

RESEARCH ARTICLE



Ellis–van Creveld syndrome and profound deafness resulted by sequence variants in the *EVC/EVC2* and *TMC1* genes

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Abstract. Ellis–van Creveld syndrome is an autosomal recessive skeletal dysplasia primarily characterized by the features such as disproportionate dwarfism, short ribs, short limbs, dysplastic nails, cardiovascular malformations, post-axial polydactyly (PAP) (bilateral) of hands and feet. *EVC/EVC2* located in head-to-head arrangement on chromosome 4p16 are the causative genes for EvC syndrome. In the study, we present two families, A and B, with Pakistani and Republic of Kosovo origin, respectively. They showed features of EvC syndrome and were clinically and genetically characterized. In family A, the affected members showed an additional feature of profound deafness. The whole exome sequencing (WES) in this family revealed two homozygous variants in *EVC2* (c.30dupC; p.Thr11Hisfs*45) and *TMC1* (c.1696-1G>A) genes. In family B, WES revealed novel compound heterozygous variants (p.Ser307Pro, c.2894+3A>G) in the *EVC* gene. This study reports first case of variants in the genes causing EvC syndrome and profound deafness in the same family.

Keywords. EvC syndrome; profound deafness; whole exome sequencing; *EVC* gene; *EVC2* gene; *TMC1* gene; biallelic variants; compound heterozygous variants.

Introduction

Ellis–van Creveld syndrome (EvC, MIM: 225500) or chondro-ectodermal dysplasia is a rare form of skeletal dysplasia, segregating in an autosomal recessive manner with an estimated prevalence of seven per million in general population. However, the disorder is more common in the old Amish population of Lancaster County, Pennsylvania, USA with a significantly increased incidence of five per thousand live births (McKusick *et al.* 1964; Shi *et al.* 2016). Primarily characterized by disproportionate dwarfism, short ribs, short limbs, dysplastic nails, PAP (bilateral) of hands and feet, and oral manifestations including enamel hypoplasia, multiple frenula, malocclusion, oligodontia and peg or misshaped maxillary incisors. Nearly two-third of all the affected individuals

have congenital heart defects, usually an atrial or atrioventricular septal defect (AVSD) (McKusick *et al.* 1964; Hills *et al.* 2011; Aziz *et al.* 2016). Other abnormalities including strabismus, genu valgum, cryptorchidism, epispadias and hypospadias, renal anomalies and pulmonary malformations have also been reported (Baujat and Le Merrer 2007; Umm-E-Kalsoom *et al.* 2010; Aziz *et al.* 2016).

Nonsense or frame-shift variants in the two genes *EVC* (MIM: 604831) or *EVC2* (MIM: 607261) are responsible for ~70% of EvC syndrome (Ruiz-Perez *et al.* 2000, 2003; Aziz *et al.* 2016). As the two genes lie in a head-to-head arrangement in close proximity, thus suggesting that they function in the same biological pathway (Ruiz-Perez *et al.* 2003). *In vivo* studies revealed that EvC null mice developed an EvC syndrome like phenotypes including short ribs, short limbs, and dental abnormalities, but lacking

cardiac malformations and polydactyly (Ruiz-Perez et al. 2007).

In vitro studies revealed that EVC and EVC2 proteins interact with each other to form a complex at the base of primary cilia membrane known as the EvC zone, which is essential for intramembranous ossification and normal endochondral growth (Valencia et al. 2009; Pacheco et al. 2012). The complex is also regarded as positive regulators of Hedgehog (Hh) signalling, and explains why mutations in either of the two genes results in indistinguishable phenotypes (Valencia et al. 2009; Pusapati et al. 2014).

In humans, heterozygous transmembrane cochlear-expressed gene 1 (*TMCI*) variants have been associated with progressive hearing loss (DFNA36), while homozygous variants in the same gene are responsible for major causes of profound recessive deafness worldwide (DFNB7/11) (Kurima et al. 2002; Kitajiri et al. 2007) accounting for 3 to 5% of deafness in European, Tunisian, Pakistani and Indian populations, and 6% in an eastern Turkish population (Kitajiri et al. 2007; Schrauwen et al. 2013).

This study shows segregation of two variants in *EVC2* and *TMCI* genes causing EvC syndrome and profound deafness, respectively in a consanguineous family of Pakistani origin and novel compound heterozygous variants causing EvC syndrome in a family collected from Kosovo.

Materials and methods

Human subjects and genomic DNA extraction

For this study, two families (A and B) segregating EvC syndrome in autosomal recessive pattern were recruited from two different countries. Family A, with three affected individuals (IV-1, IV-4 and IV-5), were of Pakistani origin, whereas family B with two affected individuals (III-2 and III-4), originated from Kosovo. Affected individuals in family A and B underwent clinical examinations at the city hospital Nawabshah, Pakistan and Technical University hospital, Munich, Germany, respectively. Family A was diagnosed with EvC syndrome and profound deafness phenotype by a classified medical physician in Hayatabad Medical Complex Peshawar Pakistan. Ethical approval for the study was obtained from the Institutional Review Board (IRB) of Quaid-i-Azam University Islamabad, Pakistan and Technical University Munich, Germany. Signed informed consents to perform the study and publication of radiographs and photographs were obtained from affected individuals and their parents. Venous blood samples were collected from seven individuals (III-3, III-4, IV-1, IV-2, IV-3, IV-4 and IV-5) of family A (figure 1a) and five individuals (II-4, II-5, III-1, III-2 and III-4) of family B (figure 2a). DNA was extracted using peripheral-blood lymphocytes by standard procedures.

