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SHORT COMMUNICATION

A clinical variant in *SCN1A* inherited from a mosaic father cosegregates with a novel variant to cause Dravet syndrome in a consanguineous family

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Summary A consanguineous family from Turkey having two children with intellectual disability exhibiting myoclonic, febrile and other generalized seizures was recruited to identify the genetic origin of these phenotypes. A combined approach of SNP genotyping and exome sequencing was employed both to screen genes associated with Dravet syndrome and to detect homozygous variants. Analysis of exome data was extended further to identify compound heterozygosity. Herein, we report identification of two paternally inherited genetic variants in *SCN1A* (rs121917918; p.R101Q and p.I1576T), one of which was previously implicated in

Abbreviations: DS, Dravet syndrome; SMEI, severe myoclonic epilepsy of infancy; *SCN1A*, voltage-gated sodium channel type I alpha subunit gene; MRI, magnetic resonance imaging; EEG, electroencephalography; ID, intellectual disability; WES, whole exome sequencing; SMEB, severe myoclonic epilepsy of infancy borderline; DTP, diphteria—tetanus—pertussis; IBD, identical by descent.

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Dravet syndrome. Interestingly, the previously reported clinical variant (rs121917918; p.R101Q) displayed mosaicism in the blood and saliva of the father. The study supported the genetic diagnosis of affected children as Dravet syndrome possibly due to the combined effect of one clinically associated (rs121917918; p.R101Q) and one novel (p.I1576T) variants in *SCN1A* gene. This finding is important given that heterozygous variants may be overlooked in standard exome scans of consanguineous families. Thus, we are presenting an interesting example, where the inheritance of the condition may be misinterpreted as recessive and identical by descent due to consanguinity and mosaicism in one of the parents.

Introduction

Dravet syndrome (DS) or severe myoclonic epilepsy of infancy (SMEI) is an epileptic encephalopathy characterized by prolonged, recurrent generalized seizures and hemiconvulsions that might be induced by fever, where frequent refractory episodes of status epilepticus usually take place (Striano et al., 2013). Heterozygous mutations in the voltage-gated sodium channel type I alpha subunit (*SCN1A*) gene are a major cause of DS explaining up to 80% of cases (Depienne et al., 2009). Almost 95% of these mutations have been presumed as *de novo* due to their absence in parents (Mulley et al., 2005). Nevertheless, this percentage may be an overestimation, since detection of mosaicism requires further analyses in which inheritance from mildly affected or asymptomatic mosaic parents has well been documented only in rare cases (Shi et al., 2012).

Herein, we report genetic and clinical findings in a consanguineous family from Turkey with two affected children resembling DS phenotypes, with the ultimate aim of delineating the associated genetic variations.

Materials and methods

Subjects

A consanguineous family from Turkey having two affected siblings along with four healthy members (Fig. 1) has been followed-up for 3 years. Physical and neurological examinations were performed for all available family members and detailed information on family history was collected. Magnetic resonance imaging (MRI) of the brain and electroencephalography (EEG) recordings were performed on the affected siblings and their asymptomatic father. Informed consents were obtained from all family members and control individuals in accordance with Istanbul University, Clinical Ethics Committee (2012/864).

The clinical course was similar in two affected siblings. The first seizures developed in the form of prolonged tonic-clonic and focal seizures with head deviation and loss of consciousness at the age of .3 months for one sibling (Patient 504) and 11 months for the other (Patient 505) following normal spontaneous vaginal deliveries. Fever-induced seizure was reported for Patient 505. For each sibling, the condition progressed into myoclonic and absence seizures, occurring as frequent as once a week. Neither of the affected siblings has been able to attend school due to emerging intellectual disability (ID).

