been reported (Sundaram 2013, Leach
17 2016, Suddaby 2019).

18 In early onset seizures (<3 months) associated with *SCN2A*, non-selective sodium
19 channel blockers are generally thought to be most effective, which is in-keeping with
20 the suggestion that most of these variants result in GoF (Wolff 2017, Sanders 2018).
21 This is not universal, and other patients display no response to sodium channel
22 blockers (Liang 2017). This is in contrast to the ASD and/or ID phenotype
23 presentations, when seizures have developed later in childhood, where non-sodium
24 channel blockers are thought to be more effective (Sanders 2018).

1 This study identified 22 patients with *SCN2A* variants through the DDD study and
2 gene matching strategies, who had not been previously reported, or had only been
3 reported as part of large exome datasets with minimal phenotypic information
4 included. Detailed phenotypic data and epilepsy treatment data were collected along
5 with evidence for pathogenicity for the variants.

6

7 **2. Materials and Methods**

8 The Deciphering Developmental Disorders study (DDD study) is a UK based
9 research study that recruited patients with severe undiagnosed neurodevelopmental
10 disorder and/or congenital anomalies, abnormal growth parameters, dysmorphic
11 features, and unusual behavioural phenotypes from 24 regional Clinical Genetics
12 centres from around the UK and Republic of Ireland between 2011 to 2015 (DDD
13 Study 2014, 2017, Wright 2014). This study used a combination of exome
14 sequencing and array-based detection of chromosomal rearrangements to identify
15 the underlying causes in these previously undiagnosed individuals. To date, over
16 4500 children have received a diagnosis from the DDD study (DDD study).

17 A complementary analysis project was applied for and granted by the DDD study,
18 allowing access to anonymised details of patients with *SCN2A* variants identified
19 through this study. From the DDD study, 28 patients were identified with pathogenic
20 or likely pathogenic *SCN2A* variants. Responsible clinicians were contacted to offer
21 recruitment to the individuals and 19 patients (or their parents/guardians) gave
22 consent for publication. An additional 3 cases were identified via gene matching
23 strategies via the DECIPHER database (DECIPHER, Firth 2009). These additional
24 patients had either a clinical exome or an ID gene panel. The ID panel was based on

1 the DDD panel (<https://www.lumc.nl/sub/4080/att/1768916> version 1, 18-02-2018,
2 1875 genes.).

3 Phenotype and genotype information was gathered from the DECIPHER and
4 additional information from the responsible clinician's routine clinical assessments
5 and examination of medical records. Phenotypes were defined as per Human
6 Phenotype Ontology terms (Köhler 2021) and International League against Epilepsy
7 definitions (Scheffer 2017). Clinical information included: medical history,
8 dysmorphology, growth parameters, developmental progress, learning, seizure
9 onset, type and progression and current or most-recent anti-epileptic drugs (AEDs)
10 prescribed. Where MRI brain imaging had been carried out, the reported findings
11 were also collected.

12 Pathogenicity evidence was collected from the DECIPHER database and referring
13 clinician. Each variant was then reviewed and classified according to ACMG criteria
14 and related publications (Richards 2015, Ellard 2020).

15 Exclusion criteria from this study were: patients with an additional proven genetic
16 diagnosis where the *SCN2A* variant was not thought to be contributory, and those in
17 whom *SCN2A* variants were classified as benign or likely benign.

18

19

20 **4 Results**

21 **Demographics**

22 22 patients (10 male, 12 female) between 2 years and 52 years (median age 10.5
23 years) were recruited according to the above methods. 19 were from the DDD study,
24 and 3 from Leiden clinical genetics services. 18 had trio exome sequencing via the
25 DDD study, 1 was diagnosed through the DDD study as the parent of a proband, 2

1 had a trio exome through routine clinical services and 1 had an ID gene panel
2 through routine clinical service. Table 1 shows a summary of the characteristics of
3 the patients with variants in *SCN2A* who were included in this study with table 1a
4 summarizing the patients with an early onset seizure phenotype associated with
5 DD/ID (DEE), and table 1b those with an ID/ASD phenotype. A comprehensive
6 summary of detailed phenotypic data is available in Supplementary data 1.

