European Journal of Medical Genetics 62 (2019) 103607

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Char Syndrome a novel mutation and new insights: A clinical report

E. Massaad[a], H. Tfayli[b], J. Awwad[c], M. Nabulsi[b], C. Farra[a,b,*]

[a] Medical Genetics Department of Pathology and Laboratory Medicine, American University of Beirut Medical Center, Lebanon
[b] Department of Pediatrics and Adolescent Medicine, American University of Beirut Medical Center, Lebanon
[c] Department of Obstetrics and Gynecology, American University of Beirut Medical Center, Lebanon

ARTICLE INFO

Keywords:
Transcription factor AP-2 beta
Mutation
Char syndrome
Patent ductus arteriosus

ABSTRACT

Transcription Factor AP-2 Beta (*TFAP2B*) functions in the differentiation of neural crest cell derivatives and contributes to the embryogenesis of the ductus arteriosus. Mutations of *TFAP2B* produces Char syndrome. Char syndrome is an autosomal dominant disorder comprising facial dysmorphism, hand anomalies, and patent ductus arteriosus (PDA). In this report, we describe a proband with a de novo *TFAP2B* frameshift mutation c.650delG p.(Gly217Alafs*32) in the basic domain. The proband presented mainly with musculoskeletal features of Char syndrome. No PDA was identified at presentation suggesting that this syndrome may prove to be phenotypically heterogeneous. This report will help illustrate the genotype/phenotype correlation of *TAFB2* mutations and better delineate the clinical features in Char syndrome.

1. Introduction

Char syndrome (OMIM 169100) is a congenital disorder characterized by patent ductus arteriosus (PDA) with facial dysmorphism and abnormal fifth digits. It was first reported by Char (1978) who described a child in whom PDA was associated with short philtrum, 'duck-bill' lips, ptosis, and low-set ears (Char, 1978). A wide spectrum of facial dysmorphology is reported including a broad forehead, prominent ears, thick and flared eyebrows, strabismus and broad nasal tip (Davidson, 1993). Char syndrome is caused by a genetic mutation in the Transcription Factor AP-2 Beta (*TFAP2B*) gene and follows an autosomal dominant mode of inheritance (Satoda et al., 1999). Complete penetrance and variable expression have been reported (Satoda et al., 1999, 2000). Various animal experimental studies showed that the *TFAP2B* gene product plays a critical role in the remodeling of the PDA as well as limb patterning (Zhao et al., 2011). Most reported pathogenic variants in *TFAP2B* gene are missense mutations in the coding region, affecting the basic domain that is critical for DNA binding (Satoda et al., 2000; Zhao et al., 2001; Mani et al., 2005; Lingaiah et al., 2011). Other reported mutations are intronic point mutations that alter splicing, or frameshift deletions (Mani et al., 2005; Chen et al., 2011). In this study we report a child with a novel heterozygous *TFAP2B* mutation c.650delG p.(Gly217Alafs*32), occurring de novo.

2. Clinical report

A 13 month-old boy presented for evaluation of failure to thrive, facial dysmorphism and clinodactyly. He was the third child of consanguineous healthy parents with no relevant family history. His two older siblings are reportedly normal (Fig. 1a).

He was born full term, small for gestational age (Weight 2.5 kg; z-score = −2SD). Transient hypoglycemia was noted at birth. He has no food allergies but was described as a slow feeder. Parents reported no delay in gross motor or cognitive development.

