Pathogenic copy number variants and SCN1A mutations in patients with intellectual disability and childhood-onset epilepsy

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Abstract

Background: Copy number variants (CNVs) have been linked to neurodevelopmental disorders such as intellectual disability (ID), autism, epilepsy and psychiatric disease. There are few studies of CNVs in patients with both ID and epilepsy.

Methods: We evaluated the range of rare CNVs found in 80 Welsh patients with ID or developmental delay (DD), and childhood-onset epilepsy. We performed molecular cytogenetic testing by single nucleotide polymorphism array or microarray-based comparative genome hybridisation.

Results: 8.8% (7/80) of the patients had at least one rare CNVs that was considered to be pathogenic or likely pathogenic. The CNVs involved known disease genes (EHMT1, MBD5 and SCN1A) and imbalances in genomic regions associated with neurodevelopmental disorders (16p11.2, 16p13.11 and 2q13). Prompted by the observation of two deletions disrupting SCN1A we undertook further testing of this gene in selected patients. This led to the identification of four pathogenic SCN1A mutations in our cohort.

Conclusions: We identified five rare de novo deletions and confirmed the clinical utility of array analysis in patients with ID/DD and childhood-onset epilepsy. This report adds to our clinical understanding of these rare genomic disorders and highlights SCN1A mutations as a cause of ID and epilepsy, which can easily be overlooked in adults.

Keywords: Array comparative genomic hybridization, Intellectual disability, Epilepsy, Copy number variation, SCN1A

Background

Copy number variants (CNVs; chromosomal deletions and duplications) have been identified as significant aetiological factors in a range of neurodevelopmental disorders including intellectual disability (ID) [1], autism [2], epilepsy [3] and psychiatric disease [4]. The detection of a causative CNV in a patient is valuable for genetic counselling and, in some cases, guiding clinical management. The observation of a rare chromosomal abnormality in a patient with a rare neurological phenotype has occasionally been the vital clue leading to the identification of genes and pathways critical to brain development [5, 6]. A limited number of previous genome-wide CNV studies have focused on patients with both epilepsy and ID [7–10]. We set out to investigate the rare CNVs present in a series of 80 patients with ID/ developmental delay (DD) and childhood-onset epilepsy. Our aims were: to determine the frequency of pathogenic CNVs in the cohort; to define the clinical features of patients...
carrying pathogenic CNVs; to identify any sub-groups of patients particularly enriched for pathogenic CNVs; and to highlight candidate genes for epilepsy and ID/DD.

**Methods**

**Study subjects**

Participants were recruited between 2010 and 2014. Participants were 80 unrelated patients (49 adults and 31 children) identified through medical genetics, learning disability and paediatric neurology clinics around Wales (see Additional file 1: Table S1 for further demographic information). Participants lacked a molecular diagnosis and had not previously undergone high resolution genome-wide cytogenetic analysis (<1 Mb resolution). The majority of participants had previously been tested by karyotype (61/80) combined with additional cytogenetic and molecular tests (Additional file 1: Table S2). Patients with known significant congenital brain malformations were excluded (e.g. malformations of cortical development, porencephaly, holoprosencephaly or intracerebral vascular malformations). CNV rates in the general population were estimated from 929 control subjects derived from the Wellcome Trust Case Control Consortium 2 National Blood Donors Cohort [11]. These were blood donors recruited by UK Blood Services and are therefore similar in ethnic origin to our mostly white British cohort. Controls were genotyped on Illumina OmniExpress single nucleotide polymorphism (SNP)-arrays.

**Ethics approval and consent to participate**

The study was approved by the Research Ethics Committee for Wales (09/MRE09/51). Informed consent for testing and publication was obtained from all participants (or their parents/legal guardians).