7

8 **Tables 1a and 1b here**

9

10 **Neurodevelopment**

11 Of the 22 patients, all had DD and 19 had also been assessed as having ID. DD was
12 mild in 3/22 (13.6%), moderate in 5/22 (22.7%), moderate-severe in 3/22 (13.6%)
13 and severe in 6/22 (27.3%) patients. In 2/22 (9.1%) patients profound DD was noted.
14 Global developmental delay (GDD) was described in 3/22 (13.6%) patients without
15 further qualification. ID was moderate in 5/22 (22.3%), moderate-severe in 3/22
16 (13.6%), severe in 4/22 (18.2%) and profound in 4/22 (18.2%) patients. 2 patients
17 were noted to have ID without further qualification.

18 Seizures were present in 15/22 (68.2%) patients, of which 10/22 had onset of
19 seizures within the first 3 months after birth, and 4 had seizure onset in childhood. In
20 patient 7, the seizures were reported at a very young age but the exact age of
21 seizure onset was not known.

22 None of the patients in this cohort were identified as having EA. In 4/22 patients
23 stereotypy was noted, another patient had choreiform movements and another
24 dystonic movements.

1 In 8/22 patients abnormalities of tone were noted; increased tone in 4/22 (18.2%)
2 and decreased or variable tone in 4/22 (18.2%). 4/22 (18.2%) had diagnoses of
3 ASD, and an additional 2 cases displayed autistic features. One patient had a
4 diagnosis of attention deficit disorder (ADD).

5 One patient had a right hemi-paresis without any identifiable anomaly on MRI, which
6 developed after an episode of status epilepticus as an infant. The onset of seizures
7 predated the hemi-paresis.

8

9 **Imaging**

10 Magnetic resonance imaging (MRI) of the brain reports were available in 12/22
11 patients. This was normal in 6/12 (50%). In cases where there were abnormal
12 findings, these included cerebral atrophy or 'thinning of the brain' in 2/12 (16.7%)
13 and hypoplasia of the corpus callosum in 3/12 (25%) patients.

14

15 **Growth**

16 Mean height and weight were on the 39.4th percentile (-0.24 standard deviation score
17 (SDs)) and 49.4th percentile (0.15 SDs) respectively. Mean occipito-frontal
18 circumference was 35.5th percentile (-1.2 SDs), with a range from <0.4th percentile to
19 92nd percentile (-6.76 to 1.43 SDs). 4/22 (18.2%) patients had a postnatal and/or
20 progressive microcephaly.

21

22 **Craniofacial Features**

23 In 6/22 (27.3%) cases, dysmorphic features were noted (see supplementary data1).
24 These were wide ranging with very little overlap between cases. Facial features were
25 described as 'coarse' in 2/22 (9.1%) cases.

1

2 **Systemic Features**

3 2/22 (9.1%) patients had joint hypermobility. One patient had a mild thoracolumbar
4 scoliosis, displayed a lordotic posture and was found to have hip dysplasia. Another
5 patient had bilateral hip dislocation.

6 Prominent fingertip pads were noted in 3/22 (13.6%) patients. 5/22 (22.7%) patients
7 had constipation. Two patients had gastro-oesophageal reflux with one of these
8 requiring a percutaneous endoscopic gastrostomy. An additional patient also
9 required a gastrostomy, without documented reflux disease. One individual had
10 Hirschsprung's disease (with a paternal history of Hirschsprung's disease and
11 therefore likely unrelated to the *de novo* SCN2A variant).

12

13 **Anti-epileptic drugs**

14 Seizures were reported in 15 individuals, of which information about treatment was
15 available in 11 cases (see table 1). Where AED treatment information was available
16 and there were early onset seizures, 5 were being managed with a combination of
17 sodium channel blockers and non-sodium channel blockers, 1 was managed on a
18 sodium channel blocker alone and 1 was managed with a single non-sodium channel
19 blocker.

20 Of the 4 cases where seizure onset was in childhood (>12 months), 1 of these was
21 managed by non-sodium channel blocker and 2 were managed by a sodium channel
22 blocker, and 1 with a combination.

