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

Running Title: *SCN2A*-related disorder

Richardson R[1], Baralle D[2,3], Bennett C[4], Briggs T[5,6], Bijlsma EK[7], Clayton Smith J[5,6], Constantinou P[8], Foulds N[2], Jarvis J[9], Jewell R[4], Johnson DS[10], McEntagart M[11], Parker MJ[10], Radley JA[12], Robertson L[13], Ruivenkamp CAL[6], Rutten J[6], Tellez J[1], Turnpenny P[14], Wilson V[1] , Wright M[1], Balasubramanian M[10, 15]

1. Northern Genetics Service, Newcastle upon Tyne Hospitals NHS Trust, Newcastle, UK
2 University Hospital of Southampton NHS Foundation Trust, Southampton, UK
3 University of Southampton Faculty of Medicine, Southamton, UK
4. Yorkshire Regional Genetics Service, The Leeds Teaching Hospitals NHS Trust, Leeds, UK
5.NW Genomic Laboratory Hub, Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University NHS Foundation Trust, Manchester, UK
6. Division of Evolution and Genomic Sciences, School of Biological Sciences, University of Manchester, UK
7. Department of Clinical Genetics, Leiden University Medical Centre, Leiden, Netherlands
8. Queen Elizabeth University Hospital, Glasgow, UK.
9. Clinical Genetics Unit, Birmingham Women's NHS Foundation Trust, Birmingham, UK
10 Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield, UK
11 South West Thames Regional Genetics Centre, St. George's Healthcare NHS Trust, St. George's, University of London, London, UK.
12 London North West Regional Genetics Service, St. Mark's and Northwick Park hospitals, Harrow, Middlesex, U.K.
13 North of Scotland Genetics Service, Aberdeen

14 Clinical Genetics Department, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK.
15 Academic Unit of Child Health, Department of Oncology & Metabolism, University of Sheffield

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Conflicts of interest

None to declare for all authors

Data Availability

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

DDD statement

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Abstract

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

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

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

Keywords

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

Introduction

Sodium voltage-gated channel α subunit 2 is a member of the sodium channel alpha subunit gene family and encodes sodium channel $Na_v1.2$ (*SCN2A* OMIM *182390). Voltage-gated sodium channel $Na_v1.2$ is one of the four major sodium channels in the brain and consists of four domains, with each domain comprising of 6 transmembrane segments (see figure 1). The S3 segment acts as the voltage sensor and the S5 and S6 segments form the ion selectivity pore (Hedrich 2019). Pathogenic variants in *SCN2A* are associated with different neurological and neurodevelopmental phenotypes including developmental and epileptic encephalopathies (DEE), self-limiting neonatal-infantile epilepsy (SLNIE), episodic ataxia (EA), developmental delay (DD), intellectual disability (ID), autism spectrum disorder (ASD), and schizophrenia (Reynolds 2020). While many *SCN2A* variants

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

DEE is an umbrella term describing diseases characterised by both delayed neurodevelopment and epilepsy. SLNIE is characterised by early onset seizures that usually resolve in early childhood with normal cognitive development and a family history suggestive of autosomal dominant inheritance (Scheffer 2017). Lauxman *et al.* characterised variants in patients with both SLNIE and DEE using whole cell patch-clamp recordings demonstrating evidence of gain of function (GoF) effects from these variants (Lauxmann 2018). Ben-Shalom *et al* demonstrated that the majority of ASD-related *SCN2A* variants resulted in a complete loss of $Na_v1.2$ function (Ben-Shalom 2017). Therefore, it would be expected that all protein-truncating variants (PTVs) (with assumed loss of function (LoF) effect) would result in an ASD and/or ID phenotype and if seizures are present, it would be expected that they are later onset (after infancy). This genotype-phenotype paradigm has been corroborated by further studies (Begemann 2019) although not unsurprisingly, occasional exceptions to this paradigm have been reported (Sundaram 2013, Leach 2016, Suddaby 2019).

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

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

2. Materials and Methods

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

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

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

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

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

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

4 Results

Demographics

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

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

Tables 1a and 1b here

Neurodevelopment

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

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

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

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

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

Imaging

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

Growth

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

Craniofacial Features

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

Systemic Features

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

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

Anti-epileptic drugs

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

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

Molecular genetics

Normal chromosomal microarray (CMA) results were found in 17/22 (77.3%) patients, and in the 5 patients where CMA findings were reported, these were interpreted as benign or likely benign. Additional single nucleotide variants were identified in 4/22 (18.1%) patients and these were found to be benign or likely benign. These additional results are summarised in Supplementary Data 1.

There were 20 *SCN2A* variants (transcript NM_001040142.2), 13 novel and 7 previously reported variants. None of the previously reported variants has been functionally characterised. The majority of the patients in this cohort (16/22, 72.7%) have missense variants in *SCN2A* which have been classified according to ACMG guidelines based on a combination of evidence including *de novo* status, multiple lines of computational evidence suggesting a deleterious effect on the protein and being absent from population databases.