WES and Sanger sequencing

WES was performed using DNA from one affected member in each of the two families. Exome enrichment was performed using the SureSelect XT Human All Exon 50 Mb kit, ver. 5 (Agilent Technologies, USA) and sequencing was performed on HiSeq 2500 systems (Illumina, San Diego, USA). Using Burrows-Wheeler Aligner (BWA ver. 0.7.5), all the reads were aligned against the human assembly hg19 (GRCh37). Subsequently Exome Depth, PINDEL (ver. 0.2.4t) and (ver. 1.0.0) SAM tools (ver. 0.1.18) were used for variant calling and filtering of variants was performed with the help of the SAM tools varFilter script and custom scripts. All the variants obtained after filtering were inserted into an in-house database for further analysis.

Identification of disease causing variants

In both the families (A and B), standard screening principles were used to search for different functional variants including nonsense, missense, indels, and splice sites in the *EVC/EVC2* genes, reported earlier to be involved in causing EvC syndrome. In addition, database was also searched for the variants causing profound hearing impairment, as this phenotype was observed in affected members in the family A. The disease causing variants, detected in the two families, were verified by Sanger sequencing as described earlier (Umair et al. 2016).

In silico analysis

The pathogenicity index of the sequence variants, identified here, was calculated using Mutation Taster (<http://www.mutationtaster.org/>), Mutation Assessor (<http://mutationassessor.org/>), Sorting Intolerant From Tolerant (SIFT, <http://sift.bii.a-star.edu.sg/>), Polymorphism Phenotyping V2 (PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/>) and CADD (<http://cadd.gs.washington.edu/>). The frequency of the variant in the general population was determined using Exome Variant Server (EVS) (<http://evs.gs.washington.edu/EVS/>), 7000 in-house controls (IHG; Germany), 1000 genomes (<http://browser.1000genomes.org/index.html>) and ExAC Browser (<http://exac.broadinstitute.org/>). Pathogenicity of splice site variant was determined using MutPred Splice (v1.3.2, <http://www.mutdb.org/mutpredsplice/about.htm>), Human Splice Finder (v2.4.1, <http://www.umd.be/HSF3/>) and Berkeley Drosophila Genome Project (BDG, http://www.fruitfly.org/seq_tools/splice.html).

Results

Clinical description of family A

In the family A, affected members IV-1, IV-4 and IV-5 were 10, 14 and 16 years old, respectively. They were born after

