***SCN1A*-related Dravet syndrome:**
Vaccinations and seizure precipitants in disease course and diagnosis

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SCN1A-related Dravet syndrome:
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SCN1A-gerelateerd Dravet syndroom:
vaccinaties en uitlokkende factoren in ziekteverloop en diagnose

(met een samenvatting in het Nederlands)

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Chapter 1

General Introduction and Outline of thesis

1.1 A CASE REPORT

A. is a healthy three months old baby when she receives her first vaccinations (Diphtheria, Tetanus, whole-cell Pertussis, inactivated Poliovirus and *Haemophilus influenza* type B). Four hours later she develops a generalized seizure while having a temperature of 39 °C. The seizure lasts 30 minutes and stops after rectal administration of diazepam by the general practitioner. Two months later she receives her second vaccination. She is prophylactically treated with diazepam and acetaminophen (paracetamol) and is admitted to the hospital for observation. Seven hours after vaccination she gets a focal seizure of the right arm and right side of the face. She remains conscious and has a temperature of 38.1 °C. The seizure stops when after six minutes rectal diazepam is administered. Her right arm remains paretic for a few hours. Twelve hours later she develops another focal seizure, but this time of the left arm. She is unresponsive and has a temperature of 37.5 °C. The seizure stops immediately when diazepam is administered intravenously. This time her left arm remains paretic for several hours. An electroencephalography (EEG) recorded a week later does not show any epileptiform abnormalities.

In the following months she continues to have generalized or focal seizures during episodes with infections or fever. She shows normal development and is considered to have febrile seizures. However, from age 12 months onwards her seizure frequency increases, seizures occur without fever as well and absences appear. She is then considered to have epilepsy and starts with valproate treatment. At age 15 months her EEG shows bilateral polyspike-waves during sleep and she has a mild developmental delay. At age 16 months she starts having myoclonias. Despite treatment with various anti-epileptic drugs in different combinations, including valproate, lamotrigine, carbamazepine, vigabatrine, topiramate and clobazam, her seizures remain difficult to control. She starts having behavioral problems, including autistic features, and her development lags further behind. At age 4 years IQ test scores are below 50. At age 4.5 years she is diagnosed with Dravet syndrome. Several months later this diagnosis is molecularly

confirmed by the identification of a pathogenic missense mutation in the *SCN1A* gene, which is absent in her parents' DNA (*de novo* mutation).

1.2 ALLEGED VACCINATION-ENCEPHALOPATHY

If a child has its first seizure following a childhood vaccination and subsequently develops a severe epilepsy syndrome, this may give the (false) impression that the epilepsy is caused by the vaccination while only a temporal association with the first seizure exists. First reports of cases with neurological symptoms after pertussis ('whooping cough') vaccinations, ranging from seizures to intellectual disability, were already published between 1933 and 1958.[1] After the implementation of national childhood vaccination programs from the 1950's onwards, the incidence of various infectious diseases and their accompanying morbidity and mortality in children dramatically decreased.[2-4] However, with this successful prevention of infections, the perceived side effects of vaccinations grew in importance.[5, 6] When in the 1970's and 1980's children with various neurological symptoms following diverse time intervals after pertussis vaccination were reported again,[7, 8] this led to severe undermining of public faith in the safety of vaccinations. Even though several children had pre-existent neurological problems, causal relationships were not distinguished from pure coincidences, and risks of neurological complications following pertussis infections were considered to be significantly higher.[9] These reports led to decreasing vaccination coverage for several decades, especially in the UK.[2, 3] Consequently, high incidences of pertussis infections, pertussis induced neurological disorders and infant deaths re-emerged.[2, 3] Furthermore, in several countries supposed vaccine injury was compensated,[3] and pre-existing neurological symptoms or epilepsy were considered relative or absolute contra-indications for pertussis vaccination.

Later studies did not reveal increased risks of acute encephalopathy following vaccinations,[10-12] but showed that most cases with neurological symptoms following vaccination had in fact complex febrile seizures, with complete recovery.[10, 13, 14] It lasted until 2006, before Berkovic et al.[15] showed that many patients with permanent encephalopathy following vaccination had a genetic disorder: in a case series of patients with alleged vaccination encephalopathy, the majority had Dravet syndrome caused by a pathogenic *SCN1A* mutation (OMIM 607208). The detection of these pathogenic *SCN1A* mutations proved that the epilepsy and neurological deterioration in these children were caused by a genetic defect, even though the vaccination could be considered the trigger of the first seizure and in theory might have had an influence on the course of disease. A later case series confirmed that many children with alleged vaccination encephalopathy have Dravet syndrome caused by *SCN1A* mutations.[16]

1.3 DRAVET SYNDROME, CLINICAL HISTORY

1.3.1 Epidemiology

Dravet syndrome comprises ~6% of all epilepsy cases with onset in infancy.[17] The prevalence of Dravet syndrome has been suggested to be 1 in 20,000 to 1 in 40,000 children based on studies performed before the identification of *SCN1A* mutations as its major etiology.[17, 18] Recently, the prevalence of Dravet syndrome caused by *SCN1A* mutations has been estimated to be 1 in 22,000 to 1 in 40,900 children.[19, 20]

1.3.2 History

1.3.2.1 ILAE classification and clinical criteria

Dravet syndrome has been categorized among 'Epilepsies and syndromes undetermined as to whether they are focal or generalized' in the proposal for revised classification of epilepsies and epileptic syndromes of the International League Against Epilepsy (ILAE) in 1989, among the group of 'epileptic encephalopathies' in the proposed ILAE classification of 2001, and both as 'genetic' if categorized based on underlying etiology, and as 'electroclinical syndrome with onset in infancy' if arranged on age at onset, according to the ILAE classification of 2010.[21-23]

According to the ILAE classification of 1989, the diagnostic criteria for Dravet syndrome (at that time still called Severe Myoclonic Epilepsy of Infancy, see 1.3.2.2) are:
"a family history of epilepsy or febrile convulsions, normal development before onset, seizures beginning during the first year of life in the form of generalized or unilateral febrile clonic seizures, secondary appearance of myoclonic jerks, and often partial seizures. EEGs show generalized spike-waves and polyspike-waves, early photosensitivity, and focal abnormalities. Psychomotor development is retarded from the second year of life on, and ataxia, pyramidal signs, and interictal myoclonus appear. This type of epilepsy is very resistant to all forms of treatment."[23]

With increasing knowledge and changing insights only adapted forms of the ILAE criteria are being used, with different researchers using different criteria. There is agreement that not all criteria have to be met for a clinical diagnosis of Dravet syndrome. Furthermore, "a family history of epilepsy or febrile convulsions" is no longer considered a criterion for the diagnosis, since *de novo SCN1A* mutations were identified as the major cause of Dravet syndrome. In addition, it has been advocated that sensitivity to fever should be given more emphasis when considering a diagnosis of Dravet syndrome.[24, 25] A normal neuroradiological examination or with aspecific findings only, is often added as criterion as well.[26-28]

It has been suggested that based on the (adapted) aforementioned criteria a tentative diagnosis of Dravet syndrome can be made at the end of the first year of life in most

cases, while a definite clinical diagnosis can be made at the end of the second year of life.[25]

1.3.2.2 Nomenclature

Dravet syndrome was first described in 1978 by Charlotte Dravet as 'les epilepsies myocloniques graves de l'enfant',[29] and became known in the English medical literature as Severe Myoclonic Epilepsy of Infancy (SMEI). For children lacking myoclonias or one of the other criteria for SMEI, the term borderline SMEI (SMEB) was introduced.[30, 31] The name was changed to Dravet syndrome by the ILAE in 2001,[22] because children with SMEI and SMEB have similar outcomes, not all children have a 'Myoclonic' epilepsy, and epilepsy is not limited to 'Infancy'.[32-34] However, the new name has prevailed over the original name in scientific publications only after 2007.[27] Sometimes 'mild' or 'classic' is being added to 'Dravet syndrome' to distinguish milder from more severely affected cases.

More recently, the term 'atypical' Dravet syndrome has been introduced for patients either with generalized tonic-clonic seizures but lacking myoclonic and absence seizures, or with multiple focal seizures but lacking generalized seizures and generalized epileptiform abnormalities.[35, 36] The epilepsy in the latter subgroup has previously been called Severe Infantile Multifocal Epilepsy (SIMFE).[27] In the past, Japanese children with clinical features of Dravet syndrome but with few or no myoclonias have been reported to have Intractable Childhood Epilepsy with Generalized Tonic-Clonic Seizures (ICEGTC).[37] DNA-analysis showed that ~70% of children diagnosed with this syndrome, had *SCN1A* mutations as well, and can therefore be considered as having Dravet syndrome.[38]

Children diagnosed with the in Germany described epilepsy syndrome Severe Idiopathic Generalized Epilepsy of Infancy with Generalized Tonic-Clonic Seizures (SIGEI) may also have the same clinical features as in Dravet syndrome, except for myoclonias, but with seizure onset up to five years of age.[39] In some SIGEI cases with seizure onset in the first year of life, *SCN1A* mutations have been detected as well.[40]

1.3.3 Clinical course

1.3.3.1 Seizures

In nearly all patients with Dravet syndrome first seizures occur between 2 and 12 months, with a mean age at onset of ~5-6 months.[19, 38, 41-47] Seizures in the first year may consist of febrile or afebrile, clonic or clonic-tonic, generalized or unilateral seizures, and are often prolonged.[48] Later on, multiple seizure types develop which may include myoclonias and absence, atonic, tonic or focal seizures:[48]

a. Unilateral seizures or hemi-convulsions are among the most common seizure types at onset, and may be long-lasting.[49] In Dravet syndrome they may appear on either

side in the same child, thereby forming a clue for the diagnosis. After seizure termination an ipsilateral motor deficit, i.e. Todd's paresis, may last for several hours. Unilateral seizures are reported in ~47% to 100% of Dravet syndrome children.[19, 38, 41, 42, 45, 46]

b. Generalized clonic and generalized clonic-tonic seizures (GTCS) are the most commonly reported seizure types in Dravet syndrome, i.e. in 88% to 100% of cases,[19, 38, 41, 42, 45, 46] and also among the most common seizure types at onset. However, detailed studies using video-EEG recordings demonstrate that many of these seizures are not primarily generalized, and should therefore be considered as 'falsely generalized'.[32] They are sometimes preceded by myoclonias.
c. Status epilepticus resulting from long-lasting GTCS or hemi-convulsion, occur in 65% to 100% [19, 38, 41, 42, 44-46] of children with Dravet syndrome with a median age at onset of 9 months.[19]
d. Myoclonic seizures may be isolated or grouped, and mainly affect the arms, but can also involve the axial muscles and neck.[49] Myoclonic seizures are reported in 35% to 100% [19, 38, 41-46] of patients with Dravet syndrome and occur at a median age of 14 months.[19]
e. Focal seizures with or without impaired consciousness occur in 40% to 96% [19, 38, 41, 42, 44-46] of patients with Dravet syndrome and appear at a median age of 10 months.[19]
f. Atypical absence seizures can occur with a myoclonic component or with impaired consciousness only.[49, 50] EEG recordings may show generalized, irregular spike-waves at 2-3.5 Hz. Atypical absences are reported in 17% to 76% of cases with Dravet syndrome,[19, 38, 41-46] and start at a median age of 16-21 months.[19, 50]
g. Tonic seizures are infrequent in Dravet syndrome.[49] In one study they were reported in 12% of cases,[45] but in most studies they are not reported at all.
h. Long-lasting nonconvulsive status, either classified as obtundation or complex partial status, may occur in some patients with Dravet syndrome as well.[33]

Seizures are in general resistant to pharmacotherapeutic treatment. Seizure types may come and go during the disease course.[51] Based on one study, younger children are more likely to have prolonged and focal seizures, while adolescents are more likely to have brief, generalized seizures during sleep.[52]

1.3.3.2 EEG

In most Dravet syndrome children the first interictal EEG recording is normal. The proportion of children with interictal epileptiform EEG abnormalities may increase from 17% to 50%, during the first year, and to 80% later in childhood.[19, 53] Paroxysmal abnormalities may consist of generalized spike-waves or polyspike-waves, but also of focal or multifocal spikes and polyspikes.[49] There is no relation between the localization of these

interictal paroxysms and seizure semiology. Background activity is in general normal in the first year of life, but slowing may be observed when the EEG is performed shortly after a prolonged seizure.[49] Later in the disease course, background activity becomes slow and poorly organized in ~50% of cases, but this may differ over time in the same patient.[49]

Intermittent photic stimulation (IPS) may induce epileptiform discharges, i.e. photoparoxysmal responses (PPR), in 7% to 75% of patients with Dravet syndrome.[19, 25, 35, 36, 38, 42, 44, 53-57] PPR may already be induced in the first year of life,[56, 57] but may vary between different tests moments within the same patient and depend on the type of photic stimulator used.[49, 58, 59]

1.3.3.3 Neurocognitive development

Psychomotor development is normal before seizure onset in Dravet syndrome, and slows or declines from the second year of life onwards. Development and intelligence quotients (DQ/IQ) significantly decrease with age, but are in general above 70 up to the age of three years, with a strong decrease thereafter.[45] In one study the median age at which development was noted to be abnormal was 18 months.[19] At age 5 years, ~7% to 13% of children still have developmental scores within the normal range.[19, 25, 45] It has been suggested that late onset of cognitive decline might be common in Dravet syndrome children who only have focal epileptiform EEG abnormalities and focal seizures.[35]

After age ten years intellectual disability is severe to profound in the majority of patients and mild or moderate in a smaller part.[19] Although developmental and intelligent quotients decline with age, absolute developmental scores in general continue to increase slowly.[45] However, developmental regression may be observed after episodes of status epilepticus.[60] Moreover, if the disease course is complicated by a period of acute encephalopathy, this leads to significant developmental regression and additional neurological deficits.[61-64]

Behavioral problems are reported for ~50% of Dravet syndrome children, and generally start after the second year of life.[19] These problems often include autistic features, hyperactivity and attention deficits, but not always fulfill criteria for an autistic spectrum or attention deficit disorder.[19, 45]

1.3.3.4 Motor disorders

Motor disorders are prevalent in Dravet syndrome, increase with age and are most often classified as ataxia (26 to 88% of cases).[19, 38, 41, 42, 45] Ataxic gait may already be present when a child first starts to walk, but often appears or worsens while using sodium channel blockers or after seizures.[28] Other motor abnormalities reported in Dravet syndrome are spasticity or pyramidal signs (4-7%), hypotonia (3-4%) and dyskinesia (3%).[19, 38, 45] It has recently been reported that many children with Dravet syndrome show progres-

sive gait deterioration in the second decade of life consisting of a crouch gait, which is characterized by increased hip and knee flexion and pes valgus.[65]

1.3.3.5 Mortality

Dravet syndrome is associated with significant mortality. Proportions of reported deaths vary from 3% to 21% in various cohort studies using different follow-up periods and age groups.[19, 33, 36, 57, 59, 66-69] Cumulative mortality rate by age 18 was estimated to be 7%,[69] while an annual mortality rate of ~1% has been estimated based on one small study.[70] Causes of premature deaths were most frequently status epilepticus and sudden unexplained death in epilepsy (SUDEP), and less commonly drowning or infections.[66]

1.3.3.6 Long-term outcome

In adulthood, seizure control may be better than in childhood with 8% to 20% of adult patients being seizure free for more than one year, in several cohorts.[36, 59, 71] Most adults with Dravet syndrome have a severe intellectual disability, although rarely patients with mild or no intellectual disability have been described as well.[26, 36, 59]

Motor disorders are present in 50 to 80% of adult cases and most commonly consist of crouch gait or cerebellar dysfunction including ataxic gait, dysarthria, and intention tremor.[26, 36, 59, 71] In a study of twelve adult patients, all had mild to severe flexion of the head, and eleven had mild parkinsonism responsive to levodopa in the two who were treated.[72] Since parkinsonism has also been described as an adverse effect of long-term use of valproate, it is unclear whether it is a late appearing symptom of Dravet syndrome as well.[73] In another small case series of adult patients, bradykinesia, hypomimia and perseverative behavior was described.[74] Choreoathetosis induced by phenytoin has also been described in adults.[75] Progressive cognitive or motor decline has been observed in some adult patients,[59, 67] of whom several became bedridden (~ 8% of adults in [36, 59]) Orthopedic problems, like kyphoscoliosis and flat feet, may also become more frequent than in childhood.[66]

1.3.4 Prognostic factors

Most patients with Dravet syndrome will develop moderate to profound intellectual disability. In several studies the presence of, and early onset of myoclonic, focal or absence seizures were associated with a worse developmental outcome.[19, 36, 45] Other clinical features that might be associated with a worse developmental outcome are the presence of a motor disorder, young age at onset of developmental delay, abnormal interictal EEG findings in the first year of life and status epilepticus.[19] Disappointingly, full control of seizures may not preserve normal development.[76] Adult patients with occipital alpha rhythms on EEG seem to have milder intellectual disability, than patients without these rythms.[36, 59] Dravet syndrome patients without absences and myoclonic seizures or

with no or few status epilepticus, might have a higher chance to become seizure free as adults.[36, 59]

1.3.5 Seizure precipitating factors

1.3.5.1 Fever and hyperthermia

The seizure precipitating effect of fever is one of the most striking features of Dravet syndrome.[25, 32] This effect occurs in virtually all children and generally already at low-grade fever (~38 °C or even less).[25, 32, 33, 47, 77] Fever sensitivity is most prominent in infancy with first seizures precipitated by fever in 21-77% of cases.[19, 25, 33, 55] It persists into childhood but is then less striking due to a decreasing frequency of febrile episodes.[33] However, the triggering effect of fever may also decrease or even disappear, as has been reported for adults and a small subset of children with Dravet syndrome.[36, 77]

Hot water bathing as a seizure precipitant has been reported for all children in a large Japanese study on Dravet syndrome; whole body immersion into a bath-tub filled with hot water ranging from 39 to 42 °C is a typical Japanese custom. A video-EEG study on seven Dravet syndrome children undergoing hot water immersion showed clinical overt seizures after 7-25 minutes as soon as rectal temperature rose above 38 °C.[33] This study demonstrated that children with Dravet syndrome are not only highly sensitive to the seizure precipitating effect of an increased body temperature caused by fever (an non-physiological increase of the hypothalamic-controlled temperature set point), but also by hyperthermia: a situation in which excessive heat exposure or endogenous heat production exceeds the body's ability to control the temperature at the physiological unchanged set point.

Hot or warm water bathing as a seizure precipitant has also been reported for 22% of Italian and Chinese children,[25, 47] and for first seizures in 2%, respectively 14% of British and Norwegian children with Dravet syndrome.[19, 55] Hot weather and intense physical exercise during the summer, other potential causes of hyperthermia, have occasionally been reported as seizure precipitants in Dravet syndrome as well, but have not been systematically studied.[35, 49]

1.3.5.2 Vaccinations

In 7% to 57% of children with Dravet syndrome the first seizure has occurred within three days following an inactivated vaccination in infancy, most commonly DPT (diphtheria, pertussis, tetanus) combination vaccines with or without additional vaccines.[19, 47, 78-83] These first seizures occurred at a mean age of 4.0 to 4.5 months, approximately two months earlier than first seizures with no or other precipitating factors,[15, 19, 78] and were febrile in 40% to 68% of cases.[15, 16, 78, 82] Vaccination-induced seizures have also been reported for second or subsequent seizures, and also following influenza, pneumococcal,

hepatitis B, measles or measles-mumps-rubella vaccines.[47, 79, 82] However, the frequency or risk of seizures following these various vaccinations in Dravet syndrome has not been studied yet. Overall, vaccination-associated seizures have been reported in 27% to 53% of Dravet syndrome children.[47, 79, 82]

1.3.5.3 Photosensitivity

Sensitivity to visual stimuli is a characteristic feature of Dravet syndrome as well. Seizures induced by visual stimuli have been reported in 0% to 42% of cases.[25, 33, 41, 45, 46, 51, 59] This clinical photosensitivity may disappear in adolescence or adulthood.[36, 59] Sensitivity to patterns is reported in 11% to 30% of cases.[25, 57, 59] Self-induction of seizures using visual stimuli, like TV, lamps or patterns from the interior, has been reported in up to 16% of cases.[51, 57] However, clinical photosensitivity may not always be confirmed by induction of PPR on EEG,[25, 46, 58] and sensitivity to visual stimuli may not always be observed clinically in children with EEG proven photosensitivity.[54]

1.3.5.4 Other provocative factors

Many other stimuli have been reported to provoke seizures in one or more cases with Dravet syndrome. These stimuli include fatigue, stress, excitement, physical exercise, noisy environments, music, food and menstruation, but have not been systematically studied in Dravet syndrome cohorts.[35, 46, 48, 84, 85]

1.3.6 Treatment

1.3.6.1 Maintenance treatment

Dravet syndrome in general is a therapy resistant epilepsy syndrome. Most children need polytherapy to reduce frequency of status epilepticus and other seizures. Valproate with clobazam and stiripentol is the most commonly used drug combination in European patients with Dravet syndrome.[76, 86] Stiripentol is considered to be effective,[87] since it has been shown to be efficacious as add-on to valproate and clobazam in open and randomized placebo-controlled studies.[88-91] Use of valproate, bromide, zonisamide and topiramate is possibly effective.[28, 76, 87, 92-97] Levetiracetam might be effective as adjunctive therapy based on open studies.[98, 99]

Ketogenic diet is possibly effective,[87] since it was shown to lead to a greater than 50% reduction in seizure frequency in more than half of patients with Dravet syndrome.[100-106] Vagal nerve stimulation may also lead to a greater than 50% reduction in seizure frequency in half of patients with Dravet syndrome, although the numbers are yet too small to draw firm conclusions.[107, 108]

Treatment with lamotrigine and carbamazepine, both sodium channel blockers, has been shown to aggravate seizures and induce myoclonic seizures.[109-111] Avoidance

of these and all other sodium channel blockers, including phenytoin,[87] fosphenytoin, oxcarbazepine, lacosamide and rufinamide is therefore recommended.[76] Since use of vigabatrin can lead to seizure aggravation as well, administration of this drug is also discouraged.[28] The related drug tiagabine might also be avoided, but was shown to reduce seizures in an animal model of Dravet syndrome.[112]

If Dravet syndrome is not recognized until adulthood, adjustment of treatment after the diagnosis may still improve seizure control, cognitive performance and quality of life.[67] This should be done cautiously, since withdrawal of lamotrigine led to increased frequency and duration of seizures in some patients with Dravet syndrome, and this might also occur when withdrawing other sodium channel blockers.[113, 114]

1.3.6.2 Acute seizure treatment

Since status epilepticus may lead to developmental regression or even death, all acute seizures should be treated quickly and effectively, following a three step treatment program: Firstly, when a major seizure starts, parents or caregivers should administer buccal midazolam[115] or alternatively, rectal or nasal diazepines (e.g., diazepam, clonazepam or lorazepam). Secondly, this step should be repeated if the child still convulses after five minutes. Thirdly, intravenous benzodiazepines should be given under monitoring of vital functions (at emergency department or by trained personnel).[76]

Phenytoin and fosphenytoin are often used in emergency units during prolonged seizures. In patients with Dravet syndrome phenytoin i.v. may be effective to stop a status epilepticus. Anecdotal reports say phenytoin must always be avoided in status epilepticus in patients with Dravet syndrome,[116] because it might work counterproductive in the treatment of status epilepticus or might worsen the subsequent course of seizures, and midazolam i.v. continu or levetiracetam i.v. are good alternatives for phenytoin. Use of the barbiturates, phenobarbital and pentothal might be avoided as well since they might have been responsible for acute neurological deterioration following status epilepticus in three infants.[117]

To enable effective treatment of potential status epilepticus, an individual treatment plan should be made that can include arrangements with the regional ambulance service and hospital, treatment kits or even a port-a-cath venous access device.[76]

1.3.6.3 Prevention of seizures

For parents and caregivers, knowledge on seizure precipitating factors in Dravet syndrome is important to be able to prevent seizures. In case of fever, schematic antipyretic treatment and physical means of cooling are important to apply.[76] Hyperthermia-induced seizures may be prevented by using climate control and physical means of cooling, and avoiding warm baths.[76] Children that are sensitive to patterns may benefit from strip-

ping the interior from patterns and from wearing sunglasses.[118] Sunglasses or a hat may also prevent seizures from bright light in the summer.[33, 76]

1.3.6.4 Future treatments

The search for effective treatment of seizures in Dravet syndrome is ongoing. Currently, clinical trials are underway to test the efficacy of cannabidiol, the major nonpsychoactive component of cannabis, in the treatment of Dravet and other refractory childhood epilepsy syndromes.[119, 120] Cannabis was used to treat epilepsy already in the late 19th century and many anecdotal successes have been reported.[119]

Other anecdotal successes or case reports may give direction to the search for new anti-epileptic drugs as well. For example, a recent case report described a marked reduction in seizure frequency in an adult woman treated with the selective serotonin reuptake inhibitor (SSRI) fluoxetine.[121] In a small observational study, 70% of patients with Dravet syndrome became seizure-free while using fenfluramine, a serotonin agonist, as add-on treatment.[122] Effective seizure inhibition by fenfluramine was also shown in a zebrafish model for Dravet syndrome using *Scn1Lab* knockdown by antisense morpholino oligomers, and feeding with a serotonin precursor suppressed hyperthermia-induced seizures in a Drosophila model for Dravet syndrome.[123, 124] Moreover, use of animal models is likely to be effective in developing and identifying new potential treatments,[125] as shown by a study using a *Scn1Lab* zebrafish mutant for drug screening.[126] In this study, ketogenic diet, diazepam, valproate, potassium bromide and stiripentol were confirmed to be efficacious in reducing seizure frequency, while seven other anti-epileptic drugs had no effect. Furthermore, screening of 320 other drugs revealed that clemizole, an antihistamine, reduced seizures as well, and may therefore be a potential new therapeutic for Dravet syndrome.

1.4 GENETICS OF DRAVET SYNDROME

1.4.1 *SCN1A* mutations

In 2001, Claes et al. were the first to describe *de novo* heterozygous mutations of the *SCN1A* gene in patients with Dravet syndrome.[127] The *SCN1A* gene, located on chromosome 2q24.3, consists of 26 exons and encodes the α-subunit of a neuronal voltage-gated sodium-channel ($Na_v1.1$).[128] Later studies confirmed that heterozygous mutations in this gene are the major cause of Dravet syndrome, and can be detected in 70 to 100% of patients.[19, 42, 44, 129-131] By now, over 1000 different *SCN1A* mutations have been described in Dravet syndrome,[132] including frameshift, nonsense or splice-site mutations in approximately 51% of patients, missense mutations in approximately 43% and exonic,

partial or whole gene duplications or deletions in approximately 6% (calculated based on Parihar et al.[133]).

Haploinsufficiency has been proposed as the disease mechanism of these mutations based on: a) the detection of complete gene deletions, b) the lack of translation into protein of an mRNA with a premature stopcodon,[134] and c) the reduced cell surface expression and attenuated currents recorded by patch-clamp analyses for $Na_v1.1$ channels bearing nonsense or missense Dravet mutations.[135, 136] Patients with a deletion of the *SCN1A* gene, due to a 2q24 (micro)deletion and including (part of) the surrounding *SCN* gene cluster and other flanking genes, may either present with Dravet syndrome or a more severe epilepsy phenotype, and may have dysmorphic features.[137, 138] Based on animal models, the main effects of mutations causative of Dravet syndrome are severely impaired sodium currents and action potential firing in GABAergic interneurons, thereby impairing their inhibitory function and consequently leading to hyperexcitability of excitatory pyramidal neurons.[112]

SCN1A mutations have also been described in cases with milder and other epilepsy phenotypes, especially the genetic epilepsy febrile seizures plus (GEFS+) spectrum. This spectrum ranges from febrile seizures (FS); febrile seizures plus (FS+), where febrile seizures extend after age five years and/or afebrile seizures occur; FS or FS+ combined with a range of generalized seizure types or focal seizures; to Myoclonic-Astatic Epilepsy (MAE) and Dravet syndrome at the most severe end. GEFS+ syndrome is a familial epilepsy syndrome that can be diagnosed if at least two family members have phenotypes consistent with the GEFS+ spectrum,[60, 139] but is in clinical practice also used to designate FS+ cases with *de novo SCN1A* mutations. *SCN1A* mutations detected in GEFS+ families are mainly missense mutations. Initially, mixtures of loss-of-function and gain-of-function effects were detected when these mutations were expressed in non-neuronal cells and studied with voltage clamp analysis.[140, 141] However, the *in vivo* effects of GEFS+ mutations are incomplete loss of $Na_v1.1$ channel function, as shown using animal models.[112] Only 1% of mutations detected in Dravet syndrome has also been detected in GEFS+ syndrome.[133]

In Dravet syndrome ~90% of patients has a *de novo SCN1A* mutation, while ~10% has inherited the mutation from one of the parents.[41, 131, 142, 143] These parents in general have no or a milder epilepsy phenotype, and this can be explained by somatic mosaicism for the mutation in approximately two-thirds of them.[142] The other one-third of Dravet syndrome children with inherited *SCN1A* mutations comes from multigenerational GEFS+ families, in which they represent the extreme end of the GEFS+ spectrum.[142] Even in families with a child with Dravet syndrome and an apparently *de novo SCN1A* mutation, recurrence of Dravet syndrome in younger (half)siblings has been observed as a result of germline or low-level somatic mosaicism in one of the parents.[144-147]

1.4.2 Effect of mutation type and modifier genes

The large phenotypic variability in Dravet syndrome can partly be explained by differences in *SCN1A* mutations. Missense mutations, that may give rise to $Na_v1.1$ channels with some residual function, are more frequent in milder cases, while complete loss-of-function mutations and large deletions are more prevalent in more severe cases.[133] Furthermore, loss-of-function mutations are associated with an earlier age at onset of myoclonic and atypical absence seizures in Dravet syndrome.[148]

However, also between patients or family members with the same *SCN1A* mutation phenotypes may vary considerably, even if somatic mosaicism is excluded.[149, 150] Genetic modifiers may partly explain this variable expression as is supported by mouse models for Dravet syndrome. In these mouse models, the severity of the phenotype depends strongly on strain background,[151] and can be dramatically influenced by combined expression with *Scn2a*, *Kcnq2* or *Scn8a* mutations.[152, 153] In patients with Dravet syndrome with *SCN1A* mutations, mutations modifying disease severity have been sought in other seizure susceptibility genes. Patients who also carry a variant in the *CACNA1A* gene, may have a younger age at seizure onset and more often prolonged or absence seizures,[154] while those who carry a heterozygous variant in the *POLG* gene might have an increased susceptibility for acute encephalopathy.[61] Variants in the *CACNB4* and the *SCN9A* gene have also been suggested as potential genetic modifiers for Dravet syndrome.[155-157]

1.4.3 Other genes for Dravet syndrome

In the ~30% of patients with Dravet syndrome who do not have *SCN1A* mutations, mutations in other genes have been sought (Figure 1). Initially, genes were examined that were already known to be associated with GEFS+ or other familial epilepsies, but mutations were rarely detected. Homozygous missense mutations of the *SCN1B* gene have been detected in two unrelated males diagnosed with Dravet syndrome, of whom one had a very severe course leading to tetra-pyramidal symptoms and death at age 14 months.[158, 159] Inherited, heterozygous nonsense mutations in the *GABRG2* gene have been detected in an adult patient,[26, 160] and in a dizygotic twin with Dravet syndrome.[161] *De novo* mutations in the *SCN2A* gene, a gene initially associated with Benign Familial Neonatal-Infantile Seizures (BFNIS), have been described in two unrelated patients with Dravet syndrome.[162, 163] However, recently *de novo SCN2A* mutations have been detected in several patients with epileptic encephalopathies not resembling Dravet syndrome (OMIM 613721).[164-166]

A more common cause of epilepsy resembling Dravet syndrome was detected in 2009. Heterozygous *PCDH19* mutations were then described in females with epilepsy and developmental delay who had previously been suspected of having Dravet syndrome, and in females from families with the X-linked disorder Epilepsy and Mental Retardation restricted to Females (EFMR, OMIM 300088).[167-169] This disorder is characterized by mul-

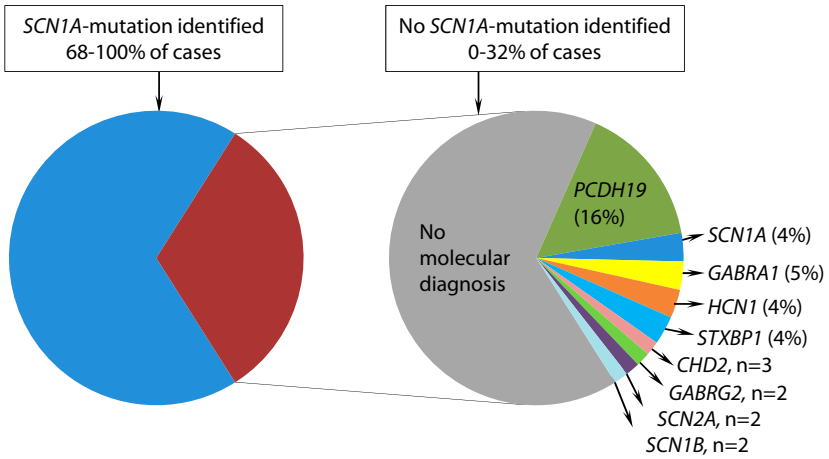

Figure 1. Schematic overview of molecular diagnoses in clinical Dravet (-like) syndrome.

The depicted proportions are global estimations based on the literature (see main text).

tiple seizure types, often associated with fever, mild to severe intellectual disability and behavioral problems, and may resemble Dravet syndrome. However, seizure onset may be later, up to five years of age,[170] seizures typically occur in clusters, and myoclonias or absences occur less frequently than in Dravet syndrome.[167] Furthermore, developmental delay is sometimes present before seizure onset.[171]

The epilepsy in children with Wolf-Hirschhorn syndrome, caused by 4p16.3 deletions, may resemble Dravet syndrome as well: seizure onset is often between 6 and 12 months of age, fever is an important seizure precipitant and most children develop multiple seizure types. In contrast to Dravet syndrome, seizures are in general well controlled and the majority of patients become seizure-free.[172] In clinical practice, diagnostic confusion with Dravet syndrome will rarely exist, since children with Wolf-Hirschhorn syndrome have additional dysmorphic features, intrauterine growth retardation, generalized hypotonia and pre-existent developmental delay. However, in children with smaller 4p deletions these symptoms may be absent or less prominent.

More recently, several new disease genes for Dravet syndrome have been identified using whole exome sequencing and other next generation sequencing methods. Mutations in the *GABRA1* and *STXBP1* gene were detected in four, respectively three patients of a group of 80 patients with Dravet syndrome without *SCN1A* mutations.[173] Previously, heterozygous mutations in the *GABRA1* gene had already been associated with childhood absence and juvenile myoclonic epilepsy (OMIM 611136),[174] while *de novo* mutations in the *STXBP1* gene had been shown in other epileptic encephalopathies.[175]

De novo missense mutations in the *HCN1* gene and *de novo* loss-of-function mutations in the *CHD2* gene have been detected in several cases with a fever-sensitive, epileptic encephalopathy resembling Dravet syndrome, as well.[176, 177] Future studies showing detailed clinical information must reveal whether patients with mutations in these newly identified disease genes show the same clinical course as in *SCN1A*-related Dravet syndrome, or only share part of the clinical features.

Interestingly, applying the same next generation sequencing techniques revealed *SCN1A* mutations that had been previously missed by Sanger sequencing or denaturing high performance liquid chromatography (dHPLC) analysis in several patients with Dravet syndrome.[173, 178] This suggests that the role of *SCN1A* mutations in the etiology of Dravet syndrome is even larger than originally thought.

1.5 CHILDHOOD VACCINATIONS

1.5.1 Seizures as adverse events following immunizations

Since fever is a common side effect of vaccinations, febrile seizures are well known adverse events following childhood vaccinations.[179-181] A causal relationship between vaccination and a febrile seizure is considered to be probable or possible, if the seizure occurs, or the fever starts within 24 hours after administration of an inactivated vaccine or 5 to 12 days after the live-attenuated measles-mumps-rubella (MMR) vaccine.[182] The incidence of febrile seizures is roughly two to three times increased within these risk periods, compared to the general incidence of febrile seizures in the pediatric population.[179-181, 183] However, the exact risk may vary depending on type of vaccine, dose number and age at administration.[184, 185] For example, the risk of febrile seizures is higher following whole-cell pertussis than acellular pertussis combination vaccines,[186-188] and following MMRV (MMR combined with Varicella) than MMR vaccines.[189] The risk of febrile seizures following vaccinations in children with epilepsy is unknown.

The risk of afebrile seizures following vaccinations is not significantly increased according to epidemiological studies.[11, 181] However, a causal relationship between vaccination and an afebrile seizure is still considered possible if the seizure occurs within 24 hours after administration of an inactivated vaccine or 5 to 12 days after the live-attenuated MMR vaccine.[182]

The risk to develop epilepsy is similar for children with first febrile seizures following vaccination and those with first febrile seizures unrelated to vaccination, and also similar for vaccinated and unvaccinated children.[184]

1.5.2 Safety surveillance

Since vaccinations induce immune responses, adverse events like local reactions or fever may occur, but serious adverse events remain extremely rare.[190] Surveillance of these adverse events following immunizations (AEFI) is propagated by the world health organization (WHO) and is important for maintaining confidence of the general public and health care providers in the vaccination program. Adverse events may either be genuine side effects or coincidental events. The causality of an association between an adverse event and vaccination is assessed based on the following criteria: consistency, strength of the association, specificity, temporal relation and biological plausibility.[191]

The safety surveillance of the Netherlands Vaccination Program (NVP) has been performed by the Centre of Infectious Disease Control of the National Institute for Public Health and the Environment (RIVM) until 2011, and is since then continued by the Netherlands pharmacovigilance centre Lareb.[192] The safety surveillance by the RIVM consisted of an enhanced passive reporting system, in which health care providers were stimulated to report adverse events.[182] Conclusions on causality assessments were always reported back to the notifying physician.

1.5.3 Netherlands Vaccination Program

The NVP was instituted in 1957, but first mass vaccinations of children had already been initiated in 1952.[191] Vaccinations in the NVP are free of charge but not mandatory. Vaccinations are given during routine visits to the Child Health Care clinics (consultatiebureaus) and the vaccination coverage is in general ~95%.[193]

The NVP consists of four vaccination moments in the first year of life, and vaccinations at age 14 months, 4 years and 9 years, and, for girls only two at age 12 years and older. New vaccines are implemented regularly and currently the NVP protects against 12 infectious diseases: diphtheria, pertussis, tetanus, polio, *Haemophilis influenza* type b, invasive pneumococcal disease caused by 10 serotypes, hepatitis B¸ meningococcal disease type C, measles, mumps, rubella and human papilloma virus. An overview of the NVP between 1990-2011, the period studied in this thesis, is given in Table 1.[191, 194] Major changes in this period were the shift of the first three infant pertussis combination vaccines from age 3, 4 and 5 months to 2, 3 and 4 months in 1999, and the replacement of the whole-cell by the less reactogenic acellular pertussis component in the infant pertussis combination vaccine in 2005.[195]

Table 1. Vaccination schedules of the NVP in the first two years of life, from 1990-2011 (adapted from chapter 4)

Age at vaccination	1990-1999		1999-2004		2005-2011	
	Injection 1	Injection 2	Injection 1	Injection 2	Injection 1	Injection 2
2 months	-	-	DTwP-IPV-Hib[b]	(HepB[c])	DTaP-IPV-Hib(-HepB)[e]	PCV7[f,g]
3 months	DTwP-IPV	Hib[a]	DTwP-IPV-Hib[b]		DTaP-IPV-Hib(-HepB)[e]	PCV7[f,g]
4 months	DTwP-IPV	Hib[a]	DTwP-IPV-Hib[b]		DTaP-IPV-Hib(-HepB)[e]	PCV7[f,g]
5 months	DTwP-IPV	Hib[a]	-		-	
11 months	DTwP-IPV	Hib[a]	DTwP-IPV-Hib[b]		DTaP-IPV-Hib(-HepB)[e]	PCV7[f,g]
14 months	MMR	-	MMR	Men C[d]	MMR	Men C

DTwP-IPV: diphtheria, tetanus, whole-cell pertussis, inactivated polio; aP: acellular pertussis; Hib: *Haemophilus influenza* type b; HepB: hepatitis B; MMR: measles-mumps-rubella; Men C: Meningococcal serogroup C; PCV7: 7 valent conjugated pneumococcal.

[a] since 1993; [b] since 2003 DTwP-IPV was mixed with Hib; [c] since 2003 children with a parent from a country where hepatitis B is moderately or highly endemic and children with the mother positive for Hepatitis B surface Antigen (HBsAg) received DTwP-IPV-Hib+HepB; [d] since 2002; [e] since October 2011 DTaP-IPV-Hib-HepB replaced DTaP-IPV-Hib for all infants; [f] since 2006; [g] since May 2011 PCV10 replaced PCV7.

1.6 OUTLINE OF THESIS

Dravet syndrome is a severe genetic epilepsy syndrome with a wide phenotypic variability. This thesis focuses on Dravet syndrome caused by *SCN1A* mutations only. The aim of this study was to further delineate the relationship with vaccinations, specify the role of seizure precipitants, and evaluate the diagnostic process in Dravet syndrome.

Although *SCN1A*-related Dravet syndrome has been acknowledged as a major cause of alleged vaccination-encephalopathy, its prevalence among children with seizures or epilepsy after vaccination had not been studied yet. In **chapter 2** the prevalence of Dravet syndrome is described among children reported with seizures following vaccination at the safety surveillance system of the RIVM between 1997 and 2006. Furthermore, differences in seizure characteristics between children with and without Dravet syndrome are explored.

In **chapter 3** the prevalence of Dravet and other epilepsy syndromes is estimated among children with epilepsy onset following vaccination (derived from the same RIVM cohort). Since knowledge about underlying causes of epilepsy with onset after vaccination, other than *SCN1A*-related Dravet syndrome, barely exists, the etiology of these other epilepsy syndromes is described as well.

An earlier seizure onset triggered by vaccination may theoretically influence disease course in Dravet syndrome. To study this possible effect, the disease course of children with and without vaccination-associated seizure onset is compared in a cohort of chil-

dren with Dravet syndrome described in **chapter 4**. Since the frequency of subsequent seizures following vaccinations in Dravet syndrome had not been studied yet, the risk of seizures following various childhood vaccinations after seizure onset is estimated as well in this cohort.

Other seizure precipitants have also been described in Dravet syndrome, but have not been systematically studied. In **chapter 5** the prevalence of these other seizure precipitating factors in Dravet syndrome is estimated using parental questionnaires and compared with two other Dutch epilepsy cohorts. Although photosensitivity has been recognized as an important characteristic of Dravet syndrome since its first description in 1978, its reported prevalence differs widely between studies. In **chapter 6** the prevalence of photosensitivity among children with Dravet syndrome is studied by re-evaluating PPR on EEGs. In addition, EEG characteristics predicting PPR occurrence are explored. Since clinical photosensitivity and EEG proven photosensitivity do not always correlate, PPR data are compared with data on sensitivity to visual stimuli from medical files and parental questionnaires as well.

Since a diagnosis of Dravet syndrome has implications for the choice of anti-epileptic drugs, early recognition of this syndrome is important to improve seizure control, and possibly disease course. In **chapter 7** the diagnostic process and factors delaying diagnosis in Dravet syndrome are described. Based on these results recommendations are made to promote an early diagnosis in children. In **chapter 8** the importance of recognizing Dravet syndrome in adults is illustrated by a description of two unrelated families in which two men were considered to have mild Dravet syndrome only after they had fathered a child with Dravet syndrome. The role of mosaic *SCN1A* mutations as an explanation for a milder disease course is also explored.

