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Prognostic, clinical and demographic features in *SCN1A* mutation-positive Dravet syndrome

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Dravet syndrome is a severe infantile onset epileptic encephalopathy associated with mutations in the sodium channel alpha 1 subunit gene *SCN1A*. To date no large studies have systematically examined the prognostic, clinical and demographic features of the disease. We prospectively collected data on a UK cohort of individuals with Dravet syndrome during a 5-year study period and analysed demographic information based on UK population and birth figures. From structured referral data we examined a range of clinical characteristics including epilepsy phenotype, seizure precipitants, electroencephalography data, imaging studies, mutation class and response to medication. Predictors of developmental outcome were determined by logistic regression. We identified 241 cases with *SCN1A* mutation-positive Dravet syndrome, 207 of which were UK-based. The incidence of mutation-positive Dravet syndrome is at least 1:40 900 UK births. Clinical features predicting a worse developmental outcome included status epilepticus (odds ratio = 3.1; confidence interval = 1.5–6.3; $P = 0.003$), interictal electroencephalography abnormalities in the first year of life (odds ratio = 5.7; confidence interval = 1.9–16.8; $P = 0.002$) and motor disorder (odds ratio = 3.3; confidence interval = 1.7–6.4; $P < 0.001$). No significant effect was seen for seizure precipitants, magnetic resonance imaging abnormalities or mutation class (truncating versus missense). Abnormal magnetic resonance imaging was documented in 11% of cases, principally with findings of non-specific brain atrophy or hippocampal changes. Sodium valproate, benzodiazepines and topiramate were reported as being the most helpful medications at the time of referral. Aggravation of seizures was reported for carbamazepine and lamotrigine. The identification of factors influencing prognosis both aids counselling and encourages early, syndrome-specific therapy. Prevention of status epilepticus with regular medication and emergency protocols is important and may influence developmental outcome.

Keywords: Dravet syndrome; *SCN1A*; severe myoclonic epilepsy of infancy; SMEI

Abbreviations: $Na_v1.1$ = voltage gated sodium channel; *SCN1A* = voltage-gated sodium channel type I alpha subunit

Introduction

Since its first description in 1978 the clinical boundaries of Dravet syndrome, also known as severe myoclonic epilepsy in infancy, have evolved significantly (Harkin *et al*., 2007). Particularly after the discovery of its primary genetic cause, a mutation in the *SCN1A* gene, there has been an increase in diagnoses in recent years (Claes *et al*., 2001; Lossin, 2009). To date no large studies have systematically examined the prognostic, clinical and demographic features of the disease.

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Dravet syndrome typically presents in the first year of life with prolonged, febrile and afebrile, generalized clonic or hemiclonic epileptic seizures in children with no pre-existing developmental problems. Other seizure types including myoclonic, focal and atypical absence seizures appear between the ages of 1 and 4 years (Dravet, 1978). The epilepsy is usually refractory to standard anti-epileptic medication and from the second year of life affected children develop an epileptic encephalopathy resulting in cognitive, behaviour and motor impairment. Seizure types within Dravet syndrome, such as status epilepticus, may be life threatening and sudden unexpected death in epilepsy can occur (Dravet *et al*., 2002). There is some evidence supporting specific treatment regimes for the epileptic seizures associated with Dravet syndrome (Oguni *et al*., 1994; Chiron *et al*., 2000; Nieto-Barrera *et al*., 2000; Kassai *et al*., 2008). As the encephalopathy is associated with cognitive decline and permanent neurological impairment it has been suggested that aggressive focused therapy should be commenced as soon as possible (Mullen and Scheffer, 2009). The majority of children (70–80%) have a mutation in the voltage-gated sodium channel type I alpha subunit gene, *SCN1A*, and recent evidence suggests that the nature of a mutation may affect the phenotype (Claes *et al*., 2009; Kanai *et al*., 2009; Zuberi *et al*., 2011). However, it is not clear whether the developmental regression is primarily caused by the genetic change itself as a consequence of the epileptic encephalopathy, or by both (Catarino *et al*., 2011). Predictors for long-term developmental outcome are not well known. A recent retrospective study based on 26 patients failed to detect any significant correlation between severity of cognitive decline; however, a number of clinical variables including age at seizure onset suggested that early absences and myoclonus are associated with poorer developmental outcome (Ragona *et al*., 2011). A further retrospective report showed that vaccination-proximate children, who had seizure onset within 2 days after vaccination, exhibited earlier seizure-onset, but developmental outcome was not different between groups (McIntosh *et al*., 2010).

Here we present a large cohort of patients with *SCN1A* mutation-positive Dravet syndrome, with the objective of identifying predictors of developmental outcome and to determine specific clinical and demographic features.

Materials and methods

We identified 355 patients with Dravet syndrome from 1023 individuals consecutively referred for *SCN1A* testing between November 2005 and February 2010. Referring clinicians completed a structured referral form for every patient prior to DNA analysis. This detailed the epilepsy phenotype (occurrence and age at onset of first seizure, prolonged febrile seizures, hemiclonic seizures, generalized tonic–clonic/clonic seizures, status epilepticus, focal seizures with impairment of awareness, myoclonic seizures and atypical absences) and any factors precipitating the first seizure. Vaccination was acknowledged as a precipitant if the referrer clearly stated this; however, we only included patients whose first seizure was within the first 2 days of vaccination. The referrer documented any EEG findings with dates, including normal interictal EEG, generalized spike and wave, photosensitivity and focal/multifocal EEG abnormalities. Details of MRI imaging and family history of febrile seizures or epilepsy were also recorded.

