Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders


*/# These authors contributed equally to this work.

Mutations in SCN2A, a gene encoding the voltage-gated sodium channel Na+1.2, have been associated with a spectrum of epilepsies and neurodevelopmental disorders. Here, we report the phenotypes of 71 patients and review 130 previously reported patients. We found that (i) encephalopathies with infantile/childhood onset epilepsies (≥ 3 months of age) occur almost as often as those with an early infantile onset (< 3 months), and are thus more frequent than previously reported; (ii) distinct phenotypes can be seen within the late onset group, including myoclonic-ataxic epilepsy (two patients), Lennox-Gastaut not emerging from West syndrome (two patients), and focal epilepsies with an electrical status epilepticus during slow sleep-like EEG pattern (six patients); and (iii) West syndrome constitutes a common phenotype with a major recurring mutation (p.Arg853Gln: two new and four previously reported children). Other known phenotypes include Ohtahara syndrome, epilepsy of infancy with migrating focal seizures, and intellectual disability or autism without epilepsy. To assess the response to antiepileptic therapy, we retrospectively reviewed the treatment regimen and the course of the epilepsy in 66 patients for which well-documented medical information was available. We find that the use of sodium channel blockers was often associated with clinically relevant seizure reduction or seizure
freedom in children with early infantile epilepsies (<3 months), whereas other antiepileptic drugs were less effective. In contrast, sodium channel blockers were rarely effective in epilepsies with later onset (≥3 months) and sometimes induced seizure worsening. Regarding the genetic findings, truncating mutations were exclusively seen in patients with late onset epilepsies and lack of response to sodium channel blockers. Functional characterization of four selected missense mutations using whole cell patch-clamping in tsA201 cells—together with data from the literature—suggest that mutations associated with early infantile epilepsy result in increased sodium channel activity with gain-of-function, characterized by slowing of fast inactivation, acceleration of its recovery or increased persistent sodium current. Further, a good response to sodium channel blockers clinically was found to be associated with a relatively small gain-of-function. In contrast, mutations in patients with late-onset forms and an insufficient response to sodium channel blockers were associated with loss-of-function effects, including a depolarizing shift of voltage-dependent activation or a hyperpolarizing shift of channel availability (steady-state inactivation). Our clinical and experimental data suggest a correlation between age at disease onset, response to sodium channel blockers and the functional properties of mutations in children with SCN2A-related epilepsy.

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**Abbreviations:** ACTH = adrenocorticotropic hormone; AED = antiepileptic drug; B(F)NIS = benign (familial) neonatal/infantile seizures; EIMFS = epilepsy of infancy with migrating focal seizures; ESES = electrical status epilepticus during slow sleep; SCB = sodium channel blocker
Introduction

The SCN2A gene encodes the voltage-gated sodium channel Naᵥ1.2, one of the major neuronal sodium channels that play a role in the initiation and conduction of action potentials. Naᵥ1.2 is expressed in axon initial segments and nodes of Ranvier of myelinated nerve fibres in early development, and in the adult brain in the axon initial segment and unmyelinated axons (Boiko et al., 2001, 2003; Kaplan et al., 2001; Liao et al., 2010b). Accordingly, SCN2A mutations have been mainly shown to affect the early developmental period (Catterall, 2014), but some mutations have also been found as causes of later onset neurological diseases (Kobayashi et al., 2012; Horvath et al., 2016), or a combination of both (Schwarz et al., 2016).

Since the first description of a patient with epilepsy caused by a SCN2A mutation and the findings of SCN2A mutations in benign (familial) neonatal/infantile seizures [B(F)NIS] (Sugawara et al., 2001; Heron et al., 2002), the phenotypic spectrum has expanded considerably. In particular, severe phenotypes with encephalopathy have been reported, including distinct epileptic syndromes such as Ohtahara syndrome (Nakamura et al., 2013; Allen et al., 2016), epilepsy of infancy with migrating focal seizures (EIMFS) (Howell et al., 2015), infantile spasms (Ogiwara et al., 2009; Wong et al., 2015) or West syndrome (Allen et al., 2013; Nakamura et al., 2013), as well as patients with unclassified severe epilepsy phenotypes. However, SCN2A mutations have also been found in patients with intellectual disability and/or autistic features without epilepsy, suggesting the possible involvement of the gene in the aetiology of autism spectrum disorders (Sanders et al., 2012; Li et al., 2016).

To date, the mechanisms for the phenotypic heterogeneity, ranging from benign to very severe clinical presentations, are poorly understood. Differences in functional effects of the mutations may account at least in part for the phenotypic diversity. In efficacy, the efficacy of anti-epileptic drugs (AEDs), especially of sodium channel blockers (SCBs), could be influenced by the way in which specific SCN2A mutations affect Naᵥ1.2 activity.

Therefore, we aimed to assess systematically the phenotypic spectrum and treatment effects in a large cohort of SCN2A-related disorders comprising 201 patients, 71 of whom were not reported previously. For some missense mutations that we selected based on specific clinical findings, and supported by previous reports from the literature, we were able to correlate phenotype and treatment responses to the specific biophysical effects of the mutations.

Materials and methods

Previously unpublished patients

Seventy-one previously unreported patients with a SCN2A mutation were included in this study. Patients were referred through a network of collaborating clinicians and geneticists. Mutations in SCN2A were identified in research or diagnostic laboratories and assumed to be pathogenic, if they were nonsynonymous, splice-site altering, nonsense or frameshift changes, predicted damaging by one or more prediction software (PolyPhen-2, SIFT and MutationTaster), seen less than twice in >60000 controls in the exome aggregation consortium browser (exac.broadinstitute.org), and either occurred de novo, or were inherited from an affected parent, an unaffected mosaic parent or previously reported as pathogenic in other patients. Sanger sequencing was used to confirm all mutations and perform segregation analysis. The study was approved by the local ethics committees.

Referring physicians were provided with a standardized phenotyping sheet to assess clinical characteristics, EEG, and neuroimaging findings. Seizures were diagnosed according to the International League Against Epilepsy commission on classification (Berg et al., 2010), and were assigned, whenever possible, to defined epileptic syndromes. Data on cognitive development and neurological features were recorded at age at onset and at last evaluation. Based on the presence and severity of epilepsy, cognitive status and age at onset of epilepsy patients were classified into the following groups: (i) B(F)NIS, defined as neonatal/infantile onset seizures with a seizure offset during infancy/early childhood, and/or autosomal-dominant inheritance, and normal cognitive development; (ii) encephalopathy with early infantile epilepsy, defined as seizure onset before the age of 3 months, and impaired cognitive development; (iii) encephalopathy with infantile/childhood epilepsy, defined as seizure onset at the age of at least 3 months, and impaired cognitive development; and (iv) intellectual disability and/or autism without epilepsy.

Antiepileptic treatment data were retrospectively assessed by standardized questionnaires. The effect on seizures was classified according to the judgement of the treating physicians into seizure freedom, seizure reduction, no effect or seizure worsening. Particular attention was given to the effects of SCBs, defined as AEDs that reduce the activity of sodium channels by stabilizing an inactivated state. SCBs included phenytoin, carbamazepine, oxcarbazepine, lamotrigine, and zonisamide. To provide a general overview in our retrospective analysis, we specifically assessed whether patients were on an SCB by the time that seizure reduction, seizure freedom, or aggravation of seizures occurred.

Frequency of SCN2A-related disorders

To estimate the frequency of SCN2A mutations causing the reported phenotypes in the general population, we used the electronic population databases of National Statistics at the Statens Serum Institute (Denmark) to calculate the birth cohort from 2007 to 2014. The Danish Epilepsy Centre is the only tertiary hospital in Denmark specialized in the treatment of epilepsy, and the majority of patients with presumed genetic epilepsy are referred to this centre for genetic testing.

Literature review

We searched PubMed using the term ‘SCN2A’ and included all relevant patient-related information in our SCN2A dataset.
Last search date was 1 June 2016. Papers not available in English, Italian or Danish were excluded. Cases with deletions and duplications spanning SCN2A as well as neighbouring genes were excluded. For patients with little or no clinical information, we listed the phenotype mentioned in the respective publication.

**Mutagenesis**

To engineer the mutations into the adult splice variant of the human Na1.2 channel, site-directed mutagenesis was performed using Quickchange® II XL (Agilent Technologies; primers are available upon request) as described previously (Schwarz et al., 2016). Transfection of the α-subunit together with pCLH-hβ1-EGFP and pCLH-hβ2-CD8 in tsA201 cells using Mirus TransIT®-LT1 reagent was performed in a standard way as described previously (Liao et al., 2010a; Lauxmann et al., 2013; Schwarz et al., 2016).

**Electrophysiology**

Standard whole-cell patch clamp recordings were performed using an Axopatch 200B amplifier, a Digidata 1320A digitizer and pClAMP 8 data acquisition software (Axon Instruments), as described before (Schwarz et al., 2016). Borosilicate glass pipettes had a final tip resistance of 1–2 MΩ when filled with internal recording solution containing (in mM): 130 CsF, 5 NaCl, 2 MgCl₂, 5 EGTA, HEPES (pH 7.4, 290 mOsm). The bath solution contained (in mM): 140 NaCl, 4 KCl, 1 MgCl₂, 2 CaCl₂, 2 HEPES, 5 dextrose (pH 7.4, 300 mOsm). We carefully checked that the maximal voltage error due to residual series resistance after up to 95% compensation was always <3 mV. Voltage clamp protocols to study channel kinetics were performed as described previously (Schwarz et al., 2016) and are provided in detail in the Supplementary material.