Patients described in chapters 4 to 8 are derived from a cohort of children with Dravet syndrome and a pathogenic *SCN1A* mutation who were referred for genetic counseling or DNA-diagnostics to the Department of Medical Genetics of the University Medical Center Utrecht. The overlap between the RIVM and Dravet syndrome cohort, and between patients in the various chapters is shown in Figure 2.

In the general discussion, implications are described for vaccination policy, diagnostics and genetic counseling in Dravet syndrome, and proposals for further research are emphasized.

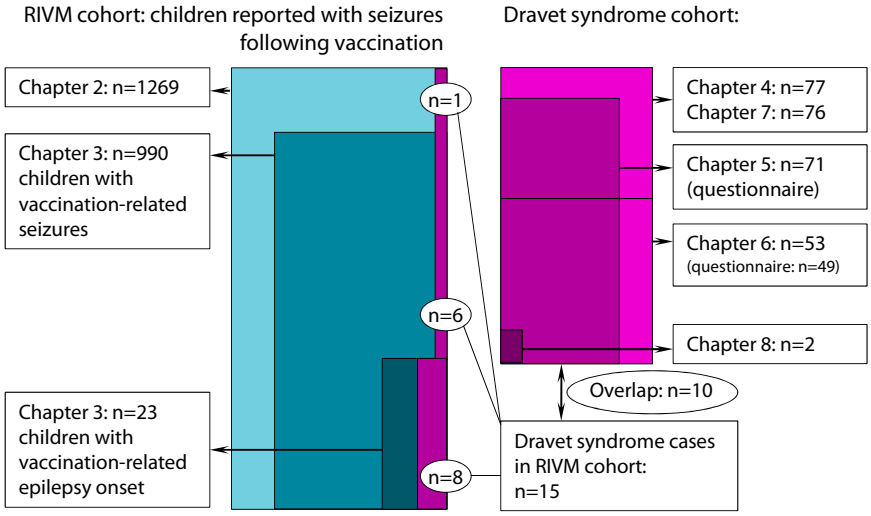

Figure 2. Overview of the RIVM and Dravet syndrome cohort and the respective number of cases included in the various chapters of this thesis.

Relative proportions in this figure are not representative of the relative sizes of the cohorts.

Chapter 2

Prevalence of *SCN1A*-related Dravet syndrome among children reported with seizures following vaccination: a population-based ten-year cohort study

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ABSTRACT

Objectives: To determine the prevalence of Dravet syndrome, an epileptic encephalopathy caused by *SCN1A* mutations, often with seizure onset after vaccination, among infants reported with seizures following vaccination. To determine differences in characteristics of reported seizures after vaccination in children with and without *SCN1A*-related Dravet syndrome.

Methods: Data were reviewed of 1,269 children with seizures following immunization in the first two years of life, reported to the safety surveillance system of the Dutch national immunization program between 1 January 1997 and 31 December 2006. Selective, prospective follow-up was performed of children with clinical characteristics compatible with a diagnosis of Dravet syndrome.

Results: In 21.9% (n=279) of children, a diagnosis of Dravet syndrome could not be excluded based on available clinical data (median age at follow-up 16 months). Additional follow-up data were obtained in 83.9% (n=234) of these children (median age 8.5 years). 15 (1.2% of 1,269; 95% CI:0.6 to 1.8%) children were diagnosed with *SCN1A*-related Dravet syndrome. Of all reported seizures following vaccinations in the first year of life, 2.5% (95% CI:1.3 to 3.6%) were due to *SCN1A*-related Dravet syndrome, as were 5.9% of reported seizures (95% CI:3.1 to 8.7%) after 2nd or 3rd DTP-IPV-Hib vaccination. Seizures in children with *SCN1A*-related Dravet syndrome occurred more often with a body temperature below 38.5 °C (57.9% vs. 32.6%, p=0.020) and reoccurred more often after following vaccinations (26.7% vs. 4.0%, p=0.003), than in children without a diagnosis of *SCN1A*-related Dravet syndrome.

Conclusions: Although Dravet syndrome is a rare genetic epilepsy syndrome, 2.5% of reported seizures following vaccinations in the first year of life in our cohort occurred in children with this disorder. Knowledge on the specific characteristics of vaccination-related seizures in this syndrome might promote early diagnosis and indirectly, public faith in vaccination safety.

INTRODUCTION

Due to the success of national immunization programs, life-threatening and disabling complications of many infectious diseases have been forgotten. Fear of alleged side-effects of vaccinations can lead to a decrease in vaccination coverage, inadequate herd immunity and subsequent epidemics of infectious diseases. Open and transparent information on side-effects of vaccinations is needed to consolidate adequate vaccination coverage.[2, 196] One of the most frightening side-effects of vaccinations might be febrile seizures. Epidemiological studies show an increased risk of febrile seizures of 25 to 34 per 100,000 Measles Mumps Rubella (MMR) vaccinations,[179] six to nine additional cases per 100,000 Diphtheria Tetanus whole cell Pertussis (DTwP) vaccine doses,[179, 181] and no or only mild increased risk after Diphtheria Tetanus acellular Pertussis (DTaP) vaccine.[184] Vaccination is not associated with an increased risk of afebrile seizures, epilepsy or irreversible neurological damage.[14, 184] However, it cannot be excluded that vaccinations might trigger seizures in certain subgroups of children with a high seizure susceptibility. In 2006, Berkovic et al., showed that among a case series of children in whom vaccination had been implicated as a direct cause of the subsequent epileptic encephalopathy, 11 out of 14 children had Dravet syndrome due to *SCN1A* mutations.[15] This showed that the epilepsy and retardation in these children was primarily due to a genetic defect and not to the vaccination.

Dravet syndrome, also known as Severe Myoclonic Epilepsy of Infancy (SMEI), is a rare epilepsy syndrome (estimated prevalence 1:20,000-40,000)[17-19] with onset in the first year of life in previously healthy children. After, often prolonged, febrile seizures or hemiconvulsions, therapy resistant afebrile seizures and neurological deterioration occur.[48] In at least 70% of children, Dravet syndrome is caused by a heterozygous mutation in the *SCN1A* gene,[19, 131] encoding the alpha-subunit of the neuronal voltage gated sodium channel. Of these mutations, 95% are spontaneous (*de novo*) mutations.[142, 143] In a small proportion of children with Dravet syndrome mutations in other genes have been identified as the cause of the epilepsy.[197] Seizures in children with Dravet syndrome are often triggered by fever or infectious diseases. Vaccinations precede first seizures in 7-57% of children with Dravet syndrome.[19, 80-83] Although Dravet syndrome is a rare disorder, among children with seizures following vaccinations the prevalence might be significant. Information on the prevalence and characteristics of early, vaccination-related seizures in Dravet syndrome, is of major importance for recognition of Dravet syndrome among children presenting with seizures following vaccinations. An early diagnosis of Dravet syndrome is important for treatment (avoidance of sodium channel blockers)[198] and genetic counseling. The knowledge that vaccination, although possibly the trigger for the first seizure, is not the cause of their child's genetic epilepsy syndrome could support immunization of other children in the family.

Our study comprises a 10-year cohort of children with seizures following vaccinations in the first two years of life, reported to the enhanced passive safety surveillance system for the Dutch national immunization program. Within this cohort, children with possible Dravet syndrome were followed up, to estimate the prevalence of *SCN1A*-related Dravet syndrome and describe the clinical characteristics of seizures following vaccinations.

METHODS

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of the University Medical Center Utrecht, Utrecht, the Netherlands (no. 07/295).

Setting: safety surveillance of immunizations

The Dutch national immunization program is a voluntary program with stable and high vaccination coverage (~95%).[199] The vaccination registry is linked to the population register and records all vaccinations at an individual level. Safety surveillance is performed by an enhanced passive reporting system, that was carried out by the National Institute for Public Health and Environment (RIVM) until 2011.

Adverse events following immunization (AEFI) were reported and registered in a database after extensive supplementation and verification. In case of serious or uncertain diagnoses and unresolved events, follow-up was done. Reporting rate was stable and high.[191] Causal relation of reported seizures with the vaccination was assessed routinely. Seizures occurring within 24 hours following administration of an inactivated vaccine (DTP-IPV, Hib or MenC),[179] or 5 to 12 days after MMR vaccination,[180] were considered to be causally related to vaccination. Temperatures were categorized as <37.5, 37.5-38.4 or ≥38.5 °C.

Study population and vaccination schedules

The total study population comprised all children with adverse events after a vaccination in the first two years of life, reported to RIVM between 1 January 1997 and 31 December 2006.

During this period the schedule included four doses of Diphtheria, Tetanus, Pertussis, inactivated Polio vaccine and Haemophilus influenzae type b (DTP-IPV(-)Hib) in the first year of life and one dose of MMR vaccine at age 14 months. Hepatitis B (HepB), Pneumococcal conjugate vaccine (PCV), Meningococcal serogroup C (MenC) and acellular Pertussis (aP) were introduced between 2001 and 2006 (see Supplementary Table 1).[191, 194, 200]

Design of study

Figure 1, schematically represents the design of the study. In stage 1 we prospectively selected all children with reported 'fits' (convulsions, atypical seizures, epilepsy), 'myoclonus', 'retardation', 'encephalopathy/encephalitis' or 'death' reported during the studied period.[191] All events, regardless of their relation to vaccination, were included if they could be classified as a febrile seizure, an afebrile seizure or an atypical seizure (see 'classification of seizures'). Reports of collapse, defined by sudden onset of pallor, limpness and hyporesponsiveness, were excluded.[201] From the RIVM database and from hard copy files, patient characteristics were collected including the ages at first seizure, last seizure and last follow-up, types of seizures, psychomotor development, comorbidity and etiology. In children diagnosed with epilepsy, data on response to treatment and results of electroencephalography (EEG) and brain scans were collected as well.

At stage 2, we contacted parents of children whose medical history met the criteria for a clinical diagnosis of Dravet syndrome (see 'case definitions'), or in whom Dravet syndrome could not be excluded on the basis of the data available at stage 1. Parents were asked to give written consent to obtain all relevant data from medical records. They could also provide follow-up data themselves.

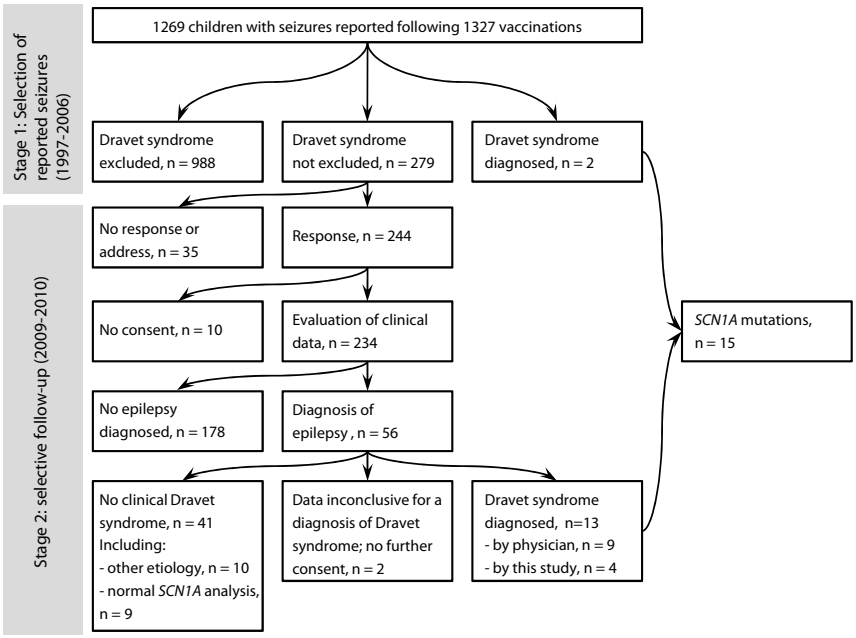

Figure 1. Design of study.

The medical records were reviewed to classify the data as possibly compatible with a diagnosis of Dravet syndrome by an experienced pediatric neurologist (FJ) and clinical geneticist (NV), independently. In case of disagreement, a second clinical geneticist (EB) also reviewed the data and consensus was reached between the three.

In children eventually diagnosed with *SCN1A*-related Dravet syndrome, the complete vaccination report was obtained from the vaccination registries.

DNA analysis of the *SCN1A* gene

DNA analysis of the *SCN1A* gene was performed if clinical criteria for Dravet syndrome were met according to the treating physician or the study group. Parents gave informed consent for the DNA analysis. DNA analysis of the *SCN1A* gene included sequence and MLPA analysis, as previously described.[202] All cases were analyzed by the laboratory for DNA diagnostics at the UMC Utrecht, except for one case who had been analyzed by the DNA Diagnostic Unit of the Neurogenetics Group, VIB Department of Molecular Genetics, University of Antwerp, Belgium.

A detected mutation was considered to be pathogenic when it had been described earlier in patients with Dravet syndrome, when it was predicted to lead to a truncated protein or haploinsufficiency, or when the mutation was not detected in the parents and therefore assigned as 'de novo' in the patient.

Statistical analysis

The outcome of interest was the proportion of seizures attributed to *SCN1A*-related Dravet syndrome among all reported seizures after vaccination in the first two years of life, and later categorized per year and per vaccination type. These proportions, were also calculated for the subgroups of 'vaccination-related' seizures and seizures reported within two months following vaccination, in order to assess the influence of delayed notifications and of seizures unrelated to vaccinations as confounders. 95% confidence intervals (95% CI) were calculated.

Differences in characteristics at stage 1, between children diagnosed with *SCN1A*-related Dravet syndrome and all other children reported with seizures, were analyzed. We used the Mann-Whitney U test to compare the two groups with respect to ages at vaccination, first seizure, notification of event, and follow-up. Differences in proportions of vaccination type (DTP-IPV or MMR), and of causality of vaccination to seizure, type of seizure (atypical or not) and temperature (< or ≥38.5 °C), categorized per vaccination type, were calculated with either Chi-Square analysis, or Fisher's Exact Test.

All statistical analyses were performed using SPSS Statistics, release 17.0 or 20, except for calculation of 95% CI (Excel).

Case definitions

1. Classification of seizures (stage 1 of study)

All events with tonic and/or clonic muscle spasms and loss of consciousness had been classified as seizures. They were further classified as afebrile, febrile (simple and complex), or atypical. Seizures occurring with a temperature of 38.5 °C or above, and those with substantial rise of body temperature but without temperature recorded, were classified as febrile seizures. Febrile seizures that were prolonged (>15 minutes), asymmetrical or recurred within 24 hours, were classified as complex febrile seizures.

Seizures were classified as atypical when the description of the seizure characteristics was insufficient for a definite classification as an epileptic seizure, febrile or afebrile. Atypical seizures occurring multiple times within 24 hours were classified as one episode of atypical seizures.

2. Selection of children with possible Dravet syndrome (stage 1 of study)

Dravet syndrome was considered in all children diagnosed with epilepsy younger than 24 months and less than eight months seizure-free at follow-up in stage 1, and a course suggestive of Dravet syndrome (see 'clinical criteria for Dravet syndrome').

In the other children a diagnosis of Dravet syndrome was considered, if the report consisted of:

a. a single seizure before twelve months of age, and a follow-up of less than six months after the seizure;
b. an afebrile or complex febrile seizure before age 18 months and a follow-up of less than three months after the seizure;
c. multiple simple febrile seizures before age 12 months or multiple other seizures before age 18 months, and less than six months of follow-up after the last seizure;
d. one or more atypical seizures, fulfilling one of the former three criteria with respect to age at time of seizures and follow-up.

3. Exclusion of Dravet syndrome in children (stage 1 and 2 of study)

Dravet syndrome was excluded in children:

a. with epilepsy of other etiology, or with electroclinical syndromes other than Dravet syndrome (e.g., West syndrome);
b. with a severe developmental delay before epilepsy onset;
c. not diagnosed with epilepsy by the age of three years or older.

4. Clinical criteria for Dravet syndrome

Dravet syndrome was diagnosed in children with:[48]

a. seizure onset within the first 12 months of life;

b. normal development before onset of seizures;
c. generalized tonic-clonic seizures or unilateral seizures as initial seizure type;
d. normal EEG in first year of life;
e. development of epilepsy with multiple seizure types, with or without myoclonus, after the first year of life;
f. a (temporarily) drug-resistant epilepsy;
g. slowing of development after first year of life;
h. exacerbation or provocation of seizures with hyperthermia; *and*
i. normal brain imaging or aspecific abnormalities only.

When only one criterion was not fulfilled, Dravet syndrome was still diagnosed.[203]

RESULTS

Study population

Within the 10-year cohort, 1,269 children (681 males; 53.7%) had been reported with seizures following 1,327 vaccinations in the first two years of life. 4.2% (n=54) of children had reported seizures after two (n=50) or three (n=4) different vaccination moments (Table 1). Of all reported AEFI in this 10-year cohort, 10.5% were classified as seizures (1,327 out of 12,634), i.e. a reporting rate of seven per 10,000 vaccinated children.

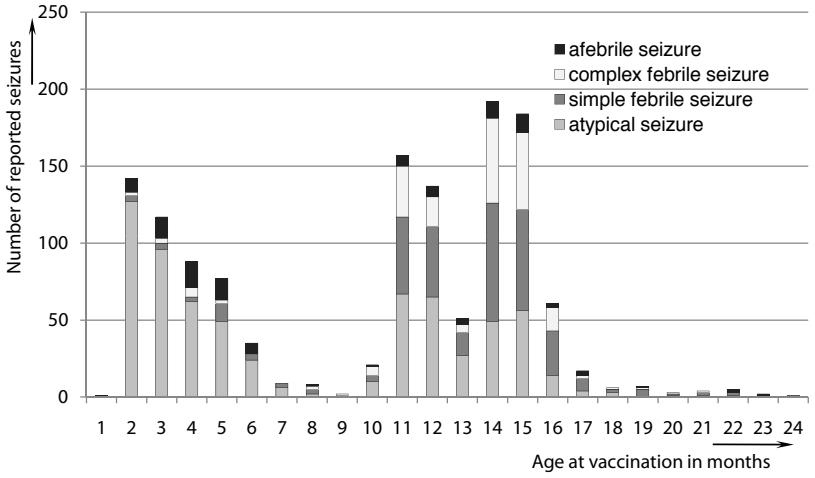

Figure 2. Classification and distribution of reported seizures following vaccinations, according to age at vaccination.

Table 1. Baseline characteristics of children with seizures following vaccinations, and with or without *SCN1A*-related Dravet syndrome

		All children reported (n=1269)		P-value	Total number
Characteristics of reports		*SCN1A*-related Dravet syndrome (n=15)	No Dravet syndrome diagnosed (n=1254)		
Number of males		10 (66.7%)	671 (53.5%)	0.310	1269
Median age in months (range), at	first seizure	4 (3-6)	11 (0-46)*	0.001	1259
	administration of first reported vaccine	4.4 (2.8-12.2)	11.5 (0.2-23.9)	0.001	1269
	notification of seizure after vaccination to RIVM	5.4 (3.9-165.9)	14.4 (0.3-174.5)	0.070	1269
	end of follow-up (stage 1)	18 (4-166)	16 (0-175)	0.619	1269
Number of children of whom first reported seizure was	vaccination-related	14 (93.3%)	956 (76.2%)	0.216	1269
	first seizure	13 (86.7%)	1157 (92.3%)	0.329	1269
	first seizure ànd vaccination-related	12 (80.0%)	862 (68.7%)	0.416	1269
Number of children with	multiple notifications of seizures after vaccination	4 (26.7%)	50 (4.0%)	0.003	1269
	multiple seizures, during follow-up (stage 1)	14 (93.3%)	375 (29.9%)	<0.001	1269
	diagnosis of epilepsy (stage 1)	10 (66.7%)	87 (6.9%)	<0.001	1269

Median age at first seizure was missing in 10 children. These were seizures unrelated and prior to, the reported vaccination.

* 4 children with first seizure after age 2.0 years, and at least 0.5 years after last vaccination

The reported vaccinations (n=1,327) included 64.5% (n=856) DTP-IPV(-)Hib vaccinations, 33.2% (n=440) MMR (+MenC) vaccinations, 1.2% (n=16) combined DTP-IPV(-)Hib/MMR vaccinations and 1.1% (n=15) other vaccinations (mainly single MenC vaccinations). 55.2% (n=732) of reported seizures occurred after a vaccination in the first year of life. Of the reported seizures 50.1% (n=665) were atypical seizures, 25.7% (n=341) simple febrile seizures, 15.5% (n=206) complex febrile seizures and 8.7% (n=115) afebrile seizures (Figure 2). In 76.3% (n=1,013) of the reported seizures a possible causal relation with the vaccination was assessed.

The median age at vaccination, was 11.5 months (m) (range 0.2 m – 23.9 m). The median time between vaccination and report of AEFI was 1.8 m (range 0 days – 14.1 years (y)). The median age at end of follow-up of stage 1 was 16 m (range 0 m – 14.6 y). At that moment,

30.7% (n=389) of children had had more than one seizure, including 7.8% (n=99) with seizures preceding the reported AEFI, and 7.6% (n=97) of children had been diagnosed with epilepsy (Table 1).

Case ascertainment in total cohort (stage 1; n=1,269 children)

In the total cohort, two children already had been diagnosed with Dravet syndrome by the treating physician, before the end of follow-up at stage 1. In 21.9% (n=279) of children, a clinical diagnosis of Dravet syndrome could not be excluded, of whom 43 children diagnosed with epilepsy. In 2.0% of children (n=25) a different etiological diagnosis was made. In the other 75.9% (n=963) of children, a clinical diagnosis of Dravet syndrome was excluded (Figure 1, Supplementary Table 2).

Identification of children with Dravet syndrome within the selected subcohort (stage 2; n=279)

Follow-up information was obtained for 83.9% (n=234) of children in whom Dravet syndrome was still considered possible (Figure 1, Supplementary Table 3). The median age at follow-up at stage 2 was 8.5 y (range 3.1 – 23.6 y).

Sixteen children had been diagnosed with epilepsy after stage 1, bringing the total number of children diagnosed with epilepsy to 56 (23.9% of 234). Of them, nine had already been diagnosed with clinical Dravet syndrome by their treating physician and pathogenic *SCN1A* mutations had been detected in eight of them (Figure 1, Table 3, Supplementary Table 3). By means of this study, an *SCN1A* mutation has been detected also in the ninth child. Based on available clinical data, we additionally diagnosed Dravet syndrome in four children, and detected pathogenic *SCN1A* mutations in all four. Of these

Table 2. Characteristics of reported seizures following DTP-IPV(-)Hib vaccination in children with and without *SCN1A*-related Dravet syndrome

	All reported seizures (n=1327), in children with		P-value	Total number
Number of reported seizures	*SCN1A*-related DS syndrome (n=20)	No DS diagnosed (n=1307)		
after DTP-IPV(-)Hib vaccination (vs. MMR, combined or other vaccines)	20 (100.0%)	836 (64.0%)	<0.001	1327
2nd or 3rd DTP-IPV(-)Hib vaccination	16 (80.0%)	255 (30.5%)	<0.001	856
vaccination-related	19 (95.0%)	637 (76.2%)	0.059	856
classified as atypical seizure (vs. febrile or afebrile seizure)	4 (20.0%)	536 (64.1%)	<0.001	856
temperature <38.5 °C	11 (57.9%)	250 (32.6%)	0.020*	787

* P=0.046 when the 69 missings were considered as temperature less than 38.5 °C.

DS = Dravet syndrome.

Table 3. Description of seizures following vaccinations in children with *SCN1A*-related Dravet syndrome.

Number	M/F	vaccination	Age at vaccination, in months	First seizure?	Seizure related to vaccination?	Time between vaccination and seizure	Delay in notification to RIVM	Classification of seizure, at time of notification	Description of seizure	Duration of seizure in min	Temperature (°C)	*SCN1A* mutation	Inheritance
1	M	DTwP-IPV2 Hib2	4.9	Yes	Yes	<24 h	6.5 years	Complex FS	GTCS	15[a]	38.6	p.Asp1239Tyr	De novo
2	M	DTwP-IPV-Hib2	3.7	Yes	No	17 days	15 months	Complex FS	GTCS	>30	38.5-39	p.Leu1717Pro	De novo
3	F	DTwP-IPV2 Hib2	3.9	Yes	Yes	8 h	7 weeks	Afebrile	GTCS	30[a]	'no'	p. Phe1535fs	ND
		DT-IPV4 Hib4	12.4	-	Yes	8.5 h	1 week	Afebrile	'Seizure'	?	37.8		
4	F	DTwP-IPV1 Hib1	3.4	Yes	Yes	5 h	4 weeks	Complex FS	GTCS	30[a]	39	p.Gly1433Glu	De novo
		DTwP-IPV2 Hib2	5.5	-	Yes	7 h	2 days	Afebrile	Focal	6[a]	38.1		
						18 h			Focal	[a]	37.5		
		DTwP-IPV3 Hib3	6.9	-	Yes	5 h	4 weeks	Simple FS	GTCS	15[a]	39		
5	M	DTwP-IPV2 Hib2	4.2	Yes	Yes	7 h	2 weeks	Atypical	GTCS?	15	38.3	p.Arg946His	ND
						8 h			Focal	<2			
6	M	DTwP-IPV Hib2	3.1	Yes	Yes	4 h	4 weeks	Atypical	GTCS?	15	38.5	p. Arg101Gln	De novo
7	M	DTwP-IPV3 Hib3	4.4	Yes	Yes	4.5 h	4 days	Afebrile	GTCS	15	'no'	duplication exon 17-20	De novo
		DTwP-IPV-Hib4	11.7	-	Yes	6 h	1 week	Afebrile	GTCS	5-10	'no'		
8	M	DTwP-IPV3 Hib3	4.8	Yes	Yes	8 h	3 days	Atypical	Hemi	5	'warm'	c.4278_4282delCAAGT	ND
						13 h			GTCS	10	>38		
						14 h			'Seizure'	?			

Table 3. Description of seizures following vaccinations in children with *SCN1A*-related Dravet syndrome. (continued)

Number	M/F	vaccination	Age at vaccination, in months	First seizure?	Seizure related to vaccination?	Time between vaccination and seizure	Delay in notification to RIVM	Classification of seizure, at time of notification	Description of seizure	Duration of seizure in min	Temperature (°C)	*SCN1A* mutation	Inheritance
9	F	DTwP-IPV3 Hib3	4.6	Yes	Yes	11.5 h	4 months	Simple FS	GTCS	10-15	38.9	p.Val1390Met	De novo
10	F	DTwP-IPV-Hib2	3.5	Yes	Yes	10 h	2 weeks	Atypical	Focal	<10	'no'	c.3880-1G>A[b]	P Mosaic[b]
		DTwP-IPV-Hib3[c]	*4.5*	-	*Yes*	*<24 h*	*1 day*	*Not recognized*	*GTCS*	*<15*	*'no'*		
11	M	DTwP-IPV-Hib2	2.8	Yes	Yes	6 h	1 month	Afebrile	GTCS	30	38.3	p.Trp384Arg	De novo
		DTwP-IPV-Hib3	4.0	-	Yes	10.5 h	1 day	Afebrile	GTCS	60	38.4		
12	M	DTwP-IPV2	4.4	Yes	Yes	8.5 h	13 years	Simple FS	GTCS	6-7	38.7	p.Ser1879fs	De novo
13	M	DT-IPV4 Hib4	12.2	No	Yes	21 h	3 months	Simple FS	GTCS	2-3	NA	p.Gly177Glu	ND
14	F	DTwP-IPV3 Hib3	6.9	No	Yes	12 h	1 day	Afebrile	GTCS	5-7[a]	~38	p.Leu331fs	ND
15	M	DTwP-IPV3 Hib3	5.1	Yes	Yes	5 h	1 week	Afebrile	GTCS	30	37	c.2176+2T>A	De novo

Children 1 - 2 had been diagnosed at stage 1 and children 3 – 11 at stage 2 of the study by their treating physician. Children 12 -15 were diagnosed at stage 2, by means of this study. DTwP-IPV-Hib = Diphtheria, Tetanus, whole-cell Pertussis, inactivated Polio vaccine, Haemophilus influenzae type b; FS= febrile seizure; GTCS= Generalized tonic clonic seizure; ND=not determined; h=hours. NA=not available.

[a] Use of valium rectiole or intravenous; [b] DNA diagnostics performed by DNA Diagnostic Unit of the Neurogenetics Group, VIB Department of Molecular Genetics, University of Antwerp; unaffected parent mosaic for mutation. [c] Seizure was reported to the RIVM, but was not recognized as a seizure by either parents or co-workers of the RIVM.

13 children with *SCN1A*-related Dravet syndrome, eight had already been diagnosed with epilepsy at stage 1 of the study. Of the other five, four had had multiple seizures and one was reported with a single seizure to the RIVM (Supplementary Table 2).

In two children with epilepsy the clinical criteria for Dravet syndrome were partly met, but no further consent for data retrieval or DNA analysis was obtained.

In the children without a clinical diagnosis of Dravet syndrome (41 with epilepsy, 178 without epilepsy), 15 (10 with epilepsy) had a different etiological diagnosis (including two with a milder form of *SCN1A*-related seizures) and in 12 (9 with epilepsy) *SCN1A* analysis had been requested by the treating physician and had shown normal results. Dravet syndrome was not further considered in patients without a diagnosis of epilepsy.

Percentage of children with *SCN1A*-related Dravet syndrome

In total, 15 children (1.2% of the total cohort (n=1269), 95% CI: 0.6 to 1.8%) were diagnosed with *SCN1A*-related Dravet syndrome (Table 1, Figure 1).

2.5% (n=18; 95% CI: 1.2 to 3.4%) of all reported seizures in the first year of life had occurred in children with *SCN1A*-related Dravet syndrome. In children with Dravet syndrome seizures following vaccination both occurred at a younger age (median age of 4.4 m vs. 11.5 m in all other children, p=0.001), and were more frequently reported after subsequent vaccinations (26.7% vs. 4.0% in all other children, p=0.003). After the second and third DTP-IPV(-)Hib vaccination the frequency of seizures due to Dravet syndrome was the highest: 5.9% (n=16; 95% CI: 3.1 to 8.7%). Only two seizures in children with *SCN1A*-related Dravet syndrome occurred after vaccinations in the second year of life, representing 0.3% (95% CI: 0.0 to 0.8%) of reported seizures in the second year of life.

The delay of notification of the seizure to the RIVM was several years in two children with Dravet syndrome (case 1 and 12) (Table 3). In one child with Dravet syndrome there was no relation in time between occurrence of the seizure and administration of the vaccine (case 2). When both reports with a delay of two months or more, as well as seizures unrelated to vaccinations were excluded as potential confounders (n=553 seizures left), the percentage of seizures attributed to Dravet syndrome was 0.5% (95% CI: 0.0 to 1.5%) for the second year, 3.9% (95% CI: 1.9 to 5.9%) for the first year and 8.7% (95% CI: 4.0 to 13.4%) for the second and third vaccination.

No children with Dravet syndrome were detected in the last two cohort years, in which whole-cell pertussis was replaced by acellular pertussis vaccine. However, there was no statistically significant difference in the proportion of children with Dravet syndrome in the last two years compared with the first eight cohort years (0/59 vs. 20/797 reports on children with Dravet syndrome per reported DTP-IPV(-)Hib vaccination, p=0.390).

Vaccination and seizure characteristics in children with *SCN1A*-related Dravet syndrome

Of the children diagnosed with *SCN1A*-related Dravet syndrome, ten were males (66.7%; p=0.31). The median age of the first seizure was 4 m (range 3 m – 6 m) (vs. 11 m, in all other children, p=0.001)(Table 1). In 80.0% (n=12) the first seizure had been vaccination-related (vs. 68.7% of all other children, p=0.416) and had occurred at a mean age of 4.1 months (Table 3).

All 20 reported seizures occurred 4 to 21 hours (h) (median 7.5 h) after DT(P)-IPV(-)Hib vaccinations, except for one seizure unrelated to vaccination (17 days in case 2) (Table 3). One reported seizure had not been recognized as a seizure by the parents or the co-workers of the safety surveillance system (case 10, Table 3). In two subjects, seizures occurred following MMR vaccinations, but were not reported to the RIVM (data not shown).

Body temperature during seizures was often below 38.5°C (57.9% in children Dravet syndrome vs. 32.6% in all other children, p=0.020; only DTP-IPV(-)Hib vaccinations analyzed). The reported seizures were generalized tonic-clonic seizures, hemiconvulsions and focal seizures. 15.0% of seizures recurred on the same day. Duration of seizures was two to 60 minutes. In 30.0% they lasted more than 15 minutes. Seizures in children with *SCN1A*-related Dravet syndrome were classified as afebrile (45.0%), simple febrile (20.0%), complex febrile (15.0%) or atypical seizures (20.0%). Atypical seizures were less frequently reported than in other children (20.0% vs. 64.1%; p<0.001; only DTP-IPV(-)Hib vaccinations analyzed) (Table 2 and 3).

All children with *SCN1A*-related Dravet syndrome had developed multiple seizure types that were temperature-sensitive. Epilepsy was refractory to treatment in all patients, except in case 9, who was seizure-free with use of anti-epileptic drugs, and in case 13, who only had a few seizures per year. In the first year of life, all children had a normal development. Most children had a moderate to severe developmental delay at stage 2. Case 7 had only mild learning difficulties.

In 86.7% (n=13) of children, the vaccination schedule was adjusted. Three children received no further vaccinations. In the other children, either one or more subsequent vaccinations did not include the pertussis component (n=4), some vaccination moments were missed (n=5) or both (n=1) (Supplementary Table 3).

DISCUSSION

In our nation-wide ten-year cohort study, we identified *SCN1A*-related Dravet syndrome as the underlying cause in 1.2% of children reported with seizures following vaccinations in the first two years of life, including 2.5% of seizures reported after vaccination in

the first year of life, and 0.3% in the second year of life. Among seizures reported after the second or third DTP-IPV(-)Hib vaccination, the proportion of *SCN1A*-related Dravet syndrome was the highest. The seizure had occurred within 24 h (median 7.5 h) after administration of DT(P)-IPV(-) Hib vaccines in the majority of children diagnosed with *SCN1A*-related Dravet syndrome.

Children diagnosed with *SCN1A*-related Dravet syndrome had a younger age at first seizure following vaccination, and more often had second and third seizures reported after subsequent vaccinations than other children. Both short or prolonged, generalized or unilateral, and febrile or afebrile vaccination-related seizures occurred in children with *SCN1A*-related Dravet syndrome. Seizures occurred more often with a body temperature below 38.5 °C, illustrating the high sensitivity also to minor temperature increase in children with *SCN1A*-related Dravet syndrome.[203]

Strengths and limitations of the study

This study is, as far as we could retrieve, the largest follow-up study on the prevalence of Dravet syndrome among children with seizures following vaccinations. The response rate in our study was high, especially among the parents of children diagnosed with epilepsy at stage 1. The long follow-up time enabled us to exclude Dravet syndrome with high certainty in the majority of cases. We have studied only the prevalence and clinical characteristics of children with clinical Dravet syndrome (including borderline SMEI) caused by *SCN1A* mutations, and have not studied cases with Dravet syndrome caused by mutations in other genes, because these latter patients probably have different clinical characteristics, including the relation between seizures and vaccinations.

Cases with milder phenotypes (for example Genetic Epilepsy Febrile Seizures plus) related to *SCN1A* mutations were not studied, because these phenotypes are variable and aspecific, the prognosis is more favorable and the chance of detecting a *SCN1A* (missense) mutation in children with these phenotypes is low (10% in case of positive family history).[44, 139, 204]

At stage 1 of the study, the cohort was selected with data from a passive surveillance system with a stable rate of reported seizures over the years. With an active survey among ≈40,000 Dutch infants, the incidence rate was similar.[195] The rate was only slightly lower than reported in an RCT including DTwP.[188, 195] The report of the AEFI was delayed several years in some children with Dravet syndrome or other epilepsies, which suggests selective reporting of events that are followed by (severe) epilepsy, especially among delayed notifications. However, when we excluded AEFI that were reported more than two months after vaccination, the proportion of children with Dravet syndrome was even slightly higher.

At stage 1 of the study, we selected children who possibly had Dravet syndrome based on available data, out of the total cohort. Only of these children follow-up was obtained

at stage 2. This selective follow-up, may have resulted in underdetection of cases with Dravet syndrome in the total cohort. However, the criteria for possible Dravet syndrome at stage 1 were broad, which makes inaccurate exclusion of children with Dravet syndrome unlikely.[203, 205]

Unfortunately, on few patients we were not fully informed or they were lost to follow-up. Therefore, the total proportion of *SCN1A*-related Dravet syndrome among children with seizures following vaccination, might be slightly higher. However, based on an estimated prevalence of Dravet syndrome of 1:20,000 to 1:40,000,[17-19] a proportion of vaccination-related seizures in children with Dravet syndrome of 27%,[82] and a proportion of detectable *SCN1A* mutations of 70%,[131] the expected number of *SCN1A*-related Dravet syndrome among children with seizures following vaccination would be between 9 and 19 in this ten-year birth cohort of 1.97 million live births.[206] This is in agreement with our results.

In none of the children reported with seizures related to MMR vaccinations, Dravet syndrome was diagnosed. There may be several explanations for this. Firstly, children with Dravet syndrome often have weekly seizures after one year of age, so the relationship with an MMR vaccine administered a week before, will not always be recognized. In our cohort, at least two of the children with Dravet syndrome had seizures within the at risk time interval after MMR vaccination but these were not reported. Secondly, children with Dravet syndrome were less often vaccinated with MMR vaccine, only 35% (5 of 14) in this study (data not shown). Thirdly, the incidence of febrile seizures in the second year of life is higher, as is the incidence of fever after MMR vaccinations (compared with the incidence after inactivated DTP-IPV-Hib vaccines), resulting in a higher frequency of (febrile) seizures after MMR vaccinations in the general population (Figure 1).[179]

Comparisons with previous studies and findings

No prospective studies on the prevalence of *SCN1A*-related Dravet syndrome among children with vaccination-related seizures have been published yet. A much smaller, retrospective study among children reported with suspected vaccination-related seizures under age six years, showed that 1.2% (4 out of 328) had *SCN1A*-related Dravet syndrome.[207] The overall results of that study are comparable with ours. However, the number of reported seizures in that study was 5-times lower when adjusted for population size, and the proportion of children diagnosed with epilepsy was higher. This suggests that in that study severe adverse events, or events which were followed by epilepsy, were more likely to be reported. Only in part of all children with clinical characteristics of Dravet syndrome, *SCN1A* analysis was performed. Together, this resulted in a similar proportion of established *SCN1A*-related Dravet syndrome as in our study.

In the 12 children with Dravet syndrome with epilepsy onset after vaccination in our cohort, the mean age at first seizure was 4.1 months. These results are comparable with

three previous studies showing a mean age of 4.0, 4.2 and 5.4 months, respectively, in patients with Dravet syndrome with seizure onset after vaccination.[15, 19, 82] All of our patients with Dravet syndrome had a seizure within 4 to 21 hours after vaccination with DTP-IPV(-)Hib vaccine, except for one in whom the seizure occurred 17 days later and was considered to be unrelated to the vaccination. In other studies, seizures occurred within 24 hours, or within 48 hours after administration of inactivated vaccines,[15, 16] but more exact time intervals were not reported. Also for the general pediatric population an increased seizure frequency has been reported only within the first 24 hours after administration of an inactivated vaccine.[179]

Although initial seizures in Dravet syndrome are often febrile, 57.9% of the seizures in our patients occurred with a temperature below 38.5°C. In the study of Berkovic et al., half of the seizures following vaccination were afebrile (defined as a body temperature below 38°C).[15] In another study one third of seizures following vaccination occurred without fever (definition of fever not reported).[82] These results emphasize that in patients with Dravet syndrome, even a mild increase in body temperature or an immune response without increased temperature, can be sufficient triggers for seizures. Moreover, this suggests that although in the general population there is no increased risk of afebrile seizures after inactivated vaccines,[179] this may be the case in subgroups of children with an increased seizure susceptibility.

Unanswered questions and future research

The DTP-IPV vaccines that were administered during the ten-year cohort of our study consisted of a whole cell pertussis (wP) component in children vaccinated in the first eight years of the cohort, and an acellular pertussis (aP) component in the last two years.[208] In several countries wP-vaccines are still used, but the majority of developed countries adopted aP-vaccines.[209] The number of reported AEFI dropped after introduction of DTaP-IPV-Hib vaccine in 2005 in the Netherlands.[208] Interestingly, all detected cases of *SCN1A*-related Dravet syndrome were reported after vaccination with wP-component (Table 3). Therefore, our results may not be representative for aP-vaccines. However, two of our cases also had seizures following vaccinations not containing pertussis (cases 3 and 12), and previously, seizures after aP-vaccines in Dravet syndrome have been described.[16, 83] When longer term follow-up data of a more recent cohort will be available, the relation between the aP-vaccines and Dravet syndrome can be studied in more detail.

For children with Dravet syndrome further studies are warranted to evaluate if adapted vaccination schedules and preventive measures are needed to protect them optimally against infectious diseases while minimizing the risk of seizures. For this purpose, it is important that physicians who make a diagnosis of Dravet syndrome in children who have earlier been reported with seizures following vaccination, report this back to the

surveillance system. For the majority of children diagnosed with Dravet syndrome in our study, this had not been done.

CONCLUSIONS AND IMPLICATIONS

Our study shows that although *SCN1A*-related Dravet syndrome is a rare disorder, 2.5% of reported seizures following vaccinations in the first year of life occurred in children with this disorder. In children with Dravet syndrome, vaccinations might induce the first and following seizures. Physicians and co-workers of immunization safety surveillance programs should have knowledge of the prevalence of Dravet syndrome among children reported with seizures following vaccinations, and of the discriminating seizure characteristics in this subgroup. This will promote earlier diagnoses of Dravet syndrome which is important for appropriate treatment and genetic counseling. Moreover, an early diagnosis will prevent parents and professionals from assuming that vaccination is the cause of the epilepsy, and will thereby promote faith and participation in immunization programs.

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Supplementary table 1: Vaccination schedules from 1997-2006

Table 1A. Vaccination schedules of the NIP from 1997-1999

Age	Injection 1	Injection 2
3 months	DTwP-IPV	Hib
4 months	DTwP-IPV	Hib
5 months	DTwP-IPV	Hib
11 months	DTwP-IPV	Hib
14 months	MMR	
4 years	DT-IPV	
9 years	DT-IPV	MMR

Table 1B. Vaccination schedules of the NIP from 1999-2006

Age	Injection 1	Injection 2[f]
2 months	DTP-IPV[a,b,c]	Hib
3 months	DTP-IPV[a,b,c]	Hib
4 months	DTP-IPV[a,b,c]	Hib
11 months	DTP-IPV[a,b,c]	Hib
14 months	MMR	Men C[d]
4 years	DT-IPV	aP[e]
9 years	DT-IPV	MMR

[a] = from 2003 onwards DTP-IPV was mixed with Hib.

[b] = from 2003 onwards children of whom at least one parent was born in a country where hepatitis B is moderately or highly endemic and children of whom the mother tested positive for Hepatitis B surface Antigen (HBsAg) received DTP-IPV-Hib-HepB.

[c] = from 2005 onwards DTwP-IPV-Hib was replaced by DTaP-IPV-Hib. [d] = from 2002 onwards.

[e] = since November 2001, from 2006 onwards mixed with DT-IPV. [f] = from April 2006 onwards infants received 7 valent conjugated pneumococcal vaccine simultaneously with DTaP-IPV-Hib(-HepB) at 2, 3, 4 and 11 months.

