Journal of Genetics (2022)101:30
https://doi.org/10.1007/s12041-022-01371-0

RESEARCH NOTE

C12orf57 pathogenic variants: a unique cause of developmental encephalopathy in a south Indian child

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Received 5 February 2022; revised 17 March 2022; accepted 1 April 2022

Abstract. Open reading frame variants which lack stop codons such as *C12orf57* variants are known to cause Temtamy syndrome, an extremely rare disorder characterized by intellectual disability, seizures, facial dysmorphism and agenesis of corpus callosum. *C12orf57* was initially reported to be required for human corpus callosum development. We report the first child who is of Indian origin with developmental and epileptic encephalopathy (DEE) with a unique phenotypic evolution as focal onset reflex seizures. We performed whole exome sequencing of genomic DNA isolated from peripheral blood samples of proband and his parents. Two pathogenic compound heterozygous variants, a start loss variant (Chr12:7053285:c.1A>G) and a premature stop gain variant (Chr12:7053327:c.43C>T), involving the *C12orf57* gene were identified in the proband. Our case report which details genotyping in this rare syndromic developmental encephalopathy, with no prior cases reported from India, expands the ethnic spectrum of patients.

Keywords. developmental encephalopathy; agenesis of corpus callosum; epilepsy; *C12orf57*; compound heterozygous.

Introduction

Temtamy syndrome is a rare disorder, which is caused by mutations involving open reading frame 57 (*C12orf57*) in chromosome 12. It was first described in 1991 and is phenotypically characterized by intellectual disability (ID), epilepsy, craniofacial dysmorphism, agenesis of the corpus callosum, and optic coloboma (Temtamy *et al.* 1991, 1996). No prior reports from the Indian subcontinent exist in literature focussing on potential mutations in this syndrome that lead to epileptogenesis and callosal anomalies. Pathogenic variants were first reported in 2013 in four siblings of a Saudi Arabian family with a deleterious nucleotide change (c.1A>G; p.Met1Val) and eight pathogenic variants have been reported till date (Salih *et al.* 2013; Alrakaf *et al.* 2018; Wang *et al.* 2020) of which seven are of Middle Eastern and one of Asian (China) ethnicity. Homozygous and compound heterozygous mutations in *C12orf57* have been described (Platezer *et al.* 2014). We report the first Indian male with genetically proven Temtamy syndrome who as diagnosed using whole exome sequencing in trios to have compound heterozygous variants involving *C12orf57*. Informed consent for publication was obtained from the parents.

Case presentation

A male, born of south Indian nonconsanguineous parentage, presented to us in early childhood with global developmental delay and no history of perinatal insults. There was a history

Published online: 14 June 2022

of seizures since 84 days of life which had a phenomenology consistent with symmetric myoclonic jerks. Overall initial electroclinical features were consistent with 'symptomatic' myoclonic epilepsy of infancy and he was initiated on levetiracetam. At 2 years follow up, the child was noted to have global developmental delay. Developmental gains were noted and he was able to speak 10–15 words and was able to walk with support with a developmental age reaching 12 months. Although his epilepsy was initially well controlled with levetiracetam, at 3 years he had two episodes of focal-onset seizures of left opercular semiology both related to drug default. He was subsequently noted to have autistic traits with hand dyspraxia. At 4 years of age, he developed a new semiology of seizures characterized by left hemispheric asymmetric tonic seizures which frequently were associated with auditory and visual reflex triggers with no overt myoclonus or ataxia. Sodium valproate followed by lamotrigine was added and ketogenic diet was initiated. He continues to have brief nondisabling reflex startle-induced spasms with no major consciousness impairing events. There was family history of pharmacoresponsive focal epilepsy of temporal plus origin with hippocampal sclerosis on MRI and hearing impairment respectively among two paternal relatives and psychiatric illness in maternal grandmother (figure 1a).

Physical examination revealed facial dysmorphism characterized by microcephaly (head circumference = 41 cm), hypertelorism, depressed nasal bridge, strabismus, full cheeks, thick lips, dental anomalies and open mouth (figure 1b). He had truncal hypotonia with no overt limb spasticity, dystonia or ataxia. He had good auditory and visual regard with normal ophthalmological examination. His routine blood investigations including thyroid profile was normal. Metabolic workup including arterial blood gas analysis, ammonia, lactate and pyruvate were within normal limits. His echocardiogram showed a 3-mm ostium secondum atrial septal defect. His initial CT head showed agenesis of corpus callosum. Developmental assessment using the revised Denver developmental scale (Denver II Technical Manual 1990; Denver developmental materials, Inc.) showed (developmental quotient being 20 on gross motor, eight on fine motor, 34 on language and 20 on personal social domains). His EEG showed occasional interictal epileptiform discharges (IED) over bilateral temporal regions along with mild to moderate degree of nonspecific disturbance in electrophysiological function intermittently over left temporal region. A follow up EEG after the onset of tonic seizures and reflex epilepsy showed multifocal IED predominantly in the form of bilateral independent frontotemporal sharp waves with sleep showing activation in a pseudo periodic fashion along with asynchronous background activity, fairly formed sleep spindles and focal paroxysmal fast activity (figure 1c). Brain MRI confirmed partial agenesis of corpus callosum and incomplete rotation of hippocampi (figure 1d).

