European Journal of Human Genetics (2014) 22, 1145–1148

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SHORT REPORT

Whole-exome sequencing links TMCO1 defect syndrome with cerebro-facio-thoracic dysplasia

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Whole-exome sequencing (WES) is a type of disruptive technology that has tremendous influence on human and clinical genetics research. An efficient and cost-effective method, WES is now widely used as a diagnostic tool for identifying the molecular basis of genetic syndromes that are often challenging to diagnose. Here we report a patient with a clinical diagnosis of cerebro-facio-thoracic dysplasia (CFTD; MIM#213980) in whom we identified a homozygous splice-site mutation in the transmembrane and coiled-coil domains 1 (*TMCO1*) gene using WES. *TMCO1* mutations cause craniofacial dysmorphism, skeletal anomalies characterized by multiple malformations of the vertebrae and ribs, and intellectual disability (MIM#614132). A retrospective review revealed that clinical manifestations of both syndromes are very similar and overlap remarkably. We propose that mutations of *TMCO1* are not only responsible for craniofacial dysmorphism, skeletal anomalies and mental retardation syndrome but also for CFTD.
European Journal of Human Genetics (2014) **22,** 1145–1148; doi:10.1038/ejhg.2013.291; published online 15 January 2014

Keywords: TMCO1; cerebro-facio-thoracic dysplasia; whole-exome sequencing and splice donor mutation

INTRODUCTION

Cerebro-facio-thoracic dysplasia (CFTD; MIM#213980) is an extremely rare autosomal recessive condition with developmental delay/intellectual disability (DD/ID), costovertebral and central nervous system anomalies and distinctive recognizable facial features. About 15 cases have been reported worldwide from nine different population groups.[1–10] Although there are major overlapping findings, each reported case has additional and/or unique clinical features, which have been summarized in Table 1.

Previously, a homozygous frameshift mutation in the transmembrane and coiled-coil domains 1 (*TMCO1*, NG_032004) gene was reported by Xin *et al*[11] in a large Amish family containing 11 affected individuals with craniofacial dysmorphism, skeletal anomalies and mental retardation (MIM# 614132). Caglayan *et al*[12] reported a different homozygous nonsense mutation in *TMCO1* with similar clinical features in a singleton non-Amish case (Table 1). Neither report drew any inference regarding clinical overlap with CFTD.

We applied whole-exome sequencing to a patient with the clinical diagnosis of CFTD. Analysis showed a novel homozygous splice donor site mutation, c.323 + 3 G > C (chr1:g.165721336 G > C [hg19]), in *TMCO1*. Interestingly, extensive clinical evaluation revealed that these two syndromes (CFTD and TMCO1 defect syndrome) essentially represent the same clinical entity, with significant overlapping features.

MATERIALS AND METHODS

Patient

A 12-month-old boy was referred to the Center for Genetics Diagnosis at Zeynep Kamil Maternity and Children's Training and Research Hospital because of multiple congenital anomalies and developmental delay. He was born at 39 weeks of gestation with a birth weight of 3.74 kg (50–75th centile), birth length of 51 cm (50th centile), OFC of 36 cm (75th centile) and an Apgar score of 9 at 1 and 9 at 5 min. His prenatal follow-up was irregular and polyhydramnios had been detected during pregnancy. There was no history of infection and/or exposure to teratogens during pregnancy. Family history revealed that the parents were not consanguineous; however, they were from the same small village (Figure 1a). The product of the first pregnancy died *in utero* in the 6th month of gestation because of multiple life-threatening congenital anomalies including renal agenesis, hydrocephaly, thoracovertebral anomalies and heart defects. There is a healthy female sibling born from the second pregnancy (Figure 1a).

Physical examination revealed weight of 9700 g (25–50%), length of 82cm (90–97%) and OFC of 47 cm (25–50%). He had hypotonia, macrocephaly, brachycephaly, a narrow forehead, a flat face, frontal bossing, hypertelorism, epicanthal folds, synophrys, upslanting palpebral fissures, long eyebrows, long philtrum, a short, broad nose tip, high palate, micrognathia, low-set ears, posteriorly rotated ears, a short neck, narrow shoulders, *pes planus*, clinodactyly and a low posterior hairline (Figures 1b and c). Chest x-ray showed a segmentation defect in the thoracic vertebrae as well as rib anomalies including irregularities and bifid costa chondrae (Figure 1d). Abdominal ultrasound showed a hypoplasic left kidney measuring 22.5 × 8 mm. Denver developmental screening test II at age 10 months revealed that development was comparable to 6 months of age. Karyotype analysis revealed a normal 46,XY. Echocardiography was normal. Cranial MRI revealed frontotemporal atrophy, dilated lateral ventricles and a short, dysgenetic corpus callosum (Figures 1e and f).