**Data and statistical analysis**

Traces were displayed off-line with Clampfit software of pClamp 10.0 (Axon Instruments). Graphics were generated using a combination of Microsoft Excel, and Origin (version 9.1; OriginLab Inc., USA) software, statistics were performed using SigmaStat 3.1 (Systat Software GmbH, Germany). All data were tested for normal distribution. For statistical evaluation, ANOVA on ranks (Kruskal-Wallis-test) with Dunn’s post hoc test for not normally distributed data or one-way ANOVA (Bonferroni post hoc test) was used when datasets were normally distributed. All data are shown as means ± standard error of the mean (SEM), n gives the number of cells. We applied the χ² test to estimate the significance of the differences in AED treatment effects in the two groups of epilepsy with encephalopathy with early and late onset.

**Results**

In the present study, we report 71 unpublished patients with pathogenic SCN2A mutations and review the phenotypes of 130 previously reported ones (see Tables 1–4, Supplementary Table 1 and Supplementary Fig. 1). The distribution of phenotypes of the 66 previously unpublished patients with epilepsy and of the total number of patients are displayed in Fig. 1.

**Phenotypic features**

**Benign (familial) neonatal/infantile seizures**

We identified nine unpublished patients (Table 1) and 24 probands from the literature with B(F)NIS due to a SCN2A mutation, as well as 109 mutation-positive family members (Berkovic et al., 2004; Striano et al., 2006; Herlenius et al., 2007; Heron et al., 2010; Liao et al., 2010b; Lemke et al., 2012; Lauxmann et al., 2013; Zara et al., 2013; Grinton et al., 2015; Johannesen et al., 2016; Schwarz et al., 2016). The mutations occurred de novo in 6/33 of the probands. Age at seizure onset ranged from the first day of life to 23 months. Approximately half of the children had seizure onset within the first month of life. Seizure types were predominantly focal clonic, tonic, and generalized tonic-clonic, frequently occurring in clusters. Interictal EEG showed mostly focal or multifocal spikes, but was normal in some cases. All children became seizure-free at a median age of 5 months (range 3 days to 2 years), and remained seizure-free with normal cognitive development until last follow-up at a median age of 5.5 years (range 7 months to 34 years, data available from 28 cases). A single proband developed a second epilepsy phenotype during later childhood with marked activation of multifocal spikes during sleep and partial cognitive deterioration, resembling electrical status epilepticus during slow sleep (ESES), and two had isolated seizures until the age of 2 and 14 years, respectively. Five children with two recurrent mutations (A263V and R1882G) exhibited episodic ataxia later in life (Liao et al., 2010a; Johannesen et al., 2016; Schwarz et al., 2016).

**Encephalopathy with early infantile epilepsy**

Twenty-eight new patients (Table 2) and 43 previously published ones (Ogiwara et al., 2009; Dhamija et al., 2013; Nakamura et al., 2013; Touma et al., 2013; Baasch et al., 2014; Martin et al., 2014; Matalon et al., 2014; Zerem et al., 2014; Fukasawa et al., 2015; Howell et al., 2015; Allen et al., 2016; Trump et al., 2016) had epilepsy onset within the first 3 months of life. Thirty-one had an identifiable epilepsy syndrome, i.e. Ohtahara syndrome (18 cases) or EIMFS (13 cases). The remaining 40 patients had unclassifiable epilepsies. The predominant seizure types in these were focal, tonic, and tonic-clonic seizures or spasms. Initial EEGs showed a suppression burst pattern in 25 cases (18 with Ohtahara syndrome, two with EIMFS, and five with unclassifiable epilepsies), and multifocal spikes in the majority of the remaining cases. Regardless of the epileptic syndrome, all patients fulfilled the criteria of encephalopathy as they had intellectual disability, being severe in 54/71 cases. Six children had autism...
spectrum disorder. Additional features included muscular hypotonia \((n = 32)\), microcephaly \((n = 15)\), marked dystonic or choreatic movement disorders \((n = 8)\), spasticity \((n = 3)\), or dysautonomia \((n = 5)\). Seven patients in this subgroup were deceased at time of follow-up, and causes of death included severe infections and status epilepticus.

**Encephalopathy with infantile/childhood epilepsy**

This group included 29 unpublished (Table 3) and 29 previously published patients (Haug et al., 2001; Sugawara et al., 2001; Kamiya et al., 2004; Ogiwara et al., 2009; Shi et al., 2009; Kobayashi et al., 2012; Allen et al., 2013; Nakamura et al., 2013; Sundaram et al., 2013; Hackenberg et al., 2014; D’Gama et al., 2015; Howell et al., 2015; Mercimek-Mahmutoglu et al., 2015; Samanta and Ramakrishnaiah, 2015; Wong et al., 2015; Dimassi et al., 2016; Horvath et al., 2016). Sixteen presented with West syndrome or infantile spasms, which evolved into Lennox-Gastaut syndrome in 5/16 patients. Two cases were diagnosed as Dravet syndrome, two as Lennox-Gastaut syndrome and two as myoclonic-atonic epilepsy. The majority of the remaining patients with seizure onset after 3 months of age had unclassifiable epilepsies mainly with generalized seizure types including generalized tonic-clonic \((n = 36\), occurring in clusters in four\), absence \((n = 12)\) and myoclonic seizures \((n = 8)\). EEG showed mainly generalized spikes and waves or multifocal spikes. Interestingly, four patients with unclassifiable epilepsies (Patients 41, 61, 62 and 65) and one patient (Patient 50) with West syndrome showed an ESES-like marked

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**Figure 1 Distribution of patients according to phenotype and age at epilepsy onset.** (A) Epilepsy phenotypes in the previously unpublished cohort \((n = 66)\). ‘Patients with Lennox-Gastaut syndrome’ refers to patients with Lennox-Gastaut syndrome not evolving from West syndrome. (B) Phenotypes in the overall cohort \((n = 201)\). LGS = Lennox-Gastaut syndrome; MAE = myoclonic-atonic epilepsy; OS = Ohtahara syndrome; WS = West syndrome.
Table 1: Clinical characteristics and treatment response of the previously unpublished patients: B(F)NIS

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation/inheritance</th>
<th>Age at epilepsy onset</th>
<th>Initial seizure type</th>
<th>Other seizure types</th>
<th>EEG features</th>
<th>MRI features</th>
<th>Cognition before seizure onset</th>
<th>Seizure-free follow-up</th>
<th>Treatment effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A202V/maternals</td>
<td>4 y 6 m</td>
<td>BNS</td>
<td>MF spikes</td>
<td>ADS</td>
<td>NA</td>
<td>Normal</td>
<td>2 m</td>
<td>PB, PH, TPM, CBZ, LEV</td>
</tr>
<tr>
<td>2</td>
<td>G288V/de novo</td>
<td>6 m</td>
<td>BNS</td>
<td>MF spikes</td>
<td>ADS</td>
<td>NA</td>
<td>Normal</td>
<td>2 m</td>
<td>PB, PH, TPM, CBZ, LEV</td>
</tr>
<tr>
<td>3</td>
<td>Q153K/de novo</td>
<td>14 m</td>
<td>MF spikes</td>
<td>MF spikes</td>
<td>ADS</td>
<td>NA</td>
<td>Normal</td>
<td>2 m</td>
<td>PB, PH, TPM, CBZ, LEV</td>
</tr>
<tr>
<td>4</td>
<td>Q153K/de novo</td>
<td>14 m</td>
<td>MF spikes</td>
<td>MF spikes</td>
<td>ADS</td>
<td>NA</td>
<td>Normal</td>
<td>2 m</td>
<td>PB, PH, TPM, CBZ, LEV</td>
</tr>
<tr>
<td>5</td>
<td>D434G/de novo</td>
<td>18 m</td>
<td>MF spikes</td>
<td>MF spikes</td>
<td>ADS</td>
<td>NA</td>
<td>Normal</td>
<td>2 m</td>
<td>PB, PH, TPM, CBZ, LEV</td>
</tr>
<tr>
<td>6</td>
<td>F207S/de novo</td>
<td>18 m</td>
<td>MF spikes</td>
<td>MF spikes</td>
<td>ADS</td>
<td>NA</td>
<td>Normal</td>
<td>2 m</td>
<td>PB, PH, TPM, CBZ, LEV</td>
</tr>
<tr>
<td>7</td>
<td>V261M/de novo</td>
<td>20 m</td>
<td>MF spikes</td>
<td>MF spikes</td>
<td>ADS</td>
<td>NA</td>
<td>Normal</td>
<td>2 m</td>
<td>PB, PH, TPM, CBZ, LEV</td>
</tr>
<tr>
<td>8</td>
<td>V261M/de novo</td>
<td>3 y</td>
<td>MF spikes</td>
<td>MF spikes</td>
<td>ADS</td>
<td>NA</td>
<td>Normal</td>
<td>2 m</td>
<td>PB, PH, TPM, CBZ, LEV</td>
</tr>
<tr>
<td>9</td>
<td>R36G/maternals</td>
<td>5 y 5 m</td>
<td>MF spikes</td>
<td>MF spikes</td>
<td>ADS</td>
<td>NA</td>
<td>Normal</td>
<td>2 m</td>
<td>PB, PH, TPM, CBZ, LEV</td>
</tr>
</tbody>
</table>

Encephalopathy with unspecified onset of epilepsy
In 10 of the previously published patients (Need et al., 2012; Wang et al., 2012; Carvill et al., 2013; Saitoh et al., 2015; Li et al., 2016), data were limited, and age at seizure onset was not available. One case was classified as Lennox-Gastaut syndrome, the others as encephalopathies with epilepsy that were not further characterized.