**Microarray analysis**

Genomic DNA was extracted from blood (n = 73) or saliva (n = 7). Samples were tested on one of three platforms: (i) Illumina610-Quad SNP-array (n = 20); (ii) Illumina OmniExpress SNP-array (n = 36); or (iii) microarray-based comparative genomic hybridization (array CGH) using a BlueGnome CytoChip ISCA 8x60k v2.0 array (n = 24). Validation testing was performed by fluorescent in situ hybridisation, multiplex ligation-dependent probe amplification (MLPA) or by testing on a second array platform. The method for identifying CNVs depended on the array platform. SNP-array data was called using PennCNV [12]. Called CNVs were filtered by probe number (10 or more) and gene content (at least one). We excluded CNVs which had 50 % or greater overlap with a CNV in the control cohort. However, for key genomic regions known to harbour recurrent CNVs associated with neurodevelopmental disorders which demonstrate incomplete penetrance (1q21.1, 15q11.2, 15q13.3, 16p11.2 and 16p13.11) we allowed CNVs to be present at low frequency in controls (≤1 %). Analysis focused on deletions and duplications larger than 100 kb and 250 kb respectively (50 kb for disease regions). Array CGH data was referenced against same sex control DNA (Promega) and analysed using Illumina BlueFuse Multi (v3.1) software, with data filtered on consecutive probes (3 or more) and size (as above). Imbalances detected by array CGH were interpreted by comparison with data from the Database of Genomic Variants, International Standards for Genomic Arrays consortium and local laboratory data. Coordinates are based on hg19/GRCh37. Statistical comparisons were made using Fisher’s exact test calculated with an online tool [13]. Parents and additional family members were analysed, where available, to determine if a CNV had arisen de novo or segregated with disease in a family. We assessed the clinical significance of CNVs based on their size, type, inheritance and whether they contained known disease genes. We were guided by the approach set out in previous publications [7, 14]. Based on this assessment some CNVs were annotated as ‘pathogenic’ (e.g. a de novo deletion of a proven disease gene/region) or ‘likely pathogenic’ (e.g. large CNVs containing genes/regions previously linked to disease). Other CNVs were considered to be of unknown significance.

**SCN1A gene testing**

A subgroup of patients was tested for intragenic SCN1A mutations. Sequencing of the complete coding region and flanking sequence of the gene was performed by bidirectional Sanger sequencing (n = 4) or by targeted next-generation sequencing (NGS) (n = 11). Sequencing (Sanger or NGS) covered all the coding sequence of SCN1A along with 20 bp of flanking intron or untranslated region (UTRs). Sequencing did not cover the promoter, deep intronic regions or the rest of the UTRs. In silico analysis of detected variants included PhyloP [15], SIFT [16], Grantham distance [17], PolyPhen-2 [18] and CADD [19]. We also searched the Exome Aggregation Consortium (ExAC) database [20], dbSNP [21], ClinVar [22] and an SCN1A-mutation-specific database [23]. Nucleotide and protein positions are based on NCBI Reference Sequences NM_001165963.1 and NP_001159435.1 respectively [24].

**Results and discussion**

The 80 patients had a range of epilepsy phenotypes including epileptic encephalopathy (EE, n = 25), non-lesional focal epilepsies (n = 22), and genetic generalised epilepsy with ID (GGE-ID, n = 22) (Table 1). In the remainder, the epilepsy phenotype was unclassified or
unknown. We found 22.5% (18/80) of the cohort carried at least one rare CNV (Table 2). Three patients had more than one rare CNV. The average size of the CNVs was 647 kb (median 488 kb). We identified 8 at least one rare CNV (Table 2). Three patients had Table 1

| Syndrome                                         | Number |
|--------------------------------------------------|--------|
| Epileptic encephalopathy (EE)                    | 9      |
| Lennox-Gastaut syndrome                           | 3      |
| Dravet syndrome                                   | 2      |
| West syndrome                                     | 1      |
| Myoclonic astatic epilepsy                        | 2      |
| Epilepsy of infancy with migrating focal seizures | 1      |
| Ohtahara syndrome                                 | 1      |
| Epilepsy with continuous spikes and waves during sleep | 1   |
| Unclassified EE with onset in infancy             | 5      |
| Genetic generalised epilepsy with intellectual disability (GGE-ID) | 18 |
| Myoclonic epilepsy                                | 3      |
| Progressive myoclonic epilepsy                    | 1      |
| Other GGE-IDs                                     | 22     |
| Non-lesional focal epilepsies                     | 2      |
| Unclassified epilepsy                             | 9      |
| Total                                            | 80     |