Whole exome sequencing and bioinformatic analysis

Genomic DNA was isolated from the peripheral blood samples of proband and his parents using salting out method

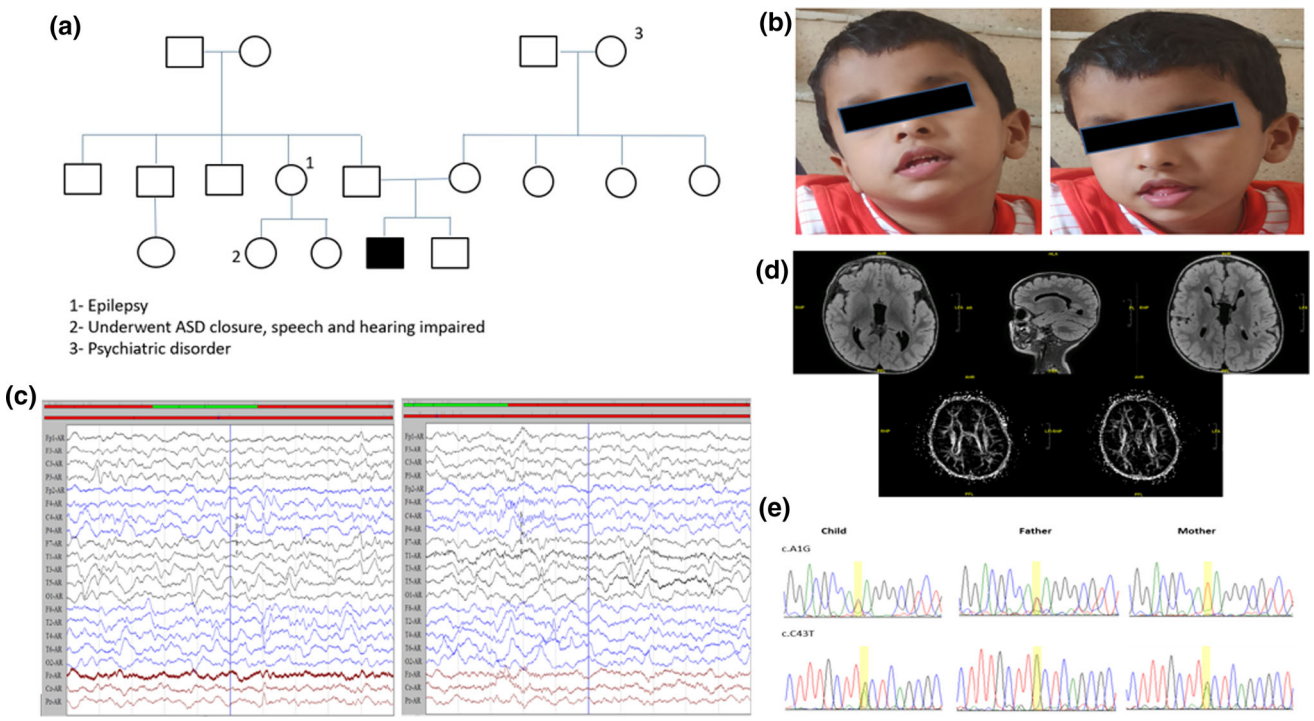

Figure 1. (a) Pedigree analysis, (b) photograph of the patient, (c) EEG images (d) MRI images and (e) Sanger sequencing electrogram of compound heterozygous variants in *C12orf57*.

Table 1. Details of compound heterozygous variants identified in the proband.

Variant	Gene	Zygosity	CADD score	SIFT prediction	PolyPhen2 prediction	Mutation-taster prediction	REVEL score	OMIM phenotype	ACMG classification
Chr12:7053285; exon1:c.A1G: p.Met1Val; (Platzer *et al.* 2014)	*C12orf57*	Het	21.9	Deleterious	Benign	Deleterious	0.492	Temtamy syndrome (MIM number: 218340)	Pathogenic (PVS1, PS3, PM2, PP5)
Ch12:7053327; exon1:c.C43T:p.Q15X	*C12orf57*	Het	60	–	–	Deleterious	–		Pathogenic (PVS1, PM2, PP5)

of DNA extraction. Sequencing libraries were generated using Agilent SureSelect Human All ExonV6 kit (Agilent Technologies) and paired end sequenced on Illumina HiSeq X Ten (Illumina) for 150 cycles according to manufacturer's instructions. Raw fastq files were aligned to human reference genome (hg19/GRCh37) using Burrows-Wheeler Aligner (Li and Durbin 2010). The SNPs and INDELs were called using Genome Analysis Toolkit's (GATK) best practice guidelines (Van der Auwera *et al.* 2013). The variants identified were annotated using ANNOVAR (Wang *et al.* 2010).

