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A splice site variant in *TCTN3* underlies an atypical form of orofaciodigital syndrome IV

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Abstract

Orofaciodigital syndrome (OFD) is clinically heterogeneous and is characterized by abnormalities in the oral cavity, facial features, digits, and central nervous system. At least 18 subtypes of the condition have been described in the literature. OFD is caused by variants in several genes with overlapping phenotypes. We studied a consanguineous Pakistani family with two affected siblings with an atypical form of OFD type 4 (OFD4). In addition to the typical features of OFD4 that include limb defects and growth retardation, the siblings displayed rare features of scaphocephaly and seizures. Exome sequencing analysis revealed a novel homozygous splice site variant c.257-1G>A in *TCTN3* that segregated with disease. This homozygous splice site variant in *TCTN3* is most likely the underlying cause of the atypical form of OFD4 observed in this family. Our results contribute to the phenotypic spectrum of *TCTN3* associated ciliopathies and will facilitate better clinical diagnosis.

KEYWORDS
orofaciodigital syndrome, scaphocephaly, seizures, *TCTN3*

1 | INTRODUCTION

Orofaciodigital syndrome (OFD) comprises a heterogeneous group of disorders characterized by short stature, micrognathia, hypertelorism, cleft lip and palate, polydactyly, and various brain structural anomalies. Variable expressivity and appearance of atypical features can also occur making it difficult to diagnose.

Based on genetic etiology and clinical description, OFD has been classified into 18 subtypes. At least 10 of these subtypes display an autosomal recessive (Bruel et al., 2017; Thevenon et al., 2016) and two an X-linked recessive/

dominant mode of inheritance (Bouman et al., 2017; Edwards et al., 1988). Additionally, there are few cases for which the classification status of OFD subtype is uncertain (Saari et al., 2015; Thevenon et al., 2016). Nineteen genes involved in OFD etiology have been reported, some of which are involved in ciliogenesis, apoptosis, and RNA metabolism (Bruel et al., 2017; Yamada et al., 2019). Variants in some of these genes also cause other conditions including Joubert syndrome (MIM: 614615) (Srour et al., 2012), Meckel syndrome (MIM: 617562) (Shaheen et al., 2015), short-rib thoracic dysplasia with or without polydactyly (MIM: 615630) (Halbritter et al., 2013), Bardet–Biedl syndrome (MIM: 615992) (Kim et al., 2010), and Simpson–Golabi–Behmel syndrome (MIM: 300209) (Budny et al., 2006).

In this study, we have clinically and genetically characterized a consanguineous family segregating OFD type 4 (OFD4) (MIM: 258860) with an autosomal recessive mode of inheritance. Exome and Sanger sequencing was used to identify a novel homozygous splice site variant in *TCTN3* that segregates with OFD4.

2 | MATERIALS AND METHODS

2.1 | Ethical approval

The study was approved by Institutional Review Board (IRBs) at Quaid-i-Azam University, Islamabad, Pakistan (IRB# QAU-155) and Columbia University (IRB# AAAS3421). Members of the consanguineous family (BD432) segregating with orofaciodigital syndrome type 4 were recruited from the Punjab province of Pakistan (Figure 1a). Informed written consent to conduct clinical and genetic studies was obtained from adult family members and parents consented for their children. Genomic DNA from blood samples of two affected (IV-1, IV-2) and four unaffected (IV-3, IV-4, III-1, III-2) family members were extracted using QIAamp DNA Mini DNA extraction kit (QIAGEN).

2.2 | Exome sequencing

Genomic DNA obtained from affected family member (IV-2) underwent exome sequencing (Figure 1a). Exome library preparation was performed using the SureSelect Human All Exon V6 kit (60.45 MB). Barcoded libraries were pooled, and paired-end sequencing was performed on an Illumina HiSeq with average on-target coverage of 130×. Reads were aligned to GRCh37/Hg19 using Burrows–Wheeler Aligner (BWA-MEM) (Li & Durbin, 2009). Duplicate removal was performed using Picard (Picard toolkit, 2019) and indel-realignment, quality recalibration, and single nucleotide and small insertion/deletion variant detection and calling were performed using the Genome Analysis Toolkit (GATK) (McKenna et al., 2010).

