

Novel NKX2-5 germline mutation in a Moroccan child with transitional atrio-ventricular septal defect (tAVSD)

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Atrioventricular septal defect is a complex congenital heart defects (CHD) with a prevalence of approximately 4% of all CHDs. Transitional form of atrio-ventricular septal defect (tAVSD) associates ostium primum atrial septal defect, common atrioventricular annulus with distinct atrioventricular valvar orifices in addition of restrictive ventricular septal defect. We describe in this report clinical and molecular features of a Moroccan boy that carries a novel NK2 homeobox 5 (NKX2-5) germline mutation (Pro141Ala), and exhibits a transitional atrio-ventricular septal defect. This phenotype has never been reported in association with NKX2-5 germline mutations. Pro141Ala is a non-reported pathogenic mutation that alters the nuclear localization signal sequence, leading to disruption of NKX2-5 nuclear translocation mechanism. Such alteration would decrease nuclear transcriptional activity of NKX2-5 and impair cardiogenesis process. The present report comes to widen the phenotypic spectrum of congenital heart disease caused by NKX2-5 germline mutations, and highlights as well the importance of the nuclear localization system in NKX2-5 activity.

Key words: atrio-ventricular septal defect (AVSD), NKX2-5, nuclear localization site (NLS)

Atrioventricular septal defect ([OMIM 606215; AVSD]) is one of the common complex congenital heart defects (CHDs) with an incidence around 4% of all CHDs observed at childhood. Atrio-ventricular septal defect (AVSD) is characterized by common atrioventricular annulus (with one or two AV valvar orifices), associated with large interatrial shunting at the primum septum, with or without interventricular communication. Depending on which of these conditions are combined, four AVSD types could be distinguished: complete (cAVSD), intermediate (iAVSD), transitional (tAVSD), and partial AVSD (pAVSD).¹ Transitional form of AVSD associates primum atrial septal defect and common atrioventricular annulus with distinct atrioventricular valvar orifices in addition of restrictive ventricular septal inlet.²

Non-syndromic AVSD was demonstrated to be associated with pathogenic germline mutations in cardiac transcription factors such as GATA4 (GATA-binding protein 4) and TBX5 (T-Box5)^{3,4}, and also with mutations in the cell adhesion molecule CRELD1 (cysteine-rich protein with EGF-like domains 1).⁵ In contrast, AVSD cases caused by deficient NKX2-5, were rather associated with somatic mutations. Indeed, although the spectrum of CHDs caused by NKX2-5 germline mutations is quite large, comprising ASD with or without AV block, tetralogy of Fallot (TOF), ventricular septal defect (VSD), double-outlet right ventricle (DORV), interrupted aortic arch (IAA), hypoplastic left heart syndrome (HLHS) amongst others, no NKX2-5 germline missense mutations were detected to date in

AVSD patients.⁶⁻⁹

We report in this paper a case of a Moroccan patient that carries a novel *NKX2-5* germline mutation, and exhibits a transitional atrio-ventricular septal defect (tAVSD), a phenotype usually associated with *NKX2-5* somatic mutations.

Case Report

The patient is a 2-year-old boy, born to healthy, non-consanguineous parents from the central North Morocco. The father and the mother were respectively 38 and 26 years old. In the neonatal period, he suffered from jaundice and recurrent respiratory infections. At two months of age, he was admitted to pediatric hospital due to afebrile acute dry cough associated with dyspnea and cyanosis of the lips and fingertips. At the admission, his weight and height was respectively 4.5 kg (-3 SD) and 54 cm (-3 SD). At auscultation, the infant showed systolic heart murmur and tachycardia with pulse reaching 160/min and respiratory rate of 60/min. Echocardiography revealed a transitional AVSD consisting of a large ostium primum atrial septal defect with left-to-right shunting and restrictive ventricular septal defect (Fig. 1).