The child weighed 6 kg (z-score < -3SD) upon presentation. His length and head circumference were (z-score < -3SD) and 45 cm (z-score = -1SD), respectively. He had distinctive clinical features with triangular face, prominent forehead, low set ears, a nose with a broad flattened tip, short philtrum, thick lips, and wideset eyes with blue sclerae (Fig. 1b). Bilateral fifth finger clinodactyly and simian creases were noted (Fig. 1c). The rest of the physical exam was unremarkable. Developmental milestones were concordant with the child's chronological age. At the age of 2 years, color doppler echocardiography revealed a patent foramen ovale (PFO) with no PDA (Fig. 1d). At the age of 2 years 4 months, he was referred for evaluation of growth failure. He weighed 7.6 kg (z-score < -3SD) and his length was 77 cm (z-score < -3SD). His hematologic and renal profiles (including renal ultrasound) were normal. An X-ray of the left hand and wrist showed a bone age of 1 year 6 months, below 2 standard deviations of chronological age. Clinodactyly was noted with no osteopenia or other

* Corresponding author. Department of Pathology and Laboratory Medicine, Faculty of Medicine, American University of Beirut Medical Center, Beirut, Lebanon.
E-mail address: cf07@aub.edu.lb (C. Farra).

https://doi.org/10.1016/j.ejmg.2018.12.012
Received 17 October 2018; Received in revised form 12 December 2018; Accepted 19 December 2018
Available online 21 December 2018
1769-7212/

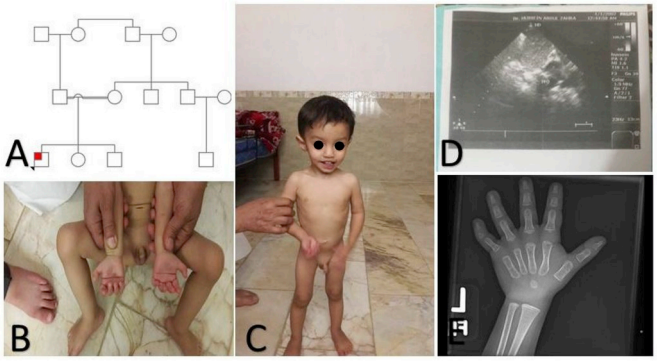

Fig. 1. A. Pedigree of the proband family. Double line indicates consanguinity. Red symbol indicates proband diagnosed with Char Syndrome. **B**. Single palmar crease and fifth finger clinodactyly in both hands. **C**. Facial dysmorphism featuring broad high forehead, triangular face, broad nose with flattened tip and tick lips. **D**. Echocardiography showing a patent foramen ovale. **E**. Xray of the frontal view of the left hand and wrist showing bone age is just below 2 standard deviations of the chronological age (Chronological age: 2 years 4 months, Assigned phalangeal bone age per the standards of Gruelich and Pyle:1 year 6 months). Clinodactyly of the left 5th, characterized by hypoplastic middle and distal phalanxes and radial deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

structural bone abnormalities (Fig. 1e). Insulin like growth factor 1 (IGF-1), IGF Binding protein-3, thyroid stimulating hormone (TSH) and free T4 concentrations were within normal limits.

Blood Karyotype GTG banding analysis at a resolution of 550 was characteristic of a chromosomally normal male. Whole Exome sequencing (CentoXome Gold) was performed for the proband and both parents. The proband's DNA revealed a heterozygous likely pathogenic deletion c.650delG p. (Gly217Alafs*32) in the *TFAP2B* gene (NM_003221.3) consistent with the diagnosis of Char syndrome (169100, AD), and with his clinical presentation (Fig. 2). This variant was not present in either parent and was therefore considered a de novo mutation. The variant was submitted in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar) with the accession number SCV000853288.