Estimated frequency of SCN2A mutations in the Danish population
Via the electronic population databases of National Statistics at the Statens Serum Institute (Denmark), we calculated the birth cohort from 2006–14, giving 550,261 births. In the same period at least seven Danish children with an SCN2A mutation causing the reported phenotypes were born, making a total minimum frequency of approximately 1/78,608 births.

Seizure outcome and treatment effects
Antiepileptic treatment effects on seizures were analysed in all unpublished patients with epilepsy for which sufficiently detailed clinical information was available (n = 66, Tables 1–4). Besides classical AEDs, corticosteroids or adrenocorticotropic hormones (ACTH) were tried in 19 children, ketogenic diet in 13, vagal nerve stimulation in three, and immunoglobulins in one.

Benign (familial) neonatal/infantile seizures
All children became seizure-free at a median age of 3 months (range: 5 days to 2 years). All but one remained
Table 2: Clinical characteristics and treatment response of the previously unpublished patients: encephalopathy with early onset epilepsy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation/inheritance</th>
<th>Age at Epilepsy seizure syndrome seizure type</th>
<th>Initial Age</th>
<th>Other seizure types</th>
<th>EEG</th>
<th>MRI</th>
<th>Neurological features</th>
<th>Additional features</th>
<th>Age at last follow-up</th>
<th>Seizure Outcome (offset: age)</th>
<th>Treatment effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>V423L/ de novo</td>
<td>1 d</td>
<td>OS</td>
<td>M, AP</td>
<td>T</td>
<td>NA</td>
<td>MBF spikes</td>
<td>NA/SD</td>
<td>34 m (deceased)</td>
<td>Intractable</td>
<td>BR</td>
</tr>
<tr>
<td>11</td>
<td>E999K/ de novo</td>
<td>1 d</td>
<td>OS</td>
<td>other T</td>
<td>M, TCS</td>
<td>SB-β-f spikes</td>
<td>NA/SD</td>
<td>Dysautonomia, irritability</td>
<td>3 y</td>
<td>Sz free (1 m, relapses with low PHT levels)</td>
<td>PHT</td>
</tr>
<tr>
<td>12</td>
<td>Q181E/ de novo</td>
<td>1 d</td>
<td>OS</td>
<td>other T</td>
<td>M, TCS</td>
<td>SB-β-f spikes</td>
<td>NA/SD</td>
<td>Dysautonomia, irritability</td>
<td>3 y</td>
<td>Sz free (4 y)</td>
<td>PHT</td>
</tr>
<tr>
<td>13</td>
<td>M548V/ de novo</td>
<td>1 d</td>
<td>OS</td>
<td>W S</td>
<td>T</td>
<td>NA</td>
<td>MBF spikes</td>
<td>NA/SD</td>
<td>18 m</td>
<td>Intractable</td>
<td>PHT</td>
</tr>
<tr>
<td>14</td>
<td>I237N/ de novo</td>
<td>1 d</td>
<td>other F</td>
<td>MBF spikes, slowing</td>
<td>F (variable onset)</td>
<td>NA/SD</td>
<td>Hypotonia</td>
<td>Poor eye contact</td>
<td>3 y 9 m</td>
<td>Intractable</td>
<td>PHT</td>
</tr>
<tr>
<td>15</td>
<td>V887A/ de novo</td>
<td>1 d</td>
<td>OS</td>
<td>W S</td>
<td>S</td>
<td>NA</td>
<td>MBF spikes</td>
<td>NA/SD</td>
<td>15 m</td>
<td>Sz free (6 m, relapse with low PHT levels)</td>
<td>PHT</td>
</tr>
<tr>
<td>16</td>
<td>GB32U/ de novo</td>
<td>1 d</td>
<td>EIMFS</td>
<td>unilateral Tc r/l</td>
<td>T</td>
<td>N</td>
<td>MF spikes, icctal pattern r/l</td>
<td>NA/SD</td>
<td>10 m</td>
<td>Intractable</td>
<td>PHT, LCM, ZNS</td>
</tr>
<tr>
<td>17</td>
<td>I1640S/ de novo</td>
<td>1 d</td>
<td>Other T</td>
<td>E TC</td>
<td>NA</td>
<td>NA</td>
<td>MBF spikes, slowing</td>
<td>NAV</td>
<td>9 y</td>
<td>Sz free (7 y)</td>
<td>LCM</td>
</tr>
<tr>
<td>18</td>
<td>K908E/ de novo</td>
<td>1 d</td>
<td>Other M</td>
<td>S</td>
<td>NA</td>
<td>NAV</td>
<td>Nystagmus</td>
<td>MC</td>
<td>8 y</td>
<td>Sz free (7 y 6 m)</td>
<td>LTG, TPM</td>
</tr>
<tr>
<td>19</td>
<td>R1882Q/ unknown</td>
<td>1 d</td>
<td>Other NA</td>
<td>F → C</td>
<td>Subtle CD</td>
<td>NA/SD</td>
<td>Nystagmus</td>
<td>MC</td>
<td>10 y 6 m</td>
<td>Intractable</td>
<td>CBZ</td>
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<tr>
<td>20</td>
<td>V1627M/ de novo</td>
<td>2 d</td>
<td>EIMFS</td>
<td>T r/l migrating</td>
<td>E AP</td>
<td>NA</td>
<td>MBF spikes, slowing</td>
<td>NA/SD</td>
<td>14 m</td>
<td>Sz free (2 m)</td>
<td>VGB</td>
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<tr>
<td>21</td>
<td>R856Q/ de novo</td>
<td>2 d</td>
<td>EIMFS</td>
<td>T r/l migrating</td>
<td>NA</td>
<td>NAV</td>
<td>Connatal sinus thrombosis</td>
<td>3 m (deceased)</td>
<td>Intractable</td>
<td>MDZ</td>
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<tr>
<td>22</td>
<td>A1500T/ de novo</td>
<td>2 d</td>
<td>Other F, T</td>
<td>NA/SD</td>
<td>C</td>
<td>HB</td>
<td>MBF spikes</td>
<td>Poor eye contact, MC</td>
<td>13 y</td>
<td>LTG, VPA (3 m), ACTH (13 m)</td>
<td>PHT, CBZ, LTG, BR</td>
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<tr>
<td>23</td>
<td>M1545V/ de novo</td>
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<td>EIMFS</td>
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<td>E TCS</td>
<td>NA/SD</td>
<td>MBF spikes, slowing</td>
<td>NA/MD</td>
<td>12 m</td>
<td>Sz free (3 m, relapses with low PHT levels)</td>
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<td>24</td>
<td>E4303A/ de novo</td>
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<td>M</td>
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<td>NA</td>
<td>MBF spikes, slowing</td>
<td>NA/MD</td>
<td>2 y 8 m</td>
<td>Sz free (12m)</td>
<td>PHT</td>
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<tr>
<td>25</td>
<td>S1536R/ de novo</td>
<td>2 d</td>
<td>Other T</td>
<td>AP, SE, TCS MF spikes, occ, slowing N</td>
<td>T</td>
<td>NA/SD</td>
<td>Dysautonomia, irritability</td>
<td>3 y</td>
<td>Intractable</td>
<td>PHT</td>
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<td>26</td>
<td>F1597L/ de novo</td>
<td>2 d</td>
<td>EIMFS</td>
<td>T r/l migrating</td>
<td>T-S</td>
<td>NA</td>
<td>SB-β-f spikes, slowing</td>
<td>At, Sclerocalcifications</td>
<td>3 y</td>
<td>Sz free (15 m)</td>
<td>CBZ</td>
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<td>27</td>
<td>V4292U/ de novo</td>
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<td>Other F</td>
<td>M</td>
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<td>NA/MD</td>
<td>2 y 8 m</td>
<td>Sz free (3 m)</td>
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<td>3 y</td>
<td>Sz free (15 m)</td>
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<td>3 d</td>
<td>Other T</td>
<td>M</td>
<td>NA</td>
<td>NA</td>
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<td>3 y</td>
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<td>M</td>
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<td>NA</td>
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<td>NA/MD</td>
<td>3 y</td>
<td>Sz free (3 m)</td>
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<td>14 m</td>
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<td>32</td>
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<td>NA</td>
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<td>Sz free (4 m)</td>
<td>PHT</td>
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Table 2 Continued

