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Homozygous variants in the GDF1 gene related to recurrent right isomerism and complex CHD in two Indian families

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Brief Report

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Abstract

Disorders of laterality are often associated with complex CHD. There is considerable debate about the appropriate terminology to describe these conditions. As our understanding of the genetic basis of these disorders improves, it is likely that terminology will be dictated by the genetic aetiology. The genetic basis of laterality disorders in the Indian population has not been studied. We report two families with autosomal recessive inheritance of isomerism and homozygous variants in the GDF1 gene in affected family members.

The disorders of abnormal lateralisation of bodily organs represent a diverse and fascinating subset among hearts with complex congenital morphologies. These disorders are characterised by isomerism of the atrial appendages and are termed left and right isomerism. They have also been referred to by the vague terminologies 'heterotaxy' and 'situs ambiguous' in the past.[1] Controversies regarding the appropriate nomenclature of these disorders continue to this day.[2,3] Our expanding knowledge on the genetics of these conditions are likely to provide clues regarding the embryological origins of these diseases. A vast majority of these disorders or sporadic but Mendelian inheritance has been noted in approximately 10% of individuals.[4] All forms of Mendelian inheritance including autosomal dominant with variable penetrance, autosomal recessive, and X-linked inheritance have been reported and several genes have been previously implicated.[5] The most well characterised of these genes is the zinc finger protein of the cerebellum 3 (ZIC 3) which is implicated in most families with X-linked isomerism.[6] More recently, growth/differentiation factor 1 (GDF1) has been implicated in autosomal recessively inherited right isomerism.[7] There are no available reports on the genetic characterisation of these disorders in the Indian population. We report two consanguineous families in whom more than one member was diagnosed with a complex CHD. Homozyogous loss of function variants in the GDF1 gene were identified in the affected children from both families.

Family 1

The proband was referred to us for cardiac evaluation at 4 months of age due to cyanosis. He was the second child of second-degree consanguineous parents (Fig 1a). His oxygen saturation was 75% in room air. The cardiac impulse was palpable in the right side of the chest. The second heart sound was single, and an ejection murmur was heard at the base of the heart. His liver was in the midline. The spleen could not be imaged. The gall bladder was in the midline. The cardiac mass was in the right side of the chest with the apex pointing to the right. The superior and inferior caval veins drained into the right-sided atrium. The pulmonary veins appeared to come together behind the atrial mass and then drain into the left-sided atrium in an unobstructed fashion. There was a large primary atrial septal defect. There was biatrial but univentricular atrioventricular communication to a dominant ventricle through a common atrioventricular valve. The dominant ventricle appeared to be a morphological left ventricle. The atrio-ventricular valve was competent. A small and incomplete right ventricle was imaged anterior and to the left of the dominant ventricle. There were discordant ventriculo-arterial connections. The aorta arose from the incomplete right ventricle and was located anterior and to the left of the pulmonary trunk. The pulmonary trunk arose from the dominant ventricle. There was severe pulmonary outflow obstruction.

His elder sister had been detected to have a congenital cyanotic heart disease and underwent superior cavo-pulmonary anastomosis at 9 months of age. Her subsequent clinical course had been hampered by an arterial ischaemic stroke involving the right middle cerebral artery at 2 years of age. Her echocardiogram revealed the same morphology identified in her sibling.

An autosomal recessive inheritance of right isomerism was suspected from the pedigree. Blood samples were obtained from the siblings as well as the mother. The father's sample could not be obtained. Whole-exome sequencing of the isolated DNA was performed using Novaseq 6000 sequencer (Illumina Truseq exome enrichment kit). This revealed a homozygous nonsense

