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Dravet syndrome as epileptic encephalopathy: evidence from long-term course and neuropathology

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Dravet syndrome is an epilepsy syndrome of infantile onset, frequently caused by *SCN1A* mutations or deletions. Its prevalence, long-term evolution in adults and neuropathology are not well known. We identified a series of 22 adult patients, including three adult post-mortem cases with Dravet syndrome. For all patients, we reviewed the clinical history, seizure types and frequency, antiepileptic drugs, cognitive, social and functional outcome and results of investigations. A systematic neuropathology study was performed, with post-mortem material from three adult cases with Dravet syndrome, in comparison with controls and a range of relevant paediatric tissue. Twenty-two adults with Dravet syndrome, 10 female, were included, median age 39 years (range 20–66). *SCN1A* structural variation was found in 60% of the adult Dravet patients tested, including one post-mortem case with DNA extracted from brain tissue. Novel mutations were described for 11 adult patients; one patient had three *SCN1A* mutations. Features of Dravet syndrome in adulthood include multiple seizure types despite polytherapy, and age-dependent evolution in seizure semiology and electroencephalographic pattern. Fever sensitivity persisted through adulthood in 11 cases. Neurological decline occurred in adulthood with cognitive and motor deterioration. Dysphagia may develop in

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or after the fourth decade of life, leading to significant morbidity, or death. The correct diagnosis at an older age made an impact at several levels. Treatment changes improved seizure control even after years of drug resistance in all three cases with sufficient follow-up after drug changes were instituted; better control led to significant improvement in cognitive performance and quality of life in adulthood in two cases. There was no histopathological hallmark feature of Dravet syndrome in this series. Strikingly, there was remarkable preservation of neurons and interneurons in the neocortex and hippocampi of Dravet adult post-mortem cases. Our study provides evidence that Dravet syndrome is at least in part an epileptic encephalopathy.

Keywords: *SCN1A*; Na^+ channel; epilepsy; neuropathology; encephalopathy

Abbreviations: Cx43 = connexin 43; GFAP = glial fibrillary acidic protein; HLA = human leucocyte antigen; $Na_v1.1$ = voltage-gated sodium channel type 1.1

Introduction

Dravet syndrome (severe myoclonic epilepsy of infancy; MIM 607208), first described ~30 years ago, is a severe epilepsy with onset in infancy (Dravet, 1978; Dravet *et al*., 2005). Dravet syndrome includes severe myoclonic epilepsy of infancy and severe myoclonic epilepsy of infancy-borderland, where one or two cardinal features of severe myoclonic epilepsy of infancy may be missing. Dravet syndrome is characterized by onset of recurrent febrile and/or afebrile hemiclonic or generalized seizures, or status epilepticus, in a previously healthy infant, followed by appearance of multiple seizure types generally resistant to anti-epileptic drugs with developmental arrest or regression (Dravet *et al*., 2005; Jansen *et al*., 2006; Wolff *et al*., 2006). Onset up to 15 months of age may occur (Depienne *et al*., 2009*b*). Mortality may be up to 15% by 20 years (Dravet *et al*., 2005).

Of the cases with Dravet syndrome, 70–80% are caused by *SCN1A* mutations, 90% of which occur *de novo* (Depienne *et al*., 2009*b*; Marini *et al*., 2009; Mullen and Scheffer, 2009). Haploinsufficiency is thought to be the mechanism underlying most cases (McArdle *et al*., 2008; Depienne *et al*., 2009*b*; Mullen and Scheffer, 2009). Genetic modifiers (Meisler *et al*., 2010) and environmental factors probably contribute to the variable phenotype of patients with *SCN1A* mutations. Other genes involved in Dravet syndrome include *SCN1B* (Patino *et al*., 2009) and *GABRG2* (Harkin *et al*., 2002). *PCDH19* (Dibbens *et al*., 2008; Depienne *et al*., 2009*a*) and *SCN2A* (Kamiya *et al*., 2004; Shi, 2009) mutations, and deletions involving the chromosome 2q *SCN* cluster (Pereira *et al*., 2004; Davidsson *et al*., 2008; Lossin, 2009; Meisler *et al*., 2010) have been reported in Dravet syndrome-like syndromes.

SCN1A knockout or -in animal models of Dravet syndrome manifest spontaneous seizures, motor deficits, ataxia and premature death (Yu *et al*., 2006; Kalume *et al*., 2007; Ogiwara *et al*., 2007; Tang *et al*., 2009; Martin *et al*., 2010). Sodium currents are significantly reduced in inhibitory interneurons in both hippocampus and cortex, but less so in hippocampal pyramidal cells (Yu *et al*., 2006; Ogiwara *et al*., 2007). In a knock-in mouse model of Dravet syndrome, excitatory cortical pyramidal neurons were shown to be mostly unaffected, while inhibitory cortical interneurons had impaired sodium channel activity (Martin *et al*., 2010). Reduced sodium currents in hippocampal and cortical GABAergic interneurons led to altered firing patterns and overall hyperexcitability (Yu *et al*., 2006; Catterall *et al*., 2008; Tang *et al*., 2009; Martin *et al*., 2010). The reduced expression of voltage-gated sodium channel type 1.1 ($Na_v1.1$) in Purkinje cells, leading to abnormal sodium flux, may contribute to ataxia observed in animal models (Yu *et al*., 2006). Further parallels between animal models and human Dravet syndrome include sensitivity to body temperature elevation, causing seizures and interictal epileptiform discharges, and age dependence of seizure frequency and severity (Oakley *et al*., 2009).

Immune-inflammatory mediators have received attention in epileptogenesis, febrile seizures and some chronic epilepsies (Vezzani and Granata, 2005; Ravizza *et al*., 2008; Vezzani *et al*., 2008). Dravet syndrome may provide a model to advance understanding of inflammation in epileptogenesis and fever as a seizure-provoking factor (Baulac *et al*., 2004; Oakley *et al*., 2009). The influence in Dravet syndrome of additional environmental factors such as vaccination may provide another window into investigation of immune factors in epileptogenesis (Berkovic *et al*., 2006; McIntosh *et al*., 2010).

In childhood, Dravet syndrome has been well studied. Dravet syndrome is comparatively uncommon, with an estimated incidence of <1:40 000 children (Hurst, 1990; Dravet *et al*., 2005), but important to diagnose because it is considered at least in part an epileptic encephalopathy, though other factors may contribute to outcomes (Ragona *et al*., 2011). Thus, seizures and frequent epileptiform activity on EEG are held in part responsible for cognitive, behavioural and other impairments (Dravet *et al*., 2005); both seizures and interictal discharges are potentially treatable and their control might improve outcomes in Dravet syndrome (Scheffer *et al*., 2009). In contrast to this knowledge in children with Dravet syndrome, the place of Dravet syndrome in adults with epilepsy is less well understood. Dravet syndrome is under-diagnosed and under-reported in adulthood (Scheffer *et al*., 2009). For patients with chronic epilepsy who are long-standing attendees at clinic, details of the early history may become obscured, and the diagnosis of Dravet syndrome may not be considered. The long-term course of Dravet syndrome has therefore not been fully characterized, particularly in patients in their forties and over.

We aimed to gather more information on Dravet syndrome in adults in order to inform management. We undertook an observational study that was not intended to be a systematic study of prevalence in adults with severe epilepsy. Using both post-mortem and surgical brain tissue resources, we also aimed to undertake a detailed systematic neuropathological investigation of Dravet syndrome. We hypothesized that in the long term, Dravet syndrome would cause further broad neurological decline, and that years of encephalopathy would eventually lead to associated brain tissue damage and loss identifiable on neuropathological examination. By analysing clinical and neuropathological data, we sought to determine if Dravet syndrome could still be considered an epileptic encephalopathy later in life.

Materials and methods

This project was approved by the relevant local (Human) Research Ethics Committees with appropriate consent, or assent from relatives or legal guardians in the case of minors, adults with intellectual impairment and study of post-mortem tissue.

Patient ascertainment and phenotyping

We included adult patients from National Hospital for Neurology and Neurosurgery clinics. All available clinical and investigational information was reviewed.

Genetic testing

Details of DNA extraction and molecular analysis of the *SCN1A* gene with DNA sequencing and gene dosage analysis are given in the supplementary material. Parents of patients with a mutation were tested where possible with direct sequencing of the mutated *SCN1A* region or multiplex ligation-dependent probe amplification.

Genotype–phenotype analysis

The design of our study limits such analysis. We divided our cohort into: (i) paediatric cases with Dravet syndrome, with death before 12 years; (ii) adult cases with Dravet syndrome with death after 45 years; (iii) living adult Dravet patients; and (iv) living children with generalized epilepsy with febrile seizure plus. We looked at each of these groups for type of *SCN1A* mutations, and distribution of *SCN1A* missense mutations.

Neuropathology

The whole brain of three adult post-mortem cases with Dravet syndrome [who all met established criteria for Dravet syndrome (Commission, 1989)], two adult post-mortem disease controls with hippocampal sclerosis and three adult post-mortem controls with no known neurological disease were studied. Adult disease cases were former residents at the National Society for Epilepsy, Chalfont (Sander *et al*., 1993). As comparators for older post-mortem cases, we studied four paediatric post-mortem cases with Dravet syndrome, one anterior temporal lobectomy specimen from a child with intractable childhood epilepsy with generalized tonic–clonic seizures, left hippocampal sclerosis, operated at 12 years and a *SCN1A* mutation (referred to as $SCN1A^+$ surgical case; Livingston *et al*., 2009), and one post-mortem brain from a child with severe febrile seizures in the genetic epilepsy with febrile seizures plus spectrum. We also had access to a brain biopsy obtained in childhood from an individual ascertained as an adult (Case 4).

Studies were undertaken to look for subtle malformations, hippocampal sclerosis (using standard qualitative, quantitative and immunohistochemical examination), cortical neuronal loss (qualitative examination), loss of specific cell populations (qualitative and semi-quantitative immunohistochemistry for interneurons), abnormalities of brainstem nuclei or tracts, distribution and quantitation of cells labelled with antibodies to $Na_v1.1$ and for evidence of inflammatory and other disease processes [examination with antibodies to human leucocyte antigen (HLA)-DR and connexin-43 (Cx-43)].

Formalin-fixed post-mortem whole brains were sliced coronally along the anteroposterior axis and each slice was carefully re-examined for macroscopic abnormalities. Systematic histological sampling using blocks of 5 mm thickness were taken from several regions where possible: frontal (F1/F2), parietal, temporal and occipital cortex, insula, cingulate gyrus, cerebellum, hippocampus, amygdala, thalamus, basal ganglia, midbrain, pons, medulla and spinal cord at the cervical level. For two adult post-mortem cases with Dravet syndrome (Cases PM1/EP039 and PM3/EP099), additional blocks were taken from medial and orbital frontal cortex (Brodmann areas 6, 8 and 11), and insula. For the paediatric post-mortem cases, available sample blocks are shown in the supplementary material. Surgically resected temporal neocortex and hippocampal tissues were available for the $SCN1A^+$ surgical case.

All blocks were processed in alcohol then xylene and embedded in paraffin within 1 week of sampling. Haematoxylin and eosin and Luxol fast blue stains were performed on sections from all regions.

Immunohistochemistry was performed on the post-mortem hippocampal, frontal cortical (F1/F2), cerebellar, pontine, medullary and spinal cord sections, and the surgically resected hippocampal and temporal neocortical sections. Details of the techniques and primary antibodies, including the panel used as markers of neurodegenerative processes, are given in the supplementary material.

Quantitative analysis

Pyramidal cell density was stereologically evaluated in the hippocampal cornu ammonis-1 and cornu ammonis-4 subfields of the adult post-mortem cases with Dravet syndrome and post-mortem hippocampal sclerosis controls. Areal $Na_v1.1$-immunopositive counts were also undertaken in the hippocampal formation (dentate gyrus, cornu ammonis and subiculum) and one gyrus of the frontal cortex in the same cases. To obtain more information on patterns of cell loss, given that Dravet syndrome is considered an interneuronopathy (Mullen and Scheffer, 2009), we undertook interneuron counts. Details of methods are in the supplementary material.

Results

Demographic and clinical data are summarized in Table 1. Median age at last follow-up for the 22 adult cases with Dravet syndrome was 39 years (range 20–66 years). Detailed case histories of the adult post-mortem cases are given in the supplementary material.

For 11 patients with Dravet syndrome, a close temporal relation of seizure onset with vaccination (Table 1) was documented, as previously described (Berkovic *et al*., 2006; McIntosh *et al*., 2010).

Family history

There was a family history of epilepsy and/or febrile seizures in nine adult patients (Supplementary Table 3), and another adult patient had a sibling who had had one isolated seizure. Case 20 comes from a family with genetic epilepsy with febrile seizures plus. Case 6 has a 15-year-old sister with microcephaly,

Table 1 Demographic and clinical features of the 22 adult (PM1-3, 4-22) and four paediatric cases with Dravet syndrome (PM23-26), and two other *SCN1A* mutation-carrying paediatric cases with other epilepsy syndromes (PM27, and 28/*SCN1A*$^+$ surgical case)

Case ID	Gender/age at follow-up or ‡age at death (yrs)	Age at onset (months), seizure type at onset	Identifiable trigger at seizure onset	Seizure types in childhood	Seizure types in adulthood	Development/autistic features/behavioural problems	Psychometry data	Intellectual outcome at last follow-up[d]	Other neurological signs	Functional outcome at last follow-up	*SCN1A* mutation/deletion
PM1/EP039	F/46‡	3, GTC	Vaccination (no further details)	GTC, CP	GTC, My, Fo, 'drops', SE, NCSE/fever sensitivity	Development delayed after seizure onset/ no autistic features/ behavioural problems	No formal neuropsychometry data	Severe	Pyramidal signs	Deceased	Missense
PM2/EP213	M/66‡	11, GTC	None	My, GTC, NCSE	GTC, My, 'drops', NCSE	Development delayed after seizure onset/ no autistic features/ behavioural problems	Progressive cognitive decline, dementia from 55 yrs	Severe	Progressive ataxia, parkinsonism, dementia, cerebellar signs	Deceased	Not possible
PM3/EP099	M/46‡	18, GTC	None	My, GTC, Fo	GTC, My, Fo	Development delayed after seizure onset/ autistic features/ behavioural problems	10 yrs, FSIQ 77, 17 yrs, FSIQ 57	Severe	Cognitive slowing, dysarthria, ataxia	Deceased	Not possible
4	M/39	6, ND	Vaccination (whooping cough, 24 h)	GTC, My, hemiclonic, dyscognitive	GTC, My, CP, 'drops', dyscognitive, NCSE	Development regression after seizure onset/no autistic features/behavioural problems	No formal neuropsychometry data	Severe	Extra-pyramidal signs (choreoathetosis, dystonia), fixed contractures	No speech, institutionalized, full care, PEG, incontinent, wheelchair-bound	None detected
5	M/25	10, FS, hemiclonic	Fever	CP, My, GTC, SE, Dyscognitive, 'drops'	GTC, CP, 'drops', My, dyscognitive, SE	Development regression after seizure onset/autistic features/no behavioural problems	No formal neuropsychometry data; progressive, slow cognitive decline	Severe	Pyramidal signs (spasticity)	Lives at home with parents, behavioural problems, minimal speech (only repeats words)	Missense
6	M/60	12, GTC	Vaccination (whooping cough, 8 h)	GTC, clonic, dyscognitive, 'drops', SE	GTC, CP, My, T, SE, NCSE	Development regression from 6 yrs/ autistic features/behavioural problems	At 6 yrs went to mainstream school; At 27 yrs, VIQ 51, PIQ 58	Severe	Not documented	Recognizes basic words, able to tell the time, PEG, recurrent respiratory infections, wheelchair-bound, incontinent, institutionalized	Truncating, del
7	M/41	9, GTC	Slight increased temperature	GTC, MJ, dyscognitive, SE	GTC, dyscognitive	Development regression from 15 mo/no autistic features/ behavioural problems	No formal neuropsychometry data	Severe	Marked scoliosis, gait abnormality	Walks unaided, with stooped posture and legs in semi-flexion; performs one-stage command	Splice donor, del
8	F/43	12, ND	No trigger documented	Dyscognitive, My	CP, dyscognitive, My	Development delayed after seizure onset/ autistic features/ behavioural problems	No formal neuropsychometry data	Severe		Lives with parents, walks unaided but uses wheelchair for longer distances, speaks in short phrases, but mainly sign language, eats unaided, with spoon, recurrent respiratory infections	Missense
9	F/27	8, FS	Fever	GTC, CP, dyscognitive, My, F, NCSE	GTC, dyscognitive, My, T	Development delayed after seizure onset/ autistic features/ behavioural problems not documented	No formal neuropsychometry data, Cognitive decline in adulthood	Severe	Truncal ataxia, pyramidal signs, hand tremor, wide-based gait		Missense

