



A de novo splicing variant supports the candidacy of *TLL1* in ASD pathogenesis

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Abstract

Congenital heart disease (CHD) is the most common type of birth defects with family- and population-based studies supporting a strong hereditary component. Multifactorial inheritance is the rule although a growing number of Mendelian forms have been described including candidates that have yet to be confirmed independently. *TLL1* is one such candidate that was proposed in the etiology of atrial septal defect (ASD). We describe a girl with congenitally corrected transposition of the great arteries (ccTGA) and ASD secundum whose whole-exome sequencing (WES) revealed a de novo splicing (c.1379-2A>G) variant in *TLL1* as well as an inherited truncating variant in *NODAL*. The identification of this dual molecular diagnosis both supports the candidacy of *TLL1* in ASD pathogenesis and highlights the power of WES in revealing multilocus cardiac phenotypes.

Introduction

Congenital heart disease (CHD) is the most common birth defect, affecting ~2% of liver births (1.35 million newborns worldwide) and accounting for nearly a third of major congenital malformations, and is the leading cause of infant mortality in western countries [1–3]. Causes of CHD are often classified into genetic and nongenetic categories (e.g., environmental teratogens including maternal exposure to medications or infectious agents) [4]. Our current understanding of CHD pathogenesis, however, is based on a multifactorial model that incorporates a multilocus genetic risk in a permissive environment [5].

Historically, the clinical genetic evaluation of CHD has focused on “syndromic” individuals i.e., those with additional birth defects, developmental delay or intellectual

disability, who account for approximately one quarter of cases [6]. Thousands of syndromes that are currently listed in OMIM feature CHD, and while classical positional mapping approaches were key to deciphering their genetic causes in the past, the use of genome sequencing over the past decade has now become a standard approach that greatly accelerated the rate of discovery [7].

Genome sequencing, given its relative simplicity and independence on multiplex pedigrees, has also been employed in the study of nonsyndromic CHD. In outbred populations, this approach has revealed a remarkable contribution of de novo variants [8]. In consanguineous populations, however, several autosomal recessive forms of nonsyndromic CHD have also been identified [9, 10]. One limitation of these approaches is the potential to reveal many candidate genes based on single mutational events, which highlights the need for follow-up confirmatory studies. Nonetheless, these studies have greatly contributed to more precise recurrence risk estimates and provided new insights into how the heart develops and how dysregulation of heart development leads to disease.

TLL1 is a gene proposed to play a role in ASD pathogenesis prior to the genome sequencing era. This was based on simple sequencing in a small cohort of ASD patients and the finding of three missense variants of unknown significance and unknown inheritance pattern [11]. In this study, we report the first de novo likely loss of function variant in *TLL1* in a patient with ASD as the strongest proof

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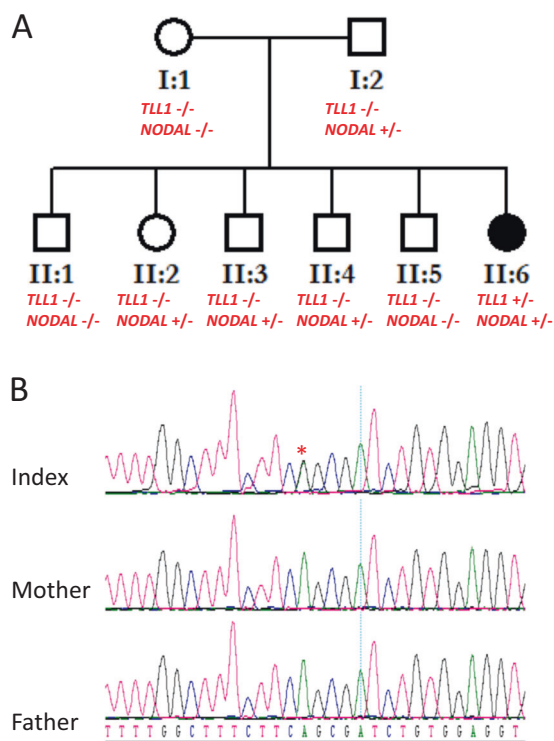
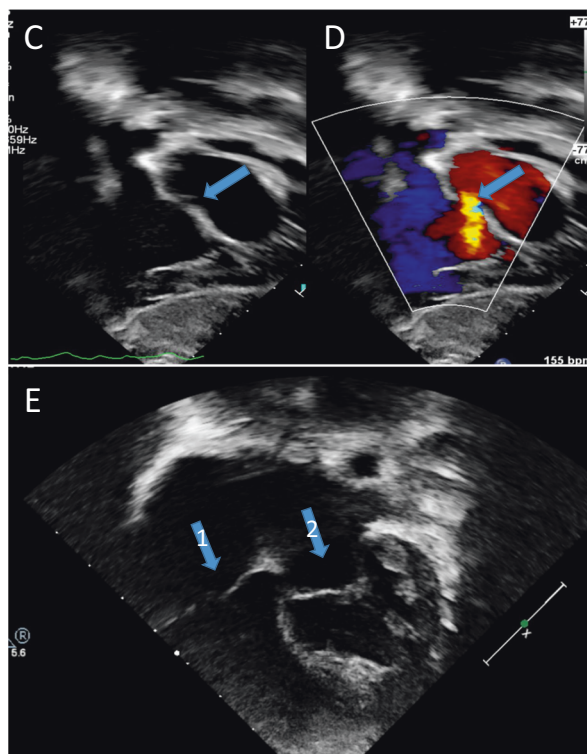