The developmental status was classified by the referring clinician using a five-point Likert scale as 1 = normal, 2 = mild learning disability, 3 = moderate learning disability, 4 = severe learning disability and 5 = profound learning disability. The raters had expertise in the assessment of developmental status including rating of gross and fine motor skills, communication and cognitive abilities and age appropriate adaptive behaviour. The age at which the development noted to be abnormal was recorded in months. The presence of acquired autistic features (yes/no), behaviour problems (yes/no) and acquired motor disorder, including hypotonia, ataxia, spasticity or dyskinesia (yes/no) was documented. The response to treatment was detailed by the referring clinician as to whether any medication increased seizure frequency or whether any medication reduced seizure frequency.

To maintain diagnostic consistency, phenotypes were assessed by the same child neurologist (S.M.Z.) prior to genetic testing, and diagnostic criteria for Dravet syndrome included: seizure onset in infancy, mainly triggered by fever and often prolonged; later occurrence of various other seizure types (febrile and afebrile) including focal seizures; atypical absences; tonic–clonic seizures; normal cognitive and motor development prior to seizure onset with subsequent slowing including plateauing or regression of skills. Consistent with previous reports (Depienne *et al*., 2009*b*) we regarded the presence of myoclonic seizures and ataxia as highly characteristic for Dravet syndrome; however, their absence did not exclude that diagnosis. Patients without the full phenotype have been described as having a 'mild form' of Dravet syndrome by other authors (Guerrini and Oguni, 2011).

Patients who did not have a *SCN1A* mutation were excluded from the phenotypic description and prediction analysis, as these individuals may have an alternative genetic diagnosis resembling *SCN1A* related Dravet syndrome, such as the X-linked protocadherin 19 (*PCDH19*) gene mutation (Depienne *et al*., 2009*a*).

Blinded independent review of disease classification was undertaken by two child neurologists (S.M.Z. and S.M.) among a subset of 100 consecutive patients referred to the service. An inter-rater analysis to determine consistency among raters demonstrated excellent reliability ($\kappa = 0.90$; $P < 0.001$) and outstanding agreement between raters.

Mutation analysis included standard sequencing of all 26 exons of the *SCN1A* gene. Mutations were classified as nonsense, frameshift, splice-site, missense or in frame insertion or deletions. Missense mutations were classed as causative if they had previously been reported in Dravet syndrome, or if they predicted a significant (non-conservative) amino acid change with significant physicochemical difference and high Grantham score in a residue conserved through evolution. Sequence changes were distinguished from databases of coding single nucleotide polymorphisms and specific missense mutations were further validated by comparing them to a panel of anonymous blood donors as a control population. In cases where a *SCN1A* mutation was found, available parental DNA was directly searched by DNA sequencing to determine whether a mutation occurred *de novo* or was inherited. In point mutation-negative cases, when the phenotype suggested an *SCN1A*-related epilepsy, multiplex ligation-dependent probe amplification (MLPA) was performed to detect large scale rearrangements of *SCN1A*.

Standard protocol approvals, registrations and patient consents

This study, including retrospective review of anonymized clinical referral data and mutation findings, was approved by the West of Scotland Regional Ethics Service.

Data analysis

Patients with missing data were excluded from the relevant analyses. Ages at seizure onset are given as median with semi-interquartile ranges and chi-square statistics were used to determine categorical differences in the cross-sectional evaluation of developmental parameters.

We used an ordinal logistic regression model to predict developmental outcome. For this analysis we only included children who were $\geqslant 3$ years of age at the time of assessment ($n = 152$) as developmental status would be difficult to determine in children younger than 3 years of age. In this selected cohort, there were only six children identified as having normal development—a number too small to be included in the logistic regression. These six were therefore grouped together with the category of mild learning disability. This resulted in four ordered categories: 'normal/mild disability' ($n = 29$); 'moderate disability' ($n = 45$); 'severe disability' ($n = 57$); and 'profound disability' ($n = 21$). As children were assessed at different ages, this was identified as a potential confounder and the age at assessment was adjusted for and held constant in the prediction models.

The Mann–Whitney U test and Kruskal–Wallace test were used to compute phenotypic differences between groups. Significance was tested at the 5% level and analysis was performed using SPSS version 15.0 (SPSS Chicago).

Results

Of the 355 individuals with Dravet syndrome 241 (68%) had a *SCN1A* mutation and were included in the phenotypical description and prediction analysis. The age range was 6 months to 42 years, 135 out of 241 were male (56%) and a family history of febrile seizures or epilepsy was reported in 65/223 cases (29%). The closest family members were first-degree relatives in 26 cases, second-degree relatives in 23 cases and third and fourth degree relatives in the remaining cases. Among first and second degree family members there were 29 relatives with febrile seizures and 38 relatives with epilepsy.

A total of 111 (46%) had a missense mutation, 43 (18%) nonsense, 46 (19%) frameshift, 20 (8%) splice site, six (3%) inframe insertion/deletion and 15 (6%) gross rearrangements. The inheritance could be determined in 115 (48%) cases where both parental samples were available. One hundred and four occurred *de novo*, six were maternal and five paternal (Zuberi *et al*., 2011).