(continued)

Table 1. Continued

Case ID	Gender/age at follow-up or ‡age at death (yrs)	Age at onset (months), seizure type at onset	Identifiable trigger at seizure onset	Seizure types in childhood	Seizure types in adulthood	Development/autistic features/behavioural problems	Psychometry data	Intellectual outcome at last follow-up[d]	Other neurological signs	Functional outcome at last follow-up	*SCN1A* mutation/deletion
10	M/20	7.5, FS	Fever, vaccination (whooping cough, hours)	CP, GTC	CP, GTC, dyscognitive, SE/fever sensitivity	Development delayed after seizure onset/ autistic features/ behavioural problems	5yrs: FSIQ 63. 12yrs: VIQ 55, PIQ 68. 16yrs: FSIQ 40. 20yrs: moderately impaired learning range, limited expressive language, very poor comprehension, very weak working memory, unable to carry out two-step commands	Moderate	Cerebellar signs, truncal and gait ataxia, action and postural tremor	Lives with parents, needs constant one-to-one care	Splice site
11	F/29	7, Feb SE	Fever, whooping cough infection	GTC, CP, SE, 'drops'	GTC, dyscognitive, CP, T, SE, NCSE	Development delayed after seizure onset/ autistic features/no behavioural problems	No formal neuropsychometry data	Severe	Abnormal gait, pyramidal signs (hyper-reflexia)	Lives with parents, requires help for activities of daily living; able to walk unaided, occasional single words	Missense
12	M/43	7, GTC	Vaccination (whooping cough, timeline not documented)	GTC, CP	GTC, CP	Development delayed after seizure onset/ no autistic features/ no behavioural problems	At 42 yrs, MMSE = 20/30	Mild	Extra-pyramidal signs (dystonic tremor, hypomimia, bradykinesia)	Lives with parents, self-caring with some help	None detected
13	M/21	12, ND	Vaccination (third dose of triple vaccination, 12 h)	My, GTC	GTC, dyscognitive, CP	Development regression after seizure onset/autistic features/behavioural problems	At 19 yrs, MMSE = 13/30	Moderate	Not documented	Residential care, still behavioural problems	None detected
14	F/40	15, GTC	Vaccination (measles vaccination, several days)	GTC, My, dyscognitive	GTC, My, 'drops', dyscognitive	Development regression after seizure onset/autistic features/behavioural problems	No formal neuropsychometry data	Severe	Kyphosis, pyramidal signs	Residential care, speaks one or two words, performs simple orders, walks unaided	None detected
15	M/31	6, GTC	Vaccination (triple vaccine, 9 days)	GTC, dyscognitive, NCSE, My	GTC, dyscognitive, My	Development regression after seizure onset/autistic features/behavioural problems	No formal neuropsychometry data, But gradual decline	Severe	Gait ataxia	Nursing home, minimal communication, walks with help	None detected
16	F/48[a]	2.5, hemiclonic	Vaccination (triple vaccine, 2 days)	Hemiclonic, CP, My, GTC	GTC, My, hemiclonic, 'drops', T, NCSE	Development delayed after seizure onset/ no autistic features/ behavioural problems	No formal neuropsychometry data, But gradual decline	Severe	Pyramidal signs	Deceased	None detected
17	M/21	3, FS	Fever	GTC, dyscognitive, 'drops', My	GTC, My, dyscognitive, SE, NCSE	Development delayed after seizure onset/ no autistic features/ no behavioural problems	No formal neuropsychometry data	Moderate	Action tremor, extra-pyramidal signs	Residential care, does basic domestic chores with prompting	None detected
18	F/26	3, FS	Fever, vaccination (no details)	My, CP, 'drops', T	GTC, T, CP	Development delayed after seizure onset/ autistic features/behavioural problems	No formal neuropsychometry data	Severe	Intention tremor	Institutionalized	None detected
19	F/44	6, FS	Fever vaccination (pertussis, 2 days)	GTC, dyscognitive	GTC, CP	Development regression after seizure onset/autistic features/behavioural problems	No formal neuropsychometry data	Severe	Gait ataxia	Lives with parents, has carers, entirely dependent, doubly incontinent	Missense

(continued)

Table 1. Continued

Case ID	Gender/age at follow-up or ‡age at death (yrs)	Age at onset (months), seizure type at onset	Identifiable trigger at seizure onset	Seizure types in childhood	Seizure types in adulthood	Development/autistic features/behavioural problems	Psychometry data	Intellectual outcome at last follow-up[d]	Other neurological signs	Functional outcome at last follow-up	*SCN1A* mutation/deletion
20	F/39	10, FS	Fever	FS, GTC, 'drops', My	GTC, My, 'drops', T, SE	Development delayed after seizure onset/ autistic features/behaviour problems	At 40 yrs, MMSE = 14/30	Moderate	None documented	Institutionalized, feeds herself, requires help with domestic chores	Missense
21	F/23	4.5, Feb SE	Fever	CP, GTC, dyscognitive, 'drops'	GTC, T, CP	Development delayed from 9 mo/autistic features/behavioural problems	No formal neuropsychometry data	Severe	Kyphosis	Institutionalized, speech limited to one or two phrases, able to walk independently	One splice site del + two missense
22	M/33	4, GTC	No trigger documented	GTC, CP, My	T, GTC, My	Development delayed from 3 yrs/autistic features/behavioural problems	No formal neuropsychometry data; at 23 yrs, no speech, carries out some one-step commands	Severe	Wide-based gait	Institutionalized; no speech, walks with help, requires help with all activities of daily living	Delins
PM23	M/2	5, a febrile GTC	No trigger documented	GTC, My. No FS	Not applicable	Development delayed from 18 mo	No formal neuropsychometry data	Mild global cognitive delay. Limited expressive language	None documented	Deceased	Whole gene deletion
PM24[a]	F/10	2, Feb SE	Fever	FS, My, CP, Abs, GTC, SE, At, Hemiclonic	Not applicable	Development never normal, regression at 5 yrs	No formal neuropsychometry	Severe (nonverbal)	Crouch gait	Deceased	Truncation
PM25[a]	M/11	8, SE	No trigger documented	GTC, recurrent SE, My, At, T (nocturnal), My Status, Fo	Not applicable	Developmental regression with seizure onset/autistic features/behavioural problems	No formal neuropsychometry	Severe	Ataxia and spasticity	Deceased	Splice site
PM26	F/11	10, FS	Fever	FS, Abs, My, GTC, SE, CP, Hemiclonic	Not applicable	Developmental slowing from 10 months 4 yrs/behavioural problems	No formal neuropsychometry	Severe	Ataxia and tremulous	Deceased	No mutation detected; MLPA not done
PM27[b]	M/5	18, Feb SE	Fever	FS, Fo, GTC, SE	Not applicable	Normal development	No formal neuropsychometry	Normal	None	Deceased	Missense
28/*SCN1A*$^+$ surgical[c]	M/12	10, FS	Fever	GC, CPS, F, GTC/ fever sensitivity	Not applicable	Development delayed clear from 3 yrs/ autistic features/behavioural problems	No formal neuropsychometry data	Moderate	None documented	In a special school, moderate global intellectual disability	Missense

Abs = absence; At = ; CP = complex partial; delins = deletion/insertion; 'drops' = 'drop attacks'; F = female; Feb SE = febrile status epilepticus; Fo = focal; FS = febrile seizure; FSIQ = full-scale IQ; GC = generalized clonic; GTC = generalized tonic–clonic; HS = hippocampal sclerosis; IED = interictal epileptiform discharges; M = male; MJ = myoclonic jerks; MLPA = Multiplex Ligation-dependent Probe Amplification; mo = months; My = myoclonic; NCSE = non-convulsive status epilepticus; PEG = percutaneous endoscopic gastrostomy; PIQ = performance IQ; PM = post-mortem; SE = convulsive status epilepticus; T = tonic; VIQ = verbal IQ; WM = white matter; yrs = years; ND = undetermined seizure type.

a Described in Wallace *et al*., 2003.

b Described in Harkin *et al*., 2007; and Deng *et al*., 2007.

c Described in Livingston *et al*., 2009.

d Classification of intellectual outcome at last follow-up as described in McIntosh *et al*., 2010.

‡age at death.

quadriparesis, profound cognitive impairment and spasms, who is on anti-epileptic drugs, but does not carry the *SCN1A* mutation found in her brother (Case 6), nor *SCN1A* deletion or duplication.

Clinical condition and evolution in adulthood

From onset in infancy, there was no period of seizure freedom recorded. In two patients, recognition of a false 'seizure-free period' in childhood led to anti-epileptic drug cessation, but increased seizure severity and frequency led to recommencement of anti-epileptic drugs, and in retrospect the parents could recognize subtle seizures had never ceased to occur. All patients had multiple anti-epileptic drugs with differential control of different seizure types (Table 2), but not complete seizure freedom.

There was an evolution of seizure semiology and predominance of certain seizure types with time (Supplementary Table 3). There was no single pattern for seizure evolution for all patients.

All patients had multiple seizure types in adulthood (Table 1). For 10 patients, seizures were mostly nocturnal and comprised brief tonic or tonic–clonic seizures. Seizures were recorded in video-EEG telemetry for 10 adults; seizures observed were complex motor, dyscognitive, tonic or secondarily generalized with focal EEG onset pattern or no recognizable EEG change. Myoclonus was not prominent in adulthood, though its frequency may have been under-reported. No adult patient in our series had documented absences; all 'absence-like' (dyscognitive) seizures recorded in adulthood had focal EEG onset or no EEG change documented. Fever sensitivity persisted into adulthood, with even slight variations of temperature sufficient to trigger seizures in nine patients. No patient had any meaningful seizure-free period. Non-convulsive status epilepticus was documented with EEG on at least one occasion in seven patients. Triggers included inter-current infections and slight increases in body or ambient temperature.

Behavioural problems or 'autistic-like' features were observed at some time of the evolution in most patients in our series (Table 1).

At last follow-up, the oldest living patient was 60 years of age. Sixteen patients were in residential care; the remainder lived at home with support. Neurological deterioration continued throughout life in all patients, with further impairment of speech, mobility and ability for daily activities (Table 1 and Fig. 1). Kyphoscoliosis was documented in six patients. Cerebellar signs were found in five patients, pyramidal signs in seven and extra-pyramidal in four patients. Non-ictal urinary incontinence occurred late in the evolution. The majority (18/22) of adult patients had severe intellectual disability (as classified in McIntosh *et al*., 2010) at last follow-up (Table 1).

Recurrent respiratory infections were documented in six patients. Dysphagia emerged as a late feature in five patients, documented in or after the fourth decade of life, leading eventually to percutaneous endoscopic gastrostomy. One adult patient died during the follow-up period from repeat aspiration pneumonia. No post-mortem brain tissue was available for review from this case.

Anti-epileptic drugs and non-pharmacological treatments are listed for each case in Table 2, as well as changes to anti-epileptic drugs after the diagnosis of Dravet syndrome was made, and their impact on seizure control, cognitive function and quality of life. Seven patients had already had drug changes instituted following diagnosis, but in only three had sufficient follow-up elapsed to evaluate the effect of the changes. There was improvement in seizure control even after years of drug resistance in three cases, with significant additional improvement in cognition and quality of life in adulthood in two. In the four of the seven patients who had had drug changes with a shorter period of follow-up, some early indication of benefit for some seizure types at least was apparent in three (the one patient with no or minor change in seizure frequency had stopped carbamazepine, but not yet started any new anti-epileptic drug).

At last follow-up, most patients were on anti-epileptic drug polytherapy. No patient was seizure-free, but in several cases secondarily generalized seizures were controlled with medication.

Causes of death in our adult series (Table 3) included three cases of bronchopneumonia, and one case of sudden unexplained death in epilepsy. In the paediatric Dravet syndrome group, three died from sudden unexplained death in epilepsy and one had global ischaemic brain injury; it is unclear for the latter case whether there was a seizure followed by cardiorespiratory arrest. No adult case in our series died of convulsive status epilepticus.

Neuroimaging findings

MRI with or without light sedation was successful in all but four of the adult cases with Dravet syndrome. Most frequently, brain imaging was normal, or showed non-specific findings, including cerebral and cerebellar atrophy, or cerebellar atrophy alone (Fig. 2A). One adult case with *SCN1A* mutation had unilateral hippocampal sclerosis on MRI performed at 22 years of age (Fig. 2B). Evidence of the anterior thalamotomy performed at the age of 16 years was seen for Case 6 (Fig. 2C and D).

Electroencephalography findings

Serial EEG data were available for 21 adult patients. At least 1 seizure was recorded with video-EEG for 10 patients; seizure types recorded included tonic, focal motor, dyscognitive and secondarily generalized. Focal EEG features (Fig. 3A–D) were recorded in 17 of our adult cases. Ictal EEG onset was maximal in the fronto-central regions in four cases (Fig. 3C).

Interictal EEG in all adult cases showed slow background activity. For 10 adults, childhood EEG data were available: four had one previous EEG in early childhood with generalized epileptiform discharges. No generalized epileptiform discharges were seen in the EEGs in adulthood; focal features were seen (focal or multifocal interictal epileptiform discharges; focal ictal discharges; Supplementary Table 3).

Non-convulsive status epilepticus was documented on video-EEG in two patients, for whom subtle seizures with predominant impairment of consciousness had previously been confused with behavioural problems.