Pathogenic CNVs

The five clearly pathogenic CNVs were all de novo deletions. We found a de novo 127 kb deletion of 2q23.1 in a woman with moderate ID, mildly dysmorphic facial features (long face, thin upper lip, slightly upslanting palpebral fissures, long nose) and seizures. The deletion disrupted the first two non-coding exons of the MBDS5 gene. MBDS5 encodes a member of the methyl-CpG-binding domain family. The MBDS5 protein binds to methylated DNA and is thought to regulate gene expression by controlling chromatin modification [25]. Deletions of the 5′-UTR of MBDS5 result in reduced expression of the gene [26]. Common clinical features in MBDS5 patients include ID/DD, seizures, language impairment, microcephaly, mild craniofacial dysmorphism and autism spectrum disorders (ASD) [26–28]. Interestingly, patients with CNVs confined to the 5′-UTR (like R911) have phenotypes similar to patients with larger 2q23.1 deletions. This highlights the critical impact of non-coding sequence at the locus [29]. We observed a de novo 182 kb deletion at 9q34.3 involving EHMT1 in an adult male (R660) with moderate-to-severe ID, dysmorphic features (hypertelorism, mid face hypoplasia, prognathism), aggressive behaviour, autisttic features, depression and epilepsy. Deletions at 9q34 involving EHMT1 are responsible for Kleefstra syndrome [30]. EHMT1 encodes a histone methyltransferase involved in transcriptional repression. EHMT1 is known to interact with MBDS5 and they work together to regulate gene expression [25]. Characteristic features of Kleefstra syndrome include ID/DD, microcephaly, psychiatric disorders, severe behavioural problems, dysmorphic features, hypotonia, heart defects and seizures [31]. In addition to truncating EHMT1 the 9q34 deletion involved the adjacent CACNA1B gene. CACNA1B encodes a subunit of a voltage-dependent calcium channel expressed on neurons. Mutations in other N-type voltage-dependent calcium channel subunits have been linked to a wide range of paroxysmal disorders including periodic paralysis [32], familial hemiplegic migraine [33], myoclonus-dystonia syndrome [34], childhood absence epilepsy [35] and idiopathic generalized epilepsy [36]. Therefore, it is possible that haploinsufficiency of CACNA1B may have contributed to the patient’s epilepsy phenotype. Patient R660 also had a paternally-inherited 1.3 Mb duplication involving the FHIT gene (considered to be likely pathogenic). The FHIT gene is a member of the histidine triad gene family. FHIT encodes diadenosine 5′,5′′-P1,P3-triphosphate hydrolase, an enzyme involved in purine metabolism. Rare CNVs involving FHIT have previously been described in autism [37, 38]. R660 carried a third rare CNV, a maternally-inherited 465 kb deletion at 3p22.1 involving ULK4. ULK4 encodes a serine/threonine kinase. Expression of the ULK4 gene is neuron-specific and developmentally regulated [39]. This third CNV was considered to be a VUS, although deletions in ULK4 have recently been reported as a potential risk factor for schizophrenia [39]. The third clearly pathogenic CNV was a de novo 603 kb 16p11.2 deletion in a girl with mild DD, ASD and infantile spasms (seizure free following treatment). Seizures are a common feature of 16p11.2 deletion syndrome along with ASD, ID/DD, psychiatric disease and increased risk of obesity [40, 41]. The reciprocal duplications at 16p11.2 locus have also been associated with epilepsy including infantile spasms [7, 42]. The last two
### Table 2 Rare CNVs detected in 80 patients with ID/DD and epilepsy