Whole-exome sequencing of the index case

We applied whole-exome sequencing to the index case at Baylor College of Medicine, Human Genome Sequencing Center. The exome capture reagent was VCRome, as previously described.[13] The mutation identified in this patient was

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Received 25 July 2013; revised 6 October 2013; accepted 31 October 2013; published online 15 January 2014

Table 1 Clinical features of CFTD and TMCO1 defect syndrome

Feature	*Reported CFTD*									*TMCO1 defect syndrome*		*Present case*
	Pascaul-Castroviejo et al[1]	*Philip et al*[2]	*Guion-Almeida et al*[3]	*Kanaka-Gantenbein et al*[4]	*Rufo-Campos et al*[7]	*Bouzas et al*[10]	*Cilliers et al*[6]	*Smigiel et al*[8]	*Cortesi et al*[9]	*Xin et al*[11]	*Caglayan et al*[12]	*Pehlivan et al*
Number of cases	3	2	1	2	1	2	2	1	1	11	1	1
AR inheritance (consanguinity)	+	+	−	−	−	NA	+	−	+	+	+	+
Polyhydroamnios	NA	+	−	NA	−	NA	−	−	−	+	−	+
Short stature	NA	+		+	+	NA	−	+	−		+	+
Birth weight ≥90th centile	NA	+	+	NA	−	NA	−	−	−	−	−	+
Macrocephaly/macrocephalic appearance	NA	+	−	NA		NA	−	−	+	+	+	+
Brachycephaly	+	+	−	NA	NA	NA	+	−	+	+	−	+
Flat face	NA	NA		NA	+	NA	+	+	−	+	−	+
Low-set ears	NA	+	+	NA	+	NA	+	+	+	+	+	+
Low posterior hairline	+	+	+	NA	+	NA	+	NA	+	+	+	+
Hypertelorism	+	+	+	NA	+	+	+	+	+	+	+	+
Synophrys	+	−	+	NA	+	NA	−	−	+	+	+	+
Brushy eyebrows	+	−	−	NA	−	NA	−	−	+	+	NA	+
Epicanthic folds	NA	+	−	NA	−	+	+	+	+	−	NA	+
Up/down slanting palpebral fissures	NA	+	+	NA	NA	NA	−	+	+	−	NA	+
Short, broad nose	+	+	+	NA	+	NA	+	+	+	+	NA	+
Cleft lip-cleft palate/high arched palate	NA	+	+	+	−	NA	+	+	−	+	−	+
Short neck	+	+	+	NA	+	NA	+	+	+	+	+	+
CNS malformation	NA	+	+	NA	+	NA	+	+	+	+	+	+
Hypotonia	NA	+	+	NA	+	NA	−	+	−	−	+	+
Psychomotor retardation	+	+	NA	+	+	+	+	+	+	+	+	+
Rib and vertebra anomalies	+	+	+	+	+	+	+	+	+	+	+	+
Genitourinary anomaly	NA	+	+	+	+	NA	+	−	−	+	−	+

NM_019026: c.323 + 3G > C (chr1:g.165,721,336 G > C [hg19]) and has been deposited in the Leiden Open Variation Database (www.lovd.nl/TMCO1 with the identifier of 00002641). All experiments and analyses were performed according to previously described methods.[14]

Mutation analysis of the cDNA

To determine the effect of the identified splice donor (SD) substitution on cDNA structure and mRNA levels, we extracted RNA from whole blood and performed RT-PCR using primers in exons 4 and 7 and exons 5 and 7 of *TMCO1* (exons are numbered according to NG_032004).