Table 2 Anti-epileptic drug history

Case ID	Anti-epileptic drug changes after diagnosis of Dravet syndrome	Improvement with anti-epileptic drug changes after diagnosis (seizure control/cognition)	All known previous anti-epileptic drug history	Other treatment	Improvement[a] (seizure types)	Documented worsening[a] (seizure types)
PM1/EP039	N/A	N/A	CBZ, CLB, GBP, LTG, PB, PHT, VPA		PHT (GTC), VPA	PHT (My)
PM2/EP213	N/A	N/A	CBZ, CLB, PB, PHT, PRM, VPA			CBZ, PHT
PM3/EP099	N/A	N/A	ACZ, CBZ, CLB, PB, PHT, PRM, VGB, VPA			
4	No new anti-epileptic drug started	N/A	CBZ,CLB, CNZ, LTG, PHT, PRM, SLT, VGB, VPA		PB, VPA	
5	Stopped CBZ; reintroduced VPA; started STP + VPA; decreased LTG	Seizure control improved cognition	CBZ, GBP, LEV, LTG, OXC, PHT, STP, TGB, TPM, VGB, VPA		PHT (GTC), STP + VPA	CBZ, OXC ('drop attacks')
6	Started LEV; reduced CBZ	Seizure control improved cognition improved	CBZ, GBP, PB, PHT, PRM, SLT, VGB, VPA	Stereotactic anterior thalamotomy, mephenytoin, phenacemide, benuride	LEV (GTC), PRM,VPA	
7	Stopped CBZ	Seizure control unchanged cognition N/A Short follow-up	CBZ, CNZ, DZP, LEV, PB, PHT, PRM, VPA	VNS	CNZ	
8	No changes made	N/A	PRM, TPM, VPA		PRM, VPA	
9	No new anti-epileptic drug started	N/A	CBZ, CLB, LEV, LTG, NTZ, OXC, PB, TPM, VGB, VPA	KD	CLB, KD, VPA	
10	Increased ZNS: suggested STP, not yet started	N/A	CBZ, CLB, ESX, LEV, LTG, PB, PGB, PHT, TPM, VGB, VPA, ZNS		VPA, ZNS	LTG ('drop attacks'), PGB
11	No new anti-epileptic drug started	N/A	ACZ, CBZ, ESX, GBP, LEV, LTG, NTZ, PB, PHT, PRM, VGB, VPA	ACTH, corticosteroids, VNS, KD, GOS exclusion diet	TPM	LTG
12	No new anti-epileptic drug started	N/A	ACZ, CBZ, CNZ, LEV, LTG, PB, PHT, PRM, SLT, VGB, VPA		SLT, VPA	
13	No new anti-epileptic drug started	N/A	CLB, LEV, LTG, OXC, VGB, VPA	Prednisolone	VPA, LEV (stopped GTC)	
14	N/A	N/A	CBZ, CNZ, DZP, ESX, LTG, NTZ, PHT, VGB	KD, ethotoin		
15	No new anti-epileptic drug started	N/A	CBZ, CLB, CNZ, ESX, LTG, NTZ, PB, VPA		CLB, ESX (dyscognitive), LEV, VPA	
16	N/A	N/A	CBZ, CLB, DZP, LEV, LTG, NTZ, OXC, PB, PGB, PHT, VPA		CBZ, VPA	OXC (My)
17		N/A		pyridoxine, biotin	VPA (GTCS)	

(continued)

Table 2. Continued

Case ID	Anti-epileptic drug changes after diagnosis of Dravet syndrome	Improvement with anti-epileptic drug changes after diagnosis (seizure control/cognition)	All known previous anti-epileptic drug history	Other treatment	Improvement^{a} (seizure types)	Documented worsening^{a} (seizure types)
	No new anti-epileptic drug started		CLB, CNZ, DZP, ESX, LEV, LTG, TPM, VPA, PIR			
18	Started VPA	Seizure control unchanged Short follow-up	CBZ, CLB, GBP, LEV, LTG, TPM, VPA		VPA	
19	No new anti-epileptic drug started. Stopped LCM; suggested STP, not yet started	N/A	CBZ, CLB, LCM, LEV, LTG, VPA, TPM, ZNS			LCM^{a}
20	No new anti-epileptic drug started	N/A	CBZ, CLB, CNZ, ESX, LEV, LTG, NTZ, PB, PHT, PIR, TPM, VGB, VPA	KD		
21	Started STP (+ CLB), later stopped, tapered RUF; restarted VPA.	Seizure control unchanged Short follow-up	CBZ, CLB, CNZ, GBP, LEV, LTG, PB, PHT, RUF, STP, TGB, TPM, VGB, VPA	pyridoxine	TPM, VPA, STP	RUF^{a}, VGB^{a}, CBZ^{a}, LTG^{a}
22	Stopped PGB; started ZNS	Seizure control improved cognition improved	ACZ, CBZ, CLB, CNZ, DZP, GBP, LEV, LTG, NTZ, PGB, PIR, VGB, VPA, ZNS		CBZ, (GTC), CLB, LEV, PIR (My), VPA, ZNS	CBZ (My), GBP (My), LTG^{a}, PGB^{a}
PM23	N/A	N/A	VPA		VPA (My)	
PM24	N/A	N/A	CLB, CNZ, LEV, LTG, STP, TPM, VPA	pyridoxine	STP	LTG
PM25	N/A	N/A	CBZ, CNZ, DZP, LTG, PB, PHT, STP, TPM, VGB, VPA	Steroids, VNS, KD	STP, VNS	–
PM26	N/A	N/A	CBZ, CNZ, GBP, LTG, TPM, VPA	None	LTG	GBP
PM27	N/A	N/A	LTG, VPA	None		
28/ *SCN1A* + surgical	N/A	N/A	No data available	Ant TLx		

a Data on which specific seizure types improved or worsened are not always available for every antiepileptic drug.

Abs = absences; ACTH = adrenocorticotrophic hormone; ACZ = acetazolamide; Ant TLx = anterior temporal lobectomy with amygdalo-hippocampectomy; CBZ = carbamazepine; CLB = clobazam; CNZ = clonazepam; DZP = diazepam; ESX = ethosuximide; GBP = gabapentin; GOS = Great Ormond Street; GTC = generalized tonic–clonic; KD = ketogenic diet; LCM = lacosamide; LEV = levetiracetam; LTG = lamotrigine; My = myoclonic; N/A = not available; NTZ = nitrazepam; OXC = oxcarbazepine; PB = phenobarbital; PGB = pregabalin; PHT = phenytoin; PIR = piracetam; PRM = primidone; RUF = rufinamide; SLT = sulthiame; STP = stiripentol; TGB = tiagabine; TPM = topiramate; VGB = vigabatrin; VNS = vagal nerve stimulator; VPA = sodium valproate; ZNS = zonisamide.

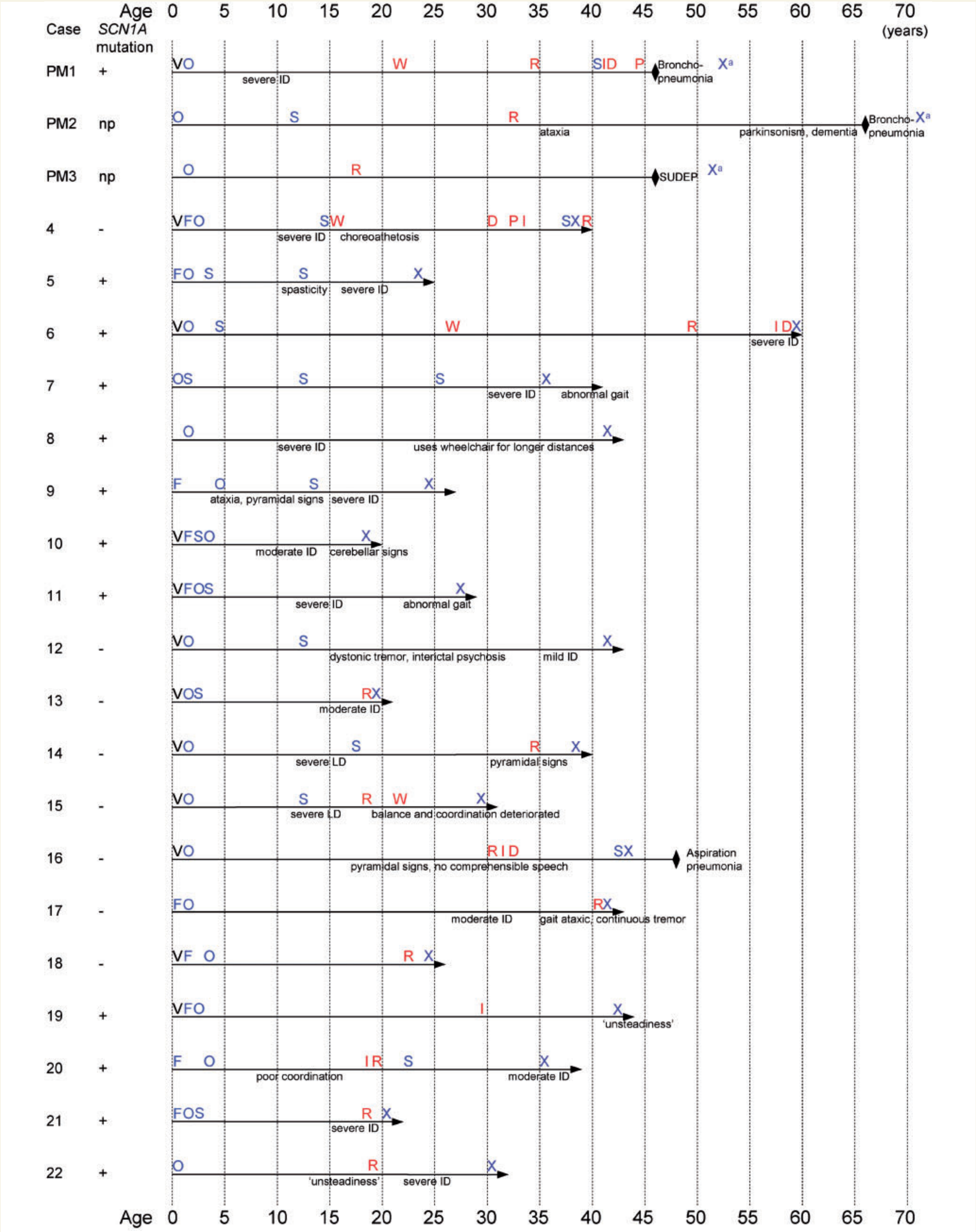

Figure 1 Timelines in Dravet syndrome—milestones in disease evolution. D = dysphagia; F = febrile seizure; I = incontinence; ID = intellectual disability; np = not possible; O = onset of afebrile seizures; P = percutaneous endoscopic gastrostomy (PEG); R = residential care; S = status epilepticus; SUDEP = sudden unexplained death in epilepsy; V = vaccination; X = diagnosis; W = wheelchair-dependent; a = diagnosis made after death; black diamond = death; horizontal arrow = living patient; + = SCN1A change found; − = no SCN1A change found.

Table 3 Summary of neuropathological findings: macroscopic findings, and results of histological staining with haematoxylin and eosin, Luxol fast blue and cresyl violet

Case ID	Macroscopic findings (brain weight post-fixation)	Cortex: frontal (F1/F2, medial, orbital), parietal, temporal and occipital	Medial and subcortical structures: hippocampus, amygdala, thalamus, basal ganglia	Cerebellum: vermis and cerebellar hemispheres	Brainstem: midbrain, pons, medulla, and cranial nerve nuclei; cervical spinal cord	Cause of death (age at death, in years)
PM1/EP039	Cerebellar atrophy, with preferential involvement of the anterior lobe and vermis (1331 g)	Normal	Normal	Loss of Purkinje cells	Myelin loss in dorsal columns of spinal cord	Bronchopneumonia and recurrent NCSE (46 yrs)
PM2/EP213	Mild cerebellar atrophy; discolouration and loss of periventricular white matter; old frontobasal contusion (1100 g)	Focal periventricular white matter and myelin loss	Normal	Mild Purkinje cells loss	Myelin loss in dorsal columns of spinal cord	Bronchopneumonia (66 yrs)
PM3/EP099	Cerebellar atrophy (1380 g)	Frontopolar, dorsal frontal and occipital cortex, with 'micro-columnar' architecture	Normal	Loss of Purkinje cells	Normal	Sudden unexplained death in epilepsy (46 yrs)
PM23	Normal. Some leptomeningeal congestion (1273 g)	Normal	Mild bilateral endfolium hippocampal gliosis. No mossy fibre sprouting.	Mild patchy gliosis but no discernable Purkinje cell loss.	Normal brainstem. Cord not available	Sudden unexplained death in epilepsy (2 yrs)
PM24	Normal (1062 g)	Frontal and occipital cortex: normal	Hippocampus (one side): no sclerosis, cornu ammonis-1 hyperconvoluted.	Purkinje cells preserved. Mild vacuolation of white matter noted.	Normal	Sudden unexplained death in epilepsy during a 46°C day in Australia (10 yrs)
PM25	Swollen brain with herniation (1300 g[a])	Frontal and temporal: widespread ischaemic neurons. No MCD or evidence of chronic atrophy	Not all subfields available for histology. Cornu ammonis-1 shows acute neuronal changes but no evidence of chronic sclerosis	Acute injury of Purkinje cells superimposed on mild chronic loss	No malformation. Ischaemic neurons noted in medulla	Sudden unexplained death in epilepsy (11 yrs)
PM26	Swollen brain (1245 g[a])	Frontal and temporal. No MCD and no atrophy	No sclerosis (mild endfolium gliosis)	Autolytic changes but no evidence of chronic atrophy	No histology	Global ischaemic brain injury (11 yrs)
PM27	Leptomeningeal congestion and uncal grooving but no tonsillar herniation (1266 g)	Frontal cortex: normal architecture but pan cortical necrosis and reactive changes consistent with cerebral infarction of 10 days	Hippocampus (one side): no evidence of chronic hippocampal sclerosis but acute anoxic changes to end-folium neurons	Autolytic changes but no evidence of atrophy/Purkinje cell loss	Normal	Convulsive status epilepticus (5 yrs)
28/*SCN1A*+ surgical[b]	Not applicable	Normal temporal neocortex	Pyramidal cell loss in left hippocampus	Not applicable	Not applicable	Not applicable
Control 1/EP296	Modest dilatation of lateral ventricles, left hippocampal formation significantly smaller than right (1156 g)	Normal	Pyramidal cell loss in the left hippocampus	Loss of Purkinje cells	Normal	Sudden unexplained death in epilepsy (49 yrs)
Control 2/EP038	Not available	Cell loss in upper cortical layers of parietal and temporal cortices	Pyramidal cell loss in both hippocampi	Loss of Purkinje cells	Normal	Pulmonary oedema (74 yrs)
Control 3	Normal (1185 g)	Normal	Normal	Normal	Normal	Cardiac arrest (36 yrs)
Control 4	–	Normal	Normal	Normal	Normal	Not available (58 yrs)
Control 5	Normal (1540 g)	Normal	Normal	Loss of some Purkinje cells	Normal	Not available (57 yrs)

[a] For these cases, pre-fixation brain weight is presented, no post-fixation brain weight available.

[b] For case 28/*SCN1A*$^+$ surgical, only the resected hippocampus and temporal neocortex were available for study.

MCD = malformation of cortical development; NCSE = non-convulsive status epilepticus; yrs = years.

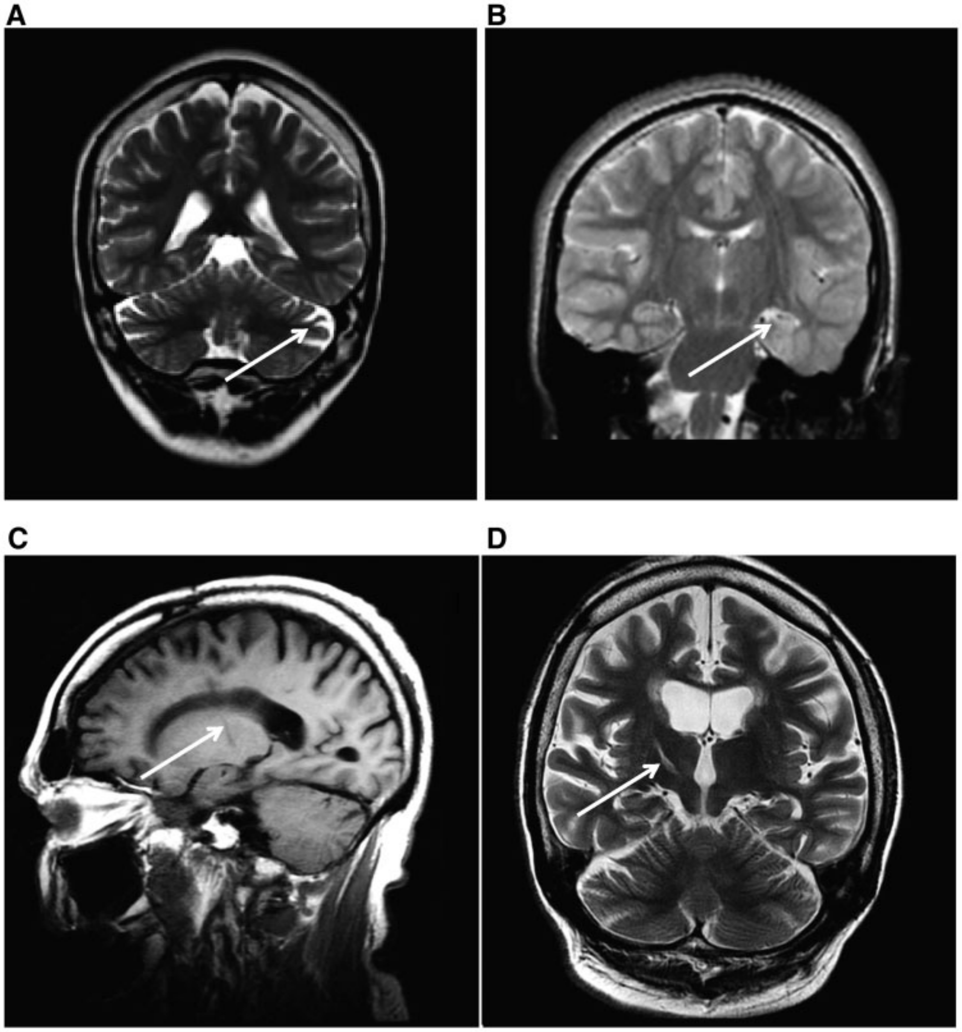

Figure 2 Brain MRI findings in adults with Dravet syndrome and *SCN1A* mutation. Cerebellar atrophy (**A**, sagittal T_1, Case 6) was a feature in some cases. Case 21 was the only adult case with Dravet syndrome in our series with hippocampal sclerosis (left in this case) evident on MRI (**B**, coronal T_2). Case 6 had a stereotactic thalamotomy at the age of 16 years (**C**, sagittal T_1 and **D**, coronal T_2). Arrows show the location of the main abnormalities in each image.

