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Original Research Article

Tbx5 variants associated with non-Holt Oram syndrome (HOS) cardiac septal defect patients

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Abstract

Congenital cardiac septal defect (CSD) is the most common of congenital heart anomalies. The T-box transcription factor TBX5 gene is important in mammalian cardiac development either cardiac septation or morphogenesis. We aimed in this study to characterize the TBX5 variants in non-HOS patient with CSD. This case-controlled study was conducted on 30 unrelated affected children with non-syndromic isolated and non-isolated cardiac septal defects and 28 apparently healthy children with matching age and sex and without a family history of cardiac diseases as a normal control group. We detected reported intronic variant (g.18738) in 12 cases and coding variant (p.L135R) in one case. These results provide further insight into the pivotal molecular role of Tbx5 variants in cardiac development and pathogencity. This in turn could contribute to the therapeutic strategies for CSD.

Keywords: Tbx5 gene, non-Hlot oram syndrome cases, Genetic variants.

INTRODUCTION

Infant morbidity and mortality are largely due to congenital heart defects (CHD). Understanding the etiology of CHD will benefit clinical management and treatment of the patient.

In most patients, CHDs occur as an isolated malformation, and isolated defects of cardiac septation are the most common form of non-syndromic CHDs and account for approximately 50% of all CHD (Van der Linde et al., 2011). While the underlying molecular basis of most cases of nonsyndromic septal defects is unknown, autosomal dominant pedigrees have been identified and have proven to be useful for identification of genes or chromosomal loci. Many of these genes have been shown to be essential for cardiac development. Tbx5 gene is one of the important factors contributing in such development (Ware and Jefferies, 2012).

The majority of TBX5 mutations causes both severe cardiac and skeletal phenotypes. It has been suggested that severe cardiac but milder skeletal abnormalities due to missense mutations at the amino terminus of the DNA-binding domain (Basson et al., 1999). However, this relation has been questioned by recent cohort population dependent studies (Brassington et al, 2003; Dreßen et al., 2016; Eker et al., 2016). Variants in TBX5 gene are found in patients with complex cardiac malformations as ASD, VSD and AVSD (Reamon-Buettner and Borlak, 2004). However, TBX5 gene variants are rarely independently studied among Egyptian CHD patients.

Therefore, we went further to study Tbx5 variants in Egyptian congenital cardiac septal defect patients. We detected intronic and coding variants in non-HOS CSD patients with positive predictive impact using bioinformatics tools.

SUBJECTS AND METHODS

This research was approved by the Research Ethics Committee of the National Research Centre and Benha University according to the "World Medical Association Declaration of Helsinki". A written informed consent was obtained from the parents of all participants.

Patients

In this case controlled study, we recruited 30 unrelated affected children with non-syndromic isolated and non-

isolated cardiac septal defects consisting of 16 females and 14 males with age ranged from days to 5 years with mean age (1.4± 1.3). Twenty eight apparently healthy children with matched age and sex and without family history of cardiac diseases were taken as normal control group. Patients are diagnosed according to guidelines (Helmut Baumgartner et al., 2010).

Methods

All patients were subjected to the full history retrieved from their parents or medical records, complete clinical general examination, cardiac examination and echocardiography. TBX-5 genotyping were done by Sanger sequencing technique. Uniform PCR conditions with 0.5U recombinant Taq DNA polymerase (2U/ul) (Thermo Fisher Scientific Inc., USA) were carried out. PCR amplicons were purified by using The MEGAquick-spin Total Fragment DNA Purification Kit (iNtRON Biotechnology, Inc., Kyeonggi do, Korea).

The sequencing chromatograms were checked by manual inspection of characteristic double peaks and analyzed by DNA Baser Sequence Assembler v4.7.0 (2013), Heracle BioSoft. Prediction of the disease causing mutations and putative effect of the mutations were identified using the MutationTaster, SNPnexus tools and Variant Effect Predictor (VEP), Ensemble genome browser v83.38, assembly GRCh38.p5 (Genome Reference Consortium Human Build 38).

RESULTS

The clinical and cardiac examination showed that the 30 studied non-HOS CSD cases comprise of 9 cases (30%) with VSD, 8 cases (26.7%) with ASD, 7 cases with AVSD (23.3%) and 6 cases (20%) with TOF. Using Direct Sanger sequencing of whole exonic and exon-intron boundary regions of T-box DNA binding domain of TBX5 gene, we identified g.18738G>T (rs2236017), reported intronic variant with uncertain clinical significance, in 12 affected CSD case (40%) and in 9 healthy individuals (32.1%) as shown in Figure (1).

