CASE REPORT

Genetic diagnosis of severe myoclonic epilepsy of infancy (Dravet syndrome) with SCN1A mutations in the Hong Kong Chinese patients

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Epilepsy is a clinically and genetically heterogeneous group of disorders. The advent of molecular genetics brings unprecedented advancement in diagnostic molecular pathology and reduces over-reliance on traditional clinical classification. Severe myoclonic epilepsy of infancy or Dravet syndrome is a catastrophic infantile-onset epilepsy. We report two unrelated Hong Kong Chinese patients with this condition presenting with febrile seizures, epilepsy with different semiologies, psychomotor retardation, and recurrent status epilepticus. Two different mutations were characterised, viz NM_001165963.1: c.680T>G; NP_001159435.1: p.I227S and NM_001165963.1: c.3953T>G; NP_001159435.1: p.L1318R (novel). Genetic characterisation conveys a definitive diagnosis and is important from the perspective of selecting anti-epileptic drug therapy and genetic counselling.

Introduction

Epilepsy is a clinically and genetically heterogeneous group of disorders. Mutations in genes encoding ion channels in brain neurons have been identified in various epilepsy syndromes. The advent of molecular genetics brings unprecedented advancement in terms of diagnostic molecular pathology and reduces over-reliance on traditional clinical classification. An accurate genetic diagnosis enables personalised medicine in this heterogeneous group of disorders. Severe myoclonic epilepsy of infancy (SMEI) or Dravet syndrome (MIM #607208) is a catastrophic infantile-onset epilepsy affecting about 1 in 20 000 to 40 000 children with a two-fold preponderance in males.[1,2] After a period of normal development, affected infants develop febrile and afebrile generalised tonic-clonic (GTC) with onset usually within 1 year of age. Some progress into multiple seizure types such as focal, absence, and myoclonus. The course may run relentlessly with psychomotor retardation, ataxia, recurrent status epilepticus and death. Up to half of the patients have a positive family history of febrile seizures and epilepsy. Electroencephalography (EEG) findings are not definitive. Genetic testing provides a more decisive diagnosis and is crucial in guiding the anti-epileptic drugs selection and genetic counselling.

Severe myoclonic epilepsy of infancy shows loci heterogeneity with at least *SCN1A, SCN2A, SCN9A* and *GABRG2* as the culprit genes.[3,4] Depienne et al[5] conducted the largest genotyping study in 333 Dravet patients with 73% harbouring mutations in *SCN1A*. To date, there were 595 mutations reported with 59% missense/nonsense mutations, 9% splicing, 23% small insertions/deletions/indels, and 8% gross insertions/deletions/complex rearrangements (Human Gene Mutation Database, Professional 2010.2 accessed on 23 August 2010). However, most mutations were characterised without functional studies, as in practice most laboratories cannot undertake such assessments. Their pathogenicity is commonly documented by disease phenotype cosegregation, conservative nature of the affected amino acid among different species as well as the absence of the variants from screening of ethnically matched normal subjects. Therefore, reporting various phenotype-genotype data from more SMEI patients can increase the level of confidence about what is suspected. On the other hand, genotypic data in Chinese are limited.[6,7] We report the first two cases of SMEI molecular analysis in two unrelated Hong Kong Chinese patients.

Case reports

Case 1

A 9-year-old boy first presented at the age of 5 months with a GTC for 20 minutes during an episode of fever in September 2002. Within the next 24 hours, he had an atypical febrile convulsion with another generalised seizure lasting 7 minutes. The seizure was aborted with rectal diazepam. General investigations including EEG, brain computed tomography (CT) and magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) culture, protein