3. Discussion

TFAP2B gene encodes proteins classified as neural crest derived transcription factors that form homo- and heterodimers (Gelb and Chin, 2012). *TFAP2B* has been recently reported to be involved in the pathogenesis of cardiac ductal patency (Zhao et al., 2011). It is expressed in the smooth muscle cells of the developing duct where it induces expression of genes involved in cell migration and differentiation for ductus arteriosus closure (Zhao et al., 2011; Ivey et al., 2008; Moser et al., 1997). *TFAP2B* also seems to regulate bone morphogenetic protein (BMP) expression, which plays a very important role in the development of both the cardiovascular system and the limbs, and may contribute to the heart-limb defects in the *TFAP2B* knockout mice (Zhao et al., 2011; Robert, 2007). Mutations in *TFAP2B* are associated with Char syndrome (MIM 169100), an autosomal dominant disorder characterized by facial dysmorphism, 5th finger abnormalities, and PDA. TFAP2B has 3 main functional domains; an *N*-terminal transactivation domain, and two highly conserved domains, a basic domain and a *C*-terminal helix-span-helix (HSH) domain involved in DNA binding and dimerization (Wankhade et al., 2000). To date, most *TFAP2B* -associated Char syndrome variants are missense mutations affecting the basic domain (Satoda et al., 2000; Zhao et al., 2001; Mani et al., 2005; Lingaiah et al., 2011; Khetyar et al., 2008). Functional analyses of two of these mutations revealed that they prevent binding to *TFAP2B* target sequence and act in a dominant negative manner (Zhao et al., 2001). Other missense mutations were identified in the PY motif of the transactivation domain, altering interactions between transcription factors and coactivators (Lingaiah et al., 2011). The intron 3 pathogenic variants cause aberrant splicing of exon 3 with exon skipping, resulting in a frameshift that creates a premature stop codon, likely resulting in nonsense-mediated decay of the transcript (Mani et al., 2005; Lingaiah et al., 2011). Our patient was found to have a deletion Gly217Alafs*32 located in the basic domain of the *TFAP2B* gene. This frameshift mutation creates a premature stop codon that produces a truncated protein