Molecular analysis

The proband was assigned to genetics laboratory for molecular testing. After obtaining the informed consent as well as the approval of the local ethic committee (under the report number Réf.06/14), the blood sample was taken and genomic DNA was extracted. Then, we

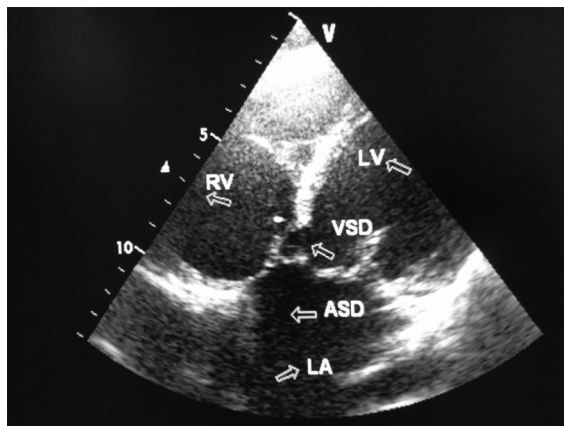


Fig. 1. Echocardiogram of the proband showing a tAVSD phenotype with a large ostium primum atrial septal defect and a restrictive ventricular septal defect.

carried out polymerase chain reaction (PCR) amplification of the two *NKX2-5* coding exons and their respective boundaries according to the standard protocol. The PCR product was directly sequenced using 3500Dx Genetic Analyser (Applied Biosystems, Massachusetts, MA, USA). The Bioinformatics analysis of the obtained sequences revealed a C to G transition at position 421 (c.421C>G). This missense mutation located in the exon 2 leads to amino acid substitution p.Pro141Ala within the *NKX2-5* homeodomain (HD) (Fig. 2).

Discussion

NKX2-5 is a member of *NKX2* transcription factor family. This highly conserved homeobox gene is widely expressed in cardiac cells throughout the heart development. *NKX2-5* interacts with *GATA4* and *TBX5* to regulate the downstream genes involved in several cardiogenesis processes.¹⁰

Germline mutations affecting this transcription factor (*NKX2-5*) were proved to cause a wide range of congenital heart defects, concerning mainly ASD, TOF, and VSD.^{10,11} The current literature confirms that *NKX2-5* mutations identified in AVSD subjects were rather reported in a somatic context.⁶⁻⁹ Thus, the present article reports the novel *NKX2-5* germline mutation Pro141Ala that was found, for the first time in association with transitional atrio-ventricular septal defect (tAVSD).

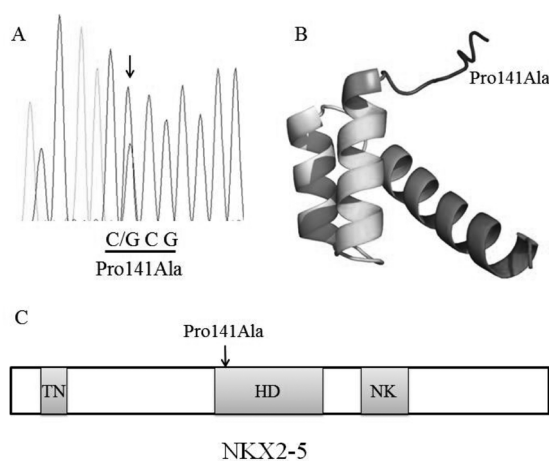


Fig. 2. Sequencing chromatogram of the detected *NKX2-5* mutation c.421C>G A) Three-dimensional (3D) model of *NKX2-5* homeodomain according to Phyre2, B) Organization of *NKX2-5* domains with the detected mutation site; C) HD: homeodomain, TN: Tinman domain.