The variants which passed the depth and quality filter were considered for inheritance pattern models includes homozygous recessive, autosomal dominant, compound heterozygous, X-linked and *de novo* variants. The variant reporting was restricted to 2413 genes, extracted from literature searches and databases, which are associated with epilepsy, intellectual disability, developmental delay and autism spectrum disorder. The potential damaging effects of the variants were assessed with the *in silico* prediction tools CADD (http://cadd.gs.washington.edu/), SIFT (http://sift.jcvi.org/), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org/) and REVEL (https://sites.google.com/site/revelgenomics/) score. The variants with a CADD score of greater than 20 and ExAC (http://exac.broadinstitute.org/) allele frequency less than 1% were filtered out. Based on the phenotypes of proband, the variants were prioritized by manually assessing the evidence from various databases including ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), OMIM (https://www.omim.org/), HPO (https://hpo.jax.org) and also the literature searches. Sanger sequencing was performed to validate the genotypes of the proband and his parents.

Results

From the whole exome sequencing analysis, 188,598 variants were identified after alignment and variant calling, which were then filtered against various databases. Twenty-three thousand six hundred and nine variants were filtered out from the epilepsy associated genes which includes both SNPs and INDELs. From this list of variants, 55 variants were then picked out with CADD score > 20 and MAF ≤ 0.01. Autosomal dominant variants identified were present in either of the unaffected parent. The proband was identified with two potentially damaging compound heterozygous variants in *C12orf57* gene; loss of start codon variant and (NM_138425.4:c.1A>G; rs587776954; Chr12:7053285,exon1:c.1A>G:p.Met1Val) premature stop gain variant (NM_138425.4:c.43C>T; rs1565574197; chr12:7053327, exon1:c.C43T:p.Q15X). His father was a heterozygous carrier of chr12:7053285, A>G variant and mother carried another variant chr12:7053327, C>T. The pathogenicity classification of variants by American College of Medical Genetics and Genomics (ACMG) guidelines suggested that

Table 2. Genotype and phenotype data of patients identified with Temtamy syndrome from literature and the present study.

	Temtamy *et al.* (1996)	Alrakaf *et al.* (2018)	Wang *et al.* (2020)	Present study
Country	North Africa	Middle East	East Asian	India
Developmental delay	GDD	GDD	GDD	GDD
Behavioural abnormalities	–	Autistic features	NIL	Autistic features
Seizures	–	GTCS, MS, FS, tonic infantile spasms	GTCS	MS, focal onset auditory reflex seizures, asymmetric tonic seizure
Congenital anomalies	Talipes equinovarus, flat feet, moderate dilatation of aorta	ASD, VSD	ASD	ASD, flat feet
Dysmorphic features	Iris coloboma, myopia, hypertelorism, frontal bossing, elongated face, arched eyebrows, antimongoloid slanting of eyes, beaked nose, low set simple lop ears, long philtrum, short upper lip, micrognathia, dental anamolies	Frontal bossing, low set, posteriorly rotated ears, depressed nasal bridge, hypertelorism, micrognathia, epicanthal folds, and upslanted palpebral fissures	Colobomatous microphthalmia, frontal bossing, low set ears, depressed nasal bridge, ocular hypertelorism, micrognathia, single transverse palmar crease	Microcephaly, frontal bossing, hypertelorism, depressed nasal bridge, strabismus, full cheeks, thick lips, open mouth, low-set ears, dental anamolies
MRI findings	Dilated cerebral ventricles, complete agenesis of CC	Abnormal CC, abnormal septum pellucidum, ventriculomegaly	Expanded lateral ventricles, agenesis of CC	Agenesis of CC
C12orf57 mutation	–	c.1A>G, p.(Met1?), c.53-2A>G, c.-3_2delinsG, c.43C>T, p.(Gln15*), c.229+2T>C	c.3G>C (p.Met1Ile)	Loss of start codon variant exon1:c.1A>G:p.Met1Val Premature stop gain variant exon1:c.C43T:p.Q15X

GDD, global developmental delay; GTCS, generalised tonic clonic seizures; MS, myoclonic seizures; CPS, complex partial seizures; FS, febrile seizures; ASD, atrial septal defect; PS, pulmonary stenosis; CC, corpus callosum.

both chr12:7053285;c.A1G and chr12:7053327;c.C43T are pathogenic variants (table 1). Both the missense variants prediction tools, SIFT and MutationTaster, predicted the first variant (c.1A>G) as a deleterious variant. It prevents the translation of *C12orf57* by demolishing the initiation codon (Salih *et al.* 2013). The second variant (c.C43T) has a high CADD score and it establishes a stop codon which ends the translation of *C12orf57* prematurely (Platzer *et al.* 2014). We confirmed the variants in the proband and his parents by Sanger sequencing (figure 1e).