Variants were annotated using ANNOtate VARiation (ANNOVAR) (Wang et al., 2010). Variants selected for further analysis were those affecting structure of exons and splice sites (±12 bp). The filtering was performed using a population specific minor allele frequency (MAF) of ≤ 0.005 in every population included in the Genome Aggregation Database (gnomAD) (Karczewski et al., 2020) and the Greater Middle East Variome Project (GME) (Scott et al., 2016) along with Kaviar allele count of ≤10. Homozygous and compound heterozygous variant was selected following an autosomal recessive or X-linked pattern of inheritance. Homozygous variants present in local exomes were excluded. A CADD-Phred score of ≥ 20 and at least one pathogenicity prediction from MutationTaster, PolyPhen-2 or SIFT obtained from dbnsfp35a (Liu et al., 2016) and dbscSNV1 (Jian et al., 2014) was used. Finally, literature review of gene functions and phenotypic reports in human and mice helped in prioritizing variants to be tested for segregation in all six family members using Sanger sequencing.

3 | RESULTS

3.1 | Clinical description

Affected male family member (IV-1) was 3 years and 8 months of age at the time of recruitment. He was born to a consanguineous healthy couple. He had a mild degree of short stature, scaphocephaly (Figure 1b(ii)) and metatarsus adductus in the right foot caused mainly by shortening of the medial cuneiform bone (Figure 1b(vi)). He also has bent radii (Figure 1b(vii) and growth retardation of skeletal elements on the right side of the body which is prominent in tarsals, carpals, and greater trochanter bone (Figure 1b(vi—viii)). Skull radiograph revealed beaten copper skull appearance (Figure 1b(iv)). His affected brother (IV-2) displayed similar features including a mild degree of short stature, scaphocephaly, and metatarsus adductus in both feet. He also had unclassified seizures.

3.2 | Exome analysis

Exome analysis revealed homozygous variants in two genes: *TCTN3* (NM_015631.6:c.257-1G>A) and *ARHGAP17* (p.(Arg35His); Table S1). Both variants were tested for segregation using Sanger sequencing which showed that only the *TCTN3* splice site variant [NM_015631.6: c.257-1G>A]