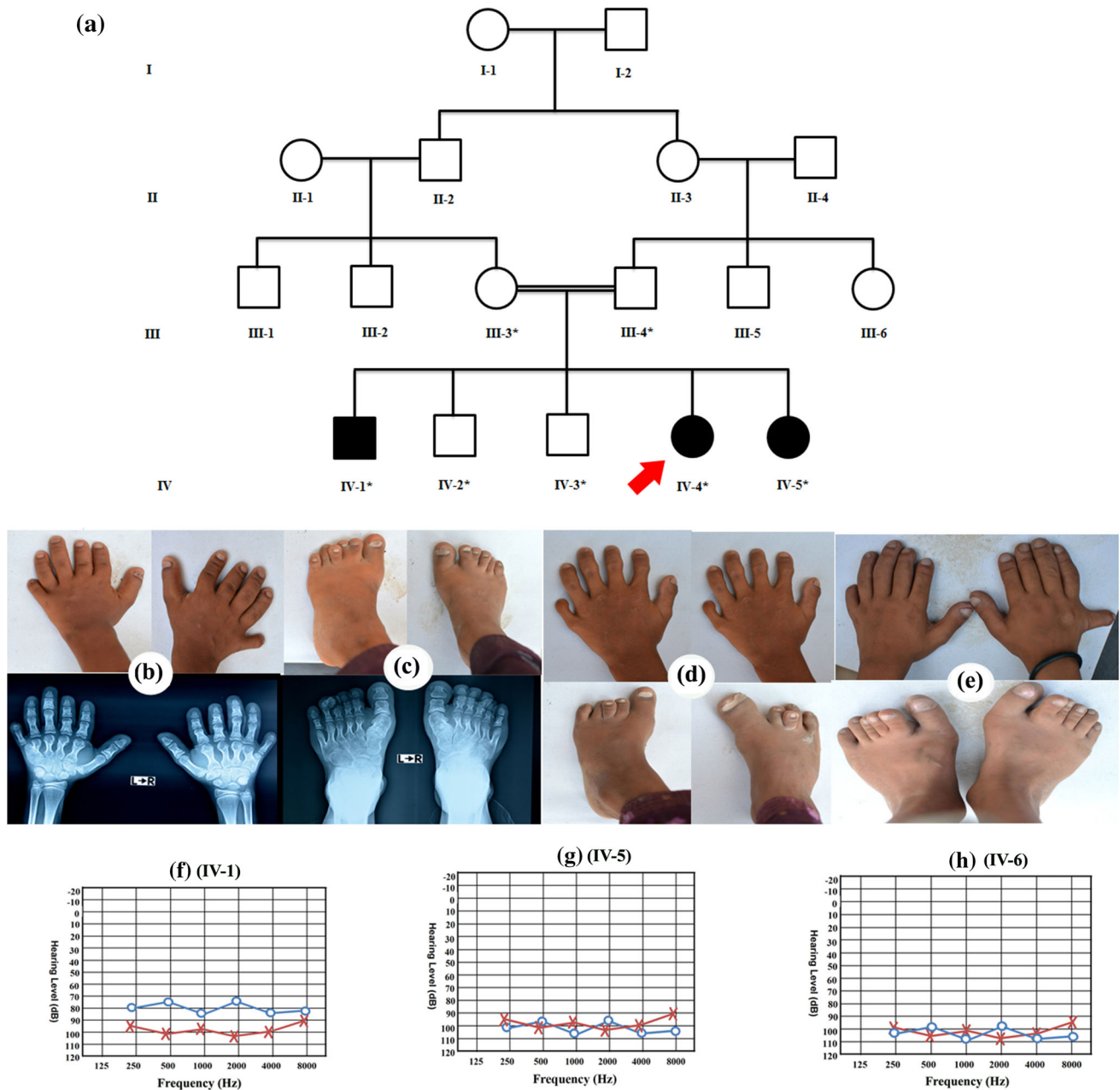


Figure 1. (a) Pedigree of family A, segregating EvC syndrome and profound deafness phenotypes. Pedigree clearly depicts autosomal recessive mode of inheritance. Circles and squares represent females and males, respectively. Shaded symbols represent affected individuals and empty symbols represent normal individuals. Double line represents consanguineous union. The individual numbers labelled with asterisks represent those which were available for this study and red arrow indicates the index patient for whom WES was performed. (b) Images and radiograph of affected individual IV-4, showing bilateral PAP in hands, brachydactyly and dysplastic nails. Radiographs also revealed bilateral PAP, and extra digit originating from 5th metacarpal. (c) Feet (IV-4) also showing PAP, cutaneous syndactyly of 2nd–3rd toe and radiographs also revealed bilateral PAP, well developed extra metatarsal along with extra toe and dysplastic nails. (d) Individual IV-5 having bilateral PAP in hands, dysplastic nails, right foot showing syndactyly of 1st–2nd toe not oriented properly and normal left foot. (e) Affected individual IV-1, having bilateral PAP in both hands and feet along with unique feature of digital clubbing with broad nails. Air conduction audiograms for affected individuals IV-5, IV-6 and IV-1. Red crosses, right ear; blue circles, left ear. Age (years) at the time of audiometry (IV-5, 16; IV-6, 14; IV-1, 10). (f–h) All the three affected individuals had profound hearing loss across all frequencies.

