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Original Article

Clinical and molecular characterisation of Holt–Oram syndrome focusing on cardiac manifestations

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Abstract *Background:* Holt–Oram syndrome is characterised by CHD and limb anomalies. Mutations in *TBX5* gene, encoding the T-box transcription factor, are responsible for the development of Holt–Oram syndrome, but such mutations are variably detected in 30–75% of patients. *Methods:* Clinically diagnosed eight Holt–Oram syndrome patients from six families were evaluated the clinical characteristics, focusing on the cardiac manifestations, in particular, and molecular aetiologies. In addition to the investigation of the mutation of *TBX5*, *SALL4, NKX2.5,* and *GATA4* genes, which are known to regulate cardiac development by physically and functionally interacting with *TBX5*, were also analyzed. Multiple ligation-dependent probe amplification analysis was performed to detect exonic deletion and duplication mutations in these genes. *Results:* All included patients showed cardiac septal defects and upper-limb anomalies. Of the eight patients, seven underwent cardiac surgery, and four suffered from conduction abnormalities such as severe sinus bradycardia and complete atrioventricular block. Although our patients showed typical clinical findings of Holt–Oram syndrome, only three distinct *TBX5* mutations were detected in three families: one nonsense, one splicing, and one missense mutation. No new mutations were identified by testing *SALL4, NKX2.5,* and *GATA4* genes. *Conclusions:* All Holt–Oram syndrome patients in this study showed cardiac septal anomalies. Half of them showed *TBX5* gene mutations. To understand the genetic causes for inherited CHD such as Holt–Oram syndrome is helpful to take care of the patients and their families. Further efforts with large-scale genomic research are required to identify genes responsible for cardiac manifestations or genotype–phenotype relation in Holt–Oram syndrome.

Keywords: Holt–Oram syndrome; CHD and skeletal malformations; cardiac arrhythmia; genetic loci

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CHD IS ONE OF THE MOST COMMON BIRTH anomalies, detected in 4–50 of every 1000 live births, and genetic factors have been suggested to underlie its development.[1–3] Recent advances in genetics have led to the identification of several disease-causing genes in syndromic disorders associated with CHD.[4] Holt–Oram syndrome (HOS; OMIM142900) is a prototype of CHD and caused by mutations in the *TBX5* gene. *TBX5* is a transcriptional factor involved in heart and limb development.[5–7] Germline *TBX5* mutations have been identified in 30–75% of Holt–Oram syndrome patients.[8–10]

Holt–Oram syndrome is characterised by cardiac and upper- limb anomalies. It is a very rare condition, affecting 1 in 100,000 live births and inherited in an autosomal dominant manner.[5,8,10–12] Its phenotypic penetrance and expressivity have been known to vary among patients. However, upper-limb malformations are almost always present, and the preaxial radial ray distributed area including the radial, carpal, or thenar bones are mainly affected.[9,12]

The prevalence of CHD in Holt–Oram syndrome patients has been reported to range from 75 to 95%,

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with atrial septal defect and ventricular septal defect being the most common CHDs in such patients. Some patients also develop cardiac rhythm disturbances such as sinus bradycardia or various degrees of atrioventricular conduction abnormalities.[6,12–14]

In our current study, we describe the clinical characteristics of eight patients with Holt–Oram syndrome from six families focusing on cardiac manifestations. Germline *TBX5* mutations were screened in all patients, and the *SALL4* gene was analysed in patients without a *TBX5* mutation to rule out Okihiro syndrome, which shows similar radial ray defects to Holt–Oram syndrome.[15] Furthermore, given that *TBX5* is involved in cardiac septal formation by interacting with other cardiac transcriptional genes, such as *NKX2.5* or *GATA4*, we also investigated these two genes as potential new genetic causes of Holt–Oram syndrome.[16–20]

Patients and methods

A total of eight patients with Holt–Oram syndrome from six unrelated Korean families were included. We reviewed the clinical findings of each patient: demographic data, structural cardiac anomalies, electrocardiographic findings, skeletal anomalies, and surgical history. The clinical diagnosis of Holt–Oram syndrome was based on clinical or radiographic evidence of radial ray defect with associated cardiac anomalies. This study was approved by the Institutional Review Board of the Asan Medical Center, Seoul, Korea.