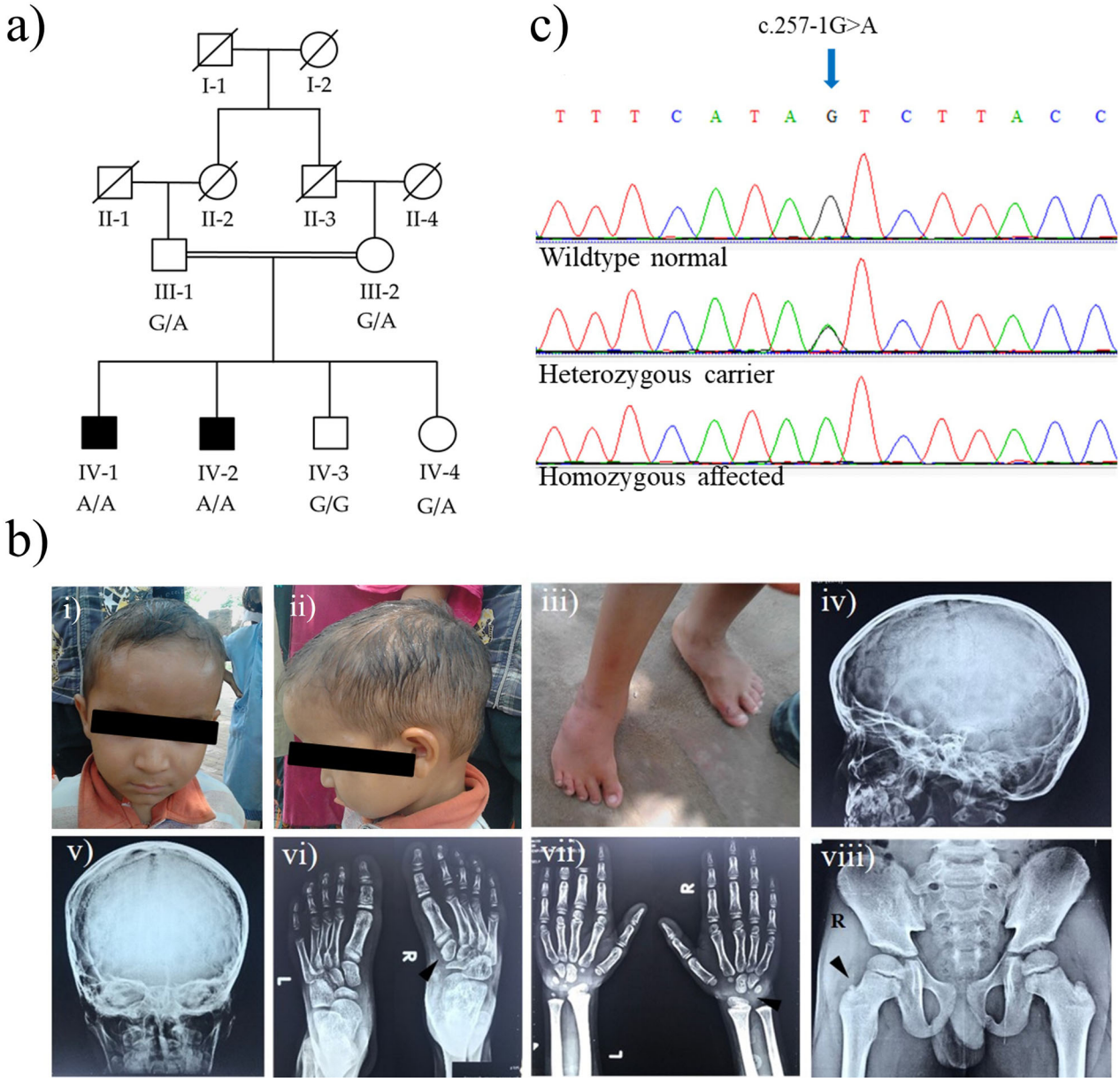

FIGURE 1 Genetic and clinical data. Pedigree drawing for consanguineous Pakistani pedigree BD432 segregating OFD4 (Panel a). Two affected individuals (IV-1 and IV-2) were homozygous for the *TCTN3* variant [c.257-1G>A]. Three individuals (III-1, III-2, and IV-4) were carriers and IV-3 was homozygous wildtype. Variant genotypes are shown under each individual with an available DNA sample. Pictures and x-rays for affected individual IV-1 (Panel b). Pictures showing scaphocephaly (i-ii) and metatarsus adductus (iii). X-rays of affected individual IV-1 displaying scaphocephaly and beaten copper skull appearance (iv-v), smaller cuneiform bone of right foot (vi), short carpal bones in the right hand and bowed radii (vii) and hypoplastic greater trochanter of right femur (viii). Sanger sequencing chromatograms showing splice site variant [c.257-1G>A] for a wildtype normal (IV-3), a heterozygous carrier (III-1) and a homozygous affected (IV-2) individual (Panel c)

(ClinVar accession number SCV001162780.1) segregated with the phenotype (Figure 1a,c; Table S2). This variant was not observed in gnomAD and GME. It has a CADD score of 25.1 and a GERP++_RS score of 5.03. The variant is located at a conserved canonical 3′ acceptor splice site in the first intron of the gene. It is predicted to be disease causing by MutationTaster and to impact splicing by both dbscSNV1.1 (ADA: 1.00; RF: 0.95), human splicing finder v3.1 (Desmet et al., 2009) and SpliceAI (Jaganathan et al., 2019). SpliceAI predicted a complete loss of the canonical acceptor site, c.257-1G>A, and a gain of an acceptor site within intron 1 at position −37. It is predicted to lead to retention of 36 bp of intronic sequence in the mRNA which translates 12 amino acids longer protein without a frameshift (Figure S1). The splice site variant in *TCTN3* was classified as pathogenic (PVS1, PM2, PP1) according to the American College of Medical Genetics (ACMG) guidelines (Richards et al., 2015).