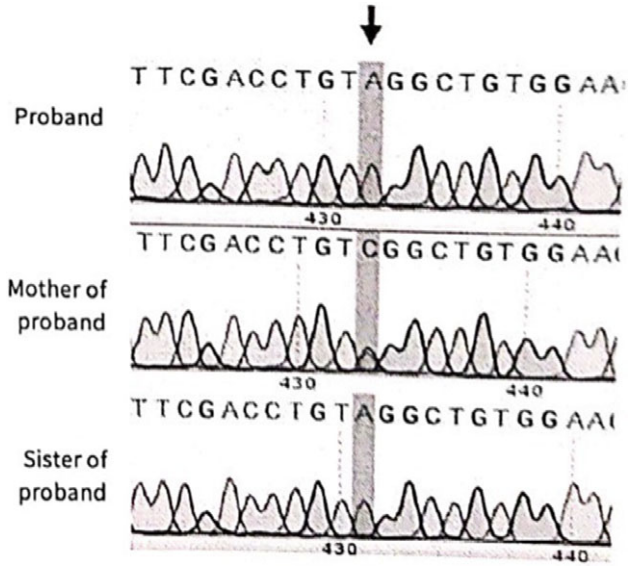

Figure 1. The chromatogram depicting capillary sequencing results for the variant in GDF1 detected in the proband of Family 1 as well as his sister and mother.

variant in the GDF1 gene (c.401C>A; p.S134X) resulting in premature truncation of the protein. This was subsequently confirmed by Sanger sequencing (Fig 1). The variant was not found in publicly available population datasets and in silico prediction algorithms suggested a deleterious effect. The variant was detected in a homozygous state in the sibling and in a heterozygous state in the mother.

Family 2

A second-degree consanguineous couple was referred to us for cardiac evaluation at 13 weeks' gestational age. Their first pregnancy had been terminated at 20 weeks' gestational age due to a CHD detected in the fetus which had been termed as single ventricle with pulmonic stenosis. A. detailed fetal cardiac and abdominal evaluation had not been performed, and no genetic testing or tissue sampling had been offered. The fetal echocardiogram in the current pregnancy demonstrated a midline liver with the stomach on the left side of the abdomen. The heart was in the right side of the chest cavity with the apex pointing to the right. A left-sided superior caval vein and the inferior caval vein drained into the left-sided atrium. The pulmonary veins appeared to form a confluence behind the heart before draining into the right-sided atrium. There was a large primary atrial septal defect. There was univentricular but biatrial atrio-ventricular communication through a common atrioventricular valve. A large dominant ventricle was imaged, but an additional incomplete ventricle could not be adequately imaged because of the early gestational age. There was pulmonary atresia. A large aortic trunk was seen arising from the ventricular mass. The aortic arch was left-sided with normal branching pattern. A vertical arterial duct provided blood flow to the pulmonary artery branches. As the previous pregnancy was also complicated by a similar heart disease and the findings were consistent with right isomerism, an autosomal recessive inheritance of right isomerism was considered.

The mother underwent amniocentesis, and the amniotic fluid was subjected to isolation of the fetal DNA. The isolated DNA was then sequenced. Analysis revealed a homozygous missense variant (c 952G>A; p. Ala318Thr) in the GDF1 gene (Fig 2). The alanine residue was noted to be conserved across species. The in silico prediction algorithms suggested a deleterious effect, and the variant was classified as likely pathogenic in the ClinVar database based on a single entry. Subsequent Sanger sequencing of the parents' blood detected the variant in a heterozygous state in both the parents.

Discussion

Isomerism of the right atrial appendages (more commonly referred as right isomerism) is a disorder of abnormal left–right pattern development in early fetal life. The condition is associated with complex congenital heart anomalies which in most cases require staged single-ventricle palliation. The embryonic origins of these disorders are still being understood.