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and glucose measurements, all of which yielded no abnormality. He was managed as atypical febrile convulsion and roseola infantum on discharge. However, he continued suffering from repeated attacks. Thus, at the age of 8 and 9 months, he had afebrile focal seizures starting with right upper limb twitching going on to four limbs convulsive movements. Before the age of 1 year, he had had six convulsions that were usually associated with fever and/or infections. An interictal EEG at the age of 6 months was normal but the one performed at the age of 10 months revealed bifrontal sharp waves, especially over right side. The patient enjoyed normal development until he was 1 year old. Psychomotor retardation was detected from the age of 2 years. When assessed at the age of 31 months, his mental age was only 9 to 12 months, and his gross motor age was 18 months. He suffered from status epilepticus when he was 5 years old and subsequently endured regression in motor and cognitive function. His brain MRI performed at 10 months old was normal, but when repeated at the age of 5 years, it showed mild cerebral atrophy. He became bed-bound; developed limb rigidity, dystonia and later still choreoathetoid movements, whilst continuing to suffer from frequent (mainly partial) seizures, despite treatment with multiple anticonvulsants. His current medications included: sodium valproate, levetiracetam, topiramate and clobazam. He had no family history of epilepsy or febrile convulsions.

Case 2

An 18-year-old boy, first presented when aged 2 months with an afebrile GTC lasting for 45 minutes in December 1992. Septic work-up revealed an *Escherichia coli* urinary tract infection. He suffered an afebrile focal seizure with left eye blinking and left limbs twitching for 45 minutes at the age of 5 months, and then five attacks of GTC followed by transient right hemiparesis at the age of 6 months. He had frequent seizures in various forms (eye blinking, myoclonic, focal, and GTC), and were easily provoked by fever, infection and sometimes defaecation. There was a strong family history of febrile convulsions in three paternal aunts and one paternal cousin. The patient showed global delay, first noticed when 11 months old. Griffiths Mental Development Scales at the age of 25 months revealed an overall development to age 12 to 17 months only. Routine investigations (liver and renal function tests, complete blood picture, and metabolic screening) were normal. His EEG, brain CT, MRI, and MR angiography at the age of 1 year were all normal. However, subsequent EEG studies at the age of 5 years showed epileptiform discharges on both sides and spikes/polyspikes during photic stimulation, while the latest EEG at the age of 10 years showed bifrontal epileptiform discharges. An interictal single-photon emission computed tomography (SPECT) scan examination performed when he was 8 years old revealed slightly reduced tracer uptake over right temporal lobe, but subtraction ictal SPECT images could not be obtained. Brain MRI when he was aged 15 years remained normal. The response to treatment with multiple antiepileptics was poor; he currently takes topiramate, valproate, and clobazam.

From both patients, peripheral blood samples were collected after obtaining informed consent. Genomic DNA was extracted using a QIAamp blood kit (Qiagen, Hilden, Germany). All the coding exons and the 10-bp flanking regions of the *SCN1A* gene were amplified by polymerase chain reaction and direct DNA sequencing. Primer sequence and protocols are available upon request. Two different mutations were identified—in case 1, NM_001165963.1: c.3953T>G; NP_001159435.1: p.L1318R (novel); in case 2, NM_001165963.1: c.680T>G; NP_001159435.1: p.I227S. The p.L1318 amino acid was conserved among species. In silico analysis by SIFT and PolyPhen predicted it to be intolerant and possibly damaging, respectively. Parents of case 1 did not carry p.L1318R. The other mutation was previously reported in another SMEI patient (p.I227S).[8] Samples from the parents of case 2 were not available for genetic analysis.

因香港華籍嬰兒嚴重肌陣性癲癇（Dravet綜合症）相關性SCN1A突變的基因診斷

癲癇是一種臨床上與遺傳學上都有廣泛不同表現的疾病。分子遺傳學的出現為分子病理學帶來前所未有的發展，亦避免對傳統臨床的分類過份依賴。嬰兒嚴重肌陣性癲癇（又稱Dravet綜合症）於嬰孩時期開始發病。本文報告分別兩名香港華籍患者，病發時出現高熱驚厥、不同的癲癇症狀、精神運動發育遲緩，以及復發性癲癇持續狀態。為病人確認出分別兩種不同的突變：NM_001165963.1: c.680T>G；NP_001159435.1:p.I227S及NM_001165963.1: c.3953T>G；NP_001159435.1: p.L1318R （新發現）。遺傳學特徵分析有助確診，並對於選擇抗癲癇藥物治療及遺傳諮詢扮演著很重要的角色。