Genetic findings

Twenty adult patients had genetic analysis: *SCN1A* mutations were found in 12 adult cases (Table 4; Figure 11). The mutations were all different, and all but one patient had novel mutations. One patient (Case 21) was found to have three *SCN1A* mutations, which to the best of our knowledge has not been previously described in the literature. We have not screened other genes for mutations in our patients. For the four adults where both parents have been tested, the mutations were *de novo*. We were unable to extract DNA of adequate quality from formalin-fixed paraffin-embedded brain tissue for two adult cases (PM2/EP213 and PM3/EP099). Of the four paediatric post-mortem cases with Dravet syndrome, two had *SCN1A* mutation, one had a whole gene deletion and one was not found to have a mutation but has not yet been checked for deletions. The two other paediatric cases, one surgical case with intractable childhood epilepsy with generalized tonic–clonic seizures, and one post-mortem case in the genetic epilepsy with febrile seizures plus spectrum, both had *SCN1A* mutations previously documented (Table 4).

Genotype–phenotype associations are summarized in Table 5. In the paediatric Dravet post-mortem subgroup, we did not observe missense mutations (Table 5); in the adult Dravet deceased subgroup for whom genetic analysis was possible, 50% had an *SCN1A* missense mutation. Both children with genetic epilepsy with febrile seizures plus phenotype had missense mutations. For the 17 adult patients living with Dravet syndrome, eight had missense mutations. Additional information is provided in the supplementary material.

Neuropathology

The macroscopic findings and results from histological and immunohistochemical studies are summarized in Tables 3 and 6, respectively.

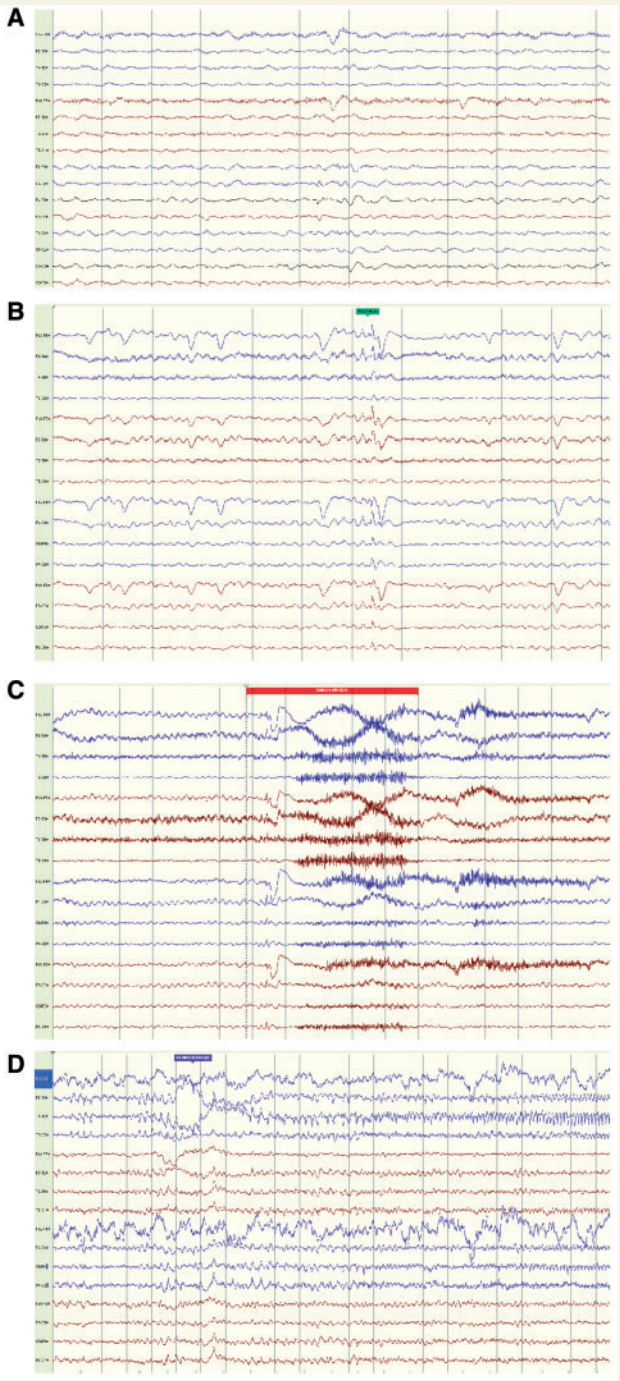

Figure 3 EEG findings. For Case 6, routine EEG showing background of bilateral diffuse slow activity at 3–5 Hz, and very rare low amplitude sharp waves/spikes, more apparent in frontal regions, right > left (**A**, bipolar montage). For Case 5, video–EEG telemetry at the age of 26 years, showed bihemispheric cortical dysfunction and bifrontal interictal epileptiform discharges (**B**, bipolar longitudinal montage). Several complex motor seizures were recorded, some with non-lateralized frontocentral EEG onset (**C**, combined longitudinal and transverse bipolar montage). Electrographic seizures were also recorded with right posterior temporal pattern (**D**, bipolar longitudinal montage).

Routine histological stains

The frontal cortex of two adult post-mortem cases with Dravet syndrome (PM1/EP039 and PM2/EP213) showed an ordered and preserved hexalaminar architecture with no neuronal cell loss, similar to the frontal cortex of post-mortem controls with no known neurological disease (Fig. 4A and B). The cortex in the frontopolar, dorsal frontal and occipital regions of one adult post-mortem case with Dravet syndrome (PM3/EP099) showed a 'micro-columnar' architecture, with exaggeration of the vertical alignment of cortical neurons (Fig. 4C), but these changes did not amount to focal cortical dysplasia type I (Blümcke *et al*., 2011). The cytoarchitecture of the parietal, temporal and occipital cortices of all adult post-mortem cases with Dravet syndrome and controls appeared normal, apart from cell loss observed in the upper cortical layers of the parietal and temporal cortex of the hippocampal sclerosis post-mortem control case (Control 2/EP296). The temporal cortex of the *SCN1A*$^+$ surgical case was well preserved, retaining hexalaminar architecture with no neuronal cell loss noted.

The hippocampi of all adult post-mortem cases with Dravet syndrome showed preservation of neurons in all cornu ammonis subfields, similar to post-mortem controls with no known neurological disease (Fig. 5A and B), and distinct from hippocampal sclerosis post-mortem controls (Fig. 5C) and the *SCN1A*$^+$ surgical case (Fig. 5D). Neuronal preservation in Dravet syndrome hippocampi was confirmed by stereological quantification of cresyl violet-stained pyramidal cells in cornu ammonis-1 and cornu ammonis-4 (Fig. 5E). The dentate gyrus of all adult post-mortem cases with Dravet syndrome also appeared normal, with a distinct, densely packed granule cell layer, as in post-mortem controls with no known neurological disease (Fig. 5A and B). In contrast, the granule cell layer of the hippocampal sclerosis post-mortem controls and the *SCN1A*$^+$ surgical case showed dispersion of granule cells into cornu ammonis-4 and dentate molecular layer (Fig. 5C and D).

We investigated the interneuronal population within the hippocampi of all adult post-mortem cases with Dravet syndrome using immunohistochemistry for calbindin, calretinin, parvalbumin and neuropeptide Y. The appearance and localization of the calbindin-, calretinin-, parvalbumin- and neuropeptide Y-immunopositive interneurons in adult post-mortem cases with Dravet syndrome were similar to that observed in the post-mortem controls with no known neurological disease (Fig. 10). While case numbers are obviously small, 2D counts of calbindin-, calretinin-, parvalbumin- and neuropeptide Y-immunopositive cells in cornu ammonis-1 and cornu ammonis-4 showed no clear difference between adult post-mortem cases with Dravet syndrome and post-mortem controls (Fig. 5F), in keeping with evidence of neuronal preservation in the Dravet syndrome hippocampi on the basis of total cell counts. Other subcortical structures (amygdala, thalamus, basal ganglia), of all adult post-mortem cases with Dravet syndrome were intact.

Routine histological stains and calbindin- and parvalbumin immunohistochemistry confirmed cerebellar atrophy with Purkinje cell loss and gliosis in all adult post-mortem cases with Dravet syndrome (Fig. 6 and Table 3). Cerebellar atrophy (without

Table 4 ***SCN1A* structural variation identified in this study**

Case ID	Nucleotide changes	Exon/intron	Mutation type	Inheritance	Amino acid change	Protein domain	Variation in the same position on the *SCN1A* variant database (http://www.molgen.ua.ac.be/SCN1AMutations)
PM1/EP039	c.677C > A	Exon 5	Missense	Not determined (parents unavailable)	p.Thr226Lys	DI-S4	c.677C > T, p.Thr226Met, *de novo* (Harkin *et al*., 2007)
5	c.4913T > C	Exon 26	Missense	*De novo* (parents and one sister analysed)	p.Ile1638Thr	DIV-S4	None in that position; one c.4911_4914delGATC,p.I1638Vf-sX11 (Depienne *et al*., 2009*b*)
6	c.992delT	Exon 7	Truncating	Not determined (no parent analysed)	p.Leu331X	DI-S5-S6	Two: c.992dupT,p.Leu331fs, *de novo*; 992[T]993ins,L331fsX339 (Mancardi *et al*., 2006)
7	c.264 + 3delAGTG	Intron 1	Splice donor, deletion	Not determined (no parent analysed)	p.?	–	One c.264 + 5G > A, de novo (Mancardi *et al*., 2006)
8	c.5639G > A	Exon 26	Missense	Not determined (one parent analysed, mother negative)	p.Gly1880Glu	COOH terminal	None found in this position
9	c.3797A > C	Exon 19	Missense	*De novo*	p.Glu1266Ala	DIII-S2	None found in this position
10	c.603-2A > G	Intron 4	Splice site	*De novo*	p.?	–	None found in this position
11	c.4384T > C	Exon 23	Missense	*De novo*	p.Tyr1462His	DIII-S6	one c.4385A > G,p.Tyr1462Cys (Zucca *et al*., 2008)
19	c.2792G > A[a]	Exon 15	Missense	Not determined	p.Arg931His	DII-S5-S6	Löfgren and DeJonghe, personal communication, 2010
20	c.4568T > C	Exon 24	Missense	Not determined (no parent analysed)	p.Ile1523Thr	DIII-DIV	None found in this position
21	c.80G > C; c.3749C > T; c.3706-2A > G[b]	Intron 18	Missense; missense; one splice acceptor mutation	Not determined (no parent analysed)	p.Arg27Thr; p.Thr1250Met; aberrant splicing (p.?)	N-terminal; DIII- S2; -	None found in this position; none found in this position; c.3706-2A > G, inheritance not determined (Singh *et al*., 2009; Löfgren and DeJonghe, personal communication, 2010)
22	c.2717_2727delinsAC	Exon 15	In-frame deletion mutation	Not determined (no parent analysed)	p.Val906_Met909delinsAsp	DII-S5	None found in this position
PM23	N/A	Whole *SCN1A* gene	Whole *SCN1A* gene deletion	*De novo*	N/A	N/A	(Marini *et al*. 2009; Depienne *et al*., 2009*b*)
PM24	c.5536_5539delAAAC	Exon 26	Truncation	*De novo*	p.Lys1846fsX1856	COOH terminal	(Case previously reported in Wallace *et al*., 2003). Claes *et al*., 2001; Kearney *et al*., 2006; Mancardi *et al*., 2006; Harkin *et al*., 2007; Zucca *et al*., 2008; Depienne *et al*., 2009*b*; Löfgren and DeJonghe, personal communication, 2010)
PM25	IVS22-14T > G	Intron 22	Splice site	*De novo*	p.?	DIIIS5-S6	(Case previously reported in Wallace *et al*., 2003)
PM27	c.4970G > A	Exon 26	Missense	*De novo*	p.Arg1657His	DIV-S4	(Case previously reported in Harkin *et al*., 2007; Deng *et al*., 2007)
28/*SCN1A*$^{+}$ surgical	c.652T > C	Exon 5	Missense	Inherited (mother and sister have the same mutation)	p.Phe218Leu	DI-S4	(Case previously reported in Livingston *et al*., 2009)

SCN1A variant database (http://www.molgen.ua.ac.be/SCN1AMutations) (Claes *et al*., 2009).
Intronic changes nomenclature: ex. c.xx + 1G > C refers to the +1 intron position following coding base xx, with + or − sign denoting the intronic 5′-beginning or 3′-ending, respectively. p.? denotes an unknown effect on the protein, an effect is expected but difficult to predict.
All mutations found are novel, except: a c.2792G > A, previously reported by Löfgren A, DeJonghe P, personal communication, 2010.
b c.3706-2A > G (Singh *et al*., 2009).
del = deletion; dup = duplication; ins = insertion; N/A = not applicable or not available.

Table 5 Genotype-phenotype analysis: *SCN1A* mutation type, and distribution of *SCN1A* missense mutations

Case ID	Type of *SCN1A* mutation	Distribution of *SCN1A* missense mutations
Children with Dravet syndrome, death between 2 and 11 years ($n = 4$, PM23–PM26)	Truncating—1 Whole-gene deletion—1 Splice site—1 No mutation, no result yet for deletion—1[a]	No missense mutation found
Children with genetic epilepsy with febrile seizures plus, one alive, 12 years, one death at 5 years ($n = 2$, 28 and PM27)	Missense—2	S4—2
Adults with Dravet syndrome, death between 46 and 66 years ($n = 4$, PM1–PM3 and 16)	Missense—1 No mutation, no deletion—1 No genetic analysis possible—2[b]	S4—1
Adults with Dravet syndrome, alive, 20–60 years ($n = 18$, Patients 4–15 and 16–22)	Missense—8[c] Truncating deletion—1 Splice site —3[c] Insertion/deletion—1 No mutation or deletion found—7	S4—2 S5–S6—1 S6—1 Others—4[c] – S2—2[c] – DIII–DIV—1 – C-terminal—1

a For one child with Dravet, who died, the result was not available regarding the presence of deletion, after a negative mutation analysis.
b For two adults with Dravet, who died, it was not possible to perform genetic analysis on the post-mortem material.
c Patient 21 had three *SCN1A* mutations found, two missense and one splice acceptor.
D = (SCN1A protein) domain; genetic epilepsy with febrile seizures plus = genetic epilepsy with febrile seizures plus; S = (SCN1A protein) segment.

reported ataxia in life) was also noted in both hippocampal sclerosis post-mortem controls (Control 1/EP296, Control 2/EP038), and in one post-mortem control with no known neurological disease (Control 5).

The paediatric post-mortem case, PM23, showed mild bilateral end folium gliosis only. For the other paediatric post-mortem cases, only preterminal event-associated changes were found at neuropathological examination of the brain with no other significant abnormality (Table 3).