Molecular characterisation

Genetic analysis was performed using genomic DNA from peripheral blood using the Quick Gene Blood kit (Fujifilm, Tokyo, Japan). A total of nine exons of the *TBX5* gene and their respective exon–intron boundaries were evaluated. The *SALL4* gene was analysed in patients without a *TBX5* mutation to rule out Okihiro syndrome. Two candidate genes, *NKX2.5* and *GATA4*, were also evaluated. The primers of each gene were designed based on Primer3web (version 4.0.0; http://primer3.wi.mit.edu) of the Whitehead Institute: *TBX5* (GenBank accession number: NT_009775.14.), *NKX2.5* (NM_004387), *GATA4* (NM_002052), and *SALL4* (NT_011362.10) (Supplementary table S1). DNA sequencing was performed using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, United States of America). Multiple ligation-dependent probe amplification analysis was also performed for the detection of deletions and duplications in the *TBX5*, *NKX2.5*, *GATA4*, and *SALL4* genes. A multiple ligation-dependent probe amplification probe mix (P180/P311, MRC, Amsterdam, The Netherlands) was used according to the manufacturer's instructions.

Results

Patient characteristics

A total of eight patients, including three men and five women, were clinically diagnosed with Holt–Oram syndrome. Of the patients, four – patients 1, 2, 3, and 4 – had familial cases of Holt–Oram syndrome. In two patients, patients 5 and 6, Holt–Oram syndrome was suspected during the foetal period by abnormal foetal sonographic findings including ventricular septal defect and radial agenesis. Another four patients – patients 1, 3, 7, and 8 – were diagnosed postnatally because of cardiac and/or limb anomalies. Patient 2, father of patient 1, and patient 4, a daughter of patient 3, were identified by familial screening (Table 1). All patients had cardiac malformations: partial atrioventricular septal defect in one patient, combined ventricular septal defect and atrial septal defect in five patients, ventricular septal defect in one patient, and secundum atrial septal defect in one patient. The type of ventricular septal defect was perimembranous (four patients), muscular (one patient), or perimembranous combined with multiple muscular (one patient). The type of atrial septal defect was secundum in all patients. Combined limb anomalies were isolated thumb anomalies or various combined lesions including triphalangeal thumb (one patient), thumb hypoplasia (four patients), thumb aplasia (one patient), first to second digits syndactyly (one patient), complete radial aplasia (two patients), radial hypoplasia (one patient), metacarpal bone hypoplasia (one patient), or aplasia (one patient). These anomalies were bilateral in three patients (Fig 1).

Clinical outcomes of cardiac manifestations

The total follow-up period was 8.2 ± 5.4 (range 2.7–14.9) years. Of the patients, seven underwent cardiac surgery at the average age of 4.0 ± 4.7 (range 0.2–11.0) years. Early surgical intervention was required in three patients (patients 1, 5, and 8) owing to severe pulmonary hypertension accompanying ventricular septal defect and atrial septal defect. Of them, one patient (patient 5) underwent pulmonary artery banding as staged palliation owing to multiple apical muscular ventricular septal defects and poorly formed apical septum. The other patients underwent one-stage total correction and there were no residual shunt or significant valve regurgitation in these patients during follow-up.

In terms of arrhythmias, patient 3 showed sinus node dysfunction with severe sinus bradycardia after

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Table 1. Clinical characteristics of the study patients.