Discussion

The pathogenesis of non-syndromic epilepsy has been excitingly unravelled in recent years. The channelopathy involves more than 10 genes encoding various neuronal channels and neurotransmitter receptors. The SMEI caused by *SCN1A, SCN2A*, and *GABRG2* is exemplary. Both the clinical phenotypes and genetic aetiologies of SMEI are heterogeneous and can be overlapping. Dravet syndrome is characterised by multiple seizure types. It can manifest as GTC, myoclonic, myoclonic-astatic, or absences. The seizures are relatively refractory to anti-epileptic treatment, and are usually preceded by febrile convulsion at early age in an otherwise normal infant. Patients may suffer from subsequent

developmental delay and cognitive impairment. Hattori et al[9] proposed a clinical scoring system for screening high-risk patients for genetic testing of Dravet syndrome. There are seven clinical predictive risk factors, namely onset within the first 7 months of life, more than five seizures, hemiconvulsions, focal seizures, myoclonic seizures, prolonged seizures, and hot water–induced seizures. Our patients had onset at 2 and 5 months old respectively, and suffered from multiple attacks of refractory seizures with different semiologies. The seizures scored more than six marks (ie belonged to high-risk groups) and we detected *SCN1A* mutations in both patients. Since the genetic analysis of *SCN1A* is quite laborious, it is useful to have a clinical screening score to guide genetic tests.

Dravet syndrome is inherited as an autosomal dominant, so 50% offspring are at risk of the disease. In fact, majority of *SCN1A* mutations are de-novo; only about 5% are inherited. Parents of case 1 did not carry the p.L1318R mutation according to the DNA sequencing results, suggesting a de-novo mutation. However, it is noteworthy that parental mosaicism has been reported.[10-12] Thus, a mutant load in blood of less than 15% may not be detected by direct DNA sequencing. The possibility of mosaic mutations must be addressed for genetic counselling and prenatal diagnosis. Proper genetic counselling and family screening should be carried out in all at-risk subjects.

A DNA-based diagnosis enables accurate and early diagnosis to formulate an optimal management plan and can avoid patient suffering from unnecessary investigations or treatment. It is common to try different anti-epileptic drugs in patients with intractable epilepsy without certain diagnosis. A definitive diagnosis offered by molecular genetics definitely saves time with respect to drug that should be tried and reduces the risks of undesirable choices. Early recognition and diagnosis of the *SCN1A* mutation in Dravet syndrome could enhance appropriate selection of anticonvulsants. Phenobarbitone, valproate, benzodiazepines, zonisamides, bromides, stiripentol, topiramate, levetiracetam and ketogenic diet are more effective in controlling these types of seizure, while treatment with sodium channel blockers (phenytoin, carbamazepine, oxcarbazepine, lamotrigine) should be avoided.[13,14] Both of our patients had an increase in seizure attacks on lamotrigine. Knowing the exact mutation is helpful particularly for the premature stop codon. Ataluren (PTC124) is an emerging genetic therapy targeting genetic disorders caused by nonsense mutations.[15] Nonsense mutations create premature protein truncation. Ataluren is able to induce selective ribosomal read-through of the premature stop codon, but not the normal stop codon, thus enabling a continuing mRNA transcription. Ataluren is now in clinical trials mainly for treating patients with cystic fibrosis and Duchenne muscular dystrophy. In the future, it may be applicable to SMEI patients with nonsense mutations. In addition, patients should be educated to avoid triggering factors such as hot water in hyperthermia-sensitive seizures.

In summary, we report the clinical and mutational findings of the two cases of SMEI in Hong Kong Chinese and illustrate the importance of molecular genetics in the diagnosis and personalised medical management of patients with Dravet syndrome.

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