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<th>Age at last follow-up</th>
<th>EEG</th>
<th>MRI</th>
<th>Cognition/Neurological features</th>
<th>Treatment effects</th>
<th>Treatment outcomes</th>
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<th>Treatment effects</th>
<th>Treatment outcomes</th>
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<td>OS</td>
<td>F</td>
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<td>T2H, NA/SD</td>
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<td>3 w</td>
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<td>S</td>
<td>T, GTC; Atypical HA = MF spikes, slowing</td>
<td>At, HM, N/SD</td>
<td>Spasticity, MC, scoliosis</td>
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<td>GTCS</td>
<td>C, SE; MF spikes</td>
<td>N, N/MID</td>
<td>Hypotonia</td>
<td>9 m</td>
<td>Intractable</td>
<td>PHT</td>
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<td>R1319Q/de novo</td>
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<td>other</td>
<td>GTC</td>
<td>FC; SW, slowing</td>
<td>HM, N/MID</td>
<td>N</td>
<td>ASD</td>
<td>3 y 10 m</td>
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<td>other</td>
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<td>N, SD/SD</td>
<td>Hypotonia</td>
<td>4 y 8 m</td>
<td>Intractable</td>
<td>VGB, PB, TPM, VPA, CLB</td>
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AA = atypical absences; A = atonic; AB = absence; ADS = attention deficit disorder; ASD = autism spectrum disorder; AU = autonomic seizures; AP = apnoeic seizures; At = atrophy; Bifr = bifrontal; Bil = bilateral; C = clonic; CC = corpus callosum; CD = cortical dysplasia; Ce = central; DA = drop attacks; ED = epileptiform discharges; F = focal; FD = focal dyscognitive; FC = febrile convulsion; fr = frontal; Gen = generalized; GTC = generalized tonic-clonic; HA = hypsarrhythmia; HC = hemiclonic; HM = hypomyelination; IS = infantile spasms; L = left; m = months; MD = mild intellectual disability; MOD = moderate intellectual disability; M = myoclonic; MC = microcephaly; MF = multifocal; N = normal; NA = not applicable; NAV = not available; NCSE = non-convulsive status epilepticus; Occ = occipital; OS = Ohtahara syndrome; Par = parietal; R = right; S = spasms; SE = status epilepticus; SB = suppression burst; SD = severe intellectual disability; SW = spike and waves; Sz = seizures; T2H = T2-hyperintensities; TCS = tonic-clonic seizures; T = tonic; TM = temporal; w = week; y = years; = change to.

Treatment (sodium channel blockers are highlighted in bold): AZA = acetazolamide; B6 = vitamin B6; BR = bromide; CBZ = carbamazepine; CLB = clobazam; CLZ = clonazepam; CS = corticosteroids; ESM = ethosuximide; FBM = felbamate; GBP = gabapentin; IMG = intravenous immunoglobulins; KD = ketogenic diet; LCM = lacosamide; Li = lidocaine; LTG = lamotrigine; LEV = levetiracetam; MDZ = Midazolam; ME = metilexine; MSX = mesuximide; OXC = oxcarbazepine; PB = phenobarbital; PHT = phenytoin; PP = pyridoxal phosphate; RGB = retigabine; RU = rufinamide; ST = sulthiame; STP = stiripentol; TPM = topiramate; VGB = vigabatrin; VNS = vagal nerve stimulation; VPA = valproate; ZNS = zonisamide.
seizure-free until last follow-up at a median age of 20 months (range: 14 months to 11 years) \((n = 9, \text{Table 1})\). Patient 4 developed an ESES-like picture at the age of 8 years, and became seizure-free again at the age of 10 years. Seizures stopped spontaneously in three patients, and with AED treatment in six. The sequence of events strongly suggested that seizure freedom was reached by treatment and not by natural history in those cases. Phenytoin was effective in two patients, oxcarbazepine in two, zonisamide in one, and clobazam in one. Initial AED treatment failed in five in two patients, oxcarbazepine in two, zonisamide in one, and clobazam in one. Initial AED treatment failed in seven cases, and six received more than two AEDs (mean 4.3) before the seizures stopped.

**Encephalopathy with early infantile epilepsy with onset younger than 3 months**

Seizure freedom has so far been achieved during the first year of life in 11 children, and during childhood in another six (median observation period after seizure freedom: 2.5 years, range 1 month to 18 years) \((n = 28, \text{Table 2})\). Effective AEDs in terms of seizure freedom included phenytoin \((n = 8)\), ACTH \((n = 2)\), and carbamazepine, lacosamide, vigabatrin, topiramate as well as a combination of lamotrigine, valproate and levetiracetam in single cases each. One child with Ohtahara syndrome (Patient 11) and one with EIMFS (Patient 23) became rapidly seizure-free after application of phenytoin at the age of 1 and 3 months, respectively, and the burst suppression pattern on EEG disappeared. In Patient 23, low plasma levels of phenytoin \((< 13 \text{ mg/l})\) resulted in seizure relapse on several occasions. With a high dosage of phenytoin \((15 \text{ mg/kg/d, divided in three daily doses})\) higher phenytoin serum levels were obtained, and seizure freedom was finally achieved. A switch to high dose carbamazepine \((45 \text{ mg/kg/d})\) was successful during follow-up. Seizure relapses to low phenytoin plasma levels were also seen on several occasions in Patient 11 and three other children (Patients 15, 25 and 35) during follow-up. Patient 26 showed prompt resolution of burst suppression pattern and temperature instability with phenytoin, but had ongoing seizures although with markedly reduced frequency. AED-related seizure reduction was evident in another 16 cases, most frequently with topiramate \((n = 6)\), phenytoin \((n = 3)\), and carbamazepine \((n = 4)\). Ineffective AEDs included phenobarbital \((n = 18)\), levetiracetam \((n = 17)\), topiramate \((n = 13)\), and valproate \((n = 11)\) (Fig. 2A and Table 2).

**Encephalopathy with infantile/childhood epilepsy with onset at 3 months or older**

Ten children became seizure-free during follow-up (median observation period after seizure freedom: 3.5 years, range 1 month to 16 years) \((n = 29, \text{Table 3})\). Eight of nine patients with West syndrome were resistant to treatment, including steroids or ACTH in six. One child (Patient 46) responded to ACTH, but later developed drug-resistant Lennox-Gastaut syndrome. Among the patients with other epilepsy phenotypes, different AEDs were effective in single cases each, including levetiracetam \((n = 2)\) and valproate \((n = 2)\). Seizure reduction occurred most frequently with levetiracetam \((n = 9)\), benzodiazepines \((n = 9)\), and valproate \((n = 7)\). Ineffective AEDs included lamotrigine \((n = 10)\), valproate \((n = 10)\), phenobarbital \((n = 9)\), and topiramate \((n = 9)\). Drug-induced aggravation of seizures occurred in seven children with carbamazepine \((n = 3)\), oxcarbazepine \((n = 2)\), phenytoin, lamotrigine and rufinamide, and remitted after discontinuation of the respective AED (see also Fig. 2B and Table 3). Atypical seizures were the predominant seizure types in these. In a boy with myoclonic-atonic epilepsy (Patient 52), the frequency of drop attacks and tonic seizures increased markedly after introduction of oxcarbazepine, and lamotrigine provoked episodes of status epilepticus.

**Response to sodium channel blocker versus non-sodium channel blocker treatment**

We tested the significance of effects of SCBs versus non-SCBs on seizure outcome of patients with encephalopathy and epilepsy with onset <3 months and \(\geq 3\) months by applying a \(\chi^2\) test (Supplementary Table 3). Treatment with phenytoin and carbamazepine or with all SCBs considered together showed a significantly better response for patients with onset of epilepsy <3 months than for those with onset \(\geq 3\) months \((P < 0.01 \text{ and } P < 0.001, \text{respectively})\). In contrast, patients with epilepsy onset \(\geq 3\) months responded significantly better to non-SCBs \((P < 0.001)\).

**Impact of the genetic diagnosis on treatment decisions**

In most patients, genetic diagnosis was only made late during follow-up and had no impact on treatment decisions, because patients were already seizure-free or many AEDs had been tried before. In some cases, however, suspicion or confirmation of a mutation in SCN2A led to specific treatment trials with SCBs: three children (Patients 15, 23 and 25) with severe early onset epilepsies and one (Patient 2) with de novo BNIS became seizure-free with phenytoin, and three children with severe early onset EIMFS (Patients 26 and 30) or Ohtahara syndrome (Patient 33) showed partial responses to some SCBs, whereas other types of AEDs had failed before. In contrast, three children with late onset epilepsies (Patients 39, 40 and 41) showed no effect on SCB trials.

**Genetic findings**

Mutations were missense in all children with B(F)NIS or encephalopathies with epilepsy onset <3 months. The majority of the missense mutations (both inherited and de novo) affects highly evolutionarily conserved amino acids and no obvious correlation between the position of the mutation and the severity of the associated phenotype was observed. In the subgroup of infantile/childhood epilepsies, mutations were missense in 45, early stop codons in six, frameshift in five (both predicting truncated proteins), and altered splice-sites in two. All non-missense mutations
Table 3 Clinical characteristics and treatment response of the previously unpublished patients: encephalopathy with late onset epilepsy