Serial sections through the brainstem, including midbrain, pons and medulla, of all adult post-mortem cases with Dravet syndrome, showed no significant pathology. In two adult post-mortem cases with Dravet syndrome, PM1/EP039 and PM2/EP213 (Table 3), loss of myelin in the dorsal columns of the medulla and cervical spinal cord was apparent (Fig. 7A). Both cases had dysphagia and ataxia. Further immunohistochemical investigation using CD68 and neurofilament antibodies showed focal macrophage infiltration (Fig. 7B) and axonal swelling (Fig. 7C and D) in the pathological areas of both cases with Dravet syndrome.

Immunohistochemistry

The frontal cortex (F1/F2), hippocampus and cerebellum of all adult post-mortem cases with Dravet syndrome and controls, and the temporal neocortex and hippocampus of the *SCN1A*$^+$ surgical case, were examined using immunohistochemistry for a range of neuronal, interneuronal, inflammatory, vascular and neurodegenerative markers. In particular, the frontal cortex was examined as ictal electroclinical patterns suggested frontal onset in several adult cases with Dravet syndrome (Fig. 3C and Table 1).

Neocortex—neuronal and interneuronal markers

Neuronal nuclei-immunopositive neurons were organized in a well-defined, hexalaminar structure in the frontal cortex of all adult post-mortem cases with Dravet syndrome or post-mortem controls. No focal neuronal loss in the frontal cortex was evident in any cases with Dravet syndrome and post-mortem controls with no known neurological disease. Round, small to medium-sized calretinin-, calbindin- and parvalbumin-immunopositive cells were predominantly found in cortical layers II–IV of all adult post-mortem cases with Dravet syndrome with similar distribution and morphology to post-mortem controls (Fig. 8A–C; CR, CB and PV). Neuropeptide Y-immunopositive cells and fibres were observed throughout the frontal cortex of all adult post-mortem cases with Dravet syndrome and post-mortem controls (Fig. 8A–C, NPY). In particular, one adult post-mortem Dravet syndrome case, PM2/EP213, and both hippocampal sclerosis post-mortem controls showed higher numbers of neuropeptide Y-immunopositive cells and fibres in the frontal cortex compared with post-mortem controls with no known neurological disease. $Na_v1.1$-immunopositive pyramidal cells were detectable throughout the frontal cortex of all adult post-mortem cases with Dravet syndrome (Fig. 9A), hippocampal sclerosis post-mortem controls and post-mortem controls with no neurological disease. A population of small, intensely labelled $Na_v1.1$ cells was noted in the lower cortical layers and in the white matter of all adult post-mortem cases with Dravet syndrome and post-mortem controls (Fig. 9B). The number of these intensely labelled $Na_v1.1$-immunopositive cells in the grey and white matter of the frontal cortex was not obviously different between cases with Dravet syndrome, hippocampal sclerosis post-mortem controls and post-mortem controls with no known neurological disease (Fig. 9C). To confirm the nature of intensely

Table 6 Summary of neuropathological findings: immunohistochemistry

Case ID	Brain region	Neuronal nuclei	$Na_v1.1$	Calretinin	Calbindin	Parvalbumin	Neuropeptide Y	GFAP	HLA-DR	Cx43	von Willebrand factor	Dynorphin
PM1/EP039	Brainstem: midbrain, pons, medulla, cervical spinal cord	ND	ND	+	+	+	ND	ND	ND	ND	ND	ND
PM2/EP213		ND	ND	+	+	+	ND	ND	ND	ND	ND	ND
PM3/EP099		ND	ND	+	+	+	ND	ND	ND	ND	ND	ND
28/*SCN1A*$^+$ surgical[a]		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Control 1/EP296		ND	ND	+	+	+	ND	ND	ND	ND	ND	ND
Control 2/EP038		ND	ND	+	+	+	ND	ND	ND	ND	ND	ND
PM1/EP039	Cerebellum: one of the cerebellar hemispheres	*loss	+	+	*loss	+	+	+ +	+ +	+	+	ND
PM2/EP213		+	+	+	+	+	+	+	+	+	+	ND
PM3/EP099		*loss	+	+	*loss	+	+	+ +	+ +	+	+	ND
28/*SCN1A*$^+$ surgical[a]		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ND
Control 1/EP296		*loss	+	+	*loss	+	+ +	+	+	+	+	ND
Control 2/EP038		*loss	+	+	*loss	+	+	+ +	+ +	+	+	ND
PM1/EP039	Hippocampus	+	+	+	+	+	+	+	+	+ +	+	+
PM2/EP213		+	+	+	+	+	+	+ +	+	+ +	+	+
PM3/EP099		+	+	+	+	+	+	+	+	+ +	+	+
28/*SCN1A*$^+$ surgical[a]		*loss	+	+	+	*loss	+ +	+ +	+ +	+ +	+	+ +
Control 1/EP296		*loss	+	+	+	+	+ +	+ +	+ +	+ +	+	+
Control 2/EP038		*loss	+	+	+	+	+ +	+ +	+ +	+ +	+	+ +
PM1/EP039	Frontal cortex: F1,F2	+	+	+	+	+	+	+	+	+	+	ND
PM2/EP213		+	+	+	+	+	+	+	+	+	+	ND
PM3/EP099		+	+	+	+	+	+	+	+	+	+	ND
28/*SCN1A*+ surgical[a]		+	+	+	+	+	+ +	+	+	+	+	ND
Control 1/EP296		+	+	+	+	+	+ +	+	+	+	+	ND
Control 2/EP038		*loss	+	+	+	+	+ +	+ +	+	+	+	ND

Immunolabelling appeared increased (+ +), similar (+) or decreased (−) compared with controls.
*loss = cell loss; N/A = tissue unavailable for examination; ND = immunohistochemistry not performed.
a For *SCN1A*+ surgical case, only the resected hippocampus and temporal neocortex were available for study.

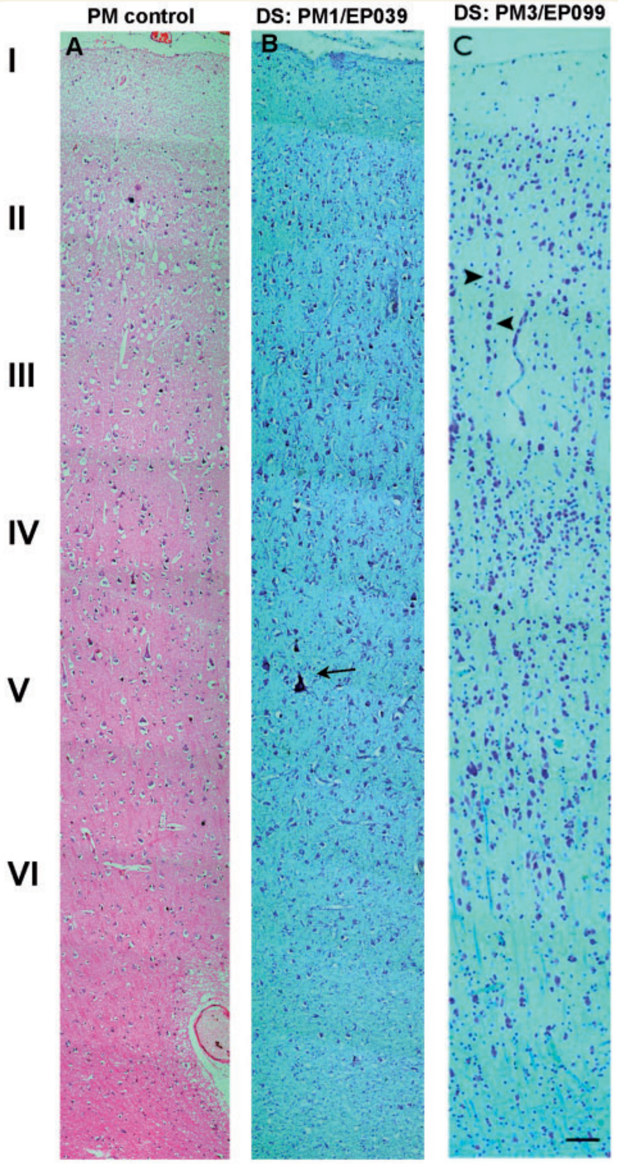

Figure 4 Frontal cortex—histological staining. (**A**) Haematoxylin and eosin shows the normal frontal cortex from a post-mortem control with no known neurological disease. (**B**) Cresyl violet shows the motor cortex of the adult Dravet syndrome (DS) case, PM1/EP039, with good preservation of the cortical laminae and Betz cells (arrow). (**C**) Cresyl violet and Luxol fast blue show the frontal cortex from the adult post-mortem Dravet syndrome case, PM3/EP099, with a focal 'micro-columnar' appearance (arrowheads to columnar alignment). Haematoxylin and eosin-stained section is 7 μm thick while Luxol fast blue and cresyl violet-stained sections are 14 μm. Scale bar = 100 μm.

labelled Na$_v$1.1-immunopositive cells in the frontal cortex specifically, double-labelling immunofluorescent studies were undertaken with three different markers of interneurons (glutamic acid decarboxylase, neuropeptide Y and parvalbumin), confirming that these cells are likely to be inhibitory cells (Fig. 9D–F).

Neocortex—connexin and inflammatory markers

Multipolar, connexin 43 (Cx43-) and glial fibrillary acidic protein (GFAP-) immunopositive cells were observed throughout the frontal cortex of all adult post-mortem cases with Dravet syndrome and controls, particularly in the subpial regions and cortical layer 1 (Fig. 8A–C). The distribution and morphology of HLA-DR-immunopositive microglial cells in the frontal cortex of all adult post-mortem cases with Dravet syndrome were similar to post-mortem controls with no known neurological disease (Fig. 8A and B, HLA-DR). In comparison, HLA-DR-immunopositive cells in the frontal cortex of both hippocampal sclerosis post-mortem controls appeared larger, more intensely labelled (Fig. 8C, HLA-DR) and formed clusters.

Neocortex—vascular cells and neurodegeneration processes

von Willebrand factor-immunopositive blood vessels were observed in all adult post-mortem cases with Dravet syndrome, hippocampal sclerosis post-mortem controls and controls with no known neurological disease, and the distribution and appearance of immunopositive vessels were not markedly different between cases (Fig. 8A–C, vWF). Immunohistochemistry using neurodegenerative process markers was not performed on frontal cortical tissue.

The same panel of markers as used to study adult post-mortem cases with Dravet syndrome showed that the temporal cortex of the *SCN1A*$^+$ case retained a normal, hexalaminar cytoarchitecture with no focal neuronal cell loss. There were a higher number of neuropeptide Y-immunopositive cells and processes throughout the temporal cortex of the *SCN1A*$^+$ case compared with post-mortem controls with no known neurological disease, and similar to immunolabelling evident in hippocampal sclerosis post-mortem controls. The immunoreactivity of Cx43, GFAP, HLA-DR and von Willebrand factor was not markedly different between temporal cortex of the *SCN1A*$^+$ case and post-mortem controls with no known neurological disease.

Hippocampus—neuronal and interneuronal markers

The expected loss of large calretinin-, calbindin-, parvalbumin- and neuropeptide Y-immunopositive cells in the cornu ammonis-4 region, and loss of calbindin-immunopositive cells in the granule layer, was detected in the hippocampus of the *SCN1A*$^+$ surgical case and the hippocampal sclerosis post-mortem controls, but not in any adult post-mortem cases with Dravet syndrome, or the controls with no known neurological disease (Fig. 10A–C), again demonstrating the preservation of neurons in this adult post-mortem Dravet syndrome series. Immunoreactivity for dynorphin (DYN), a marker of mossy fibre sprouting (Vezzani *et al*., 1999; Thom *et al*., 2009), was not observed in the molecular layer of the adult post-mortem cases with Dravet syndrome and controls with no known neurological disease, but was present in the *SCN1A*$^+$ surgical case and both hippocampal sclerosis post-mortem cases (Fig. 10A–C; DYN). Na$_v$1.1-immunopositive labelling was observed in the hippocampal pyramidal and granule cells of the cases with Dravet syndrome (Fig. 9B), and controls, and the *SCN1A*$^+$ surgical case. A population of small, intensely labelled Na$_v$1.1 cells, similar to those noted in the frontal cortex,

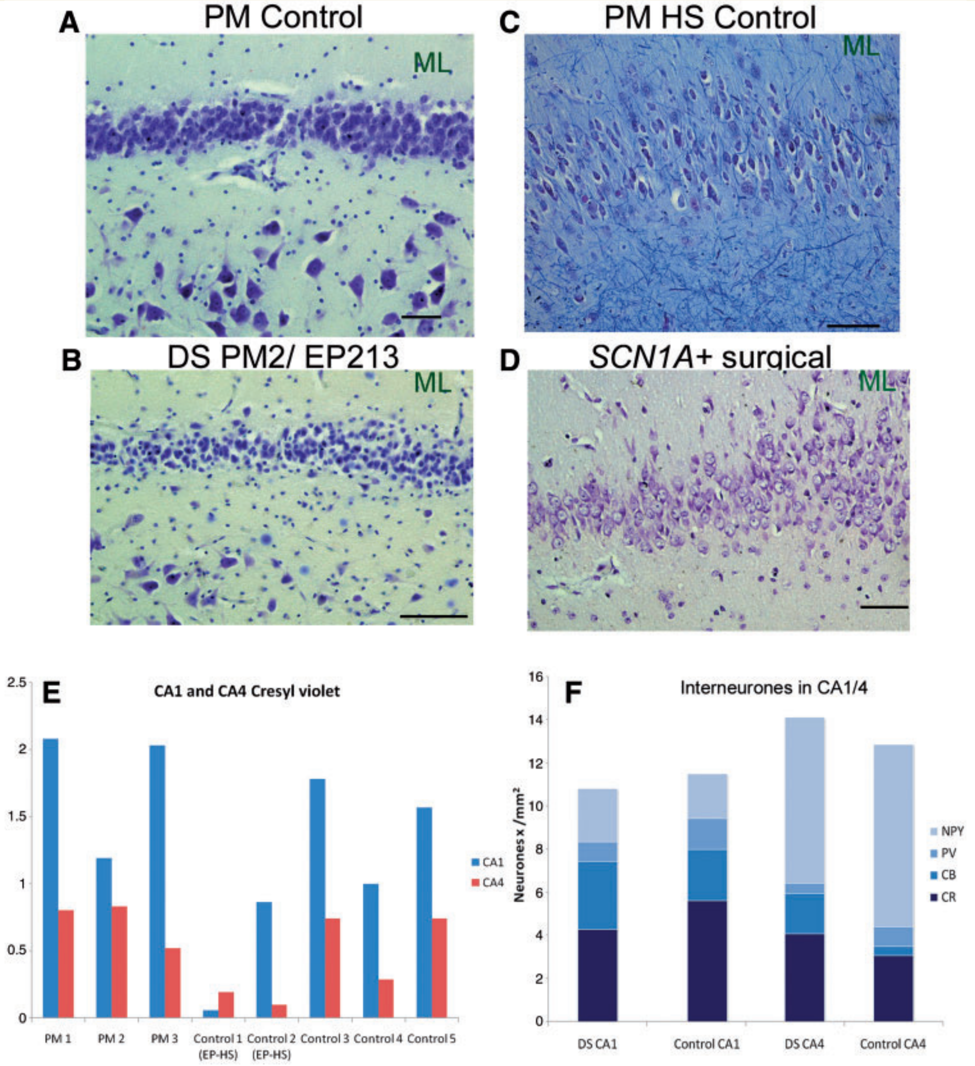

Figure 5 Hippocampus, histological staining and interneuronal cell counts. Cresyl violet shows the normal hippocampus from a post-mortem (PM) control with no known neurological disease (**A**), and the adult post-mortem case with Dravet syndrome (DS), PM2/EP213 (**B**). In contrast, pyramidal cell loss in the left cornu ammonis-4 and granule cell dispersion are seen in the hippocampal sclerosis post-mortem (PM HS) control (**C**), and the *SCN1A*$^+$ surgical case (**D**). (**E**) Stereological quantification of cresyl violet-stained neurons shows lower numbers of pyramidal cells in cornu ammonis-1 and -4 for hippocampal sclerosis post-mortem controls (Control 1 and 2 EP-HS) compared with adult post-mortem cases with Dravet syndrome (PM1–3) and post-mortem controls with no known neurological disease (Controls 3–5). (**F**) Areal 2D counts of calbindin (CB), calretinin (CR), parvalbumin (PV) and neuropeptide Y (NPY)-immunopositive cells in the cornu ammonis-1 and -4 show that the average number of hippocampal interneurons in the adult post-mortem Dravet syndrome ($n = 3$) and controls with no known neurological disease ($n = 2$) is not markedly different. Refer to Fig. 10 (hippocampus immunolabelling) for images of calbindin, calretinin, parvalbumin and neuropeptide Y immunoreactivities in the hippocampus of cases with Dravet syndrome and controls. Scale bar = 50 µm. CA = cornu ammonis; ML = molecular layer.

was also found scattered throughout the hippocampal formation (dentate gyrus, cornu ammonis subfields and subiculum) of the *SCN1A*$^+$ surgical case, adult post-mortem cases with Dravet syndrome and post-mortem controls. Quantification of these cells revealed that the number of small, intensely labelled Na$_v$1.1 cells was lower in the hippocampus compared with the frontal cortex, within each case, but not markedly different between adult post-mortem cases with Dravet syndrome, hippocampal sclerosis post-mortem controls and controls with no known neurological disease (Fig. 9C).