Supplementary Table 2. Follow-up results of children according to classification of seizures at stage 1

Number of children according to classification of seizures in stage 1	Single seizure		Multiple seizures, classification				Total
	atypical seizure	seizure*	atypical seizures	febrile seizures	not further classified**	Epilepsy	
Stage 1							
- Seizures reported after vaccinations	505	375	108	163	21	97	1269
- ***SCN1A*-related Dravet syndrome**	0	0	0	0	0	**2**	**2**
- Other etiological diagnosis	3	4	1	3	1	13	25
- No etiological diagnosis	502	371	107	160	20	82	1242
- Dravet syndrome possible	63 (12.5%)	84 (22.4%)	4 (3.7%)	77 (47.2%)	8 (38.1%)	43 (44.3%)	279 (22.0%)
Stage 2							
- Follow-up data available	49 (77.8%)	70 (83.3%)	3 (75.0%)	67 (87.0%)	5 (62.5%)	40 (93.0%)	234/279 (83.9%)
- Multiple seizures	7 (14.3%)	23 (32.9%)	-	-	-	-	30/119 (25.2%)
- Diagnosed with epilepsy	3 (6.1%)	3 (4.3%)	1 (33.3%)	6 (9.0%)	3 (60.0%)	-	16/194 (8.2%)
- ***SCN1A*-related Dravet syndrome**	**1 (2.0%)**	**0**	**0**	**2 (3.0%)**	**2 (40.0%)**	**8 (20.0%)**	**13/234 (5.6%)**
- Other etiological diagnosis	0	4 (5.7%)	0	3 (4.5%)	0	8 (20.0%)	15/234 (6.4%)
- Inconclusive for Dravet syndrome	1 (2.0%)	0	0	0	0	1 (2.5%)	2/234 (0.9%)

* afebrile, simple febrile, and complex febrile seizures combined; ** combination of atypical, afebrile or febrile seizures.

Supplementary Table 3. Clinical features of children with Dravet syndrome.

	M/F	Age at onset of seizures	Seizure types	Development delay	Neurological symptoms	Vaccination scheme	Age at DNA diagnosis	*SCN1A* mutation	Ref
1	M	5 mo	GTCS, H, SE, M, F	ID	Autism, ataxia, hemiplegia	Incomplete: several vaccinations and without pertussis component	8 years	p.Asp1239Tyr	131
2	M	4 mo	GTCS, H, SE, A, M,	Mild ID (IQ 71 at 7 y)		Stopped	2 years	p.Leu1717Pro	-
3	F	4 mo	GTCS, F, SE, M, A, H, At	Moderate ID (TIQ 50 at 6 y)	Ataxia	Incomplete: without pertussis component	8 years	p.Phe1535fs	-
4	F	3.5 mo	GTCS, H, F, A, M	Severe ID		Complete	5 years	p.Gly1433Glu	210
5	M	4 mo	GTCS, GT, SE, F	Delayed		Complete	3 years	p.Arg946His	15 [b]
6	M	3 mo	GTCS, SE, M, A, F	4 y DQ<50	Autism, ataxia, hypotonia	Incomplete: one vaccination	11 years [a]	p.Arg101Gln	43 [b]
7	M	4 mo	GTCS, M, Ast, A, SE	Mild		Incomplete: several vaccinations	5 years [a]	duplication exon 17-20	-
8	M	5 mo	GTCS, H, GT, SE, M, F, A	Severe		Incomplete: several vaccinations	3 years	c.4278_4282delCAAGT	-
9	F	4.5 mo	GTCS, At, F, SE	Mild	Attention deficit	Incomplete: several vaccinations	2 years	p.Val1390Met	210 [b]
10	F	3.5 mo	GTCS, SE, H, F	Moderate-severe		Incomplete: one vaccination	2 years	c.3880-1G>A	211
11	M	3 mo	GTCS, SE, H, A, F	Delayed		Stopped	1 years	p.Trp384Arg	-
12	M	4.5 mo	GTCS, H, A, M	Severe ID		Incomplete: without pertussis component	23 years	p.Ser1879fs	-
13	M	6 mo	GTCS, H, GT	Moderate ID	Autism	Incomplete: without pertussis component	13 years	p.Gly177Glu	41 [b]
14	F	4 mo	GTCS, F, A, M	Moderate-severe	Autism	Incomplete: without pertussis component	11 years	p.Leu331fs	-
15	M	5 mo	GTCS, H, SE, F, A, M	Severe	Autistic, ataxia	Stopped	8 years	c.2176+2T>A	-

Abbreviations: A = (atypical) absences, At = atonic seizure, Ast = Astatic seizure, F = focal seizure, GTCS = Generalized tonic-clonic seizure, GT = Generalized tonic seizure, H = hemi-convulsions, M = myoclonus, SE = status epilepticus. Ref = references of previously described patients with identical mutations. ID = Intellectual Disability.

[a] Age at first referral for DNA diagnostics was younger, but only after re-analysis (case 6) or use of additional techniques (case 7) an *SCN1A* mutation was detected. [b] Mutation has been described in several other publications as well.

Chapter 3

Etiologies for seizures around the time of vaccination

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ABSTRACT

Objectives: This study was an assessment of the incidence, course and etiology of epilepsy with vaccination-related seizure onset in a population-based cohort of children.

Methods: The medical data of 990 children with seizures after vaccination in the first 2 years of life, reported to the National Institute for Public Health and Environment in the Netherlands in 1997 through 2006, were reviewed. Follow-up data were obtained of children who were subsequently diagnosed with epilepsy and had had seizure onset within 24 hours after administration of an inactivated vaccine or 5 to 12 days after a live attenuated vaccine.

Results: Follow-up was available for 23 of 26 children (median age 10.6 years) with epilepsy onset following vaccination. Twelve children developed epileptic encephalopathy, 8 had benign epilepsies and 3 had encephalopathy prior to seizure onset. Underlying causes were identified in 15 children (65%) and included *SCN1A*-related Dravet syndrome (formerly severe myoclonic epilepsy of infancy) or genetic epilepsy febrile seizures plus syndrome (n=8 and n=1, respectively), a protocadherin 19 mutation, a 1qter microdeletion, neuronal migration disorders (n=2) and other monogenic familial epilepsy (n=2).

Conclusions: Our results suggest that in most cases, genetic or structural defects are the underlying cause of epilepsy with onset after vaccination, including both cases with preexistent encephalopathy, or benign epilepsy with good outcome. These results have significant added value in counseling of parents of children with vaccination-related first seizures and they might help to support public faith in vaccination programs.

INTRODUCTION

Parental fear of vaccination-induced neurological deterioration has led to decreasing vaccination coverage and subsequent outbreaks of preventable infectious diseases in various countries.[2] However, development of neurological deficits or epileptogenesis could not be related to vaccinations, in several independent epidemiological studies.[10, 13, 14, 179, 184] In the general pediatric population, the risk of febrile seizures is twofold to fivefold increased on the day of administration of an inactivated vaccine and from day 5 up to 2 weeks after administration of a live attenuated vaccine.[14, 179, 180, 184, 212, 213] A seizure within this risk period might be the first of an epilepsy syndrome, and parents might misinterpret the vaccination as the primary cause of the epilepsy.

In a retrospective study of 14 children with epileptic encephalopathy assumed to be caused by vaccination, 11 had Dravet syndrome due to *de novo* neuronal sodium channel 1 subunit (*SCN1A)* gene mutations.[15] Dravet syndrome (formerly severe myoclonic epilepsy of infancy) is a rare epilepsy syndrome with seizure onset in the first year of life often triggered by fever, infectious diseases, or vaccinations in a previously healthy child. In the second year, multiple intractable seizure types evolve, and neurodevelopment slows.[48] In ~80% of children with Dravet syndrome a mutation in the *SCN1A* gene is detected.[131] These mutations occur *de novo* in ~95%.[142, 143]

The detection of a pathogenic *SCN1A* mutation in children with alleged vaccination encephalopathy proved that, although the vaccination might have triggered the first seizure, the epilepsy and subsequent intellectual disability were caused by a genetic defect.[15] Most published case series and retrospective cohort studies confirming *SCN1A*-related Dravet syndrome as a cause of alleged vaccination encephalopathy [16, 83, 207, 214] have focused on epileptic encephalopathy. Only a few case reports have shown that occasionally other genetic causes of epilepsy also present with seizures after vaccination.[215, 216] To assess underlying causes in children with any epilepsy syndrome with onset after vaccination, we studied a nationwide 10-year cohort of children reported having possible epileptic seizures after vaccination.

PATIENTS AND METHODS

Selection of the study cohort (stage 1)

We selected a cohort of children (n=1,269) with possible epileptic seizures following vaccination in the first two years of life, reported to the safety surveillance system of the Dutch National Immunization Program between January 1, 1997, and December 31, 2006.[182, 214] This cohort was previously described in detail in a prevalence study of Dravet syndrome that was part of a project on vaccinations and Dravet syndrome approved

by the medical ethical committee of the University Medical Center Utrecht, Utrecht, Netherlands (no. 07/295).[214] Seizures with a temporal relation to vaccination, defined by occurrence within the risk interval of 24 hours after administration of an inactivated vaccine or 5 to 12 days after a live attenuated vaccine (i.e., measles-mumps-rubella [MMR] vaccine),[179-181, 217] were considered to be vaccination-related. Children without vaccination-related seizures were excluded from the present study.

Within the study period, the vaccination schedule in this age group included 4 doses of diphtheria, tetanus, pertussis, inactivated polio vaccine, *Haemophilus influenzae* type b vaccines in the first year of life and 1 dose of MMR vaccine at age 14 months. Additional vaccines were introduced between 2001 and 2006 (Supplementary Tables 1 and 2).[182, 191]

Database of adverse events following immunizations

During the study period, safety surveillance of the national immunization program was performed by the National Institute for Public Health and Environment (RIVM) using an enhanced passive reporting system for adverse events following immunizations (AEFI).[182] AEFIs were reported by either child health clinic staff (~80%), who routinely inquire about adverse events at the next clinic visit after vaccination (according to national guidelines for notification of AEFIs), or by other physicians or parents. The reporting rate of seizures as AEFIs was stable over the years, and similar to the incidence rate within an active study.[195] Reported adverse events were registered in a database after extensive supplementation by obtaining detailed eyewitness accounts, medical history from clinic charts, and information from general practitioners.[182] From this database, we extracted the following data: administered vaccine, ages at vaccination, time of reporting of the AEFI and last follow-up, ages at first and last seizure, classification of seizure(s), body temperature at time of seizure (categorized as <37.5, 37.5-38.4 or ≥38.5 °C.), family history of seizures, and results of genetic, metabolic and imaging studies.

Selection of children with epilepsy and vaccination-related onset (stage 2)

From the cohort of vaccination-related seizures, children in whom this reported seizure had been the first seizure, and who had been diagnosed with epilepsy, were selected. The parents of these children were contacted for written consent to retrieve additional data from medical files. The following additional data were collected: ages at last seizure and last follow-up, types of seizures, response to treatment, results of electroencephalography (EEG), psychomotor development before and after seizure onset, comorbidity, family history, and results of physical examination and genetic, metabolic and imaging studies.

Classification of epilepsy syndromes

Seizure types, epilepsy syndromes, and etiology were classified by a pediatric neurologist (F.E.J.) and a clinical geneticist (N.E.V.) according to the proposed International League Against Epilepsy classification of 2010 based on data from medical files and EEG reports.[21] Children were further categorized as follows: group I, children with a preexistent encephalopathy, defined as developmental delay preceding the seizure onset; group II, children with epileptic encephalopathy, defined as (temporarily) refractory seizures and developmental decline related to recurrent seizures; and group III, children with relatively benign epilepsy, defined as normal development and good seizure control.

Statistical analysis

We compared the characteristics of reported seizures between children with and without epilepsy with vaccination-related onset in our cohort, and between the different subgroups of children with epilepsy. The Mann-Whitney *U* test or Kruskal-Wallis test was used to analyze continuous variables (i.e., ages at first vaccination-related seizure, report of event, last follow-up). Binary variables (i.e., proportions of types of vaccination [inactivated or live attenuated] and body temperature at the time of seizure [< or ≥38.5 °C]) were calculated with either Pearson's chi-squared test, or the Fisher exact test. A threshold of $\alpha = 0.05$ was considered significant.

RESULTS

Baseline characteristics of study cohort

Within the studied 10-year period, 990 children were reported with 1,022 possible epileptic seizures occurring in a temporal relation to either an inactivated vaccine (n=695 [68.0%]), or live attenuated vaccine (n=327 [32.0%]) administered in the first 2 years of life. The median age at end of stage 1 follow-up was 15 months (Table 1). In these 10 years, 1.9 million infants received >7.5 million diphtheria, tetanus, pertussis, inactivated polio vaccine, *Haemophilus influenza* type b combination vaccines, and 1.8 million toddlers received their first MMR vaccine.

Of the 990 reported children (Figure 1), 45 (4.5%) had been diagnosed with epilepsy during stage 1 follow-up; 26 (2.6%) had vaccination-related seizure onset and 19 (1.9%) had seizure onset before the reported vaccination-related seizure. A total of 945 children had febrile, afebrile, or atypical seizures but were not diagnosed with epilepsy during stage 1 follow-up. In 14 (1.4%) of the 990 children, an underlying etiology related to the seizures or seizure susceptibility had been reported to the RIVM during stage 1 follow-up. Those reports did not include any of the 26 children with epilepsy with vaccination-related onset (Figure 1, Table 2).

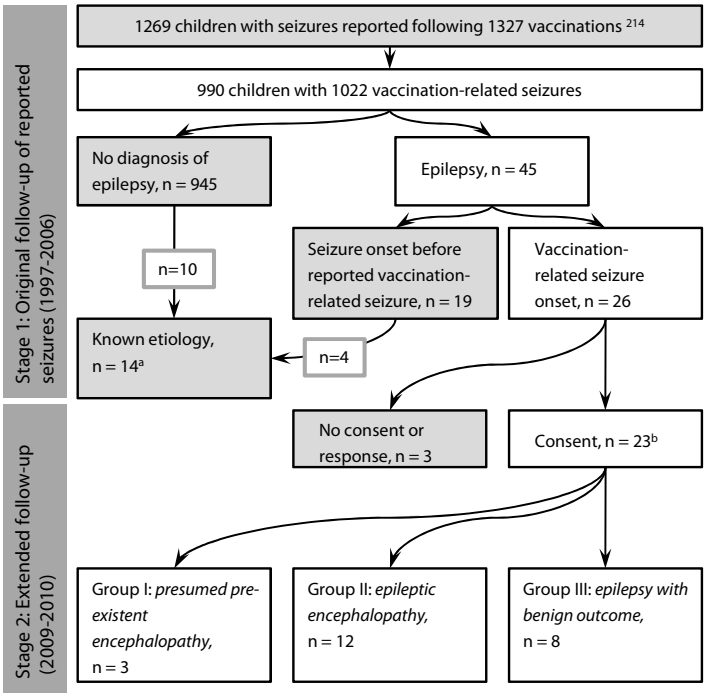

Figure 1. Design of study and overview of results.

[a] See Table 2. [b] See Table 3.

The 26 children with epilepsy with vaccination-related onset (median age at epilepsy diagnosis: 11.5 months; age range 4-41 months) were older at follow-up than the other children (median: 19 vs 15 months; p=0.009). They more often had subsequent vaccination-related seizures (23.1% vs 2.4% in all other children; p<0.001) and more often had body temperatures <38.5 °C during the reported seizures (54.8% vs 20.8%; chi-square test, p<0.001) (Table 1).

Follow-up of children with epilepsy and vaccination-related onset

Parents of 23 (88%) of the 26 children with epilepsy with vaccination-related onset provided informed consent for retrieval of extended follow-up (stage 2) information from medical files. There were no significant differences in baseline characteristics between children with and without extended follow-up (data not shown). The median age at this extended follow-up was 10.6 years (range: 5.6 – 23.6 years). Genetic testing had been performed in 14 (61%) of 23 children. Clinical characteristics and results of ancillary investigations are summarized in Table 3.

Table 1. Baseline characteristics of children with vaccination-related seizures, with or without vaccination-related epilepsy onset

Characteristics of reports	All children (n=990)		*P*	Total No.	Children with vaccination-related epilepsy onset and stage 2 follow-up (n=23)			*P*	Total No.
	Vaccination-related epilepsy onset (n=26)	Other children (n=964)			Group I: Presumed preexistent encephalopathy (n=3)	Group II: Epileptic encephalopathy (n=12)	Group III: Benign epilepsy (n=8)		
Median age in months (range), at									
First reported vaccination	5.6 (2.2-16.2)	11.6 (0.2-22.2)	0.154	990	6.4 (5.6-15.1)	4.7 (2.3-12.1)	13.6 (4.7-16.2)	0.005	23
Report of seizure to safety surveillance system	10.8 (4.3-166)	14.2 (0.3-73.4)	0.860	990	11.9 (5.7-19.9)	6.9 (4.3-166)	16.4 (6.5-25.3)	0.171	23
End of stage 1 follow-up	19 (7-166)	15 (0-105)	0.009	990	18 (13-21)	19 (9-166)	23.5 (7-45)	0.847	23
Number of children with, (%)									
Male gender	11 (42.3)	509 (52.8)	0.290	990	1 (33.3)	5 (41.7)	4 (50.0)	0.685[a]	23
Multiple reports of seizures after vaccinations	6 (23.1)	23 (2.4)	<0.001	990	1 (33.3)	4 (33.3)	1 (12.5)	0.369[a]	23
Number of reported seizures	33	989		1022	4	17	9		30
Inactivated vaccine (vs. attenuated vaccine)	27 (81.8)	668 (67.5)	0.090	1022	3 (75)	17 (100)	5 (55.6)	0.019[a]	30
Temperature <38.5 °C	17 (54.8)	193 (20.8)	<0.001*	961	3 (75)	10 (66.7)	3 (33.3)	0.114[a]	28

*p=0.001 for inactivated vaccines and P=0.018 for live attenuated vaccines; p=0.025 for inactivated vaccines, when known patients with Dravet syndrome were excluded.

[a] Fisher exact test, group III versus groups I and II.

Group I: Presumed preexistent encephalopathy

Three (13%) of the 23 children with vaccination-related epilepsy onset already had developmental delay before seizure onset and developed mild to severe intellectual disability. They were therefore presumed to have preexistent encephalopathy. They developed fever-sensitive epilepsy with focal seizures and had abnormal results on brain MRI scans. One child (case 1) had a terminal microdeletion of chromosome 1q and the second child (case 2) had bilateral periventricular nodular heterotopias (BPNH) without a *FLNA* mutation detected by DNA sequencing. Both children had additional multiple congenital malformations and dysmorphic features. In the third child a distinct developmental delay preceded the seizure onset, but genetic analyses had not been performed.

Group II: Epileptic encephalopathy

Twelve (52%) of the 23 children with vaccination-related epilepsy onset were considered to have epileptic encephalopathy. All but 2 had intractable seizures. In 10 of the 12 children, an underlying cause was determined. Eight children (cases 4-11) were diagnosed with Dravet syndrome (previously described in [214]) and had a pathogenic *SCN1A* mutation.

One girl (case 12) had epilepsy and mental retardation restricted to females (EFMR) and a *de novo* protocadherin 19 (*PCDH19)* mutation (described as case 7 in [170]). One child (case 13) died unexpectedly at age 19 months. Postmortal microscopic examination of brain tissue showed a bilateral perisylvian neuronal migration disorder. Genetic analyses, performed with DNA extracted from postmortal tissue, failed.

One child (case 14) had fever-sensitive epilepsy, which was drug-resistant in infancy but relatively well controlled later on. Results of extensive metabolic and molecular testing, including analysis of the *SCN1A* and the *PCDH19* gene and testing for Angelman syndrome, did not reveal an underlying cause. The last child (case 15) was diagnosed with West syndrome, which later developed into Lennox-Gastaut syndrome. Extensive metabolic and genetic analyses, including analysis of the *SCN1A* gene did not reveal an underlying cause.

Group III: Benign epilepsy

Eight (35%) of the 23 children with vaccination-related epilepsy onset were considered to have relatively benign epilepsy. Seven of the 8 children were seizure-free without use of antiepileptic drugs at follow-up. The median age at last seizure was 4 years (range: 1.3-8.5 years). An underlying genetic etiology was identified or was plausible on the basis of the family history in 3 of the 8 children.

Five of the 8 children had fever-sensitive epilepsy classified as febrile seizures plus (cases 16 and 19-22). In 3 children, a (grand)parent had had febrile seizures as well, consistent with a diagnosis of genetic epilepsy febrile seizure plus (GEFS+) syndrome. In only

Table 2. Underlying causes of seizures related to vaccination, reported at stage 1 follow-up (14 of 990 children)

Type of etiology according to ILAE 2010	Etiology	Type of seizures	Gender	Vaccination	Age at vaccination in months
Children with vaccination-related seizure onset					
Genetic	Sotos syndrome	multiple simple FS	M	MMR	15.5
Genetic	Down syndrome	atypical seizure	M	dh1	3.0
Genetic	Down syndrome	atypical seizure	M	dh3	6.9
Genetic	Wolf-Hirschhorn syndrome	simple FS	M	MMR	14.8
Structural	Dandy walker cyst and congenital hydrocephaly	simple FS	M	dh4	21.5
Acute symptomatic	Acute arteria media infarct due to thrombus in cardiomyopathy	afebrile, focal seizure	F	dh2	3.7
Acute symptomatic	Human Herpes virus 6 infection	complex FS	F	MC	14.6
Acute symptomatic	Invagination	atypical seizure, not epileptic	F	MC	15.5
Children with seizure onset before vaccination-related seizure					
Genetic	GEFS+ syndrome due to *SCN1A* mutation	multiple complex FS	F	MC	15.4
Structural	Perinatal intracerebral hemorrhage	epilepsy	M	dh3	5.3
Structural	Congenital toxoplasmosis infection	epilepsy	F	dh3	6.5
Structural	Perinatal intracerebral hemorrhage	epilepsy	M	dh3	6.8
Structural	Pyridoxin-dependent epilepsy	epilepsy	F	dh4	11.1
Structural	Previous stroke	multiple simple FS	M	d4	16.3

dh, dtp-ipv(-)hib vaccine; FS, febrile seizure; F, female; M, male; MC, MMR and meningitis C vaccine.

1 of those 5 children had genetic testing been performed (case 16). In this child, DNA analysis of the *SCN1A* gene showed a pathogenic mutation, inherited from her affected father (previously described in [218]).

The 3 other children had epilepsy without fever-sensitivity (cases 17, 18 and 23). Two of these 3 children (cases 17 and 18) had multiple, similarly affected family members and were either diagnosed with benign familial infantile epilepsy or unclassified familial epilepsy (Figure 2). Both pedigrees were compatible with autosomal dominant inherited epilepsies, but neither family opted for genetic testing. Case 17 had mild learning problems, as did several other family members with and without epilepsy. The clinical history and EEG results were not compatible with a diagnosis of epileptic encephalopathy. Case 23 had unclassified epilepsy with a negative family history for seizures.

Table 3. Characteristics of epilepsy and development and underlying causes in children with vaccination-related epilepsy onset, at stage 2 follow-up

Number	Gender	Age at vaccination, in months	Reported vaccinations	Seizure classification	Fever sensitivity	Electroclinical syndromes	Outcome	Etiology	Inheritance	CT/MRI scan of brain	Pre-existent DD	DD	ID	Malformations[a]	Molecular testing	Family history[b]
Group I: Presumed preexistent encephalopathy																
1	F	6.4	dh3, 4	GTCS SE At F	+	Epilepsy due to chromosomal disorder	I	Genetic; 1qter deletion	DN	atrophic cortex	+	+	+	+	+	-
2	M	5.6	dh3	F GTCS	+	Epilepsy due to neuronal migration dis.	C	Structural; BPNH		BPNH	?	+	+	+	+	-
3	F	15.2	m	F	+	Unclassified epilepsy	I	Unknown		white matter lesions	+	+	+	-	-	-
Group II: epileptic encephalopathy																
4	F	3.9	dh2, 4	GTCS F SE M A H At	+	Dravet syndrome	I	Genetic; *SCN1A*	DN	normal	-	+	+	-	+	+
5	F	3.4	dh1, 2, 3	GTCS H F A M	+	Dravet syndrome	I	Genetic; *SCN1A*	DN	normal	-	+	+	-	+	+
6	M	4.4	d2	GTCS H A M	+	Dravet syndrome	I	Genetic; *SCN1A*	DN	normal	-	+	+	-	+	-
7	M	5.2	dh3	GTCS H SE F A M	+	Dravet syndrome	I	Genetic; *SCN1A*	DN	aspecific	-	+	+	-	+	+
8	M	4.8	dh3	GTCS H T SE M F A	+	Dravet syndrome	I	Genetic; *SCN1A*	ND	aspecific	-	+	+	-	+	+
9	M	4.9	dh2	GTCS H SE M F	+	Dravet syndrome	I	Genetic; *SCN1A*	DN	normal	-	+	+	-	+	-
10	F	4.6	dh3	GTCS At F SE	+	Dravet syndrome	C	Genetic; *SCN1A*	DN	mild atrophia	-	+	+	-	+	-
11	M	4.4	dh3, 4	GTCS M At A SE	+	Dravet syndrome	I	Genetic; *SCN1A*	DN	normal	-	+	+/-	-	+	-
12	F	12.1	dh4	GTCS H T M A	+	Epilepsy and mental retardation limited to females (EFMR)	I	Genetic; *PCDH19*	DN	normal	-	+	+	-	+	-
13	F	2.3	dh1, 2	F GTCS SE	+	Epilepsy due to neuronal migration disorder	†	Structural; BPSMD (pathology)		atrophia, white matter lesions	?	+	U	+	-	-
14	F	6.4	dh3	GTCS SE F	+	Unclassified epilepsy	C	Unknown		arachnoid cyst	-	+	+	-	+	-
15	F	5.3	dh3	IS F GTCS M A At	-	West syndrome; Lennox-Gastaut	I	Unknown		normal	-	+	+	-	+	-

Table 3. Characteristics of epilepsy and development and underlying causes in children with vaccination-related epilepsy onset, at stage 2 follow-up (continued)

Number	Gender	Age at vaccination, in months	Reported vaccinations	Seizure classification	Fever sensitivity	Electroclinical syndromes	Outcome	Etiology	Inheritance	CT/MRI scan of brain	Pre-existent DD	DD	ID	Malformations[a]	Molecular testing	Family history[b]
Group III: epilepsy with good outcome																
16	F	13.0	dh4, m	GTCS F	+	Febrile seizures plus	SF	Genetic; *SCN1A*	P	NA	-	-	-	-	+	+
17	F	4.7	dh3	F	-	Unclassified, familial epilepsy	SF	Genetic; ND		normal	-	+	-	-	-	+
18	M	5.5	dh3	GTCS	-	Benign familial infantile epilepsy	SF	Genetic: ND		NA	-	-	-	-	-	+
19	F	11.2	dh4	GTCS A SE	+	Febrile seizures plus	C	Unknown		NA	-	-	-	-	-	+
20	M	14.5	mc[c]	F A	+	Febrile seizures plus	SF	Unknown		NA	-	-	-	-	-	-
21	F	14.3	m	GTCS SE	+	Febrile seizures plus	SF	Unknown		normal (CT)	-	-	-	-	-	-
22	M	16.2	m	GTCS F A	+	Febrile seizures plus	SF	Unknown		normal	-	-	-	-	-	+
23	M	14.6	mc	F	-	Unclassified	SF	Unknown		NA	-	-	-	-	-	-

A, absence; At, atonic; BPNH, Bilateral periventricular nodular heterotopias; BPSMD, bilateral perisylvian migration disorder; C, controlled with anti-epileptic drugs; CT, computed tomography; d, diphtheria, tetanus, whole-cell pertussis, inactivated polio vaccine; DD, developmental delay; DN, de novo; F, focal seizure; GTCS, generalized tonic-clonic seizure; h, *Haemophilus influenzae* type b, H, hemiconvulsion, I, intractable; ID, intellectual disability; IS, infantile spasms; ND, not determined; M, myoclonia; m, measles mumps rubella (MMR); mc, MMR and meningococcal serogroup C; P, paternal; SE, status epilepticus; SF, seizure free; T, generalized tonic seizure; U, unknown; †, died;

[a] congenital malformations and dysmorphic features; [b] First or second-degree relative with febrile seizures or epilepsy; [c] Seizure categorized as temporally related to meningitis C vaccination because of 13-hour interval.

Differences in characteristics between subgroups of children with epilepsy with vaccination-related onset

In children with presumed preexistent (group I) or epileptic (group II) encephalopathy, seizures occurred more often after administration of inactivated vaccines (75% and 100%, respectively) and started at younger ages (median: 6.4 and 4.7 months) than in children with benign epilepsy (group III: 55.6% [p=0.019, Fisher exact test for group I/II versus group III], and 13.6 months [p=0.005, Kruskal-Wallis test]). There were no statistically significant differences between the 3 subgroups in body temperature at the time of seizure, delay between seizure and time of report, or age at original follow-up by the RIVM (Table 1).

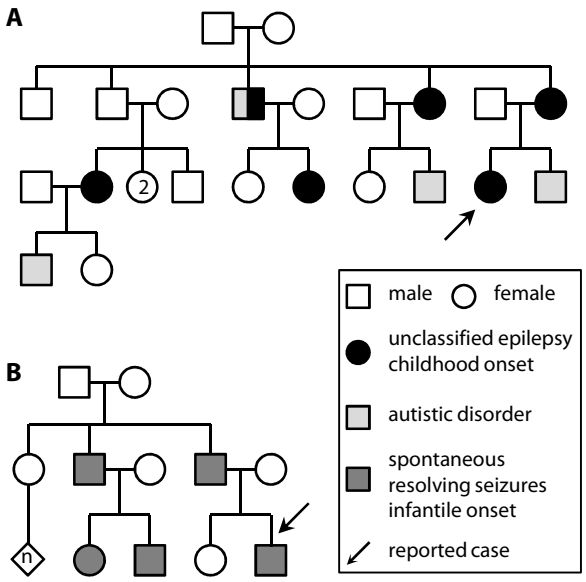

Figure 2. Family pedigrees.

A. Case 17 with unclassified epilepsy. B. Case 18 with benign familial infantile epilepsy. The inheritance pattern of epilepsy was compatible with autosomal dominant transmission in both families.

DISCUSSION

In this Dutch, nationwide, 10-year cohort of children reported with possible epileptic seizures in a time frame related to a vaccination, 26 children (2.6%) were identified in whom the reported seizure was the first of an epilepsy syndrome. Extended follow-up of 23 of these children found that 12 had developed epileptic encephalopathy, 3 had preexisting encephalopathy and 8 had well-controlled epilepsy with normal cognitive outcome.

Underlying causes of the epilepsy syndromes were detected in two-thirds of children, were genetic in the majority of these children, and were likely genetic in 2 additional cases because of the presence of cortical malformations, dysmorphic features and other congenital anomalies. In children with identified genetic causes, these fully explained the electroclinical syndromes and other clinical features. Although no underlying cause was detected in one-third of children with epilepsy with vaccination-related onset, a genetic basis of epilepsy in these children is still possible: genetic analyses were incomplete, some children had positive family histories for seizures, and molecular defects underlying many genetically determined epilepsies have yet to be discovered. In the past, the absence of a detectable underlying cause in children with vaccination-related seizure onset led to the assumption that the vaccination itself was the cause of the subsequent neurological deterioration in some. However, the large variability in electroclinical syndromes and corresponding cognitive outcomes in our study, further support the hypothesis that predisposing factors within the child, and not the vaccination, cause the observed neurological deterioration.[15]

The administered vaccines could have acted as a trigger for the first seizure, thereby unmasking the genetic seizure predisposition in the children in our cohort. Seizure precipitation by vaccination or fever is a hallmark of *SCN1A*-related Dravet syndrome.[80-83] Although fever is a known seizure precipitant in children with a *PCDH19* mutation, a 1q-terminal deletion and *SCN1A*-related GEFS+ syndrome,[139, 167, 219, 220] our study is the first to acknowledge vaccination-related onset in the latter three syndromes. A chance association is unlikely because vaccination-related seizures recurred after a second vaccination in both the GEFS+ and 1qter case, occurred in an additional case of *SCN1A*-related GEFS+ syndrome with only febrile seizures (Table 1), and have previously been reported in another girl with a *PCDH19* mutation and 2 GEFS+ cases.[78, 215]

The majority of children with vaccination-related onset of epilepsy in our study were reported to have fever-sensitive epilepsy at follow up. Interestingly, these children more often had vaccination-related seizures with body temperature <38.5 °C, which was significantly lower than in all other children, confirming previous observations in patients with Dravet syndrome.[214] In this study, we found that significance is sustained after exclusion of children diagnosed with Dravet syndrome (data not shown), suggesting that many children with vaccination-related epilepsy onset have an increased sensitivity to even mild body temperature elevations.

One-quarter of our cases with vaccination-related epilepsy onset had seizures reported after subsequent vaccinations as well. This finding raises the question as to whether these children should receive further vaccinations. The present study was not designed to test the influence of further vaccinations on disease course. However, a study on Dravet syndrome found no differences in outcome between children who did or did not receive further vaccinations.[83] In the groups of children with presumed

preexistent and epileptic encephalopathy, all except one reported seizure occurred after inactivated instead of live attenuated vaccines. Because the estimated increased risks of seizures following both vaccine types are roughly the same,[179] this difference is probably largely explained by the higher number of administered inactivated vaccines (ratio: 4 to 1 live attenuated vaccine) in the first 2 years of life. However, currently, developed countries mainly use less reactogenic acellular pertussis vaccines.[195] Moreover, cancellation of vaccination increases the risk of a vaccine-preventable disease (e.g., pertussis, measles). These diseases may also induce seizures or cause other, more severe complications. All these aspects must be taken into account when reviewing current vaccination guidelines for children with epilepsy.

In our study, approximately one-third of all children with epilepsy with vaccination-related onset had benign epilepsy. This information is important for clinicians and parents because previous studies focused mainly on epileptic encephalopathy with onset after vaccination. This method may have given the false impression that vaccination-related seizure onset in general has a bad prognosis. The proportion of children with benign epilepsy in our study may even be underestimated because we selected a cohort from an enhanced passive reporting system for AEFI. The parents of children with more severe epilepsies may be more likely to consider vaccinations causative of the epilepsy and more willing to report seizures as an AEFI.[221, 222] Because the time interval between the first seizure and the diagnosis of epilepsy is probably longer, and our initial follow-up period was limited, a diagnosis may not have been made within the follow-up period and thus have led to an underestimation of the proportion of benign epilepsy in our study. However, the Dutch vaccine adverse event surveillance system has a very high reporting rate, with limited underreporting of severe adverse events like seizures.[182, 195] Further bias toward a specific subgroup is probably limited because of the high response rate, long prospective follow-up, similar length of initial follow-up and similar delay in reporting the vaccination-related seizure in the 3 subgroups of epilepsy with vaccination-related onset.

We confirm the high proportion of children with *SCN1A*-related Dravet syndrome (8 of 12 cases) within the subgroup of patients with epileptic encephalopathy that was previously described.[15] In our study, West syndrome was a much less frequently identified epilepsy syndrome after vaccination, in contrast to another study,[207] probably because we used strict criteria for a temporal relation between vaccination and seizures.

CONCLUSIONS

In our cohort, underlying genetic or structural causes were identified in 65% of children with epilepsy with vaccination-related onset. These underlying causes were not limited

to *SCN1A*-related Dravet syndrome, but extended to other genetically determined fever-sensitive epilepsies. In addition, one-third of cases had relatively benign epilepsy with good outcome, showing that vaccination-related epilepsy onset does not necessarily have a poor prognosis. These results imply that early genetic testing should be considered in all children with vaccination-related onset of epilepsy and might help to support public faith in vaccination programs.

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Supplementary Table 1 Vaccination schedules of the NIP from 1997-1999

Age	Injection 1	Injection 2
3 months	DTwP-IPV	Hib
4 months	DTwP-IPV	Hib
5 months	DTwP-IPV	Hib
11 months	DTwP-IPV	Hib
14 months	MMR	
4 years	DT-IPV	
9 years	DT-IPV	MMR

Supplementary Table 2 Vaccination schedules of the NIP from 1999-2006

Age	Injection 1	Injection 2[f]
2 months	DTP-IPV-[a,c]	(HepB[b])
3 months	DTP-IPV-[a,c]	
4 months	DTP-IPV-[a,c]	
11 months	DTP-IPV-[a,c]	
14 months	MMR	Men C[d]
4 years	DT-IPV	aP[e]
9 years	DT-IPV	MMR

[a] since 2003 onwards DTP-IPV was mixed with Hib.

[b] since 2003 children with a parent from a country where hepatitis B is moderately or highly endemic and children with the mother positive for Hepatitis B surface Antigen (HBsAg) received DTP-IPV-Hib+HepB.

[c] since 2005 DTwP-IPV-Hib was replaced by DTaP-IPV-Hib.

[d] since September 2002.

[e] since 2002, from 2006 onwards combined with DT-IPV.

[f] since April 2006 infants received 7 valent conjugated pneumococcal vaccine simultaneously with DTaP-IPV-Hib (-HepB) at 2, 3, 4 and 11 months.

Chapter 4

Effect of vaccinations on seizure risk and disease course in Dravet syndrome

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ABSTRACT

Objectives: To study the effect of vaccination-associated seizure onset on disease course and estimate the risk of subsequent seizures after infant pertussis combination and measles, mumps, rubella (MMR) vaccinations in Dravet syndrome (DS).

Methods: We retrospectively analyzed data from hospital medical files, child health clinics, and the vaccination register for children with DS and pathogenic *SCN1A* mutations. Seizures within 24 hours after infant whole-cell, acellular or nonpertussis combination vaccination or within 5 to 12 days after MMR vaccination were defined as 'vaccination-associated'. Risks of vaccination-associated seizures for the different vaccines were analyzed in univariable and in multivariable logistic regression for pertussis combination vaccines and by a self-controlled case series analysis using parental seizure registries for MMR vaccines. Disease courses of children with and without vaccination-associated seizure onset were compared.

Results: Children who had DS (n=77) with and without vaccination-associated seizure onset (21% and 79%, respectively) differed in age at first seizure (median 3.7 vs 6.1 months, $p < 0.001$) but not in age at first nonvaccination-associated seizure, age at first report of developmental delay, or cognitive outcome. The risk of subsequent vaccination-associated seizures was significantly lower for acellular pertussis (9%; odds ratio 0.18, 95% confidence interval [CI] 0.05-0.71) and nonpertussis (8%; odds ratio 0.11, 95% CI 0.02-0.59) than whole-cell pertussis (37%; reference) vaccines. Self-controlled case series analysis showed an increased incidence rate ratio of seizures of 2.3 (95% CI 1.5-3.4) within the risk period of 5 to 12 days following MMR vaccination.

Conclusions: Our results suggest that vaccination-associated earlier seizure onset does not alter disease course in DS, while the risk of subsequent vaccination-associated seizures is probably vaccine-specific.

INTRODUCTION

Childhood vaccinations in general induce only mild adverse events such as fever. Although the incidence of febrile seizures is increased 2- to 3-fold within specific time intervals after various vaccinations,[181, 182, 188] febrile seizures remain uncommon adverse events in the general pediatric population. However, vaccination might precipitate a seizure in a child with a previously unrecognized genetic predisposition for Dravet syndrome (DS) or other genetic epilepsy syndrome.[15, 223]

DS, formerly known as severe myoclonic epilepsy of infancy, is a rare and severe epilepsy syndrome caused by heterozygous mutations in the *SCN1A* gene in at least 70% of cases.[131] Seizures are often precipitated by infectious disease or fever.[32] First seizures precipitated by vaccination are reported in 7% to 57% of DS cases and occur approximately 2 months earlier than other first seizures, without an evident effect on cognitive outcome.[19, 47, 78-80, 82, 83] Subsequent seizures precipitated by vaccination have also been reported in DS,[78, 82, 83] but the risk of these seizures is unknown. We therefore estimated the risk of subsequent seizures after infant whole-cell (wP), acellular (aP) and nonpertussis (non-P) combination vaccination, and measles, mumps, and rubella (MMR) vaccination in a nationwide DS cohort. Since the question remains whether earlier seizure onset affects epileptogenesis, we also studied the effect of vaccination-associated seizure onset on disease course in DS.

METHODS

Patient cohort

The study cohort consisted of children with *SCN1A*-related DS referred for genetic counseling or DNA diagnostics to the Department of Medical Genetics of the University Medical Center Utrecht. The majority of Dutch patients with *SCN1A*-related DS are known at this center because until 2012 it was the only one to perform *SCN1A* analysis in the Netherlands. *SCN1A* mutations were considered pathogenic when previously reported in patients with DS, predicted to lead to haploinsufficiency or occurring "de novo" in the patient. Novel missense mutations inherited from a milder affected parent were considered to be likely pathogenic. Children with *SCN1A* mutations, who had seizure onset within the first 12 months of life and subsequently developed a drug-resistant epilepsy with multiple seizure types and neurodevelopmental slowing, were considered to have DS.

Parents were invited by their physician to participate in this study if the child was younger than 19 years at study inclusion (2009 -2013).

Standard protocol approvals, registrations, and patient consents

Written informed consent was obtained from the parents of all children, for data retrieval from medical records and the vaccination registry, and additionally for participation in a questionnaire. The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht (07/295).

Changes in the Netherlands Vaccination Program

The Netherlands Vaccination Program (NVP) includes 4 doses of pertussis combination vaccine in infancy, with or without simultaneous other vaccines (Table 1). The first 3 infant pertussis combination vaccinations were advanced 1 month to ages 2, 3, and 4 months (subcohorts wP3 vs wP2) in 1999. Whole-cell (wP) pertussis combination vaccine was replaced by acellular (aP) pertussis combination vaccine (subcohort aP2) in 2005.[182, 191, 195] Until the late nineties children diagnosed with epilepsy regularly received infant combination vaccines without the pertussis (non-P) component, because epilepsy was considered a relative contraindication for wP vaccination[224] but not an absolute contra-indication for any vaccination. MMR vaccine is administered at age 14 months, since 2002 simultaneously with a conjugated meningococcal serogroup C vaccine.[182]

Table 1: Vaccination schedules of the NVP in the first 2 years of life, from 1990 to 2011

Age at vaccination	1990-1999, wP3		1999-2004, wP2		2005-2011, aP2	
	Injection 1	Injection 2	Injection 1	Injection 2	Injection 1	Injection 2
2 months	-	-	DTwP-IPV-Hib[b]	(HepB[c])	DTaP-IPV-Hib(-HepB)[e]	PCV7[f,g]
3 months	DTwP-IPV	Hib[a]	DTwP-IPV-Hib[b]		DTaP-IPV-Hib(-HepB)[e]	PCV7[f,g]
4 months	DTwP-IPV	Hib[a]	DTwP-IPV-Hib[b]		DTaP-IPV-Hib(-HepB)[e]	PCV7[f,g]
5 months	DTwP-IPV	Hib[a]	-		-	
11 months	DTwP-IPV	Hib[a]	DTwP-IPV-Hib[b]		DTaP-IPV-Hib(-HepB)[e]	PCV7[f,g]
14 months	MMR	-	MMR	Men C[d]	MMR	Men C

DTwP-IPV: diphtheria, tetanus, whole-cell pertussis, inactivated polio; aP: acellular pertussis; Hib: *Haemophilus influenza* type b; HepB: hepatitis B; MMR: measles-mumps-rubella; Men C: Meningococcal serogroup C; PCV7: 7 valent conjugated pneumococcal.

[a] since 1993 [b] since 2003 DTwP-IPV was mixed with Hib; [c] since 2003 children with a parent from a country where hepatitis B is moderately or highly endemic and children with the mother positive for Hepatitis B surface Antigen (HBsAg) received DTwP-IPV-Hib+HepB; [d] since 2002; [e] since October 2011 DTaP-IPV-Hib-HepB replaced DTaP-IPV-Hib for all infants; [f] since 2006; [g] since May 2011 PCV10 replaced PCV7.

Collection of vaccination data

Individual vaccination schedules were retrieved from the National Vaccination Registry for all children. Because only month and year of vaccination had been registered until 2005, exact vaccination dates were retrieved from child health care files or parental copies of the infant vaccination cards, when available. Seizures within 24 hours after

administration of an inactivated (pertussis combination) vaccine or within 5 to 12 days after administration of the live attenuated MMR vaccine[179, 180, 183, 184, 214] were classified as 'vaccination-associated' if exact dates for both vaccination and seizure were known or if this relationship was reported by at least 2 different sources, i.e., medical files, child health care files, parents, or report of the safety surveillance of the NVP.