4 | DISCUSSION

We report on a novel homozygous splice site variant in *TCTN3* causing OFD4 in a consanguineous family with two affected children. Previously, a compound heterozygous and five homozygous *TCTN3* variants with OFD4 have been reported in families of different ancestries (Al-Dewik et al., 2019; Thomas et al., 2012; Yadava & Ashkinadze, 2019). Variants in *TCTN3* have also been reported to be involved in the etiology of Joubert syndrome and congenital heart defects (Chen et al., 2019; Huppke et al., 2015). Frameshift and nonsense variants have been observed more often with OFD4, whereas missense variants are more closely associated with other disorders. (Table S3).

TCTN3, located on chromosome 10q24.1, encodes a 607 amino acid transmembrane protein which localizes to the ciliary transition zone and regulates protein trafficking into the cilium (Garcia-Gonzalo et al., 2011). The cilium has essential role in osteogenesis and patterning of skeletal elements (Haycraft et al., 2007). Additionally, it also acts as a regulator of apoptosis (Park et al., 2007). The role of *TCTN3* in the sonic hedgehog and apoptotic pathways was uncovered in *Tctn3*-knockout mice that displayed neuronal apoptosis with malformations of neural tube and limbs (Wang et al., 2018). Neuronal apoptosis has been associated with epileptic seizures (Méndez-Armenta et al., 2014), implicating the role of *TCTN3* in seizures observed in this family.

OFD is a severe condition, with a few subtypes reported to be lethal. OFD type 1, is X-linked male-lethal (Bouman et al., 2017). The phenotype in the presented family is less severe than the earlier reports with *TCTN3* variants, polydactyly and oral abnormalities being absent and long bone hypoplasia being milder (Thomas et al., 2012). In the affected family members of BD432 two clinical features scaphocephaly and seizures were observed that are not usually associated with OFD4. Scaphocephaly was reported in a patient with OFD4 in a clinical report, for which the underlying genetic cause is unknown (Rosing et al., 2008). Epilepsy and seizures have been reported in patients with other subtypes of OFD but not with OFD4 (Degner et al., 1999; Wentzensen et al., 2016). It should be noted that since seizures were only observed in one brother (IV-2) it is possible that this phenotype may be unrelated to OFD4. Metatarsus adductus, observed in both affected family members, is emerging as a pathognomonic feature of OFD4 along with talipes and bowing and hypoplasia of long bones (Bruel et al., 2017). Previously, talipes has been associated with three out of six variants in *TCTN3* reported with OFD4 (Thomas et al., 2012; Yadava & Ashkinadze, 2019). The absence of typical features of OFD such as cleft lip/plate, tongue hamartoma, and polydactyly and the presence of features not usually observed in OFD make this case a unique clinical entity.

The presence of consanguinity suggests the role of rare homozygous variants in the disease pathogenesis. These variants may be coding or noncoding and have variable effects on the phenotype. However, the nature of variant in the key gene is important in defining the overall phenotype. The splice site variant in *TCTN3* was predicted to lead to a longer protein which might have a different effect on the protein function compared to both loss of function and missense variants, explaining the atypical presentation of the syndrome in this family.

In conclusion, we have identified a novel homozygous splice site variant in *TCTN3* segregating with a rare presentation of OFD4 in a consanguineous family. The phenotype expansion will be helpful in aiding accurate clinical diagnosis in the future.

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AUTHOR CONTRIBUTIONS

SH drafted the manuscript and performed experimental work, SN prepared samples for Sanger sequencing, HK performed clinical evaluation, AA prepared samples for exome sequencing, IS analyzed exome sequence data analysis, WA and SML designed the study and revised the manuscript. All authors read and approved the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data obtained for this study is available from the corresponding author.

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SUPPORTING INFORMATION

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