# De novo SCN1A mutations in Dravet syndrome and related epileptic encephalopathies are largely of paternal origin

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*J Med Genet* 2010 47: 137-141 originally published online July 8, 2009
doi: 10.1136/jmg.2008.065912

# De novo *SCN1A* mutations in Dravet syndrome and related epileptic encephalopathies are largely of paternal origin

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▶ Supplementary tables 1–3 are published online only at http://jmg.bmj.com/content/vol47/issue2

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Received 23 December 2008
Revised 29 June 2009
Accepted 30 June 2009
Published Online First 8 July 2009

## ABSTRACT

**Background** Dravet syndrome is a severe infantile epileptic encephalopathy caused in approximately 80% of cases by mutations in the voltage gated sodium channel subunit gene *SCN1A*. The majority of these mutations are de novo. The parental origin of de novo mutations varies widely among genetic disorders and the aim of this study was to determine this for Dravet syndrome.

**Methods** 91 patients with de novo *SCN1A* mutations and their parents were genotyped for single nucleotide polymorphisms (SNPs) in the region surrounding their mutation. Allele specific polymerase chain reaction (PCR) based on informative SNPs was used to separately amplify and sequence the paternal and maternal alleles to determine in which parental chromosome the mutation arose.

**Results** The parental origin of *SCN1A* mutations was established in 44 patients for whom both parents were available and SNPs were informative. The mutations were of paternal origin in 33 cases and of maternal origin in the remaining 11 cases. De novo mutation of *SCN1A* most commonly, but not exclusively, originates from the paternal chromosome. The average age of parents originating mutations did not differ from that of the general population.

**Conclusions** The greater frequency of paternally derived mutations in *SCN1A* is likely to be due to the greater chance of mutational events during the increased number of mitoses which occur during spermatogenesis compared to oogenesis, and the greater susceptibility to mutagenesis of the methylated DNA characteristic of sperm cells.

## INTRODUCTION

Dravet syndrome (DS), or severe myoclonic epilepsy of infancy (SMEI) (OMIM #607208) is a severe epileptic encephalopathy with onset around 6 months of age. Patients typically present with febrile hemiclonic or generalised tonic-clonic status epilepticus, followed by the development of other seizure types including myoclonic, focal, absence and atonic seizures between 1–4 years. Development is normal in the first year of life with subsequent developmental slowing and sometimes regression. Cognitive outcome is poor with ongoing refractory seizures; ataxia and spasticity may evolve.[1] Some patients who lack one or more of the clinical features have been regarded as having the borderland variant of SMEI (SMEB). Recent work led to the suggestion that both the classical and the borderland variants of SMEI be incorporated under the eponym of DS as they share clinical features and may be caused by the same mutations.[2 3]

The majority of cases of DS are caused by de novo loss of function mutations in the voltage gated sodium channel α1 subunit gene *SCN1A* (OMIM *1823890).[3–6] Approximately 80% of patients with DS have point mutations or small insertions or deletions in the *SCN1A* gene.[3] Large deletions affecting one or more exons or the entire gene and adjacent sequences are also seen in a small proportion of cases.[6–9] Similar *SCN1A* mutations may also cause other infantile epileptic encephalopathies including severe infantile multifocal epilepsy, cryptogenic generalised epilepsy and, in a few patients, myoclonic astatic epilepsy.[3 6 10]

The parental origin of mutations has been studied for a number of disorders caused by de novo mutations, including various forms of craniosynostosis,[11–13] achondroplasia,[14] Rett syndrome,[15 16] haemophilia A,[17] neurofibromatosis I[18] and neonatal diabetes.[19] Data from many of these studies has been collated in the imprinted gene and parent-of-origin effect database (http://www.otago.ac.nz/IGC).[20] In almost all of the diseases studied, there is a bias towards paternal origin of the de novo mutations, with between 65–100% of mutations arising on the paternal chromosome. Exceptions are von Hippel–Lindau disease,[21] tuberous sclerosis[22] and haemophilia B,[23 24] where mutations originate with approximately equal frequency on the paternal and maternal alleles.

The aim of this study was to determine the parent-of-origin of de novo *SCN1A* mutations in patients with DS and other infantile encephalopathies, and to determine whether there is any relationship between parental age, mutagenesis in *SCN1A*, and the occurrence of DS and related disorders.

## METHODS

### Patients and mutation screening

Patients with DS or other epileptic encephalopathies with de novo *SCN1A* mutations were included if DNA was available from one or both parents. Ninety-one patients were studied, 84 of whom have been described elsewhere.[3 8 25 26] The study was approved by the human research ethics committees of Austin Health and the Women's and Children's Hospital.