Demographically, 207 out of 241 patients (86%) were based in the UK. To determine the incidence of mutation-positive Dravet syndrome in the UK we chose a 5-year birth cohort from 2003 to 2007 ($n = 88$). During this period our laboratory was the only centre in the UK to perform this test and we diagnosed a mean number of 17.6 (range = 15–23) children per birth year. Given that the average number of live births in the UK for this time interval was 720 000 per year (Office of National Statistics), we estimate that at least 1:40 900 children is affected. Within this cohort of children aged 3 to 7 years ($n = 88$) we have had five reported deaths (6%) at a median age of 5 years. The cause of death included status epilepticus ($n = 2$) and sudden unexpected death in epilepsy ($n = 3$).

Phenotypical features of mutation positive Dravet syndrome patients ($n = 241$) are listed in Table 1. The interictal EEG findings in the first and second year of life were specified as generalized spike and wave in 53/81 (66%) of cases and focal/multifocal EEG abnormalities in 51/81 (63%) of cases. Among the most commonly recorded MRI abnormalities were non-specific atrophic changes in 7/22 cases (32%) and temporal lobe/hippocampal changes in 12/22 cases (55%). When we compared patient ages at seizure onset in relation to the precipitant type (fever/illness, no precipitant or vaccination), we found that the vaccination group had a significantly earlier onset of seizures with 4.0 ± 1.0 months (median $\pm$ semi-interquartile ranges) compared to the fever/illness group with 6.0 ± 1.0 months and those without a precipitant 5.0 ± 1.5 months [$\chi^2 = 7.83$, degrees of freedom (df) = 2, $P = 0.020$). The most commonly reported medication responses (increase or reduction in seizure frequency) among the mutation positive Dravet syndrome patients are detailed in Table 2.

In a cross-sectional analysis of outcome measures in different age groups we divided the entire sample ($n = 241$) into seven age categories: 1st = first year of life ($n = 15$), 2nd = second year of life ($n = 44$), 3rd–4th = third and fourth year of life ($n = 49$), 5th–7th = fifth to seventh year of life ($n = 35$), 8th–10th = eighth to tenth year of life ($n = 32$), 11th–14th = eleventh to fourteenth year of life ($n = 34$) and 15th + = fifteenth year of life and older ($n = 32$). Figures 1 and 2 illustrate the acquired developmental outcome variables 'autistic features', 'behaviour problems', 'acquired motor disorder' and 'developmental status' over time.

Chi-squared analysis showed significant differences across all four variables: acquired autistic features ($\chi^2 = 39.53$, $df = 6$, $P < 0.001$), behaviour problems ($\chi^2 = 54.05$, $df = 6$, $P < 0.001$) and acquired motor disorder ($\chi^2 = 37.19$, $df = 6$, $P < 0.001$) all increased significantly with age. Similarly we observed a significant increase in the degree of learning disability seen in the older age groups ($\chi^2 = 184.45$, $df = 24$, $P < 0.001$).

To identify predictors of developmental outcome we performed an ordinal logistic regression analysis including the clinical features listed in Table 1 and the mutation class (truncating/missense). The presence of a motor disorder, abnormal interictal EEG findings in Year 1, status epilepticus and early focal seizures with impairment of awareness ($\leqslant 24$ months), were each positively associated with the tendency of a worse developmental outcome (Table 3). A young age at onset of myoclonic seizures and a young age at onset of developmental delay were each associated with the tendency for worse developmental outcome. The different seizure precipitants, photosensitivity, MRI abnormalities or mutation class had no significant effect on developmental outcome.

Discussion

We present the first systematic UK population-based evaluation estimating the incidence of *SCN1A* mutation-positive Dravet syndrome at 1:40 900. This figure, however, might be an underestimate, as we have recently seen an increase in genetically proven cases, including cases of germinal and somatic mosaicism, and a widening of the spectrum (Harkin *et al*., 2007). Therefore the mutation status only reflects those mutations we are able to identify with current techniques. Considering the 30% mutation negative cases, the overall incidence of Dravet syndrome might be

Table 1 Phenotypical features in *SCN1A* mutation-positive Dravet syndrome ($n = 241$)

Feature	Age at onset in months (median ± semi-IQR)	Occurrence number/ total (%)
First seizure	6.0 ± 1.5	241/241 (100)
Prolonged febrile seizure (>10 min)	7.0 ± 2.0	168/232 (72)
Hemiclonic seizure	7.0 ± 3.5	161/225 (72)
Generalized tonic–clonic/clonic seizures	8.0 ± 3.0	216/231 (94)
Status epilepticus	9.0 ± 3.5	188/235 (80)
Focal seizure with impairment of awareness	10.0 ± 5.8	122/200 (61)
Myoclonic seizure	14.0 ± 7.5	161/232 (69)
Atypical absence	21.0 ± 7.5	112/218 (51)
Age at which development noted to be abnormal	18.0 ± 6.0	N/A
First seizure precipitated by:		
Fever/illness		134/230 (58)
No precipitant		75/230 (33)
Vaccination		17/230 (7)
Bath		4/230 (2)
Abnormal interictal EEG in first 6 months		8/47 (17)
Abnormal interictal EEG in months 7–12		45/90 (50)
Abnormal interictal EEG in months 13–24		34/55 (62)
Abnormal interictal EEG in months 25–36		30/38 (79)
Photosensitivity		34/211 (16)
Autistic features		69/208 (33)
Behaviour problems		98/213 (46)
Motor disorder		77/214 (36)
Hypotonia		6/214 (3)
Ataxia		56/214 (26)
Spasticity		15/214 (7)
Dyskinesia		6/214 (3)
MRI abnormalities		22/200 (11)

IQR = interquartile range; N/A = not applicable.