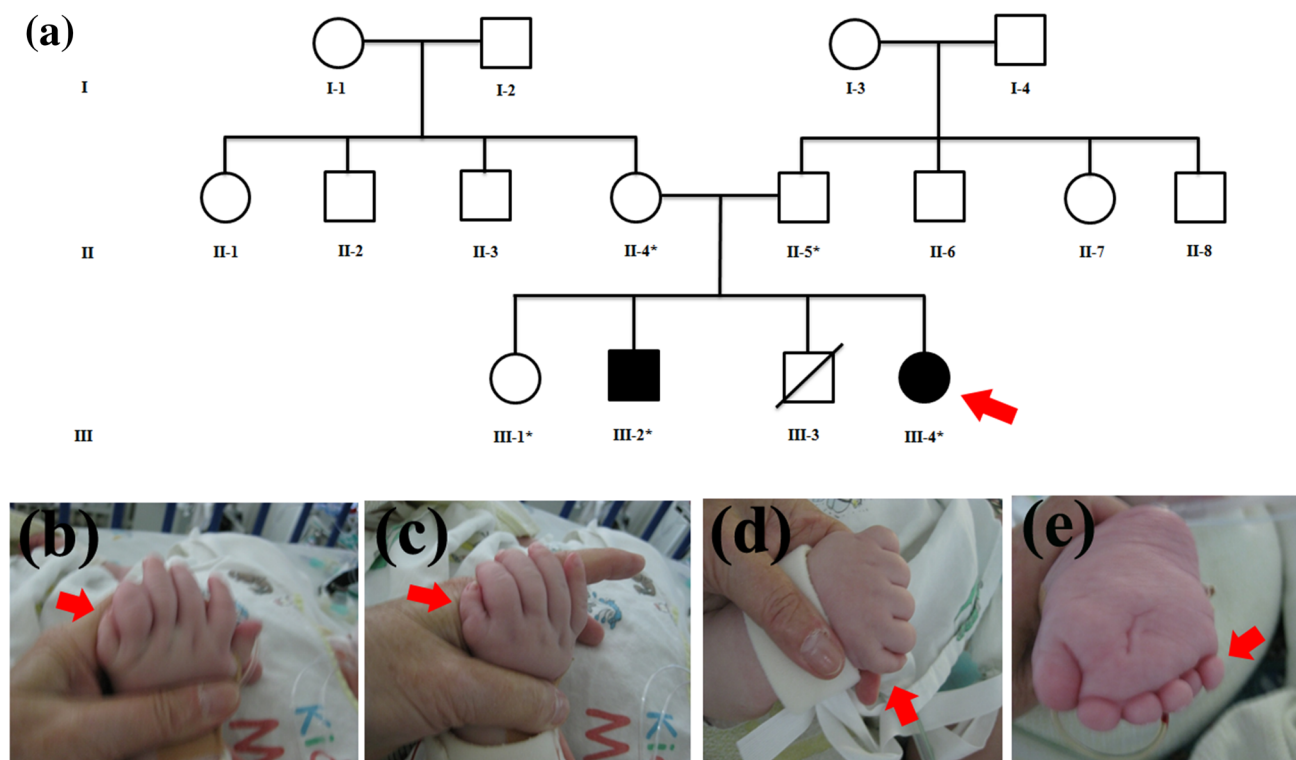


Figure 2. (a) Pedigree of family B depicting autosomal recessive inheritance suffering from EvC syndrome, red arrow indicating the affected individual (III-4) subjected to WES. (b–d) Affected individual (III-4), having bilateral PAP, indicated by red arrow, (e) PAP in right foot, indicated by red arrow.

normal pregnancy/delivery. Both parents did not reveal any previous history of dwarfism, dental, cardiac, hearing impairment and skeletal malformations.

All three affected members (IV-1, IV-4 and IV-5) were short statured (95 to 101 cm), had bilateral PAP in hands and/or feet. Syndactyly of 2nd–3rd toe and 1st–2nd toe in the right foot was observed in affected member IV-5 and IV-4 (figure 1, c&d), respectively. This condition of syndactyly was found missing in the third affected member (IV-1) of the family (figure 1e). Features including narrow shoulders, broad hands with brachydactyly, cubitus valgus in the upper limbs, and rhizomelic shortening of limbs with genu valgum (knock-knee) were observed in all the three affected members. Varied nail abnormalities were observed in the affected members. In two affected members (IV-4 and IV-5), the nails were dystrophic while in the third affected member (IV-1) it developed in to clubbed shaped (figure 1e). In two affected members (IV-4 and IV-1) feet were broad, swollen and the great toes were laterally deviated (hallux valgum, figure 1, c–e; table 1). Cardiac abnormality was observed only in an affected member IV-1, however ECG was not available. All three affected members had profound deafness (figure 1, f–h). Facial dysmorphism, thoracic deformity, sweating, hair and dental anomaly were not observed in the affected members.

Radiological study of an affected member (IV-5) revealed cone shaped epiphyses, shortening of the metacarpal and metatarsal bones, duplication of 5th metacarpal and metatarsal in both hands and feet, bilateral PAP in hand and feet along with capitate and hamate bones fusion (figure 1, b&c). Radiographs of other two affected members (IV-1 and IV-5) were not available for the study.

Clinical description of family B

In family B, affected individuals including 9-year-old boy (III-2) and his 2-year-old sister (III-4) were born after normal pregnancies. Their parents were healthy, non-consanguineous and belonged to Republic of Kosovo, a country in Southeast Europe. Parents of affected individuals (II-4 and II-5) did not reveal any previous history of malformations of skeletal, dental, polydactylies, cardiac, etc. An affected individual (III-4) was born with AVSD, and had bilateral PAP in both hands (figure 2, b–d) and unilateral in the right foot (figure 2e). The second affected individual (III-2) also had the same phenotypes and was operated successfully (table 1). Both the affected individuals did not reveal any additional clinical feature. Radiographs of the affected members were not available for the present study.

Table 1. Detail clinical description of affected individuals of family A and B.

	Family A			Family B	
Clinical features	Individual IV-1	Individual IV-4	Individual IV-5	Individual II-2	Individual II-4
Ethnic origin	Pakistani	Pakistani	Pakistani	Kosovo	Kosovo
Sex	Male	Female	Female	Male	Female
Age (yr)	10	14	16	9	2
Height (cm)	91.74	101.49	107.89	NA	NA
Weight (kg)	22.3	24.5	30	NA	NA
PAP of hands	+	+	+	+	+
PAP in feet	+	—	+	+	+
Preaxial fused polydactyly in feet	—	+	—	—	—
Syndactyly in feet	—	+	+	—	—
Short stature	+	+	+	—	—
Deafness*	—	+	+	—	—
Nail clubbing*	+	—	—	—	—
Speech problems	—	+	+	—	—
Distal limb shortening	+	+	+	—	—
Dysplastic nails	—	+	+	—	—
Short broad nose	+	+	+	—	—
Long philtrum	—	—	—	—	—
Hypodontia	—	—	—	—	—
Neonatal teeth	—	—	—	—	—
Lip notch	—	—	—	—	—
Cardiac defects	+	+	+	+	+
Macroglossia	—	—	—	—	—
Ankyloglossia	—	—	—	—	—
Fusion of capitates and hamate bones	No data	No data	+	—	—
Cone-shaped epiphysis	No data	No data	+	—	—
Narrow chest	+	+	+	—	—

*Novel finding; — a feature is absent; + a feature is present. NA, not available.