Hippocampus—connexin and inflammatory markers

Cx43-immunoreactivity was not detected in the hippocampus of any post-mortem controls with no known neurological disease (Fig. 10A, Cx43). In contrast, Cx43-immunopositive cells were observed in the cornu ammonis regions, particularly cornu ammonis-4 and granule cell layer border, of adult post-mortem cases with Dravet syndrome, hippocampal sclerosis post-mortem controls (Fig. 10B and C, Cx43) and the *SCN1A*$^+$ surgical case. The immunoreactivity of GFAP in the hippocampus of adult post-mortem cases with Dravet syndrome and controls with no

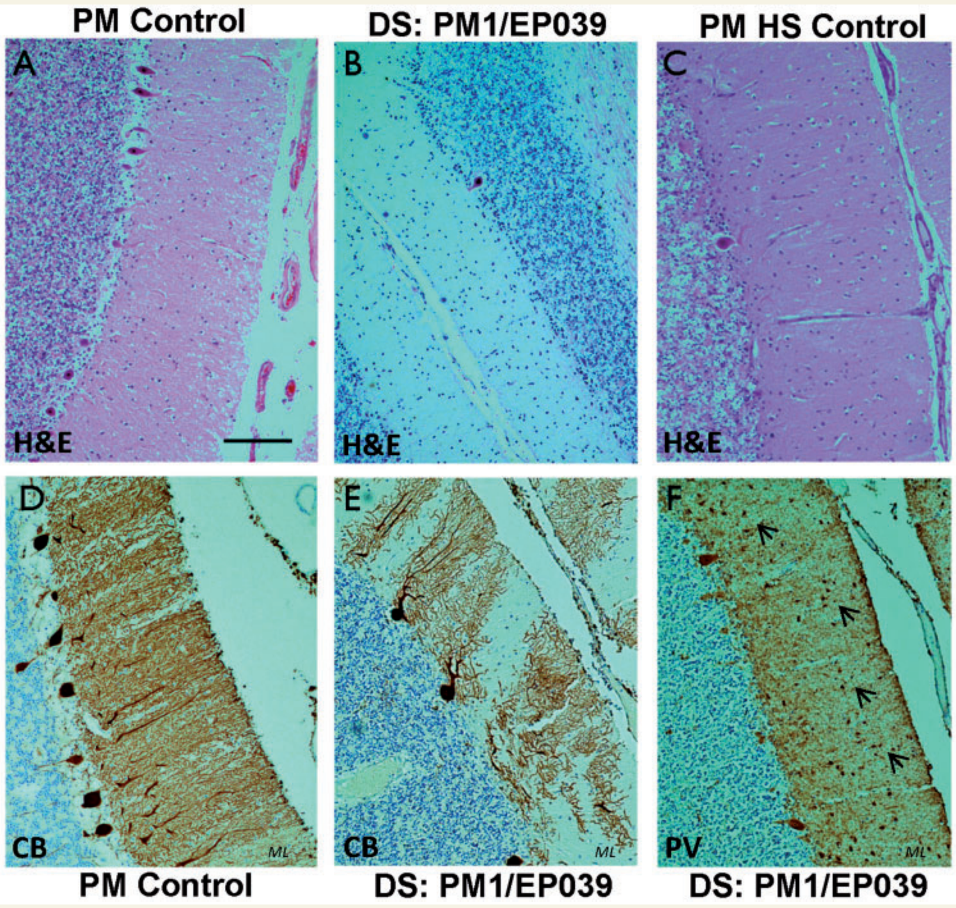

Figure 6 Cerebellum, histological staining and immunolabelling. (**A**) Haematoxylin and eosin (H&E) shows a normal cerebellum from a post-mortem control with no known neurological disease. The same stain shows Purkinje cell loss in the cerebellum of the adult post-mortem Dravet syndrome case, PM1/EP039 (**B**), and a hippocampal sclerosis post-mortem control (**C**). The loss of Purkinje cells and their processes, which normally extend into the molecular layer as observed in **D**, is evident in calbindin- and parvalbumin-immunolabelled cerebellar sections from the case with Dravet syndrome, PM1/EP039 (**E** and **F**). Small, parvalbumin-immunopositive cells are still observed in the molecular layer of the Dravet syndrome cerebellum (**F**, arrows). Scale bar = 100 μm. ML = molecular layer.

neurological disease was not markedly different (Fig. 10A and B, GFAP); scattered GFAP-immunopositive cells were observed throughout the hippocampal formation. GFAP-immunopositive cells and a dense matrix of GFAP-immunopositive fibres were detected in the hippocampus of hippocampal sclerosis post-mortem controls (Fig. 10C, GFAP) and *SCN1A*$^+$ surgical case. The distribution and morphology of HLA-DR immunopositive microglial cells in the hippocampus of Dravet syndrome and post-mortem controls with no known neurological disease were similar, while larger and more clustering of HLA-DR immunopositive cells were observed in hippocampal sclerosis post-mortem controls (Fig. 10A–C, HLA-DR) and the *SCN1A*$^+$ surgical case.

Hippocampus—vascular cells and neurodegeneration processes

The immunoreactivity of von Willebrand factor was not markedly different between adult post-mortem cases with Dravet syndrome, controls (Fig. 10A–C, vWF), and the *SCN1A*$^+$ surgical case. There were infrequent AT8-immunopositive neurons in the hippocampi of all adult post-mortem cases with Dravet syndrome (all Braak Stage 2 or less). There were no neuronal inclusions or plaques noted with any of the markers in any adult post-mortem cases with Dravet syndrome. Immunohistochemical labelling with markers for dementia and neurodegeneration (Supplementary Table 1) of post-mortem Dravet syndrome hippocampi was not markedly different from post-mortem controls.

Cerebellum—neuronal and interneuronal markers

Focal reduction in calbindin- and parvalbumin-immunopositive Purkinje cells and dendrites was confirmed in the cerebellum of two adult cases with Dravet syndrome (PM1/EP039 and PM3/EP099; Fig. 6A and B), both hippocampal sclerosis post-mortem controls and one control with no known neurological disease (Control 5). Loss of calbindin- and parvalbumin-immunopositive Purkinje cells was only occasionally observed in the cerebellum of the Dravet syndrome case, PM2/EP213. Calretinin-immunopositive cells in the Purkinje and granule cell layers were preserved in adult post-mortem cases with Dravet syndrome as in controls. Similarly, small, parvalbumin-immunopositive cells were retained in the Purkinje and molecular layers of all adult

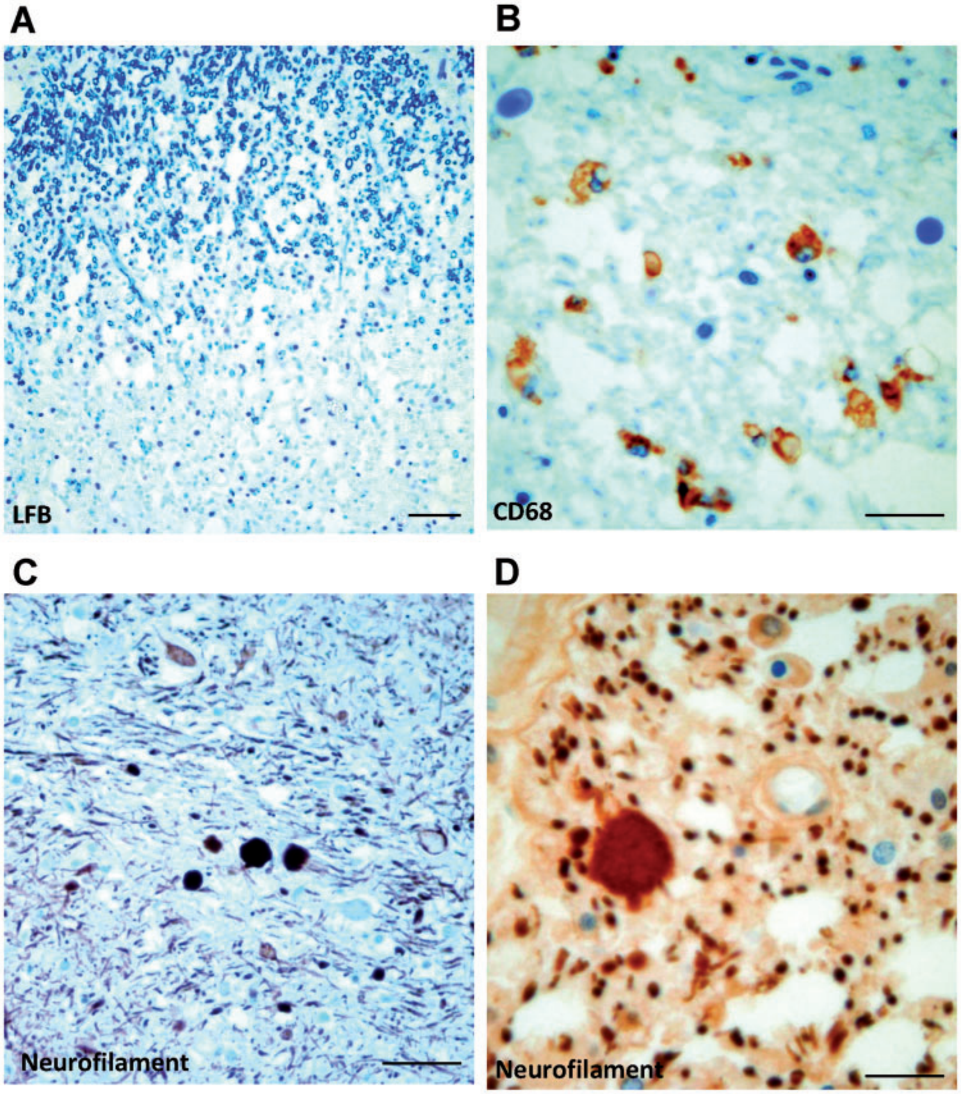

Figure 7 Brainstem and spinal cord—histological staining and immunolabelling. (**A**) Luxol fast blue (LFB) section shows a cord area with myelin pallor in the dorsal column of the adult post-mortem Dravet syndrome case, PM1/EP039, where no myelin debris is observed. (**B**) The same area immunolabelled with the CD68 antibody shows infiltration of CD68-immunopositive macrophages into the myelin pallor. Neurofilament immunohistochemistry shows axonal swelling in the spinal cord of the Dravet syndrome case, PM1/EP039, which is presented here, in low (**C**) and high (**D**) magnification. The other Dravet case, PM2/EP213, shows similar findings as PM1, while the spinal cord was normal for Dravet syndrome case PM3/EP099 (data not shown). Scale bar = 50 μm (**A** and **C**); 25 μm (**B** and **D**).

post-mortem cases with Dravet syndrome, even in regions of cerebellar atrophy (Fig. 6F). Neuropeptide Y-immunoreactivity was not observed in the cerebellum of any adult post-mortem cases with Dravet syndrome or controls, except in one hippocampal sclerosis post-mortem control (Control 1/EP296), which showed a small number of neuropeptide Y-immunopositive cells and processes in the granule cell layer. While $Na_v1.1$-immunopositive Purkinje cells were observed in the cerebellum of all cases (Fig. 9A), the small, intensely labelled $Na_v1.1$-immunopositive neurons, which were observed in the frontal cortex and hippocampus, were not found in the cerebellum of any adult post-mortem cases with Dravet syndrome or post-mortem controls.

For the paediatric post-mortem cases with Dravet syndrome, the cerebellum was preserved; in some cases, there were 'acute' changes related to pre-terminal cerebral events, and in one (PM25), there was mild Purkinje cell loss (Table 3).

Cerebellum—connexin and inflammatory markers

A few Cx43-immunopositive cells were observed only in the cerebellar molecular layer of post-mortem cases with Dravet syndrome and controls. GFAP- and HLA-DR-immunopositive cells were mainly observed in the granule cell layer and white matter of the cerebellum of post-mortem cases with Dravet syndrome and controls. The distribution and appearance of these cells were not markedly different between post-mortem cases with Dravet syndrome and controls. In post-mortem cases with Dravet syndrome, PM1/EP039 and PM3/EP099, hippocampal sclerosis controls and one control with no known neurological disease (Control 5) that

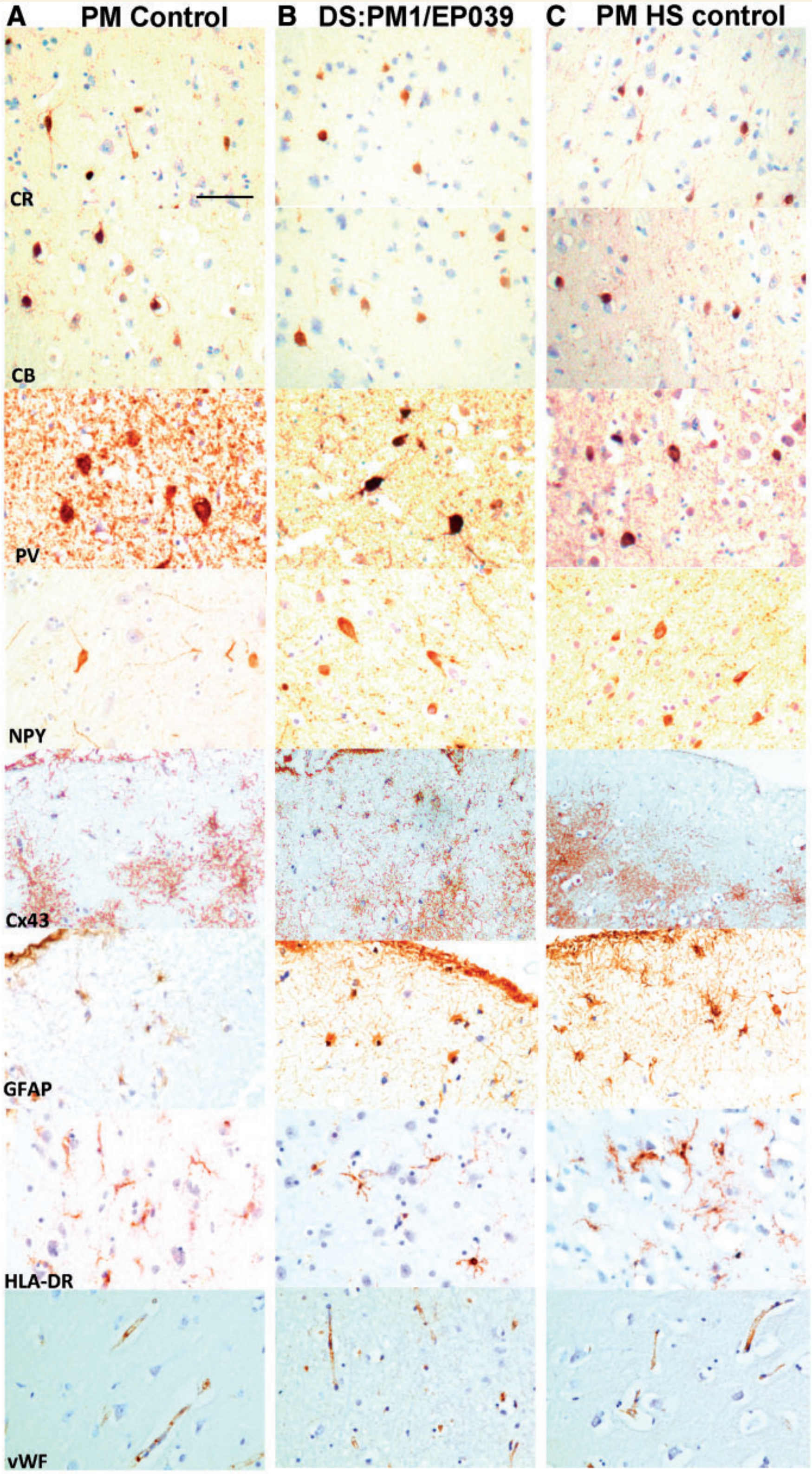

Figure 8 Frontal cortex—immunolabelling. The frontal cortex of a post-mortem control with no known neurological disease (**A**), the adult post-mortem Dravet syndrome case, PM1/EP039 (**B**) and a hippocampal sclerosis post-mortem control (**C**) is immunolabelled with a panel of interneuronal, inflammatory and vascular markers. The distribution and morphology of immunolabelled cells in the frontal cortex are not markedly different between post-mortem cases with Dravet syndrome and controls. Apart from images of Cx43 and GFAP immunolabelling, which are taken from subpial or layer I, images for all other markers are taken in frontal cortical layers II and III of the post-mortem cases with Dravet syndrome and control. Scale bar = 50 µm. CB = calbindin; CR = calretinin; NPY = neuropeptide Y; PV = parvalbumin; vWF = von Willebrand factor.