Family number	Patient number	Gender	Cardiac anomaly	Cardiac operation	Age at operation	Associated cardiac arrhythmias	Limb anomaly
1	1	F	pmVSD, multiple ASD, PDA, PHT	VSD, ASD patch closure, PDA ligation	2 months	None	Lt. triphalangeal thumb
	2	M	pmVSD	Patch closure	11 years	None	Both thumb hypoplasia
2	3	F	Secundum ASD	Patch closure	9 years	Severe Sinus bradycardia	Lt. thumb hypoplastic
	4	F	pmVSD, secumdum ASD	VSD, ASD patch closure	1 year	CAVB → PPM insertion	Lt. thumb hypoplasia
3	5	M	Large pmVSD multiple muscular VSD, secundum ASD PHT	PAB	12 days	None	Rt. metacarpal bone hypoplasia, Rt. hypoplastic thumb
4	6	M	multiple muscular VSD secundum ASD, PDA	None	–	None	Rt. radial aplasia, Rt.thumb hypoplasia
5	7	F	pAVSD, secundum ASD	Repair with single patch technique	22 months	CAVB → PPM insertion	Lt. radial apalsaia, both metacarpal bone aplasia
6	8	F	Large pmVSD Secundum ASD bilateral SVC, PHT	VSD, ASD patch closure	2 months	CAVB → PPM insertion	Both radial and first metacarpal bone hypoplasia club hands, both thumb agenesis, Lt. syndactyly

ASD = atrial septal defect; CAVB = complete atriaventricular block; Lt = left; PAB = pulmonary artery banding; pAVSD = partial atrioventricular septal defect; PDA = patent ductus arteriosus; PHT = pulmonary hypertension; pmVSD = perimembranous ventricular septal defect; PPM = permanent pacemaker; Rt = right; SVC = superior vena cava

atrial septal defect operation. Initially it was mild and not symptomatic. However, after 10 years of operation, she intermittently experienced fatigue and dizziness. On routine regular health examination, severe bradycardia was detected and she was recommended to insert permanent pacemaker. It brought her to our hospital to further evaluation. Her treadmill exercise test showed no significant abnormality, and holter monitoring showed sinus bradycardia with intermittent premature atrial complex. Therefore, we decided to just observe and regularly follow-up. Her daughter (patient 4) also experienced arrhythmias after ventricular septal defect and atrial septal defect patch closure. In her case, complete atrioventricular block was developed postoperatively and required permanent pacemaker insertion. Complete atrioventricular block was noted in patient 8 postoperatively, which was thought as complication after large perimembranous ventricular septal defect patch closure operation. Preoperatively, she suffered from significant congestive heart failure requiring mechanical ventilation. She underwent total correction on day 67 with body weight of 3.5 kg at the operation. She performed permanent pacemaker insertion 24 days after total correction. Patient 7 also showed complete atrioventricular block after operation. During immediate postoperative periods, she had normal sinus rhythm with intermittent first-degree atrioventricular block. However, it progressed to 2:1 atrioventricular block with heart rate of 110 beats/minute at the time of discharge. On postoperative follow-up, it progressed to complete atrioventricular block that she underwent permanent pacemaker insertion on postoperative day 48.

Molecular characterisation of candidate genes for Holt–Oram syndrome

There were three different *TBX5* mutations detected in three of the six families (50%): c.709C > T (p.Arg237Trp), c.880G > T (p.Glu294X), and c.755 + 2T > C (IVS7(+2)T > C). In the three patients with no *TBX5* mutations, whole exons and their exon–intron boundaries of the *SALL4* genes were investigated, but no mutations were found. Mutation analyses of *NKX2.5* and *GATA4* genes were negative (Table 2). Multiple ligation-dependent probe amplification detected no significant deletions or duplications in the *TBX5*, *NKX2.5*, *GATA4*, and *SALL4* genes.

Discussion

Holt–Oram syndrome is characterised by distinctive cardiac and upper-limb skeletal abnormalities.