23

24 **Molecular genetics**

1 Normal chromosomal microarray (CMA) results were found in 17/22 (77.3%)
2 patients, and in the 5 patients where CMA findings were reported, these were
3 interpreted as benign or likely benign. Additional single nucleotide variants were
4 identified in 4/22 (18.1%) patients and these were found to be benign or likely
5 benign. These additional results are summarised in Supplementary Data 1.
6 There were 20 *SCN2A* variants (transcript NM_001040142.2), 13 novel and 7
7 previously reported variants. None of the previously reported variants has been
8 functionally characterised. The majority of the patients in this cohort (16/22, 72.7%)
9 have missense variants in *SCN2A* which have been classified according to ACMG
10 guidelines based on a combination of evidence including *de novo* status, multiple
11 lines of computational evidence suggesting a deleterious effect on the protein and
12 being absent from population databases.
13 From this cohort, 6 patients had a presumed LoF variant and of these 2 patients had
14 nonsense variants, 2 had frameshift variants and 2 had splice site variants. These
15 were classified as pathogenic or likely pathogenic variant based on a combination of
16 evidence including *de novo* status and absence from population databases. Of the 6
17 presumed LoF variants identified, none were associated with a neonatal-onset
18 seizure phenotype. Seizures were seen in 2/6 cases and were later onset (after 2
19 years). ASD was diagnosed in 4 of the presumed LoF variant cases, with ID and
20 autistic features in one patient and isolated ID in one patient.
21 A summary of the variants, ACMG criteria and classification is provided in table 2.

22

23 **Table 2 here**

24

25 Figure 1 shows the position of the variants on a schematic representation of *SCN2A*.

1 The 20 variants identified were spread along the gene, in all domains. The 6 protein
2 truncating variants (from 6 patients) associated with ASD or ID were throughout the
3 gene. 6/10 of the missense variants (7/11 patients) associated with DEE appear to
4 cluster near the voltage sensor. 3/4 of the missense variants (3/5 patients)
5 associated with ASD/ID clustered near the pore.

6

7 **Figure 1 here**

8

9 **Inheritance and mosaicism**

10 In 17/22 (77.3%) cases, the *SCN2A* variant was found to be *de novo*. In 2 patients
11 inheritance was unknown. In 2 patients the variant was inherited from an affected
12 parent (patients 3 and 6).

13 Patient 3, who had absence seizure onset at 3 months, and moderate DD and ID,
14 inherited the variant from an affected mother (see **figure 2b**), who was also reported
15 to have seizures (although the age of onset is unknown) and a 'specific learning
16 disability'. This variant is absent from gnomAD, lies with a constrained region of the
17 gene (assessed via DECIPHER database) and *in silico* tools predict this to be
18 deleterious. The only previous report of this variant is referring to this same patient
19 (Fitzgerald 2015). Currently, therefore, this remains a VUS with a posterior
20 probability of 81.2%.

21 Patient 6 inherited the variant from his father who was also reported to have onset of
22 seizures at a very young age (although exact age unknown), which resolved by
23 around age 4 -5 years and learning difficulties. (see **figure 2a**). A full sibling of patient
24 6 did not have the *SCN2A* variant, but had infantile onset seizures with a milder ID
25 but with an antenatal and perinatal history suggestive of vascular risk, and so with a

1 plausible alternative explanation. Wider cascade testing of the *SCN2A* variant in this
2 family was not possible. Given the presence of this pathogenic *SCN2A* variant, with
3 no other class 3,4 or 5 variants from the trio exome analysis and no CNVs on CMA
4 in this patient, this was concluded to be the likely explanation for the patients'
5 phenotype.

6

7 One family (patients 16 and 17, **see figure 2c**) had presumed gonadal mosaicism:

8 Two maternal half siblings were identified to have the same pathogenic *SCN2A*

9 variant, which was absent from the mother's blood and buccal DNA and absent from

10 patient 16's father. It is therefore presumed that this is due to maternal gonadal

11 mosaicism.