Collection of medical data

The following data were retrospectively collected from medical records: ages at first and second seizure, DNA diagnosis, start of antiepileptic drug (AED) treatment, first report of developmental delay, and last follow-up or death; duration of and body temperature at first and second seizures; *SCN1A* mutation type (missense vs other); number of intensive care (ICU) admissions related to vaccinations or infections in the first 2 years of life; any hospital admission related to infection; and neuropsychological test results. Cognitive outcome was dichotomized as IQ <50 or >50 (moderate/severe vs mild/borderline intellectual disability).

Parental questionnaire

Parents received a questionnaire by mail or e-mail and answered the questions during a structured telephone interview. The questionnaire included: date and body temperature of first and second seizure, seizures related to vaccination, first notice of developmental delay, and neuropsychological test results. Parents who kept a seizure diary were asked to provide a copy for the 6 weeks following MMR vaccination in the second year of life.

Statistical analysis

Differences in baseline characteristics and parameters for disease course between children with DS with and without vaccination-associated seizure onset and differences between the 3 subcohorts were calculated with either Pearson's chi-square test or Fisher's exact test for binary variables and with Mann-Whitney *U* test or Kruskal-Wallis test for continuous variables, as appropriate. Differences in observed risk of subsequent vaccination-associated seizures between wP, aP, and non-P combination vaccinations in the first 2 years of life, were calculated with Fisher exact test. The influence of potential confounders on these risks was tested in multivariable logistic regression analysis. Variables with a p value <0.2 in univariable analysis were included in the multivariable model. Results are presented as odds ratios (ORs) with 95% confidence interval (CIs).

Children without any vaccination, with start of vaccination after age 6 months, or with non-Dutch vaccination schedules were excluded from the subcohort and pertussis combination vaccine risk analyses. Children who had been diagnosed with DS only as a result of our previous study because they had been reported with vaccination-associated seizures[214] were also excluded from these analyses to avoid selection bias.

To estimate the seizure risk following MMR vaccinations, the incidence rate ratio (IRR) for seizures was calculated in self-controlled case series (SCCS) design with days 5-12 after vaccination set as risk window and days 1-4 and 13-42 as control period.[225] Patients without seizures between days 1 and 42 could not be included in this analysis. Data were analyzed using Access, Excel, IBM SPSS Statistics 20, and SAS 9.3. In all analyses, a *p* value <.05 was considered statistically significant.

RESULTS

Baseline characteristics of DS children

Parents of 77 of 88 children (88%) (43 males, 56%) fulfilling inclusion criteria, gave consent for retrieval of medical and vaccination registry files (Figure 1). Median age at study was 9.8 (range 1.9-20) years. DNA was analyzed at a median age of 4.4 (range 0.8-18.8) years and showed missense mutations in 31 (40%) and other mutations in 46 (60%) children. Copies of child health care files or vaccination cards were available for 61 (79%) children. Parents of 71 children (92%) (median age 10.7 [range 1.9-22.8] years) were interviewed.

Vaccination-associated seizure onset and disease course in DS

Seventy-three children (95%) started their vaccinations according to the NVP schedule, 1 (1%) at a later age, 2 (3%) according to non-Dutch schedules, and 1 (1%) child did not receive any vaccination. Overall, 16 of 77 children (21%) had their first seizure within 24 hours of a pertussis combination vaccination (Table 2). Of the other 61 children, 4 had their first seizure before any vaccination.

Vaccination-associated first seizures occurred at a significantly younger age than first seizures unrelated to vaccination, but body temperature (63% vs 61% ≥38 °C), seizure duration, and recurrence rate of seizures within 24 hours were not different (data not shown). Children with and without vaccination-associated seizure onset had similar age at first nonvaccination-associated seizure, age at first report of developmental delay, cognitive outcome, and baseline characteristics, but AEDs were started at a significantly younger age in children with vaccination-associated seizure onset. For the single child without any vaccinations, the ages at first seizure (4.5 months) and first report of developmental delay (18 months) did not differ from other children with DS (Table 2).

Effect of vaccination schedules on vaccination-associated seizure onset in DS

The wP3, wP2 and aP2 subcohorts included 21, 26, and 24 children, respectively (Table 3). Six of 77 children (8%) were excluded because of no vaccinations or different schedules (n=4) or a DS diagnosis as a result of our previous study on DS and vaccinations (n=2) (Figure 1).[214] The proportion of vaccination-associated first seizures was the highest in

wP3 and the lowest in aP2, but this difference was not statistically significant. Despite a younger age at first vaccination in wP2 and aP2 then in wP3, the ages at vaccination-associated first seizures were not different. Following seizure onset, use of alternative vaccination schedules including non-P combination vaccines was the highest for wP3 and lowest for aP2.

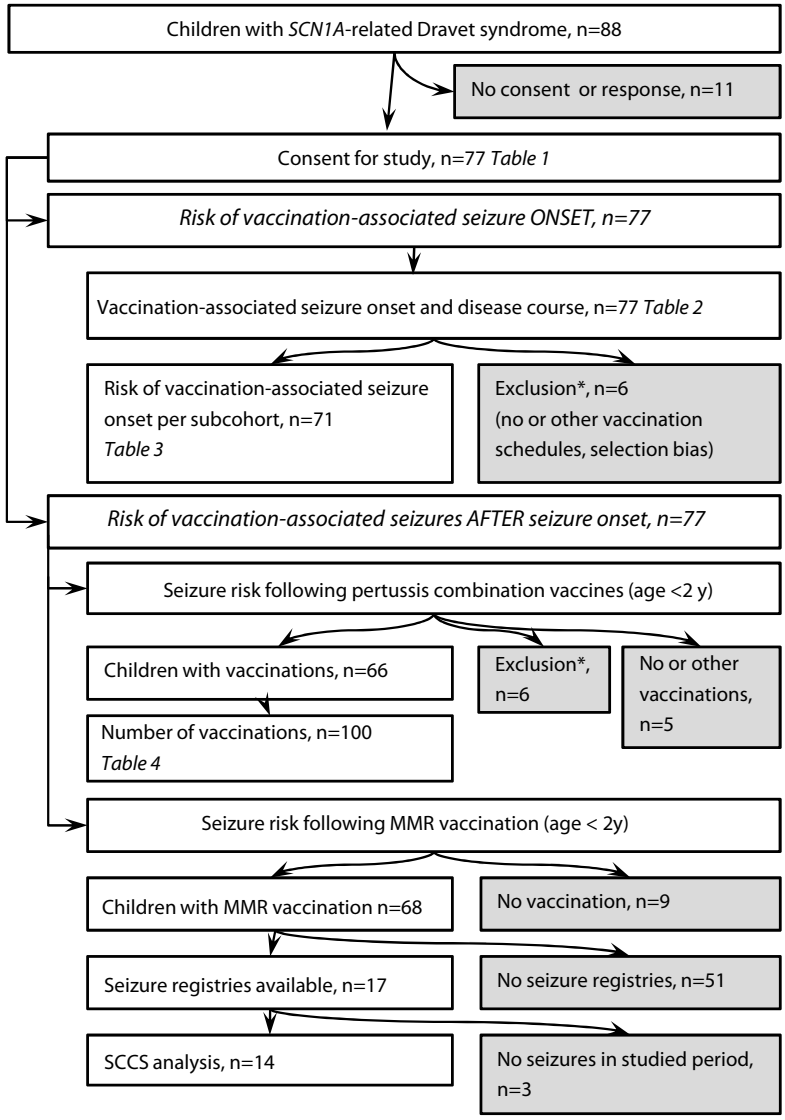

Figure 1. Design of study.

* = same exclusion criteria

Table 2. Disease progression in children who had DS with and without vaccination-associated seizure onset

	Children with DS				
	First seizure vaccination-associated (n=16, 21%)	No. available	First seizure not vaccination-associated (n=61, 79%)	No. available	*p* value
Event, median age (range), mo					
- First seizure	3.7 (2.0-4.9)	16	6.1 (0.8-12)	61	<0.001[a]
- First nonvaccination-associated seizure	5.5 (3.6-9.0)	15	6.1 (0.8-12)	61	0.449
- Start of AED treatment	6.1 (3.4-15)	16	9.1 (2.6-25)	61	0.008[a]
- First developmental delay reported in medical files	23 (16-52)	15	24 (6-68)	57	0.939
- First developmental delay noticed by parents	24 (6-84)	15	21 (6-72)	53	0.688
Outcome of children with DS, n (%)					
- Subsequent vaccination-associated seizures	11 (69)	16	26 (44)	59	0.08
- Cognitive, IQ < 50	11 (73)	15	34 (59)[b]	58	0.379
- Deceased[c]	0 (0)	16	3 (5)	61	1.00

Abbreviations: AED = antiepileptic drug; DS = Dravet syndrome. [a] Significant values. [b] IQ<50 in 16/26 (62%) of those with and 16/30 (53%) of those without subsequent vaccination-associated seizures; p=0.536, Chi-square test. [c] Causes of death: status epilepticus (n=1), sudden unexpected death in epilepsy (n=2).

Risk of vaccination-associated subsequent seizures for infant (pertussis) combination vaccines in DS

After seizure onset, 66 of the 71 children (93%) eligible for the subcohort analysis received a total of 100 infant combination vaccinations (Table 4). Median age at vaccination was 11.2 (range 2.1 – 23.1) months. Of these 100 vaccinations, 62 (62%) were the fourth dose and 59 (59%) were administered while using AEDs. Two (2%) were administered after an *SCN1A* mutation had been identified.

For 21 vaccinations (21%), an associated seizure had been reported (Table 4). The risk of vaccination-associated seizures was significantly lower for aP and non-P than for wP combination vaccines. ORs were 0.18 (95% CI 0.05-0.71) for aP and 0.11 (95% CI 0.02-0.59) for non-P combination vaccines with wP set as reference, adjusted for vaccination-associated seizure onset (OR 3.2, 95% CI 0.96-10.4). Sex, mutation type, use of AED, age at vaccination, and vaccine dose (first to third vs fourth) were not independent predictors of seizure risk.

Table 3. Vaccination-associated seizure onset and vaccine uptake in children with DS according to vaccination schedule

	Year of birth/vaccination, vaccination schedule, and subcohort			
	1990-1999, whole-cell 3m, wP3 (n=21)	1999-2004, whole-cell 2m, wP2 (n=26)	2005-2011, acellular 2m, aP2 (n=24)	*p* value
Vaccination-associated first seizure, median age (range), mo	3.7 (3.1-4.9)	4.0 (2.9-4.6)	4.3 (3.4-4.5)	0.901
Children with DS, n (%)				
Vaccination-associated first seizure	6 (29)	5 (19)	3 (12)	0.355[a]
- First dose	3 (50)	0 (0)	0 (0)	
- Second dose	3 (50)	2 (40)	1 (33)	
- Third dose	0 (0)	3 (60)	2 (66)	
Use of alternative[b] vaccination schedule after seizure onset	18 (86)	13 (50)	7 (29)	0.001[c]

[a] Fisher exact test, aP2 vs. wP2 and wP3.

[b] For example, discontinuing all vaccinations, discontinuing pertussis vaccinations, postponing vaccinations.

[c] Significant value.

Risk of seizures after MMR vaccinations in DS

Sixty-eight of 77 children (88%) received MMR (with or without meningococcal serogroup C) vaccination in the second year of life. In 20 (29%), a seizure within the risk window of days 5 to 12 after MMR vaccination was reported in their medical files, but this temporal relation was only recognized by physicians in 13 of 20 (65%) and recalled by 4 of 18 (22%) interviewed parents.

Seizure diaries for the 6 weeks after MMR vaccination (median age 15 months) were available for 17 children (25%), of whom 14 (82%) had at least one registered seizure in this period. The seizure IRR was 2.3 (95% CI 1.5-3.4) for the risk window of days 5 to 12 after MMR vaccination (Supplementary Figure 1).

Infectious diseases and hospital admissions in DS

Infections preventable by regular NVP-vaccines were not reported in the medical files of the 77 children with DS. Complications of other infections led to hospital admission in 52 children (68%) and included 43 ICU admissions within the first 2 years of life in 24 children (31%). In 2 of these ICU admissions, not only infection but also vaccination may have contributed to seizure aggravation. Two additional ICU admissions were only vaccination-associated.

Table 4. Risk of vaccination-associated seizures after seizure onset for (pertussis) combination vaccines in the first 2 years of life in Dravet syndrome

	Type of combination vaccine				
	All	Whole-cell (wP)	Acellular (aP)	Without pertussis (non-P)	*p* value (Chi-square)
No. of vaccines (%)					
Administered	100	43	33	24	
With seizure	21 (21)	16 (37)	3 (9)	2 (8)	0.001[a]
OR (95% CI)					
Crude	--	Ref.	0.17 (0.04-0.64)	0.15 (0.03-0.74)	
Adjusted[b]	--	Ref.	0.18 (0.05-0.71)	0.11 (0.02-0.58)	

Abbreviations: CI = confidence interval; OR = odds ratio; Ref. = reference.

[a] wP vs. aP/non-P combination vaccine. [b] adjusted for vaccination-associated seizure onset.

Vaccinations: wP: DTwP-IPV had been combined or simultaneously administered with Hib at 41 (95%) vaccinations, and simultaneously administered with HepB at one (2%) vaccination. aP: DTaP-IPV was combined with Hib at 31 (94%) and combined or simultaneously administered with HepB at 7 (21%) vaccinations, and simultaneously administered with PCV at 18 (55%) vaccinations. Non-P: DT-IPV had been simultaneously administered with Hib at 17 (71%) vaccinations.

DISCUSSION

This retrospective cohort study shows that although vaccination-associated seizure onset in DS occurs at a significantly younger age, this does not lead to a younger age at first seizure unrelated to vaccination and onset of developmental delay. This provides direct evidence that there is no effect of vaccination-associated seizure onset on disease course in DS, supporting previous studies that did not show a relation between vaccination-associated, earlier seizure onset and cognition.[19, 78, 83] However, patients with earlier, vaccination-associated seizure onset started earlier with AED-treatment, which might have prevented deterioration. The relation between timing of AED introduction and disease course in DS has not been studied and is unknown. Of note, advancing the start of vaccination schedules from age 3 to age 2 months did not influence age at vaccination-associated seizure onset. This is in line with a reported onset of seizures after age 2 months in most cases of DS,[47, 131] probably resulting from age-dependent *SCN1A* expression.[226]

Several cohort studies have described subsequent seizures following pertussis combination vaccines,[47, 82, 214] and our study provides novel data on the quantitative risk of vaccination-associated seizures after seizure onset in DS. Our study shows that replacement of wP by aP combination vaccines leads to fewer vaccination-associated seizures in DS. A previous study failed to show differences between these vaccines, but analyzed only first seizures in a smaller cohort.[83] Our finding of lower risks following aP vaccines is in agreement with the reduced risk of febrile seizures in the general pediatric

population,[186, 187] even though vaccination-associated seizures in DS frequently occurred without fever in our and previous studies.[78, 82, 83, 214] These reductions in seizure risk are probably explained by lower reactogenicity of aP vs wP vaccines.[195] Seizures in the risk period following MMR vaccinations have only occasionally been reported in DS[82, 214, 227] but were very common in our cohort. Our estimated seizure IRR following MMR vaccines in DS does not differ from the relative seizure risk in the general pediatric population.[179-181, 185, 212] These findings suggest that although absolute seizure risks following various vaccinations are substantial in DS, vaccine-specific *relative* seizure risks for the general population might also apply to patients with DS.

Our study has a number of strengths. We used multiple sources to validate both vaccination status and its temporal relation to seizures. We collected several important determinants in the multivariable regression to identify possible confounders. To study the effect of vaccination-associated seizure onset on disease course in DS, we measured a number of different parameters instead of only age-dependent cognitive outcome.[45] Our study has also some limitations. Since the purpose of the study was known to parents, a selective participation of children with vaccination-associated seizures cannot be ruled out, which may have resulted in an overestimation of seizure risks. However, we expect this effect to be limited since the participation rate was high, vaccination-associated seizures occurred also in children who were eligible but not included in the study (data not shown), and the percentage of vaccination-associated seizure onset in our cohort (21%) is in agreement with previous reports (7-57%, median 23%).[19, 47, 78-80, 82, 83] Another limitation is the small number of patients eligible for the SCCS analysis. This may have introduced inclusion bias to those with a high seizure frequency, since children were only eligible when parents had kept a seizure diary and had had at least one seizure in the studied period. A bias towards children with MMR-associated seizures is less likely because only few parents recalled MMR-associated seizures and all except 2 had seizures outside the MMR risk window as well. Since each case served as its own control, and the seizure IRR was similar to the relative risk in the general pediatric population, we think that the IRR may apply to the whole group of patients with DS.

Differences in detected vaccination-associated seizure risks between various pertussis combination vaccines might have been influenced by other differences between older and younger patients with DS: first, other changes in vaccination schedule were made as well, including introduction of simultaneous other vaccinations (e.g., pneumococcal conjugate vaccine) or vaccine combinations, shifts of vaccine producers, and changes on an individual basis;[182] Second, data on vaccination-associated seizures may have been less complete in older patients because of the retrospective nature of the study; and finally, younger children may have been less severely affected because improved awareness may have increased diagnosis of milder DS cases over time. However, we

believe that these differences between subcohorts have had limited effect, because the risk following wP vaccines administered in the oldest patients was highest and the less reactogenic aP and non-P combination vaccines administered to respectively the youngest and oldest patients gave strikingly similar seizure risks. To define these vaccination-associated seizures, we used an interval of 24 hours, while some other studies[78, 83] included the day after vaccination as well. However, this difference has not influenced our results, since all reported seizures on the day after vaccination in our cohort occurred within 24 hours after vaccination. Even if we had used a longer interval of up to 72 hours,[82] this would have led to the inclusion of only 2 more reported seizures.

Because of low numbers in the subcohorts, the reduced risk of vaccination-associated seizure onset after replacement of wP by aP vaccines did not reach statistical significance. Both this finding and the estimated seizure risk following MMR vaccines need replication in a larger cohort. A prospective observational study determining exact risks of vaccination-associated seizures would overcome the limitations of our study but may be challenging to design for vaccinations in infancy because DS is often diagnosed only after the first year of life.

Since vaccination-associated seizure onset was associated with an increased risk of subsequent vaccination-associated seizures, some children with DS might be more prone to vaccination-associated seizures than others. Since this risk was not influenced by *SCN1A* mutation type, it might be interesting to study whether individual variation in immune responses determines risk of vaccination-associated seizures. Future studies in DS might also address complication rates following both vaccination and infection, underlying mechanisms of afebrile vaccination-associated seizures, and the effect of prophylactic treatment with antipyretics on these risks.

Based on our and previous findings, physicians may reassure parents that vaccination-associated seizures in general probably do not affect disease course or cognitive outcome. A diagnosis of DS is not a contra-indication for further vaccinations[228] and there is no evidence for an increased incidence of other adverse events following vaccinations in DS. Moreover, because natural infections probably have higher complication rates than vaccinations in DS[227] and often led to seizure aggravation and hospital admission in our study, children with DS may significantly benefit from vaccinations. However, seizures in DS in general may progress to status epilepticus, which may, in some patients, lead to acute encephalopathy with severe neurological deterioration,[32] and this might also apply to vaccination-associated seizures. Therefore, precautions to reduce vaccination-associated seizure risk such as use of vaccines with lower seizure risk when available, should be considered.

Our study shows that vaccination-associated seizure onset does probably not affect disease course in DS, while the risk of subsequent vaccination-associated seizures seems vaccine-specific.

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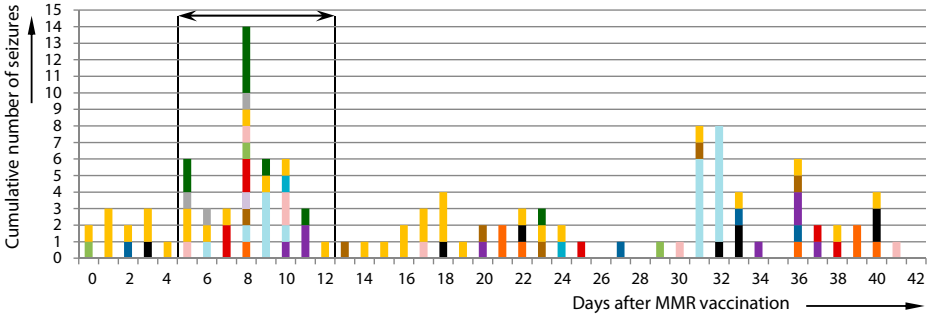

Supplementary Figure 1. Cumulative number of seizures according to seizure diaries for the six week following MMR vaccination in 14 children with Dravet syndrome.

Day 0 is the day of administration of the vaccine. The two sided arrow indicates the risk window of day 5 to 12 following vaccination. Each colour represents a different child.

Chapter 5

Seizure precipitants in Dravet syndrome: What events and activities are specifically provocative, compared with other epilepsies?

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ABSTRACT

Objectives: This study aimed to describe seizure precipitants in Dravet syndrome (DS) compared with other epilepsies.

Methods: Seizure precipitants as reported in a Dutch cohort of patients with DS with pathogenic *SCN1A* mutations (n=71), were compared with those of a cohort with childhood epilepsy (n=149) and of a community-based cohort with epilepsy (n=248); for all three Dutch cohorts, the same type of questionnaire was used. Seizure precipitants were categorized as 'fever', 'visual stimuli', 'sleep deprivation', 'stress, including physical exercise', 'auditory stimuli' and 'other'.

Results: For 70 (99%) of 71 patients with DS, at least one seizure precipitant was recalled by parents. Seizure precipitants that were reported in more than half of the cohort with DS were as follows: having a fever (97%), having a cold (68%), taking a bath (61%), having acute moments of stress (58%), and engaging in physical exercise (56%). Seizure precipitants freely recalled by parents were often related to ambient warmth or cold-warmth shifts (41%) and to various visual stimuli (18%).

Patients with DS had more positive seizure precipitant categories (median 4) compared with the cohort with childhood epilepsy (median 2) and the community-based cohort with epilepsy (median 0) ($p < 0.001$) and showed the highest percentage in each category (all $p < 0.001$). Within the category 'stress, including physical exercise', physical exercise was more often reported to provoke a seizure in stress-sensitive patients in the cohort with DS than in the cohort with childhood epilepsy (78% vs. 35%, $p < 0.001$). In the cohort with childhood epilepsy, physical exercise was more often reported in fever-sensitive children than in other children (25% vs. 12%, $p = 0.042$).

Conclusions: Our study shows a high prevalence of a range of seizure precipitants in DS. Our results underscore elevated body temperature as an important seizure precipitant, whether caused by fever, warm bath, ambient warmth, or physical exercise. Knowledge of these seizure precipitants may improve preventive strategies in the otherwise difficult treatment of DS.

INTRODUCTION

Dravet syndrome (DS) is a severe epilepsy syndrome with onset of seizures in the first year of life and subsequent evolution to polymorphous and therapy-resistant seizures.[24] Fever is the best-known and most characteristic seizure precipitant in DS, especially in early childhood. Vaccinations and hot water baths have been shown to be common precipitants for first and subsequent seizures in DS as well.[24, 25, 33, 77] A variety of other factors, including flickering sunlight, striped patterns, music, fatigue, stress, and physical exercise, have been reported to provoke seizures in individual cases but have not been studied systematically in cohorts with DS.[24, 35, 48, 49, 58, 84, 85, 118, 229] As seizures in patients with DS are often pharmacoresistant, other treatment strategies, including life style adjustments, are important to improve seizure control or to prevent seizures. To be able to accurately counsel and advise patients and their caregivers with respect to life style, identification of precipitating factors is essential.

In this study, we aimed to assess the frequency of various seizure precipitants in a large cohort of Dutch patients with DS with *SCN1A* mutations and detect a Dravet-specific precipitant spectrum using two other Dutch epilepsy cohorts for comparison.

MATERIALS AND METHODS

Dravet cohort

In this study, we included patients with DS with pathogenic *SCN1A* mutations who had been referred for genetic counseling or DNA diagnostics to the Department of Medical Genetics of the University Medical Center Utrecht. The majority of Dutch patients with *SCN1A*-related DS are known at this center because until 2012 it was the only center to perform *SCN1A* analysis in the Netherlands. Dravet syndrome was diagnosed in patients with seizure onset within the first 12 months of life, pharmacoresistant epilepsy with multiple seizure types, and slowing of development after seizure onset. *SCN1A* mutations were considered pathogenic when previously reported in patients with DS, predicted to lead to haploinsufficiency or occurred *de novo* in the patient. Novel missense mutations inherited from a milder affected parent were considered to be likely pathogenic. Mutations were categorized as 'missense' or 'loss-of-function' mutation.

Parents of all patients diagnosed with DS, who were under the age of 19 years at the time of study inclusion (2009-2013), were invited by their treating physician to participate in this study. Parents provided written informed consent for active participation via a questionnaire and data retrieval from medical records. Data on the following variables were extracted: sex, age at completion of questionnaire, age at first seizure, seizure frequency, neuropsychological test results, or intellectual disability (defined as IQ < 70).

The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht (no. 07/295) and performed in 2013.

Cohort with childhood epilepsy and community-based cohort with epilepsy

To detect Dravet-specific reported seizure precipitants, we compared these in the cohort with DS with precipitants reported in two cohorts recently studied: a cohort with childhood epilepsy from the same University Medical Centre and a community-based cohort with epilepsy from the same geographical region.[230, 231]

In the cohort with childhood epilepsy, all children 2–16 years of age with active epilepsy (i.e., a definite clinical diagnosis of epilepsy, and seizures within the two years prior to data collection), who consulted one of three pediatric epileptologists of the UMC Utrecht between 2008 and 2010, were selected. Of the 345 patients fulfilling the selection criteria, 153 (44%) had responded. The purpose of this questionnaire-based study was to address the relationship between stress, among other seizure precipitants, and epilepsy.[230]

In the community-based cohort with epilepsy, all patients of at least 12 years of age, with at least two antiepileptic drug (AED) prescriptions in the last two years, were electronically selected from 30 public pharmacies in the (sub)urban area 'het Gooi-Utrecht' in the central part of the Netherlands in 2010 and approached by mail.[231] After a single mail approach to 1894 patients, 575 (30%) responded, and, in 248 of them, a definite epilepsy diagnosis was confirmed by evaluation of medical data and questionnaires.[232] Both studies were approved by the institutional medical ethics committee. From both cohorts, we excluded patients with DS (n=4 and n=0, respectively) for data comparisons.

Questionnaire for general and specific seizure precipitants

Questionnaires used for the three cohorts were send via mail or e-mail. The DS questionnaire was completed through telephone interviewing (NV), while responses to the questionnaires for the cohort with childhood epilepsy and the community-based cohort with epilepsy were written and send via postal mail. The three questionnaires were largely, but not completely, overlapping with respect to seizure precipitating factors (Table 1). Seizure precipitants from all three questionnaires were classified into the categories 'fever', 'visual stimuli', 'sleep deprivation', 'stress, including physical exercise', 'auditory stimuli' and 'other'. Additionally, the questionnaires for the cohort with DS and the cohort with childhood epilepsy included detailed questions on specific stressors as seizure precipitants (Supplementary Table 1). Patients who scored positive for the category 'stress, including physical exercise' were considered to be stress-sensitive.

Table 1. Comparison of questionnaires used in the cohort with Dravet syndrome and in two other Dutch cohorts with epilepsy.

Precipitating factors asked	Dravet syndrome cohort	Childhood epilepsy cohort[230]	Community-based epilepsy cohort[231]
General questions			
Are there any circumstances/precipitants that often provoke seizures, in the present or in the past?	+	-	-
Are there any factors that can provoke seizures?	-	+	-
Are there any circumstances/precipitants that most often provoke seizures?	-	-	+
Category Fever			
Fever	+	+	+
Category Visual stimuli			
Flashing lights	-	+	-
Flashing sunlight	+	-	+
TV	+	-	+
Video games	+	-	+
Disco	-	-	+
Disco lights	+	-	-
Category Sleep deprivation			
Sleep deprivation	+	+	+
Category Stress, including physical exercise [a]	[a]	[a]	
Stress	-	-	+
Stressful periods	+	+	-
Acute moments of stress	+	+	-
Physical exercise	+	+	-
Category Auditory stimuli			
Sound/Music	+	-	+
Sound	-	+	-
Category Other			
Other (freely recalled)	+	+	+
Having a cold	+	-	-
Taking a bath	+	-	-
Defecating	+	-	-
Being tired	-	+	-
Drinking alcohol	-	-	+
Having menstruation	-	-	+

[a] Detailed questions about specific stressors as seizure precipitants are shown in Supplementary Table 1.

Statistical analysis

Differences in variables between the cohort with DS and the other two cohorts with epilepsy were calculated with Kruskal-Wallis test for continuous variables and Pearson's chi-square (or Fisher's exact test in case of low expected numbers) for categorical variables. Univariable linear regression was used to test if sex, mutation type, intellectual disability, age at seizure onset, age at study or seizure frequency were related to the number of positive seizure precipitants categories within the cohort with DS. To detect Dravet-specific precipitant categories, we used logistic regression to assess relative differences in prevalence of positive categories in general between the three cohorts; seizure precipitant category was used as the dependent variable, and study cohort and the overall number of other positive precipitant categories as independent variables.

Differences in the various stress-related seizure precipitants between the cohort with DS and the cohort with childhood epilepsy were calculated with Pearson's chi-square (or Fisher's exact test in case of low expected numbers) for categorical variables. To correct for the overall higher prevalence of reported seizure precipitants in DS, the differences in stress-related seizure precipitants were also calculated for only the stress-sensitive patients in the cohort with DS and the cohort with childhood epilepsy.

All statistical analyses were performed using IBM SPSS Statistics 20. A threshold of $\alpha = 0.05$ was considered statistically significant. The Bonferroni method was used to correct for multiple testing.

RESULTS

Baseline characteristics of the cohort with DS

The parents of 71 (81%) of 88 patients with DS, who fulfilled the criteria for inclusion, signed consent forms for the study and completed the questionnaire on precipitating factors. The median age of the patients at the time of study was 10.7 (range: 1.9-22.8) years.

The median age at first seizure was 5.3 (range: 0.8-12) months. All patients subsequently developed multiple seizure types with a median reported frequency of 530 (interquartile range: 52-1951) seizures per year at the time of completion of the questionnaire. All except one child two years of age at the time of study had slowing of development. This resulted in mild to severe (n=67) intellectual disability in all children above three years of age, except for two patients with IQs between 70 and 85. *SCN1A* mutations were classified as missense in 29 (41%) and loss-of-function mutations in 42 (59%) patients and included one likely pathogenic mutation.

Reported precipitating factors in DS

For 70 (99%) of 71 patients with DS, at least one seizure precipitant was recalled by parents, with a median number of four positive seizure precipitant categories. The number of positive seizure precipitant categories was not related to sex, mutation type, intellectual disability, age at seizure onset, age at study, or seizure frequency (all $p > 0.05$). Results on precipitating factors are given in Table 2 (for categories) and Supplementary Table 2 (for individual factors).

Five seizure precipitants were reported in more than half of patients with DS: having a fever (97%), having a cold (68%), taking a bath (61%), having acute moments of stress (58%) and engaging in physical exercise (56%). Interestingly, parents of five patients (7%; median age: 12.9 years) spontaneously mentioned that the tendency to have seizures with fever had subsided with aging. Parents of 52 (73%) patients freely recalled precipitants not mentioned in the questionnaire (Supplementary Table S2).

Table 2. Reported seizure precipitants categories in the cohort with Dravet syndrome compared with the other two Dutch cohorts with epilepsy

	Cohort with Dravet syndrome (n=71)	Cohort with childhood epilepsy (n=149)[230]	Community-based cohort with epilepsy (n=248)[231]	p-value
Baseline characteristics				
Male (%)	39 (55%)	78 (52%)	135 (54%)	0.904
Median age in years (range) at				
- Seizure onset	0.4 (0.1-1.0)	2.5 (0.0-12.7)	27 (0.0-84)	<0.001
- Study	10.7 (1.9 – 23)	8.8 (2.2-16.9)	53 (12-91)	<0.001
Intellectual disability (IQ < 70) (%)	67 (94%)	70 (47%)	NA	<0.001
Categories of seizure precipitants, n (%)				
Median number of positive precipitant categories (range)	4 (0-6)	2 (0-6)	0 (0-6)	<0.001
Fever	**69 (97%)**	**48 (32%)**	**10 (4%)**	**<0.001**[a]
Stress (including physical exercise) (Fig. 1)	51 (72%)[b]	80 (54%)[b]	83 (34%)	<0.001
Sleep deprivation	35 (49%)	50 (34%)	62 (25%)	<0.001
Visual stimuli	30 (42%)[b]	26 (17%)	43 (17%)[b]	<0.001
Auditory stimuli	16 (23%)	15 (10%)	13 (5%)	<0.001
Other precipitant factors[b]	**66 (93%)**	**93 (62%)**	**61 (25%)**	**<0.001**[a]
No precipitating factors	1 (1%)	22 (15%)	131 (53%)	<0.001

[a] Remains statistically significant after correction for number of other positive precipitant categories using binary logistic regression.

[b] Calculated based on subcategories. NA = not available.

Baseline characteristics of the two non-DS Dutch epilepsy cohorts

After exclusion of four children with DS, the cohort with childhood epilepsy consisted of 149 subjects with a median age at study of 8.8 (range: 2.2-16.9) years.[230] The etiology of the epilepsy had been classified according to the proposed ILAE 2010 classification as genetic (16%), structural (43%), metabolic (11%) or of unknown etiology (30%). The community-based cohort with epilepsy consisted of 248 subjects (no patients with DS to exclude) with a median age of 53 (range: 12-91) years.[231] The epilepsy had been classified according to the ILAE 2001 classification as localization-related in 47%, generalized in 12.5%, and unclassified in 41% of the patients.

The cohort with DS differed significantly from the other two cohorts in age at seizure onset, age at study, and number of patients with intellectual disability but not in sex distribution (Table 2).

Comparison of precipitating factors between the cohort with DS and the two cohorts with non-Dravet epilepsy

In patients with DS, significantly more categories with positive seizure triggers were reported (median: 4) than in the cohort with childhood epilepsy cohort (median: 2) or in the community-based cohort with epilepsy (median: 0) ($p < 0.001$, Table 2). Furthermore, in all categories, patients with DS showed the highest percentages of reported seizure precipitants. This remained significant even after Bonferroni correction to correct for multiple testing.

The category 'fever' remained more often reported in the cohort with DS than in the cohort with childhood epilepsy (OR: 0.018 [95% CI: 0.004-0.079]) and the community-based cohort with epilepsy (OR: 0.002 [95% CI: 0.000-0.009]), when corrected for the higher number of all other positive categories (OR: 1.74 [95% CI: 1.37-2.21] in DS) using logistic regression analysis.

Similarly, the category 'other' remained more often reported in DS. Within this category, spontaneously reported seizure precipitants were more often related to ambient warmth or cold-warmth shifts in the cohort with Dravet syndrome than in the cohort with childhood epilepsy and the community-based cohort with epilepsy, both when comparing complete cohorts (42% vs. 3% and 1%, $p < 0.001$, data not shown) and when comparing subjects with freely recalled precipitants only (58% vs. 9% and 5%, $p < 0.001$, data not shown). Spontaneously recalled precipitants were also more often related to various visual stimuli in the cohort with DS than in the other two cohorts with epilepsies (18% vs. 3% and 1%, for complete cohorts, $p < 0.001$, data not shown).

Comparison of stress-related precipitating factors including physical exercise between the cohort with DS and the cohort with childhood epilepsy

The responses to detailed questions on specific stressors within the category 'stress, including physical exercise', are shown in Figure 1 for the stress-sensitive patients from the cohort with DS (n=51) and the cohort with childhood epilepsy (n=80). In the cohort with DS, physical exercise was more often reported to provoke seizures than in the cohort with childhood epilepsy, both when comparing complete cohorts (56% vs. 19%, p<0.001, data not shown) and when comparing stress-sensitive patients only (78% vs. 35%, p<0.001, Figure 1).

Interestingly, within the cohort with childhood epilepsy, physical exercise provoked seizures more often in fever-sensitive children (12 of 48, 25%) than in other children (12 of 101, 12%, p=0.042).

Stressful periods were less often reported to provoke seizures in patients with DS than in patients from the cohort with childhood epilepsy, both for stress-sensitive patients (29% vs. 75%, p<0.001, Figure 1) and for all patients (21% vs. 40%, p=0.005, data not shown). Anxiety, a subcategory of acute stress, was also less frequently reported as a seizure precipitant in the stress-sensitive patients with DS (p=0.044, Figure 1). It is noteworthy that parents of approximately half of the patients with DS responded that their child was not able to perceive anxiety or stressful periods.

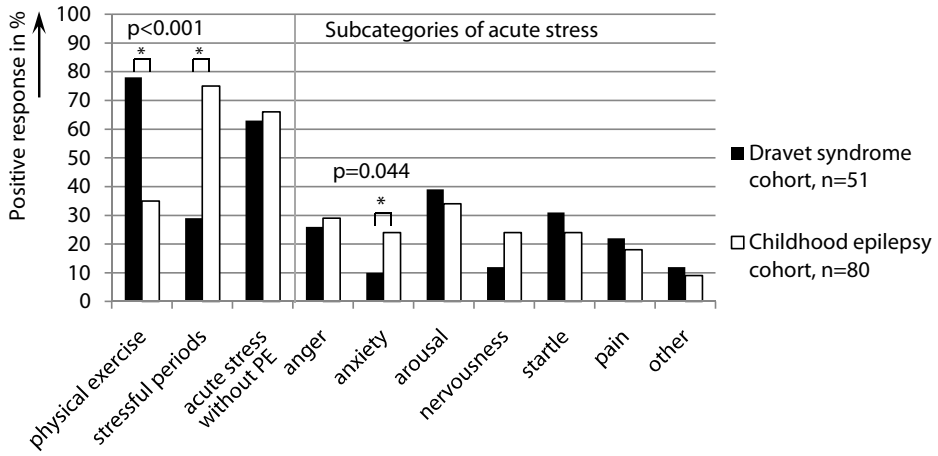

Figure 1. Percentage of stress-sensitive patients reported to have seizures after specific precipitants related to stress and/or physical exercise.

*=statistically significant difference; PE= physical exercise.

DISCUSSION

Comparison with previous studies

In this study, we determined the seizure precipitant spectrum in patients with *SCN1A*-related DS by comparing seizure precipitants in these patients with those in two cohorts with non-Dravet epilepsy: we found a high prevalence as well as a large variety of common precipitants in DS, including fever, ambient warmth, and physical exercise.

Our study confirms that fever is a seizure precipitant for nearly all patients with DS and may subside with aging in some of them.[25, 33, 36, 77] Our results underline the threat of elevated body temperature in general as a seizure precipitant in DS, whether caused by fever or hyperthermia: a (warm) bath was reported by 61%, and environmental heat or temperature changes were spontaneously mentioned as seizure precipitants by 42% of the parents of patients with DS. Hot water baths as seizure precipitant have previously been reported in 22% of Chinese and Italian children with DS,[25, 47] but is most frequently reported in Japanese children with DS children,[33] who are bathed at relatively high temperatures (39 to 42 °C). Seizures induced by environmental heat have been reported in some cases with DS as well.[35, 76] Illness or fever was also the most common seizure precipitant in two previous studies with patients with epilepsy and intellectual disability or children with intractable epilepsy, but seizures related to ambient warmth were uncommon in these surveys.[233, 234]

Our data further indicate that physical exercise is recognized as a common seizure precipitant in DS and may also act by hyperthermia, since (1) physical exercise was more frequently reported as a seizure precipitant in children with fever-sensitive epilepsy, i.e., more frequently in DS than in other childhood epilepsies and within the latter group more frequently in fever-sensitive children; (2) physical exercise, especially during warm weather, has been previously reported as a seizure precipitant;[24, 46, 49, 235] (3) a body temperature of more than 38°C is sufficient to induce seizures in children with DS, as shown by a study with hot water immersion;[33] and (4) exercise-induced hyperthermia, with a mean rectal temperature of 38.5 °C, has been shown to be a common phenomenon in children.[236]

Stress and sleep deprivation were the most common seizure precipitants in the other two cohorts with epilepsy, as reported in other epilepsy studies,[237, 238] but not in the patients with DS. However, this does not necessarily mean that the intrinsic sensitivity to these precipitants is lower in DS than in other epilepsies; some of our patients with DS were less often exposed to or did not perceive several of these stressors due to their intellectual disability, according to their parents, or these stressors may have went unnoticed. The absolute percentage of patients with DS with stress or sleep deprivation as a precipitant was still higher than in the other two cohorts with epilepsy. Seizures during (birthday) parties were previously reported to be very common and probably acting

through stress,[76] or may have been influenced by sleep deprivation. Seizures during sleep are common in DS[52] and may affect sleep quality, but its effect on further seizure generation is unknown.

Surprisingly, 'visual stimuli' was the second lowest seizure precipitant category in DS, while photosensitivity is considered a hallmark of DS.[48] This may mean either that the reported photosensitivity in up to 75% in DS[56] is limited to EEG proven photosensitivity and may have been biased towards cases with self-induction or that our questions were not specific enough. The latter may be true since parents spontaneously recalled various other visual stimuli to provoke seizures as well. Provocative visual stimuli may vary largely and depend on light intensity, frequency of flickering, and pattern. Future studies using more detailed questionnaires and correlating answers with results of EEG studies may elucidate the true extent of photosensitivity in DS.

Strengths and limitations

To our knowledge, this is the first study specifically determining the prevalence of seizure precipitants in DS with proven *SCN1A* mutations. Previous DS studies mainly focused on precipitants for the first seizure and inclusion was not always restricted to patients with *SCN1A* mutation-positive DS. In this large cohort of patients with DS, we were able to detect a Dravet-specific precipitant spectrum by comparing the results with other populations with epilepsy. Our cohort is probably representative for children and adolescents with DS, since the majority of patients with diagnosed DS in the Netherlands is known at our center and the response rate was high.

However, there are also some weak points in our study: our questionnaire had not been validated, and there were slight differences between the different cohorts, including the variable number of questions within some precipitant categories and the different ways the questionnaire was completed, i.e., written or during telephone interview. Another difference between questionnaires was the general question inquiring after any precipitant in the cohort with childhood epilepsy and after precipitants that 'often' or 'most often' provoke seizures in the cohort with DS and the community-based cohort with epilepsy, respectively. This might explain the low prevalence of seizure precipitants in the latter cohort compared with the cohort with childhood epilepsy. However, since active epilepsy was not a selection criterion for the community-based cohort, differences in seizure frequency between cohorts might be a more likely factor influencing prevalence of precipitants. Because of lack of exact seizure frequency data, we could not correct for this factor. Because of these differences between study cohorts, the higher overall prevalence of seizure precipitants in DS compared with the other two cohorts with epilepsy might not be a Dravet-specific feature. However, fever and the category 'other' remained more often reported as seizure precipitants in DS than in the other two cohorts with epilepsy when corrected for the higher number of other positive pre-

cipitant categories in DS using logistic regression analysis. Furthermore, a high overall prevalence of various precipitating situations has been previously found for patients with epilepsy and intellectual disability of other etiologies as well.[234]

Inherent to all questionnaire studies, our study was probably also subject to recall bias. To test which factors objectively provoke seizures in DS, prospective studies using diaries or video-EEG studies while exposing children to a variety of precipitants in a standardized and controlled way, are needed; the latter study design would, however, be difficult to perform because of the often severe epilepsy and concomitant behavioral and cognitive problems.