WES and Sanger sequencing

WES was performed on DNA from an affected member from each of the two families (A and B) at the Institute of Human Genetics, Helmholtz Zentrum Munchen, Germany, as described previously (Umair *et al.* 2017). Variants screening was performed on the basis of autosomal recessive inheritance of the disease, clinically for EvC syndrome, and profound deafness phenotype. Variants with a frequency higher than 0.1% were excluded in 7000 in-house control exomes (IHG, Munich, Germany) and different public databases.

To screen for homozygous and/or compound heterozygous variants, a step-wise filtering process was followed (table 2). In family A, two homozygous sequence variants including a duplication (c.30dupC; p.Thr11Hisfs*45) in exon 1 of the *EVC2* (NM_147127.4) and a homozygous splice acceptor site (c.1696-1G>A) in intron 18 of *TMCI* gene (NM_138691) were identified.

In family B, compound heterozygous variants comprising c.919T>C (p.Ser307Pro) in exon 7 and c.2894+3A>G in exon 20 of the *EVC* gene (NM_153717.2) were revealed.

The variants identified in both families were predicted to be highly pathogenic by different *in silico* tools.

To exclude the nonpathogenic nature of the novel variants identified in family A, exon 1 of the *EVC2* and intron–exon junction of exon 19 of the *TMCI* gene were Sanger sequenced in 150 ethnically matched control individuals. In addition, the sequence variants identified in the two families here were not present in homozygous or compound heterozygous state in the dbSNP, Exome Variant Server and 1000 Genome SNPs, 7000 in-house control exomes (IHG) and 50 in-house control exomes (HMG, Pakistan). WES was followed by Sanger sequencing to validate segregation of the variants in both the families. Primers for PCR-amplification of the exons of the three genes (*EVC*, *EVC2* and *TMCI*) were designed using Primer 3 (<http://frodo.wi.mit.edu/primer3/>) and Exon Primer (<http://ihg.gsf.de>).

Discussion

Two families A and B originated from Pakistan and Republic of Kosovo, respectively were investigated at

Table 2. Filtering steps followed after WES to search for the candidate variant in affected individuals of families A (IV-4) and B (III-4).

Filtration methods	Number of variants detected in family A	Number of variants detected in family B
Total variants detected in affected individual	70,992	81,523
Total heterozygous variants detected	39,456	40,684
Total homozygous variants detected	31,528	40,839
Total variants after dbSNP exclusion	3,234	2,896
Total homozygous frame-shift variants detected	120	131
Total homozygous indels detected	124	127
Total homozygous missense variants detected	59	86
Total homozygous nonsense variants detected	2	1
Total homozygous splice site variants detected	65	56
Total homozygous near splice site variants detected	19	22
Total homozygous synonymous variants detected	40	28
Total homozygous unknown variants detected	20	16
Total homozygous 3' and 5' UTR variants detected	274	266
Total homozygous variants identified after applying different filters (NHLBI-ESP; 1000 Genomes; ExAC) with MAF > 0.01	53	67
Total compound heterozygous variants identified after applying different filters (NHLBI-ESP; 1000 Genomes; ExAC) with MAF > 0.01	96	86
Homozygous variant identified in <i>EVC2</i> or <i>EVC</i> and other known genes and segregating with EvC syndrome	1	Nil
Compound heterozygous variant identified in <i>EVC2</i> or <i>EVC</i> and other known genes and segregating with EvC syndrome	Nil	1
Homozygous variant identified in deafness related genes and segregating with profound deafness phenotypes	1	Nil

clinical and genetical levels in the present study. Both the families presented features representing EvC syndrome (Ruiz-Perez *et al.* 2000; Cahuana *et al.* 2004; Aziz *et al.* 2016; Shi *et al.* 2016). Bilateral PAP in hands and/or feet and cardiac abnormalities were observed in affected members of both the families. In family A, the affected members had nails clubbing and profound deafness, features not reported previously in EvC syndrome. Some of the features including short stature, bilateral PAP, short and broad fingers, short thick tubular bones, and hypoplastic nails, reported previously in patients with EvC syndrome, were also observed in affected members of the family A (Umm-E-Kalsoom *et al.* 2010; Aziz *et al.* 2016). However, clinical features such as dental anomalies, long tongue (Macroglossia), fusion of upper lip, ankyloglossia, microdontia, and enamel hypoplasia, reported previously in several studies were not observed in affected members of families A and B (Cahuana *et al.* 2004; Umm-E-Kalsoom *et al.* 2010; Gopal *et al.* 2014; Aziz *et al.* 2016). Genitourinary abnormalities including vulvar-atresia, nephrocalcinosis, megaureters and renal agenesis reported by Alves-Pereira *et al.* (2009) and Gopal and Belavadi (2014) were not observed in the two families.