| Subject | Age | Sex | Clinical features | Seizure onset | Syndrome | Seizure types | Cytoband | CNV Type | Coordinates | Size (Kb) | Tests | Status | Interpretation | Genes |
|---------|-----|-----|-------------------|--------------|----------|--------------|----------|----------|-------------|----------|-------|--------|----------------|-------|
| R125    | 10 m | F   | Severe DD, cleft palate | 3 m | EIMFS | FE, EBCS, CSE | 2q24.3 | Del | 163823021–167958723 | 4,136 | c/f | DN | Path | SCN3A, SCN2A, SCN1A, SCN9A, SCN7A & 8 others |
| R351    | 15y  | M   | Moderate DD, poor coordination, joint contractures, mildly dysmorphic | 3 m | Dravet | FS, GTCS, CSE, M | 2q24.3 | Del | 166842637–166918932 | 76 | c/d | DN | Path | SCN1A |
| R404    | 7y   | F   | Mild DD, ASD | 8 m | West | IS, Abs | 16p11.2 | Del | 29595483–30198151 | 603 | b/e,f | DN | Path | DOC2A, KIF22, MAPK3, PRRT2, QPRRT, SE2DL2 & 24 others |
| R660    | 21y  | M   | Mod-severe ID, challenging behaviour, ASD, depression, dysmorphic | 8 m | GGE-ID | Abs, M, FDS, EBCS | 9q34.3 | Del | 140707889–140890373 | 182 | b/e | DN | Path | CACNA1B, EHMT1 |
| R111    | 22y  | F   | Mod ID, small head, mildly dysmorphic | 10y | FE | FDS, GTCS | 2q22.3 | Del | 148691873–148818437 | 127 | b/e | DN | Path | MBDS, ORC4 |
| R913    | 20y  | M   | Mod-severe ID, challenging behaviour, ASD | 10 m | FE | FS, FDS, EBCS | 16p13.11 | Del | 15512574–16262571 | 750 | b/e | Likely | ABC1, C16orf45, FOPNL, KAAA0430, MIR848, MYH11, NDE1 |
| R345    | 27y  | F   | Mild ID, dysmorphic | <6y | GGE-ID | M, Abs, GTCS | 2q13 | Del | 111392259–113094793 | 1,703 | b/e | Pat | Likely | BUB1, BCL2L11, ANAPC1, MERTK, FBNL7 & 5 others |
| R58     | 26y  | F   | Severe ID, scoliosis | <8y | GGE-ID | At, Abs, M | 1q21.1 | Del | 145625979–145723645 | 98 | a/e | Mat | VUS | CD160, RNF115 |
| R74a    | 51y  | F   | Mild-mod ID, depression | 3 m | FE | FS, FE, EBCS | 1p21.1 | Del | 104167778–104297687 | 130 | a/e | U | VUS | AMY1A, AMY1B, AMY1C, AMY2A |
| R101    | 32y  | M   | ID, seizures | <16y | U | U | 11q22.3 | Del | 109173027–109325299 | 152 | b/e | Pat | VUS | C11orf87 |
| R198    | 19y  | M   | Severe ID, ASD, mild right hemiparesis | 7 m | LGS | FE, IS, Abs, NCS, GTCS, At, FDS | Xq28 | Del | 150589930–150811921 | 222 | c/nd | U | VUS | PASD1 |
| R528    | 23y  | M   | Severe ID, challenging behaviour, ASD, dysmorphic, regression | 11y | FE | FE, Abs | 15q13.3 | Del | 32019919–32514341 | 494 | b/e | U | VUS | CHRNA7 |
| R605    | 41y  | M   | ID, seizures | <16y | U | U | 15q11.2 | Del | 22383292–23227733 | 889 | b/e | Pat | VUS | CYP1P1, NIPA1, NIPA2, TUBGCP5 & 8 others |
| R622a   | 28y  | F   | Moderate ID, challenging behaviour | 6 m | GGE-ID | IS, GTCS, M | 18p11.22 | Del | 10042023–10581304 | 539 | b/e | Mat | VUS | APCDD1, NAPG |
### Table 2 Rare CNVs detected in 80 patients with ID/DD and epilepsy (Continued)

| Ref | Age | Sex | Clinical features | Seizure types | CNV Type | chromosome position | Status | Tests | Interpretation |
|-----|-----|-----|-------------------|---------------|----------|---------------------|--------|-------|----------------|
| R650 | 21y | M   | Mild ID, thin habitus, depression | Abs, M, GTCS | 15q13.3 | Dup 32029693–32514926 | 485 | a/nd U | VUS CHRNA7 |
|     |     |     |                   |               | 15q14 Del 34700297–34807869 | 108 | a/nd U | VUS GOLGA8A |
| R786 | 9y  | M   | Moderate DD, Leg hypertonia, dystonia | Abs, At | 21q21.3 | Del 27715263–27955385 | 240 | a/e Mat | VUS CYYR1 |
| R931 | 15y | F   | Severe DD, ASD, dysmorphic, microcephaly | GTCS | 7q11.22 | Del 71815170–72305671 | 491 | b/e Pat | VUS CALN1, MIR4650-1, MIR4650-2, SBDSP1, TYW18 |
| R981 | 5y  | F   | Severe DD, regression, ASD, leg hypertonia | Abs, At, M | 3p26.3 | Dup 726675–1301830 | 575 | c/nd U | VUS CNTN6 |