Clinical implications

The recognition of seizure precipitants in individual patients is important to guide (self)management of epilepsy, and thereby may improve seizure frequency and quality of life. Therefore, physicians should inform parents of pediatric patients with newly diagnosed DS about the large variation of potential seizure precipitants, especially the seizure precipitating effect of hyperthermia and physical exercise. Seizures induced by physical exercise, a warm bath or warm weather, might be prevented if hyperthermia is impeded. Hyperthermia caused by warm weather might be prevented by leaving the child in the shade and using protective clothing or climate control.[76] The use of a cooling vest or cap to prevent hyperthermia-induced seizures has been advocated by parents of children with DS as well.[239] However, a seizure reducing or hyperthermia-preventing effect of these cooling devices should be proven first before they are generally applied in patients with DS.

In some patients with DS, the nature of a specific seizure precipitant may not be directly clear, e.g., bright sunlight may trigger a seizure by photosensitivity, hyperthermia or a combination of the two. Seizures may, thus, be prevented by either wearing sunglasses or cooling down. To determine the nature of a seizure precipitant, video-EEG studies may be very useful as described in a child with DS with musicogenic seizures.[85]

CONCLUSION

In conclusion, our study shows a high prevalence of a range of seizure precipitants in DS. Our results underscore elevated body temperature as an important seizure precipitant, whether caused by fever, warm bath or ambient warmth. Physical exercise is a common seizure precipitant and probably acts by hyperthermia as well. Knowledge of these seizure precipitants may lead to preventive strategies in the otherwise difficult treatment of DS.

ACKNOWLEDGEMENTS

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Supplementary Table 1. Questions on acute and chronic stressors as seizure precipitants

	Dravet syndrome cohort	Childhood epilepsy cohort[230]
Subcategory Acute stress		
Does your child (sometimes) experience epileptic seizures precipitated by acute stress?	+	-
Does your child experience epileptic seizures precipitated by acute stress (psychological stress or physical exercise)?	-	+
Which moments can precipitate seizures?	+	-
In which situations has this occurred?	-	+
Anger	+	+
Anxiety	+	+
Arousal	+	+
Nervousness	+	+
Startle	+	+
Pain	+	+
Physical exercise	+	+
Other, that is...	+	+
Subcategory Stressful periods		
Does your child experience more epileptic seizures in stressful periods?	+	-
Which stressful periods can increase seizure frequency?	+	-
Does your child experience more epileptic seizures in positive stressful periods?	-	+
Which positive stressful periods were accompanied by an increase in seizure frequency?	-	+
Does your child experience more epileptic seizures in negative stressful periods?	-	+
Which negative stressful periods were accompanied by an increase in seizure frequency?	-	+
His/her birthday	+	+
Sinterklaas	+	+
A party	+	+
Argument / fight at home	+	+
Being bullied	-	+
Death of a relative/pet	+	-
Death of a relative	-	+
Death of a pet	-	+
Moving homes	-	+
Moving homes by a friend	-	+
Tests at school	+	+
Other, that is...	+	+

Supplementary Table 2. Results on seizure precipitants for Dravet syndrome

Seizure precipitants recalled by parents	Number of DS children (n=71)
Listed seizure precipitants	
Fever	69 (97%)
Having a cold	48 (68%)
Taking a bath	43 (61%)
Acute moments of stress	41 (58%)
Physical exercise	40 (56%)
Sleep deprivation	35 (49%)
Flashing sunlight	20 (28%)
Sound/Music	16 (23%)
Stressful periods	15 (21%)
TV	12 (17%)
Defecating	10 (14%)
Disco lights	7 (10%)
Videogames	6 (8%)
Freely recalled seizure precipitants	52 (73%)
Median number of precipitants (range)	1 (1-5)
Cold - warmth shifts	11 (15%)
Warmth in general	9 (13%)
Warm weather	8 (11%)
Tiredness	5 (7%)
Bustle	4 (6%)
Teething	3 (4%)
Patterns	3 (4%)
Bright sunlight	3 (4%)
Flashing light of camera	2 (3%)
Showering (with closed door)	2 (3%)
Infections, without fever	2 (3%)
Sun bathing	1 (1%)
Fireworks	1 (1%)
Light in some shops	1 (1%)
Quickly opening of curtains	1 (1%)
Pulling clothes over head	1 (1%)
Darkness	1 (1%)
Going to bathroom at night	1 (1%)
Going to bathroom in general	1 (1%)
Defecating after constipation	1 (1%)
Constipation	1 (1%)
Leg cramps	1 (1%)

Supplementary Table 2. Results on seizure precipitants for Dravet syndrome (continued)

Seizure precipitants recalled by parents	Number of DS children (n=71)
Combination tiredness, warmth, exercise	1 (1%)
Earache	1 (1%)
Waking up	1 (1%)
Sleeping	1 (1%)
Hunger	1 (1%)
Specific foods	1 (1%)
Wind	1 (1%)
Touching hand	1 (1%)
Trampoline jumping	1 (1%)
Swimming	1 (1%)
Rain falling on face	1 (1%)

Chapter 6

Photosensitivity in patients with *SCN1A*-related Dravet syndrome is underrecognized and related to prognosis

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Submitted

ABSTRACT

Objectives: To detect determinants for photoparoxysmal EEG response (PPR) in *SCN1A*-related Dravet syndrome (DS) and to study its correlation with clinical features.

Methods: Data were studied from nationwide retrieved medical histories and EEGs of patients with DS (n=53; 31 males, age range 2-19 years) with pathogenic *SCN1A* mutations. Detailed questionnaires and interviews on visual stimuli were completed by parents (n=49).

Results: PPR was found in 22 (42%) patients (12 males; median age 1.25 years), and repeatedly in 17% of patients. PPR was induced in 17% of 249 EEGs with intermittent photic stimulations (IPS) and occurred more often when using optimal photic stimulation protocols (OR 2.11 [95% CI 1.09-4.13]) and in EEGs showing spontaneous epileptiform abnormalities (OR 5.08 [95% CI 2.05-12.55]). Patients with PPR tended to be younger at first seizure (p=0.072), were significantly younger at second seizure (p=0.049), more often showed moderate to severe intellectual disability (p=0.042), and spontaneous occipital epileptiform abnormalities on EEG (p<0.001). Clinical visual sensitivity was reported in medical files in 22% and by parents in 43% of patients, and included self-induction in 24% of cases. Combined, clinical or EEG proven sensitivity to visual stimuli was noted in 65% of cases.

Conclusions: Photosensitivity is an important feature in DS: it is already present at a young age and seems to be related to the severity of the disorder. Sensitivity to visual stimuli is often noticed while this is not confirmed by EEG. Detection of PPR can be improved by repetitive testing with use of accurate photic stimulation protocols.

INTRODUCTION

Dravet syndrome (DS) is a severe epilepsy syndrome caused by heterozygous *SCN1A* mutations in at least 70% of cases.[131] Onset is in the first year of life with febrile or afebrile, generalized or unilateral seizures. Later on various other seizure types appear and psychomotor development slows. Eventually this results in intellectual disability, motor problems and therapy resistant epilepsy in the majority of cases.[32] Photosensitivity has been noticed as a typical characteristic of DS, since the first description of the syndrome by Charlotte Dravet in 1978.[29] However, the prevalence of photosensitivity is unclear and has been reported to vary from 0% to 75% in various DS cohorts (Table 1),[19, 25, 33, 36, 38, 41, 42, 44-46, 51-53, 55-57, 59, 81, 205, 240-242] with even wide variation within the same country: 8-32% in Japan[33, 36, 38, 42, 51, 59, 205] and 30-75% in Italy.[25, 53, 56, 241, 242]

Clinically, photosensitivity may be noticed by induction of eyelid myoclonia, myoclonic jerks or absences by eye closure, watching television or fixation on patterns.[49] Some patients may respond with epileptiform EEG discharges to intermittent photic stimulation (IPS)[25, 33] and pattern stimulation.[59] This photoparoxysmal EEG response (PPR) can also be evoked in about 5% of patients with other, especially generalized, epilepsy types.[243] Based on family studies, PPR is highly heritable with evidence for linkage at various loci.[244] Recently, mutations in the *CHD2* gene have been found in a rare epileptic encephalopathy with prominent clinical photosensitivity, while *CHD2* variants were also associated with other photosensitive epilepsies.[245, 246] In generalized epilepsies, photosensitivity predominates in females with a peak occurrence in adolescence.[243] Photosensitivity in DS is, however, already observed in the first years of life, without any known sex preference. Furthermore, PPR does not always occur in patients who clinically respond to visual stimuli with seizures[58, 247, 248] and may disappear and reappear over time in the same patient.[24, 51, 59]

Recognition of photosensitivity in patients with DS may provide parents and physicians with important tools to reduce seizure frequency, as treatment with occlusive patch therapy or blue-tinted lenses, has been shown to decrease seizure frequency drastically in some photosensitive patients with DS.[118, 229] Furthermore, removal of patterns from the home interior may reduce seizure frequency in pattern-sensitive children.

To be able to accurately advise patients and caregivers about these life-style adjustments, accurate recognition and diagnosis of photosensitivity is needed. The atypical finding of reported photosensitivity in patients with DS at a very young age combined with many inconclusive results in the literature on prevalence, prognosis as well as impact of photosensitivity, may hamper this. This led us to investigate photosensitivity within a nationwide DS cohort with *SCN1A* mutations. We thereby tried to answer if certain EEG characteristics or use of antiepileptic drugs (AED) predict PPR occurrence, if clinical and EEG proven photosensitivity correlate, if PPR positive and negative patients differ clinically, and how photosensitivity impacts daily life and prognosis in DS.

Table 1. Review table with original studies reporting on photosensitivity in Dravet syndrome including the present study

Study		Baseline Characteristics				Reported prevalence of photo- and pattern sensitivity					
Reference	Country	No. of cases	Males	*SCN1A* mutation	Age at study, range in years	Photosensitivity PPR	Photosensitivity Clinical	Pattern-sensitivity	Self-induction	Disappearance of photosensitivity	Remarks
This study	**Netherlands**	**53/49**	**59%**	**100%**	**0-19**	**42% (22/52)**	**49% (24/49)**	**6% (3/49) clinical**	**24% (12/49) clinical**	**1/9 with multiple PPRs**	
						65% combined (32/49)					
Dalla Bernardina *et al.*, 1982	Italy	20	75%	NR	0.5-17	75%					A
Dravet *et al.*, 1992	France	63	67%	NR	0.3-27	49%		11% clinical	16% clinical		
Ohki *et al.*, 1997	Japan	14	71%	NR	0.5-18	21%	21%		14% clinical	1/3, clinical, at age 3 yrs	
						29% combined					
Singh *et al.*, 2001	Australia, Canada, UK	12	58%	NR	2.5-26	17%					
Oguni *et al.*, 2001	Japan	84	45%	NR	0.5-28	7%	7%	8% PPR		1/14, PPR, at age 7 yrs	B
Caraballo & Fejerman, 2006	Argentina	53	64%	NR	4-14	26%				3/14, PPR	C
Nabbout *et al.*, 2003	France, Italy	93	42%	35%	NR		42%				D
Bremer *et al.*, 2012	Norway	22	64%	68%	2-17	46%					
Specchio *et al.*, 2012	Italy	22	59%	73%	5-15	41%					E
Ragona *et al.*, 2011	Italy	26	50%	77%	5-19	50%	31%				
Ohmori *et al.*, 2003	Japan	28	42%	82%	0.4-36	32%		14% PPR			F
Ragona *et al.*, 2010	Italy	37	43%	84%	0.5-18	19% (4/21)	30%	30% clinical			G
Hattori *et al*, 2008	Japan	46	43%	85%	mean 17	32%					H
Fujiwara *et al.*, 2003	Japan	35	57%	86%	2-31	14%			3% clinical, EEG		
Akiyama *et al.*, 2010	Japan	31	39%	86%	0.5-43		19%	19%	3% EEG	6/7, clinical, <age 20 yrs	I

Table 1. Review table with original studies reporting on photosensitivity in Dravet syndrome including the present study (continued)

Study		Baseline Characteristics				Reported prevalence of photo- and pattern sensitivity					
Reference	Country	No. of cases	Males	*SCN1A* mutation	Age at study, range in years	Photosensitivity		Pattern-sensitivity	Self-induction	Disappearance of photosensitivity	Remarks
Nabbout *et al.*, 2013	France	67	58%	87%	mean 9		22%				
Kim *et al.*, 2015	USA	23	56%	87%	0.9-19	17%					J
Takayama *et al.*, 2014	Japan	64	47%	92%	1-16			11% clinical		7/7, clinical	
Marini *et al.*, 2007	European	39	44%	100%	3-10	45% (17/38)					
Zucca *et al.*, 2008	Italy	12	?	100%	2-29	36% (4/11)					
Brunklaus *et al.*, 2012	UK	241	56%	100%	0.6-42	16% (34/211)					K
Lee *et al.*, 2014	Taiwan	37	57%	100%	2-26	0%	35%				

NR: not reported. A: Appearance of PPR before age 1.0 years in four, between 1.0 and 2.0 years in six and after 2.0 years in five; IPS was performed routinely at all EEGs. B: Photosensitive epilepsy 12/39 typical and 2/45 borderline cases; six sensitive to constant light. C: median age PPR 3 y (10 m - 48 m), 4 (8%) patients with focal myoclonias induced by photic stimulation or eyelid closing. D: clinical photosensitivity was defined as triggering of absence seizures by photic stimulation. E: PPR was 9% at onset, but progressively increased during study period. F: PPR only in typical, not in borderline cases. G: PPR appeared between age 18 and 30 months. H: PPR only in typical not in borderline cases. PPR appeared at age: <1 years in one, between 1-2 years in four, between 2-3 years in four, after age 3 in seven patients. I: photo- or pattern-sensitive seizures only in typical, not in borderline cases. J: PPR more frequently in younger children. K: No significant effect of PPR on developmental outcome.

MATERIALS AND METHODS

Patient selection

We included patients with DS and a pathogenic *SCN1A* mutation who 1) were referred for genetic counselling or DNA diagnostics to the Department of Medical Genetics of the University Medical Center Utrecht and 2) had at least one EEG with an IPS test recorded at the University Medical Center Utrecht, or at one of the nationwide epilepsy centers. DS was diagnosed in patients with seizure onset within the first 12 months of life, showing pharmaco-resistant epilepsy with multiple seizure types, and slowing of development after seizure onset. *SCN1A* mutations were considered pathogenic when previously detected in patients with DS and reported as pathogenic, predicted to lead to haploinsufficiency or occurred 'de novo' in the patient. Novel missense mutations inherited from a milder affected parent were considered to be likely pathogenic.

Parents of patients with DS were invited by their treating physician to participate in this study. Parents provided written informed consent for data retrieval from medical records and additionally, for active participation through a questionnaire. The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht (no. 07/295).

Data collection: clinical history

All available medical records were collected from hospitals throughout the country. Data on the following variables were extracted: sex, ages at first and second seizure, ages at start of AED, study of medical files, and diagnosis; mutation type (missense or loss-of-function [i.e. frameshift, splice site, nonsense, deletion or duplication]), severity of intellectual disability (moderate/severe [IQ<50] versus mild/borderline [IQ 50-85]), clinical history of photosensitivity and self-induction, age at report of clinical photosensitivity.

Data collection and evaluation of EEG reports

Copies of official reports were requested for all EEG recordings mentioned in the medical records. The following items were scored for all EEGs with IPS or other visual stimuli: age at EEG, stimulus (IPS or other visual stimuli), use of any antiepileptic drug at time of EEG, use of optimal IPS protocol, background slowing, spontaneous epileptiform activity, driving response, PPR, and asymmetry of EEG (in background, epileptiform activity, driving or PPR). PPR and optimal IPS protocols (i.e. using flash frequencies up to 60 Hz and eye closure condition) were defined according to the definitions of the European consensus guidelines.[249, 250] A distinction was furthermore made between PPRs confined to the occipital area and those that were generalized.

In addition, the following items were scored per patient: number of EEGs available, age at first and last available EEG; any EEG with interictal epileptiform abnormalities, localization of predominant epileptiform abnormality (frontal, centrotemporal or occipital), age at first EEG with interictal epileptiform abnormalities; tested with other visual stimuli, number of IPS tests, age at first and last IPS-EEG, any test using an optimal IPS protocol, any PPR induced, generalized or focal PPR, number of positive IPS tests, age at first and last PPR-EEG.

Questionnaire for visual stimuli as seizure precipitants

The questionnaires were send by (e-)mail and responses were completed through telephone interviewing (NV). The following questions were answered:

a. Does your child experience sensations/irritations (pain, tiredness, dizziness, headache etc.) while watching: TV programs, videogame on TV, computer program, sunlight (between trees, on water), disco lights, TL lights, escalators, striped clothes or wallpaper, shutters (horizontal), shutters (vertical), or undulating surfaces (*control question*)?
b. Has it occurred more than once that your child experienced a seizure while watching: (similar items as question 1)?
c. Does your child induce seizures him/herself by, for example, eye blinking, looking at patterns or watching TV?
d. What helps to prevent self-induced seizures (wearing sunglasses, increase distance to TV, medication, reduce stress, other…)?
e. Have you, as parent, experienced the self-induction in your child as problematic?

Based on the specificity of the parental answers 'clinical photosensitivity' was scored as 'no', 'possible' or 'definite' (DK). Outcomes were compared to results from EEG studies in the same patients.

Statistical analysis

Differences in variables between PPR positive and negative patients with DS, were calculated with Mann-Whitney *U* test for continuous variables, and Pearson's chi-square or Fisher's exact test (in case of low expected numbers) for categorical variables. A threshold of $\alpha = 0.05$ was considered significant. The association of other EEG characteristics with induction of PPR was tested with logistic regression for all IPS-EEGs using the following items as independent variables: optimal IPS protocol, spontaneous epileptiform abnormality, driving response, asymmetry in EEG and slow background. Variables with $p<0.2$ in univariable analysis, were tested in a multivariable analysis as well. A threshold of $\alpha=0.20$ was considered significant. All statistical analyses were performed using IBM SPSS Statistics 20.

RESULTS

Baseline characteristics

Fifty-three patients out of a nationwide cohort of 77 patients with DS, fulfilled the criteria for inclusion in this study. Baseline and clinical characteristics are shown in Table 2. The

Table 2. Baseline, clinical, EEG and IPS characteristics of patients with DS (n=53) with and without PPR

	PPR positive (n=22)	PPR negative (n=31)	p value
Baseline characteristics			
Male (%)	12 (55%)	19 (61%)	0.623
Missense vs. loss-of-function mutations (%)	8 (36%)	15 (48%)	0.384
Median age (range) in years at,			
- Study of medical files	11.9 (2.2-19.1)	10.9 (2.5-18.5)	0.381
- Parental questionnaire	12.4 (2.2-22.8)	11.0 (2.7-18.9)	0.366
Disease progression			
Median age (range) in months at,			
- First seizure	4.0 (0.8-9.9)	5.8 (2.0-12.0)	0.072
- Second seizure	5.8 (1.1-13.7)	7.9 (2.7-15.3)	0.049[a]
- Start anti-epileptic drug treatment	8.7 (2.6-24.9)	8.9 (2.9-25.0)	0.639
Moderate/severe vs. mild/borderline Intellectual Disability (%)[W]	17 (81%)	16 (53%)	0.042[a]
EEGs			
Median number (range)	9.5 (4-23)	7 (2-14)	0.064
Median age (range) in years, at			
- First available EEG	0.5 (0.0-1.1)	0.8 (0.2-4.5)	0.035[a]
- First interictal epileptiform	1.1 (0.2-4.7)	1.8 (0.6-5.5)	0.004[a]
- Last EEG	7.8 (1.8-17.9)	6.3 (1.0-12.7)	0.295
Interictal Epileptiform EEG (%)	22 (100%)	30 (97%)	1.000[F]
Predominant epileptiform localisation with or without generalisation:			
- Occipital localisation (%)	18 (82%)	8 (27%)	<0.001[F a]
- Frontal or centrotemporal	4 (18%)	22 (73%)	
IPS tests			
Number of patients with other visual stimuli tested	10 (46%)	1 (3%)	<0.001[F a]
Number of patients with at least one optimal IPS test	14 (64%)	16 (52%)	0.384
Median number (range) IPS tests	4.5 (1-14)	3 (1-8)	0.124
Median age (range) in years, at			
- First IPS-EEG	0.7 (0.2-8.9)	0.9 (0.2-4.5)	0.351
- Last IPS-EEG	5.1 (1.2-17.9)	4.9 (0.6-12.7)	0.334

[W] in both groups data were missing for one patient [F] Fisher exact [a] Significant value.

median age of the patients was 3.1 (range 1-18) years when a diagnosis of DS was first considered, 4.9 (range 1-18) years at time of DNA diagnosis and 11.5 (range 2-19) years at time of study. Missense mutations in the *SCN1A* gene were detected in 23 (43%) patients and loss-of-function mutations in 30 (57%) patients. The parents of 49 patients (92%, median age 11.1 [range 2-22] years) completed the questionnaire on visual stimuli as seizure precipitants.

A total of 455 EEGs were available, including 249 (55%) EEG-reports with IPS results and 14 (3%) EEG-reports with tests of other visual stimuli like TV and pattern. The number of performed IPS tests declined with age (Figure 1A): IPS tests were performed at a median age of 2.1 (range 0.2-17.9) years, and before age 5 years in 79%. Hundred-seventy-four (70%) IPS tests had been performed before a diagnosis of DS was considered and 201 (81%) before the diagnosis was confirmed by DNA results. Two-hundred-one (81%) IPS tests were performed under AED treatment (Figure 1B; monotherapy n=61, polytherapy n=140, Figure 2). Eighty-five (34%) of the IPS-tests were performed using an optimal IPS protocol.

PPR and other EEG characteristics

PPR was induced in 43 (17%) of all 249 IPS-EEGs (Figure 1A). PPR was not suppressed by AED treatment: PPR was detected in 40 (20%) of the 201 IPS-EEGs performed under AED treatment (Figure 1B) and 3 (6%) of the 48 IPS-EEGs without AED treatment (p=0.025, Chi-square). PPR was induced most frequently in the second year of life. PPR occurred more often when using optimal IPS protocols (p=0.027, OR 2.11 [95% CI 1.09-4.13] univariable; p=0.008, OR 8.11 [95% CI 1.71-38.36] multivariable) and when EEGs showed spontaneous epileptiform abnormalities (p<0.001, OR 5.08 [95% CI 2.05-12.55] univariable; p=0.078, OR 6.58 [95% CI 0.81-53.45] multivariable) or any asymmetric characteristic (p=0.013, OR 2.36 [95% CI 1.20-4.65], univariable only) (Table 3). Additionally, PPR was induced in 9 (64%) of 14 EEG-reports with tests using other visual stimuli such as TV or black and white striped patterns.

Table 3. Results of logistic regression analysis for PPR in IPS-EEGs (n=249)

EEG characteristics	Univariable analysis		Multivariable analysis	
	OR (95% CI)	p value	OR (95% CI)	p value
Spontaneous epileptiform abnormality	5.08 (2.05-12.55)	<0.001 [a]	6.58 (0.81-53.45)	0.078
Optimal protocol	2.11 (1.09-4.13)	0.027 [a]	8.11 (1.71-38.36)	0.008 [a]
Driving response	2.83 (0.91-8.76)	0.071	-	
Asymmetry in EEG	2.36 (1.20-4.65)	0.013 [a]	-	
Slow background	1.81 (0.79-4.15)	0.161	-	

[a] Significant value

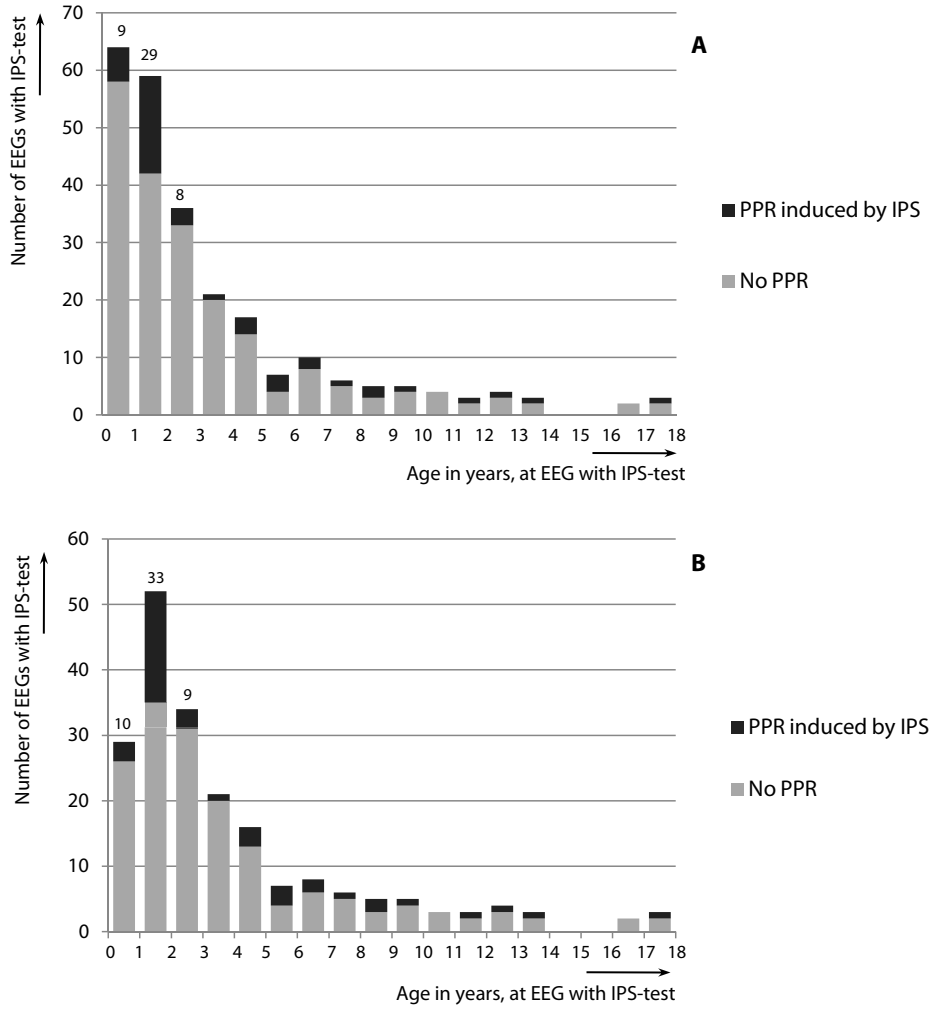

Figure 1. Photoparoxysmal response (PPR) in Dravet syndrome according to age in years.

A. Results of all intermittent photic stimulation (IPS) tests.

B. Results of IPS tests under antiepileptic drug treatment. Percentage of PPR positive IPS tests in the first three years are indicated above the bars.

PPR in patients with DS

The median number of IPS-EEGs available per patient was four (range 1-14), and for 11 (21%) patients one or more tests using other visual stimuli were available (Table 2). PPR was induced at least once in 22 (42%) of 53 patients by IPS (n=20) or other visual stimuli (n=7). The median age at first PPR induced by IPS was 1.25 (range 0.4-8.9) years and by other visual stimuli 3.6 (range 0.6-7.9) years.

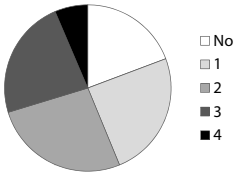

Figure 2. Number of antiepileptic drugs used during intermittent photic stimulation tests.

Monotherapy: valproate, lamotrigine, levetiracetam, phenobarbitone, ethosuximide, carbamazepine, vigabatrine or topiramate. Polytherapy: combination of the latter drugs and clobazam, clonazepam, nitrazepam, lorazepam, stiripentol, felbamate or phenytoin.

In nine (41%) of the 22 PPR positive patients, PPR was induced repeatedly in various EEGs (n=8 for IPS-tests only), but in none of them in all. In eight of these nine patients, PPR was still induced at their last IPS test (at a median age of 4.1 [range 1.5-17.9] years) (Figure 3). PPR was always generalized in 11 (50%), always focal in six (27%) patients, and either generalized or focal in five (23%) patients. Seizure types associated with PPR showed overlap with seizure types induced by visual stimuli as reported in medical files (Table 4).

DS patients with and without PPR

Differences between PPR positive and negative patients are described in Table 2. PPR positive patients showed a trend towards a younger age at first seizure and had a significant earlier age at second seizure, first available EEG and first epileptiform EEG

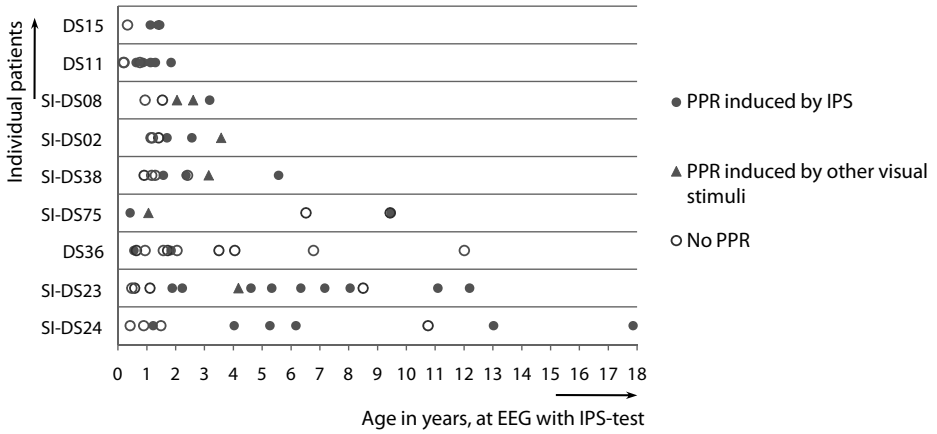

Figure 3. Photoparoxysmal response (PPR) according to age in patients with Dravet syndrome with at least twice induced PPR (n=9).

Both results of intermittent photic and other visual stimulation tests are shown.
SI = self-induction reported.

and more often had moderate to severe intellectual disability (ID) than PPR negative patients. Patients with moderate or severe ID also had younger ages at first and second seizure than patients with mild or borderline ID (p=0.06 resp. p=0.002, Mann-Whitney U test, data not shown). PPR positive patients more often had an occipital localization of their dominant spontaneous epileptiform abnormality than PPR negative patients (82% vs. 27%). There were no significant differences in baseline characteristics like sex, mutation type and age at study.

Clinical photosensitivity: questionnaire results and medical files

Parental questionnaires on photosensitivity were completed for 49 (92%) of the 53 patients with DS. Sensitivity to visual stimuli was reported in medical files (median age at first report 1.4 years [range 0.2-4.0] years) or was definite based on parental questionnaire for 24 (49%) of 49 patients with DS (Table 5). This included 14 (64%) of 22 PPR positive patients and 10 (37%) of 27 PPR negative patients, making the total number of patients with clinically or EEG proven sensitivity to visual stimuli 32 (65%) out of 49. Surprisingly, only six (12%) patients were considered visual sensitive by both questionnaire results, medical files as well as IPS results.

For 15 (47%) of the 32 visual sensitive patients the type of seizures precipitated by visual stimuli was reported in EEG reports (n=5), medical files (n=5), or both (n=5) (Table 4).

Table 4. Seizure types precipitated by visual stimuli, according to EEG reports and medical files

Case	PPR	During EEG	Clinical
DS03	+, O	M (TV, light intensity)	M
DS08	+, I/O	A (pattern)	A, M, GTCS, SE (SI, pattern)
DS23	+, I/O	EM, A (TV)	EM, A (SI, TV)
DS24	+, I	EM, M	EM, M (SI)
DS45	+, I	EMA, M (SI)	EMA (SI)
DS38	+, I/O	EM (SI), M (TV)	-
DS09	+,I	GTCS	-
DS11	+, I	M	-
DS15	+, I	M	-
DS02	+, I/O	A (TV)	-
DS26	+, I	-	EMA
DS74	+, I	-	EM (SI), M (disco)
DS75	+, I/O	-	EM (SI, TV)
DS63	-	-	M, EMA, GTCS (bright light)
DS77	-	-	A, M, GTCS (SI)

I: IPS, O: other visual stimuli; A: atypical absence, EM: eyelid myoclonia, EMA: eyelid myoclonia with absence, M: myoclonus, GTCS: generalized tonic-clonic seizure, SE: status epilepticus, SI: self-induction.

Seizures had occurred at least twice when watching TV in 13 (27%) of 49 patients, including seven PPR negative patients, according to parents.

Self-induction in DS

Self-induction was reported for 12 (24%) of 49 patients, either in medical files (n=10) or in parental questionnaires (n=8) (Table 5). Parents considered self-induction problematic in three (6%) patients. This included one patient for whom all five IPS tests had been negative. Pattern-induced seizures were reported in three (6%) patients, who also showed self-induction. Self-induction was mild in one patient with a single negative IPS test. In the other two patients self-induction was problematic and had even resulted in a status epilepticus in one of them (DS08, Table 4). Spontaneous and self-induced seizures were significantly reduced on dark days or when wearing dark sunglasses in this patient.

According to the parents of patients with self-induction, effective measurements to reduce self-induction were: removal of visual stimuli (including patterns and 50 Hz TV), stress reduction, and distracting or addressing behaviour when the patient started self-inducing.

Table 5. Photosensitivity in Dravet syndrome: photoparoxysmal response (PPR) compared with data from questionnaires and medical files

	All patients (n=49)	PPR positive (n=22, 45%)	PPR negative (n=27, 55%)	p value
Reported Photosensitivity	24 (49%)	14 (64%)	10 (37%)	0.064
- In medical files	11 (22%)	9 (41%)	2 (7%)	0.007[F a]
- By parents (questionnaire)	21 (43%)	11 (50%)	10 (37%)	0.362
TV-induced seizures	13 (27%)	6 (27%)	7 (26%)	0.915
Pattern-induced seizures	3 (6%)	2 (9%)	1 (4%)	0.581[F]
Reported Self-induction	12 (24%)	10 (46%)	2 (7%)	0.003[F a]
- In medical files	10 (20%)	9 (41%)	1 (4%)	0.003[F a]
- By parents	8 (16%)	6 (27%)	2 (7%)	0.117[F]
Problematic Self-induction	3 (6%)	2 (9%)	1 (4%)	0.581[F]

[a] Significant value [F] Fisher's exact test.

DISCUSSION

The 42% PPR positive patients in our DS cohort were younger at their first seizures and had more severe intellectual disability than our PPR negative patients. This novel finding is in line with a) previous remarks of a possible correlation between photosensitivity and the severity of DS,[32, 56] b) a higher prevalence of PPR in typical versus borderline DS cases,[33, 42, 59, 205] and c) an earlier age at seizure onset in photosensitive versus non-

photosensitive cases with other types of epilepsy.[251] However, in another DS study photosensitivity did not relate to cognitive outcome, but the reported prevalence of photosensitivity of 16% was relatively low.[19]

In our cohort, onset and prevalence of PPR was most prominent in the second year of life, but PPR was neither induced consistently by all IPS tests within cases, nor in all clinically photosensitive cases. This young age at first PPR and the discrepancy between clinical and EEG-proven photosensitivity are in agreement with previous studies (Table 1).[25, 46, 51, 53, 56, 81, 205] In contrast to some studies, PPR did not subside at follow-up in our cohort,[36, 59] possibly due to lack of follow-up into adulthood in our study. Alternatively, reported disappearance of PPR in other studies may be a misinterpretation of single negative IPS tests without tests repeated, or of subsiding clinical instead of EEG-proven photosensitivity.

In our cohort we found a higher PPR rate when a standardised IPS protocol was used, and in some of our cases PPR appeared when using other visual stimuli like TV or pattern. Performing systematic and standardised photic stimulation with 'eye closure' and 'eyes closed' may be more difficult in this young and disabled patient group, thus reducing the likelihood of evoking a PPR considerably.[249] Induction of PPR may also depend on the type of photic stimulator used, as previously reported in a clinical photosensitive child with DS.[58] This dependence might be explained by differences in the quantity of light, since PPR in DS has been shown to depend on quantity-of-light instead of wavelength.[118, 252] So, differences in photic stimulator type, use of other visual stimuli and applied IPS protocols may significantly influence PPR induction rate in DS and may thereby largely explain the variability in reported prevalence of PPR in DS cohorts, of 0% to 75%.[19, 25, 33, 36, 38, 41, 42, 44-46, 51-53, 55-57, 59, 81, 205, 240-242] Furthermore, PPRs confined to the posterior area's might not have been considered as PPR by some investigators, although details in most studies are lacking. Additionally, some variability may also be explained by selection bias towards more severe or typical DS cases in some studies, or by inclusion of cases with clinical Dravet syndrome but other etiologies like *CHD2* mutations in studies not limited to *SCN1A*-related DS, thereby leading to higher rates of photosensitivity.[177, 246] Furthermore, a higher background prevalence of PPR in the general epilepsy population in Europe compared to Asia may partly explain differences in prevalence-range between countries (8-32% in Japan versus 30-75% in Italy).[243, 253]

Although AEDs are considered to reduce or abolish PPR,[254] the likelihood of detecting photosensitivity in this cohort was increased when using AEDs. The appearance of PPR seemed to be related to disease progression with spontaneous epileptiform discharges and asymmetries in the EEG being present, and consequently AEDs being used.

Self-induction was reported for 24% of our patients with DS, which is higher than the 3% to 16% reported in other cohorts,[38, 51, 57, 59] and also occurred in some of our PPR negative patients. However, in some of our cases self-induction was reported either in

medical files or by parents, suggesting that self-inducing behaviour has decreased (when reported in older medical files only), or increased (when reported by parents only) over time, or might not always be considered very problematic. Self-induction was reported for all our pattern-sensitive patients, but the proportion of reported pattern-sensitivity was lower than in previous studies.[25, 42, 57, 59]

Strengths and limitations of study

Our study has several strong points. To our knowledge, our study is the only study so far to use a combination of EEGs and a detailed questionnaire to evaluate photosensitivity, and to correlate PPR with other EEG characteristics in DS. Other strong points are a high number of included IPS EEGs and standardised re-evaluation of all EEG reports. EEGs were from a limited number of hospitals and therefore quite uniformly performed, and represent the normal clinical situation.

A weak point is the retrospective data collection. Since the applied IPS protocols and number of IPS tests varied within our DS cohort, several PPR negative patients in our study might have been sensitive to photic stimulation using other test conditions. More severely affected cases may have been referred to epilepsy centers or tertiary hospitals at an earlier age, and consequently may have had more and better IPS-EEGs performed. This could have led to a higher detection rate of PPR in this group, and perhaps also in other DS studies. Prospective follow up studies, systematically applying standardised IPS protocols,[249] varying light intensity and including tests for pattern sensitivity, are essential to overcome the limitations of our study and detect the true extent and nature of photo- and pattern-sensitivity in DS. However, these studies can be performed only centralized and after a diagnosis of DS has been suspected or confirmed, which may still occur after the age of two years in many cases.[55]

Interpretation

In familial generalized epilepsies, PPR may segregate independently from the epilepsy and might be considered a genetic risk factor for development of epilepsy. However, the high prevalence, early expression and lack of sex preference of PPR suggest that in DS photosensitivity, like fever-sensitivity, is intrinsically related to the etiology of the syndrome. This idea is also proposed for other genetic epilepsies like Unverricht-Lundborg disease and *CHD2*-encephalopathy, and is further supported for DS by the correlation between PPR and spontaneous epileptiform EEG abnormalities and the correlation between PPR and disease severity in our cohort. However, on the basis of our data we cannot distinguish between PPR as a marker for intrinsically more severely affected cases, or as an independent prognostic factor influencing cognitive outcome in DS. In the latter case, prevention of seizures induced by visual stimuli might improve outcome in these patients.

Clinical implications

The recognition of photosensitivity in individual patients with DS is important, since this has consequences for patient management that hopefully result in reduced seizure frequency and improved quality of life. In photosensitive children the use of blue-tinted sunglasses or an eye-patch, the removal of stripes and patterns from the interior, and replacement of 50 Hz TV screens can significantly reduce seizure frequency and even prevent status epilepticus.[118, 229] Since photosensitivity in general, and even self-induction, may easily remain unrecognized or vary over time, physicians need to repetitively address this when taking a history. In our cohort, EEGs had been performed mainly before DS was diagnosed. However, even after diagnosing DS or when treated with AEDs, repeated (video-)EEG tests with IPS and other visual stimuli such as patterns and TV, may remain useful. Video-EEG studies could be used to determine the effect of life-style adjustments like the use of sunglasses or replacement of TV screens. Since photosensitivity had appeared in the second year of life in half of PPR positive cases, appearance of PPR may prompt an earlier diagnosis of DS in a subset of patients.

CONCLUSION

The majority of patients with DS are photosensitive from a very early age, but photosensitivity remains easily underrecognized. PPR in DS seems to be related to the severity of the disorder. Interictal spontaneous epileptiform discharges, occipital localization of spontaneous discharges and asymmetries are EEG variables indicative of occurrence of PPR. Furthermore, detection of PPR depends on repetitive testing with use of accurate photic stimulation protocols, and also occurs while using AEDs.

We advocate improved awareness of sensitivity to visual stimuli in patients with DS, to enable prevention of seizures and stimulate effective management of this severe disorder.

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Chapter 7

Diagnostic odyssey in Dravet syndrome: a retrospective cohort analysis

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In preparation

ABSTRACT

Objectives: To asses factors associated with a longer time to diagnosis in *SCN1A*-related Dravet syndrome (DS).

Methods: *SCN1A* analysis became available in the Netherlands in January 2004. In 76 children with DS (born 1990-2011), we assessed the diagnostic interval: the time between the first possibility to request *SCN1A* analysis (either date of first seizure, or January 2004) and the actual request. In addition, we evaluated factors related to a longer diagnostic interval and use of contra-indicated antiepileptic drugs (AED), and the sensitivity of proposed DS screening tools.

Results: *SCN1A* analysis was requested at a median age of 3.65 (IQR 1.6-7.9) years with a median diagnostic interval of 1.45 (IQR 0.7-3.0) years. Earlier birth year (B=-0.043, p=0.001) and later first consultation of a pediatric neurologist (B=0.026, p=0.019) were predictors for a longer diagnostic interval. Use of contra-indicated AED occurred in 87% of patients, was higher with longer diagnostic interval (B=1.1, p=0.027) and lower with later birth year (B=-0.67, p=0.001).

In 87% of patients, the clinical diagnosis of DS was established after molecular confirmation (median age 4.4 [IQR 2.7-8.6] years). In 11% of cases, an initial diagnosis of febrile seizures plus (FS+) was changed into DS after developmental delay was noticed (median age 2.0 [IQR 1.5-3.0] years). Before *SCN1A* analysis was performed, 11 (15%) children had alternative diagnoses, most often mitochondrial disorders. Criteria for the screening tests of Fountain-Capal, Hattori and LeGal et al. were met by respectively 100%, 72% and 60% of children with DS, at a median age of respectively 11.3, 6.1 and 11.0 months.

Conclusions: Since the introduction of *SCN1A* analysis, age at diagnosis of DS has decreased significantly in our country. An early diagnosis is important to prevent prescription of contra-indicated AEDs. Application of screening tools and awareness of the variable presentation of DS may further improve its early recognition.

INTRODUCTION

Dravet syndrome (DS), previously known as severe myoclonic epilepsy of infancy (SMEI), is a genetic epilepsy syndrome characterized by onset of seizures in the first year of life, usually consisting of generalized or unilateral clonic seizures, which are often prolonged and provoked by fever.[29] Later, other seizure types, including myoclonias, atypical absences and focal seizures may occur. Psychomotor development is normal before seizure onset, but slows down, usually from the second year of life onwards.[32] According to the International League against Epilepsy (ILAE) in 1989, other criteria for DS include the appearance of generalized spike-waves and polyspike-waves, early photosensitivity and focal abnormalities on the electroencephalogram (EEG); ataxia, pyramidal signs, and interictal myoclonus; resistance to all forms of treatment; and a family history of febrile seizures or epilepsy.[23] When one or more criteria are lacking, a diagnosis of borderline, atypical or mild DS may be considered,[27] but minimum criteria that must be met have not been established.