Table 2 Medication response in *SCN1A* mutation positive Dravet Syndrome ($n = 241$)

Medications reported to have reduced seizure frequency (five most common)	Number/total (%)
Valproate	81/160 (51)
Clobazam/clonazepam	55/160 (34)
Topiramate	45/160 (28)
Levetiracetam	21/160 (13)
Stiripentol	20/160 (13)
Medications reported to have increased seizure frequency (three most common)	**Number/total (%)**
Carbamazepine	36/60 (60)
Lamotrigine	26/60 (43)
Valproate	4/60 (7)

as frequent as 1:28 600. Previous American and French findings from smaller cohorts had approximated similar incidence figures ranging from 1:20 000 to 1:40 000 making Dravet syndrome a rare disease (Hurst, 1990; Yakoub *et al.*, 1992). We observed a slightly higher male to female ratio of 1.27:1, similar to recent figures obtained from a large parent survey (Skluzacek *et al.*, 2011). The inheritance figures for febrile seizures and epilepsy in this series are close to those established by a case control study among relatives of patients with mutation-positive Dravet syndrome and matched controls (Mancardi *et al.*, 2006). The authors found that febrile seizures and epilepsy occurred in 20 out of 74 (27%) families with severe myoclonic epilepsy in infancy, and did not differ significantly from controls where 13 out of 70 (18.5%) families were affected. Similar to our data there were more relatives with epilepsy than with febrile seizures.

The estimated mortality of 6% at 5 years of age highlights that children with Dravet syndrome face a substantial risk of early epilepsy-related death compared with children with idiopathic epilepsy. A recent population-based study found that subjects with epilepsy and cognitive impairment had a significantly higher mortality risk than subjects with epilepsy without cognitive impairment (Sillanpaa and Shinnar, 2010). In the latter group the risk of sudden unexplained death was 7% at 40 years and there were no deaths in subjects younger than 14 years of age. A population-based survey from Japan found that 63 out of 623 patients with Dravet syndrome (10%) had died of mainly epilepsy-related causes: sudden unexplained death in epilepsy 53%, status epilepticus 36% and accidental drowning 10% (Sakauchi *et al.*, 2011). Longitudinal studies are required to verify these data in the future.

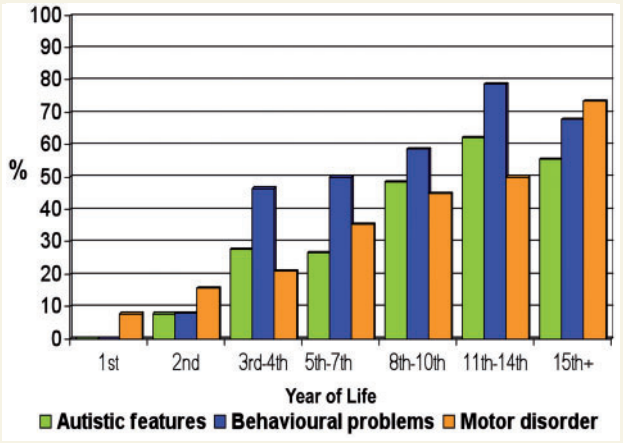

Figure 1 Cross-sectional analysis of acquired autistic features, behavioural problems and motor disorder over time.

Our analysis of clinical features reveals an evolution of different seizure types over the first 3 years that corresponds to early descriptions by Charlotte Dravet (Dravet, 1978). The majority of seizures had been precipitated by fever or illness, however, one-third had no precipitant and 7% had been triggered by vaccination. As we only included cases that were vaccinated within 2 days of their first seizure, our vaccination figures are conservative compared to a previous report that allowed longer time intervals between vaccination and seizure occurrence, stating a figure of 16% (Tro-Baumann *et al.*, 2011). Pertussis vaccination had been alleged to cause 'vaccine encephalopathy' in the past, however, recent evidence demonstrated that the encephalopathy is, in the majority of cases, truly a presentation of Dravet syndrome caused by a *SCN1A* gene mutation (Berkovic *et al.*, 2006). In keeping with a recent retrospective study of 40 patients with Dravet syndrome, we found that vaccination-triggered seizures presented significantly earlier than those without precipitant or