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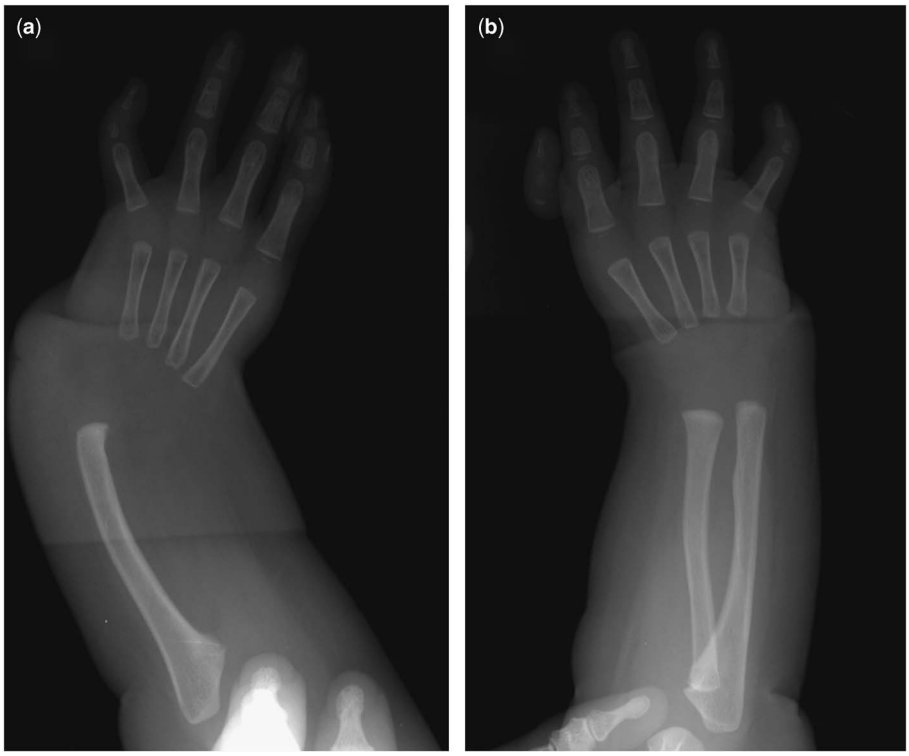

Figure 1.
*Plain X-ray findings for patient 7. (**a**) Radial aplasia, first metacarpal bone aplasia, and thumb hypoplasia in the left forearm. (**b**) Aplasia of the right first metacarpal bone.*

Table 2. Molecular characterisation of Holt–Oram syndrome candidate genes using direct sequencing and multiple ligation-dependent probe amplification.

Family number	Patient number	*TBX5*	*SALL4*	*NKX2.5*	*GATA4*
1	1	c.880G > T (p.Glu294X)			
	2 (Patient's father)	c.880G > T (p.Glu294X)			
2	3	c.755 + 2T > C (IVS7(+2)T > C)			
	4 (Patient's first baby)	(c.755 + 2T > C (IVS7(+2)T > C))			
3	5	Normal*	Normal*	Normal*	Normal*
4	6	Normal*	Normal*	Normal*	Normal*
5	7	c.709C > T (p.Arg237Trp)			
6	8	Normal*	Normal*	Normal*	Normal*

*Normal, no exonic, and intron–exon boundary sequence variant, deletion, or duplication was found

Although various cardiac anomalies such as isolated left atrial isomerism, inferior caval vein interruption with hemiazygous continuation to the left superior vena cava, and agenesis of pericardium and hypoplastic left heart syndrome have been reported in association with Holt–Oram syndrome. Holt–Oram syndrome most commonly presents as atrial septal defect or ventricular septal defect.[5,14,21] In our present study, all eight patients showed cardiac septal defects. Various electrocardiographic abnormalities have also been reported in Holt–Oram syndrome patients with a wide range in incidence (18–71%). An abnormal electrocardiographic finding with no structural cardiac anomaly has been found in some patients (39%),[13,22] whereas others present with severe atrioventricular block requiring permanent pacemakers.[5,14,23] Half of the patients (four cases) experienced conduction abnormalities after cardiac surgery, and three of them ended up with performing permanent pacemaker insertion, which indicates the importance of careful evaluation and management of cardiac arrhythmias during follow-up.

The cardinal manifestations of limb anomalies accompanied by Holt–Oram syndrome are preaxial

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radial ray abnormalities without ulnar ray anomalies, as was noted in all of the patients in our present series. Some patients with *SALL4*-related disorders including Okihiro syndrome can also present with radial ray malformations. Townes–Brocks syndrome shares an overlapping phenotype with Holt–Oram syndrome, but the former condition is caused by *SALL1* mutations. However, extra-cardiac or limb anomalies, including in the eye, kidney, and anus, are more common in *SALL4*-related disorders and Townes–Brocks syndrome than in Holt–Oram syndrome, which are important clues in the differential diagnosis.[15,24] On the basis of these characteristics, all of our patients were diagnosed with Holt–Oram syndrome.