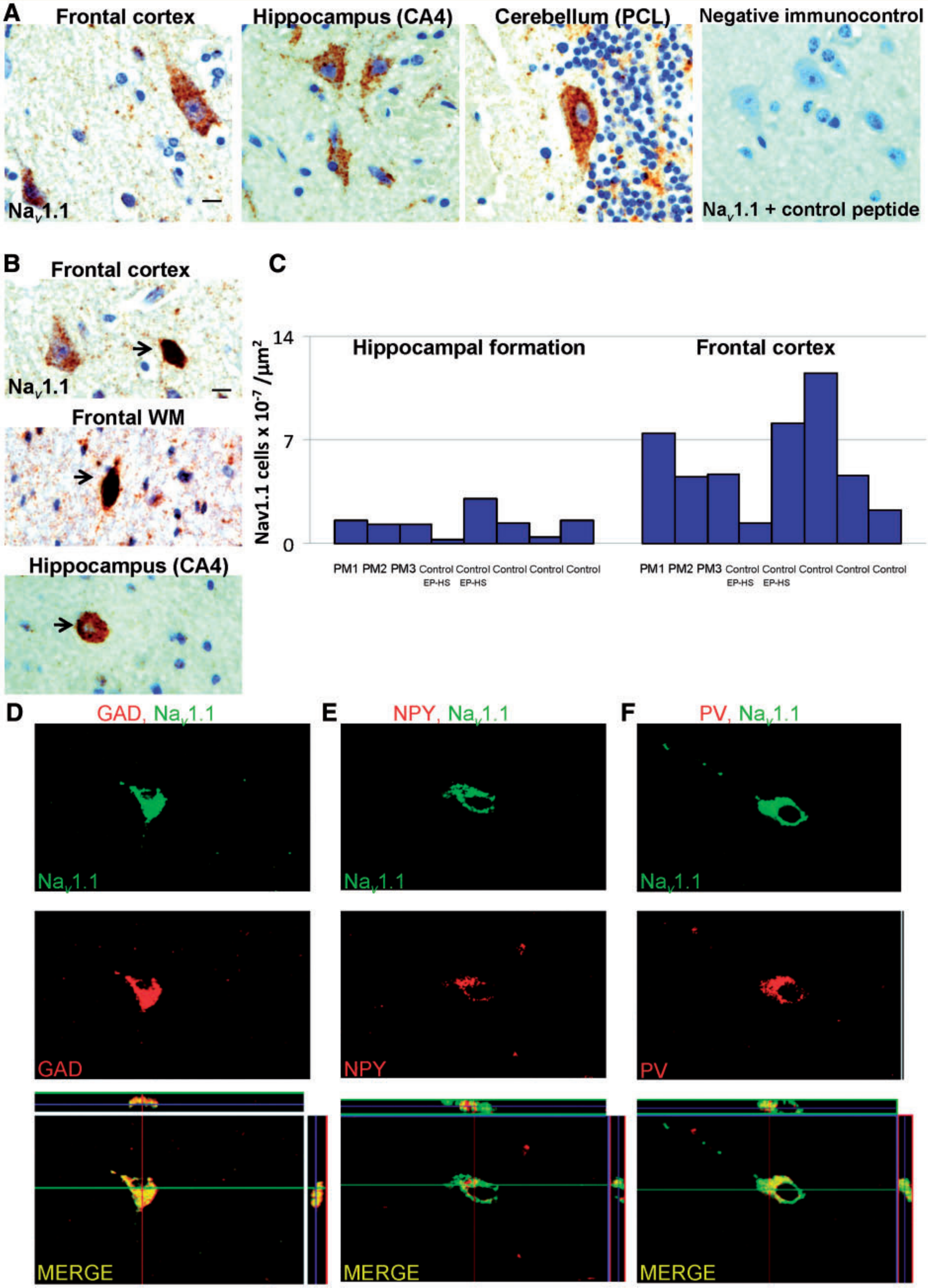

Figure 9 Na$_v$1.1-immunoreactivity in frontal cortex, hippocampus and cerebellum. (**A**) Na$_v$1.1-immunolabelling is observed in the cytoplasm of pyramidal cells in frontal cortex, hippocampal pyramidal cells, and cerebellar Purkinje cells, in all adult post-mortem cases with Dravet syndrome. No Na$_v$1.1-immunopositive cells are observed in sections that are incubated with primary Na$_v$1.1 antibody solution pre-mixed with control peptide. (**B**) A number of small, intensely labelled Na$_v$1.1-immunopositive cells (arrows) are also found in the

(continued)

had marked Purkinje cell loss, GFAP and HLA-DR-immunopositive cells were also observed in the cerebellar molecular layer.

Cerebellum—vascular cells and neurodegeneration processes

The immunoreactivity of von Willebrand factor was similar between all cases. Immunohistochemistry using neurodegenerative markers was not performed in the cerebellum.

Brainstem

The immunoreactivities of the calcium-binding proteins, calretinin, calbindin and parvalbumin, were not markedly different between adult post-mortem cases with Dravet syndrome and controls. Immunohistochemistry for GFAP, ubiquitin, α-synuclein and non-phosphorylated neurofilaments did not reveal any pathological inclusions in the brainstem nuclei, neuronal loss or gliosis, in the adult post-mortem cases with Dravet syndrome.

Discussion

Dravet syndrome is an important and paradigmatic epilepsy syndrome, being among the first genetic epilepsy syndromes for which the molecular basis has been unravelled, enabling functional studies and animal models to reveal fundamental insights into the underlying pathophysiology (Catterall *et al*., 2008). Dravet syndrome is thought to be underestimated in prevalence and under-diagnosed in adults (Scheffer *et al*., 2009). There are many gaps in the understanding of the clinical evolution of Dravet syndrome in later ages, particularly after the fourth decade of life, as for many years Dravet syndrome has been considered to be of the remit of the child neurologist. As children with Dravet syndrome were prospectively followed, it became clear that some did reach adulthood (Dravet *et al*., 2005). More recently, adult patient series have been characterized (Jansen *et al*., 2006; Akiyama *et al*., 2010), but most adults were under 35 years of age at last follow-up. Surviving adults, over 35 years of age, with Dravet syndrome may have missed out on a diagnosis as the syndrome was only described 30 years ago (Dravet *et al*., 1978) and the diagnosis is often not considered in adult clinics.

We show that diagnosis even late(r) in life, in patients previously labelled as having drug-resistant epilepsy with intellectual disability of unknown cause, can carry important implications for affected patients; rational treatment changes can be instituted, with possible benefit as we and others have shown, even after years of drug resistance. In addition, recognition of the changes in language, cognition, swallowing and gait, and determining whether specific patterns exist, may help to improve diagnostic and prognostic information and may reinforce a mandate for treatment changes.

We identified 22 adult patients with Dravet syndrome who had not been diagnosed in childhood. Two-thirds were over 39 years of age at last follow-up, a greater proportion than for other studies to date (Table 7). Two adult cases with Dravet syndrome reached their sixties; survival to the seventh decade had not been previously reported. Ours is not a systematic evaluation of the prevalence of Dravet syndrome or *SCN1A* mutation in adults with severe epilepsy, but an observational study of a highly selected patient group from a tertiary centre. Together with the very detailed clinical records available and the neuropathology evaluation, this provided a unique opportunity for a study on the long-term follow-up and outcome of adult patients with Dravet syndrome.

Genotype–phenotype analyses are often complex (Kanai *et al.*, 2009; Scheffer, 2011; Zuberi *et al*., 2011), and more so in our selected series. Caution is required in interpretation. Considering the type of *SCN1A* mutations in the two extremes of age at death, a pattern may seem to emerge: in the four children with Dravet who died early, there were no missense mutations (Table 5); of the patients who died after the age of 45 years, out of the 2 in whom genetic analysis was possible, 1 had 1 *SCN1A* missense mutation, and the other was found not to have an *SCN1A* mutation or deletion. No truncating mutations were found in this group. Compared with published data, there seem to be more missense than truncating *SCN1A* mutations in the older Dravet group. We must emphasize limitations (ascertainment bias; selection bias; small numbers; predominance of paediatric cases in published data), but one could hypothesize that missense mutations are more frequent in patients with longer survival, testable with a prospective longitudinal study.

We acknowledge important limitations in our study. Though it includes some longitudinal data, it is a cross-sectional study. The numbers of post-mortem cases are small. We were unable to obtain DNA of sufficient quality from the two older post-mortem cases without a molecular genetic result (no frozen tissue was available). Neuropathological analyses at other levels, for example the electron microscopic, were not possible, as no appropriately fixed material was available. We cannot fully disentangle the natural history of Dravet syndrome and what may relate to other aspects, such as the chronic effects of anti-epileptic drugs. We note that for Unverricht-Lundborg disease, for example, previously reported progressive neurological deterioration was later attributed to the use of phenytoin (Eldridge *et al*., 1983); and avoidance has meant life expectancy may approach normal (Kalviainen *et al*., 2008). Despite these factors, the data available do generate new insights.

Features of Dravet syndrome in adults include drug-resistant seizures with a seizure repertoire that differs from that in childhood. Atypical absences and generalized interictal epileptiform

Figure 9 Continued
frontal lower cortical layers, frontal white matter, and hippocampal cornu ammonis-4, but not in the cerebellum. (**C**) The number of small, intensely labelled $Na_v1.1$-immunopositive cells in frontal cortex and hippocampus is not markedly different between cases with Dravet syndrome, hippocampal sclerosis post-mortem controls and post-mortem controls with no known neurological disease. (**D–F**) Double-labelled immunofluorescent studies show small, intensely labelled $Na_v1.1$ cells in the frontal cortex and hippocampi of cases with Dravet syndrome co-express glutamic acid decarboxylase (**D**), neuropeptide Y (**E**) and parvalbumin (**F**). Scale bars = 10 μm (A–C). CA = cornu ammonis; GAD = glutamic acid decarboxylase; PCL = Purkinje cell layer; WM = white matter.

Table 7 Adults with Dravet syndrome in the literature

Authors	Number of cases aged 18 yrs or older (total number in study)	Age range in study (yrs)	Dravet syndrome subtypes	*SCN1A* structural variation
Rossi *et al*., 1991	Not specified (15)	9-24 (mean 15)	SMEI	Not mentioned
Dravet *et al*., 2005	Not specified (105)	2.5-33.6 (median 11.5)	SMEI and SMEB	Not mentioned
Jansen *et al*., 2006	14	18-47 (median 26.5)	SMEI and SMEB	10/14 mutations (+1 *GABRG2* mutation)
Berkovic *et al*., 2006	2	17.5, 47	1 SMEI and 1 SMEB	2/2 mutations
Depienne *et al*., 2006	4	23–40	SMEI	4/4 mutations
Fujiwara *et al*, 2006	2	19, 19	SMEI	Not mentioned
Striano *et al*., 2007*a*	Not specified (58)	0.3–25	SMEI	Not mentioned
Striano *et al*., 2007*b*	Not specified (28)	3–23 (mean 9.4)	SMEI	Not mentioned
Zucca *et al*., 2008	1	28	SMEI	1/1 deletions
Kassai *et al*., 2008	Not specified (64)	3–20	SMEI	Not mentioned
Akiyama *et al*., 2010	31	18–43 (median 22)	14 SMEI and 17 SMEB	25/31 mutations
Marini *et al*., 2009	2	26 and 30	SMEI	One duplication exon 26, one amplification exon 26
Andrade *et al*., 2009	2	19, 34	SMEI	Not mentioned
Ragona *et al*., 2010	Not specified (37)	0.5–28 (mean 16)	SMEI	37/37 mutations

SMEB = severe myoclonic epilepsy of infancy-borderland; SMEI = severe myoclonic epilepsy of infancy.

discharges seen in childhood were not documented in our adult series. In many of our adult patients, the predominant seizures are nocturnal with focal semiological features and sometimes secondary generalization; focal onset was often documented on ictal EEG. This concurs with the findings of Akiyama *et al*. (2010), whose recent series of adult Dravet syndrome showed 35/40 apparently generalized seizures had frontal origin, with or without secondary generalization in the ictal EEG. No single clinical characteristic in our series allowed the distinction between *SCN1A* mutation-positive and -negative adult cases, but our numbers are small for subgroup comparisons.

Although long life is possible, long-term functional, seizure-related, cognitive and social outcomes appear unfavourable, with cognitive and physical decline, gait disturbance and later dysphagia, incontinence and increasing dependence for all activities of daily life. We cannot say how earlier recognition and treatment might influence these outcomes.

Dysphagia has emerged as a shared dysfunction in older cases with Dravet syndrome. This is a novel observation in Dravet syndrome, and not a feature of other chronic epilepsies, except some of the progressive myoclonic epilepsies, epilepsies associated with cerebrovascular disease and Lennox-Gastaut ‘syndrome’ (Ogawa *et al*., 2001). Dysphagia may manifest with unexplained cough, or recurrent respiratory infections, which may lead to neurological deterioration, and weight loss. Notably, for homozygous null *Scn1a*$^{-/-}$ knock-out mice, manual feeding extends survival (Yu *et al*., 2006). Awareness and early diagnosis of dysphagia may prevent complications, which include worsening of seizure control, poor nutrition and fluid intake, poor quality of life and life-threatening aspiration pneumonia. The neuropathological basis of the dysphagia is unclear, though visible changes in the brainstem were noted in two patients with Dravet syndrome and dysphagia.

The neuropathology of human Dravet syndrome has not been previously well characterized. To our knowledge, this is the first systematic neuropathological study in Dravet syndrome. We included three adult and four paediatric post-mortem cases with Dravet syndrome, and two other *SCN1A* mutation-carrying paediatric cases with other syndromes. Several findings are of interest.