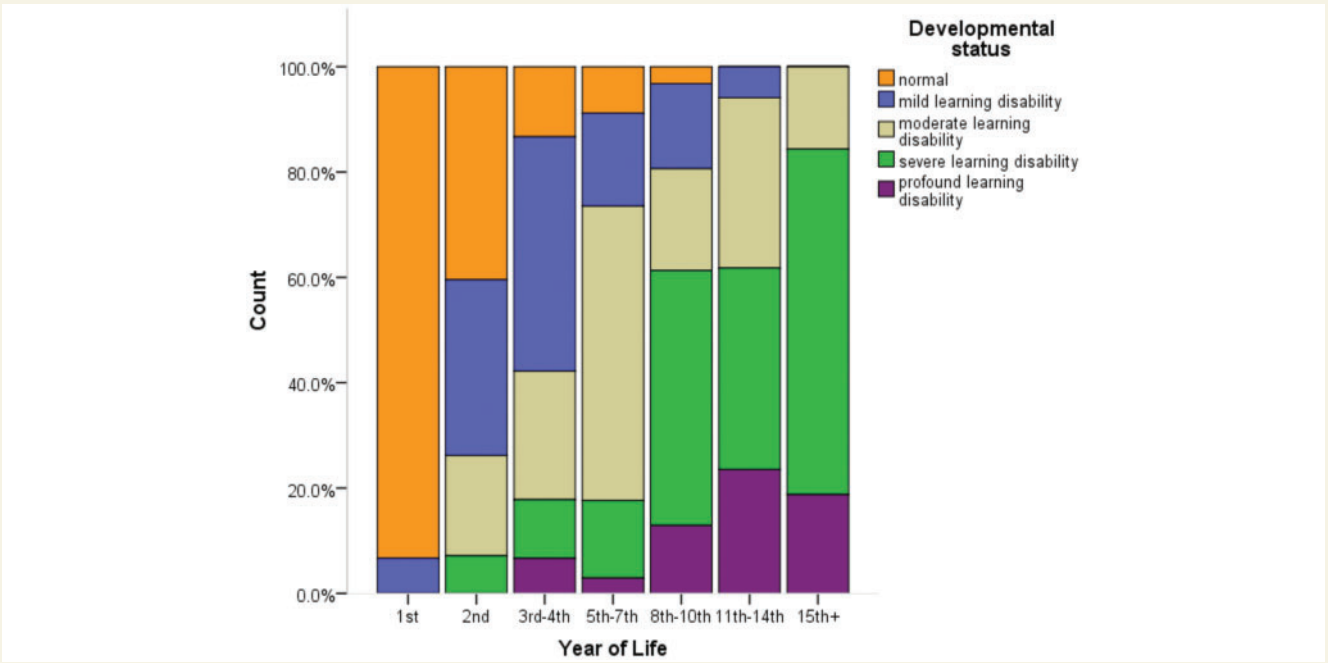

Figure 2 Cross-sectional analysis of developmental status over time.

Table 3 Ordinal univariate logistic regression analysis for variables predicting worse developmental outcome (*n* = 157; adjusted for age at assessment)

Predictor variable	*B*	Wald test χ^2	OR (95% CI)	*P*
Motor disorder (yes/no)	1.19	12.31	3.28 (1.69–6.38)	<0.001
EEG abnormalities in Year 1 (yes/no)	1.74	9.93	5.70 (1.93–16.8	0.002
Status epilepticus (yes/no)	1.12	9.09	3.07 (1.48–6.35)	0.003
Age at onset of delay (months)	−0.04	6.81	0.96 (0.94–0.99)	0.009
Early focal seizures with impairment of awareness ⩽24 months (yes/no)	1.19	4.24	3.30 (1.06–10.28)	0.039
Age at onset of myoclonic seizures (months)	−0.03	3.65	0.97 (0.94–1.00)	0.056

CI = confidence interval; OR = odds ratio.

with fever/illness, nevertheless vaccination itself had no impact on the developmental outcome (McIntosh *et al*., 2010). This supports the argument that children carrying a *SCN1A* mutation are destined to develop the disease, which in turn can be precipitated by a series of factors such as fever/illness, vaccination or a bath. However, the nature of the trigger has no effect on overall developmental outcome and thus does not seem to be responsible for the subsequent encephalopathy.

We observed a clear increase in abnormal interictal EEG recordings over time. Whereas few abnormal recordings were seen in the first 6 months, over three-quarters of children had an abnormal interictal EEG by the end of the third year. Consistent with previous reports, we principally observed generalized spike-wave and focal/multifocal EEG abnormalities (Dravet *et al*., 2002). Among our cohort we noticed that an abnormal interictal EEG recording in the first year of life predicted a worse developmental outcome. The affected voltage gated sodium channels ($Na_v1.1$) are vital for the generation of action potentials throughout the CNS, but play a specifically important role in inhibitory interneurons (Yu *et al*., 2006; Ogiwara *et al*., 2007). As the brain matures with age and adapts towards higher cognitive functioning, a defect in $Na_v1.1$ channels may become more apparent. This might present as disturbance of the ordered electrical activity measured by EEG, and the more severe the degree of malfunction, the earlier the EEG abnormality might appear and the more unfavourable the sequelae could be.

We found that at least 11% of cases had abnormal MRI imaging, a figure similar to that found in previous smaller reports (Renier *et al*., 1990; Siegler *et al*., 2005; Striano *et al*., 2007*b*). Reviewing ours and previous studies, the two most commonly reported abnormalities were non-specific brain atrophy and temporal/hippocampal changes. Given that recurrent prolonged seizures in infancy are the hallmark of Dravet syndrome, it is perhaps surprising that hippocampal changes are not seen more frequently. There is good evidence to show that prolonged febrile seizures can cause acute hippocampal injury, however, it remains unclear how many of those children will develop mesial temporal sclerosis in the future (Scott *et al*., 2003). Whether the atrophic changes are linked to the underlying channelopathy remains unclear and we were unable to establish whether these changes occurred predominantly in the older age groups. Overall, MRI abnormalities were not a predictor for worse developmental outcome and 89% of imaging had been reported as normal.