Patients were screened for mutations in *SCN1A* as previously described.[3 25] Where available, the

parents were sequenced for the mutation in the amplicon in which the mutation was detected in their child.

### Single nucleotide polymorphism identification and genotyping

The region surrounding the mutation was polymerase chain reaction (PCR) amplified and sequenced for each patient and his or her parents to genotype any single nucleotide polymorphism (SNPs) that were present. The primer sequences used for amplification are listed in supplementary table 1. Additional internal primers were used for sequencing; these were the primers used previously for *SCN1A* mutation screening.[10] Genotyping was done for certain SNPs using restriction enzyme digestion. The primer sequences and restriction enzymes used for these assays are listed in supplementary table 2.

Genotyping was done for one SNP, rs13060006, by high resolution melting analysis using a LightScanner (Idaho Technology Inc, Salt Lake City, Utah, USA). PCRs were done for high resolution melting analysis according to the manufacturer's instructions, using the primers 5′-CCACCCAGCAAAGAAGG-3′ and 5′-CAGTGGGAAAGCACCAGA-3′. Melting curve data were acquired over a temperature range of 60–98°C. The melting curves obtained were analysed using the Small Amplicon Genotyping module of the LightScanner Analysis Software.

### Allele specific PCR

The parental origin of mutations was determined by using allele specific PCR (AS-PCR), also called the amplification refractory mutation system, to amplify and analyse the patient's two alleles separately.[11] This method relies on the presence of a heterozygous SNP in the same amplicon as the mutation.

AS-PCR was done for the SNPs listed in supplementary table 3 using the corresponding primers and specific amplification conditions. Products were amplified using recombinant Taq DNA Polymerase (Invitrogen, Carlsbad, California, USA). Reactions contained 1×PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 15 ng/μl of each primer, 0.5 U Taq DNA Polymerase, and 100 ng template DNA in a 20 μl reaction volume. PCRs were cycled through 10 cycles of: denaturation at 94°C for 30 s, annealing for 30 s and extension at 72°C, then 25 cycles of: denaturation at 94°C for 30 s, annealing 30 s and extension at 72°C, followed by a final extension at 72°C for 10 min. Four sets of reactions were done for each primer pair, using annealing temperatures of 60, 62, 65 or 68°C for the first 10 PCR cycles and 55, 57, 60 or 63°C for the subsequent 25 cycles. Extension times varied according to the expected length of the PCR product and these are listed in supplementary table 3. At least one homozygous sample was included in all AS-PCRs as a control to confirm that amplification was allele specific. If allele specificity was not achieved at the annealing temperatures described above, three further sets of reactions were done with annealing temperatures of 68°C followed by 63°C, 70°C followed by 65°C, and 72°C followed by 67°C. If no product was seen in the initial PCRs, reactions were then done using lower annealing temperatures between 50°C and 60°C. AS-PCR products showing robust amplification and allele specificity were selected for sequencing. These were purified using the Qiagen Qiaquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced with BigDye v3.1 (Applied Biosystems Inc, Foster City, California, USA) using the appropriate forward and reverse primers. Sequencing reactions were analysed using an ABI 3730 capillary sequencer and traces were examined for the presence of the patient's mutation.

To summarise, patients and their parents were sequenced for the exon in which their mutation was located, and adjacent exons in cases where no informative SNPs were present in the exon containing the mutation. Twenty-six SNPs, including four not listed in dbSNP, were used for AS-PCR. Thirty-five different primer combinations were used for AS-PCR; some SNPs were used as the basis for multiple AS-PCRs. Details of the AS-PCRs performed are listed in supplementary table 3.

The parental origin was determined for large deletions by genotyping SNPs within the deletion. Non-transmission of a genotype or haplotype from a parent to the affected child indicated that the deletion originated in that parent's allele.

### Microsatellite analysis of informative families

All informative families were tested to confirm family relationships and gender of the parental samples using 12 highly polymorphic microsatellite markers. These were: D3S3680; D4S418; D6S289; D7S2560; D8S281; D13S175; D13S221; D15S117; D19S1150; DXS1113; DXS1036; DXS7423. PCRs were done using the Qiagen Multiplex PCR kit according to the manufacturer's instructions. The reverse primer of each pair was labelled with either HEX or FAM. Products were analysed on an ABI 3131 Genetic Analyzer.