The mutation detection rate of the *TBX5* gene in Holt–Oram syndrome patients is known to vary from 30 to 75%.[25] In the present analysis, the positive mutation rate was 50%. Of the three mutations, c.709C > T (p.Arg237Trp) and c.755 + 2T > C (IVS7 (+2)T > C) are those previously reported[6,25,26] whereas c880G > T (p.Glu294X) is the novel mutation that we detected in the family 1 (patients 1 and 2).[27] Large exonic deletions in *TBX5* have also been found in cases of Holt–Oram syndrome,[9,25] but such deletions were not detected in our patients.

The variable mutation detection rate of the *TBX5* gene among studies may be explained by the wide spectrum of clinical manifestations among patients with Holt–Oram syndrome. It can be argued that applying strict diagnostic criteria, including personal and/or family history of cardiac septal defect and/or conduction defects in the presence of preaxial radial ray deformity, can improve the detection rate of genetic causes of Holt–Oram syndrome.[9] However, in this study, all patients fulfilled strict diagnostic criteria, but the mutation detection rate was only 50%.

There is much controversy regarding the genotype–phenotype correlation of Holt-Oram syndrome. Basson et al[6] suggested that the location of mutations in the *TBX5* gene has an impact on the type of anomaly, that is, cardiac or limb, but this correlation was opposed by other groups. Owing to the small number of patients included in our study, evaluation of genotype–phenotype correlations was limited. Considering the three mutations identified in our study, c.880G > T (p.Glu294X), c.755 + 2T > C (IVS7(+2)T > C), and c.709C > T (p.Arg237Trp), previous studies showed that most patients with p.Arg237Trp have limb anomalies, whereas only half have cardiac anomalies;[6] in contrast, our patient harbouring p.Arg237Trp had both cardiac and limb anomalies. In addition, we were unable to identify any significant differences in phenotypic expressivity between patients with and without *TBX5* mutations.

Discovering genetic factors underlying cardiac manifestations in Holt–Oram syndrome is important not only to better understand the molecular pathology of cardiac lesion but also to help families affected by Holt–Oram syndrome, by providing them with genetically appropriate counselling and care. Genetic screening is an effective tool to identify affected family members who have no obvious manifestations of Holt–Oram syndrome. Moreover, owing to the dominant inheritance pattern of Holt–Oram syndrome, prenatal genetic counselling, based on identification of familial mutations, can be an important issue in preparing perinatal care and family planning. Therefore, further efforts are needed to identify new causative genes or genetic influences.

To increase the mutation detection rate, we screened for *SALL4* point and exonic deletion mutations, which are found in Okihiro syndrome as well as in rare cases of Holt–Oram syndrome.[15,28] However, no *SALL4* mutations were identified. The interaction of *TBX5* with other transcription factors such as *NKX2.5*, *GATA4*, *TBX20*, *TBX1*, and *MYH6* may affect cardiac embryogenesis in Holt–Oram syndrome patients.[19,29–31] Screening for interacting candidate genes may help to identify new causative genes of cardiac manifestations in Holt–Oram syndrome. We therefore screened *NKX2.5* and *GATA4*, which were known to interact with *TBX5* during cardiac septal formation, myocardial patterning, and chamber specification, in an attempt to identify new disease-causing genes in Holt–Oram syndrome, but found no positive results. Furthermore, multiple ligation-dependent probe amplification analysis did not reveal any exonic deletion or duplication in these candidate genes. It might be because of the small number of affected patients in this study.

Conclusions

Most commonly detected cardiac anomalies in Holt–Oram syndrome were cardiac septal defects. Our present data indicate the importance of careful management and monitoring for CHDs in patients with Holt–Oram syndrome, especially in those with cardiac conduction abnormalities. Although our patients showed typical clinical findings and underwent *TBX5* and other candidate gene screening, half of all Holt–Oram syndrome patients remain genetically undiagnosed. Further large-scale genomic research is needed to uncover new genetic causes and genotype correlation with cardiac manifestations in patients with Holt–Oram syndrome and understand the genetic heterogeneity of this disease.

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Conflicts of Interest

None.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1047951114001656

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