The response to medication data we present here are descriptive in nature and have to be interpreted within their limits of level 3 evidence. Sodium valproate and benzodiazepines are the two most commonly reported anti-epileptic drugs to reduce seizure frequency, which is consistent with the literature (Dravet *et al*., 2002). Two randomized controlled trials have shown that a combination of sodium valproate, clobazam and stiripentol effectively control seizures in Dravet syndrome (Chiron *et al*., 2000; Kassai *et al*., 2008). Given the diagnostic uncertainty at the time of referral, stiripentol would often not be the first line treatment, which might explain the low response figures reported for this drug. Topiramate and levetiracetam have both been reported as two of the more helpful anti-epileptic drugs in our cohort. There is evidence from several open label and retrospective studies that topiramate is useful in Dravet syndrome, however, no randomized controlled trials have been performed yet (Nieto-Barrera *et al*., 2000; Ceulemans *et al*., 2004; Kröll-Seger *et al*., 2006). Levetiracetam has been shown to be efficacious as add-on therapy in Dravet syndrome (Striano *et al*., 2007*a*) and has been reported to be helpful in treating juvenile myoclonic epilepsy as well as refractory epilepsy (Verrotti *et al*., 2010). Further placebo-controlled trials are required to investigate this in the future. Carbamazepine and lamotrigine have been the most common anti-epileptic drugs to have increased seizure frequency in our cohort and in previous reports have been shown particularly to aggravate myoclonic and generalized clonic seizures as well as status epilepticus (Guerrini *et al*., 1998; Wallace, 1998; Striano *et al*., 2008). Therefore preventing a child with Dravet syndrome from taking medication that might worsen seizures can only be beneficial. Given that sodium valproate has been the most commonly and successfully used drug in this cohort, it is not surprising that occasionally an individual does not respond well to it.

Physicians reported significant numbers of acquired autistic features and behavioural problems in this cohort, a finding that is consistent with our previous work showing that two-thirds of children with Dravet syndrome scored in the abnormal range (>90th percentile) of 'hyperactivity/inattention' and one-third in the abnormal range for 'conduct problems' (Brunklaus *et al*., 2011).

Several independent predictors for developmental outcome in Dravet syndrome have been identified. The earlier the delay was noticed, the more severe the outcome appeared to be, suggesting that a significant genetic change presents early and becomes more pronounced with time. Similarly, occurrence of a motor disorder indicated worse outcome. Animal models have clearly shown the important role $Na_v1.1$ plays in the functioning of cerebellar Purkinje cells and that significant *SCN1A* mutations can cause a motor disorder (Yu *et al*., 2006). Therefore the presence of a motor disorder may reflect a higher disease burden in that individual. Our results agree with a recent report suggesting that early myoclonus might be associated with a more severe phenotype with worse developmental outcome (Ragona *et al*., 2011). However, our results go further in identifying predictors that are potentially preventable such as status epilepticus, or those that might aid counselling e.g. early EEG findings or young age at onset of delay.

Interestingly EEG abnormalities in the first year of life and status epilepticus predicted worse developmental outcome, however, the mutation class did not. How do we explain these findings? Dravet syndrome is caused by a genetic change resulting in altered function of $Na_v1.1$ sodium channels in neurons clustered throughout the brain (Ogiwara *et al*., 2007). Animal models have shown how this can impact on different brain functions such as on hippocampal gamma-aminobutyric acidergic interneurons influencing seizure susceptibility and on cerebellar Purkinje cells causing a motor disorder (Ogiwara *et al*., 2007; Yu *et al*., 2006). The degree to which individuals are affected is difficult to predict. There is some evidence that the nature of mutation might influence the phenotype (e.g. earlier seizures, more severe disease) but no association with developmental outcome has yet been shown (Kanai *et al*., 2009; Zuberi *et al*., 2011). Missense mutations can vary considerably ranging from missense mutations leading to very

minor protein changes up to missense mutations leading to complete abolition of protein function. A severe missense mutation can in fact be as deleterious as a truncating mutation.

Our understanding of the functional effect of mutations is still unrefined and classification models lack accuracy to truly reflect the mutation impact. On the other hand we know that identical mutations can present in very different ways in the same family, highlighting the importance of modifying effects such as other genes and environmental factors (Kimura *et al*., 2005). In a combined model of genetic and environmental factors, a genetic change will present according to its severity and modifying factors and its effects persist throughout life making an impact on a variety of neuronal functions. This already vulnerable system may be susceptible to secondary aggravating events such as status epilepticus and our results emphasize the importance of status epilepticus as independent predictor for worse cognitive development.

Some children with Dravet syndrome have been reported to undergo an encephalopathic course following an episode of status epilepticus or acute illness (Chipaux *et al*., 2010; Takayanagi *et al*., 2010; Tang *et al*., 2011). The affected individuals had either truncating mutations or a missense mutation with significant physicochemical property difference (Grantham score > 70). Two of these were treated with lamotrigine, which might have exacerbated the disease course. Follow-up imaging clearly showed new MRI changes concordant with significant disease deterioration affecting cortical and cerebellar regions. Barbiturate treatment for status epilepticus may have further contributed to brain damage in three affected patients (Chipaux *et al*., 2010). These cases highlight how susceptible children with Dravet syndrome are to sudden deterioration and every effort should be made not to expose affected children to potential trigger factors.