Discussion

This report highlights compound heterozygous variants involving the *C12orf57* gene in a patient of south Indian ethnicity with atypical presentation of Temtamy syndrome with respect to clinical and radiological features. The diagnosis in this patient was established by whole exome sequencing performed in trios and confirmed by Sanger sequencing. Two previously reported heterozygous variants were identified in our patient with concordant phenotypic features (Platzer *et al.* 2014; Alrakaf *et al.* 2018). *C12orf57* gene is ubiquitously expressed in human tissues and is essential for the development of human corpus callosum (Akizu *et al.* 2013). A few pathogenic variants in *C12orf57* are reported in literature causing Temtamy syndrome of which the c.1A>G pathogenic variant in start codon is the most frequent causing severe reduction in protein levels, suggesting a loss of function (Salih *et al.* 2013). The c.3G>C pathogenic variant also affects the same start codon, but at different bases and affects protein expression level resulting in clinical manifestations (Wang *et al.* 2020). The Q15X variant is a pathogenic variant and predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay (Zahrani *et al.* 2013; Platzer *et al.* 2014; Alrakaf *et al.* 2018) (https://www.ncbi.nlm.nih.gov/clinvar/variation/620193). It has been noted that the *C12orf57* is a three-exon gene that encodes a 126-residue protein (Akizu *et al.* 2013). A recent functional study reports that *C12orf57* controls synaptic scaling in excitatory neurons (Jiang 2019), however the mechanisms behind epileptogenesis are poorly understood as in Aicardi syndrome in which differential methylation patterns in several neurodevelopmental networks are hypothesized to be putative, leading to phenotypic heterogeneity (Piras *et al.* 2017). As opposed to Aicardi syndrome in which cortical abnormalities are described, our patient did not demonstrate epileptogenic lesions on MRI and reflex sensitivity could represent a consequence of neuronal hyperexcitability of the sensory or association cortices, with a synchronized discharge spreading to functionally connected cortical or subcortical structures through white matter tracts. It is also possible given the increased synaptic activity postulated with this variant, physiological responses are responsible for the induction of synchronization of larger networks or functionally connected epileptogenic cortex, although this remains to be conclusively proven (Jiang 2019).

Phylogenetic analysis indicated that the protein is highly conserved as a single copy across evolution. Other genes which have been implicated in agenesis of corpus callosum include *MCOLN1*, *HERC2*, *DCLK2*, *CACNA1A* and *KCNH3* (Meloche *et al.* 2020). This child had global developmental delay, truncal hypotonia and symptomatic epilepsy with reflex seizures which is refractory to sodium valproate and lamotrigine favouring a developmental encephalopathy. Dysmorphic features in Temtamy syndrome are not distinct from patients with intellectual disability and syndromic agenesis of corpus callosum such as Aicardi syndrome (Donnai and Barrow 1993; Aicardi 2005; Ganesh *et al.* 2005). The current patient's phenotype was similar to the literature reports of craniofacial anomalies consisting of arched eyebrows, antimongoloid slant of the eyes, beaked nose, low-set and simple lop ears, full cheeks, long philtrum, short upper lip, and micrognathia (Temtamy *et al.* 1991). Previously reported ocular abnormalities such as coloboma and microphthalmia were not found in this child. The most frequent congenital heart defect, i.e. ASD was also observed in the present case. The phenotype and genotype of previously reported cases of Temtamy syndrome in comparison to our poband is detailed in table 2. The epilepsy phenotype with focal-onset seizures, startle-induced tonic seizures, spasms and occasional secondary generalized seizures suggests a diffuse network centered on cortical hyperexcitability with multifocal IEDs on EEG. This phenotype has however been reported with other callosal-agenesis syndromes such as Aicardi syndrome (Grosso *et al.* 2007) suggesting the role of the pathogenic variant in leading to dysfunction in epileptogenic pathways centred on reflex seizures. This case report expands the ethnic spectrum of patients with this rare syndrome and highlights the utility of WES in trios in rare DEE phenotypes.

Acknowledgements

Authors thank the family for their enthusiastic participation. This study was supported by Indian Council for Medical Research, India, vide grant no. 2018- 2670.

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Corresponding editor: Ashwin Dalal