From this cohort, 6 patients had a presumed LoF variant and of these 2 patients had nonsense variants, 2 had frameshift variants and 2 had splice site variants. These were classified as pathogenic or likely pathogenic variant based on a combination of evidence including *de novo* status and absence from population databases. Of the 6 presumed LoF variants identified, none were associated with a neonatal-onset seizure phenotype. Seizures were seen in 2/6 cases and were later onset (after 2 years). ASD was diagnosed in 4 of the presumed LoF variant cases, with ID and autistic features in one patient and isolated ID in one patient.

A summary of the variants, ACMG criterions and classification is provided in table 2.

Table 2 here

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

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

Figure 1 here

Inheritance and mosaicism

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

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

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

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

One family (patients 16 and 17, see figure 2c) had presumed gonadal mosaicism: Two maternal half siblings were identified to have the same pathogenic *SCN2A* variant, which was absent from the mother's blood and buccal DNA and absent from patient 16's father. It is therefore presumed that this is due to maternal gonadal mosaicism.

figure 2 here (pedigrees)

Discussion

Here we report detailed phenotypic information for 22 cases of *SCN2A*-related disorders that have not previously been reported with detailed phenotypic data. Among them there were 13 novel variants. The majority of these data supports the previously proposed genotype-phenotype paradigm and add to the understanding of gonadal mosaicism risk and inherited variants.

Protein-truncating variants (PTV) in *SCN2A* have been shown to be situated throughout the gene, as expected by their resulting functional effect. Previous functional assessments have found that missense variants leading to LoF tend to be situated at the pore loop or N-terminus (Ben-Shalom 2017) whereas missense

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

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

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

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

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

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

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

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

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

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

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

It is hypothesed that *SCN2A* variants in DEE vs ASD/ID have opposing effects of the $Na_V1.2$ function (Ben-Shalom 2017), and this study provides some evidence to supports this, with all the PTVs associated with ASD/ID.

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

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

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

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

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

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

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

References

AlSaif S, Umair M, Alfadhel M. (2019). Biallelic *SCN2A* Gene Mutation Causing Early Infantile Epileptic Encephalopathy: Case Report and Review. *Journal of Central Nervous System Disease*. 11:1179573519849938, doi: 10.1177/1179573519849938.

Baasch AL, Huning I, Gilissen C, Klepper J. *et al*. (2014) Exome sequencing identifies a *de novo SCN2A* mutation in a patient with intractable seizures, severe intellectual disability,optic atrophy, muscular hypotonia, and brain abnormalities. *Epilepsia*. 55:e25–e29. doi:10.1111/epi.12554

Begemann A, Acuna MA, Zweier M, Vincent M *et al*. (2019). Further corroboration of distinct functional features in *SCN2A* variants causing intellectual disability or epileptic phenotypes. *Molecular Medicine* 25:6. doi: 10.1186/s10020-019-0073-6.

Ben-Shalom R, Keeshen CM, Berrios KN, An JY, , Sanders SJ, Bender KJ (2017) Opposing effects on NaV1.2 function underlie differences between *SCN2A* variants observed in individuals with autism spectrum disorder or infantile seizures. *Biol Psychiatry.* 82: 224–232. doi: 10.1016/j.biopsych.2017.01.009.

Bernardo S, Marchionni E, Prudente S, *et al.* (2017) Unusual association of *SCN2A* epileptic encephalopathy with severe cortical dysplasia detected by prenatal MRI. *Eur J Paediatr Neurol*.;21:587–590.doi: 10.1016/j.ejpn.2017.01.014.

ClinVar. Landrum MJ, Lee JM, Benson M, Brown GR*, et al:* (2018) ClinVar:improving access to variant interpretations and supporting evidence. *Nucleic Acids Res*. 46:D1062-D1067. doi: 10.1093/nar/gkx1153. (RCV001028090.1) (accessed 01/05/2020)

Deciphering Developmental Disorders Study. (2014) Large-scale discovery of novel genetic causes of developmental disorders. *Nature*. 519;;223-8. doi: 10.1038/nature14135.

Deciphering Developmental Disorders Study. (2017) Prevalence and architecture of *de novo* mutations in developmental disorders. *Nature*. 542:433-438. doi: 10.1038/nature21062.

DECIPHER. DECIPHER v9.24: https://decipher.sanger.ac.uk/

D'Gama AM, Pochareddy S, Li M, Jamuar SS, *et al*. (2015). Targeted DNA Sequencing from Autism Spectrum Disorder Brains Implicates Multiple Genetic Mechanisms. *Neuron.* 88:910-917. doi: 10.1016/j.neuron.2015.11.009.