Exome sequence analysis revealed two homozygous disease causing variants including a duplication (c.30dupC; p.Thr11Hisfs*45) in *EVC2* and a splice acceptor site

variant (c.1696-1G>A) in *TMC1* genes in the family A and compound heterozygous variants including p.Ser307Pro and c.2894+3A>G in *EVC* gene in the family B. To date, 62 variants in *EVC2* and 69 in *EVC* genes have been reported in patients with EvC and Weyers syndromes (HGMD; <http://www.hgmd.cf.ac.uk/ac/index.php>). These included 34 nonsense (23 in *EVC2* and 11 in *EVC*), 10 missense (three in *EVC2* and seven in *EVC*), 44 small frame-shift insertion/deletions (25 in *EVC2* and 19 in *EVC*), three in-frame deletions in *EVC*, 24 splice site (six in *EVC2* and 18 in *EVC*), 11 large deletions including 520-kb homozygous deletion (five in *EVC2* and six in *EVC*) causing EvC syndrome with borderline intelligence (Temamy *et al.* 2008). Largely, mutations in *EVC2* and *EVC* are of frameshift or nonsense nature predicting loss of function and nonsense mediated decay. In family A, a novel C nucleotide duplication (c.30dupC) caused a frameshift and created a premature stop codon 45 amino acids downstream (p.Thr11Hisfs*45) in the *EVC2* gene (figure 3a). The variant is most likely to result in loss of function of the *EVC2* protein either through nonsense-mediated mRNA decay (NMD) or producing a truncated *EVC2* protein. In the same family, a novel splice acceptor site variant (c.1696-1G>A) detected in the *TMC1* gene was found to change the 3' splice acceptor site of exon 19 from AG to AA (figure 3b). Analysis of the flanking sequences of the