It has been suggested that based on clinical symptoms a tentative diagnosis of DS can be made at the end of the first year of life.[25] A tentative diagnosis of DS can be confirmed by early genetic testing of *SCN1A*, the gene involved in 70 to 80% of patients with DS.[131] An early DS diagnosis is important for several reasons. First, certain antiepileptic drugs (AED), especially sodium channel blockers, should be avoided in patients with DS, because these have been found to aggravate seizures.[76, 109] Diagnosing DS in infancy may therefore help clinicians to choose appropriate medication.[87] Furthermore, establishing a diagnosis may help parents coping with their child's disease. Finally, identification of a genetic cause may prevent additional invasive diagnostic procedures and genetic counseling can be provided.[255]

Because of these potential benefits of an early diagnosis, in various study populations tools were developed to determine which infants need to be screened for *SCN1A* mutations. Key symptoms within these screening tools are among others, seizure exacerbation with hyperthermia, seizure onset in the first year of life, occurrence of status epilepticus (SE), and normal development before seizure onset.[203, 205, 256] Despite the availability of these screening tests, a considerable delay in diagnosing DS may still occur.[55] Recognition of factors contributing to this delay may help to improve early DS diagnosis in future cases.

To identify possible factors related to the timing of diagnosis, we studied the diagnostic process in a nation-wide cohort of children with *SCN1A*-related DS. We evaluated the age at clinical and molecular diagnosis of DS, the diagnostic interval (defined as the time between the first possibility to request *SCN1A* analysis and the actual request), disease course, other ancillary investigations, and the use of contra-indicated drugs. Additionally, we evaluated the usefulness of three previously proposed screening tests for Dravet syndrome, in our cohort.[203, 205, 256]

METHODS

Patient cohort

We studied a cohort of children with *SCN1A*-related DS who had been referred for genetic counselling or DNA diagnostics to the Department of Medical Genetics of the University Medical Center Utrecht, the Netherlands. Children with an *SCN1A* mutation and seizure onset within the first 12 months of life, (temporarily) drug-resistant epilepsy with multiple seizure types, and slowing of development after seizure onset were considered to have DS and eligible for inclusion. Parents were invited by their physician to participate in this study if their child was under the age of 19 years at study inclusion (2009-2013). Parents gave written informed consent for data retrieval from medical records, and for participation in a questionnaire. The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht (no. 07/295).

SCN1A analysis

DNA analysis of the *SCN1A* gene became available in the Netherlands in January 2004, and until 2012 the laboratory for DNA diagnostics of the University Medical Center Utrecht was the only center that offered this test in the Netherlands. The *SCN1A* test consisted of analysis of coding exons including flanking splice-site consensus sequences, either indirectly, by using High Resolution Melting Curve Analysis (HRMCA) followed by Sanger sequence analysis for fragments with aberrant melting curves (September 2008-May 2011) or directly, by Sanger sequencing. Detection of deletions and duplications was performed by Multiplex Ligation Probe Analysis (MLPA) since November 2006.

Identified *SCN1A* mutations were considered pathogenic when previously reported as pathogenic in patients with DS, when predicted to lead to haploinsufficiency, or when it had occurred *de novo* in the patient. Novel missense mutations inherited from a milder affected parent were considered to be likely pathogenic.

Collection of medical data

Medical data were collected from all available medical records. A structured telephone interview with the parents was used to supplement missing data. For each child, the medical records were searched for data on the proposed 1989 ILAE criteria for DS: a) family history of epilepsy or febrile convulsions (only first degree relatives in our study); b) normal development before seizure onset; c) seizures occurring in the first year of life; d) multiple seizure types; e) epileptiform EEG abnormalities; f) delayed psychomotor development; g) ataxia, pyramidal signs or interictal myoclonus; h) AED resistance.[23] Drug resistant epilepsy was defined as epilepsy not controlled by the administration of two or more tolerated and appropriately chosen AED.[257] Seizures types, and ages at

seizure onset, first EEG that showed epileptic discharges, seizure free intervals, and first notice of developmental delay were also scored.

Additional data collection included: age at study; age at ordering DNA analysis; age at clinical diagnosis and DNA diagnosis; *SCN1A* mutation type (categorized as either missense mutations, or other mutations: deletions, intragenic duplications, frameshift, splice-site and nonsense mutations); neurological abnormalities; psychiatric diagnoses; ancillary investigations (categorized as radiological, metabolic, molecular genetics and clinical genetics [i.e. dysmorphologic examination by a clinical geneticist]), and their results; alternative diagnoses that had been considered; maintenance treatment with (contra-indicated) AED. An alternative diagnosis was defined as a different disorder, or an etiology other than the *SCN1A* mutation, which was described by the clinician as the likely cause of seizures in two or more letters in the child's medical files. We defined contra-indicated AED for DS as all sodium channel blockers, vigabatrine and phenobarbital.[76]

Diagnostic interval

For all patients, we calculated the diagnostic interval: the time between the first moment *SCN1A* analysis could have been considered, and the moment this test was actually ordered. For patients with seizure onset before 2004, the diagnostic interval was defined as the time between January 2004, when *SCN1A* testing became available in the Netherlands, and the date of ordering genetic testing by the clinician. For patients with seizure onset in 2004 or later, this interval was defined as the time between the date of first seizure and the date of ordering genetic testing.

Evaluation of screening tools

We evaluated three previously described screening tools that aim to identify children who are likely to have *SCN1A*-related DS.[203, 205, 256] Hattori et al. proposed a risk score to predict DS in the first year of life of children presenting with a febrile seizure, which includes the following symptoms (risk score per symptom in brackets): onset before seven months (2); five or more seizures (3); hemiconvulsions (3); focal seizures (1); myoclonic seizures (1); prolonged seizures of >10 minutes (3); hot water-induced seizures (2). A sum score >= 6 or a sum score >= 5 in case the effect of hot water bathing is unknown, indicates a high risk of DS.[205] We used the cut-off point of 5, because hot water bathing is not a custom in Dutch children, and defined febrile seizures as at least one seizure with temperature >= 38.0 °C. According to Le Gal et al., *SCN1A* analysis is indicated if a child has recurrent status epilepticus (SE), with the first one occurring before the age of 18 months.[256] According to Fountain-Capal et al., testing children who meet four or more of the ILAE criteria (including seizure exacerbation by hyperthermia), constitutes a high-sensitivity testing strategy.[23, 203]

We determined the risk scores of these tests for all our patients, and also determined at what age the test would have turned out positive. For patients with seizure onset in 2004 or later, we calculated the interval between the date of a positive screening test and the date of actual request of *SCN1A* analysis, i.e. the hypothetical shortening of the diagnostic interval by application of these tests.

Statistical analysis

Differences in variables between patients with DS with seizure onset before versus in or after 2004 were tested with Mann-Whitney *U* test for continuous variables and Chi-square or Fisher's exact test (in case of low expected numbers) for categorical variables.

Linear regression analysis was used to identify predictors of a longer diagnostic interval, after normalization of the distribution of diagnostic interval with Ln transformation. The following variables were tested: birth year, family history, alternative diagnosis, fever only noticed as a seizure precipitant after the first year of life, time between seizure onset and epileptiform EEG abnormalities, first notice of developmental delay and first visit to a pediatric neurologist, respectively. Variables with $p < 0.2$ in univariable analysis, were tested in a multivariable analysis using a forward model as well.

We used logistic regression to analyze the influence of the following factors on the administration of contra-indicated AED: diagnostic interval, birth year and first or second seizure being focal. Statistical analyses were conducted using IBM SPSS Statistics 20 - 22.0 ® (IBM, Armonk, NY). A p value < 0.05 was considered significant.

RESULTS

Baseline and clinical characteristics

Parents of 87 children with DS were approached and informed consent for the study was given for 76 (87%) children (42 males, 34 females). The median age at time of study was 9.8 (interquartile range [IQR] 6.4-13.6) years, and the median year of birth was 2002 (range 1990-2011, Figure 1).

The children in our cohort met a median of 7 (range 5-8) of the previously described ILAE criteria for DS, and all had seizures induced by fever or hyperthermia (only noticed after the first year of life in 16%). All children had normal development before seizure onset in the first year of life (median age 5.2 [IQR 3.9-7.3] months) and later on had developmental delay (first noticed at a median age of 24 [IQR 18-36] months) (Supplementary Table 1). All had developed multiple seizure types and (temporarily) drug resistance. Eight (11%) children had been seizure free for more than one year. In 66 (87%) children epileptiform EEG abnormalities were detected (median age at first abnormal EEG 15.3 [IQR 10.3-30.9] months) and for 66 (87%) children ataxia, pyramidal signs or (interictal) myoclonias were reported.

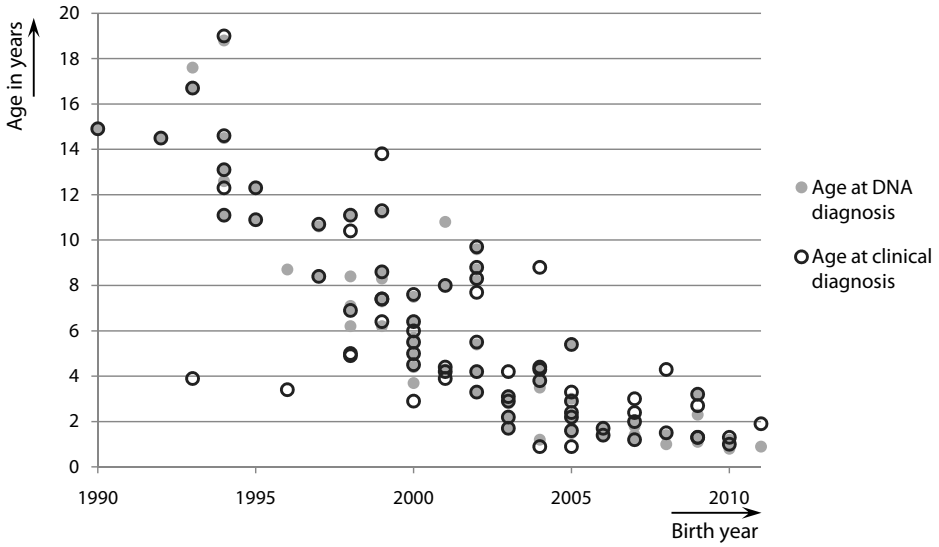

Figure 1. Age at clinical and DNA diagnosis in children with Dravet syndrome, according to birth year.
SCN1A analysis was introduced in 2004 in the Netherlands.

Genetic characteristics and family history

Thirty-one (41%) patients had missense and 45 (59%) other *SCN1A* mutations. Five (7%) probands had inherited the mutation from a milder affected or unaffected parent, with mosaicism for the mutation being identified in three parents. Overall, eight (11%) children had a first-degree relative with epilepsy or febrile seizures (Supplementary Table 2).

Diagnostic process

A clinical diagnosis of DS was considered for the first time at a median age of 3.0 (IQR 1.3-5.8) years, and at a significantly younger age in children with seizure onset in 2004 or later (Table 1). In 56 (74%) patients other electroclinical syndromes had been considered as well (Table 2). Ancillary investigations other than *SCN1A* analysis were performed in 75 (99%) patients (Table 3) with abnormal results in 46 (61%) leading to alternative diagnoses in 11 (15%) patients with DS, most commonly mitochondrial disorders. Mitochondrial dysfunction had been suspected in four cases based on elevated serum lactate and alanine levels, and in four additional cases a mitochondrial disorder had initially been diagnosed based on diminished ATP production in a muscle biopsy. Two others had initially been diagnosed with multiple acyl-CoA dehydrogenase deficiency (MADD), respectively short-chain acyl-CoA dehydrogenase deficiency (SCAD). The child without additional diagnostic testing was 1.0 year of age at time of *SCN1A* diagnosis.

A definite diagnosis of DS was made at a median age of 4.4 (IQR 2.7-8.6) years, and at a significantly younger age in children with seizure onset in 2004 or later (Figure 1).

A definite diagnosis was based on clinical characteristics before the genetic diagnosis in 10 (13%) patients, upon receiving the DNA results in 54 (71%) patients, and more than three months after receiving the results in 12 (16%) patients. Of these 12 patients, five had initially been diagnosed with (GE)FS+, in three a definite distinction between (GE)FS+ and DS had not been made yet, two had been considered to have a mitochondrial disorder and in two, the treating physician considered the epilepsy not typical for DS at the time of mutation detection.

Table 1. Overview of baseline characteristics and diagnostic process in patients with DS with seizure onset before and after 2004.

	Seizure onset < 2004 (n=45)	Seizure onset ≥ 2004 (n=31)[a]	*p* value
Baseline and clinical characteristics			
Number of males (%)	23 (51%)	19 (61%)	0.380
Number of patients with missense vs. other mutations (%)	17 (38)	14 (45)	0.520
Median age at first seizure in months (IQR)	5.1 (4.0-6.7)	6.1 (3.3-8.1)	0.440
Median age at study in years (IQR)	12.8 (10.9-15.9)	5.7 (4.1-8.1)	<0.001
Diagnostic process			
Median age in years (IQR) at			
- Diagnosis of DS, first considered	4.5 (2.6-7.9)	1.4 (1.0-2.6)	<0.001
- Request of *SCN1A* analysis	7.1 (3.9-10.8)	1.4 (1.0-2.6)	<0.001
- Definite diagnosis of DS	7.6 (4.7-11.1)	2.3 (1.4-3.4)	<0.001
Median diagnostic interval in years (IQR)	2.1 (1.2-3.5)	0.9 (0.7-2.1)	0.003
Number of patients with delay in DS diagnosis after DNA result (%)	3 (7)	9 (29)	0.012[b]
Median number of categories of ancillary investigation performed (range)	3 (1-4)	2 (0-4)	0.013
Treatment			
Number of patients with (%)			
- First AED used was contra-indicated	12 (27)	6 (19)	0.461
- Any contra-indicated AED used	45 (100)	21 (68)	<0.001[b]

Abbreviations: AED = antiepileptic drug; DS = Dravet syndrome; IQR = interquartile range.

[a] including 3 children born in 2003. [b] Fisher exact test.

Diagnostic interval for *SCN1A* analysis

SCN1A analysis was ordered at a median age of 3.65 (IQR 1.6-7.9) years. In children born before 2004, the year of introduction of *SCN1A* analysis, this median age was 7.1 years, and for children born after 2004, 1.4 years ($p < 0.001$) (Figure 1, Table 1). The median diagnostic interval was 1.45 (IQR 0.7-3.0) years. For children with seizure onset before 2004 the interval was 2.1 years, and for children with seizure onset in 2004 or later, 0.9 years.

Table 2. Electroclinical syndromes considered in patients with DS, before DS diagnosis

Type of electroclinical syndrome	Number of DS patients (n=76)
Febrile Seizures	41 (54%)
- Atypical	23 (30%)
Other childhood epilepsies	
- West syndrome	3 (4%)
- Benign Myoclonus Epilepsy	6 (8%)
- Lennox Gastaut syndrome	5 (7%)
- Doose	3 (4%)
- Other	4 (5%)
FS+/GEFS+ syndrome	8 (11%)
DS considered unlikely	4 (5%)

Univariable linear regression showed that earlier birth year (B=-0.048, p< 0.001), having an alternative diagnosis (B=0.345, p=0.056), a longer interval between seizure onset and first notice of developmental delay (B=0.012, p=0.035) and a longer interval between seizure onset and first consultation of a pediatric neurologist (B=0.033, p=0.004; missing data n=6) were associated with a longer diagnostic interval. In a multivariable model, birth year (B=-0.043, p=0.001) and interval between seizure onset and first consultation of a pediatric neurologist (B=0.026, p=0.019) were the only independent predictors for the diagnostic interval.

In three (4%) children, the initial results of the *SCN1A* analysis had been negative, due to a later introduction of MLPA to detect deletions and duplications (n=1), a sample switch (n=1) and failure of the analysis program (n=1). This led to an additive delay in DNA and clinical diagnosis of respectively 1.3, 6.9 and 6.3 years.

Screening tools

The criteria for genetic testing according to Fountain-Capal et al.,[203] were met by all children at a median age of 11.3 months (Table 4). Fifty-four (72%, one adopted child excluded) patients had a high risk score as defined by Hattori et al,[205] at a median age of 6.1 months. Forty-five (60%) children had recurrent SE with the first SE occurring before age 18 months. Their second SE, the first indication to order genetic testing according to the test by Le Gal et al.,[256] occurred at a median age of 11.0 months. When applied to the children with seizure onset in 2004 and later, these tests could have led to a median decrease of the diagnostic interval of 8.2, 10.7 and 4.6 months, respectively.

Antiepileptic treatment

In eighteen (24%) patients the first antiepileptic drug prescribed is considered contraindicated in DS: carbamazepine (n=8), phenobarbital (n=8), phenytoin (n=1), and vi-

Table 3. Overview of ancillary investigations, other than *SCN1A* analysis, and alternative diagnoses in children with Dravet syndrome

Patients with	Radiological	Metabolic	Molecular genetics	Clinical genetics	Miscellaneous	Total
Ancillary investigations performed, % (n/cases)	99% (75/76)	71% (54/76)	47% (36/76)	26% (20/76)	-	99% (75/76)
Abnormal findings, % (n/cases)	33% (25/75)	19% (10/54)	14% (5/36)	30% (6/20)	5% (4/77)	61% (46/75)
Description of findings (n)	aspecific findings (23)	mitochondrial dysfunction[a] (8)	microdeletion (2)	neurocutaneous syndromes considered (3)	immunological disorders considered (3)	
	cavernoma[a] (1)	MADD[a] (1)	familial translocation (2)	ectodermal dysplasia[a] (1)	enterovirus meningitis[a] (1)	
	sinus falciforme[a] (1)	SCAD[a] (1)	hemophilia A (1)	other syndromes considered[a] (3)		
Alternative diagnosis based on findings, % (n/cases)	3% (2/75)	11% (6/54)	0% (0/36)	10% (2/20)	1% (1/77)	15% (11/75)

Abbreviations: MADD = multiple acyl-CoA dehydrogenase deficiency; SCAD = short-chain acyl-CoA dehydrogenase deficiency.

[a] Findings that had been considered as the etiology for the epilepsy, before *SCN1A*-related Dravet syndrome was diagnosed.

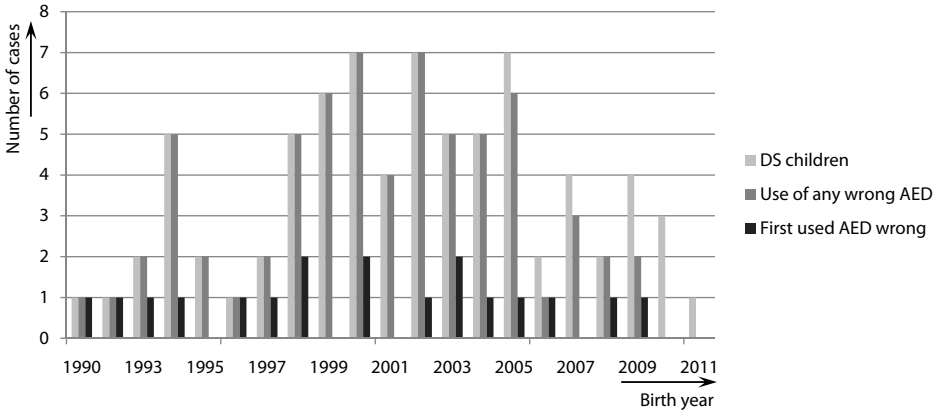

Figure 2. Use of contra-indicated antiepileptic drugs in children with Dravet syndrome, according to birth year.

Total number of children with Dravet syndrome, number of children that have used contra-indicated AEDs, and number of children in whom the first used AED was contra-indicated in Dravet syndrome, per birth year.

gabatrine (n=1). Sixty-six (87%) patients had received maintenance treatment with one or more contra-indicated AED (Figure 2). In three children (4%) a diagnosis of DS had already been considered before prescription of the first AED and none of them received any contra-indicated AED.

Univariable logistic regression showed that the risk of receiving contra-indicated AED decreased with later birth year, (B=-0.67, p=0.001) and increased with longer diagnostic interval (B=1.1, p=0.027). In a multivariable model only birth year showed statistical significance.

DISCUSSION

In this Dutch DS cohort, we show that age at clinical diagnosis of DS has decreased significantly after introduction of DNA analysis of the *SCN1A* gene. Although DS is a clinical diagnosis, the majority of our patients received a definite DS diagnosis only after detection of an *SCN1A* mutation. This was not limited to very young patients as suggested by a previous questionnaire study,[255] but was also seen in our older patients. Other studies have stated that a tentative diagnosis of DS on clinical grounds is possible in the first year of life.[25, 205] In our cohort, DS was first considered only at a median age of 3.0 years and diagnosed at 4.4 years, and this occurred at even later ages in a Norwegian study.[55] Since ages at diagnosis have decreased in both countries since the introduction of *SCN1A* analysis, awareness of the clinical entity DS seems to have strongly increased over the past years, but recognition may remain difficult at an early stage of the disease.[34]

Table 4. Test characteristics of three proposed screenings tests for Dravet syndrome, in our and original studies.

	Fountain-Capal et al.[203]	Hattori et al.[205]	Le Gal et al.[256]	
Chosen model	Cut-off 4 out of 9	Model 4	One model	Combined
Our cohort, n=75 available [a]				
Number of positive patients (sensitivity)	75 (100%)	54 (72%)	45 (60%)	
Median age at positive test in months (IQR)	11.3 (8.9-15.2)	6.1 (4.6-8.2)	11.0 (8.9-13.9)[b]	
Patients in younger subcohort, n=30				
- with positive test (%)	30 (100%)	19 (63%)	20 (67%)	30
- positive test before *SCN1A* request (%)	23 (77%)	18 (60%)	11 (37%)[c]	28
Hypothetical decrease of diagnostic interval by positive test, in months (IQR)	8.2 (2.8-20.7)	10.7 (7.0-24.9)	4.6 (1.3-20.2)	9.8 (3.6-23.7)
Previously reported test characteristics	Reference [203]	Reference [205]	Reference [256]	
Test population, children with	*SCN1A* gene analyzed	febrile seizures	status epilepticus	
Number of patients				
- in tested population	69	96	71	
- with identified *SCN1A* mutation	16	45 (39 with DS)	12 (10 with DS)	
- diagnosed with Dravet syndrome	16	46	10	
Sensitivity	100%	96%	90% [d]	
Specificity	~14% [d]	88%	89% [d]	
Positive predictive value	26%	88%	56% [d]	
Negative predictive value	100%	96%	98% [d]	

Abbreviations: DS = Dravet syndrome; IQR = interquartile range; [a] adopted child excluded from analysis; [b] age was lacking for 3 children; [c] age lacking for two; [d] calculated based on data in original study.

Both a longer diagnostic interval and an earlier birth year were associated with the use of contra-indicated AED in our cohort. Our findings confirm a questionnaire study in which clinicians responded that *SCN1A* analysis allowed them to make an earlier diagnosis and adapt their treatment accordingly.[255] However, use of contra-indicated AEDs as the first prescribed drug remained substantial in our cohort. Overall, prescription of contra-indicated AED had occurred in nearly 90% of our patients with DS, all patients from a Norwegian study and a quarter of patients from a French study.[45, 55]

Nearly all children in our study underwent other ancillary investigations before DS was molecularly confirmed. However, the number of ancillary investigations was lower in the younger subgroup, and the child in whom an *SCN1A* mutation was detected at age one year did not have brain magnetic resonance imaging or other ancillary investigations. These findings show that an early DS diagnosis may indeed prevent (invasive) ancillary investigations, as has been postulated previously.[255] In 15% of our cases with DS, an alternative etiological diagnosis had been reported, most frequently a mitochon-

drial disorder. Mitochondrial dysfunction has repeatedly been reported in patients with *SCN1A*-related DS; a primary mitochondrial disorder, confirmed by molecular genetic testing (showing bi-allelic mutations in the *POLG* gene), as diagnosed in one of these children, is very rare.[258-261] The high prevalence of reported mitochondrial dysfunction in our cohort, suggests that these are secondary to either the *SCN1A* mutation itself or oxidative stress caused by recurrent seizures, as has been suggested previously.[262]

Although, according to the ILAE description of DS,[23] psychomotor development is "retarded from the second year of life on" developmental delay was noticed only after the second year and even up to age seven years, in half of our patients. Although this was later than in a previous study, in which development delay was noticed at a median age of 18 months,[19] our findings are in line with other studies describing cognitive decline after age five years or preserved cognitive functions in a subset of patients with DS.[19, 25-27, 35, 76, 263] The perception that all cases with DS will fulfill the established ILAE criteria may be an important barrier to an early diagnosis, according to a previous study.[69]

Strengths and limitations

Our study has a number of strengths. Our study was performed in a large and nationwide, and thereby a probably representative cohort of *SCN1A*-related DS. We not only evaluated the diagnostic process, but also diverse screening tools and their usefulness in improving early diagnosis. One of the limitations of our study is the retrospective data collection from medical records. However, the effect of this retrospective data collection is probably limited since data were complemented with information from parental questionnaires when necessary, and the clinical characteristics of our cohort were largely similar to those in previously reported DS cohorts from other countries.[19, 38, 41, 42, 44-46] Furthermore, our estimated sensitivity of the screening tool by Hattori et al. was similar to a previous study.[25]

Another limitation of our study may be that we have failed to include patients from the most recent birth years in whom the molecular diagnosis, or the distinction with febrile seizures plus had not yet been made at time of study inclusion, thereby possibly introducing an inclusion bias. This could have led to an underestimation of the diagnostic interval in our younger subgroup. However, the effect on the overall results will be limited, because the most recent birth year forms only a small proportion of the cohort.

Implications

The variability in clinical presentation and early course of DS, with focal or generalized seizures, febrile or afebrile seizures, and a long interval between first seizure and developmental slowing, or epileptiform EEG abnormalities, may hamper the early recognition of the syndrome in a subset of children with DS. As a consequence many children may receive an AED that is contra-indicated in DS, when treatment is initiated. Use of sodium

channel blockers, like lamotrigine and carbamazepine, often leads to seizure aggravation, but its relation to disease course is unknown.[76, 109] As long as there are alternative treatment options, such as levetiracetam, use of sodium channel blockers might be avoided in all young patients with focal seizures,[87, 264] until Dravet syndrome is excluded clinically.

The younger the patients were at the time of molecular genetic diagnosis, the larger the number of patients who had initially been diagnosed with (GE)FS+. We speculate that both onset of developmental delay only after detection of the *SCN1A* mutation, and a reasonable seizure control when using appropriate AEDs may have contributed to these misclassifications. Since a misdiagnosis of (GE)FS+ can give parents false ideas about the prognosis, we support the use of the term *SCN1A*-related epilepsy for young children in whom the diagnosis of GEFS+ and DS cannot be distinguished yet,[60] as has been advocated previously.[34] This is especially important because increasing use of next generation sequencing techniques will result in younger ages at molecular diagnosis in children with seizures. Future studies are needed to detect early predictors of more and less favorable outcomes in *SCN1A*-related epilepsies.

As long as broad genetic testing is no first line ancillary investigation in children with seizures,[87] application of three previously proposed screening tools for DS might help less experienced clinicians to diagnose DS at an early age. Especially the test proposed by Fountain-Capal et al. may be useful, since the sensitivity in our cohort was 100%, and the positive predictive value in their own study 26%.[203] The tool by Hattori et al.,[205] is probably more specific, but fails to detect DS in children without distinct febrile seizures in the first year of life, or with a seizure onset around 12 months of age. The screening tool by Le Gal et al. is useful for the subset of children with DS with SE at a young age, which formed only 60% of our cohort.[256] The use of these three screening tools could advance recognition of DS in a large set of cases, but is inaccurate to exclude a DS diagnosis at an early age.

CONCLUSION

Since the introduction of *SCN1A* analysis, age at diagnosis of DS has decreased significantly in the Netherlands. An early diagnosis is important to prevent prescription of contra-indicated AEDs. Application of screening tools and awareness of the variable presentation of DS may further improve its early recognition.

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Supplementary Table 1. Clinical characteristics of children with Dravet syndrome (n=76) with *SCN1A* mutations.

#	Sex	Age at study (yrs)[I]	Age clinical diagnosis DS (yrs)	Age at molecular diagnosis (yrs)	Age at first seizure (mo)	Seizure types[II]	Epileptiform EEG	Therapy resistant	>1 yr seizure free	Age at first report of developmental delay (mo)[III]	Intelligence quotient[IV]	Psychiatric diagnosis[V]	Motoric problems[VI]	Mutation[VII]	Number of ILAE criteria met
1	F	7.9	4.2	4.2	7.1	G H F A M AT^{P} T SE	+	+	-	50	50-70	ASD	-	c.2593C>T p.(Arg865*)	7
2	F	12	4.5	4.4	6.0	G H F A M T SE	+	+	-	30	50-70	-	GA	c.982insG p.(Glu328fs)	7
3	M	15†	10.7	10.6	5.2	G H F A M AT^{P} T SE	+	+	-	23	<50	-	GA U	c.4942C>T p.(Arg1648Cys)	7
4	M	11	5.5	5.4	4.4	G H F A M AT T	+	+	-	84^{P}	70-85	-	GA O	c.3430-?_4002+?dup, duplication exon 17-20	7
5	M	13	10.4	7.1	11.9	G F SE	+	+	-	56	50-70	ADHD	GA HT U	c.2356_2358delinsTTT p.(Ala786Phe)	6
6	M	9.3	1.7	1.7	2.9	G H F A M T SE	+	+	-	21	<50	-	GA AX HT U	c.1150T>C p.(Trp384Arg)	7
7	M	14	6.9	6.9	4.6	G H F A M AT^{P} T^{P} SE NC^{P}	+	+	-	13	<50	-	GA AX HT U	c.2792G>A p.(Arg931His)	7
8	M	5.3	1.2	1.2	2.0	G H F A M T^{P} SE NC	+	+	-	11	<50	ASD	GA	c.3706-1G>A p.(?)	7
9	F	9.4	3.1	3.0	3.5	G H F A M AT^{P} SE	+	+	-	31	<50	-	GA U	c.3880-1G>A p.(?)	7
10	F	6.5	1.7	1.7	8.6	G H F A M AT^{P} SE C	+	+	-	24	50-70	-	AX O U	c.4219C>T p.(Arg1407*)	7
11	M	9.8	2.2	2.2	0.8	G H F A M T SE NC	+	+	-	21	<50	-	GA AX U	c.726_728delinsATTACA, p.(Ser243delinsLeuHis)	7
12	M	8.5	3.8	3.8	3.3	G H F A^{P} M AT SE C	-	+	-	48	50-70	ADHD ASD	GA CG O U	c.5536_5539del p.(Lys1846fs)	6
13	F	6.2	2.2	2.2	10.1	G F A M SE	+	+	-	21	ID	-	O	c.2837G>A p.(Arg946His)	8
14	F	8.1	2.9	1.7	8.0	G H^{P} F^{P} A M AT T SE NC	+	+	-	9	<50	-	GA CG	c.383-?_601+?del, deletion exon 3-4	7
15	F	16	3.4	8.7	3.9	G H F A M T SE	+	+	-	21	<50	-	GA AX HT O	c.4605insT p.(Phe1535fs)	7
16	M	12	2.9	3.7	3.9	G H F A M AT T SE C NC	+	+	-	15	<50	ASD	GA HT O U	c.3542del p.(Phe1181fs)	7

Supplementary Table 1. Clinical characteristics of children with Dravet syndrome (n=76) with *SCN1A* mutations. (continued)

#	Sex	Age at study (yrs)[I]	Age clinical diagnosis DS (yrs)	Age at molecular diagnosis (yrs)	Age at first seizure (mo)	Seizure types[II]	Epileptiform EEG	Therapy resistant	>1 yr seizure free	Age at first report of developmental delay (mo)[III]	Intelligence quotient[IV]	Psychiatric diagnosis[V]	Motoric problems[VI]	Mutation[VII]	Number of ILAE criteria met
17	M	6.4	1.4	1.4	4.3	G H^{P} F A^{P} M AT T SE C^{P}	+	+	-	39	50-70	-	GA	c.580G>A p.(Asp194Asn)	7
18	F	11	4.2	4.2	5.1	G H F A M AT^{P} SE	+	+	-	42	<50	-	GA U	c.4904dup p.(Arg1636fs)	7
19	M	16	8.4	8.4	6.2	G F A M T SE NC	+	+	-	18	<50	-	GA AX HT U	c.1510_1514del p.(Arg504fs)	7
20	M	12	6.4	6.4	9.9	G H^{P} F^{P} A M AT T SE NC	+	+	-	36^{P}	<50	ASD	GA AX HT CG O U	c.5674C>T p.(Arg1892*)	7
21	M	17	10.9	10.9	5.8	G H F^{P} A MT^{P} SE NC	+	+	-	11	<50	ASD	GA AX O U	c.1129C>T p.(Arg377*)	7
22	F	15	11.1	11.1	4.4	G F A MT^{P} SE	-	+	+	52	50-70	-	GA AX O U	c.964+1G>A p.(?)	6
23	F	14	5.0	6.2	3.4	G H F A M ATT SE	+	+	-	16	<50	ASD	GA HT O U	c.4298G>A p.(Gly1433Glu)	8
24	F	19	14.9	14.9	5.0	G H F A M ATT SE C	+	+	-	36	<50	-	GA O U	c.2343T>A p.(Asn781Lys)	7
25	F	18	12.3	12.6	3.1	G H F A M ATT^{P} SE	+	+	-	17	<50	-	GA AX O	c.4797T>G p.(Tyr1599*)	7
26	M	2.2†	0.9	1.2	2.1	G H^{P} F A^{P} M SE C	+	+	-	12	ID	-	GA	c.2684T>G p.(Leu895Arg)	7
27	M	8.1	8.0	8.0	6.1	G H F ATT SE	-	+	-	36	50-70	ADHD ASD	-	c.5488_5489del p.(Gln1830fs)	5
28	M	4.8	3.3	3.0	4.7	G H A M T SE	+	+	-	29	ID	-	AX HT O U	c.622_657delinsT p.(Asp208fs)	7
29	F	19	16.7	16.7	3.1	G H F A M AT^{P} T SE C	+	+	-	28	<50	ASD	GA AX O U	c.2904C>A p.(Cys968*)	7
30	M	20	3.9	17.6	5.4	G H F A M SE	-	+	+	14	<50	-	GA AX HT C O	c.1738C>T p.(Arg580*)	6
31	F	9.8	7.7	3.3	4.6	G F A M ATT^{P} SE C	+	+	+	34	50-70	ADHD	GA HT O U	c.4168G>A p.(Val1390Met)	7
32	M	3.2	1.3	1.3	6.9	G H F A^{P} SE	-	+	-	26	<50	-	GA AX O	c.603-1G>T p.(?)	6
33	M	13	4.9	8.4	4.9	G H F A M SE C	+	+	-	25	<50	ADHD ASD	GA AX S O U	c.3715G>T p.(Asp1239Tyr)	7

Supplementary Table 1. Clinical characteristics of children with Dravet syndrome (n=76) with *SCN1A* mutations. (continued)

#	Sex	Age at study (yrs)[I]	Age clinical diagnosis DS (yrs)	Age at molecular diagnosis (yrs)	Age at first seizure (mo)	Seizure types[II]	Epileptiform EEG	Therapy resistant	>1 yr seizure free	Age at first report of developmental delay (mo)[III]	Intelligence quotient[IV]	Psychiatric diagnosis[V]	Motoric problems[VI]	Mutation[VII]	Number of ILAE criteria met
34	M	9.5	2.9	2.8	4.2	G H F A M AT SE NC	+	+	-	28	50-70	ASD	GA AX HT O U	c.5150T>C p.(Leu1717Pro)	7
35	F	9.9	9.7	9.6	7.2	G H F A M AT[P] T SE C	+	+	-	24	<50	-	GA HT O U	c.1520_1523dup p.(Gln509fs)	7
36	F	18	14.5	14.5	3.3	G H F M AT SE	+	+	-	20	<50	ASD	GA AX O U	c.2791C>T p.(Arg931Cys)	7
37	F	4.4	1.3	1.1	3.3	G H F A M SE C	+	+	-	25	<50	-	GA U	c.5348C>T p.(Ala1783Val)	7
38	M	10	8.8	8.7	4.0	G F A[P] M T SE	+	+	-	22	<50	-	GA HT O U	c.4229del p.(Asn1410fs)	7
39	M	12	3.9	4.3	4.5	G H F A M AT T SE NC	+	+	-	18	<50	-	GA AX HT CG O U	c.769T>C p.(Cys257Arg)	7
40	F	4.1	3.2	3.2	2.8	G F A M[P] AT T[P] SE	+	+	-	<15[S]	<50	-	GA U	c.4834G>A p.(Val1612Thr)	6
41	M	5.7	2.4	1.9	8.1	G H F A M T SE	+	+	-	18	<50	-	GA	c.2584C>T p.(Arg862*)	7
43	M	18	14.6	14.5	7.3	G H F A M[P] AT[P] T SE	+	+	-	38	<50	-	GA AX HT O U	c.4219C>T p.(Arg1407*)	8
44	M	8.7	4.4	4.4	9.8	G A M T SE	+	+	-	30	<50	ADHD ASD	GA O U	c.1186G>T p.(Gly396Trp) [LP,pat]	8
45	F	13	8.6	8.6	9.5	G F A M SE[P]	+	+	-	34	50-70	ASD	-	c.100dup p.(Ala34fs)	7
46	F	13	6.0	5.7	7.0	G H F A[P] M T SE	+	+	-	24	<50	ASD	GA AX CG O	c.1360C>T p.(Gln454*)	7
47	M	4.6	0.9	1.5	5.8	G H F A M AT T SE	-	+	-	11	<50	ASD	GA U	c.1537del p.(Glu513fs)	7
48	M	18	13.1	13.1	4.1	G H F[P] M SE	+	+	+	54	<50	ADHD	O	c.2251del p.(Ser751fs)	7
49	M	3.3	1.3	1.2	4.5	G H A M SE	-	+	-	21	<50	-	HT	c.2244G>A p.(Trp748*)	6
50	F	8.3	8.3	8.2	9.8	G F M AT[P] T SE	+	+	-	42	50-70	ASD	O U	c.1178G>A p.(Arg393His)	7
51	F	11	5.5	5.4	10.3	G F SE[P] C	+	+	-	48	50-70	-	GA AX O U	c.2836C>T p.(Arg946Cys)	7

Supplementary Table 1. Clinical characteristics of children with Dravet syndrome (n=76) with *SCN1A* mutations. (continued)

#	Sex	Age at study (yrs)[I]	Age clinical diagnosis DS (yrs)	Age at molecular diagnosis (yrs)	Age at first seizure (mo)	Seizure types[II]	Epileptiform EEG	Therapy resistant	>1 yr seizure free	Age at first report of developmental delay (mo)[III]	Intelligence quotient[IV]	Psychiatric diagnosis[V]	Motoric problems[VI]	Mutation[VII]	Number of ILAE criteria met
52	F	4.9	1.5	1.4	3.3	G H F A SE	+	+	-	19	50-70	ASD	GA HT O U	c.657_658del p.(Arg219fs)	6
53	F	13	7.6	7.5	5.0	G H F A AT SE	+	+	-	45	50-70	ASD	O	c.812G>A p.(Gly271Asp)	6
54	M	4.1	2.7	2.3	7.6	G H F A M T[P] SE	+	+	-	21	<50	-	GA U	c. 1738C>T p.(Arg580*)	7
55	M	9	4.3	4.2	6.1	G H[P] F A M[P] AT[P] SE NC	+	+	-	18	50-70	ADHD ASD	AX O U	c.5164A>G p.(Thr1722Ala)	7
56	F	7	5.4	5.4	11.9	G A AT[P] SE	+	+	-	46	50-70	ASD	GA HT U	c.5193del p.(Ile1733fs)	6
57	F	3	1.0	1.0	7.0	G H A M T SE	+	+	-	14[P]	50-70	-	GA O	c.265-?_383+?del, deletion exon 2	7
58	M	5.9	2.0	1.8	10.4	G H F A[P] M AT[P] T[P] SE	+	+	-	17	50-70	-	GA AX O U	c.1118dup p.(Leu373fs)	8
59	F	12	11.3	11.2	5.8	G H F A M SE	+	+	-	17	<50	ASD	GA O U	c.2792G>A p.(Arg931His)	7
60	M	14	6.4	6.2	2.0	G H F T SE	+	+	-	23	ID	-	AX HT O U	c.3712G>T p.(Glu1238*)	7
61	M	2.5	-	0.8	4.1	G H F A M AT[P] SE	-	+	-	24	50-70	-	GA	c.775A>C p.(Ser259Arg)	6
62	F	12	5.0	4.9	6.8	G H F A M[P] AT T[P] SE C	+	+	+	39	50-70	ASD	GA O	c.4979T>C p.(Leu1660Phe)	6
63	F	4.7	4.3	1.0	2.5	G H[P] F A M SE[P]	+	+	-	17	<50	-	GA	c.5266T>C p.(Cys1756Arg)	7
64	M	7.1†	7.4	7.4	10.8	G H F A M T[P] SE	+	+	+	68	50-70	ADHD ASD	AX	c.4262_4275del p.(Gly1421fs)	8
65	F	18	12.3	12.3	6.1	G H F A M T SE	+	+	-	38	<50	ASD	O	c.140del p.(Asn47fs)	7
66	M	7.6	1.6	1.5	4.7	G H A M SE	+	+	-	25	70-85	ASD	GA AX	c.150del p.(Lys50fs)	7
67	F	13	11.1	11.1	4.9	G H F A M AT[P] T[P] SE	+	+	-	18	<50	ASD	GA	c.987_988delinsC p.(Leu331fs)	7
68	M	8.8	8.8	3.5	9.3	G H F SE	+	+	+	47	50-70	ASD	AX HT U	c.241G>A p.(Asp81Asn)	7

Supplementary Table 1. Clinical characteristics of children with Dravet syndrome (n=76) with *SCN1A* mutations. (continued)

#	Sex	Age at study (yrs)[I]	Age clinical diagnosis DS (yrs)	Age at molecular diagnosis (yrs)	Age at first seizure (mo)	Seizure types[II]	Epileptiform EEG	Therapy resistant	>1 yr seizure free	Age at first report of developmental delay (mo)[III]	Intelligence quotient[IV]	Psychiatric diagnosis[V]	Motoric problems[VI]	Mutation[VII]	Number of ILAE criteria met
69	M	5.6	3.0	1.4	6.7	G H^{P} F SE	-	+	-	15	50-70	-	-	c.4841del p.(Leu1614fs)	5
71	F	7	2.4	2.3	11	G H F A AT T SE^{P}	+	+	-	24	<50	ASD	GA CG U	c.5269G>A p.(Gly1757Arg)	6
72	M	11	3.3	3.3	5.8	G H F A M T SE	+	+	-	27	<50	ADHD ASD	GA AX O U	c.5734C>T p.(Arg1912*)	7
73	F	9.5	4.2	3.1	8.1	G H^{P} F A M AT^{P} T SE	+	+	-	30	<50	-	GA AX HT CG O U	c.4633A>G p.(Ile1545Val)	7
74	M	19	19	18.8	6.5	G H F A M AT^{P} SE	+	+	-	42	<50	ASD	GA HT O U	c.4266T>A p.(Tyr1422*)	7
75	M	12	4.4	10.8	3.1	G H F A M AT T SE C NC	+	+	+	36	<50	ADHD ASD	GA AX HT	c.302G>A p.(Arg101Gln)	7
76	M	14	7.4	7.3	5.2	G H F AT SE	+	+	-	33	<50	-	GA U	c.2177-9_2189del p.(?)	6
77	M	14	13.8	8.3	2.8	G H F A M AT^{P} T SE C NC	+	+	-	13	<50	-	GA AX O	c.3986G>T p.(Arg1329Leu)	8
78	F	1.9	1.9	0.9	7.5	G H F^{P} A M AT^{P} T^{P} SE	-	+	-	23	N	-	GA O	c.3145A>T p.(Lys1049*)	6

I: †: deceased, age of death; II: G: generalized tonic clonic seizures, H: hemiconvulsion, F: focal seizure, A: absence, M: myoclonia, AT: atonic, T: tonic, SE: status epilepticus, C: clonic, NC: non-convulsive status epilepticus, P: parental information; III: P: parental information, S: child adopted at age 15 months, no information on early development was available; IV: ID: intellectual disability without IQ scores available, N: developmental scores within normal ranges; V: ADHD: attention deficit hyperactivity disorder, ASD: autism spectrum disorder; VI: GA: gait abnormalities reported by parents, medical files: AX: ataxia, S: spasticity, HT: hypotonia, CG: crouching gait, O: other motor disorders, U: unspecified neurological problems; VII: LP, pat: likely pathogenic mutation, inherited from milder affected parent.