The family was enrolled into the study when Patients 504 and 505 were 9 and 7 years old, respectively. Physical examinations revealed normal motor functions with the ability to walk independently. However, their weight and height measurements were below the third percentile compared to their age and sex groups. Patient 504 had normal EEG patterns. His brain MRI showed mild asymmetric widening of the posterior horn of the left ventricle, adjacent to slight non-specific white matter change in T2 weighted images, at the age of 9 years (Supplementary Fig. 1). The brain MRI of Patient 505 was within normal limits. Her EEG exhibited generalized epileptiform activity with no distinctive photosensitivity (Supplementary Fig. 2).

The affected siblings have continually received a combination of anticonvulsant drugs with the active ingredients of valproic acid, clonazepam, and topiramate. Under this treatment, Patient 505 was seizure-free for almost 1 year, whereas Patient 504 has rarely experienced mild seizures that do not interfere with his daily life activities.

The family also had a deceased elder child who developed vaccination-induced febrile tonic-clonic and focal seizures when she was 2 months old and passed away due to status epilepticus when she was 1.5 years old. The father and other family members do not have a history of seizures. They all have past the mean age of onset observed in the family and did not reveal any remarkable findings in their neurological examinations.

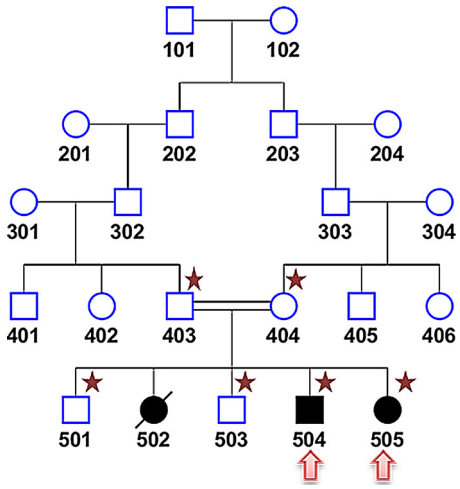

Figure 1 The consanguineous family recruited for this study is depicted. A star sign indicates the available family members for genetic analyses. DNA from affected children shown with arrows was used for whole genome exome sequencing.

Table 1 Variant filtering results.

Filtering condition/affected individuals	504	505
Quality filtering		
Total number of variants	154,525	166,229
Genotype quality ≥15 and coverage ≥4	108,708	108,673
GMAF <0.01 in 1000Genome project and ESP6500[a]	23,145	23,287
Not found in segmental duplication regions	16,991	17,088
Not found in in-house exome database (n = 368) of varying disorders	13,501	13,558
Filtering for known genes of DS		
Disease related genes: *GABRA1, GABRG2, SCN1A, SCN1B, STXBP1, PCDH19*	65	
Frameshift/non-frameshift INDEL, non-synonymous, stop gain/lost or splicing variants	6 (*SCN1A, SCN1B*)	
Selected variants	*SCN1A* (c.302G>A and c.4727T>C)	
Filtering for recessive homozygous variants		
Homozygous in two individuals		1318
Common variants in shared homozygous regions detected by HomSI: (Chr1:245-250Mb, Chr2:0-7Mb, Chr4:75-82Mb, Chr7:26-31Mb, Chr9:136-141Mb)		44
Frameshift/non-frameshift INDEL, non-synonymous, stop gain/lost or splicing variants		3 (*NAAA*, *CAMSAP1*, *NACC2*)
Selected variants		(1) *NAAA*:c.1024A>C; p.S342R (2) *CAMSAP1*:c.2294T>C; p.I765T (3) *NACC2*:c.1736C>T; p.P579L
Filtering for potentially compound heterozygous variants		
Heterozygous in two individuals	8634	
Frameshift/non-frameshift INDEL, non-synonymous, stop gain/lost or splicing variants	486	
Compound heterozygous in two individuals	1 gene (*SCN1A*)	
Selected variants	*SCN1A* (c.302G>A and c.4727T>C)	

[a]NHLBI exome sequencing project.

Genetic and bioinformatic analyses

Whole exome sequencing (WES) along with SNP genotyping were performed in selected family members. Further details are presented in Supplementary methods online.