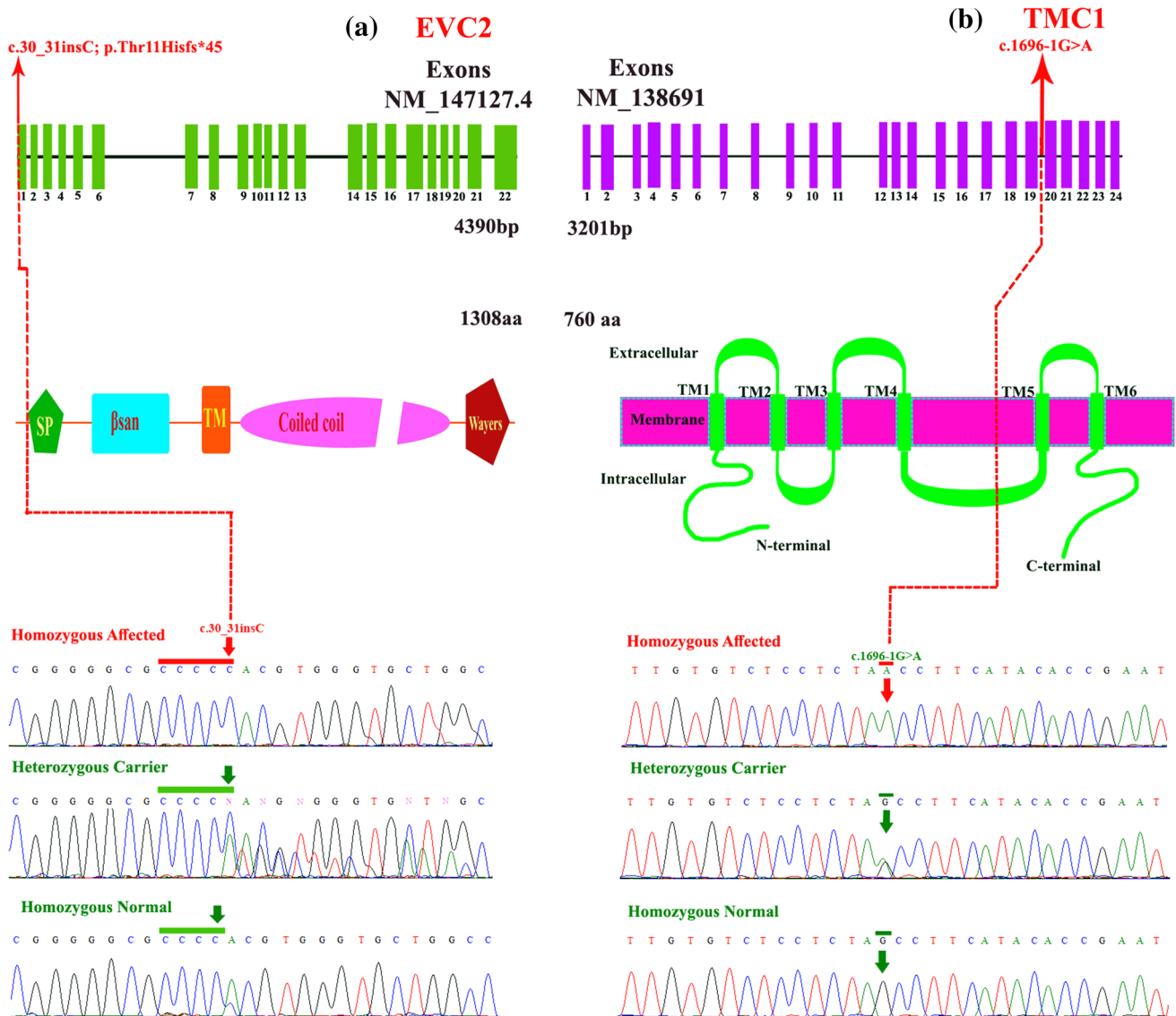


Figure 3. *EVC2* gene structure with known protein domains of the gene. (a) Sanger sequencing electrograms of the identified variant (c.30dupC) in homozygous affected, heterozygous carrier and homozygous normal individual. Red arrows showing the position of variants identified in the *EVC2* gene. (b) *TMC1* gene structure with known protein domains of the gene. Sanger sequencing electrograms of the identified variant (c.1696-1G>A) in homozygous affected, heterozygous carrier and homozygous normal individual. Red arrows showing the position of variants identified in the *TMC1* gene. Intronic regions are not drawn to scale.

variant (c.1696-1G>A) suggested a possible use of either of the two cryptic splice acceptor sites that could lead to production of a mutant *TMC1* protein (Maquat 1996). The variant was predicted to be deleterious by the majority of *in silico* tools. Due to nonavailability of the patient mRNA, further experimental study on both the variants was not possible. However, previous studies by Valencis *et al.* (2009) and Ganapathy *et al.* (2014) described the pathogenic effect of different variants in *EVC2* and *TMC1* genes both *in vivo* and *in vitro*.

TMC1 encodes a transmembrane protein mostly expressed in neurosensory hair cells of the mouse cochlea (Kurima *et al.* 2002). In the *Tmc1*^{dn/dn} mice (homozygous), the cochlear responses are completely absent,

while cochlear hair cells development is normal. In the *Tmc1*^{dn/dn} mice, 10 days after birth, the primary hair cell of the organ of Corti degenerates as a result vestibular saccular macule degeneration is observed (Bock and Steel 1983). Regardless of this observation, vestibular deficits phenotypes are not associated with both mutant mice (homozygous *Tmc1*^{dn/dn} and heterozygous *Tmc1*^{Bth/+}) (Kurima *et al.* 2002; Vreugde *et al.* 2002; Zhao *et al.* 2014). Further, the studies performed on the *Beethoven* (*Bth*) and *deafness* (*dn*) mice supported the role of *Tmc1* in the inner-hair and outer-hair cells. This may regulate proper differentiation of immature hair cells into fully functional auditory receptors or may help other membrane proteins trafficking in these cells (Marcotti *et al.* 2006).