H.sapiens	141	PRVLFSQAQVYELEERRFKQORYLSAPERDQLASVLKLTSTQVKIWFQNR	190	NP_004378.1
P.troglodytes	141	PRVLFSQAQVYELEERRFKQORYLSAPERDQLASVLKLTSTQVKIWFQNR	190	XP_518104.2
M.mulatta	141	PRVLFSQAQVYELEERRFKQORYLSAPERDQLASVLKLTSTQVKIWFQNR	190	XP_001096796.1
C.lupus	140	PRVLFSQAQVYELEERRFKQORYLSAPERDQLASVLKLTSTQVKIWFQNR	189	NP_001010959.1
B.taurus	140	PRVLFSQAQVYELEERRFKQORYLSAPERDQLASVLKLTSTQVKIWFQNR	189	NP_001039908.1
M.musculus	140	PRVLFSQAQVYELEERRFKQORYLSAPERDQLASVLKLTSTQVKIWFQNR	189	NP_032726.1
R.norvegicus	140	PRVLFSQAQVYELEERRFKQORYLSAPERDQLASVLKLTSTQVKIWFQNR	189	NP_446103.1
G.gallus	122	PRVLFSQAQVYELEERRFKQKYLSPERDHLANVLKLTSTQVKIWFQNR	171	NP_990495.1
D.rerio	144	PRVLFSQAQVYELEERRFKQKYLSPERDHLANVLKLTSTQVKIWFQNR	193	NP_571496.1
X.tropicalis	128	PRVLFSQAQVYELEERRFKQKYLSPERDHLANVLKLTSTQVKIWFQNR	177	NP_001116891.1

Fig. 3. Multiple alignment of NKX2-5 protein sequences across species showing the Pro141 residue conservation among species.

Missense mutation c.421C>G, that causes the Pro141Ala amino acid change, is localized in the amino terminus of NKX2-5 DNA-binding homeodomain (HD). This 60-amino-acids domain plays a critical role in transactivation of the down-stream target genes through binding to their 5'TC/TAAGTG consensus binding sites.¹² Multiple alignments of NKX2-5 protein sequences across species exhibit the strong conservation of this residue through the different species (Figure 3), which suggests an important role in the homeodomain function.

Disease-causing potential of Pro141Ala mutation assessed through different functional consequence prediction tools shows that this mutation is predicted to have a disease-causing effect according to Mutation Taster, probably-damaging effect according to PolyPhen, and deleterious effect on protein function according to SIFT prediction tool.

The pathogenicity of this mutation could be explained by the particular importance of codon Pro141. Indeed, this residue lies particularly within the NKX2-5 nuclear localization signal (NLS) sequence, which is a short consensus sequence at the homeobox amino terminus that is crucial for transporting the protein to the nuclear pore. Therefore, mutations affecting this sequence disrupt the nuclear translocation mechanism, and thus reduce the NKX2-5 transcriptional activity.¹³

Pro141Ala is a novel mutation that was not reported in any published work. Moreover, this pathogenic mutation is not mentioned in the well-known human mutation databases, such as NCBI SNP database (dbSNP) at <http://www.ncbi.nlm.nih.gov/snp>, 1000 genomes at <http://browser.1000genomes.org>, Exome

Aggregation Consortium (ExAC) at <http://exac.broadinstitute.org>, Human Genome Database (HGMD) at <http://www.hgmd.cf.ac.uk>, and NHLBI GO Exome Sequencing Project (ESP) at <http://evs.gs.washington.edu/EVS>.

Taken together, these findings suggest that Pro141Ala is a novel pathogenic mutation that seems to be responsible of the transitional AVSD observed in our proband.

It would have been more interesting if we had screened the parents and siblings' DNA to find out whether the mutation is sporadic or familial, as well as its segregation mode, but unfortunately, parental and sibling DNA samples were not available.

In conclusion, our report emphasizes particularly the importance of nuclear localization system in NKX2-5 activity and provides further evidence that NKX2-5 homeodomain alteration lead to an aberrant cardiac development. On the other hand, the reported transitional AVSD case comes to widen the spectrum of congenital heart disorders caused by NKX2-5 germline mutations.

Finally, in order to deepen the understanding of the molecular mechanism through which the mutation causes the observed phenotype, further functional studies of Pro141Ala would be of great interest.

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