WES and SNP genotyping

WES was performed for the two affected siblings on an Illumina HiSeq-2000 system using the TruSeq SBS Kit v3-HS. All available family members were SNP genotyped using Illumina Human HumanCytoSNP-12 BeadChip kit.

Prioritization of exome variants for segregation analysis

All variants were initially filtered for quality control according to the criteria listed in Table 1. Quality filtering was followed by three different filtering approaches in order to detect variants common both to Patients 504 and 505 in (i) DS related genes (Carvill et al., 2014; Le Gal et al., 2014), (ii) homozygous variants within regions detected by HomSI (Görmez et al., 2014) and (iii) potentially compound heterozygous variants. Additionally, non-captured exons of DS-related genes were screened in affected siblings *via* Sanger sequencing. Haplotypes constructed using SNP genotypes were utilized for rejecting segregation of exome variants within the family, whereas Sanger sequencing in familial segregation was performed in uninformative haplotypes, whenever possible. Presence of the candidate variants was further screened in two distinct control sets from Turkey representing 468 distinct individuals collectively.

Results and discussion

WES data was initially screened for six DS related genes (Table 1, Supplementary Table 1 for non-filtered exome variants in these genes). Two heterozygous variants were

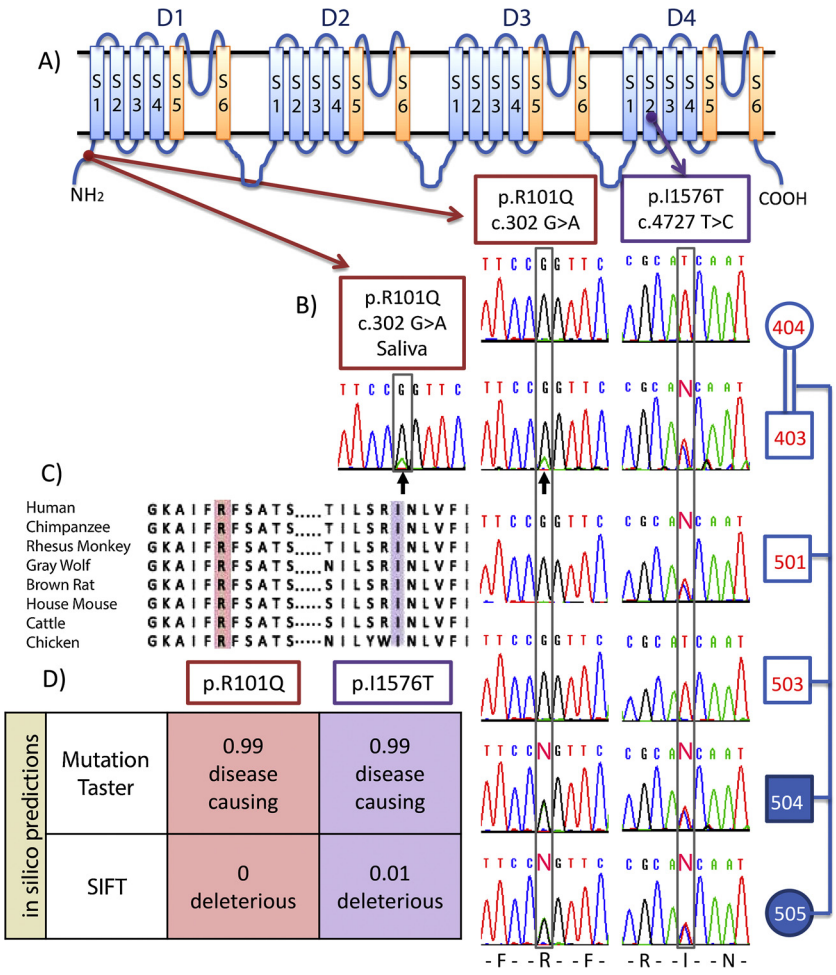

Figure 2 Genetic variants identified in *SCN1A*. (A) Schematic diagram of SCN1A protein that is composed of four internally homologous domains (D1—D4) each with six transmembrane segments (S1—S6) (Kanai et al., 2004). S4 has a designated role as voltage sensor, where the pore region is formed between S5 and S6 (Kanai et al., 2004). The red and purple dots show the location of the two genetic variants investigated. (B) Chromatograms showing segregation of the two variants in the family. The clinical variant p.R101Q (c.302G>A; rs121917918) is heterozygous in the affected siblings, while the father displays reduced expression of the mutant A allele in both his blood and saliva samples, indicated with black arrows. The novel p.I1576T (c.4727T>C) variant is carried in the heterozygous form by the affected siblings, the father and the healthy child 501. Both variations are shown with the letter ''N'' in the chromatograms, when in full heterozygous forms. (C) The evolutionary conservation of variant amino acids. (D) *In silico* prediction results of variant amino acids using SIFT and mutation taster. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