## RESULTS

The parental origin of the mutations was definitively determined for 44/91 unrelated patients; 43 with de novo mutations, and one sibship (table 1: patient 44) of two sisters whose originating parent was thought to have gonadal mosaicism because the same mutation was observed in both sisters and neither parent. Thirty-three cases were of paternal origin and 11 of maternal origin. The mutations, parental origins, ages of the originating parents at birth and countries of origin for these 44 independent cases are listed in table 1. In an additional five cases paternal DNA samples were not available (table 1: patients 45–49), and the mutations were shown not to originate from the maternal allele. Thus, a paternal origin was presumed for these mutations but it could not be definitely established that they arose de novo. Although de novo origin from the paternal allele is highly probable, given that these patients have DS and their fathers were unaffected with no family history of epilepsy, these patients were excluded from analyses of parental age and mutation type. Microsatellite analysis of informative families confirmed family relationships and results for the X chromosome markers indicated there had been no inadvertent switching of paternal and maternal samples.

The 44 patients for whom parent-of-origin was determined had 43 different mutations: one mutation recurred in two unrelated patients (table 1: patients 31 and 32), one of whom had paternal and the other maternal origin. The mutations seen in these 44 patients included 33 nucleotide substitutions, seven small (1–4 bp) deletions, one 7 bp insertion and three large deletions. Of the nucleotide substitutions, 25 (74%) were paternal and eight were maternal. Seven (88%) of the small deletions and insertions were paternal while one was maternal. Two large deletions were of maternal origin and one was paternal.

The largest subset of patients (25) were of Australian origin. When the parental ages for the Australian families were compared with the median parental ages from the general Australian population for the years in which their children were born (data collated from Australian Bureau of Statistics publications 3301.0, Births: Australia from 1998 to 2004), no significant difference in ages was seen (data not shown).

## DISCUSSION

We were able to determine the parental origin of the mutation in more than half of the patients we studied. It was necessary to