Ellard S, Baple E, Callaway A, Berry I, *et al.* ACGS Best Practice Guidelines for Variant Classification in Rare Disease (2020). Association for Clinical Genomic Science. https://www.acgs.uk.com/quality/best-practice-guidelines/. Accessed 01/05/2020

Firth HV, Richards SM, Bevan AP, Clayton S. *et al.*, (2009) DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources. *Am.J.Hum.Genet* 84, 524-533. doi: 10.1016/j.ajhg.2009.03.010

Fitzgerald TW, Gerety SS, Jones WD, van Kogelenberg M, *et al*. (2015). Large-scale discovery of novel genetic causes of developmental disorders. *Nature*. 519: 223–228. doi: 10.1038/nature14135.

Hedrich UBS, Lauxmann S, Lerche H. *SCN2A* channelopathies: Mechanisms and models. (2019) *Epilepsia*. 60 Suppl 3:S68-S76. doi: 10.1111/epi.14731.

Heyne HO, Baez-Nieto D, Iqbal S, Palmer DS, *et al* (2020) Predicting functional effects of missense variants in voltage-gated sodium and calcium channels. *Sci Transl Med*. 12(556):eaay6848. doi: 10.1126/scitranslmed.aay6848.

Howell, K.B., McMahon, J.M., Carvill, G.L., Tambunan, D. *et al*. (2015) SCN2A encephalopathy: A major cause of epilepsy of infancy with migrating focal seizures. *Neurology*. 85:958-966. doi: 10.1212/WNL.0000000000001926.

Köhler S, Gargano M, Matentzoglu N, Carmody LC, *et al*. (2021) The Human Phenotype Ontology in 2021. Nucleic Acids Res. 49(D1):D1207-D1217. doi: 10.1093/nar/gkaa1043.

Kamiya. K, Kaneda M, Sugawara T, Mazaki E, *et al* (2004). A Nonsense Mutation of the Sodium Channel Gene SCN2A in a Patient with Intractable Epilepsy and Mental Decline *The Journal of Neuroscience*. 24:2690 –2698. doi: 10.1523/JNEUROSCI.3089-03.2004.

Kong Y, Yan K, Hu L, Wang M, *et al* (2018). Data on mutations and Clinical features in *SCN1A* or *SCN2A* gene. *Data Brief*. 30;22:492-501.doi: 10.1016/j.dib.2018.08.122.

Kosmicki JA, Samocha KE, Howrigan DP, Sanders SJ, *et al*. (2017). Refining the role of *de novo* protein truncating variants in neurodevelopmental disorders using population reference samples. *Nat Genet*. April ; 49: 504–510. doi: 10.1038/ng.3789.

Lauxmann, S, Verbeek NE, Liu Y, *et al.*(2018) Relationship of electrophysiological dysfunction and clinical severity in *SCN2A*-related epilepsies. *Human Mutation*.39:1942–1956. doi: 10.1002/humu.23619.

Law S, (2017). SCN2A Variant Viz 6.0 https://public.tableau.com/profile/sindy.law#!/vizhome/SCN2AVariantViz6_0/Dashboard1 (accessed 1/05/2020)

Leach EL, van Karnebeek CDM, Townsend KN, Tarailo-Graovac M, Hukin J, Gibson WT. (2016) Episodic ataxia associated with a *de novo SCN2A* mutation. *Eur. J. Paed. Neurol.* 20. 772-776. doi: 10.1016/j.ejpn.2016.05.020.

Liang JS, Lin LJ, Yang MT, Wang JS, Lu JF. (2017) The therapeutic implication of a novel *SCN2A* mutation associated early-onset epileptic encephalopathy with Rett-like features. *Brain Dev*. 39:877–881.doi: 10.1016/j.braindev.2017.06.003.

Lindy AS, Stosser MB, Butler E, Downtain-Pickersgill C, *et al.* (2018). Diagnostic outcomes for genetic testing of 70 genes in 8565 patients with epilepsy and neurodevelopmental disorders. *Epilepsia*.59:1062-1071. doi: 10.1111/epi.14074.

Lord J, Gallone G, Short PJ, McRae JF, *et al*. (2019) Pathogenicity and selective constraint on variation near splice sites. *Genome Res.* 29: 159-170. doi: 10.1101/gr.238444.118.

Monies D, Abouelhoda M, AlSayed M, Alhassnan Z, *et al*. (2017).The landscape of genetic diseases in Saudi Arabia based on the first 1000 diagnostic panels and exomes.*Hum Genet*.136:921–939. doi: 10.1007/s00439-017-1821-8.

Ogiwara I, Ito K, Sawaishi Y, Osaka, H. *et al.* (2009) *De novo* mutations of voltage-gated sodium channel alpha II gene *SCN2A* in intractable epilepsies. *Neurology*.;73:1046–1053. doi: 10.1212/WNL.0b013e3181b9cebc.

Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. MIM Number: 182390 : 09/01/2020: URL: https://omim.org/

Reynolds C., King MD., and Gorman KM. (2020). The phenotypic spectrum of *SCN2A*-related epilepsy. Eur. J.Paed.c Neurol. 24 117-122.doi: 10.1016/j.ejpn.2019.12.016.

Richards, S., Aziz, N., Bale, S., Bick, D. et al. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetic Med.* 17 :405-24. doi: 10.1038/gim.2015.30.

Sanders SJ, Campbell AF, Cottrell JR, Moller RS *et al.* (2018). Progress in understanding and treating *SCN2A*-mediated disorders. *Trends Neurosci*.; 41: 442–456. doi: 10.1016/j.tins.2018.03.011.

Scheffer IE, Berkovic S, Capovilla G, Connolly MB *et al*. (2017) ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia.;58:512-521. doi: 10.1111/epi.13709.

Schwarz N, Bast T, Gaily E, Golla G, *et al*.,(2019) Clinical and genetic spectrum of *SCN2A*-associated episodic ataxia. *Eur J Paediatr Neurol*. 23:438-447. doi: 10.1016/j.ejpn.2019.03.001.

Stahl EA, Breen G, Forstner AJ, McQuillin A, *et al*. Genome-wide association study identifies 30 loci associated with bipolar disorders. *Nat Genet*. 2019 May; 51: 793–803. doi: 10.1038/s41588-019-0397-8.

Staněk D, Laššuthová P, Štěrbová K, Vlčková M. *et al*. (2018). Detection rate of causal variants in severe childhood epilepsy is highest in patients with seizure

onset within the first four weeks of life. *Orphanet Journal of Rare Diseases*. 13:71. doi: 10.1186/s13023-018-0812-8.

Stosser MB, Lindy AS, Butler E, Retterer K, *et al*.,(2018). High frequency of mosaic pathogenic variants in genes causing epilepsy-related neurodevelopmental disorders. *Genet. Med.* 20.:403-410doi: 10.1038/gim.2017.114.

Suddaby, JS, Silver J and So J. (2019), Understanding the schizophrenia phenotype in the first patient with the full *SCN2A* phenotypic spectrum. *Psychiatric Genetics*, 29:91–94. doi: 10.1097/YPG.0000000000000219.

Sundaram SK, Chugani HT, Tiwari VN, Huq AH. (2013) *SCN2A* mutation is associated with infantile spasms and bitemporal glucose hypometabolism. *Pediatr Neurol*. 49:46–49. doi: 10.1016/j.pediatrneurol.2013.03.002.

Takezawa Y, Kikuchi A, Haginoya K, Niihori T. *et al*. (2018) Genomic analysis identifies masqueraders of full-term cerebral palsy. *Ann Clin Transl Neurol*.5:538–551. doi: 10.1002/acn3.551.

Wang T, Guo H, Xiong B, Stessman HAF, *et al*, (2016). *De novo* genetic mutations among a Chinese autism spectrum disorder cohort. *Nature Communications.* 7:13316. doi: 10.1038/ncomms13316.

Wei CM, Xia GZ, Ren RN. (2018) [Gene mutations in unexplained infantile epileptic encephalopathy: an analysis of 47 cases]. Zhongguo Dang Dai Er Ke Za Zhi. 20:125-129. Chinese. (abstract in English). doi: 10.7499/j.issn.1008-8830.2018.02.009.

Wolff, M, Johannesen, KM, Hedrich UBS, Masnada S. *et al.* (2017) Genetic and phenotypic heterogeneity suggest therapeutic implications in *SCN2A*-related disorders. *Brain*. 140: 1316-36. doi: 10.1093/brain/awx054.

Wolff M, Brunklaus A, Zuberi SM.(2019) Phenotypic spectrum and genetics of *SCN2A* related disorders, treatment options, and outcomes in epilepsy and beyond.*Epilepsia*.;60(S3):S59–S67. doi: 10.1111/epi.14935.

Wright CF, Fitzgerald TW, Jones WD, Clayton S, *et al*., (2014).Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data. *Lancet* (London, England) 385;9975;1305-14. doi: 10.1016/S0140-6736(14)61705-0.

Zeng Q, Yang X, Zhang J, Liu A, *et al*. (2018). Genetic analysis of benign familial epilepsies in the first year of life in a Chinese cohort. *J Hum Genet.* 63:9-18. doi: 10.1038/s10038-017-0359-x.

Zerem A, Lev D, Blumkin L, Goldberg-Stern H. *et al.* (2014). Paternal germline mosaicism of a *SCN2A* mutation results in Ohtahara syndrome in half siblings. Eur. J. Paed.Neurol. 18. 567-571. doi: 10.1016/j.ejpn.2014.04.008.