RESULTS

Whole-exome sequencing

Targeted exome capture identified a homozygous NM_019026: c.323 + 3G > C (chr1:g.165,721,336 G > C [hg19]) nonsynonymous substitution in the *TMCO1* gene located on 1q23.3. The mutation is in a 4.6 Mb block of AOH (Figure 2a) and changes the SD site immediately downstream of exon 5 to a nonconsensus SD (Figure 2b). This mutation has not been reported in the 1000 Genomes Project (http://www.1000genomes.org) or other large-scale exome sequencing projects including the Exome variant server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA, USA (http://evs.gs.washington.edu/EVS/). In addition, the *TMCO1* mutation was not identified in our 'in-house'-generated exomes from 1500 individuals at the Baylor College of Medicine (BCM) Human Genome Sequencing Center and BCM Whole Genome Laboratory Database (MGL; http://www.bcm.edu/geneticlabs/), with over 1000 individuals tested for diagnostic purposes, nor in the Atherosclerosis Risk in Communities Study (ARIC) Database (http://drupal.cscc.unc.edu/aric/).

cDNA analysis

The resultant cDNA sequence analysis from these experiments demonstrated that exon 5 is preferentially skipped in patient BAB 4427 RNA, fusing exon 4 to exon 6 (Figures 2B andD) and leading to a premature termination codon early in exon 6. However, a small amount of patient RNA is correctly spliced, indicated by light bands in the exon 5–7 PCR (carried out for 40 cycles) and the sequencing of this cDNA. As the mutation leads to a premature termination codon in the beginning of exon 6 (156 bp in length) and there are 7 exons in *TMCO1*, it is likely that this RNA would be subject to nonsense-mediated decay (introduces a stop codon more than 50 bp from the 3′ end of the penultimate exon). The parents contained both normally spliced alleles and the aberrant product present in the patient (Figure 2c).

DISCUSSION

In this study, we report the third homozygous *TMCO1* mutation that we now identify in a Turkish patient with the initial referring diagnosis of CFTD. A careful literature and clinical database search from OMIM and PubMed revealed that the TMCO1 defect syndrome and CFTD appear to be one and the same, with important clinical overlapping features. We compared clinical features of both syndromes in Table 1. Although there are many additional features, the major clinical features of this syndrome are craniofacial dysmorphia, skeletal anomalies especially in the thoracic vertebrae and ribs, growth delay and central nervous system anomalies. There are additional relatively rarely associated anomalies, including cardiac defects (atrial septal defect and patent ductus arteriosus), genitourinary anomalies (inguinal hernia, shawl scrotum, renal pelvic/distal ureter dilation), skin and hair anomalies (sacral dimple, synophrys, hypertrichosis,

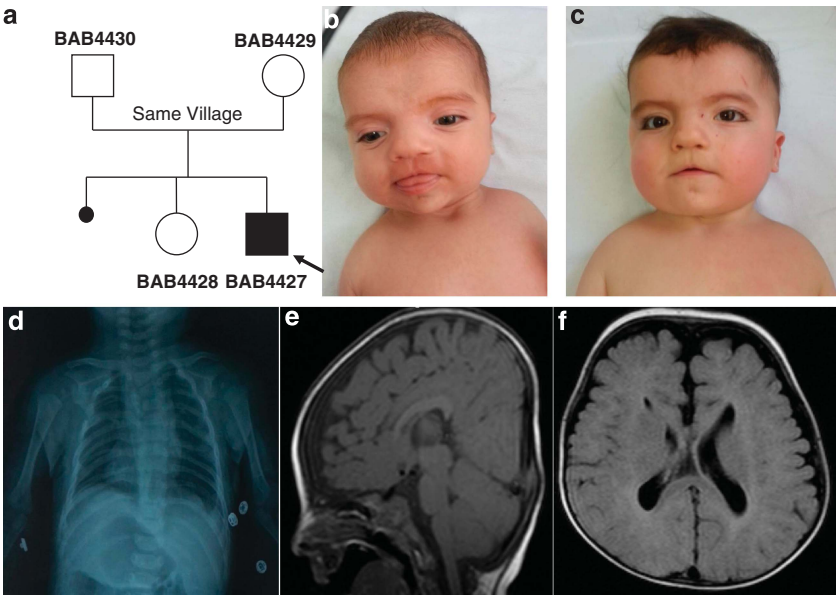

Figure 1 Pedigree, photographs and chest X-ray of the patient. (**a**) Pedigree of the family. Black arrow indicates the proband. Parents are not consanguineous but originate from the same small village. (**b**, **c**) Color photograph of the proband at age 4 and 12 months, respectively. Facial dysmorphic features of the patient includes flat face, frontal bossing, hypertelorism, epicanthal folds, synophrys, upslanting palpebral fissures, long eyebrows, long philtrum, a short, broad nose tip, and micrognathia. (**d**) Chest X-ray of the patient showing rib irregularities and bifid costa. (**e**, **f**) Midsagittal and axial view of cranial MRI of the patient showing frontotemporal atrophy, corpus callosum dysgenesis and enlarged ventricles.