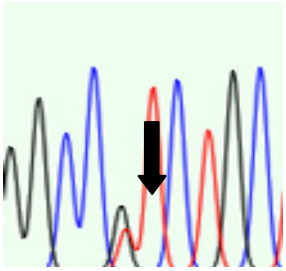

Figure 1. Partial sequence chromatogram displaying the Tbx5 gene for affected case presented rs2236017.

A second variant was missense variant, p. L135R, as shown in Figure (2), and located in the N - terminal domain, it was found in one case (3.3%) displaying isolated Atrioventricular septal defects (AVSD) and no upper limb defects, thumb aplasia or hypoplasia were found. According to the bioinformatics analysis, this variant located in highly conserved region, and might led to a new donor splicing site gain causing alternative spliced isoforms.

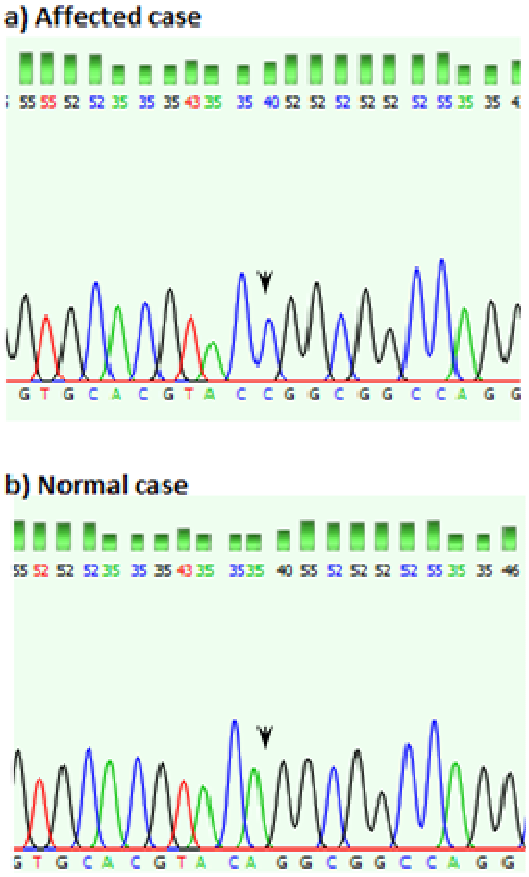

Figure 2. Partial sequence chromatogram displaying the Tbx5 gene for normal case for [AA] and affected case presented [CC].

DISCUSSION

The etiology of CHDs includes both genetic and environmental factors still under investigation, because that the related-genes variants can only explain a small fraction of CHD cases. The pathogenesis of sporadic cases, the most common form of CHD, remains poorly understood (Wang et al., 2017).

Approximately 85% of TBX5 gene mutations are novel either missense or nonsense variants locating mostly at the T-box domain, and lead to loss-of-function, either by

lack of protein or diminished DNA binding, or gain-of-function (Heinritz et al., 2005; Postma et al., 2008, Baban et al, 2014 and Eker et al., 2016). A lot of non-coding variants of TBX5 located in intronic region, promoter and enhancer regions were reported to be associated with CHDs (Liu et al., 2009; Shan et al., 2012 and Smemo et al., 2012).

In the present study, we detected reported p.L135R as missense variant leading to alteration in protein conformation or splicing events (Fayez et al, 2017), and intronic variant (g.18738) in 13 out 30 studied non-HOS CSD cases (43.3%).

Basson et al.. (1999) pointed that missense point variant near the amino-terminal end of the T-box domain produces very significant cardiac malformations with mild or no limb deformations. In contrast, missense point variant at the carboxyl end of the T-box domain produces severe limb abnormalities with minor CHDs. Al-Qattan and Abou Al-Shaar (2015) agreed with Basson et al. (1999) and added that extended protein variants are more inclined to cause severe bilateral skeletal malformations and more severe cardiac anomalies.

In contrast, Brassington et al. (2003), Eker et al. (2016) and Dreßen et al. (2016) pointed that neither the type of mutation nor the location of a mutation are determinant factors for severity of HOS or CHD phenotypes. Baban et al. (2014) postulated that gain-of-function variant might lead to deleterious effects on heart development, but not on the upper limb development.

Postma et al. (2008) pointed that the gain-of-function TBX5 variants lead to phenotype distinctly different from loss-of-function TBX5 mutations, indicating a difference in dosage sensitivity in the heart and limbs for TBX5.

CONCLUSION

Our data are consistent with the hypothesis of dosage sensitivity and variant location so far. We conclude that these data warrant the inclusion of the TBX5 variant detection in future case-control studies for isolated CHD. We recommend further broad differential Tbx5 variant based studies among HOS and non-HOS heart malformed patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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