**Table 1** Details of patients for whom parent-of-origin was identified

| Patient | Phenotype | Mutation | Origin | Parental age* | Country of origin |
|---|---|---|---|---|---|
| 1[a] | Dravet | c.41delT (p.F14fsX91) | Maternal | 33.5 | Australia |
| 2[b] | Dravet | c.111delC (p.P37fsX91) | Paternal | 28.2 | Australia |
| 3[a] | Dravet | c.251A→G (p.Y84C) | Paternal | 29.1 | Canada |
| 4[a] | Dravet | c.265-1G→A | Paternal | 32.4 | New Zealand |
| 5[a] | Dravet | c.302G→A (p.R101Q) | Paternal | 34.0 | Canada |
| 6[a] | Dravet | c.495-496-ins7 bp (p.T166fsX170) | Paternal | 28.4 | Australia |
| 7[a] | Dravet | c.512T→A (p.I171K) | Paternal | 24.1 | New Zealand |
| 8[a] | Dravet | c.523G→A (p.A175T) | Paternal | 45.3 | UK |
| 9[a] | Dravet | c.602+1G→A | Paternal | 29.8 | Australia |
| 10[a] | Dravet | c.680T→G (p.I227S) | Maternal | 25.5 | UK |
| 11[a] | Dravet | c.1197C→A (p.Y339X) | Paternal | 32.1 | Australia |
| 12 | Dravet—Mild | c.1216G→T (p.V406F) | Paternal | 33.5 | Australia |
| 13[a] | Dravet | c.1237T→A (p.Y413N) | Paternal | 38.0 | Canada |
| 14[a] | Dravet | c.1662+2T→C | Paternal | 38.3 | Canada |
| 15[a] | SIMFE | c.1724delT (p.F575fsX622) | Paternal | 32.7 | Australia |
| 16[a] | Dravet | c.2348T→C (p.L783P) | Paternal | 29.3 | Canada |
| 17[a] | Dravet | c.2562delA (p.G854fsX876) | Paternal | 41.9 | Canada |
| 18[a] | Dravet | c.2589+3A→T | Paternal | 36.3 | New Zealand |
| 19[a] | Dravet | c.2831T→A (p.V994E) | Paternal | 32.5 | Australia |
| 20[a] | Dravet | c.2837G→A (p.R946H) | Paternal | 33.4 | Australia |
| 21[a] | Dravet | c.2849G→A (p.G950E) | Paternal | 39.0 | Australia |
| 22[a] | CGE | c.2946+1G→T | Paternal | 35.8 | Canada |
| 23[a] | Dravet | c.3022G→T (p.E1008X) | Maternal | 24.4 | Australia |
| 24[b] | Dravet | c.3672+1G→A | Paternal | 26.4 | Australia |
| 25 | Dravet | c.3968delC (p.P1323fsX1325) | Paternal | 41.2 | Australia |
| 26[a] | Dravet | c.4186T→G (p.C1396G) | Maternal | 22.1 | Canada |
| 27[a] | Dravet | c.4219C→T (p.R1407X) | Paternal | 29.8 | Australia |
| 28[a] | Dravet | c.4279C→T (p.Q1427X) | Paternal | 32.1 | Australia |
| 29[b] | Dravet | c.4339-14T→G | Paternal | 30.6 | Australia |
| 30 | Dravet | c.4348C→A (p.N1450K) | Paternal | 45.3 | New Zealand |
| 31[a] | Dravet | c.4547C→A (p.S1516X) | Maternal | 25.9 | Australia |
| 32 | Dravet | c.4547C→A (p.S1516X) | Paternal | 35.0 | Australia |
| 33 | Dravet | c.4558delC (p.G1520X) | Paternal | 26.0 | UK |
| 34[a] | Dravet | c.4633A→G (p.I1545V) | Paternal | 38.4 | Australia |
| 35[a] | LGS | c.4907G→A (p.R1636Q) | Maternal | 25.6 | Australia |
| 36[a] | Dravet | c.4934G→A (p.R1645Q) | Maternal | 26.6 | Australia |
| 37[a] | CFE | c.4970G→A (p.R1657H) | Paternal | 25.4 | Australia |
| 38[a] | Dravet | c.5176T→C (p.W1726R) | Maternal | 36.9 | Canada |
| 39[a] | Dravet | c.5347G→A (p.A1783T) | Paternal | 27.7 | Australia |
| 40[a] | Dravet | c.5536-5539delAAAC (p.K1846fsX1856) | Paternal | 38.4 | Australia |
| 41[a] | Dravet | c.5674C→T (p.R1892X) | Maternal | 37.9 | UK |
| 42[c] | Dravet | IVS20-IVS21del4,499bp | Paternal | 32.2 | USA |
| 43[c] | Dravet | Del exons 21–26 | Maternal | 30.4 | Australia |
| 44[d] | Dravet | Del exons 1–22 | Maternal (known gonadal mosaicism) | | Israel |
| 45[a] | Dravet | c.580G→A (p.D194N) | Paternal† | | New Zealand |
| 46[a] | Dravet | c.602+5G→A | Paternal† | | Australia |
| 47[a] | Dravet | c.1811G→A (p.R604H) c.4573C→T (p.R1525X) | Paternal† | | Australia |
| 48[a] | Dravet | c.2833T→A (p.F945L) | Paternal† | | Australia |
| 49[a] | Dravet | c.3096delA (p.E1032fsX1045) | Paternal† | | USA |

*Parental age refers to age at birth of child, given in years to one decimal place.
†DNA for the patient's father is unavailable, therefore it is not certain that these are de novo mutations. [a]Originally described by Harkin *et al*[3]; [b]originally described by Wallace *et al*[25]; [c]originally described by Mulley *et al*[8]; [d]originally described by Marini *et al*.[26]
CFE, cryptogenic focal epilepsy; CGE, cryptogenic generalised epilepsy; LGS, Lennox–Gastaut syndrome; SIMFE, severe infantile multifocal epilepsy.

assume de novo origin since a small percentage of gonadal mosaicism is known in DS,[27 28] which cannot be detected in the absence of affected siblings. The determination of the parent-of-origin of these putative de novo mutations relies on the presence of a heterozygous SNP in the same amplicon as the mutation, and also requires that it is possible to determine from which parent each allele of the SNP was inherited. Therefore, it is not possible to determine the parent-of-origin for every patient with a de novo mutation. The proportion of patients for whom we were able to determine the parent-of-origin was similar to that in studies done for other genes using similar methods.[11–15 19]

De novo mutations in *SCN1A* arise most often, but not exclusively, on the paternal chromosome. Of the 44 mutations studied, 75% were paternally derived and 25% were maternally derived. Similar proportions of small insertions and deletions (88%) and nucleotide substitutions (74%) were paternally derived. It is not possible to draw any definitive conclusions about the parental origin of large deletions compared to other

mutation types as only three patients with large deletions were included in the study, and one of these was known to be the result of gonadal mosaicism.