This has further implications on treatment demanding prompt and focused therapy, as prevention of status epilepticus with regular medication and emergency protocols may influence developmental outcome. Finally identification of factors influencing prognosis both aids counselling and encourages early, syndrome specific therapy.

Limitations

There are several limitations to this study. The percentage of vaccine-related cases might be underestimated in our sample as we were not able to review original hospital notes and vaccination records. Although we included consecutively referred individuals, it is possible that the more severe cases might have been referred from the older age groups compared to the younger ones. However, we addressed this by controlling for the age at referral in the logistic regression model. Data on cognitive outcome were obtained from professionals with expertise in the assessment of developmental status but were not derived from formal psychometric testing and data might be subject to bias due to inter-rater differences. Finally the benefit of 'optimal' treatment has only been available for the last 5–6 years and we do not know how these individuals will develop in the future. Prospective studies are needed to assess the effect of treatment on development in the long-term.

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References

Berkovic SF, Harkin L, McMahon JM, Pelekanos JT, Zuberi SM, Wirrell EC, et al. De-novo mutations of the sodium channel gene *SCN1A* in alleged vaccine encephalopathy: a retrospective study. Lancet Neurol 2006; 5: 488–92.

Brunklaus A, Dorris L, Zuberi SM. Comorbidities and predictors of health related quality of life in Dravet syndrome. Epilepsia 2011; 52: 1476–82.

Catarino CB, Liu JY, Liagkouras I, Gibbons VS, Labrum RW, Ellis R, et al. Dravet Syndrome as epileptic encephalopathy: evidence from very long-term course and neuropathology. Brain 2011; 134: 2982–3010.

Ceulemans B, Boel M, Claes L, Dom L, Willekens H, Thiry P, et al. Severe myoclonic epilepsy of infancy: toward an optimal treatment. J Child Neurol 2004; 19: 516–21.

Chipaux M, Villeneuve N, Sabouraud P, Desguerre I, Boddaert N, Depienne C, et al. Unusual consequences of status epilepticus in Dravet syndrome. Seizure 2010; 19: 190–4.

Chiron C, Marchand MC, Tran A, Rey E, D'athis P, Vincent J, et al. Stiripentol in severe myoclonic epilepsy in infancy: a randomised placebo-controlled syndrome-dedicated trial. STICLO study group. Lancet 2000; 356: 1638–42.

Claes LR, Deprez L, Suls A, Baets J, Smets K, Van Dyck, et al. The *SCN1A* variant database: a novel research and diagnostic tool. Hum Mutat 2009; 30: E904–20.

Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium channel gene *SCN1A* cause severe myoclonic epilepsy of infancy. Am J Hum Genet 2001; 68: 1327–32.

Depienne C, Bouteiller D, Keren B, Cheuret E, Poirier K, Trouillard O, et al. Sporadic infantile epileptic encephalopathy caused by mutations in *PCDH19* resembles Dravet syndrome but mainly affects females. PLoS Genet 2009a; 5: e1000381.

Depienne C, Trouillard O, Saint-Martin C, Gourfinkel-An I, Bouteiller D, Carpentier W, et al. Spectrum of *SCN1A* gene mutations associated with Dravet syndrome: analysis of 333 patients. J Med Genet 2009b; 46: 183–91.

Dravet C. Les epilepsies graves de l'enfant. Vie Med 1978; 8: 543–8.

Dravet C, Bureau M, Oguni H, Fukuyama Y, Cokar O. Severe myoclonic epilepsy in infancy (Dravet Syndrome). In: Roger J, Bureau M, Dravet Ch, Genton P, Tassinari CA, Wolf P, editors. Epileptic syndromes in infancy, childhood and adolescence. London: John Libbey; 2002. p. 81–103.

Guerrini R, Dravet C, Genton P, Belmonte A, Kaminska A, Dulac O. Lamotrigine and seizure aggravation in severe myoclonic epilepsy. Epilepsia 1998; 39: 508–12.

Guerrini R, Oguni H. Borderline Dravet syndrome: a useful diagnostic category? Epilepsia 2011; 52 (Suppl 2): 10–2.

Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM, et al. The spectrum of SCN1A-related infantile epileptic encephalopathies. Brain 2007; 130: 843–52.

Hurst DL. Epidemiology of severe myoclonic epilepsy of infancy. Epilepsia 1990; 31: 397–400.

Kanai K, Yoshida S, Hirose S, Oguni H, Kuwabara S, Sawai S, et al. Physicochemical property changes of amino acid residues that accompany missense mutations in *SCN1A* affect epilepsy phenotype severity. J Med Genet 2009; 46: 671–9.

Kassai B, Chiron C, Augier, Cucherat M, Rey E, Gueyffier F, et al. Severe myoclonic epilepsy in infancy: a systematic review and a meta-analysis of individual patient data. Epilepsia 2008; 49: 342–8.

Kimura K, Sugawara T, Mazaki-Miyazaki E, Hoshino K, Nomura Y, Tateno A, et al. A missense mutation in *SCN1A* in brothers with severe myoclonic epilepsy in infancy (severe myoclonic epilepsy in infancy) inherited from a father with febrile seizures. Brain Dev 2005; 27: 424–30.