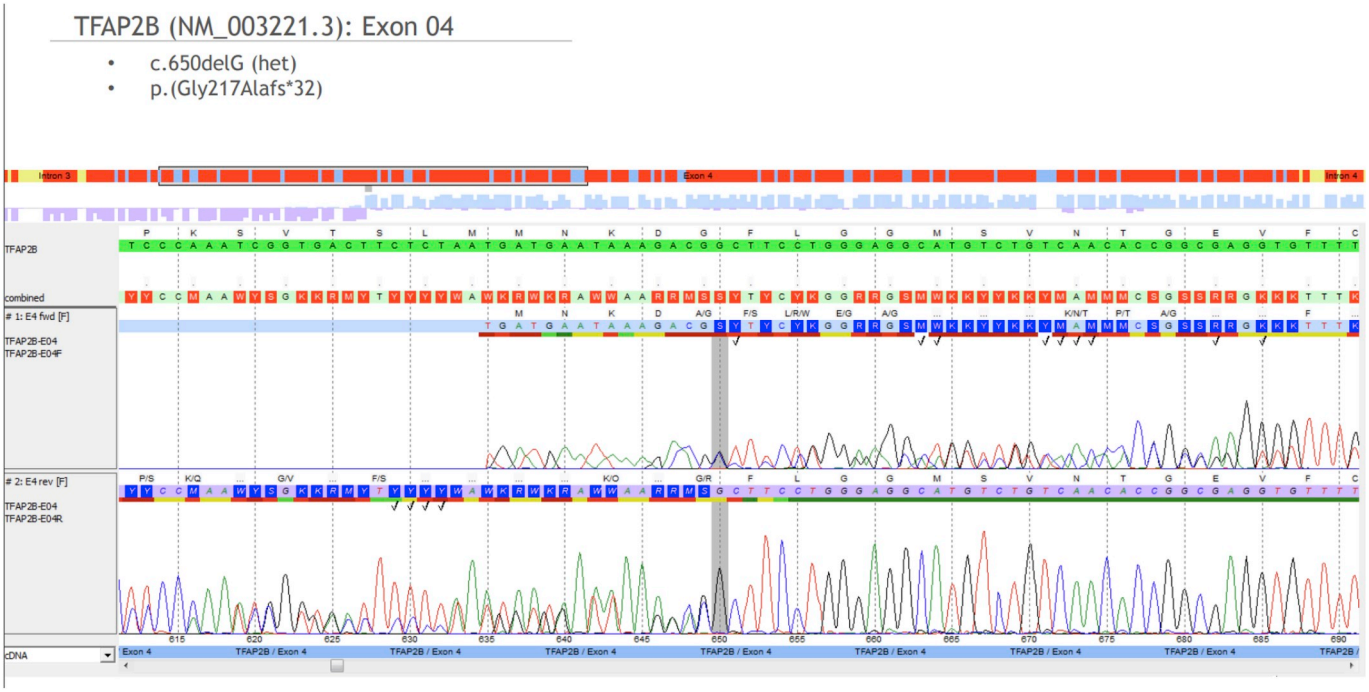

Fig. 2. Sequencing electropherogram showing a novel heterozygous Transcription Factor AP-2 Beta (TFAP2B) frameshift mutation c.650delG p.(Gly217Alafs*32) in both directions (forward and reverse).

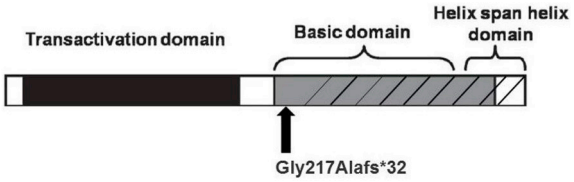

Fig. 3. Deletion mutation c.650delG p.(Gly217Alafs*32) located in the basic domain of the transcription factor Transcription Factor AP-2 Beta (TFAP2B). Striped area present the resulting truncated portion of the protein. *Source (reference 8–9)*.

with a partially transcribed basic domain and no transcription of the HSH domain (Fig. 3). He presented with dysmorphic features including a broad forehead, flat nasal bridge, short philtrum, thick lips, low-set ears as well as clinodactyly of the 5th fingers. Examination of genotype–phenotype correlations among several *TFAP2B* mutations revealed that those located in the DNA-binding domain of the protein produce the full Char phenotype while those in the trans-activation domain are associated with PDA but only mild facial and no limb abnormalities (Mani et al., 2005). Moreover, the allele A of TFAP2B (rs2817399), characterized by a mutation between exon 4 and 5 (DNA binding domain), has been reported to be associated with low mRNA levels of calcium-and potassium-channel genes that are involved in construction and closure of PDA (Waleh et al., 2010). In our patient, the mutation is in exon 4 of the DNA-binding domain and although it causes a truncated protein we do not have documentation of a PDA. Given that this patient's mutation resides in the DNA-binding domain of the protein, we can speculate that this patient might have had a small PDA responsible for his failure to thrive at presentation, which closed spontaneously to become undetected on echocardiography at the age of 2 years. Review of the reported patients with Char syndrome reveals the variability of the phenotype, particularly in regards to the presence or absence of PDA and limb deformities and its correlation with the genetic mutations (Nyboe et al., 2018; Sweeney et al., 2000). Although an important clinical feature of Char syndrome diagnosis, PDA identification maybe overlooked or missed during clinical assessment because of its transient nature. No clear association between increased occurrence of PFO and Char Syndrome has been so far reported. Further reports will add to the understanding of the genotype/phenotype correlation of *TAFP2B* mutations and a better delineation of clinical features.

Ethics approval

All procedures were reviewed and approved by the Institutional Review Board of the American University of Beirut. Oral consents were obtained from both parents. Peripheral blood samples of the proband and his parents were collected after obtaining informed consent. Genomic DNA was extracted according to standard protocols. Whole Exam sequencing (CentoXome Gold) was performed for proband and parents by Centogene, Germany.