Patients with Dravet syndrome often have autism-like behavioural features, and autism spectrum disorder has been associated with seizures in the first year of life (Saemundsen *et al*., 2008). In a recent report, the neuropathological examination of one Dravet paediatric case, who died of sudden unexplained death in epilepsy, showed multifocal micronodular dysplasia of the left temporal cortex and bilateral endfolium gliosis (Le Gal *et al*., 2010). We did not find other subtle malformation as reported in abstract form by Hayashi *et al*. (2004). In one of our adult cases, there was an exaggerated columnar architecture, or radial alignment of neurons involving frontal and occipital regions. This patient had a history of autistic spectrum disorder. Although studies in autism have also described abnormalities of cortical minicolumns (Casanova *et al*., 2010), neuropathological data in Dravet syndrome remain very limited; we simply make this observation, but cannot draw general conclusions from our single case.

One adult case with Dravet syndrome had unilateral hippocampal sclerosis on a MRI brain scan performed in his 20's; his previous MRIs were not available for review. The *SCN1A*$^{+}$ surgical case had unilateral hippocampal sclerosis. Previous studies have shown that in a small proportion of patients with Dravet syndrome, hippocampal sclerosis is observed (Striano *et al*., 2007*a*), and this may not be present in the early childhood scans (Siegler *et al*., 2005). Prospective MRI studies in Dravet syndrome are required. It is of note that even on quantitative analysis, there was no neuropathological evidence of neuronal loss in the post-mortem adult cases with Dravet syndrome, showing that Dravet syndrome *per se*, and *SCN1A* mutation (one post-mortem adult case with Dravet syndrome), are not sufficient to cause hippocampal neuronal loss despite decades of drug-resistant seizures and recurrent episodes of status epilepticus. Rarely, significant clinical and imaging

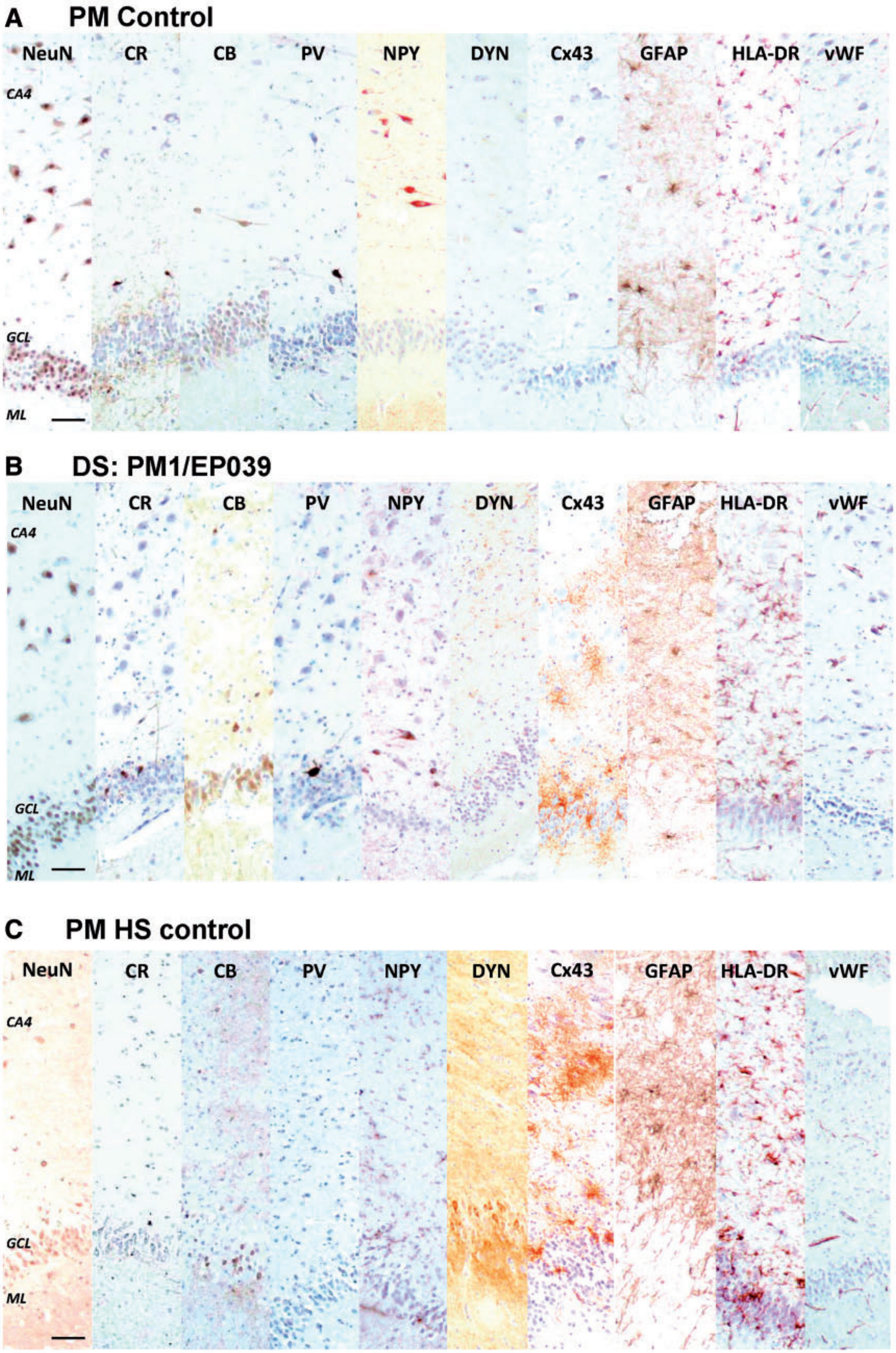

Figure 10 Hippocampus—immunolabelling. The hippocampi of a control with no known neurological disease (**A**), the adult post-mortem Dravet syndrome case, PM1/EP039 (**B**), and a hippocampal sclerosis post-mortem control (**C**), are immunolabelled with a panel of interneuronal, inflammatory and vascular markers. The distribution and morphology of neuronal nuclei, calretinin, calbindin, parvalbumin, and neuropeptide Y-immunopositive cells in the hippocampus are similar between the case with Dravet syndrome and post-mortem

(continued)

changes have been reported in Dravet syndrome following status epilepticus (Sakakibara *et al*., 2009; Chipaux *et al*., 2010; Tang *et al*., 2011). There may be age-dependent vulnerability of the brain to injury induced by seizures (Haut *et al*., 2004), but it is difficult to separate out effects of seizures on the brain from the effects of the disease process *per se*, and the effects of drugs and other factors. It has been suggested that *SCN1A* mutation may protect hippocampal neurons (Auvin *et al*., 2008), but more research is needed to determine whether (and which, if any) *SCN1A* mutations (or other causes of Dravet syndrome) are actually neuroprotective, and it should be noted that Dravet syndrome is not primarily a hippocampal epilepsy.

Cerebellar atrophy was a frequent finding in cases with Dravet syndrome but did not differ, either in pattern or distribution, to that previously described in patients with chronic epilepsy without Dravet syndrome (Crooks *et al*., 2000). The exact mechanism of selective Purkinje cell loss, as well as the potential relationship to observed ataxia, requires further study. In contrast to a previous post-mortem report in a paediatric Dravet syndrome case (Renier and Renkawek, 1990), no cerebellar dysplasia was seen in any of our cases.

Vacuolar demyelinating myelopathy of the dorsal columns of the cervical cord was noted in two patients with Dravet syndrome. This is not a typical finding in patients with epilepsy, and although a toxic or metabolic cause remains possible, future studies in patients with Dravet syndrome may elucidate if this is a feature specific to Dravet syndrome. It is of interest that ataxia can be observed in Dravet syndrome. More data are required to establish whether the vacuolar myelopathy is its basis and whether such myelopathy will respond to, or be prevented by, better seizure control or modulation of $Na_v1.1$ function; we note in passing that $Na_v1.1$ channels are expressed in white matter astrocytes (Black *et al*., 1994) in close relationship with oligodendrocytes (Waxman and Black, 1984).

We found no significant alterations in the distribution and morphology of inhibitory interneuronal subsets in cortex, cerebellum, brainstem or hippocampus in adult Dravet syndrome, even with quantitative analysis. The prevalence of small, intensely-labelled $Na_v1.1$-immunopositive cells was not different in adult post-mortem cases with Dravet syndrome and post-mortem controls with no known neurological disease. This, of course, does not exclude putative functional abnormalities in any of these cell types, as reported for the mouse models (Yu *et al*., 2006; Ogiwara *et al*., 2007).

The clinical association between seizures and febrile episodes was not underpinned by any evidence of persistent excessive neuroinflammatory pathology or microglia in cases with Dravet syndrome. Cx43, GFAP and HLA-DR immunoreactivities in the frontal cortex were not different between adult post-mortem cases with Dravet syndrome and controls. In the hippocampus, higher numbers of Cx43-immunopositive cells in adult post-mortem cases with Dravet syndrome and hippocampal sclerosis post-mortem controls were observed, compared with post-mortem controls with no neurological disease, where no Cx43-immunolabelling was seen in the hippocampus. Previous studies have suggested that the upregulation of Cx43 in medial temporal lobe epilepsy with hippocampal sclerosis may facilitate seizure propagation (Fonseca *et al*., 2002; Kielian, 2008). GFAP and HLA-DR immunoreactivities were similar between adult post-mortem cases with Dravet syndrome and controls with no neurological disease, in contrast with a greater expression in hippocampal sclerosis post-mortem controls. In the cerebellum, Cx43 immunoreactivity was similar between adult post-mortem cases with Dravet syndrome and controls (low immunoreactivity). The immunoreactivity of GFAP and HLA-DR is mainly observed in the granule cell layer and white matter of adult post-mortem cases with Dravet syndrome and controls, with higher immunoreactivity in the molecular layer of cases, with loss of Purkinje cell and processes.

Overall, we have not identified any histopathological hallmark of Dravet syndrome. In fact, a striking feature was the conspicuous preservation of neurons and interneurons, including within the hippocampus, and cortex in the frontal, temporal and occipital regions, despite decades of poorly controlled seizures. Where extensive changes were seen, in the paediatric post-mortem cases, these were compatible with their agonal states; in the paediatric sudden unexplained death in epilepsy cases, there were no neuropathological abnormalities beyond changes common to chronic epilepsy. Therefore, in neither paediatric nor adult post-mortem cases, at the levels we examined the blocks available for study, were there any pathological changes to explain the observed cognitive/developmental arrest or decline.

Seizure freedom was not attained in any of our adult patients, but seizure control was significantly improved in the three cases with sufficient follow-up after specific post-diagnosis anti-epileptic drug changes, with use of appropriate drugs and withdrawal of others previously described to worsen control, such as lamotrigine, carbamazepine, vigabatrin (Guerrini *et al*., 1998;

Figure 10 Continued
control with no known neurological disease, while expected loss of these cells is detected in the hippocampal sclerosis post-mortem control. The immunoreactivity of dynorphin (DYN), a marker that demonstrates mossy fibre sprouting, which is often associated with hippocampal sclerosis, is intense in the inner to outer molecular layer of the hippocampal sclerosis post-mortem case but not in the case with Dravet syndrome or the post-mortem control with no neurological disease. The immunoreactivity of Cx43, a gap junction marker that has been reported to be upregulated in astrocytes from resected epileptic human brain tissue, is higher in the hippocampus of the case with Dravet syndrome and the hippocampal sclerosis post-mortem control compared with the post-mortem control with no neurological disease. The immunoreactivity of GFAP, HLA-DR and von Willebrand factor is not greatly different between cases with Dravet syndrome and post-mortem controls, whilst GFAP and HLA-DR differ from the hippocampal sclerosis post-mortem control. Scale bars = 50 μm. CA = cornu ammonis; CB = calbindin; CR = calretinin; GCL = granule cell layer; ML = molecular layer; NeuN = neuronal nuclei; NPY = neuropeptide Y; PV = parvalbumin.

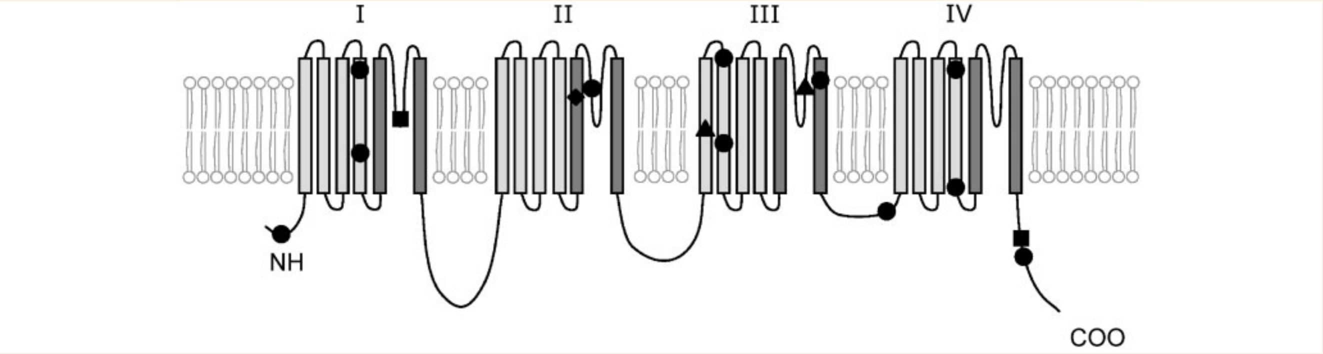

Figure 11 Schematic representation of the *SCN1A* mutations found in our study (Table 4). SCN1A protein scheme adapted from Harkin *et al*. (2007). The protein has four domains, I–IV, each consisting of six transmembrane segments, S1–S6. Circle = missense; square = truncating; triangle = splice-site mutation; diamond = in-frame deletion. Positioning of the mutations within segments is approximate.

Perucca *et al*., 1998), phenytoin and oxcarbazepine (Table 2), which may have different effects on different seizure types in Dravet syndrome. Even if the patient had had drug-resistant seizures for many years, the suppression of at least one seizure type was possible for at least several months, as also shown in a recent report (Akiyama *et al*., 2010). For our oldest living patient, at 60 years, rational drug changes proved possible once the clinical diagnosis, with confirmation from molecular genetics (which was important in this case given the lack of literature on long-term features of Dravet syndrome), gave carers confidence in such anti-epileptic drug changes. A previous anti-epileptic drug change had led to status epilepticus and strong reluctance to entertain further changes. Subsequent drug changes led to significant benefits, even after 60 years of drug-resistant seizures: convulsive seizures were controlled and the patient began speaking again for the first time for over 5 years (Video in Supplementary material).

Dravet syndrome has been considered an 'epileptic encephalopathy' in the International League Against Epilepsy classification (Engel *et al*., 2001) and a syndrome carrying higher risk of epileptic encephalopathy in the recent reorganization (Berg *et al*., 2010), but controversy exists as to whether the seizures and interictal discharges themselves are responsible for the cognitive decline (Dravet *et al*., 2005). Our data show Dravet syndrome is indeed at least partly an epileptic encephalopathy: extensive neuropathology has not shown any consistent cerebral structural abnormalities, cell loss or other neurodegeneration, and clinically, even after many decades of drug-resistant seizures, medication changes may improve seizure control, and be associated with cognitive improvement. Necessarily, the pathological components of our study are cross-sectional, not longitudinal. We must therefore await long-term follow-up of newly diagnosed infants and children with Dravet syndrome, who are appropriately treated, to determine formally whether effective control of seizures and interictal discharges prevents encephalopathy and other co-morbidities (Scheffer *et al*., 2009)—not only cognitive decline, but also the additional features that we and others have reported. However, we have shown that the neurological substrate, at least at the levels we have examined, appears largely intact and therefore potentially capable of maintaining normal function—if seizures at least can be controlled. That unexpected longevity is possible further mandates efforts at earlier diagnosis and prompt effective treatment. We also conclude that Dravet syndrome may be found in older and younger adults and is a diagnosis that needs consideration in this group, because it has management implications. Dravet syndrome is an important example of the value of study of an apparently rare epilepsy, and the value of clinical acumen in syndrome discovery and clinical diagnosis.

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Supplementary material

Supplementary material is available at *Brain* online.

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