12 **figure 2 here** (pedigrees)

13

14

15

16 **Discussion**

17 Here we report detailed phenotypic information for 22 cases of *SCN2A*-related

18 disorders that have not previously been reported with detailed phenotypic data.

19 Among them there were 13 novel variants. The majority of these data supports the

20 previously proposed genotype-phenotype paradigm and add to the understanding of

21 gonadal mosaicism risk and inherited variants.

22 Protein-truncating variants (PTV) in *SCN2A* have been shown to be situated

23 throughout the gene, as expected by their resulting functional effect. Previous

24 functional assessments have found that missense variants leading to LoF tend to be

25 situated at the pore loop or N-terminus (Ben-Shalom 2017) whereas missense

1 variants with GoF effect tend to be located in the transmembrane segments or in
2 cytoplasmic loops near to these or in the cytoplasmic loop containing the inactivation
3 gate (Hedich 2019).

4 This variants found in this study showed some correlation with these previous
5 findings. 6/10 of the missense variants associated with DEE (and therefore predicted
6 to result in GoF) and 3/4 of the missense variants associated with ASD or ID (and
7 therefore predicted to result in LoF) being positioned as previously seen (Hedich
8 2019, Wolff 2019).

9 There was a range of DD and/or ID within this cohort, from mild to profound in-
10 keeping with previous case series (Alsaif 2019, Schwarz 2019). There was a range
11 of seizure phenotypes reported. No cases were reported with SLNIE, which is likely
12 to be reflective of the cases selected for the DDD study, which will have been biased
13 towards patients with more severe developmental phenotypes. None of the patients
14 in this cohort were identified as having EA. As this is an uncommon later finding in
15 *SCN2A*, this is not unexpected.

16 One adult patient was reported with bipolar disorder. It is interesting that this patient
17 with a LoF variant and ASD went on to have this diagnosis in adulthood. Bipolar
18 disorder-associated loci have been identified in GWAS studies, which include
19 *SCN2A* (Stahl 2019) but it remains to be seen if there is a causal link here. There
20 were no patients with a diagnosis of schizophrenia. Overall, awareness of
21 vulnerability to secondary psychiatric disorders in patients with *SCN2A*- related ASD
22 should lead to a lower threshold for assessment in any adult displaying symptoms.

23 No specific facial gestalt was recognisable, although 6/22 (27.3%) patients had
24 dysmorphic features. Cases of *SCN2A*-related disorder are likely to be made with
25 panel-based or next generation sequencing (NGS) based approaches to

1 investigation, rather than on clinical assessment alone. The presence of dysmorphic
2 features may require consideration of a separate or additional genetic diagnosis but
3 may be part of the phenotypic presentations of *SCN2A*-related disorder.

4 Other previously reported associated phenotypes include structural brain
5 abnormalities including severe cortical dysplasia (Bernardo 2017), cerebral atrophy
6 (Ogiwara 2009, Baasch 2014), and hypoplastic corpus callosum (Baasch 2014). In
7 50% of the patients in this study who had cranial imaging an abnormality was
8 identified including cerebral atrophy and hypoplasia of the corpus callosum.

9 Spasticity and other movement disorders such as dystonia, chorea, stereotypies,
10 opisthotonus and oculogyric crises have also been noted in *SCN2A* disorders
11 (Takezawa 2018, Howell 2015) and these were also observed in this cohort (6/22
12 patients).

13 Perhaps due to the impact of the phenotype on the likelihood of an individual having
14 the capacity to have a child, in the majority of previously reported cases, the variants
15 have been *de novo* (Lindy 2018). The 2 families from this study with inherited
16 variants highlight the possibility of inheritance of *SCN2A* variants associated with a
17 non-benign phenotype. No evidence of incomplete penetrance was demonstrated in
18 these data.