Fig. 1 **a** Pedigree of the study family with the genotypes indicated for each tested member. **b** Sequence chromatogram showing the de novo *TLL1* variant (asterisk indicates site of the identified variant in this study). **c** Arrow points to a small ASD II. **d** Arrow points to left to



right shunt across ASD. **e** Apical 4-chamber echocardiography showing L-looping ventricles with normal great arteries relationship. Arrow 1 indicates mitral valve and left ventricle, Arrow 2 indicates tricuspid valve and right ventricle

to date implicating *TLL1* loss-of-function in the etiology of a Mendelian form of CHD.

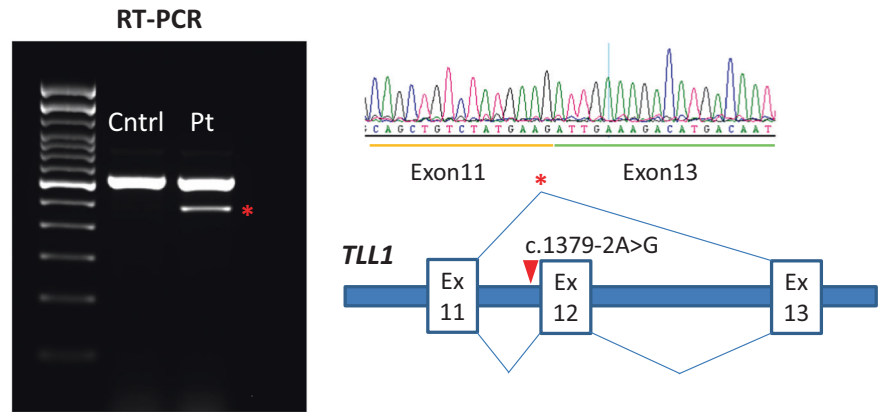
Case report

An 18-month-old girl who is known to have congenitally corrected transposition of great arteries (ccTGA) and ASD secundum status post pulmonary artery banding presented to the medical genetics clinic for workup. She was a product of preterm delivery 29 weeks of gestation via cesarean section, and birth weight was 1.25 kg. The presence of murmur on newborn examination prompted a full cardiac workup, which revealed ccTGA and fenestrated ASD (Fig. 1). Pulmonary artery banding for left ventricle training surgery was performed and patient has been treated with diuretics and anti-failure medications until normal cardiac function is achieved. She remains clinically stable and a double-switch cardiac surgery is planned in the future. She has no symptoms of ciliary dyskinesia. When evaluated in the medical genetics clinic, her clinical exam showed non-dysmorphic face and normal growth parameters (weight 10.2 kg (50th centile), length 77 cm (10th centile), and head circumference 45.5 cm (31st centile)). Apart from a sternotomy scar, her

dysmorphism examination was normal. Parents (Saudi Arabian) are unrelated. Clinical whole-exome sequencing (WES) was requested on the index given the complex nature of the CHD and it revealed the following two heterozygous variants: *TLL1* (NM_012464.4:c.1379-2A>G, NC_000004.11:g.166964424A>G, ClinVar ID: SCV000992315) and *NODAL* (NM_018055.4:c.555dupC (p.Thr186HisfsTer92), NC_000010.10:g.72195378dupG, ClinVar ID: SCV000992316) (Fig. 1). While the *TLL1* variant was absent in gnomAD, the *NODAL* variant was present at a very low frequency (two heterozygotes, MAF 0.00000798). No other clinically relevant variants were identified.