Age (at recruitment) and seizure onset in y(ears), m(onths) or w(eeks). Clinical features: ID intellectual disability, DD, developmental delay, ASD autism spectrum disorder

Syndrome, electroclinical syndrome or main epilepsy type at recruitment: Dravet, Dravet syndrome; EIMFS, epilepsy of infancy with migrating focal seizures; FE focal epilepsy, GGE-ID, genetic generalised epilepsy with ID, LGS Lennox-Gastaut syndrome, U unknown, West West syndrome

Seizure types: Abs absence, At atonic, CSE convulsive status epilepticus, EBCS evolution to bilateral or convulsive seizures, FDS focal dyscognitive seizures, FS febrile seizures, GTCS generalised tonic-clonic seizures, IS infantile spasms, M myoclonic, NCS non-convulsive status epilepticus, T tonic, seizure type at presentation is underlined (when known)

CNV type, Duplication or Deletion. Coordinates, chromosome position of first/last abnormal probes based on hg19/GRCh37. Tests, primary array/confirmation method: (a) Illumina610-Quad SNP-array, (b) Illumina OmniExpress SNP-array, (c) BlueGnome CytoChip array CGH, (d) quantitative PCR, (e) Illumina Exome BeadChip or custom Illumina SNP array, (f) fluorescence in situ hybridization, and (nd) not done. Status: DN, de novo; inherited Paternally; Maternally or U(known). Interpretation (of clinical significance): Path(ogenic); Likely, likely pathogenic; VUS, variant of uncertain significance

*Patients R622 and R74 had pathogenic SCN1A mutations which suggest these two CNVs are likely to be benign*
clearly pathogenic CNVs were both de novo deletions at 2q24.3: one was 76 kb in size and deleted exons 4 to 28 of the SCN1A gene; the other was 4.1 Mb and deleted 13 genes including SCN1A. SCN1A encodes a voltage-gated sodium channel which is essential for the generation and propagation of action potentials in neurons. Mutations in SCN1A cause a spectrum of seizure disorders including familial febrile seizures, generalised epilepsy with febrile seizures plus and Dravet syndrome (severe myoclonic seizures of infancy) [43–45]. Typical features of these disorders are seizure onset in infancy with fever sensitivity. Severe manifestations of SCN1A-related disease include pharmacoresistant seizures, ID/DD, ataxia and autistic behaviour [46, 47]. Patient R125, who had the larger of the two deletions, had a severe phenotype with poor seizure control, severe DD and a cleft palate. These additional features may be due to haploinsufficiency of other genes in the region. The deletion in R125 included SCN2A, SCN3A and SCN9A. All three of these genes encode voltage-gated sodium channels which have been linked to epilepsy [48–50]. The patient’s epilepsy phenotype was considered to be epilepsy of infancy with migrating focal seizures (EIMFS). A number of patients with 2q24.3 deletions and EIMFS-like phenotypes have recently been reported [51, 52]. Patient R351, who had the smaller of the 2q24.3 deletions, had previously undergone SCN1A sequencing which had not detected their multi-exon deletion. This highlights that DNA sequencing alone is insensitive to CNVs and that dose-sensitive techniques (e.g. array CGH or MLPA) are required to detect a significant proportion of SCN1A mutations [53].

Two further likely pathogenic CNVs were found. One was a paternally-inherited 1.7 Mb deletion of 2q13 in a female patient (R345) with mild ID, small ventricular septal defect, facial dysmorphism (long face, retrognathism, broad nasal root, hypertelorism, mild facial asymmetry) and epilepsy. Deletions at 2q13, similar to the one found in patient R345 have been reported in other patients with DD/ID [54, 55]. Common manifestations include facial dysmorphism, autistic features, seizures and cardiac malformations. Previously reported 2q13 deletions have been inherited from an apparently normal parent, consistent with incomplete penetrance. Interestingly, the father of R345 shares similar facial features, but has no history of ID or epilepsy. The third likely