common to both affected siblings within *SCN1A* gene (NM_001165963.1), making them strong candidates for compound heterozygosity: a reportedly clinical variant (rs121917918: p.R101Q; c.302G>A) and a novel variant (p.I1576T; c.4727T>C) (Fig. 2a, Supplementary Table 1). These variants were also identified using a parallel approach through global filtering of WES data for compound heterozygosity (Table 1). However, familial segregation of these variants revealed paternal inheritance of both alleles, thereby rejecting true compound heterozygosity. Interestingly, the father was shown to be mosaic only for the clinical variant, which was confirmed by Sanger sequencing his DNA obtained both from blood and saliva (Fig. 2b). Identification of paternal origin for these two variants prompted us to perform a more detailed neurological examination of the father using EEG and brain MRI recordings as well as an updated family history, which was in support of an asymptomatic status for the father.

Among the variants in the *SCN1A* gene, the p.I1576T; c.4727T>C variant was absent in all available databases as well as in 468 controls from Turkey, thereby confirming its novelty with a power of 95% assuming a minor allele frequency of 0.01 (Collins and Schwartz, 2002). At the protein level, the c.4727T>C variant leads to a substitution of a non-polar isoleucine to a polar threonine residue at a strictly conserved position across species (Fig. 2c). This substitution was predicted to be damaging with high confidence by *in silico* tools predicting the effect of amino acid substitutions on protein function (Fig. 2d). Additionally, we have performed segregation analysis of all four non-synonymous

variants obtained from the unfiltered exome data of the known DS genes (Supplementary Table 2), which did not segregate with the condition.

Most of the previous reports have shown association between the clinical variant p.R101Q (c.302G>A, rs121917918) and severe myoclonic epilepsy of infancy borderline (SMEB) (Fukuma et al., 2004; Kanai et al., 2004; Marini et al., 2007; Depienne et al., 2009), except for the work conducted by Harkin et al. (2007) that reported one SMEI patient exhibiting the variant. Children who are diagnosed with SMEB tend to have similar developmental stages as in DS, except for the lack of myoclonic or atypical absence seizures and the absence of generalized spike wave activity in their EEGs (Scheffer et al., 2009).

Thus, the novel p.I1576T variant in this family may exert its effect on the clinical phenotype of DS/SMEI when it cosegregates with the clinical p.R101Q variant. Therefore, we speculate that the presence of this novel variant induces an additive effect on the clinical manifestation as DS in the affected children. Our results indicate that the presence of the novel p.I1576T variant alone is not sufficient in DS manifestation, nor is the mosaicism of the clinical p.R101Q variant observed in the father (Fig. 2b). Consistent with our findings, it was previously determined that 10% of DS patients have inherited variations from an asymptomatic or mildly affected parent, where mosaicism was shown to be protective in 7% of families with DS (Marini et al., 2006).