Supplementary Table 2. Family history in first degree relatives according to inheritance pattern of *SCN1A* mutations for 76 children with DS.

	Inherited *SCN1A* mutations	'*De novo*' *SCN1A* mutation	*SCN1A* mutations of undetermined inheritance	Total
Number of DS children	4	56	16	76
Positive family history in first degree relatives	2	5 (9%)	1 (6%)	8 (11%)
- epilepsy	2 (50%) - 2 parents with clinical milder DS, one with proven mosaic mutation [a]	4 (7%)[b] - 1 *SCN1A*-negative sib with other epilepsy type - 3 parents with other epilepsy types	1 (6%) - clinical DS in deceased father [a]	7 (9%)
- febrile seizures	0 (0%)	1 (2%) - one sib	0 (0%)	1 (1%)
Negative family history	2 (50%) - 2 parents with mosaic mutation	51 (91%)	15 (94%)	68 (89%)

[a] Previously reported in ref [202]; [b] The family history became positive in an additional proband, after diagnosis: a sib was born with the same, apparently *de novo SCN1A* mutation, seizure onset within the first year of life and normal development at age 1.9 years.

Chapter 8

Adults with a history of possible Dravet syndrome: an illustration of the importance of analysis of the *SCN1A* gene

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ABSTRACT

Most patients with Dravet syndrome have *de novo* mutations in the neuronal voltage-gated sodium channel type 1 (*SCN1A*) gene. We report on two unrelated fathers with severe childhood epilepsy compatible with a possible diagnosis of Dravet syndrome, who both have a child with Dravet syndrome. Analysis of the *SCN1A* gene revealed a pathogenic mutation in both children. One father exhibited somatic mosaicism for the mutation detected in his son. A relatively favorable cognitive outcome in patients with Dravet syndrome may be explained by somatic mosaicism for the *SCN1A* mutation in brain tissue. A mild form of Dravet syndrome in adult patients is associated with a high recurrence risk and possibly a more severe epilepsy phenotype in their offspring.

INTRODUCTION

Dravet syndrome, also known as severe myoclonic epilepsy of infancy (SMEI) is an intractable epilepsy, starting in the first year of life with febrile and afebrile hemiclonic or generalized tonic-clonic seizures, followed by myoclonic seizures, absences and partial seizures.[24] Following the first year of life, developmental delay becomes apparent, leading in a majority of cases to mental retardation. Patients lacking one of the core features of the disease can be diagnosed with borderline Dravet syndrome.[27]

Heterozygous mutations in the neuronal voltage-gated sodium channel type 1 (*SCN1A*) gene are detected in 70-80% of patients with Dravet syndrome; 89-95% of mutations are *de novo*. Five to 11% of patients with Dravet syndrome have inherited an *SCN1A* mutation from one of their parents. [142, 143] In general, these parents have either no epilepsy phenotype or a milder form of the disease, which includes febrile seizures. We report on two father-child pairs with a severe epilepsy phenotype compatible with a diagnosis of (borderline) Dravet syndrome, caused by an *SCN1A* mutation.

CASE REPORTS

Family 1

The proband was a 3-year-old boy. At the age of 5 months he had a generalized tonic-clonic seizure (GTCS), provoked by fever, that lasted for >30 min. Hereafter, he had weekly unprovoked generalized seizures or alternating hemiconvulsions lasting 20-120 min, despite treatment with sodium valproate (VPA). Seizures were not provoked by vaccination. In the subsequent months, he also developed absences, tonic-, atonic-, myoclonic- and complex partial seizures, and continued to have frequent seizures under the treatment of VPA, topiramate (TPM) and stiripentol (STP). At the age of 3 years and 7 months, his level of development was that of a child of 17 months (Bayley Scales of Infant Development, second edition [BSID II]) and he could not yet speak. His behavior was hyperactive and autistic. Up to age 4, interictal electroencephalograms (EEGs) showed background slowing, but no epileptic discharges. A magnetic resonance imaging (MRI) scan of the brain showed a small, left temporal arachnoidal cyst.

The proband's father had his first seizure, provoked by fever, at the age of 8 months. He developed refractory generalized epilepsy with daily seizures that, at the age of 3 years and 9 months, necessitated admission to an institution. No medical data on seizure semiology were available. EEGs showed generalized epileptiform abnormalities. At the age of 6 years, cognitive impairment and aggressive outbursts were noted. Anticonvulsive medication was withdrawn at the age of 11 years and 6 months. His last seizure occurred at the age of 14 years. Around the age of 12, he learned to read and write and

was subsequently educated to lower technical school level. The father was 41 years old and employed, when his son was diagnosed with Dravet syndrome. The family history of first- and second-degree relatives was negative for febrile or afebrile seizures.

Family 2

The proband is a 3-year-old girl. She had her first generalized tonic-clonic seizure (GTCS) with fever, not provoked by vaccination, at the age of 10 months. The following day she had an afebrile seizure. An interictal EEG was normal. She was seizure free and without treatment until the age of 17 months. At age 17 months she had five febrile and afebrile generalized seizures within five days. At that time, the EEG showed right frontal, sharp spike-wave complexes. Carbamazepine (CBZ) was started. Hereafter, she had weekly unprovoked tonic-clonic seizures, gradually increasing in severity and frequency. Around the age of 2 years, she developed myoclonias, myoclonic-astatic seizures, and atypical absences. At 3 years of age, she had tonic-clonic seizures every 2 weeks while under the treatment of VPA, levetiracetam (LEV) and clonazepam (CZP). Subsequent EEGs showed multifocal (poly)spike-wave complexes. An MRI scan of the brain was normal. Her psychomotor development slowed when the seizures started to occur weekly. At the age of 2.5 years, she had a developmental level of an 18-month-old.

The proband's father had his first seizure, a hemiconvulsion provoked by fever, at the age of 5 months. Hereafter he had generalized tonic-clonic seizures with a duration of around 2 minutes, and secondary generalized tonic-clonic seizures that started with a scream, deviation of the eyes and turning his head to the left. He was treated with phenobarbital (PB) and CBZ. The seizure frequency increased after CBZ was withdrawn at the age of 18 months. Myoclonic, and atonic seizures appeared and his behavior was hyperactive. EEGs showed focal and generalized spike-wave complexes. A cerebral computed tomography (CT) scan and metabolic screening were normal. PB was discontinued; VPA was started and his seizure frequency decreased to one generalized tonic-clonic seizure a year. No information was available on his psychomotor development, but, with additional support, he had followed secondary school education. During adolescence, he was diagnosed with an autism spectrum disorder. He worked in a sheltered workplace. At the age of 22, his seizure frequency increased. Clobazam and lamotrigine were introduced but resulted in a further increase in seizure frequency. At the age of 25, he had two psychotic episodes and during a hospital admission he drowned in the bath during a seizure. His family history was negative for febrile seizures and epilepsy.

MOLECULAR ANALYSIS AND RESULTS

Sequence analysis of the *SCN1A* gene in the first proband revealed a frameshift mutation (c.1537delG; p.Glu513fs). The mutation was also detected in DNA isolated from lymphocytes, hair, saliva and urine from his father. However, the level of mutant sequence was lower than the level of wild type sequence (Figure 1), indicating somatic mosaicism for the mutation in the father. The father's parents were not available for DNA analysis.

Sequence analysis of the *SCN1A* gene of the second proband showed a missense mutation (c.2837G>A, p.Arg946His). There was no DNA from the deceased father and the paternal grandparents were not available for analysis.

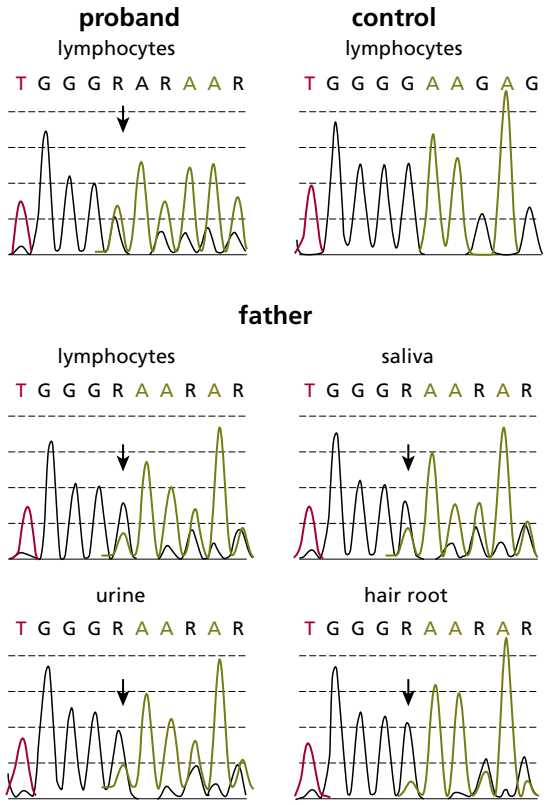

Figure 1. Sequence electropherography showing paternal mosaicism for the c.1537delG *SCN1A* mutation in family 1 with Dravet syndrome.

The arrows indicate the position of the frameshift mutation from DNA derived from the first proband's lymphocytes and a control sample, and from the father's DNA derived from different tissue samples. In the father, the level of mutant sequence differs between the tissues and is lower than the level of wild type sequence as compared with this ratio in the proband's lymphocytes. This indicates somatic mosaicism due to a postzygotic, *de novo* mutation in the father.[265]

DISCUSSION

Both the fathers had severe disabling epilepsy at a young age, but a mild form of Dravet syndrome was only considered after Dravet syndrome was diagnosed in their children and confirmed by detection of an *SCN1A* mutation. The limited information on the childhood epilepsy of the father of the first proband precludes a definite diagnosis, but may be compatible with a diagnosis of borderline Dravet syndrome. The father of the second proband fulfilled the clinical criteria for Dravet syndrome at the age of 18 months. His epilepsy improved on valproate monotherapy. Both fathers had a relatively good cognitive outcome, as reported earlier, for a minority of patients with Dravet syndrome.[26, 263] Two recent articles reported on adults who themselves had a history of severe epilepsy, who transmitted an *SCN1A* mutation to their offspring with Dravet syndrome.[142, 149]

The father of our first proband showed somatic mosaicism for the familial *SCN1A* mutation, which is a likely explanation for his favorable outcome. Somatic mosaicism was reported to be present in one of the parents of at least 7% of patients with Dravet syndrome. Parents with a higher level of mosaicism had a more severe epilepsy.[142] Because levels of mosaicism in the brain do not necessarily correlate with levels of mosaicism in other body tissues, analysis of DNA samples isolated from lymphocytes may give a false impression of a heterozygous germline mutation being present. The amount and distribution of the *SCN1A* mutation in the brain may be a major determinant for the severity of the phenotype in individuals with mosaic mutations. The phenotypic variability in Dravet syndrome is probably also influenced by other, as yet unidentified, genetic and environmental factors, for example the adequacy of the treatment received.

In children with Dravet syndrome, treatment with sodium channel blockers should be avoided,[198] because of the potential for seizure aggravation, as occurred in our second proband. This may also hold true for adult patients: the father of the second proband, whose seizures in childhood were well controlled on valproate monotherapy, developed refractory seizures again after the introduction of lamotrigine in adulthood.

Neurologists who see adult patients with a history of severe epilepsy at a young age should be aware of the possibility of milder forms of Dravet syndrome and should consider analysis of the *SCN1A* gene. If the patient has a mutation, the risk of recurrence for Dravet syndrome in their offspring is 50%. Because the phenotype severity in patients with *de novo SCN1A* mutations may be reduced due to somatic mosaicism in brain tissue, the symptoms of the disease in offspring may be more severe and hard to predict.

ACKNOWLEDGEMENTS

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Chapter 9

General Discussion

The aim of this thesis was to investigate the relationship between Dravet syndrome and vaccinations, the role of other seizure precipitants, and the pitfalls and delays in the diagnosis of Dravet syndrome, in order to identify potential targets for improved diagnosis, treatment and seizure prevention.

In this chapter the main findings are discussed in relationship to previous studies, the implications of the results for vaccination policy, diagnostics and genetic counseling in Dravet syndrome are described, and proposals on directions of future research are made.

9.1 THE RELATIONSHIP BETWEEN VACCINATIONS AND DRAVET SYNDROME

9.1.1 Dravet syndrome is a major cause of alleged vaccination-encephalopathy.

In 2006, Berkovic et al. showed that in a case series of patients with alleged vaccination encephalopathy most had a clinical course compatible with Dravet syndrome and carried pathogenic *SCN1A* mutations.[15] This finding was confirmed in a later case series.[16] The study described in **chapter 3**, confirmed that *SCN1A*-related Dravet syndrome is the most prevalent cause of (presumed) epileptic encephalopathy (defined as normal development before and developmental decline after seizure onset) following vaccination (i.e. 8 of 12 cases). When all children with epilepsy onset following vaccination in the first two years of life are considered, approximately one-third has *SCN1A*-related Dravet syndrome. The lower prevalence in our study as compared to that in previous case series[15, 16] is explained by the inclusion of children with either pre-existent encephalopathy or benign epilepsy with normal cognitive outcome. In the majority of children with epilepsy following vaccination an underlying cause can be identified. These include structural brain anomalies and other genetic causes besides *SCN1A*-related Dravet syndrome, such as the clinically related disorder epilepsy and mental retardation restricted to females (EFMR) caused by *PCDH19* mutations, and *SCN1A*-related genetic epilepsy febrile seizures plus (GEFS+) syndrome (**chapter 3**).

In clinical practice, epilepsy onset following vaccination might be rare, with an estimated prevalence of 0.2% among all patients with refractory epilepsy and 1.4% among those with additional intellectual disability.[216] In these patients ancillary investigations are needed to detect the etiology of their epilepsy, as in patients without a vaccination-associated seizure onset, but with extra focus on the possibility of a fever-sensitive genetic epilepsy syndrome, especially *SCN1A*-related Dravet syndrome.

Among all children reported with a seizure following vaccination, this seizure will be the onset of epilepsy in only a small subset (at least 2.6% according to **chapter 3**) of

them. Other children may have had prior seizures, or will develop no further seizures or only febrile seizures. When all reported children with a (possible) seizure following vaccination in the first two years of life are considered, the prevalence of *SCN1A*-related Dravet syndrome is estimated to be at least 1.2%. At least 2.5% of seizures following vaccinations in the first year of life, and 5.9% of seizures after the second or third vaccination can be attributed to *SCN1A*-related Dravet syndrome (**chapter 2**). Seizures in children with Dravet syndrome occur often with body temperatures below 38.5 °C and reoccur often after one or more subsequent vaccinations.

Other identified predispositions for seizures in children reported in our (**chapter 3**) and other studies with a seizure following vaccination include chromosomal disorders (e.g. Down syndrome), microdeletions (e.g. Angelman syndrome), metabolic diseases, other monogenic disorders (e.g. Tuberous Sclerosis Complex), and perinatally acquired brain damage.[207, 216, 266] The proportion of identified underlying causes for vaccination-associated seizures is limited by the retrospective design and incomplete genetic evaluation in our and other studies. Furthermore, some studies also included cases in which the reported seizures occurred too long after vaccination to be regarded as causally related. The true extent and nature of the genetic predisposition underlying vaccination-associated seizures can only be revealed when both next generation sequencing techniques and brain imaging are applied in large cohort studies. These studies should prospectively include children with seizures occurring within defined time-intervals following vaccination. This genetic predisposition may include factors involved in the immune response to vaccinations. This is illustrated by a genome-wide association study which showed that common variants in the interferon-stimulated gene *IF144L* and the measles virus receptor *CD46* are associated with measles-mumps-rubella (MMR) vaccine-related febrile seizures, while common variants in the *SCN1A* and *SCN2A* gene were confirmed to be associated with febrile seizures in general.[267]

If epilepsy has started after vaccination, this may give the impression that the vaccination has triggered not only the first seizure, but also any other emerging neurological symptoms or developmental delay in the child. In Dravet syndrome vaccination-associated first seizures occur at a significantly younger age than first seizures unrelated to vaccination, as shown by various studies[19, 78, 83] and confirmed in **chapter 4**. However, comparison of ages at first seizure unrelated to vaccination and at first notice of developmental delay in children with and without vaccination-associated seizure onset (**chapter 4**), indicates that their clinical course is similar. This is in line with previous studies that did not show an effect of vaccination-associated, earlier seizure onset on cognitive outcome in Dravet syndrome.[19, 78, 83] In the whole group of children with epilepsy onset following vaccination approximately one-third has benign epilepsy with normal cognitive outcome (**chapter 3)**. Overall, these findings support the idea that in children with seizure onset following vaccination, the occurrence of subsequent

seizures and additional neurological symptoms are the result of the underlying etiology of the epilepsy, while the prior vaccination can only be considered as the trigger of the first seizure. Moreover, it shows that vaccination-encephalopathy is not a true entity.

However, a negative effect of an earlier seizure onset triggered by vaccination on disease course of some metabolic or genetic disorder other than *SCN1A*-related Dravet syndrome, cannot be excluded based on current knowledge. If existent, this potential risk of infant vaccinations would most likely be extremely low in the general pediatric population, since (1) epilepsy onset following vaccination is very rare (reporting rate of ~1.4 per 100,000 vaccinated children, based on data in **chapter 3**) and (2) *SCN1A*-related Dravet syndrome is the most common cause (8 of 12) within the subgroup of (presumed) epileptic encephalopathies.

9.1.2 Implications for the national vaccination program and its safety surveillance

To support faith in the safety of vaccinations, it is important that parents of children with a vaccination-associated seizure are informed that although vaccination can trigger a seizure in susceptible children, in children who develop epilepsy the underlying etiology explains the disease and its course. This should be done uniformly by physicians as well as by professionals involved in the Netherlands Vaccination Program (NVP) and its safety surveillance.

Furthermore, to provide parents with adequate information on the risk of subsequent (vaccination-associated) seizures, early recognition of Dravet syndrome among children presenting with vaccination-associated seizures is of utmost importance. To achieve this, child health clinic staff, pediatricians and co-workers of the safety surveillance system of the NVP should be educated in the presenting symptoms, possible signs, and clinical course of Dravet syndrome. In the general pediatric population, vaccination-associated (febrile) seizures occur most frequently after the fourth infant combination vaccine at age 11 months and the MMR vaccine at age 14 months (Figure 2, **chapter 2**) and are relatively often reported to child health clinic staff and co-workers of the safety surveillance system. They should especially be aware of a possible diagnosis of Dravet syndrome if the vaccination-associated seizure occurred at a relatively young age (~4 months), as shown in **chapter 2**. If children have also had other, earlier or subsequent seizures, the characteristics of these seizures (prolonged? hemiconvulsion? triggered by a bath, infection or low-grade fever?) can provide further information on the likelihood of a diagnosis of Dravet syndrome.

Within the safety surveillance system of the NVP, the causality of all reported seizures following vaccination is assessed. It is important to acknowledge that many vaccination-associated seizures in Dravet syndrome may not fulfill case definitions for generalized convulsive seizures as adverse events following immunizations (AEFI),[201] because they

are focal. This may include seizures without loss of consciousness or with focal, postictal paresis (**chapter 2**). Therefore, some of these seizures might be more difficult to recognize as possibly being induced by vaccination. Furthermore, 30% to 60% of vaccination-associated seizures in Dravet syndrome do not fulfill criteria for febrile seizures,[268] since they occur at body temperatures below 38 °C (**chapter 2, 3** [15, 16, 78, 82]). In contrast to the general pediatric population,[11, 181] the risk of afebrile seizures following vaccination is most likely increased in children with Dravet syndrome, and possibly also in children with other genetically determined fever-sensitive epilepsy syndromes (**chapter 3**).

When clinicians or parents of a child with Dravet syndrome request information about seizure risks following vaccinations, risks after infant pertussis combination vaccines (9% within the first 24 hours) and MMR vaccine (seizure incidence rate ratio of 2.3 within day 5 to 12) can be discussed based on the results in **chapter 4**. However, data on seizure risks after other vaccines, like influenza, are not (yet) available for children with Dravet syndrome. Vaccine-specific relative seizure risks for the general population might also apply to patients with Dravet syndrome, since (1) vaccines with a relative lower febrile seizure risk in the general pediatric population also had a lower seizure risk in Dravet syndrome (acellular versus whole-cell pertussis combination vaccines), and (2) the relative increase of seizures following MMR is similar as in the general pediatric population (2-3),[179-181, 185, 212] as discussed in **chapter 4**.

In **chapter 4** we show that first seizures occurred within 24 hours after an infant combination vaccination in 21% of the described Dutch patients with Dravet syndrome. In other studies in different countries, 7 to 57% (median 23%) of patients with Dravet syndrome had their first seizure following vaccination.[19, 47, 78-80, 82, 83] Possible explanations for this large variation are differences in study design (data retrieval from either parental interviews, questionnaires for doctors or chart review) and population, and differences in national vaccination programs. Advancing the start of the NVP to the age of 2 months in 1999, has not led to an earlier age of vaccination-associated seizure onset in our cohort (age 3.7 months versus 4.0 months, both with whole-cell pertussis vaccinations, **chapter 4**). Therefore, national vaccination programs that start and finish the first three vaccinations at a younger and thereby less susceptible age, may lead to fewer vaccination-associated seizure onset in Dravet syndrome. A reduction in observed vaccination-associated seizure onset after replacement of whole-cell by acellular pertussis vaccines (19% versus 12%) did not reach statistical significance due to low numbers in the subcohorts. However, the risk of subsequent seizures was significantly lower following acellular than after whole-cell pertussis combination vaccines (9% versus 37%, **chapter 4**). Overall, use of less reactogenic vaccines in national vaccination programs (like acellular versus whole-cell pertussis,[195] or MMR+varicella (V) versus MMRV vaccines [269]) would probably reduce the incidence of vaccination-associated seizures in children with (the predisposition for) Dravet syndrome. However, adaptation of the NVP to pre-

vent vaccination-associated seizure onset in Dravet syndrome is currently not indicated, since this AEFI does not affect disease course and is very rare in the whole pediatric population.

9.1.3 Implications for vaccination of individual patients with Dravet syndrome

Although the current knowledge reveals no need for adaptation of the NVP to reduce seizure risks for children with Dravet syndrome, vaccination schedules might be adapted on an individual basis in these children. After seizure onset, nearly half of our patients with Dravet syndrome had at least one seizure following an NVP vaccination (**chapter 4**). Overall, more than half of our patients and 27% to 53% of children with Dravet syndrome from other studies have experienced any (first or subsequent) vaccination-associated seizure.[47, 79, 82] Prospective studies to establish exact seizure risks following infant vaccinations are difficult to perform in patients with Dravet syndrome, as discussed in chapter 4, because most children are diagnosed after infancy only (**chapter 7**). However, studying childhood vaccinations at age four and nine years with use of a self-controlled case series design, may provide important information on relative seizure risks in older children with Dravet syndrome. Furthermore, the influence of individual variation in immune response and use of prophylactic measures on the general vaccination-associated seizure risk could be studied.

Approximately half of our patients with Dravet syndrome had not completed the vaccination schedule or had followed adapted vaccination schedules (**chapter 4**). These patients are at increased risk of preventable infectious diseases and their complications. In approximately half of them adaptations in the vaccination schedule had been initiated by the parents (questionnaire, unpublished results). The two main reasons for parents to refrain from seasonal influenza or human papilloma virus vaccination in their child were "fear of seizures" and "ineffective/unnecessary" (questionnaire, unpublished results). Fear of seizures may not be irrational, since many patients with Dravet syndrome have a tendency to (febrile) status epilepticus, which might be induced by vaccination. Therefore, physicians and parents should make a balanced and individualized decision on further vaccinations in each child with Dravet syndrome. The following information might be helpful:

a. Many children who are not (completely) vaccinated, are still largely protected due to 'herd immunity', but will be at increased risk when inevitably new epidemics of infectious diseases like measles arise, especially when they are living in areas with a low vaccination coverage, e.g., due to decreased vaccine uptake for religious reasons.
b. Protection against certain infectious diseases, like pertussis and measles may especially be important in this patient group. Epidemic upsurges in pertussis infections occur every two to three years in the Netherlands[270] and symptoms may last 3 to 4 months. Protection against measles is important because measles lead to a

long-term immunosuppression increasing other infections and infectious disease mortality.[271]

c. Naturally occurring infections often induce seizures in Dravet syndrome, and have a significantly higher complication rate (63%) than vaccinations (7.2%), according to a previous study.[227]
d. Vaccinations may induce seizures, but in general do not influence disease course or cognitive outcome (**chapter 4**).
e. The risk of seizures is probably vaccine specific. For older children with an incomplete vaccination status who had seizures following whole-cell pertussis-combination vaccines, risk of seizures following catch-up acellular pertussis-combination vaccines is probably lower (**chapter 4**).
f. Individual differences may also exist: children with a vaccination-associated seizure onset may have an increased risk of subsequent vaccination-associated seizures (**chapter 4**).
g. In contrast to infectious diseases, timing and place of vaccinations can be controlled. Vaccinations might be given at a time of relatively low seizure frequency, and monitored during hospital admission if necessary, thereby enabling immediate seizure treatment. Furthermore, acetaminophen (paracetamol) prophylaxis could be used to reduce temperature rises and thereby probably also seizure risk. The benefits of acetaminophen (paracetamol) use must be weighed against the potential disadvantages of a less adequate immune response,[272, 273] and possibly an increased risk of transient liver toxicity when combined with valproic acid and topiramate.[274]
h. Siblings of children with *SCN1A*-related Dravet syndrome, in whom the *SCN1A* mutation is excluded, have no increased risk of vaccination-associated seizures. In the older subjects of our cohort, many siblings had not been completely vaccinated (unpublished results). Timely and complete vaccination of siblings is important to protect children with Dravet syndrome from infectious diseases.

9.2 SEIZURE PRECIPITANTS IN DRAVET SYNDROME

Parents of nearly all patients with Dravet syndrome report various other seizure precipitants, besides vaccinations. The most common seizure precipitants are related to elevated body temperature, and this probably not only includes fever or a warm bath, but physical exercise as well (**chapter 5**). Knowledge on Dravet-specific seizure precipitants is important because they may guide lifestyle adjustments that may reduce seizure frequency, as discussed in chapter 5 & 6. However, establishing the exact sensitivity to certain stimuli is difficult because provocation tests are unpractical and sometimes even unethical to perform in this patient group. An exception forms the routine testing of

sensitivity to visual stimuli during diagnostic electroencephalography (EEG). However, most EEGs are only performed before children are diagnosed with Dravet syndrome, and often without use of accurate protocols for intermittent photic stimulation tests, or without testing of other visual stimuli (**chapter 6**). This may significantly hamper recognition of photosensitivity, even though the majority of children with Dravet syndrome is sensitive to visual stimuli based on combined data from medical files, parental questionnaires, and EEGs (**chapter 6**). Why patients with Dravet syndrome are specifically sensitive to the precipitating effect of visual stimuli and elevated body temperature is largely unknown. Resolving this question may significantly add to our understanding of the pathophysiology of the syndrome and may lead to improved treatment in the future.

9.2.1 Why do fever and hyperthermia provoke seizures in Dravet syndrome?

Elevated body temperature is the most important seizure precipitant in Dravet syndrome and especially the sensitivity to even mild temperature elevation is characteristic.[25, 32] In the past, it was thought that children with Dravet syndrome more frequently had fever or infections and in some an immunodeficiency may initially be suspected (Table 3, **chapter 7**).[32] However, children with Dravet syndrome are not only sensitive to fever, but to all body temperature rises including hyperthermia, as was shown by hot water submersions.[33] Therefore, mild infections that would go unnoticed in unaffected children of the same age, may be more easily noticed in patients with Dravet syndrome because of seizure induction, giving the impression of a higher infection frequency.

This raises the question why even mild increases in body temperature can precipitate seizures in Dravet syndrome. In this context, it is noteworthy that some other diseases caused by mutations in voltage-gated sodium channels (*SCN* genes) are also temperature-sensitive: e.g., in Brugada syndrome caused by loss-of-function mutations in the cardiac expressed *SCN5A* gene, ventricular fibrillation might be provoked by fever;[275, 276] attacks in the paroxysmal extreme pain disorder (PEPD) caused by gain-of-function mutations in the *SCN9A* gene, can be triggered by cold.[277] Furthermore, hyperthermia-induced seizures are also reported in zebrafish, drosophila and mouse models for Dravet syndrome.[123, 278, 279] A possible explanation is that mutations in the *SCN1A* gene only cause defective $Na_v1.1$ channel function at higher than physiological temperatures. In *in vitro* patch clamp studies, the gating defects caused by missense mutations in the *SCN1A* gene appeared to be more severe at 40 °C as compared to 37 °C.[280] Moreover, in a knock-in drosophila model of a GEFS+ *SCN1A* mutation, the reduced repetitive firing in GABAergic local neurons was primarily linked to elevated temperatures.[278] Furthermore, a thermolabile effect of mutations has also been proposed or identified in various other disorders like fever-induced recurrent rhabdomyolysis caused by an aldolase A mutation,[281] fatal viral infection-associated encephalopathy associated with carnitine

palmitoyltransferase II variants,[282] and a functional polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene.[283]

However, most *SCN1A* mutations causing Dravet syndrome are predicted to lead to haploinsufficiency. The expected effect of these mutations would be a decrease in functional $Na_v1.1$ channels by 50%, regardless of body temperature. Since the seizures in patients with these mutation types are also temperature-sensitive, a more pronounced effect of the mutation with higher temperatures is not a sufficient explanation for the temperature-sensitivity in this syndrome. More likely, an optimal function of the $Na_v1.1$ channel is just more important at higher temperatures. This idea is supported by an, in general, earlier onset of febrile than afebrile seizures in children with Dravet syndrome,[19, 51] and an earlier appearance of hyperthermia-inducible seizures than spontaneous seizures in several animal models for Dravet syndrome.[279] Furthermore, small but significant changes at high temperature have been reported for normal $Na_v1.1$ gating function in neurons of flies.[278] Still, the pathophysiological mechanism underlying hyperthermia-induced seizures in general, remains largely unknown. Proposed mechanisms include overactivation of transient receptor potential vanilloid (TRPV4)-channels and N-methyl-d-aspartate (NMDA) glutamate receptors,[284] increased expression of inflammatory mediators like IL1-beta,[285] and $GABA_A$ receptor function changes.[286] A possible role of $GABA_A$ receptors is also supported by an increased probability and duration of hyperthermia-induced seizures in GEFS+ drosophila, which were fed with a $GABA_AR$ antagonist.[278]

Animal models provide more insights into the pathophysiology of hyperthermia-induced seizures in Dravet syndrome. In previous studies it was shown that haploinsufficiency of *scn1a* impairs excitability of the two major subtypes of GABAergic interneurons that are the major inhibitory regulators of the cortical network activity: the parvalbumin (PV) expressing and somatostatin (SST) expressing neurons.[287] A recent study shows that selective deletion of $Na_v1.1$ in either PV or SST neurons leads to susceptibility to hyperthermia-induced seizures; seizure onset is earlier and seizure duration longer in mice with either heterozygous deletion of $Na_v1.1$ in both types of neurons or homozygous deletions in PV cells.[288] Feeding with a serotonin precursor suppresses hyperthermia-induced seizures in Dravet syndrome flies, but not in GEFS+ flies.[124] Further animal studies may help to elucidate the exact pathophysiologic mechanism of hyperthermia-induced seizures. This may lead to improved understanding of the origin of febrile seizures, and thereby improve prevention and treatment of these seizures, both in children in general and those with Dravet syndrome.

A related question is why vaccinations trigger afebrile instead of febrile seizures in some children with Dravet syndrome. An explanation would be that vaccination-associated seizures are provoked merely by the induced immune response, instead of an accompanying temperature rise. However, this explanation seems unlikely, since vaccinations

are presumed to always induce immune responses, but do not always trigger seizures in children with Dravet syndrome. Furthermore, in febrile vaccination-associated seizures temperature rise on its own is sufficient to explain the seizure. Moreover, the immune response induced by vaccinations does not lead to an increase of afebrile seizures in the general population.[184]

Therefore, an alternative explanation would be that vaccination-associated seizures that are considered to be 'afebrile', are precipitated already by a small increase of body temperature. Seizures with temperatures below 38.0 °C are considered to be afebrile, but temperatures slightly below 38.0 °C might be sufficient to induce seizures in children with Dravet syndrome.[25, 32, 33, 47, 77] Furthermore, some seizures may have been classified as 'afebrile', because signs of increased temperature lacked and temperatures had not been measured at the moment of the seizure. In other seizures, tympanic measurement may have shown a temperature slightly below 38.0 °C, while rectal temperature measurement might have revealed a core temperature higher than 38.0 °C.[236] When future studies on seizure risk following vaccination in Dravet syndrome would be conducted, it would be interesting to study if prophylactic use of acetaminophen (paracetamol) would equally reduce the risk of febrile and afebrile seizures.

9.2.2 Why do visual stimuli provoke seizures in Dravet syndrome?

The high prevalence and early expression of sensitivity to visual stimuli in Dravet syndrome suggests that this is a direct result of the *SCN1A* mutation, comparable with fever-sensitivity in this syndrome, as discussed in **chapter 6**. However, little is known about the underlying biology of the photoparoxysmal response (PPR) in epilepsies in general, and in Dravet syndrome.

Only two genetic animal models of photosensitivity have been extensively studied, the *Papio papio* (Guinea baboon)[289] and the Fepi chicken strain. In the Fepi chicken, photosensitivity is caused by a homozygous splice-site mutation in the *Sv2a* gene, and light-induced seizures are initiated and generated in the brainstem.[290, 291] It was proposed that photosensitivity in Dravet syndrome might be caused by $Na_V1.1$ dysfunction in a specific subtype of GABAergic interneurons, but this assumption needs to be studied in animal models.[112] *Chd2* knockdown zebrafish larvae show increased electrographic activity during 'light ON' stimulus.[245] Therefore, *Scn1Lab* knockdown or knockout zebrafish larvae might be suitable for studies on photosensitivity in Dravet syndrome, as well as for further exploration of the intriguing observation that PPR in Dravet syndrome might be quantity-of-light instead of wavelength dependent.[118, 252]

To further elucidate mechanisms behind PPR, it would also be interesting to study whether high-frequency oscillations (HFOs) can be observed in ictal EEGs of seizures induced by light stimulation in animal models, as has been shown for hyperthermia-induced seizures.[292] HFOs are promising biomarkers of epileptic tissue. They reflect the

seizure-generating capability of the underlying tissue and are clearly linked to the seizure onset zone in focal epilepsies, suggesting a correlation of HFOs with endogeneous epileptogenicity.[293] HFOs have been studied mainly in patients with focal epilepsies and currently the clinical use of HFOs detected on scalp EEGs is studied. In the future, it would be interesting to study HFOs on scalp EEGs in children with Dravet syndrome. Detection of HFOs might improve understanding of seizure generation, involved epileptic neuronal networks,[294] and the seizure precipitating effect of various stimuli in this syndrome.

9.3 CLINICAL VARIABILITY AND DIAGNOSTICS OF DRAVET SYNDROME

Since the introduction of *SCN1A* analysis in the Netherlands, the age at clinical diagnosis of Dravet syndrome in children has significantly decreased, as shown in **chapter 7**. However, recognition is still not optimal, leading to prescription of contra-indicated antiepileptic drugs (AED) before the diagnosis is considered, or to a misclassification of febrile seizures plus (FS+) in young children with a detected *SCN1A* mutation.

Several of the patients with Dravet syndrome who had been reported with a seizure following vaccination at the National Institute for Public Health and the Environment (RIVM) in **chapter 2**, were diagnosed only by means of our study and not by their treating physician. This implies that an unknown number of patients with Dravet syndrome may not have been diagnosed yet. Under-diagnosis is currently probably largest in the adult population. This is a potential problem since contra-indicated AEDs may be prescribed, and may worsen disease course as illustrated in **chapter 8**. Furthermore, a missed diagnosis of Dravet syndrome in relatively mildly affected adults may lead to unexpectedly more severe expression in the offspring, as described in **chapter 8**.

So, there is a need for a more accurate diagnosis in both young children and adults. If better treatments become available in the future, the need of an early diagnosis will increase only further.

9.3.1 How to improve treatment?

In the first years of life, seizure frequency may increase while developmental quotients decrease in many children with Dravet syndrome.[45] Even when avoiding sodium channel blockers and other AEDs contra-indicated in Dravet syndrome, seizure frequency might be substantial. Nearly half of European patients with Dravet syndrome have four or more tonic-clonic seizures a month and each year one-third of patients have at least one status epilepticus, according to a parent-reported survey.[86] Hopefully, ongoing clinical trials and animal studies (see Introduction) will ultimately lead to better treatment and thereby improved seizure control in this patient group.

Improved seizure control would also lead to improved cognitive outcome, if Dravet syndrome would indeed be an epileptic encephalopathy, i.e. an epileptic disorder in which the seizures are considered to contribute to the cognitive decline. However, this is debated, because of the following observations:

a. Rare cases have been reported with full seizure control but decreasing developmental quotients over time;[76]
b. In various studies there was no consistent correlation between seizure characteristics and the degree of intellectual disability;[45]
c. Although developmental and intelligent quotients decline with age, absolute developmental scores in general continue to increase slowly;[45]
d. Focal knockdown of *scn1a* in the basal forebrain of adult rats leads to a spatial memory deficit, without inducing seizures;[295]
e. Treatment with a single low dose of clonazepam recovers the abnormal social behaviors and memory deficits in Dravet syndrome mice, showing that these symptoms are not caused by neuronal damage from recurrent seizures.[296]

The observed temporal relationship between increased seizure frequency and cognitive decline, that may give the impression of an epileptic encephalopathy, is therefore most likely a reflection of the expression pattern of the *SCN1A* gene. At birth, expression of $Na_v1.1$ protein is low, while expression of the $Na_v1.3$ channel encoded by the *SCN3A* gene, is at its highest level. After birth, expression of $Na_v1.3$ declines, while $Na_v1.1$ increases.[226] In children with Dravet syndrome, $Na_v1.1$ channels fail to fully replace the normal developmental loss of embryonic $Na_v1.3$ channels, leading to loss of sodium currents in GABAergic inhibitory neurons and subsequently seizures, developmental delay and other symptoms. The multiple co-morbidities in Dravet syndrome may reflect $Na_v1.1$ dysfunction in different classes of GABAergic neurons, as proposed by Catterall et al.[112] For example, autistic-like behavior is caused by haploinsufficiency of $Na_v1.1$ in PV neurons and hyperactivity by that in SST neurons as shown in a mouse model,[288] while ataxia might be caused by $Na_v1.1$ dysfunction in cerebellar Purkinje cells.[297]

Results from a questionnaire study showed that not only epilepsy severity, but also behavioral problems, learning difficulties and motor problems are predictors of a poorer health-related quality of life in patients with Dravet syndrome, with behavioral problems such as hyperactivity/inattention being the strongest predictors.[298] The various symptoms of Dravet syndrome are most likely an expression of a channelopathy, rather than an epileptic encephalopathy. So, to improve quality of life, improvement of seizure control, cognition and other outcome measures should be separate targets in the development of better treatment and prevention strategies. For example, it would be important to study if pes planus and other foot deformities, which are present in nearly half of children with Dravet syndrome,[69] are first signs of crouch gait, and if progres-

sion towards this motor disorder could be prevented. A survey by the International Dravet syndrome Epilepsy Action (IDEA) League shows that other co-morbidities like dysautonomia, growth impairment, and congenital heart abnormalities may also be overrepresented in children with Dravet syndrome and need further attention as well.[69]

Furthermore, sudden unexpected death in epilepsy (SUDEP) is an important complication of Dravet syndrome and a major threat for families with a child with Dravet syndrome.[68, 69] Studies on *Scn1a* knockout mice suggest that ictal bradycardia induced by generalized tonic-clonic seizures may cause SUDEP,[299] although altered cardiac electrical function might also contribute to SUDEP.[300] Cardiac evaluation in patients with Dravet syndrome showed an abnormal regulation of heart rate,[301] and currently clinical studies are performed to study cardiac arrhythmias in Dravet syndrome.

Another complication of Dravet syndrome that deserves full attention is acute encephalopathy. Acute encephalopathy often presents as status epilepticus followed by coma, may be associated with lesions on brain magnetic resonance imaging scan during the acute phase, and lead to significant developmental regression and additional neurological deficits or even death.[61-64] This is a relatively unknown complication, but might not be infrequent since it was observed in approximately 8% of children from a multi-center study,[63] and in at least four (5%) of the seventy-seven patients from the cohort described in chapter 4 (unpublished data). It has been described as the second cause of death in Dravet syndrome.[68] To improve prevention and recovery of acute encephalopathy, better understanding of the pathophysiology of this problem is needed, but no animal models are available yet. Retrospective studies may help to identify risk factors for this complication. One study showed that acute encephalopathy was preceded by febrile illness in all cases.[63] If high or prolonged fever would be the trigger for this complication, therapeutic hypothermia in children with febrile status epilepticus might prevent this complication or attenuate its sequelae. Furthermore, accurate AED treatment might prevent status epilepticus and thereby acute encephalopathy as well.

9.3.2 How to promote early detection of *SCN1A* mutations?

The heterogeneous onset of Dravet syndrome, with focal or generalized seizures, febrile or afebrile seizures, and a long interval between first seizure and developmental slowing, or epileptiform EEG abnormalities, may hamper the early recognition of the syndrome. The original 1989 ILAE criteria[23] are not or only later in the disease course fulfilled in many patients who are considered to have Dravet syndrome, and minimum diagnostic criteria are lacking.

9.3.2.1 Early and fast genetic testing of children with epilepsy

For children diagnosed with epilepsy, analyzing panels of selected epilepsy genes including the *SCN1A* gene, by next generation DNA sequencing techniques forms already

an important diagnostic approach.[178] Early genetic testing in all children with epilepsy may promote a diagnosis of Dravet syndrome before the full disease course is disclosed, and also that of many other genetic epilepsies. The diagnostic yield of genetic tests will grow only further with increasing numbers of epilepsy genes identified and increasing possibilities to sequence exomes and genomes.

In the near future, broad genetic testing might be performed immediately after the first seizure, as the first diagnostic test. At this moment such a 'genetic first approach' for children with seizures is not yet feasible in many countries. Therefore, specific knowledge on the early clinical course of Dravet syndrome remains important for a fast recognition and accurate diagnosis. In the future, genetic screening for Dravet syndrome might even be included in neonatal screening programs, but only when novel *SCN1A* mutations can unequivocally be interpreted as causative of either Dravet or GEFS+ syndrome, and starting treatment before seizure onset shows to significantly improve disease course and outcome.

9.3.2.2 Early recognition of children who are likely to have an SCN1A mutation

Targeted DNA analysis of the *SCN1A* gene in young and older patients with a disease course suggestive of Dravet or GEFS+ syndrome, remains an important testing strategy. In older patients, retrospective evaluation of the clinical history will reveal the characteristic evolution of clinical findings in the first years and thereby an indication for *SCN1A* analysis.[26] As long as a 'genetic first approach' is not yet in use, the general application by pediatricians and pediatric neurologists of the proposed clinical screening tests for Dravet syndrome by Hattori et al.,[205] Fountain-Capal et al.[203] and Le Gal et al.,[256] may advance *SCN1A* analysis and diagnosis of Dravet syndrome in young children, as discussed in **chapter 7**.