poliosis, sparse eyebrows/eyelashes, and low posterior hairline), gastrointestinal system anomalies (poor feeding and constipation), joint/skeletal anomalies (joint laxity/subluxation, polydactyly, *pes planus/valgus* deformity, scoliosis, vertebral fusion, craniosynostosis) and growth hormone deficiency. Beside these postnatal features/findings, there are some prenatal manifestations including decreased fetal movements and polyhydramnios, which were reported in 36% of TMCO1 defect syndrome patients and in 34% of CFTD patients.

CFTD was initially named by Pascual-Castroviejo *et al*[1] in 1975 in three unrelated patients with mental retardation, characteristic facial features and spinal anomalies. As two of the reported cases were born to consanguineous marriages, an autosomal recessive inheritance pattern was suggested for this new condition. Consanguinity between parents was present in most of the reported cases. Major diagnostic features of the reported cases are DD/ID without growth delay, brain malformation, distinctive facial features and costovertebral anomalies. Although distinctive facial features including synophrys and DD/ID resemble the Cornelia de Lange syndrome (CdLS; MIM#122470), costovertebral anomalies and brain malformation are more specific to CFTD, and even macrocephaly and normal growth are opposite to what is observed with the CdLS phenotype. The gene responsible for CFTD has not yet been described.

Xin *et al*[11] described an autosomal recessive condition in a large Old Order Amish family with major diagnostic features of craniofacial dysmorphism, including brachycephaly, high arched bushy eyebrows, synophrys, long eyelashes, low-set ears, microdontia and gingival fibromatosis, skeletal anomalies of the chest including spine, rib and scapula, and mental retardation. Using genome-wide homozygosity mapping and sequencing techniques, they identified a 2 bp homozygous frameshift mutation in exon 2 of *TMCO1* [c.139_140delAG (p.(Ser47Ter))], which segregated in the family. They named this rare condition the TMCO1 defect syndrome. Caglayan *et al*[12] reported the second case of TMCO1 defect syndrome in a male patient born to a first-degree cousin marriage. The first pregnancy resulted in a spontaneous abortion in the first trimester, and the second child was a healthy female individual. Using whole-exome sequencing, they described a homozygous nonsense mutation p.(Arg87Ter) in exon 5 of *TMCO1*. The patient's clinical findings were consistent with the TMCO1 defect syndrome, including DD/ID, distinctive facial features, costovertebral anomalies of the chest including rib and scapula anomalies and brain malformations such as corpus callosum dysgenesis and cerebellar herniation.

Interestingly, Xin *et al*[11] reported the rate of first trimester spontaneous abortion as 22% of all pregnancies in fetuses affected with the TMCO1 defect syndrome. Caglayan *et al*[12] also reported one miscarriage out of three pregnancies in the family they studied; however, they did not report whether the fetus had been affected. Spontaneous miscarriage has been reported in CFTD also.[2] The family of our index case had one pregnancy that had spontaneously aborted in the second trimester, with multiple congenital anomalies including kidney, central nervous system, heart and thoracovertebral defects. It is most likely that this unborn fetus had suffered from the same homozygous mutation as the proband, and therefore had CFTD. Although the function of *TMCO1* is not known, the gene is highly conserved throughout evolution and expressed in a wide variety of tissues in both human embryonic and adult tissues, pointing to a potential role in developmental regulation. It is also possible that manifestation of more severe phenotypes may lead to spontaneous abortions, which are frequently observed in both TMCO1 defect syndrome and CFTD families.

In this report we underscore the association and inherent similarities between CFTD and TMCO1 defect syndromes. The characteristic features of CFTD and TMCO1 defect syndrome are strikingly similar, including facial dysmorphia, central nervous system anomalies and skeletal anomalies of the thorax. Here we report a single case with the diagnosis of CFTD with a mutation in *TMCO1*; nevertheless, significant overlapping clinical features of these syndromes suggest that both syndromes are the same. However, further CFTD cases should be screened for mutations in the *TMCO1* gene to prove this hypothesis.