| Patient | Mutation/inheritance | Age at seizure onset | Epilepsy syndrome | Initial seizure type | Other seizure types | EEG | MRI | Cognition onset/offset | Neurological features | Additional features | Age at last follow-up | Seizure outcome (offset: age) | Treatment effects | Seizure-free | Sz reduction | No effect | Worsening |
|---------|----------------------|----------------------|-------------------|---------------------|-------------------|-----|-----|------------------------|----------------------|----------------------|---------------------|----------------------|-------------------------|----------------------|-------------|-------------|---------|---------|
| 38      | GB95S/de novo        | 3 m                  | Other             | HC                  | GTC, FD, AA       | Bifr SW      | N   | MSA/D                     | N                    | ASD                  | 7 y                 | Intractable           | TPM, LEV, RUF, PB, B6, VPA, LCM, OXC, ZNS, KD | LEV, ZNS        | BD, VGB, CS, ACTH, TPM, KD, PB, OXC, VPA, ESM, LTG, OXC | PB, VPA, ST, KD, LTG |
| 39      | T227S/de novo        | 3 m                  | WS                | T, AP               | S                  | HA --> post spikes, slowing | N   | SD/SD                     | Hypotonia            | MC                   | 2 y                 | Intractable           | LEV, ZNS | VPA, ESM, LTG, OXC | PB, VPA, ST, KD, LTG |
| 40      | A733T/de novo        | 3 m                  | Other             | HC r/l              | AA, DA, FD        | MF spikes, slowing | N   | MD/MD                     | N                    | Regression, ASD, 6 y | 12 y               | Intractable           | LEV, ZNS | VPA, ESM, LTG, OXC | PB, VPA, ST, KD, LTG |
| 41      | R1882P/de novo       | 4 m                  | Other             | T, C r              | MF spikes, SW- status → ESES-like | N   | NSD | N                       | Stereotypical       | N                    | 16 y               | Intractable           | VPA, PB | VPA, CLZ, RUF, TPM, LTG |
| 42      | L1665F/de novo       | 4 m                  | Other             | TS, SE              | GTC (series)      | N               | N   | NSD/D                     | N                    | Stereoyped            | 6 y 3 m            | Intractable           | CBZ, VPA  | VPA, CLZ, RUF, TPM, LTG |
| 43      | L1342P/de novo       | 6 m                  | WS                | S                   | HA --> MF spikes, slowing | At   | SD/SD | Axial hypotonia, limb spasticity, hemiparesis | N                    | N                    | 16 y               | Intractable           | VPA, PB | VPA, CLZ, RUF, TPM, LTG |
| 44      | L881P/de novo        | 6 m                  | WS --> LGS        | T, TC, AA           | HA --> MF spikes, slowing | At   | MSA/D | N                       | Hypotonia            | N                    | 16 y               | Intractable           | VPA, PB | VPA, CLZ, RUF, TPM, LTG |
| 45      | II281F/de novo       | 7 m                  | WS --> LGS        | T, TC               | HA --> MF spikes, slowing, diffuse SW | At   | MSA/D | N                       | Hypotonia            | N                    | 16 y               | Intractable           | VPA, PB | VPA, CLZ, RUF, TPM, LTG |
| 46      | R853Q/de novo        | 8 m                  | WS --> LGS        | AA, T, F (4 y)      | HA --> for bil spikes, T2H, at SW, Slowing | N   | NSD/D | Dystonia              | Preterm 28 MC, regression, ASD | 16 y | Intractable           | VPA, CLZ, TPM, LTG, OXC, KD | LEV, ZNS | VPA, ESM, LTG, OXC | PB, VPA, ST, KD, LTG |
| 47      | A1652T/de novo       | 9 m                  | WS                | S                   | atypical HA --> slowing | HM   | SD/SD | Hypotonia            | N                    | N                    | 16 y               | Intractable           | VPA, PB | VPA, CLZ, RUF, TPM, LTG |
| 48      | R1319W/de novo       | 10 m                 | WS --> LGS        | T, S                | TCS, A, AA        | HA --> spikes occur --> spikes cephalic r | N   | NSD | Hypotonia, Diabetes, ASD | 10 y               | Sz free (7 y)         | Intractable | VPA, PB | VPA, CLZ, RUF, TPM, LTG |
| 49      | E121 K / de novo     | 11 m                 | WS --> other S    | FC, SE, M           | HA --> MF spikes, slowing | N   | SD/SD | Hypotonia            | N                    | N                    | 16 y               | Intractable           | VPA, PB | VPA, CLZ, RUF, TPM, LTG |
| 50      | R853Q/de novo        | 13 m                 | WS                | S                   | T, ALI, M          | HA --> MF spikes, slowing | N   | MD/SD | Hypotonia, choreo-athetosis | 8 y                 | Intractable           | VPA, PB | VPA, CLZ, RUF, TPM, LTG |
| 51      | H930Q/de novo        | 15 m                 | MAE               | TCS                 | A, MAS, T, AA     | Slowing occ     | N   | MND | Ataxia                  | ASD                  | 6 y                 | Sz free (18 m) | LEV + TPM, VPA, OXC, CLB | VPA, OXC, CLB, LEV, ESM, B6, PB, OXC, CLB, VGB, PP, KD |
| 52      | P1652S/de novo       | 2 y                  | MAE               | MA, DA              | T, AA, M          | Spike-slow-waves to-par bil | N   | MND | Ataxia                  | ASD, MC | 3 y                 | Intractable           | TPM, LEV, RUF, PB, B6, VPA, OXC, CLB, VGB, PP, KD |
| 53      | F612S/de novo        | 2 y                  | Other             | TCS                 | M                   | Fr spikes r/l, fr delta N | N   | NSD | Hypotonia            | ASD, Regression, ASD | 7 y                 | Intractable           | CLB, LEV, RUF, BD, OXC, CLB, VGB, PP, KD |
| 54      | c.605 + 1G > T          | 2 y                  | Other             | TCS                 | M                   | Midline spikes ce, slowing to bitemporal spikes | N   | NSD | Hypotonia            | ASD, Regression, ASD | 7 y                 | Intractable           | CLB, LEV, RUF, BD, OXC, CLB, VGB, PP, KD |
| 55      | V1228Cfs7/de novo     | 3 y                  | LGS               | TCS                 | T, SE              | Spike-slow-waves, Beta (sleep) | N   | SD/SD | Spasticity            | ASD, MC | 9 y                 | Sz free (6 y) | VPA + LTG, CLZ, ESM | VPA, ACTH, TPM, LTG, PHT, OXC, CLB |
| 56      | R853Q/de novo        | 3 y                  | Other             | TS                  | S                   | HA                 | CC  | SD/SD | Hypoplasia           | Asynchronous dysmyelination | 25 y               | Intractable           | VPA, ACTH, TPM, LTG, OXC, CLB, VGB, PP, KD |
| 57      | CI 170Tfs15/de novo   | 3 y                  | Other             | TCS                 | M, AB              | Gen SW, 1 fr SW, high voltage alpha | N   | SD/SD | Pyramidal signs, hand dystonia | Hypertonic asymmetry | 17 y               | Intractable           | VPA, ACTH, TPM, LTG, OXC, CLB, VGB, PP, KD |
| 58      | G1223R/de novo       | 3 y                  | Other             | F, M                | TCS, M, A, AA, NCSE | Gen SW, PSW, N, lowing | N   | NMD | Ataxia                  | ASD, Regression | 19 y               | Intractable           | VPA, ACTH, TPM, LTG, OXC, CLB, VGB, PP, KD |
| 59      | R1235T/de novo       | 3 y                  | Other             | FC                  | A, NCSE            | Gen SW, PSW, HM | SD/MD | N | ASD                  | N                    | 14 y                 | Intractable           | VPA, ACTH, TPM, LTG, OXC, CLB, VGB, PP, KD |
| 60      | A1773V/de novo       | 3 y                  | Other             | F                   | Sharp waves fr l fr (sleep) | N   | MD/SD | N                       | Aspiration            | 14 y                 | Intractable           | VPA, ACTH, TPM, LTG, OXC, CLB, VGB, PP, KD |
| 61      | K1933M/de novo       | 4 y                  | Other             | F, TCS              | AA                  | MF spikes, bil N | SW --> ESES-like | EEG | Ataxia, parkinsonian gait | 17 y | Intractable | VPA, ACTH, TPM, LTG, OXC, CLB, VGB, PP, KD |

(continued)
Table 3 Continued

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<th>Patient</th>
<th>Mutation/inheritance</th>
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<th>Initial seizure type</th>
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<th>MRI</th>
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<th>Neuropsychological features</th>
<th>Additional features</th>
<th>Age at last follow-up</th>
<th>Seizure outcome (age effect):</th>
<th>Treatment effects</th>
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<td>Gen SW</td>
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<td>Other</td>
<td>F</td>
<td>sec. Gen. TCS</td>
<td>MF spikes→ESES-like</td>
<td>N</td>
<td>MD/MID</td>
<td>Clumsiness</td>
<td>13 y</td>
<td>Sz free (10 y)</td>
<td>ST</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>S1656F/le novo</td>
<td>8 y 11 m</td>
<td>LGS</td>
<td>GTC</td>
<td>Bilf SW; slowing</td>
<td>N</td>
<td>MD/MID</td>
<td>Hypotonia, crouched gait</td>
<td>12 y</td>
<td>Intractable</td>
<td>LLTG, VPA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AA = atypical absences; A = atonic; AB = absences; ADS = attention deficit disorder; ASD = autism spectrum disorder; AU = autonomic seizures; AP = apneic seizures; At = atrophy; Bif = bifrontal; Bil = bilateral; C = clonic; CC = corpus callosum; Ce = central; DA = drop attacks; ED = epileptiform discharges; F = focal; FD = focal dyscognitive; FC = febrile convulsion; fr = frontal; Gen = generalized; GTC = generalized tonic-clonic; HA = hypsarrhythmia; HC = hemiconic; HS = hippocampal sclerosis; HM = hypomyelination; IS = infantile spasms; L = lef t; LGS = Lennox-Gastaut syndrome; MAE = myoclonic-atonic epilepsy; m = months; MD = mild intellectual disability; MD = moderate intellectual disability; N = normal; NA = not applicable; NAV = not available; NCSE = non-convulsive status epilepticus; OcC = occipital; Par = parietal; R = right; S = spasms; SE = status epilepticus; SD = severe intellectual disability; SW = spike and waves; Sz = seizures; T = tonic; T2H = T2-hyperintensities; TCS = tonic-clonic seizures; Te = temporal; w = week; WS = West syndrome; y = years; → = change to.