Several reports indicate that variations in *SCN1A* gene may be the reason for vaccination-induced seizures, where a range of 7—57% of children diagnosed with DS accumulated their initial seizures preceding diphtheria—tetanus—pertussis (DTP) vaccination (Berkovic et al., 2006; Verbeek et al., 2013). Moreover, a recent work showed that 30% of children diagnosed with DS and shown to exhibit variations in *SCN1A* gene had vaccination-induced seizures, which was further supported by a parallel work that reported the mean age of first seizure onset as 4.1 months (McIntosh et al., 2010; Verbeek et al., 2013). Thus the deceased child in this family probably carried the same *SCN1A* gene variants reported herein, where vaccination has been the triggering factor for her in the onset of her seizures.

We have also considered a recessive, identical by descent (IBD) inheritance pattern in the family given the consanguinity and unaffected status of the parents in order to detect a novel genetic mechanism leading to a DS-like phenotype or epilepsy with ID. HomSI analysis of exome data followed by segregation and/or SNP haplotype analyses have eliminated potential contribution of homozygous variants (Supplementary Fig. 3a and b).

Conclusion

Our in-depth genetic analyses of this family confirmed the diagnosis of affected individuals as DS, where presence of the novel p.I1576T *SCN1A* gene variant may have augmented the clinical effect in individuals already carrying the clinical p.R101Q variant in full heterozygous form. We highlighted the importance of parental mosaicism in DS diagnosis and genetic counseling. This study as well identified a novel variant that could be relevant in *SCN1A* screens especially when coinherited with a pathogenic variant. It also draws attention to misinterpretation of exome data in consanguineous pedigrees where the condition is in fact associated with heterozygous variants.

Conflict of interest

None of the authors has any conflicts of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eplepsyres.2015.02.020.

References

Berkovic, S.F., Harkin, L., McMahon, J.M., Pelekanos, J.T., Zuberi, S.M., Wirrell, E.C., Gill, D.S., Iona, X., Mulley, J.C., Scheffer, I.E., 2006. De-novo mutations of the sodium channel gene SCN1A in alleged vaccine encephalopathy: a retrospective study. Lancet Neurol. 5 (6), 488—492.

Carvill, G.L., Weckhuysen, S., McMahon, J.M., Hartmann, C., Møller, R.S., Hjalgrim, H., Cook, J., Geraghty, E., O'Roak, B.J., Petrou, S., Clarke, A., Gill, D., Sadleir, L.G., Muhle, H., von Spiczak, S., Nikanorova, M., Hodgson, B.L., Gazina, E.V., Suls, A., Shendure, J., Dibbens, L.M., De Jonghe, P., Helbig, I., Berkovic, S.F., Scheffer, I.E., Mefford, H.C., 2014. GABRA1 and STXBP1: novel genetic causes of Dravet syndrome. Neurology 82 (14), 1245—1253.

Collins, J.S., Schwartz, C.E., 2002. Detecting polymorphisms and mutations in candidate genes. Am. J. Hum. Genet. 71 (5), 1251—1252.

Depienne, C., Trouillard, O., Saint-Martin, C., Gourfinkel-An, I., Bouteiller, D., Carpentier, W., Keren, B., Abert, B., Gautier, A., Baulac, S., Arzimanoglou, A., Cazeneuve, C., Nabbout, R., LeGuern, E., 2009. Spectrum of SCN1A gene mutations associated with Dravet syndrome: analysis of 333 patients. J. Med. Genet. 46 (3), 183—191.

Fukuma, G., Oguni, H., Shirasaka, Y., Watanabe, K., Miyajima, T., Yasumoto, S., Ohfu, M., Inoue, T., Watanachai, A., Kira, R., Matsuo, M., Muranaka, H., Sofue, F., Zhang, B., Kaneko, S.,

Mitsudome, A., Hirose, S., 2004. Mutations of neuronal voltage-gated Na+ channel alpha 1 subunit gene SCN1A in core severe myoclonic epilepsy in infancy (SMEI) and in borderline SMEI (SMEB). Epilepsia 45 (2), 140—148.