| Table 3 | SCN1A mutations in the cohort |
|---------|-----------------------------|
| Subject | R622 | R74 | R710 | R769 |
| Age     | 28y | 51y | 24y | 3y |
| Sex     | F   | F   | F   | F   |
| Clinical features | Moderate ID, challenging behaviour | Mild-mod ID, depression | Moderate ID, ataxia, stroke-like episodes | Mod-severe DD, poor coordination |
| Seizure onset | 6 m | 3 m | 6 m | 5d |
| Syndrome | GGE-ID | FE | PME | CSWS |
| Seizure types | IS, GTCS, M | FS, FE, EBDC | C-CSE, M, FDS, EBDC | T, GTCS, CSE, FE, At, Abs, M |
| Genomic coordinates | Chr2 g.166915177 _166915180dup | Chr2 g.166915162 G > A | Chr2 g.166913001 G > C | Chr2 g.166848780 C > T |
| cDNA | c.283_286dup | c.301C > T | c.393C > G | c.5005G > A |
| Protein | p.Gly96Glufs*24 | p.Arg101Trp | p.Ser131Arg | p.Ala1669Thr |
| Inheritance | De novo | De novo | Segregates with phenotype | De novo |
| PhyloP | - | 0.91 (highly conserved) | 0.89 (highly conserved) | 0.86 (highly conserved) |
| Grantham distance | - | 101 (moderate) | 110 (moderate) | 58 (small) |
| SIFT | - | 0 (deleterious) | 0.02 (deleterious) | 0 (deleterious) |
| PolyPhen-2 (HumVar) | - | 0.982 (probably damaging) | 0.368 (benign) | 1 (probably damaging) |
| CADD (PHRED-scaled) | - | 34 (top 0.1 %) | 22.3 (top 1 %) | 26.1 (top 1 %) |
| ExAC frequency | 0 | 0 | 0 | 0 |
| dbSNP | - | rs121917965 | - | - |

Age (at recruitment) and seizure onset in y(ears), m(onths) or d(ays). Clinical features: ID intellectual disability, DD developmental delay
Syndrome, electroclinical syndrome or main epilepsy type at recruitment: CSWS, epilepsy with continuous spikes and waves during sleep; FE focal epilepsy, GGE-ID genetic generalised epilepsy with ID, PME progressive myoclonic epilepsy
Seizure types: Abs absence, At atonic, C clonic, CSE convulsive status epilepticus, EBDC evolution to bilateral or convulsive seizures, FDS focal dyscognitive seizures, FS febrile seizures, GTCS generalised tonic-clonic seizures, IS infantile spasms, M myoclonic, T tonic, seizure type at presentation is underlined. Coordinates are based on hg19/GRCh37. Nucleotide and protein reference sequences were NM_001165963.1 and NP_001159435.1

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pathogenic CNV was a maternally-inherited 750 kb duplication of 16p13.11 in a man with mild ID, ASD, seizures and a history of aggressive episodes. We considered the 16p13.11 duplication to be likely contributory as there was a family history of childhood epilepsy in the patient’s mother and a maternal uncle (untested). Deletions in the 16p13.11 region are clear risk factors for neurodevelopmental disorders including epilepsy [3, 56]. There is also evidence that duplications at 16p13.11 predispose to neurodevelopmental disorders (ASD, schizophrenia and ID) [57–60] and have been reported in patients with epilepsy [61]. Several further CNVs at genomic ‘hot spots’ were observed (duplications at 1q21.1, 15q11.2 and 15q13.3). These duplications were all inherited from unaffected parents and overlapped CNVs in the control cohort. They were therefore considered to be VUS. It remains possible that some of these VUS have contributed to disease risk. For example, there is evidence that CHRNA7 duplications may subtly increase the risk of neurodevelopmental disorders including ID [62]. However, further large-scale epidemiological studies are required to fully define these risks. Among the non-‘hotspot’ CNVs of uncertain significance we found a 575 kb duplication involving the first 4 exons of CNTN6. This duplication was identified in a 5-year-old girl with severe DD, ASD, bilateral lower limb hypertonia and early-onset seizures. CNTN6 is an interesting candidate gene for neurodevelopmental disorders as it encodes a neural adhesion molecule that operates in the formation, maintenance and plasticity of neuronal networks. In addition, CNVs involving CNTN6 have been reported in patients with DD/DD and autistic features [2, 63–65].