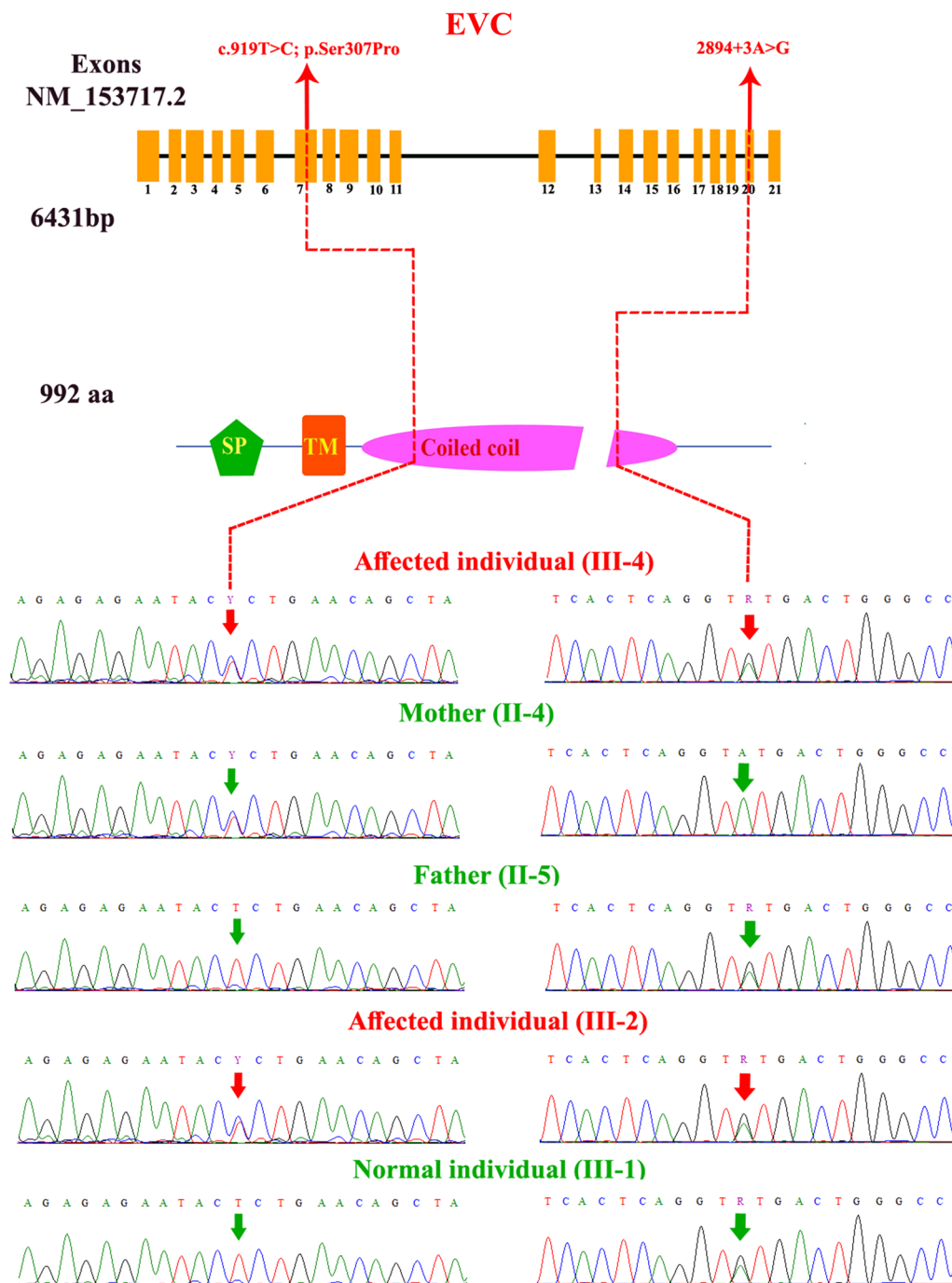


Figure 4. *EVC* gene structure with known protein domains of the gene. Sanger sequencing electrograms showing cosegregation of compound heterozygous variants (c.919T>C; p.Ser307Pro), (c.2894+3A>G) in family B. Red arrows showing the position of variants identified in the *EVC2* gene. Intronic regions are not drawn to scale.

Nakanishi *et al.* (2014) demonstrated that the function of *Tmc1* remains complex and the *Tmc1* mouse models showed that TMC1 is required for mechanoelectrical transduction (MET) and vestibular hair cells. While the *Evc*^{-/-} null mouse revealed the role of EVC protein as intercellular component of Hh signalling residing at the base of chondrocytes cilia and play important role in normal transcriptional activation of Indian Hh target genes in

bone development (Ruiz-Perez *et al.* 2007; Pusapati *et al.* 2014).

In family B, novel compound heterozygous variants (p.Ser307Pro; c.2894+3A>G) in the *EVC* gene were found segregating with the disease phenotype (figure 4). The missense variant (p.Ser307Pro) involved replacement of serine (polar) with proline (non-polar) residue at amino acid position 307 in the EVC protein. The distinctive cyclic

structure of proline side chain gives it an exceptional conformational rigidity compared to other amino acids. The proline acts as a structural disruptor in the middle of regular protein structure. Therefore, it is highly likely that p.Ser307Pro mutation disrupt the secondary structure and function of EVC protein. On the other hand, the splice donor site variant (c.2894+3A>G) might have resulted in retention of a part or full size intron, thus altering the mRNA structure that led to nonsense-mediated mRNA decay (NMD) pathway. Different splice site prediction tools indicated that the variant might affect normal splicing. Further, pathogenicity of the identified variant was also tested using different online tools.

Sequence analysis clearly showed that presence of deafness in affected members of the family A is due to mutation in the *TMC1* gene. As reported previously, pathogenic variants in the *TMC1* results in both autosomal recessive (DFNB7) and autosomal dominant (DFNA36), mapped on chromosome 9q13-q21 (Kurima *et al.* 2002). Kitajiri *et al.* (2007) reported a frequency of 3.4% in a cohort of 557 families of Pakistani origin suffering from profound deafness, carrying mutations in the *TMC1* gene. To date, 58 variants have been identified in *TMC1* gene in patients from different ethnic backgrounds. This included eight nonsense, 25 missense, 14 splice site, seven small deletions, one regulatory, one small insertion and two gross deletions (HGMD; <http://www.hgmd.cf.ac.uk/ac/index.php>).

Biallelic variants in two different genes involving mutations in the *EVC2* and *TMC1* genes resulting in EvC syndrome and profound deafness is the first such case reported. However, digenic inheritance of human diseases have been reported earlier in cases of Bardet–Biedel syndrome (BBS) (Katsanis *et al.* 2001), deafness (Ficarella *et al.* 2007), familial Parkinsonism (Funayama *et al.* 2008) amyotrophic lateral sclerosis (Butterfield *et al.* 2009), tooth agenesis (He *et al.* 2013), alport syndrome (Mencarelli *et al.* 2015) and congenital hypogonadotropic hypogonadism (Méndez *et al.* 2015). Further, recently Pedurupillay *et al.* (2016) reported a Pakistani consanguineous family having two biallelic variants in *LPAR6* and *PDECH6* causing alopecia and incomplete achromatopsia phenotypes.

In conclusion, we have reported the first case having two biallelic variants in two different genes (*EVC2* and *TMC1*) causing two phenotypes (EvC syndrome and profound deafness) in the same family. The report clearly indicated significance of exome sequencing in identifying more than one causal variant particularly in case of families showing multiple phenotypes. Pathogenic variants in *EVC2*, *EVC* and *TMC1* extend the spectrum of mutations in these genes causing EvC syndrome and profound deafness.

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