Kröll-Seger J, Portilla P, Dulac O, Chiron C. Topirimate in the treatment of highly refractory patients with Dravet Syndrome. Neuropediatrics 2006; 37: 325–9.

Lossin C. A catalog of *SCN1A* variants. Brain Dev 2009; 31: 114–30.

Mancardi MM, Striano P, Gennaro E, Madia F, Paravidino R, Scapolan S, et al. Familial occurrence of febrile seizures and epilepsy in severe myoclonic epilepsy of infancy (SMEI) patients with *SCN1A* mutations. Epilepsia 2006; 47: 1629–35.

McIntosh AM, McMahon J, Dibbens LM, Iona X, Mulley JC, Scheffer IE, et al. Effects of vaccination on onset and outcome of Dravet syndrome: a retrospective study. Lancet Neurol 2010; 9: 592–8.

Mullen SA, Scheffer IE. Translational research in epilepsy genetics: sodium channels in man to interneuronopathy in mouse. Arch Neurol 2009; 66: 21–6.

Nieto-Barrera M, Candau R, Nieto-Jimenez M, Correa A, del Portal LR. Topiramate in the treatment of severe myoclonic epilepsy in infancy. Seizure 2000; 9: 590–4.

Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, et al. Na(v)1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an *SCN1A* gene mutation. J Neurosci 2007; 27: 5903–14.

Oguni H, Hayashi K, Oguni M, Mukahira A, Uehara T, Fukuyama Y, et al. Treatment of severe myoclonic epilepsy in infants with bromide and its borderline variant. Epilepsia 1994; 35: 1140–5.

Ragona F, Granata T, Dalla Bernardina B, Offredi F, Darra F, Battaglia D, et al. Cognitive development in Dravet syndrome: a retrospective, multicenter study of 26 patients. Epilepsia 2011; 52: 386–92.

Renier WO, Renkawek K. Clinical and neuropathologic findings in a case of severe myclonic epilepsy of infancy. Epilepsia 1990; 31: 287–91.

Sakauchi M, Oguni H, Kato I, Osawa M, Hirose S, Kaneko S, et al. Mortality in Dravet syndrome: search for risk factors in Japanese patients. Epilepsia 2011; 52 (Suppl 2): 50–4.

Scott RC, King MD, Gadian DG, Neville BGR, Connelly A. Hippocampal abnormalities after prolonged febrile convulsion: a longitudinal MRI study. Brain 2003; 126: 2551–7.

Siegler Z, Barsi P, Neuwirth M, Jerney J, Kassay M, Janszky J, et al. Hippocampal sclerosis in severe myoclonic epilepsy in infancy: a retrospective MRI study. Epilepsia 2005; 46: 704–8.

Sillanpaa M, Shinnar S. Long-term mortality in childhood-onset epilepsy. N Eng J Med 2010; 363: 2522–9.

Skluzacek JV, Watts KP, Parsy O, Wical B, Camfield P. Dravet syndrome and parent associations: the IDEA League experience with comorbid conditions, mortality, management, adaptation, and grief. Epilepsia 2011; 52 (Suppl 2): 95–101.

Striano P, Coppola A, Pezzella M, Ciampa C, Specchio N, Ragona F, et al. An open-label trial of Levetiracetam in severe myoclonic epilepsy of infancy. Neurology 2007a; 69: 250–4.

Striano P, Mancardi MM, Biancheri R, Madia F, Gennaro E, Paravidino R, et al. Brain MRI findings in severe myoclonic epilepsy in infancy and genotype-phenotype correlations. Epilepsia 2007b; 48: 1092–6.

Striano P, Striano S, Minetti C, Zara F. Refractory, life-threatening status epilepticus in a 3-year-old girl. Lancet Neurol 2008; 7: 278–84.

Takayanagi M, Haginoya K, Umehara N, Kitamura T, Numata Y, Wakusawa K, et al. Acute encephalopathy with a truncation mutation in the *SCN1A* gene: a case report. Epilepsia 2010; 51: 1886–8.

Tang S, Lin JP, Hughes E, Siddiqui A, Lim M, Lascelles K. Encephalopathy and *SCN1A* mutations. Epilepsia 2011; 52: e26–30.

Tro-Baumann B, von Spiczak S, Lotte J, Bast T, Haberlandt E, Sassen R, et al. A retrospective study of the relation between vaccination and occurrence of seizures in Dravet syndrome. Epilepsia 2011; 52: 175–8.

Verrotti A, D'Adamo E, Parisi P, Chiarelli F, Curatolo P. Levetiracetam in childhood epilepsy. Pediatr Drugs 2010; 12: 177–86.

Wallace SJ. Myoclonus and epilepsy in childhood: a review of treatment with valproate, ethosuximide, lamotrigine and zonisamide. Epilepsy Res 1998; 29: 147–54.

Yakoub M, Dulac O, Jambaque I, Chiron C, Plouin P. Early diagnosis of severe myoclonic epilepsy in infancy. Brain Dev 1992; 14: 299–303.

Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. Nat Neurosci 2006; 9: 1142–9.

Zuberi SM, Brunklaus A, Birch R, Reavey E, Duncan J, Forbes GH. Genotype-phenotype assiciations in *SCN1A* related epilepsies. Neurology 2011; 76: 594–600.