**SCN1A mutations**

Struck by finding two deletions involving SCN1A we realised that this key monogenic cause of epilepsy had not been extensively pre-screened in our cohort (only 9/80). The majority of recruits were adults (n = 49) who were initially investigated before SCN1A testing was available. Furthermore, in contrast to paediatric settings, the significance of SCN1A mutations for adult patients is often neglected [66], usually because key elements of early history (e.g. age of onset, initial seizure types) are not available. We therefore selected a group of patients with early-onset epilepsy for SCN1A sequencing. Of the 38 patients with seizure onset before 12 months, 6 had previously had normal testing for SCN1A while 3 others had pathogenic CNVs. Fifteen of the remaining 29 patients were prioritized for testing based on clinical features (e.g. a history of myoclonic or febrile seizures). This found 4 pathogenic SCN1A mutations (Table 3). All four patients had seizure onset in early infancy (6 months or before) and ongoing seizures despite anticonvulsant therapy. Three of the mutations were missense mutations. The fourth was a 4 base duplication leading to a frameshift early in the gene. In silico analysis indicated the missense mutations were all deleterious changes affecting conserved residues (Table 3). One missense mutation segregated with epilepsy and ID phenotypes in the patient’s family (the proband’s two affected siblings and their mildly-affected mother) the others were all de novo. In combination with the array data these results indicate that at least 6/80 (7 %) of our cohort had SCN1A-related seizure disorders.

**Conclusions**

We have reported the range of rare CNVs found in a series of 80 Welsh patients with childhood-onset epilepsy and ID/DD. We identified clearly or likely pathogenic CNVs in 7 (8.8 %) of the patients including 5 rare de novo deletions. Our results highlight key genes for brain development including drawing attention to SCN1A mutations in adults with early-onset pharmaco-resistant epilepsy and ID. Our results contribute additional phenotypic descriptions for these rare genomic disorders and support the use of molecular cytogenetic analysis in the genetic evaluation of patients with ID/DD and epilepsy.

**Additional file**

Additional file 1: Table S1. A detailed demographic description of the cohort. Table S2. Previous cytogenetic and molecular testing in the cohort. (DOCX 17 kb)

**Abbreviations**

Array CGH: microarray-based comparative genomic hybridization; ASD: autism spectrum disorder; CNV: copy number variant; DD: developmental delay; EMPS: epilepsy of infancy with migrating focal seizures; EE: epileptic encephalopathy; ID: Intellectual disability; GGE: genetic generalised epilepsy; MLPA: multiplex ligation-dependent probe amplification; NCBI: national center for biotechnology information; NGS: next-generation sequencing; SNP: single nucleotide polymorphism; VUS: variant of uncertain clinical significance.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

MPK, DTP, GK and MJO conceived and designed the project. RT, AEF, CD, DTP and MPK coordinated the project. Participants were recruited and phenotyped by AEF, PB, CJ, HA, EM, AC, CT, SD, FG, JTWN, LH, GT, CW, JN, RHT, DTP and MPK. SKC, MIR and CD facilitated sample archiving. KM, SM, SJ and HM performed the molecular analysis. ER, PH and AEF analysed the data. AEF drafted the manuscript with input from ER, RHT, SM, HM, FG, CW, MIR, MUO, GK, DTP and MPK. All authors read and approved the final manuscript.

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**Inferences**

- CHRNA7 duplications may subtly increase the risk of neurodevelopmental disorders including ID.
- CNTN6 is an interesting candidate gene for neurodevelopmental disorders.
- SCN1A mutations were found in 4 out of 80 patients.
- Missense mutations were identified in these cases.
- The results indicate at least 6/80 (7%) of the cohort had SCN1A-related seizure disorders.

**Conclusions**

- The study identified 7 rare CNVs in 80 patients.
- SCN1A mutations were found in 5 patients.
- These results highlight key genes for brain development.

**Key Takeaways**

- The study contributes to understanding genetic causes of epilepsy.
- SCN1A mutations are a significant finding.
- Further research is needed to understand the impact of these genetic findings.
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