Treatment (sodium channel blockers are highlighted in bold): AZA = acetazolamide; B6 = vitamin B6; BR = bromide; CBZ = carbamazepine; CLB = clobazam; CLOZ = clonazepam; CS = corticosteroids; DSM = ethosuximide; FBM = felbamate; GBP = gabapentin; IVIG = intravenous immunoglobulins; KD = ketogenic diet; LCM = lacosamide; LTG = lamotrigine; MDZ = midazolam; MSX = mesuximide; OXC = oxcarbazepine; PB = phenobarbital; PHT = phenytoin; PP = pyridoxal phosphate; RGB = retigabine; RUF = rufinamide; ST = sulfonamide; STP = stiripentol; TMM = topiramate; VGB = vigabatrin; VNS = vagal nerve stimulation; VPA = valproate; ZNS = zonisamide.

Functional studies

Electrophysiological analyses were performed and compared to the wild-type channel for four of the newly identified Nav1.2 missense mutations (for location see Fig. 3A) to correlate their functional effects to the time of onset of disease. We chose the four mutations, and the treatment of onset of juvenile epilepsy with severe phenotypes and intellectual disability (patients with encephalopathy with early infantile epilepsy, patients with congenital encephalopathy, and patients with intractable seizures). We chose the four mutations, and the treatment of onset of childhood epilepsy with severe phenotypes and intellectual disability (patients with early infantile encephalopathy with early infantile epilepsy, and children with early myoclonic-ataxic encephalopathy).

Ten mutations were found at amino acid position 1882, with variable substitutions. R1882G resulted in benign in-tractability, while R1882L and R1882Q variants resulted in severe phenotype and intellectual disability. Mutations R1882G and R1882T were previously reported in two patients with early infantile encephalopathy with early infantile epilepsy. In one patient with an early infantile encephalopathy with early infantile epilepsy, we found a 12-year-old patient (Patient 26) in which the mutation was identified to have a twin pair with the same mutation, both having seizures at birth. In a twin pair with the same mutation, one of them being deceased, and the other having severe intellectual disability (Tran et al., 2013). However, in children with severe encephalopathy beyond the first year of life, in children with intellectual disability or autism, and children with early onset epileptic encephalopathy, the A263V variant (de novo) has previously been described in patients with early infantile encephalopathy, and childhood onset epileptic encephalopathy (Liao et al., 2015). In patients with intellectual disability, the A263V variant was found in nine independent families with specific phenotypes (Table 4), and some of them were associated with frame deletions (n = 2), and two cases were associated with specific phenotypes.
promptly upon phenytoin treatment. V423L recurred in two children with Ohtahara syndrome (Patients 10 and 33), both showing a peculiar severe phenotype with a high pharmacoresistance including lack of response to one or more SCBs (Table 1). G899S was selected from a child (Patient 38) with intractable infantile/childhood epilepsy with tonic-clonic seizures and absences, and mutation P1622S from a child with intractable myoclonic-atonic epilepsy (Patient 52). In both, aggravation of seizures occurred after introduction of oxcarbazepine and/or lamotrigine.

We found gain-of-function effects for the mutations V423L and F1597L, whereas the mutations G899S and P1622S showed loss-of-function effects (Fig. 3 and Supplementary Table 2). The analysis of the V423L mutation revealed a change in slope of activation (Fig. 3C) as well as a doubling of the window current and a dramatic increase in the persistent sodium current compared with the wild-type (Fig. 3B, C and F). For F1597L mutant channels, we observed a hyperpolarizing shift of the activation curve (Fig. 3C), fast inactivation-time constants were significantly larger for mutant channels (Fig. 3D) and recovery from fast inactivation was accelerated (Fig. 3E and Supplementary Table 2).

In contrast, electrophysiological analysis of the mutations G899S and P1622S revealed profound loss-of-function changes. The most prominent change for P1622S mutant channels was a significant hyperpolarizing shift of the fast inactivation curve (Fig. 3C). The effect of the G899S mutation on channel kinetics was not as pronounced as for P1622S, consisting of a depolarizing shift and a slope change of steady-state activation (Fig. 3C). Both mutations thus predict a decrease of channel availability and membrane excitability in neurons expressing mutant Na\textsubscript{v}1.2 channels, an effect that would be further enhanced by SCBs. Functional consequences of known SCN2A variants that have been reported in the literature are listed in Supplementary Table 1.

**Table 4 Clinical characteristics of the previously unpublished patients: intellectual disability and/or autism without epilepsy**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation/inheritance</th>
<th>EEG</th>
<th>MRI</th>
<th>Cognition onset/ follow-up</th>
<th>Neurological features</th>
<th>Additional features</th>
<th>Age at follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>K1387Sfs*4/de novo</td>
<td>NA</td>
<td>NA</td>
<td>MD</td>
<td>N</td>
<td>ASD</td>
<td>5 y 4 m</td>
</tr>
<tr>
<td>68</td>
<td>R1435Q/de novo</td>
<td>N</td>
<td>T2H</td>
<td>SD</td>
<td>N</td>
<td>ASD, early puberty</td>
<td>7 y 8 m</td>
</tr>
<tr>
<td>69</td>
<td>T1711Lfs*3B/de novo</td>
<td>Slowing</td>
<td>N</td>
<td>SD</td>
<td>Hypotonia</td>
<td>Rett-like, ASD</td>
<td>9 y 8 m</td>
</tr>
<tr>
<td>70</td>
<td>G1744E/de novo</td>
<td>N</td>
<td>N</td>
<td>MD</td>
<td>N</td>
<td>ASD</td>
<td>4 y</td>
</tr>
<tr>
<td>71</td>
<td>c.386+2T&gt;C/de novo</td>
<td>NA</td>
<td>NA</td>
<td>MD</td>
<td>NAV</td>
<td>Episodic ataxia, ASD</td>
<td>13 y</td>
</tr>
</tbody>
</table>

ASD = autism spectrum disorder; m = months; MD = moderate intellectual disability; N = normal; NA = not applicable; NAV = not available; SD = severe intellectual disability; T2H = T2-hyperintensities; y = years.

In the benign end of the spectrum, both familial and de novo mutations are found. These patients are characterized by a normal cognitive development and self-limited epilepsy with cessation of seizures mostly during the first year of life. Seizure semiology shows considerable variation, the typical ‘clustering’ is not always present, and family history may be negative due to the presence of de novo mutations. Seizures may be initially difficult to treat: in our cohort of unpublished patients, seizures were initially drug-resistant in 6/9 children (as discussed further below). However, EEG might help to rule out a severe epileptic phenotype, showing typically a normal background activity with or without multifocal spikes, but never a suppression burst pattern. After cessation of neonatal/infantile seizures, five children developed episodic ataxia and pain (Liao et al., 2010a; Johannesen et al., 2016; Schwarz et al., 2016).

The group with encephalopathy and epilepsy is the largest among the SCN2A carriers; 69% of the cohort falls into this category (Fig. 1). Half of those patients had a seizure onset in the early infantile period (<3 months). In the late onset group, seizure onset was usually before the age of 4 years (only five patients had a later seizure debut).
Fifty-four of the 139 children had an identifiable epileptic syndrome (32%). In the early infantile group, Ohtahara syndrome (n = 18) and EIMFS (n = 13) constitute the most important specific phenotypes. Whereas Ohtahara syndrome was the first syndrome to be reported in SCN2A-related encephalopathies, EIMFS was only recently recognized (Howell et al., 2015). In EIMFS, KCNT1 mutations have been reported as the most common underlying genetic cause so far (Barcia et al., 2012; Ohba et al., 2015). In KCNT1-related EIMFS, the prognosis seems to be uniformly poor, and affected children show severe disability with ongoing seizures during follow-up (Barcia et al., 2012;
The relative frequency of the disease groups as represented in Fig. 1A for the newly identified cases with epilepsy and in Fig. 1B for all cases probably contains biases, as (i) benign neonatal-infantile epilepsies were the first entity in which SCN2A mutations were detected suggesting a relative over-representation of those and may be also the severe early onset cases in the literature; and (ii) since cases without epilepsy are probably underdiagnosed.

**Genotype**

Recurrent mutations are seen both in the benign and severe end of the spectrum (Table 5). However, even with the same mutation, a quite large phenotypic variance is observed. A remarkable phenotype was found for R853Q mutation carriers in whom 6/9 were affected by West syndrome. Furthermore, three of the patients with an A263V mutation showed a BNIS phenotype with late onset episodic ataxia, while three others with the same mutation had more severe phenotypes. Thus, both the mutation itself and other genetic or environmental factors contribute to the individual phenotype.

Interestingly, we found only missense mutations in the early onset epilepsies, whereas truncations, splice-site and nonsense mutations were solely seen in the epilepsies with a later onset and in the group of cases without epilepsy. This observation, along with the functional effects described in the results section and discussed below, could have implications for treatment in these two major groups of SCN2A patients. Supplementary Table 1 provides an overview of all known SCN2A mutations, the associated clinical phenotypes and known functional consequences.

**Prevalence**

We estimated the frequency of SCN2A mutations causing the reported phenotypes in the Danish population to be 1/78,608. This number will most likely be an underestimate. There is not a strong tradition for a systematic genetic screening of patients with isolated autism or intellectual disability, thus there might be a recruitment bias towards patients with epilepsy.