19 Mosaicism in probands and parents is recognised in epilepsy-related
20 neurodevelopmental genes (Stosser 2018) and one previous case of paternal
21 germline mosaicism of *SCN2A* has been reported in a family with two children with
22 an Ohtahara syndrome epilepsy phenotype (Zerem 2014). This study documents the
23 first reported family with a presumed LoF phenotype (ID/DD with or without later
24 onset seizures) (patients 16 and 17) with presumed gonadal mosaicism. This has
25 important implications for counselling of families with a child with a *de novo* *SCN2A*

1 pathogenic variant. Generally, in clinical practice, recurrence risk based on gonadal
2 mosaicism rate is counselled as around 1%. This study and the previous reported
3 case of mosaicism is in-keeping with this estimated recurrence risk and there is
4 insufficient data at present to alter this practice for *SCN2A* variants.

5 It is hypothesised that *SCN2A* variants in DEE vs ASD/ID have opposing effects of the
6 Nav1.2 function (Ben-Shalom 2017), and this study provides some evidence to
7 supports this, with all the PTVs associated with ASD/ID.

8 It is possible that some variants confer a risk to a broader range of *SCN2A* related
9 phenotypes. From this study, nine patients had a previously reported variant, in six of
10 these patients there were phenotypic differences to the previous reports. For
11 example, patient 6 and 7's variant was previously reported in a patients with an
12 infantile encephalopathy phenotype (Wei 2018), with epilepsy of unknown type and
13 age of onset and neurodevelopmental disorder (Lindy 2018), as well as benign
14 familial epilepsy (Zeng 2018). Similarly, patient 4 presented with a DEE and this
15 variant has been previously reported in both DEE (Wolff 2017) and SLNIE (Kong
16 2018). As both SLNIE and DEE can be caused by a GoF variant, it is plausible that
17 in different families, the variant may result in either of these phenotypes, although it
18 appears to be consistent within each pedigree.

19 Patient's 16 and 17 both have DD and ID and with seizures in patient 16 only. This
20 variant has been previously reported with an ASD phenotypes (Wolff 2017, Wang
21 2016), Similarly, the variant observed in patient 22 has been reported with ASD, but
22 he had isolated DD and ID (D'Gama 2015). Given that ASD and ID phenotypes are
23 both associated with LOF, again it seems reasonable to conclude that this variant
24 may result in differing elements of the LOF-associated phenotypes in different
25 families.

1 These variants will be particularly interesting to functionally characterise, to see if an
2 unusual functional pattern explains the phenotypic variation between cases.

3

4 Based on the hypothesis that GoF variants cause an early onset seizure, which are
5 best treated with sodium channel blockers, and LoF variants lead to later onset
6 epilepsy, which are best treated with non-sodium channel blockers, the correlation of
7 phenotype and prescribed AED treatment was examined. The correlation of
8 response to antiepileptic drugs with phenotype or genotype was variable with 6/7
9 (85.7%) presumed GoF and 2/4 (50%) presumed LoF cases being treated with an
10 AED in-keeping with the hypothesis. There are limitations to this data, given this is
11 based on effective treatments reported within the context of routine clinical practice,
12 rather than a clinical trial setting.

13 The limited ability to predict the phenotype from the variant's type and position
14 highlights the importance of a functional assessment of each variant. A prediction of
15 function allows a prediction of phenotype and gives a degree of prognostic
16 information, although this is limited by the variability of presentation, even with the
17 same variant. However, the lack of definitive correlation of function (or presumed
18 function) and AED response both in the literature and from this study, indicates that
19 accurately assessing variant function is not necessarily sufficient to act as a
20 predictive biomarker for treatment response.

21 As whole exome or whole genome based testing is used as first line for investigation
22 of patients, more variants are identified that may be of uncertain clinical significance.
23 It has been recognised that an agnostic approach is increasingly identifying a much
24 broader phenotype in previously well-described syndromes. Routine functional work
25 as part of variant assessment is unlikely to be possible. Heyne *et al.* published data

1 from a tool that uses a machine learning model to predict LoF or GoF effects, with
2 good correlation with previously functionally tested variants (Heyne 2020). While this
3 is a tool not validated for clinical practice, this has potential to be a cost-effect
4 mechanism to predict variant function in a time-frame that is clinical useful. It
5 remains to be seen whether early and optimised therapy could also act to reduce
6 some of the presumed impact of the variant's function on the developing brain.

7

8

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