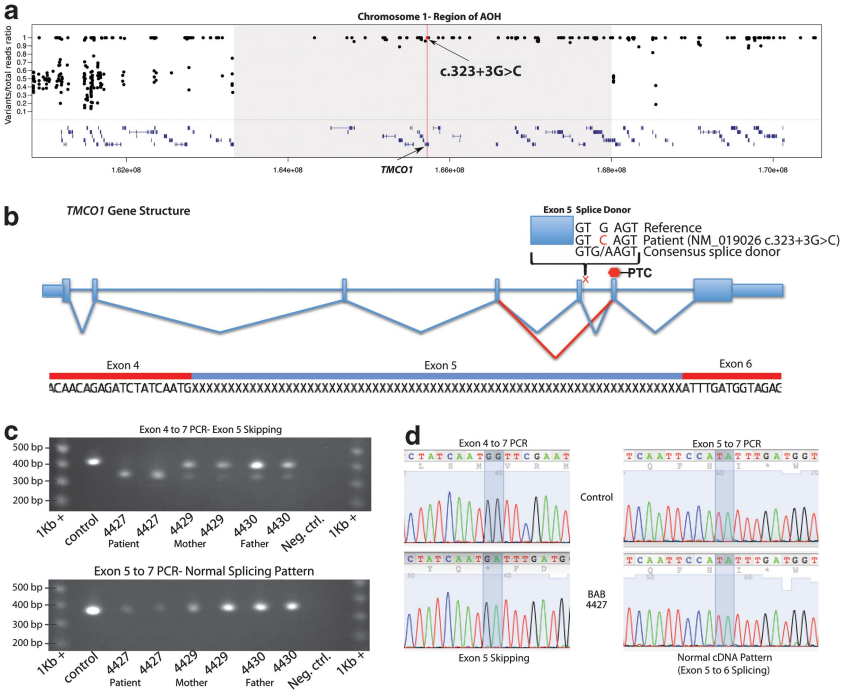

Figure 2 AOH surrounding *TMCO1* and the gene structure prior to and after splice donor mutation. (**a**) The region of AOH surrounding *TMCO1* is indicated with the gray box. (**b**) *TMCO1* gene structure is depicted, with thick blue lines indicating exons and untranslated regions of the gene. Blue thin lines indicate the canonical splicing pattern, whereas the red thin lines indicate the splice pattern in the proband. The cDNA sequence is concordant with that present in NCBI (NM _019026.4). The red 'X' indicates the location of the SD mutation. A premature termination codon occurs one amino acid after the aberrant splicing event, indicated by the stop sign in the sixth exon. The splice donor sequence from the reference human genome (hg19/GRCh37) is indicated at the top of exon 5, along with the patient sequence and the consensus SD sequence. The sequence of the cDNA skipping the fifth exon is indicated below the gene structure. (**c**) RT-PCR was conducted in the RNA of patient BAB4427, mother BAB4429, father 4430 (two separate RNA and cDNA reactions) and an unaffected control individual. PCR conducted with primers in exons 4 and 7 revealed the mis-spliced product as the only species in the patient, whereas parents had both splicing products and the control individual had a normally spliced product. PCR from exons 5 to 7 indicated the correct splice isoform in the control individual and a low level of correct product in the affected patient (after 40 cyles). (**d**) cDNA sequencing from the PCRs in **c** revealed predicted structures in the products. The correctly spliced product and exon 5 deletion product junctions are highlighted in the gray regions in the Figure. Both PCR products from the control individual contain exon 5 (top of figure), whereas the exon 4–7 PCR in BAB 4427 lacks exon 5 (bottom of the figure).

CONFLICT OF INTEREST

JRL is a paid consultant for Athena Diagnostics, has stock ownership in 23andMe and Ion Torrent Systems, and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases and bacterial genomic fingerprinting. The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from the chromosomal microarray analysis (CMA) and clinical exome sequencing offered in the Medical Genetics Laboratory (MGL; http://www.bcm.edu/geneticlabs/). Other authors have no disclosures relevant to the manuscript.

ACKNOWLEDGEMENTS

We thank the patient and his family who participated in this study. This work was supported by US National Human Genome Research Institute (NHGRI) grant U54HG006542.

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