**Seizure outcome and treatment effects**

In B(F)NIS families, seizures are reported to be controlled by AEDs (Berkovic et al., 2004; Striano et al., 2006; Herlenius et al., 2007). In the unpublished cohort, however, many patients were resistant to various AEDs, including phenobarbital, topiramate, levetiracetam and valproate, whereas SCBs (especially oxcarbazepine and phenytoin), were completely or partially effective in 6/9 cases, suggesting a specific effect of SCBs in this subgroup. However, as known from previous studies, three patients became seizure-free without any AEDs, which is part of the natural history of these patients. Thus, the effect of SCBs in these patients should be interpreted with some caution, although

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**Table 5 Recurrent mutations**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Frequency % (n cases)</th>
<th>Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>M136l</td>
<td>1 (2)</td>
<td>EE, EIMFS</td>
</tr>
<tr>
<td>V213D</td>
<td>1 (2)</td>
<td>EOE</td>
</tr>
<tr>
<td>V261l</td>
<td>1 (2)</td>
<td>BNS</td>
</tr>
<tr>
<td>A263v/T</td>
<td>3.5 (7)</td>
<td>BNS, EE, OS</td>
</tr>
<tr>
<td>V423L</td>
<td>1 (2)</td>
<td>OS</td>
</tr>
<tr>
<td>V430Q/G/A</td>
<td>1.5 (3)</td>
<td>BFIS, OS</td>
</tr>
<tr>
<td>N503K F</td>
<td>1 (2)</td>
<td>ID</td>
</tr>
<tr>
<td>R853Q</td>
<td>4.5 (9)</td>
<td>WS, EE, LGS</td>
</tr>
<tr>
<td>R856/L/Q</td>
<td>1 (2)</td>
<td>OS,EIMFS</td>
</tr>
<tr>
<td>G882R/E</td>
<td>1 (2)</td>
<td>EIMFS</td>
</tr>
<tr>
<td>K905N</td>
<td>1 (2)</td>
<td>EE</td>
</tr>
<tr>
<td>F928C</td>
<td>1 (2)</td>
<td>EIMFS, EE</td>
</tr>
<tr>
<td>R937C</td>
<td>1 (2)</td>
<td>ID</td>
</tr>
<tr>
<td>C59S</td>
<td>1 (2)</td>
<td>ASD</td>
</tr>
<tr>
<td>E999V/K</td>
<td>1 (2)</td>
<td>EIEE, OS, EOE</td>
</tr>
<tr>
<td>G1013</td>
<td>1 (2)</td>
<td>ASD</td>
</tr>
<tr>
<td>I1021Y</td>
<td>1 (2)</td>
<td>LGS, EE</td>
</tr>
<tr>
<td>E1211K</td>
<td>1 (2)</td>
<td>WS</td>
</tr>
<tr>
<td>V1282F</td>
<td>1 (2)</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>R1319Q/W</td>
<td>1.5 (3)</td>
<td>OS</td>
</tr>
<tr>
<td>V1326/D</td>
<td>1 (2)</td>
<td>EIMFS, OS</td>
</tr>
<tr>
<td>S1336Y</td>
<td>1 (2)</td>
<td>ASD</td>
</tr>
<tr>
<td>L1342P</td>
<td>2.5 (5)</td>
<td>EOE, WS</td>
</tr>
<tr>
<td>R1435</td>
<td>1 (2)</td>
<td>ASD</td>
</tr>
<tr>
<td>Q1531K</td>
<td>1 (2)</td>
<td>BFNS</td>
</tr>
<tr>
<td>T1623N</td>
<td>1 (2)</td>
<td>OS, EE</td>
</tr>
<tr>
<td>R1629/L/H</td>
<td>1 (2)</td>
<td>EE</td>
</tr>
<tr>
<td>R1882G/Q/L</td>
<td>5 (10)</td>
<td>BIS, OS, EE</td>
</tr>
</tbody>
</table>

ASD = autism spectrum disorder (without seizures); BIS = benign familial infantile seizures; BNS = benign neonatal seizures; EE = epileptic encephalopathy; EIEE = early infantile epileptic encephalopathy; EIMFS = epilepsy of infancy with migrating focal seizures; EOE = early onset epileptic encephalopathy; ID = intellectual disability (without seizures); LGS = Lennox-Gastaut syndrome; OS = Ohtahara syndrome; WS = West syndrome.

Ohba et al., 2015). In our SCN2A cohort, 2/5 patients with EIMFS became seizure-free at the age of 2 and 12 months with vigabatrin and phenytoin, respectively, and showed mild intellectual disability. Thus, prognosis seems to be more favourable in SCN2A-related EIMFS compared to those caused by KCNT1 mutations.

Epilepsies with onset beyond the early infantile period are increasingly recognized in SCN2A-related disorders. In particular, West syndrome has recently emerged as the most important specific phenotype, accounting for 16 patients so far. Of note, one recurring mutation (R853Q), which has been found in nine cases so far, is frequently associated with West syndrome (six cases), and should therefore be considered in the diagnostic work-up of children with West syndrome.

The intellectual disability/autism group without epilepsy is most likely an underestimate of the actual number of cases, since it is not common practice in all countries/hospitals to perform genetic testing in patients with intellectual disability and/or autism without seizures. This group of patients represents 16% of the cohort.
Figure 3  Functional studies reveal pronounced gain-of-function changes for the V423L and F1597L mutations and loss-of-function changes for the P1622S and G899S mutations. (A) Schematic of the human Na_{1.2} α-subunit together with β₁ and β₂ subunits showing the locations of the four functionally studied mutations (V423L green square; F1597L blue diamond; G899S orange inverted triangle; P1622S red triangle). (B) Representative current traces of whole-cell Na⁺ currents recorded from tsA201 cells transfected with either Na_{1.2} wild-type or mutant channels. (C) Voltage dependence of steady state Na⁺ channel activation and inactivation revealing a significant depolarizing shift of the activation curve for G899S (loss-of-function) as well as a significant hyperpolarizing shift of the inactivation curve for P1622S (loss-of-function) in comparison with the wild-type. Lines represent fits of Boltzmann functions. (D) Voltage dependence of the fast inactivation time constant for wild-type and mutant channels revealing a slowing of fast inactivation for F1597L and an acceleration for P1622S. (E) The time course of recovery from fast inactivation determined at −100 mV showed significant changes between wild-type and mutant channels. F1597L mutant channels showed a significantly faster recovery (gain-of-function), whereas P1622S mutant channels showed a significant slowing of the recovery from fast inactivation compared to wild-type channels (loss-of-function). Lines represent fits of exponential functions yielding the time constant \( t_{\text{rec}} \). (F) Voltage dependence of the persistent sodium current showing a large increase for V423L compared to the wild-type. Current amplitudes recorded at the end of a 70-ms depolarization were normalized to the peak current amplitude (steady state current/initial peak current). Shown are means ± SEM.
we observed a clear correlation between introduction of the drugs and seizure freedom.

Apart from B(F)NIS, we found a different pattern of seizure outcome and AED effects in our cohort according to the age at seizure onset. Of all patients with encephalopathy and epilepsy onset <3 months, 17/28 (61%) became seizure-free, 10 of them during SCB treatment, mainly with phenytoin (n = 8), whereas other standard AEDs (e.g. phenobarbital, levetiracetam) were largely ineffective. Interestingly, in five children recurrent declines of phenytoin plasma levels resulted in prompt seizure relapses that were reversible after adjusting the phenytoin dosage. These observations underline the beneficial effect of phenytoin on seizure activity in these patients.

To date, there are only few reports on treatment response in SCN2A-related epilepsies. In the series of Nakamura et al. (2013) on 15 children with early onset seizures, epilepsies were described as intractable in 12 of 15 cases. Phenytoin was tried in five children with seizure onset between 1 day and 6 weeks of age, and showed partial effects in four of them. Zonisamide showed some effects in 4/6 children. Two children became seizure-free with lamotrigine at the age of 6 months and 6 years, respectively. Howell et al. (2015) reported improvement of seizure control in 11/15 patients with early seizure onset, phenytoin was reported to be partially effective in seven children with neonatal seizure onset. Sawaishi et al. (2002) described a striking effect of lidocaine, a prototypic sodium channel blocker, in a patient with Ohtahara syndrome due to a SCN2A mutation (Sawaishi et al., 2002; Ogiwara et al., 2009).

Taken together, patients with early onset epilepsies were difficult to treat but responded relatively well to SCBs, in particular to phenytoin in appropriate dosages. The high dosages needed to control seizures completely might not have been reached in many patients who did not become seizure-free.

In contrast, only 10/29 (34%) children with infantile/childhood epilepsy became seizure-free, and seizures mostly did not respond to SCBs. In the nine children with West syndrome, seizures and hypsarrhythmia in the EEG were mostly resistant to standard treatment including steroids and ketogenic diet. Nakamura et al. (2013) reported on eight children with West syndrome in their series. All were intractable, suggesting that SCN2A-related West syndrome is particularly difficult to treat. However, some case reports describe treatment responses to ACTH (Nakamura et al., 2013), topiramate (Ogiwara et al., 2009; Sundaram et al., 2013; Samanta and Ramakrishnaiah, 2015) and transient effects of prednisolone (Matalon et al., 2014).