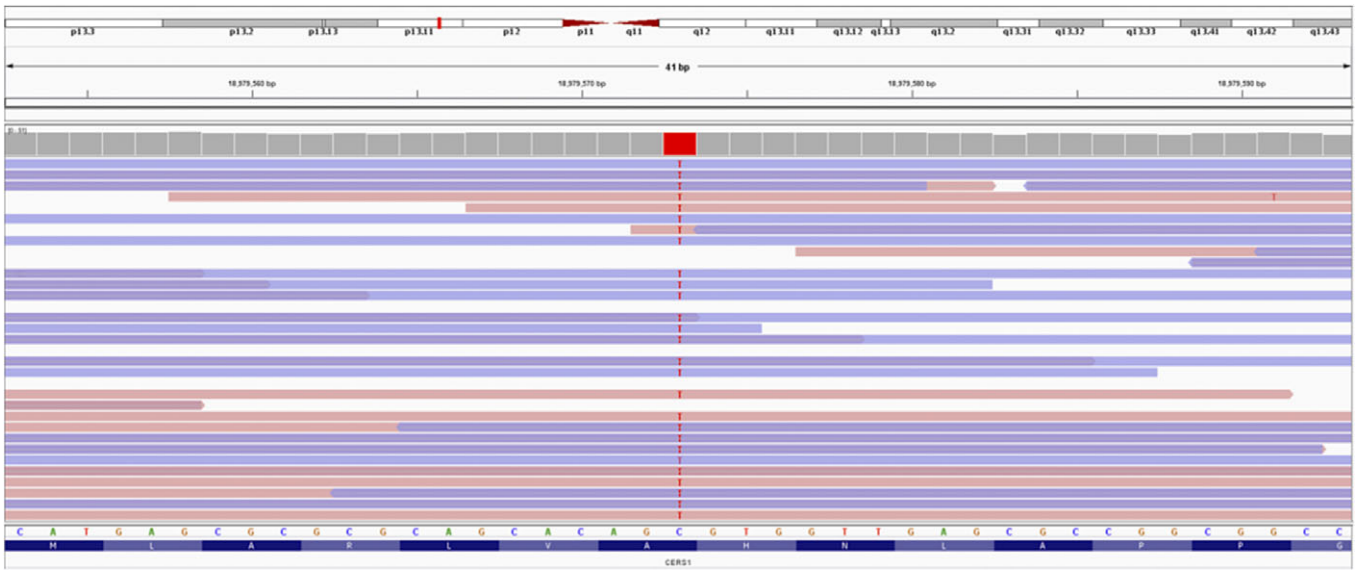

Figure 2. Integrative Genomic Viewer (IGV) snapshot depicting the homozygous variant in the GDF1 gene detected in the proband of Family 2.

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The transforming growth factor-beta (TGF-β) family of proteins play a key role in the laterality of structures in early embryonic life.[7] Nodal, a. member of the TGF-β family is vital for left-sided development. Expression of Nodal in the lateral plate mesoderm is a key step in left–right patterning.[8] GDF-1 is believed to be a key co-factor for Nodal expression in the lateral plate mesoderm. Mice embryos with biallelic GDF-1 knockout have been shown to have the absence of Nodal expression in the lateral plate mesoderm as well as right pulmonary isomerism and CHD.[9] Polymorphisms in the GDF 1 gene have been considered to increase the risk of CHD and heterozygous variants in GDF-1 have been linked to less complex heart diseases, although this association is being challenged.[10]

Biallelic loss of function variants in GDF1 was identified and considered causative in a Finnish non-consanguineous family in which five of seven children died due to right isomerism and complex CHD.[7] Each of the deceased children were identified to be compound heterozygotes for two mutations in GDF1. One variant was identified in a heterozygous state in each of the parents, and both the healthy siblings did not carry any of the variants.

In a large single-cohort study on the genetics of congenital heart defects by massive parallel sequencing, a single homozygous variant in GDF1 was identified in 10 apparently unrelated Ashkenazim with isomerism. Genetic linkage studies suggested a founder effect for this mutation from a common ancestor.[11] A similar founder effect was identified in three unrelated Arab families with right isomerism, pulmonary stenosis/atresia, and parallel great arterial trunks who shared a homozygous truncating variant in GDF 1.[10] However, in another large cohort of 323 patients with laterality defects who underwent massive parallel sequencing of all protein coding regions, heterozygous or homozygous variants in GDF1 were not identified in any patient.[12]

There is expanding evidence about the causative role of homozygous or compound heterozygous variants in the GDF1 gene in families with autosomal recessively inherited right isomerism. Our report suggests that biallelic loss of function of GDF 1 could potentially be implicated in Indian families with right isomerism and autosomal recessive inheritance.

Data availability statement. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Author contributions. SUN and MRK were involved in the clinical management of both the patients, reviewed the literature, and prepared the manuscript. MRK will act as the guarantor for the manuscript.

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Conflict of interest. The authors report no conflict of interest with regard to this work.

Ethical standards. This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained.

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