The *TLL1* variant c.1379-2A>G disrupts the invariant acceptor splice site of intron 11. Indeed, RT-PCR using urine-derived epithelial cells (RT-PCR was unsuccessful on blood or skin fibroblasts) revealed the presence of an aberrant band representing skipping of exon 12 (r.1379_1524del) predicting frameshift and premature truncation (p.(Ala460Aspfs*2)) (Fig. 2). Subsequent targeted segregation analysis by Sanger sequencing confirmed the de novo nature of this variant (Fig. 1). The *NODAL* variant, on the other hand, was observed in the father and three siblings whose follow-up echocardiography revealed normal findings.

Fig. 2 RT-PCR experiment showing an aberrant band in the index patient corresponding to complete skipping of exon 12 caused by the canonical splicing variant in *TLL1*



Discussion

Tolloid-like-1 *TLL1* is an astacin-like metalloprotease that shares structural similarity to bone morphogenetic protein-1 [12]. Studies in mouse with a disrupted *Tll1* allele suggest that *TLL1* plays multiple roles in the formation of the mammalian heart and is essential for the formation of the interventricular septum [13]. Specifically, homozygous mutants were embryonic lethal at mid-gestation and displayed cardiac failure and a constellation of developmental defects confined to the heart [13]. Constant features were incomplete formation of the muscular interventricular septum and an abnormal positioning of the heart and aorta [13].

Mechanistically, this role seems to be related to the enzymatic activity of *TLL1*. *TLL1* specifically processes procollagen C-propeptides at the physiologically relevant site, whereas *TLL2* lacks this activity [14]. *BMP1* and *TLL1* cleave chordin at sites similar to procollagen C-propeptide cleavage sites, and counteract the dorsalizing effects of chordin upon overexpression in *Xenopus* embryos [14]. Chordin is a key developmental protein and its deficiency is known to cause abnormal heart development [15].

The first link between *TLL1* and human ASD was based on targeted sequencing of *TLL1* in a cohort of 19 unrelated ASD patients and the demonstration of three missense variants of unknown significance [11]. The first case, a 35-year-old man with an isolated type I ASD, identified heterozygosity for NM_012464.5(*TLL1*):c.544A>C (p.Met182Leu) substitution at the first residue in the catalytic domain, which is conserved among all the astacins. The second case, a 70-year-old man with a type II ASD, had a heterozygous NM_012464.5(*TLL1*):c.713T>C (p.Val238Ala) substitution at a conserved residue within the catalytic domain, located just before the zinc-binding signature. The patient also had an aneurysm of the interatrial septum and atrial fibrillation requiring pacemaker implantation, and had suffered myocardial infarction as well as cerebrovascular accident. The third case, a 72-year-old man with a type II ASD had a heterozygous NM_012464.5

(*TLL1*):c.1885A>G (p.Ile629Val) substitution. The patient also had an aneurysm of the interatrial septum and bradycardia. Inheritance pattern was not established for any of these three heterozygous variants. On the other hand, a multiplex 3 generation family with ASD was found to segregate a heterozygous NM_012464.5:c.787A>G (p.Ile263Val) variant in *TLL1* [1].

Our finding of a de novo canonical splice-site variant in *TLL1* in a patient with ASD lends the strongest support to date implicating *TLL1* in the etiology of a subset of ASD patients. The mechanism of dominance, however, remains unclear because simple haploinsufficiency may be challenged by the modest pLI score of 0.37. Interestingly, our patient was also found to have a familial truncating variant in *NODAL* in what likely constitutes a dual molecular diagnosis. Multilocus phenotypes is a relatively recent concept made clear by the power of genome sequencing to interrogate all genes rather than a priori selected candidates. Estimates vary from 2–7% so although they represent a minority of cases, multilocus phenotypes nonetheless provide fresh insights into how “atypical” presentations may simply be the true amalgamation of two independent phenotypes in the same patient [10]. In our case, it seems likely that the *NODAL* variant played a role in the etiology of ccTGA despite the lack of phenotype in other family members since reduced penetrance is well known for this gene [16]. However, we cannot rule out the possibility that ccTGA may have been solely caused by the *TLL1* variant given the complex nature of cardiac malformation in *Tll1*^{-/-} mouse.

In summary, we report a patient with ASD and TGA and a de novo *TLL1* splicing variant as well as a truncating *NODAL* variant inherited from a normal father. We suggest that this further supports the candidacy of *TLL1* in the etiology of ASD. Whether TGA is related to *TLL1* or represents a hybrid phenotype caused by a reduced penetrance *NODAL* variant remains unclear.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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