Görmez, Z., Bakir-Gungor, B., Sagiroglu, M.S., 2014. HomSI: a homozygous stretch identifier from next-generation sequencing data. Bioinformatics 30 (3), 445—447.

Harkin, L.A., McMahon, J.M., Iona, X., Dibbens, L., Pelekanos, J.T., Zuberi, S.M., Sadleir, L.G., Andermann, E., Gill, D., Farrell, K., Connolly, M., Stanley, T., Harbord, M., Andermann, F., Wang, J., Batish, S.D., Jones, J.G., Seltzer, W.K., Gardner, A., Infantile Epileptic Encephalopathy Referral Consortium, Sutherland, G., Berkovic, S.F., Mulley, J.C., Scheffer, I.E., 2007. The spectrum of SCN1A-related infantile epileptic encephalopathies. Brain 130 (Pt 3), 843—852.

Kanai, K., Hirose, S., Oguni, H., Fukuma, G., Shirasaka, Y., Miyajima, T., Wada, K., Iwasa, H., Yasumoto, S., Matsuo, M., Ito, M., Mitsudome, A., Kaneko, S., 2004. Effect of localization of missense mutations in SCN1A on epilepsy phenotype severity. Neurology 63 (2), 329—334.

Le Gal, F., Lebon, S., Ramelli, G.P., Datta, A.N., Mercati, D., Maier, O., Combescure, C., Rodriguez, M.I., Seeck, M., Roulet, E., Korff, C.M., 2014. When is a child with status epilepticus likely to have Dravet syndrome? Epilepsy Res. 108 (4), 740—747.

Marini, C., Mei, D., Helen Cross, J., Guerrini, R., 2006. Mosaic SCN1A mutation in familial severe myoclonic epilepsy of infancy. Epilepsia 47 (10), 1737—1740.

Marini, C., Mei, D., Temudo, T., Ferrari, A.R., Buti, D., Dravet, C., Dias, A.I., Moreira, A., Calado, E., Seri, S., Neville, B., Narbona, J., Reid, E., Michelucci, R., Sicca, F., Cross, H.J., Guerrini, R., 2007. Idiopathic epilepsies with seizures precipitated by fever and SCN1A abnormalities. Epilepsia 48 (9), 1678—1685.

McIntosh, A.M., McMahon, J., Dibbens, L.M., Iona, X., Mulley, J.C., Scheffer, I.E., Berkovic, S.F., 2010. Effects of vaccination on onset and outcome of Dravet syndrome: a retrospective study. Lancet Neurol. 9 (6), 592—598.

Mulley, J.C., Scheffer, I.E., Petrou, S., Dibbens, L.M., Berkovic, S.F., Harkin, L.A., 2005. SCN1A mutations and epilepsy. Hum. Mutat. 25 (6), 535—542.

Scheffer, I.E., Zhang, Y.H., Jansen, F.E., Dibbens, L., 2009. Dravet syndrome or genetic (generalized) epilepsy with febrile seizures plus? Brain Dev. 31 (5), 394—400.

Shi, Y.W., Yu, M.J., Long, Y.S., Qin, B., He, N., Meng, H., Liu, X.R., Deng, W.Y., Gao, M.M., Yi, Y.H., Li, B.M., Liao, W.P., 2012. Mosaic *SCN1A* mutations in familial partial epilepsy with antecedent febrile seizures. Genes Brain Behav. 11 (2), 170—176.

Striano, P., de Jonghe, P., Zara, F., 2013. Genetic epileptic encephalopathies: is all written into the DNA? Epilepsia 54 (Suppl. 8), 22—26.

Verbeek, N.E., van der Maas, N.A., Jansen, F.E., van Kempen, M.J., Lindhout, D., Brilstra, E.H., 2013. Prevalence of SCN1A-related Dravet syndrome among children reported with seizures following vaccination: a population-based ten-year cohort study. PLOS ONE 8 (6), e65758.