A bias towards paternal origin of de novo mutations is often, but not always, observed in the genetic disorders for which parent-of-origin has been studied.[20] The proportion ranges from exclusively paternal to an approximately equal frequency of paternal and maternal origin. A preponderance of maternally derived mutations has not been seen in any disorder caused by point mutations or small insertions or deletions, although maternal origin is seen more frequently for some large deletions and chromosomal rearrangements (imprinted gene and parent-of-origin effect database).[20] The most extensively studied genes with an exclusively paternal origin of mutations are the fibroblast growth factor genes *FGFR2* and *FGFR3*. Mutations in these genes cause achondroplasia and several craniosynostosis syndromes, and 79 of 79 de novo *FGFR2* and 50 of 50 de novo *FGFR3* mutations studied were paternally derived.[11–14 20] In contrast, for the coagulation factor IX gene *F9*, 59 of 123 haemophilia B mutations studied were paternally derived, giving an approximately equal distribution of the parent-of-origin for this gene (imprinted gene and parent-of-origin effect database).[20] The distribution of the parental origin of mutations in *SCN1A* falls between these two extremes and is similar to those seen for haemophilia A, Rett syndrome and neonatal diabetes.[19 20]

The greater frequency of paternally derived mutations in *SCN1A* is likely to be due to the greater chance of mutations occurring during spermatogenesis as compared to oogenesis. This is due to the considerably higher number of mitoses that occur during spermatogenesis compared to oogenesis. Twenty-two mitoses followed by two meioses occur during oogenesis, giving a total of 23 chromosome replications. In contrast, the DNA in sperm cells produced at the onset of puberty has already undergone 30 chromosome replications, with a further 23 replications occurring each year.[29] Therefore, sperm cells have a much higher probability of having acquired mutations due to replication error, compared with eggs at oogenesis.

It is also possible that some mutations of both paternal and maternal origin are the result of somatic or gonadal mosaicism. This has been described in sibships with *SCN1A* mutations[27 28] and was observed in one of the families studied here (patient 44). In these cases, the mosaicism was detected because of the presence of the same mutation in siblings. By chance alone, however, not all parents with somatic or gonadal mosaicism would have more than one affected child and therefore these would not be detected.

The most commonly occurring of the six potential nucleotide substitutions is a C:G→T:A transition. This substitution results from the deamination either of cytosine to form uracil or of 5-methylcytosine to form thymine, followed by the incorporation of adenine into the complementary DNA strand during the next replication. Methylated cytosines in CpG dinucleotides are particularly susceptible to this form of mutagenesis. The DNA in sperm cells is potentially more susceptible to this form of mutation as it is more highly methylated than that in oocytes.[30] Of the 33 nucleotide substitutions examined in this study with a definite de novo origin, 14 (42%) were C:G→T:A substitutions and 11 of these were paternally derived. This suggests that the deamination of cytosine during spermatogenesis is a frequent mutational mechanism causing de novo mutations in *SCN1A*.

The occurrence and relative frequencies of the paternally and maternally derived mutations in *SCN1A* is similar to that seen in several other genes including *KCNJ11*, another ion channel gene.[19] The mutations in both genes have been shown to result from a mixture of replication errors during gametogenesis and postzygotic mutations leading to gonadal mosaicism. This contrasts with the exclusively paternal mutations seen in *FGFR2* and *FGFR3*. The mutations in both these genes occur in 'hot spots' at CpG dinucleotides and a pronounced paternal age effect is seen, which may be due to selection for germ cells carrying the mutation within the testis.[29] This selection only occurs for mutations in specific genes and does not appear to occur for mutations in *SCN1A*.

This is the first study of the parental origin of de novo mutations in an epilepsy related gene. De novo *SCN1A* mutations in DS and related epileptic encephalopathies confer phenotypic effects through haploinsufficiency. They are most frequently but not exclusively of paternal origin and are unrelated to parental age. Multiple mutational mechanisms are likely to be involved in the occurrence of these mutations. Study of further cases, especially those with large deletions or duplications, which may have a different proportion of paternal to maternal origins, would be of interest to help understand how this group of devastating epileptic encephalopathies arise. In addition, the large number of de novo *SCN1A* mutations now known in DS provide a useful model for the study of the mechanisms contributing to different mutation types.

**Acknowledgements** We thank the patients and their parents for participation in this study. Patients were referred by the Infantile Epileptic Encephalopathy Referral Consortium. This work was supported by the National Health and Medical Research Council of Australia.

**Funding** National Health and Medical Research Council of Australia, GPO Box 1421, Canberra ACT 2601, Australia.

**Competing interests** None.

**Ethics approval** This study was conducted with the approval of the Human Research Ethics Committees of Austin Health and the Women's and Children's Hospital.

**Provenance and peer review** Not commissioned; externally peer reviewed.

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