Conflicts of interest

All authors declare that there is no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Char, F., 1978. Peculiar facies with short philtrum, duck-bill lips, ptosis and low-set ears–a new syndrome? Birth Defects Orig. Artic. Ser. 4, 303–305.
Chen, Y.W., Zhao, W., Zhang, Z.F., et al., 2011. Familial nonsyndromic patent ductus arteriosus caused by mutations in TFAP2B. Pediatr. Cardiol. 32, 958–965.
Davidson, H.R., 1993. A large family with patent ductus arteriosus and unusual face. J. Med. Genet. 30, 503–505.
Gelb, B.D., Chin, S.E., 2012. Genetics of congenital heart disease. In: Hill, J.A., Olson, E.N. (Eds.), Muscle. Academic Press, Boston/Waltham, pp. 473–480.
Ivey, K.N., Sutcliffe, D., Richardson, J., Clyman, R.I., Garcia, J.A., Srivastava, D., 2008. Transcriptional regulation during development of the ductus arteriosus. Circ. Res. 103, 388–395.
Khetyar, M., Syrris, P., Tinworth, L., Abushaban, L., Carter, N., 2008. Novel TFAP2B mutation in nonsyndromic patent ductus arteriosus. Genet. Test. 12, 457–459.
Lingaiah, K., Sosalagere, D.M., Mysore, S.R., Krishnamurthy, B., Narayanappa, D., Nallur, R.B., 2011. Mutations of TFAP2B in congenital heart disease patients in Mysore, South India. Indian J. Med. Res. 134, 621–626.
Mani, A., Radhakrishnan, J., Farhi, A., et al., 2005. Syndromic patent ductus arteriosus: evidence for haplo insufficient TFAP2B mutations and identification of a linked sleep disorder. Proc. Natl. Acad. Sci. U. S. A. 102, 2975–2979.
Moser, M., Ruschoff, J., Buettner, R., 1997. Comparative analysis of AP-2 alpha and AP-2 beta gene expression during murine embryogenesis. Dev. Dynam. 208, 115–124.
Nyboe, D., Kreiborg, S., Darvann, T., Dunø, M., Nissen, K.R., Hove, H.B., 2018. A study of familial Char syndrome involving the TFAP2B gene with a focus on facial shape characteristics. Clin. Dysmorphol. 27, 71–77.
Robert, B., 2007. Bone morphogenetic protein signaling in limb outgrowth and patterning. Dev. Growth Differ. 49, 455–468.
Satoda, M., Pierpont, M.E., Diaz, G.A., Bornemeier, R.A., Gelb, B.D., 1999. Char syndrome, an inherited disorder with patent ductus arteriosus, maps to chromosome 6p12-p21. Circulation 99, 3036–3042.
Satoda, M., Zhao, F., Diaz, G.A., et al., 2000. Mutations in TFAP2B cause Char syndrome, a familial form of patent ductus arteriosus. Nat. Genet. 25, 42–46.
Sweeney, E., Fryer, A., Walters, M., 2000. Char syndrome: a new family and review of the literature emphasising the presence of symphalangism and the variable phenotype. Clin. Dysmorphol. 9, 177–182.
Waleh, N., Hodnick, R., Jhaveri, N., et al., 2010. Patterns of gene expression in the ductus arteriosus are related to environmental and genetic risk factors for persistent ductus patency. Pediatr. Res. 68, 292–297.
Wankhade, S., Yu, Y., Weinberg, J., Tainsky, M.A., Kannan, P., 2000. Characterization of the activation domains of AP-2 family transcription factors. J. Biol. Chem. 275, 29701–29708.
Zhao, F., Bosserhoff, A.K., Buettner, R., Moser, M., 2011. A heart-hand syndrome gene: Tfap2b plays a critical role in the development and remodeling of mouse ductus arteriosus and limb patterning. PLoS One 6, e22908.
Zhao, F., Weismann, C.G., Satoda, M., et al., 2001. Novel TFAP2B mutations that cause Char syndrome provide a genotype-phenotype correlation. Am. J. Hum. Genet. 69, 695–703.