Seizure worsening associated with SCB treatment was observed in seven children of our cohort, all of whom had seizure onset beyond the age of 3 months. Hackenberg et al. (2014) reported an increase of seizure frequency related to carbamazepine treatment in a child with seizure onset at 3 months of age. Howell et al. (2015) reported the appearance of myoclonus with vigabatrin and lamotrigine in one child. SCBs are known to aggravate seizure activity in epileptic syndromes that are caused by loss-of-function mutations in Na\textsubscript{1.1} channels, e.g. Dravet syndrome, putatively because they further reduce sodium channel activity in inhibitory neurons (Brunklaus et al., 2014) expressing Na\textsubscript{1.1} as the major sodium channel (Catterall, 2014). As discussed below, a similar mechanism may apply for loss-of-function mutations in Na\textsubscript{1.2} channels.

The statistical χ\textsuperscript{2} test confirmed our impression of differential treatment effects of SCBs and non-SCBs in early versus late onset encephalopathies with epilepsy. The late onset cases responded significantly better to non-SCBs than the early onset ones, which may indicate that the epilepsy in the early onset cases is more difficult to treat. This observation even strengthens the finding of a significantly better response to SCBs of the early onset compared to the late onset cases, and suggests that the higher seizure freedom rate of the early onset group (61%, compared to only 34% of the late onset group) is the likely consequence of a specific pharmaco-response to gain-of-function SCN2A mutations (see discussion below on functional effects).

In summary, SCBs seem to have positive effects on seizures in early infantile onset epilepsies, but are not effective or can even worsen seizure activity in epilepsies with onset at 3 months of age or later. However, these conclusions have to be taken with care, as the natural history of these conditions is unknown, our observations are purely retrospective and the exact duration from AED introduction to seizure freedom was not clear in all cases.

## Functional studies and their pharmacological and neurophysiological interpretation

Out of the diversity of SCN2A mutations that have been identified until now, only a small number have been studied functionally. SCN2A mutations can lead to either augmented or reduced Na\textsubscript{1.2} activity (Kamiya et al., 2004; Scalmanni et al., 2006; Xu et al., 2007; Ogiwara et al., 2009; Liao et al., 2010a; Liao et al., 2010b; Liao et al., 2010c; Rauch et al., 2012; Lauxmann et al., 2013; Sundaram et al., 2013; Codina-Sola et al., 2015; Schwarz et al., 2016).

Here, we functionally analysed four additional mutations, two of which were identified in patients suffering from encephalopathy with early infantile onset epilepsy. For these mutations (V423L and F1597L), a clear gain-of-function effect was found (Fig. 3). V423L particularly showed a tremendous increase in persistent sodium current, probably explaining the extremely severe phenotype with highly drug-resistant Ohtahara syndrome in both children affected by this mutation. We hypothesize that this persistent current was too large to be sufficiently reduced by SCBs in clinically relevant dosages. F1597L caused an accelerated recovery from fast inactivation, and the patient showed an EIMFS phenotype with a prompt response to phenytoin
treatment. In contrast, we have shown clear loss-of-function effects for two mutations found in children with infantile/childhood epilepsy (G899S and P1622S). The phenotype consisted of tonic-clonic seizures and absences in one and myoclonic-ataxic epilepsy in the other child, which was clearly different from the other two mutations. This difference was equally seen with regard to the treatment response, which showed seizure aggravation upon SCB treatment in both cases.

In previous studies, several disease-causing mutations in SCN2A have been functionally analysed. In children with severe early onset epilepsies, gain-of-function mutations were described (Ogiwara et al., 2009; this study, see above). Missense mutations from patients with B(F)NIS have been characterized to cause a gain-of-function (Scalmani et al., 2006; Xu et al., 2007; Liao et al., 2010a, b; Lauxmann et al., 2013; Schwarz et al., 2016) with one of these mutations (A263V) also causing severe epilepsy in some cases (see above). Few studies also indicated some biophysical features indicating a loss-of-function (Scalmani et al., 2006; Misra et al., 2008); however, studies in neurons by Scalmani et al. (2006) suggested a net gain-of-function with increased excitability for two such cases so that the main effect seems to be a gain-of-function (R223Q and R1319Q), matching the effective SCB treatment of patients carrying the R1319Q mutation. These distinct effects of the same mutation analysed in neuronal versus non-neuronal cells could be caused by post-translational modifications, phosphorylation, trafficking and protein–protein interactions (such as with β-subunits, which we also used in our study) of the channels which can be quite different in neuronal cells and heterologous expression systems (for review see Shao et al., 2009). It has been shown for mutations in Na1.1 that—besides the β-subunits as the closest interacting partners of α-subunits—ankyrin, calmodulin or other endogenous proteins can also have essential roles for intracellular trafficking and functional expression (Rusconi et al., 2007; Cestele et al., 2013). Such effects may also apply for the mutations we studied here in tsA201 cells. Furthermore, modifier genes might affect the clinical severity and the variability of phenotypes seen in epilepsy patients with gain-of-function SCN2A mutations (Bergren et al., 2005; Hawkins and Kearney, 2016).

In contrast to the observed gain-of-function effects, missense loss-of-function and nonsense mutations have been identified in patients with later onset epilepsies, although two mutations also showed a hyperpolarizing shift of the activation curve as a gain-of-function feature (Kamiya et al., 2004; Ogiwara et al., 2009; Lossin et al., 2012). Finally, autism spectrum disorder without seizures was also associated with loss-of-function mutations (Kamiya et al., 2004; Rauch et al., 2012; Sanders et al., 2012; Carvill et al., 2013; Codina-Sola et al., 2015; D’Gama et al., 2015; Howell et al., 2015; Carroll et al., 2016; Horvath et al., 2016; Li et al., 2016; Trump et al., 2016) (Supplementary Table 1).

These findings strengthen the hypothesis that there is a link between gain-of-function SCN2A mutations, early onset epilepsy, and effectiveness of SCBs on the one hand, and loss-of-function mutations, later onset epilepsy, and ineffectiveness of SCBs on the other hand. Gain-of-function versus loss-of-function mutations affecting the neuronal excitability of different neurons during specific developmental stages might explain the variation in seizure onset and the response to SCBs in early infantile syndromes. Early in development, the Na1.2 channel is highly expressed at nodes of Ranvier and axon initial segments and is partially replaced during development by the Na1.6 channel (Kaplan et al., 2001; Liao et al., 2010b), which could be confirmed in adult human hippocampal brain slices (Liao et al., 2010b). Na1.2 channels are therefore considered to contribute to action potential generation and propagation and influence the axonal firing frequency during early development. Hence, mutations causing gain-of-function effects can alter the characteristic firing patterns of Na1.2-expressing neurons and cause hyperexcitability, which can be dampened by SCB treatment and thus improve seizure outcome. In contrast, loss-of-function mutations cannot drive Na1.2-expressing cells into hyperexcitability during early development, and therefore patients may not exhibit early onset seizures. However, loss-of-function mutations seem to lead to severe epileptic phenotypes later in development. In the more mature brain, unmyelinated axons express the Na1.2 channel, such as mossy fibres projecting from the hippocampal dentate gyrus into the CA3 region (Kaplan et al., 2001; Liao et al., 2010b). The most prevalent targets of mossy fibres apart from CA3 pyramidal cells are GABAergic parvalbumin-positive interneurons. Mossy fibres contact inhibitory basket cells ~50 times more frequently than pyramidal cells mediating a powerful feedforward inhibition (Acsady et al., 1998). Therefore, a reduced excitability of dentate granule cells due to Na1.2 loss-of-function mutations can result in CA3 hyperexcitability, which can spread to subsequent hippocampal regions and further. A treatment with SCBs can therefore act in a similar way as hypothesized for loss-of-function Na1.1 mutations. Here, SCBs are predicted to further reduce the activity of inhibitory neurons expressing Na1.1 as major sodium channel. Similarly, SCBs could further reduce the activity of Na1.2-expressing dentate granule cells, which in turn would activate inhibitory neurons less effectively. As mossy fibres are not the only unmyelinated fibres within the brain, other unmyelinated inhibitory neurons expressing Na1.2 channels could also contribute to neuronal hyperexcitability. Only parvalbumin-positive inhibitory neurons within the cortical layers have been shown to be myelinated (Micheva et al., 2016). Unmyelinated inhibitory neurons expressing Na1.2 channels are controlling the activity of excitatory neurons and provide feedforward inhibition (Kepecs and Fishell, 2014). In early development, a reduced activity of these inhibitory neurons due to Na1.2 loss-of-function mutations could be of little or no consequence, as excitatory
Na\textsubscript{1.2}-expressing neurons within the cortex also show reduced excitability. As mentioned above, the Na\textsubscript{1.2} channel is partially replaced by the Na\textsubscript{1.6} channel at nodes of Ranvier and axon initial segments during development, but is still expressed in unmyelinated fibres, such as those from inhibitory neurons. Additionally, the neonatal splice variant of Na\textsubscript{1.2} has been shown to have a seizure protective role during early development (Gazina et al., 2015). A reduced excitability of unmyelinated cortical inhibitory neurons later in development could therefore lead to hyperexcitable cortical networks. A disruption of the excitatory/inhibitory balance caused by SCN2A loss-of-function mutations can therefore cause seizures and additionally underlie neuropsychiatric diseases and autism, reflected by a higher prevalence in patients with loss-of-function mutations and late onset phenotypes in our cohort.

In summary, our study reflects the large spectrum of SCN2A-related disorders and identifies SCN2A mutations as one of the most common genetic causes of epilepsy. We have established two distinct groups with seizure onset either before or after 3 months of age, which show phenotypic differences, gain-of-function versus loss-of-function Na\textsubscript{1.2} abnormalities and a likely related differential response to treatment with SCBs.

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Supplementary material

Supplementary material is available at *Brain* online.

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