However, the Hattori and Le Gal screening tests have limited sensitivity, and especially the tests of Fountain-Capal and Le Gal will often turn positive only after the moment the first AED has been prescribed. Furthermore, these tests are not designed to recognize children with *SCN1A*-related GEFS+ syndrome. Screening tests that can predict which children with febrile seizures are likely to have an *SCN1A* mutation, would be helpful. Febrile seizures occur in ~2% to 5% of children of 6 months to 5 years of age, and even more frequently in several Asian countries.[302] Detection of *SCN1A* mutations in this patient group may help to distinguish between the majority of children who will have no or few further febrile seizures and whose parents can be reassured, and the few children who will have frequent febrile seizures or will develop epilepsy, and thereby need accurate medical follow-up.

Previous studies among children presenting with a febrile seizure have already shown that a young age at onset, low degree of fever and a positive family history of febrile seizures are predictors of recurrent febrile seizures.[303, 304] Neurodevelopmental abnor-

malities, complex febrile seizures and a positive family history of epilepsy are predictors of subsequent epilepsy.[305, 306] Furthermore, a brief duration between onset of fever and the initial febrile seizure is a predictor of both recurrent febrile seizures and subsequent epilepsy.[305, 306] Interestingly, most of these predictors may also be features of GEFS+ or Dravet syndrome. This raises the question whether these are predictors of subsequent febrile or afebrile seizures in general, or predictors of an *SCN1A* mutation and thereby of subsequent seizures. This might be studied prospectively by performing *SCN1A* analysis in cohorts of children with febrile seizures, and analyzing which features are predictors of an *SCN1A* mutation, and which features remain independent predictors of recurrent febrile and afebrile seizures.

9.3.2.3 Uncertain pathogenicity of SCN1A mutations

Approximately 80% of *SCN1A* mutations has not been reported previously.[132] So, when a variant in the *SCN1A* gene is detected, the pathogenicity might not be immediately certain. Mutations that are predicted to lead to haploinsufficiency are considered pathogenic. However, pathogenicity of missense and some potential splice-site mutations can probably not be assessed based on prediction programs only. In these cases, family studies remain important. The probability that a mutation is pathogenic increases when it is *de novo* in a patient whose parents are not affected by seizures, or when it co-segregates with the phenotype in a large multigenerational (GEFS+) family with a sufficient number of informative meioses. Vice versa, when a potential pathogenic *SCN1A* mutation is shown to be inherited from an unaffected parent, pathogenicity becomes unlikely, although the variant may still represent a risk factor for a seizure disorder.[307] Furthermore, prediction of pathogenicity has to take into account the possibility of parental mosaicism,[142] and incomplete penetrance as reported for few missense or splice-site mutations.[132] In specific cases, genotyping of the grandparents might be helpful to exclude or confirm these explanations.

Presence of an identified *SCN1A* variant in the ExAC database is commonly used as an argument against pathogenicity,[308] since individuals affected by severe pediatric disease are supposed to be excluded from this database. However, the database includes a number of (likely) pathogenic *SCN1A* mutations and this might be explained by artefacts, mosaicism for these mutations, or by the inclusion of cases with an unknown or unreported history of seizures. Electrophysiological studies to test the effect of missense mutations will be helpful to clarify pathogenicity in only few cases, since these tests are time-consuming and may show artificial effects, e.g., when tested at room temperature or in non-neuronal cells.[140, 141, 280]

SCN1A variants not considered to be pathogenic might still have mild effects on $Na_v1.1$ function, as suggested by bi-allelic *SCN1A* variants in families with Dravet and GEFS+

syndrome[309] and by genome-wide association analysis that pinpoints the *SCN1A* gene as a susceptibility locus for genetic generalized epilepsies.[307]

9.3.3 How to predict prognosis of *SCN1A*-related epilepsies?

At this moment, the uncertainty of the prognosis in a young child with an identified *SCN1A* mutation, is an important genetic counseling issue and a real burden for parents. How to predict clinical expression, i.e. course of disease?

9.3.3.1 Why is prediction of disease course difficult?

Even if the pathogenicity of an *SCN1A* mutation is certain, prediction of the phenotype remains difficult. Mutations that are predicted to lead to haploinsufficiency are considered the most severe mutations and are predominantly reported in Dravet syndrome.[132, 133] However, 13% of detected mutations in GEFS+ cases also lead to haploinsufficiency.[132] Missense mutations causing Dravet syndrome are more often located in the pore region of the $Na_v1.1$ channel,[132] and have larger changes in amino acid polarity than those identified in GEFS+ syndrome.[148] However, several missense mutations have been detected both in patients with Dravet syndrome and in GEFS+ syndrome, and phenotypes may vary considerably between family members with the same missense or nonsense mutation or even intragenic *SCN1A* deletion.[142, 149, 150]

Based on clinical features only, the distinction between Dravet syndrome and (GE)FS+ in young patients with a *de novo SCN1A* mutation may also be difficult to make, as shown in **chapter 7**, since:

a. Frequent febrile seizures in a child with a normal development may initially occur in both disorders.
b. Seizure onset in the first year of life and multiple seizure types may also occur in some patients with GEFS+ syndrome.[310, 311]
c. Other neurological symptoms and developmental delay may be noticed only later in the disease course in some children with Dravet syndrome (**chapter 7**).

Even at older ages the distinction may not always be clear since neuropsychiatric problems may also occur in GEFS+ syndrome,[312, 313] and seizure control might be reasonable in some cases with Dravet syndrome.[76, 314]

9.3.3.2 What influences disease course?

Besides the severity of the *SCN1A* mutation, other exogenous or endogenous factors undoubtedly influence disease severity and prognosis. Infectious diseases and use of contra-indicated AEDs are examples of such exogenous factors.[109-111, 315] An effect of AEDs on outcome could be tested by comparing the disease course of patients who did and did not use contra-indicated AEDs. To correct for individual (endogenous) variation

in severity of the disorder, stratification for mutation type and age at seizure onset are minimum requirements.

The influence of endogenous factors, i.e. other genetic factors, on the severity of the disorder might be even larger. Studies on mouse models of Dravet syndrome with the same *scn1a* defect but different genetic backgrounds show large differences in severity of the disorder,[151] and mouse strains with a milder phenotype were shown to have less impaired sodium channel function, current density and electrical excitability of interneurons.[316, 317] Genetic factors influencing disease severity in patients with an *SCN1A* mutation might first of all consist of variants influencing expression of both the normal *SCN1A* and mutated allele, and thereby residual $Na_v1.1$ function. These potential genetic modifiers may include common variants within the *SCN1A* gene like the splice site polymorphism IVS5N+5G>A (rs3812718) that reduces the expression of the neonatal exon 5N relative to the adult exon 5A.[318] This single nucleotide polymorphism (SNP) has been associated with febrile seizures in children,[319, 320] the response to sodium channel blockers in patients with epilepsy, and the effect of carbamazepine on cortical excitability in healthy volunteers, but these effects could not always be replicated.[318, 321, 322] Other potential genetic modifiers might be variants in the promoter region of the *SCN1A* gene, and enhancers located in the 2q24.3 region around the *SCN1A* gene. Epigenetic factors may also influence expression of residual *SCN1A* expression, but this has not been studied yet.

Genotype-phenotype correlation studies in patients with a deletion of the *SCN1A* gene also including the surrounding *SCN* gene cluster (*SCN3A*, *SCN2A*, *SCN9A* and *SCN7A*) may help to understand the influence of expression of other sodium channel genes on disease severity.[137, 138] For example, natural variation in expression of the *SCN3A* gene, and thereby the level of $Na_v1.3$ channels might compensate for loss of $Na_v1.1$ function and thereby influence age at onset or disease severity. Also variants in other genes influencing either the excitability of interneurons or the excitatory neurons, for example the *SCN8A* gene, might influence epilepsy severity.

Genetic variants influencing innate and immunological responses and thereby, the frequency and height of fever, and indirectly the frequency and severity of febrile seizures, may also be modifiers. The various co-morbidities like autistic features, hyperactivity, motor disorders and sudden unexpected death might be influenced by different genetic modifiers, since they might be caused by $Na_v1.1$ dysfunction in different cell types[112, 288] with cell type specific expression of modifier genes. Several loci for genetic modifiers have been detected in mouse models with the same *scn1a* defect but different genetic backgrounds,[151] and several potential genetic modifiers have been tested in patients with *SCN1A* mutations.[61, 154-157] However, currently no clinical relevant genetic modifiers have been detected yet (see also Introduction).

When searching for genetic factors explaining the variable expression of *SCN1A* mutations, somatic mosaicism of the *SCN1A* mutation should not be overlooked as a determinant of phenotypic variation. It is known that mosaicism of an *SCN1A* mutation can explain apparent non-penetrance or a mild phenotype in a parent of a child with Dravet syndrome.[132, 142] Parents with detected mutation levels in blood of more than 25%, always had seizures, while those with mutation levels of less than 12.5%, had no reported seizures.[132] Mosaicism may explain a relatively favorable disease course in Dravet syndrome as described in **chapter 8**. High-level mosaicism might be common in *de novo SCN1A* mutations, since this was detected in 6.5% of a set of 107 presumed germline *de novo* mutations in other genes, when using amplicon-based deep sequencing in parent-proband trios.[323] A decreased ratio of mutation versus wild-type genotype detected on electropherogram when using Sanger sequencing may also reveal some cases of high-level mosaicism, especially when using DNA derived from various tissues (**chapter 8**), but might be missed easily in a diagnostic setting.

9.3.3.3 How to build a prognostic model?

To develop a model to predict epilepsy severity and cognitive outcome based on early clinical features and genetic characteristics in young children with an *SCN1A* mutation, clinical studies in large cohorts of patients with *SCN1A*-related seizures are needed.

These cohorts should consist of both mildly and more severely affected patients, and selection bias toward severe cases should be excluded as much as possible. Patients with milder disease courses may have been less frequently diagnosed with an *SCN1A*-related epilepsy syndrome. For example, cases of (GE)FS+ syndrome caused by *de novo SCN1A* mutations have hardly been reported in the literature, while in clinical practice they are not uncommon. Previous studies focusing on Dravet syndrome were probably also subject to selection bias toward more severe cases. In our own study (**chapter 7**), for example, developmental delay was an inclusion criterion. Several children with mutations predicted to lead to haploinsufficiency, normally considered causative of Dravet syndrome, had normal development at the time of our study and were therefore not included. Selection bias toward more severely affected cases may be even larger in studies on adults with Dravet syndrome. In this age group, patients are less likely to have been diagnosed in childhood, because the knowledge on the syndrome was limited at that time. More severely affected adult patients who need extensive medical care, are more likely to be diagnosed, than those with a relatively favorable outcome.

Detailed phenotyping of large cohorts of patients should not focus only on seizure semiology and frequency, but also on cognitive and motor functioning, also in cases considered to have GEFS+ syndrome. A cluster analysis of these features could reveal if the severity of *SCN1A*-related epilepsies is distributed dichotomously or plateau shaped, and if *SCN1A*-related GEFS+ and Dravet syndrome are different disorders ("splitting")

or the ends of a continuum ("lumping"). Furthermore, it can be tested whether early clinical characteristics correlate with cognitive outcome or epilepsy course, and could be considered reflections of the endogenous disease severity in a patient.[324] Previous studies have suggested that the presence and age of onset of various seizure types, age of first notice of developmental delay, and age of first abnormal interictal EEG findings are predictive of developmental outcome.[19, 36, 45] In our study (**chapter 6**), a young age at first seizures and presence of PPR seemed to correlate with cognitive outcome.

In large cohorts, deep sequencing of the *SCN1A* gene, using DNA derived from blood and other tissues, can be used to reveal the prevalence of high-level somatic mosaicism. In addition, genetic modifiers as predictors of outcome could be studied. Preferentially, genetic modifiers would be searched for in patients with similar mutations who have a phenotype either at the mild or severe end of the GEFS+/Dravet spectrum, including patients from GEFS+ families with a large intrafamilial variation. Detection of potential genetic modifiers may either be performed by using a targeted or a whole genome approach. The effect of detected potential genetic modifiers on residual sodium current density and excitability of neurons might be further evaluated in animal models, or neurons generated from induced pluripotent stem cell (iPSCs) of patients with Dravet syndrome.[325]

9.3.4 What is the recurrence risk of Dravet syndrome?

Estimating the recurrence risk of Dravet syndrome may be an important genetic counseling issue for families with *SCN1A*-related seizures. For Dravet syndrome families in which the *SCN1A* mutation is also detected in a parent, the risk of transmitting the *SCN1A* mutation to another child is up to 50%. However, in some families the severity of the disorder might differ between siblings and may be milder than Dravet syndrome.[142, 326] Vice versa, in families with GEFS+ syndrome, a child may be born with Dravet syndrome.[142] These familial differences in severity probably result from the inheritance of more or less favorable combinations of genetic modifiers. At this moment, data are lacking to predict disease severity in a new, affected family member. However, the chance that a child is more severely affected, may be higher for a mildly affected parent with a *de novo SCN1A* mutation, because somatic mosaicism may have ameliorated the parent's phenotype (as discussed in **chapter 8**).

In most patients with Dravet syndrome, the *SCN1A* mutation is not detected in DNA, derived from blood, of the parents. Even in families with a child with an apparently *de novo* mutation, recurrence risk of Dravet syndrome in a sibling is not zero, because of the possibility of undetected, low-level somatic or germline mosaicism in one of the parents.[144-147] Although several cases of recurrence have been described, exact risks are not available. The recurrence risk of genetic disorders caused by apparently *de novo* mutations is often considered to be less than 1%, but a recurrence risk of 4.3% was

estimated for apparently de *novo* mutations in the *DMD* gene.[327] Probabilistic modeling studies also show that recurrence risks may be substantially higher than 1%.[328, 329]

In our cohort, recurrence of an apparently *de novo* mutation had occurred in one sibling of 56 cases with an apparently *de novo* mutation (Supplementary Table 2, chapter 7). These 56 children with Dravet syndrome had 26 younger siblings, 3 unaffected fraternal twins and 2 younger half-sibs (unpublished results). The observed recurrence risk in case of an apparently *de novo SCN1A* mutation is therefore 1 in 30 (3%) in our cohort. Although the sample size of this study is too small to establish exact recurrence risks, the results support a recurrence risk of more than 1% in apparently *de novo SCN1A* mutations. Furthermore, deep amplicon resequencing has detected low-level mosaicism in 8.6% of 174 parental couples with a child with Dravet syndrome and an apparently *de novo SCN1A* mutation.[330, 331] Future studies need to establish empirical recurrence risks in families with and without identifiable low-level parental mosaicism.

As long as these specified data are not available, a recurrence risk of over 1% and consequently the option of prenatal diagnostics (with risk of spontaneous abortion of ~0.3%) should be counseled. For those families at increased recurrence risk because of proven germline or somatic mutation in the father, methods to detect the *SCN1A* mutation in fetal DNA circulating in maternal plasma (non-invasive prenatal diagnostics) would be desired to circumvent the accompanying risks of invasive prenatal diagnostics.[332, 333]

9.4 CONCLUDING REMARKS

Based on the findings in this thesis, physicians and professionals involved in the NVP can inform parents of children with a vaccination-associated seizure that although vaccination can trigger a seizure in susceptible children, the underlying etiology explains the disease course. Parents of children with Dravet syndrome can be reassured that vaccination-associated seizure onset does not affect outcome and can be advised on further vaccinations in their child. Furthermore, they can be informed about the seizure precipitant effect of various forms of elevated body temperature and visual stimuli. The findings in this thesis may also improve the early recognition of Dravet syndrome in young children, whether presenting with or without a vaccination-associated seizure onset. An earlier diagnosis may prevent prescription of contra-indicated AEDs and unnecessary ancillary investigations.

Ongoing trials with new drugs and animal studies may eventually lead to improved seizure control and better treatment of cognitive impairment, behavioral problems and motor disorders in patients with Dravet syndrome. Furthermore, acute encephalopathy and premature death might be prevented. With better treatment results, the necessity of an early diagnosis will increase only further. In the future, rapid testing of epilepsy

genes immediately after seizure onset may optimize the diagnostic process. Diagnostic testing for somatic mosaicism and genetic modifiers may provide additional input for a more accurate prediction of the disease severity and recurrence risk in patients with *SCN1A*-related epilepsy. Hopefully, these efforts will lead to an improved quality of life in families affected by Dravet syndrome.

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Summary

Febrile seizures are well known adverse events following childhood vaccinations. When a seizure following vaccination is the first of an evolving epilepsy syndrome, the vaccination might be misinterpreted as the primary cause of the epilepsy. In 2006, it was shown that most children with epileptic encephalopathy, assumed to be caused by vaccination, had Dravet syndrome due to *de novo SCN1A* mutations.[15] Dravet syndrome (formerly known as severe myoclonic epilepsy of infancy, or SMEI) is a rare epilepsy syndrome with seizure onset in the first year of life often triggered by fever, infectious diseases or vaccinations, in a previously healthy child. In the second year or later, multiple seizure types evolve and neurodevelopment slows down. Eventually, most patients will have therapy resistant epilepsy and intellectual disability. In at least 70% of patients with Dravet syndrome, the syndrome is caused by heterozygous mutations in the *SCN1A* gene. These mutations occur *de novo* in about 90% of cases.

The aim of the studies in this thesis was to further delineate the relationship with vaccinations, specify the role of seizure precipitants, and evaluate the diagnostic process in *SCN1A*-related Dravet syndrome.

In **chapter 2** we studied the prevalence of Dravet syndrome among 1,269 children reported with seizures following vaccination in the first two years of life, which was estimated to be at least 1.2%. Of all reported seizures following vaccinations in the first year of life, 2.5% were due to Dravet syndrome. Seizures in children with Dravet syndrome occurred more often with a body temperature below 38.5 °C (58% versus 33%) and reoccurred more often after following vaccinations (27% versus 4%), than in children without this diagnosis.

In **chapter 3** we describe that of all children reported with seizures following vaccination, 2.6% had been diagnosed with epilepsy and had a vaccination-associated seizure onset. Of them, 13% already had encephalopathy prior to seizure onset, 52% had developed an epileptic encephalopathy and 35% had benign epilepsy. Of the children with a (supposed) epileptic encephalopathy, two-thirds had *SCN1A*-related Dravet syndrome. Overall, underlying causes had been identified in 65% of children, including monogenic epilepsy disorders, a microdeletion and neuronal migration disorders.

In **chapter 4** the effect of vaccination-associated seizure onset on disease course and the risk of subsequent seizures after various vaccinations were studied in 77 children with Dravet syndrome. The age at first seizure was younger in the 21% of children with a vaccination-associated seizure onset, but children with and without vaccination-associated seizure onset did not differ in age at first non-vaccination-associated seizure, age at first report of developmental delay, or cognitive outcome.

After seizure onset, the risk of a seizure within 24 hours following an acellular pertussis combination vaccination was 9%, significantly lower than the 37% risk after whole-cell pertussis vaccines. Within the risk period of 5-12 days following MMR vaccination, a 2.3-fold increased incidence rate of seizures was shown using a self-controlled case series analysis.

Based on the findings in **chapters 2, 3 and 4** parents of children with a vaccination-associated seizure can be informed that although vaccination can trigger a seizure in susceptible children, the further clinical course depends on the underlying etiology and is not explained by the vaccination. Our findings may help to improve early recognition of Dravet syndrome as such an underlying etiology. Parents of children with Dravet syndrome can be reassured that vaccination-associated seizure onset does not affect outcome and can be advised on further vaccinations in their child.

The importance of seizure precipitants in Dravet syndrome, other than vaccinations, is studied in **chapter 5**. Responses to a questionnaire of parents of 71 patients with Dravet syndrome were compared with responses to questionnaires in a childhood epilepsy cohort and a community-based epilepsy cohort. For 99% of patients with Dravet syndrome at least one seizure precipitant was reported, with fever, a cold, a bath, acute stress and physical exercise each reported in more than half of them. For each of the seizure precipitants categories 'fever', 'visual stimuli', 'sleep deprivation', 'stress, including physical exercise', 'auditory stimuli', and 'other' the percentage of patients scoring positive was higher in the cohort with Dravet syndrome than the other cohorts with epilepsy. Physical exercise was more often reported to provoke a seizure in stress-sensitive children with Dravet syndrome than in stress-sensitive children with other epilepsies (78% vs. 35%).

Chapter 6 focuses on visual stimuli as seizure precipitants in Dravet syndrome. We re-evaluated photoparoxysmal response (PPR) on EEGs, and collected data on sensitivity to visual stimuli from medical files and parental questionnaires in 53 patients. PPR was induced in 17% of EEGs with intermittent photic stimulations (IPS) and occurred more often when using optimal IPS protocols. The 42% of patients with PPR tended to be younger at seizure onset and to have worse cognitive outcomes. Overall, sensitivity to visual stimuli, clinical or EEG proven, was noted in 65% of cases.

Based on the findings in **chapter 5** and **6** the seizure precipitant effect of elevated body temperature with various causes including physical exercise and of visual stimuli might be better recognized, which may contribute to prevention of seizures in patients with Dravet syndrome.

Since a diagnosis of Dravet syndrome has implications for the choice of antiepileptic drugs (AED), early recognition of this syndrome is important to improve seizure control, and possibly disease course. In **chapter 7** the diagnostic process was studied in a cohort of 76 patients with *SCN1A*-related Dravet syndrome. The clinical diagnosis of DS was established at a median age of 4.4 years and in 87% of patients after DNA confirmation only. Eleven percent of cases had initially been diagnosed with *SCN1A*-related febrile seizures plus (FS+), that was changed into Dravet syndrome after notice of development delay at a median age of 2.0 years. The age at clinical diagnosis decreased significantly after introduction of *SCN1A* analysis in the Netherlands. Before *SCN1A* analysis was performed, 11 (15%) children had received a different diagnosis, most commonly mitochondrial disorders. Use of contra-indicated AED occurred in 87% of patients and decreased with a shorter diagnostic interval and later birth year. Criteria to select children likely to have *SCN1A*-related Dravet syndrome, as proposed by respectively Fountain-Capal et al., Hattori et al. and Le Gal et al.,[203, 205, 256] were met for respectively 100%, 72% and 60% of the children in our cohort, at median ages of 11, 6 and 11 months.

In **chapter 8** the importance of recognizing Dravet syndrome in adults is illustrated by the case histories of two unrelated fathers with severe childhood epilepsy in whom a relatively mild form of Dravet syndrome was only considered after they had fathered a child with *SCN1A*-related Dravet syndrome. Since somatic mosaicism of the *SCN1A* mutation was proven in one of the fathers, somatic mosaicism in brain tissue might be an explanation for a relatively favourable disease course in Dravet syndrome. The disease course in the other father deteriorated after prescription of a sodium channel blocker.

The findings in **chapter 7** and **8** stress the necessity of an early and accurate diagnosis of Dravet syndrome. Application of screening tools and awareness of the variable presentation of Dravet syndrome may improve early recognition. This may prevent prescription of contra-indicated AEDs and unnecessary ancillary investigations.

In **chapter 9** is discussed that in the future, rapid next-generation DNA-sequencing of epilepsy genes immediately after seizure onset may optimize the diagnostic process. Testing for somatic mosaicism and genetic modifiers may improve prediction of disease course and clinical management and more precisely define recurrence risk in families with *SCN1A*-related epilepsy.

Samenvatting

(Summary in Dutch)

Het Rijksvaccinatieprogramma beschermt kinderen tegen verschillende ernstige infectieziekten. Vaccinaties geven in het algemeen slechts milde bijwerkingen, zoals koorts. Koorts, ongeacht of deze het gevolg is van een vaccinatie, kan bij jonge kinderen een koortsstuip uitlokken. Ongeveer 2% tot 5% van de kinderen maakt ooit een of meer koortsstuipen door. Een klein deel van de kinderen met een koortsstuip ontwikkelt later epilepsie. Als een kind een eerste koortsstuip krijgt vlak na een vaccinatie en vervolgens epilepsie ontwikkelt, kan dit de indruk wekken dat de epilepsie veroorzaakt is door de vaccinatie. In 2006 werd in een onderzoek beschreven dat de meeste kinderen met een ernstige vorm van epilepsie die een gevolg leek van een vaccinatie, het Dravet syndroom hadden door een spontane, aangeboren fout in hun erfelijk materiaal.

Het Dravet syndroom is een zeldzame vorm van epilepsie die in 1978 voor het eerst beschreven werd door de Franse neurologe Charlotte Dravet. Het syndroom komt naar schatting voor bij 1 op de 20.000 tot 1 op de 40.000 kinderen. De eerste aanvallen treden op in een ogenschijnlijk gezonde, zich normaal ontwikkelende baby. Deze aanvallen kunnen worden uitgelokt door koorts, infectie, vaccinatie of een warm bad. Na het eerste levensjaar treden ook andere soorten epileptische aanvallen op en blijkt de epilepsie moeilijk te behandelen zijn. Ook ontstaat er een ontwikkelingsachterstand en uiteindelijk ontwikkelt zich meestal een verstandelijke beperking. Het syndroom wordt bij minimaal 70% van de patiënten veroorzaakt door een mutatie (ziekteveroorzakende verandering) in het *SCN1A*-gen. Bij ongeveer 90% van de patiënten is dit een spontane mutatie, dat wil zeggen dat deze niet bij een van de ouders wordt teruggevonden.

Hoofddoel van het onderzoek dat in dit proefschrift wordt beschreven, was het verkrijgen van meer inzicht in de relatie tussen vaccinaties en het verloop van het door *SCN1A*-mutaties veroorzaakte Dravet syndroom. Daarnaast werden ook andere factoren die aanvallen kunnen uitlokken onderzocht, en werd het traject dat voorafgaat aan het stellen van de diagnose Dravet syndroom onderzocht om na te gaan hoe de diagnose tijdiger gesteld kan worden.

In het onderzoek dat in **hoofdstuk 2** wordt beschreven, werd bepaald hoe vaak Dravet syndroom voorkomt bij kinderen met een (mogelijk) epileptische aanval na een vaccinatie in de eerste twee levensjaren. In de periode 1997 tot 2006 waren 1.269 kinderen met een dergelijke aanval gemeld bij het Rijksinstituut voor Volksgezondheid en Milieu (RIVM). De medische gegevens werden opgevraagd van kinderen die mogelijk verschijnselen van het Dravet syndroom hadden. Bij 11 kinderen bleek dat de diagnose Dravet syndroom inmiddels gesteld was. Bij 4 andere kinderen werd alsnog de diagnose Dravet syndroom gesteld op grond van het verloop van de verschijnselen en werd met DNA-onderzoek een *SCN1A*-mutatie gevonden. In totaal werd het Dravet syndroom aangetoond bij 1,2% van de kinderen gemeld met een aanval na vaccinatie. Uit dit onderzoek

blijkt ook dat kinderen met Dravet syndroom vaker een aanval bij een lichaamstemperatuur van minder dan 38,5 °C en vaker opnieuw een aanval na een volgende vaccinatie hadden, dan gemelde kinderen zonder de diagnose Dravet syndroom.

In het onderzoek dat in **hoofdstuk 3** wordt beschreven, werd onderzocht hoe vaak kinderen met een aanval na vaccinatie epilepsie ontwikkelen. Ook het verloop en onderliggende oorzaken van de epilepsie werden onderzocht. Uit de bij het RIVM beschikbare gegevens bleek dat van de gemelde kinderen met een aanval na een vaccinatie, 2,6% de eerste aanval had gekregen vlak na een vaccinatie èn vervolgens epilepsie had ontwikkeld. De medische gegevens van deze kinderen werden opgevraagd en beoordeeld. Uit deze gegevens bleek dat 13% al een ontwikkelingsachterstand had voorafgaand aan de eerste aanval. 35% van de kinderen had juist een voorbijgaande epilepsie zonder ontwikkelingsproblemen. Bij de overige 52% van de kinderen ontstond een ontwikkelingsachterstand na de eerste epileptische aanvallen. In deze laatste groep bleek tweederde van de kinderen het Dravet syndroom te hebben. In de gehele groep van kinderen met epilepsie was bij 65% een onderliggende, aangeboren oorzaak voor de epilepsie vastgesteld. Naast *SCN1A*-mutaties waren dit een chromosoomafwijking, andere erfelijke vormen van epilepsie, of een aanlegstoornis van de hersenen.

In **hoofdstuk 4** worden de resultaten beschreven van een onderzoek naar het vóórkomen van vaccinatie-gerelateerde aanvallen en de invloed daarvan op het ziekteverloop bij het Dravet syndroom. In een groep van 77 kinderen met Dravet syndroom werd onderzocht hoe vaak vaccinatie een eerste aanval uitlokte en of dit het ziekteverloop beïnvloedde. Bij 21% van de kinderen trad de eerste aanval vlak na een vaccinatie op. Deze groep was jonger ten tijde van de eerste aanval dan de groep overige kinderen. Dit vroegere begin van de epilepsie beïnvloedde het verloop van de aandoening echter niet: er waren geen verschillen tussen de twee groepen in a) leeftijd waarop de eerste, niet door een vaccinatie uitgelokte aanval optrad; b) de leeftijd waarop voor het eerst een ontwikkelingsachterstand werd opgemerkt; en c) het uiteindelijke bepaalde intelligentieniveau.

Het voorkomen van aanvallen na een vaccinatie, nà het begin van de epilepsie, werd in dezelfde groep van 77 kinderen met Dravet syndroom onderzocht. In Nederland worden kinderen in het eerste levensjaar viermaal gevaccineerd tegen onder andere difterie, kinkhoest, tetanus en polio (DKTP). De samenstelling van het kinkhoestvaccin is in 2005 gewijzigd: tot die tijd werd het zogenoemde whole-cell kinkhoestvaccin gebruikt, en sindsdien een acellulair kinkhoestvaccin. Eerdere onderzoeken toonden al aan dat acellulaire kinkhoestvaccins in het algemeen minder vaak bijwerkingen geven dan whole-cell kinkhoestvaccins. De resultaten in hoofdstuk 4 tonen aan dat bij kinderen met Dravet syndroom de kans op een epileptische aanval binnen 24 uur na een

DKTP-vaccinatie duidelijk lager was na acellulaire dan whole-cell kinkhoestvaccinaties, namelijk 9% tegenover 37%.

In Nederland worden kinderen op de leeftijd van 14 maanden gevaccineerd tegen onder andere bof, mazelen en rode hond (BMR). Het is bekend dat eventuele bijwerkingen, zoals koorts, 5 tot 12 dagen na de vaccinatie optreden. De resultaten van het onderzoek in hoofdstuk 4 tonen aan dat bij kinderen met Dravet syndroom de kans op aanvallen binnen deze periode ruim twee keer hoger was dan buiten deze periode.

Op grond van de bevindingen in de **hoofdstukken 2, 3 en 4** kan aan ouders van een kind met een (koorts)stuip na vaccinatie worden uitgelegd dat vaccinatie een aanval kan uitlokken in daarvoor gevoelige kinderen; het optreden van eventuele volgende aanvallen wordt niet veroorzaakt door de vaccinatie maar door een onderliggende aanleg in het kind zelf. De bevindingen kunnen daarnaast bijdragen aan de vroegtijdige herkenning van het Dravet syndroom door o.a. kinderartsen, consultatiebureau-artsen en medewerkers van het Rijksvaccinatieprogramma. Aan ouders van kinderen met het Dravet syndroom kan worden uitgelegd dat het ziekteverloop niet wordt beïnvloed door een aanval uitgelokt door vaccinatie. Ook kunnen zij geïnformeerd worden over de kans op (hernieuwde) aanvallen na volgende vaccinaties.

Het belang van andere factoren die aanvallen kunnen uitlokken bij het Dravet syndroom, werd bestudeerd in het onderzoek dat beschreven staat in **hoofdstuk 5.** Ouders van 71 patiënten met het Dravet syndroom beantwoordden voor dit onderzoek een vragenlijst. De resultaten daarvan werden vergeleken met antwoorden uit twee andere onderzochte patiëntengroepen met epilepsie. Van alle ouders van een kind met het Dravet syndroom noemde 99% minimaal één uitlokkende factor. Koorts, een verkoudheid, in bad gaan, acute stress en lichamelijke inspanning werden elk als uitlokker genoemd bij meer dan de helft van de patiënten met het Dravet syndroom. Alle onderzochte categorieën van uitlokkende factoren, d.w.z. zowel 'koorts', 'lichtprikkels', 'slaaptekort', 'stress, met inbegrip van lichamelijke inspanning', 'geluidsprikkels' en 'overige' werden bij de groep patiënten met het Dravet syndroom vaker vermeld dan bij de twee andere groepen patiënten met epilepsie. Binnen de categorie 'stress, met inbegrip van lichamelijke inspanning' werd lichamelijke inspanning vaker genoemd als uitlokker bij kinderen met het Dravet syndroom, dan bij kinderen met een andere vorm van epilepsie.

In het in **hoofdstuk 6** beschreven onderzoek werd het vóórkomen van lichtprikkels (lichtflitsen en patronen) als uitlokkende factor bij het Dravet syndroom bestudeerd. Het effect van lichtflitsprikkeling op de hersenactiviteit werd beoordeeld op grond van opgevraagde verslagen van gemaakte EEGs (hersenfilms). Daarnaast werden opmerkingen in de medische dossiers over de gevoeligheid voor lichtprikkels verzameld en

beantwoordden ouders een vragenlijst. Op basis van deze gegevens was 65% van de patiënten met het Dravet syndroom gevoelig voor lichtprikkels. Bij 42% van de patiënten werd gevoeligheid voor lichtflitsprikkeling op het EEG aangetoond. De kans om dit vast te stellen was hoger bij optimaal uitgevoerde lichtflitsprikkeling en als spontane epileptische activiteit op het EEG zichtbaar was. Patiënten bij wie gevoeligheid voor lichtflitsprikkeling was aangetoond waren jonger ten tijde van hun eerste aanvallen en hadden uiteindelijk een ernstigere verstandelijke beperking. Patiënten met aangetoonde gevoeligheid voor lichtflitsprikkeling leken dus gemiddeld ernstiger aangedaan dan degenen zonder aangetoonde gevoeligheid.

Op basis van de bevindingen in **hoofdstuk 5** en **6** kan het effect van diverse lichtprikkels en diverse oorzaken van een verhoogde lichaamstemperatuur op het ontstaan van aanvallen hopelijk beter worden herkend. Dit zou kunnen bijdragen aan het verminderen van het aantal aanvallen bij patiënten met Dravet syndroom.

Het vaststellen van het Dravet syndroom heeft gevolgen voor de keuze van anti-epileptica (medicijnen om de epilepsie mee te behandelen). Van bepaalde anti-epileptica is namelijk bekend dat deze specifiek bij het Dravet syndroom de aanvallen kunnen verergeren. Vroegtijdige herkenning van de aandoening is dus belangrijk om de aanvalsbehandeling te verbeteren, en daarmee wellicht ook het verdere verloop van de aandoening.

In het in **hoofdstuk 7** beschreven onderzoek werd het diagnostisch proces, dat wil zeggen het traject dat voorafgaat aan het stellen van de diagnose, bestudeerd in een groep patiënten met Dravet syndroom als gevolg van een *SCN1A*-mutatie. Het uitvoeren van DNA-onderzoek van het *SCN1A*-gen is in Nederland mogelijk sinds 2004. In de bestudeerde groep patiënten was de diagnose Dravet syndroom op een leeftijd van 4 jaar en 5 maanden bij de helft gesteld. Op grond van de verschijnselen had de diagnose al eerder gesteld kunnen worden, maar dat gebeurde bij 87% van de patiënten pas nadat de *SCN1A*-mutatie was aangetoond. De leeftijd waarop de diagnose werd gesteld, daalde aanzienlijk na het beschikbaar komen van DNA-onderzoek van het *SCN1A*-gen in Nederland.

Nadat de *SCN1A*-mutatie was vastgesteld, werd bij 11% van de kinderen aanvankelijk gedacht dat zij een mildere vorm van koortsgevoelige epilepsie hadden. De diagnose Dravet syndroom werd alsnog gesteld nadat een ontwikkelingsachterstand was ontstaan. Het vaststellen van een ontwikkelingsachterstand gebeurde bij de helft van alle kinderen pas na hun tweede verjaardag. Voordat de *SCN1A*-mutatie was vastgesteld, had 15% van de kinderen een andere diagnose als vermeende verklaring voor de epilepsie gekregen.

Van de hele groep patiënten had 87% minimaal één anti-epilepticum gebruikt waarvan bekend is dat het de aanvallen bij het Dravet syndroom kan verergeren. Dit was minder vaak het geval bij kinderen bij wie al op jongere leeftijd de *SCN1A*-mutatie was vastgesteld en bij kinderen uit recentere geboortejaren.

In **hoofdstuk 8** wordt het belang van onderkenning van het Dravet syndroom bij volwassenen geïllustreerd door middel van een beschrijving van twee families. Bij twee volwassen mannen werd alsnog aan een milde vorm van het Dravet syndroom gedacht, nadat ze zelf een kind met Dravet syndroom hadden gekregen. Zij waren beiden als kind behandeld voor een ernstige vorm van epilepsie. Bij één vader kon mozaïcisme voor de *SCN1A*-mutatie worden vastgesteld, dat wil zeggen dat de mutatie slechts in een deel van zijn lichaamscellen aanwezig was. Mozaïcisme voor de *SCN1A*-mutatie in de hersenen bij een patiënt met Dravet syndroom zou een verklaring kunnen zijn voor een relatief gunstig verloop van de aandoening.

Bij de andere vader verliep de ziekte als kind gunstig. Als volwassene verslechterde het ziekteverloop, mede nadat met een anti-epilepticum werd gestart waarvan bekend is dat het aanvallen kan verergeren bij het Dravet syndroom. De diagnose Dravet syndroom was op dat moment echter nog niet bij hem overwogen.

De bevindingen in **hoofdstuk 7** en **8** benadrukken de noodzaak van een vroege diagnose van het Dravet syndroom. Het verbeteren van de kennis bij artsen over de variabele presentatie van het syndroom zou vroegtijdige herkenning kunnen bevorderen. Daarmee zou zowel het voorschrijven van voor het Dravet syndroom ongeschikte anti-epileptica, als het uitvoeren van belastende onderzoeken naar andere oorzaken voor de epilepsie, verminderd kunnen worden.

In **hoofdstuk 9** worden de richtingen voor verder wetenschappelijk onderzoek bij het Dravet syndroom aangegeven. Uiteraard is er behoefte aan een betere behandeling. Als een betere behandeling beschikbaar komt, wordt het stellen van de diagnose Dravet syndroom op een jonge leeftijd des te belangrijker. Het vaststellen van deze diagnose kan versneld worden, indien bij jonge kinderen met epileptische aanvallen direct en snel DNA-onderzoek van bekende epilepsie-genen verricht zou worden. Met jongere leeftijden waarop *SCN1A*-mutaties worden opgespoord, stijgt het belang van een goede inschatting van het te verwachten ziekteverloop. Verschillen in verloop zouden verklaard kunnen worden door mozaïcisme voor de *SCN1A*-mutatie, maar ook door veranderingen in andere delen van het erfelijk materiaal.

Toekomstig wetenschappelijk onderzoek zou zich moeten richten op het ontwikkelen van testen die deze factoren kunnen opsporen en die het ziekteverloop kunnen voorspellen. Ook is er meer kennis nodig over factoren die de herhalingskans op het Dravet

syndroom bij een volgend kind in een gezin bepalen dan wel voorspellen, zodat ouders hierover beter voorgelicht kunnen worden.

List of Publications

THIS THESIS

Effect of vaccinations on seizure risk and disease course in Dravet syndrome.
Verbeek NE, van der Maas NA, Sonsma AC, Ippel E, Vermeer-de Bondt PE, Hagebeuk E, Jansen FE, Geesink HH, Braun KP, de Louw A, Augustijn PB, Neuteboom RF, Schieving JH, Stroink H, Vermeulen RJ, Nicolai J, Brouwer OF, van Kempen M, de Kovel CG, Kemmeren JM, Koeleman BP, Knoers NV, Lindhout D, Gunning WB, Brilstra EH.
Neurology 2015; 85: 596-603.

Seizure precipitants in Dravet syndrome: What events and activities are specifically provocative compared with other epilepsies?
Verbeek NE, Wassenaar M, van Campen JS, Sonsma A, Gunning B, Knoers N, Lindhout D, Jansen FE, Leijten F, Brilstra EH, Kasteleijn-Nolst Trenité D.
Epilepsy Behav 2015; 47: 39-44.

Etiologies for seizures around the time of vaccination.
Verbeek NE, Jansen FE, Vermeer-de Bondt PE, de Kovel CG, van Kempen MJ, Lindhout D, Knoers NV, van der Maas NA, Brilstra EH.
Pediatrics 2014; 134: 658-666.

Prevalence of SCN1A-related dravet syndrome among children reported with seizures following vaccination: a population-based ten-year cohort study.
Verbeek NE, van der Maas NA, Jansen FE, van Kempen MJ, Lindhout D, Brilstra EH.
PLoS One 2013; 8: e65758.

Adults with a history of possible Dravet syndrome: an illustration of the importance of analysis of the SCN1A gene.
Verbeek NE, van Kempen M, Gunning WB, Renier WO, Westland B, Lindhout D, Brilstra EH.
Epilepsia 2011; 52: e23-25.

OTHER SCIENTIFIC PUBLICATIONS

Characterization of ANKRD11 mutations in humans and mice related to KBG syndrome.
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Curriculum Vitae

Nienke Verbeek was born in 1977 in Hengelo (O), the Netherlands. In 1995 she obtained her high school diploma at the Lyceum de Grundel (Hengelo) and began to study Biomedical Sciences at the University of Leiden. In 1998 she started medical school at the same university. She graduated *cum laude* in Biomedical Sciences in 2001 and obtained her medical degree *cum laude* in 2003.

In 2003 and 2004 she worked as a resident not in training (ANIOS) at the Department of Internal Medicine at the Medical Center Haaglanden, location Leidschendam. She then worked as ANIOS in Clinical Genetics of the Erasmus MC in Rotterdam. In 2005 she started her residency clinical genetics at the Department of Medical Genetics at the University Medical Center Utrecht, under the supervision of Prof. F.A. Beemer and subsequently Prof. D. Lindhout. Since December 2010 she has been registered as a clinical geneticist and has continued to work at the same department. During her residency the research that has led to this thesis was initiated under supervision of Dr. E.H. Brilstra.

Nienke Verbeek lives in Utrecht with Jeroen and their two sons Geert and Guus.

Nienke Verbeek werd geboren in 1977 te Hengelo (O). In 1995 behaalde zij haar VWO-diploma aan het Lyceum de Grundel (Hengelo) en begon zij met de studie Biomedische Wetenschappen aan de Universiteit Leiden. In 1998 startte zij met de studie Geneeskunde aan dezelfde universiteit. Het doctoraalexamen Biomedische Wetenschappen en het artsexamen legde zij *cum laude* af in respectievelijk 2001 en 2003.

In 2003 en 2004 werkte zij als arts niet in opleiding tot specialist (ANIOS) bij de afdeling Interne Geneeskunde van het Medisch Centrum Haaglanden, locatie Leidschendam. Vervolgens werkte zij als ANIOS bij de afdeling Klinische Genetica van het Erasmus MC te Rotterdam. In 2005 startte zij met de opleiding tot klinisch geneticus aan de afdeling Medische Genetica van het Universitair Medisch Centrum Utrecht, met als opleider Prof. dr. F.A. Beemer en vervolgens Prof. dr. D. Lindhout. Tijdens de opleiding begon zij onder leiding van dr. E.H. Brilstra met het onderzoek dat geleid heeft tot dit proefschrift. De opleiding tot klinisch geneticus rondde zij af in december 2010. Sindsdien is zij als klinisch geneticus werkzaam in het UMC Utrecht.

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