

## Holt-Oram syndrome in two generations with translocation t(9;15)(p12;q11.2)

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**H**olt-Oram syndrome (HOS), first described by Holt and Oram in 1960,<sup>1</sup> is transmitted in an autosomal dominant mode of inheritance that is highly penetrant, with variable expression and characterized by upper limb anomalies that are always present, including mainly preaxial ray and congenital heart defects and/or cardiac conduction anomalies (OMIM 142900). The clinical manifestations vary and range from subclinical radiographic findings to overt, life-threatening disease. HOS occurs in approximately 1:100 000 live births; 85 percent of cases are attributed to new mutations in the *TBX5* gene.<sup>2</sup>

The upper limb anomalies may be unilateral or bilateral and involve structures derived from the embryonic radial ray, typically the radial, carpal, and thenar bones. Aplasia, hypoplasia, fusion, and anomalous development of these structures produce a wide spectrum of phenotypes, including triphalangeal or absent thumbs, foreshortened arms, and phocomelia. They affect preferentially the left rather than the right side without affecting the lower limbs at any time.<sup>3</sup>

The formation of a limb involves numerous genes.<sup>3</sup> Between the fourth and sixth weeks of fetal development primary limb and heart differentiation occurs. In this condition, skeletal anomalies always affect the upper limbs. The lower limbs are not affected in HOS. The appearance of the upper limb buds before the lower limb buds may explain the preferential involvement of the upper limbs seen in this syndrome.<sup>4</sup>

In the majority of cases, cardiac defects like atrial septal defects or ventricular septal defects are found. More complex cardiovascular abnormalities such as tetralogy of Fallot or isolated pulmonary arterial hypoplasia are rare.<sup>1,5-9</sup> The gene locus for HOS has been mapped to chromosome 12q24,<sup>1</sup> and mutations of the *TBX5* gene have been identified as the underlying gene defect. *TBX5* is a member of the T-box transcription factor family and members of the T-box family of transcription factors regulate a variety of developmental

processes in vertebrates and invertebrates, including specification of mesoderm, development of the heart, vasculature, and limbs and tumorigenesis.<sup>10-12</sup> *TBX5* is expressed in the embryonic heart and forelimbs and regulates transcription of downstream genes, such as those encoding atrial natriuretic factor and fibroblast growth factor 10 (*FGF10*) by binding to *TBX*-binding DNA elements.<sup>13-18</sup> Various mutations in this gene cause HOS, with the described abnormalities in hand and heart development. Different mutations, each introducing a premature stop codon, have been identified, but no correlation of position of mutation and its phenotype has been observed.<sup>4,19-21</sup>

We report the case of an infant with upper limb anomalies and total anomalous venous return. The father and two brothers of the infant have the same upper limb anomaly but not the cardiac defect. The karyotype analysis revealed translocation 46,XX, t(9;15)(p12;q11.2). To our knowledge, this is the first case of translocation between chromosomes 9 and 15. This strongly suggests a new "heart-hand" locus on one of these chromosome. To our knowledge, translocation 46,XX, t(9;15)(p12;q11.2) has not been previously reported in HOS in the English literature.

### CASE

A four-day old female infant was referred to us as a case of HOS because of respiratory distress and an upper limb anomaly (Figure 1). She was born by cesarean delivery at 38 weeks gestation to a 37-year-old gravid a 3 para 1 mother. Her father and two of her brothers have upper limb anomalies (Figure 2). The parents were non-consanguineous (Figure 3). On physical examination the weight was 1890 g (<10th centile), the length was 44 cm (<10th centile), and head circumference was 32 cm (10th centile). She was noted to have a bilateral hypoplastic forearm, cyanosis, tachypnea and II/IV murmur. Echocardiography showed total anomalous venous return and an atrial septal defect (Figure 4). The variety

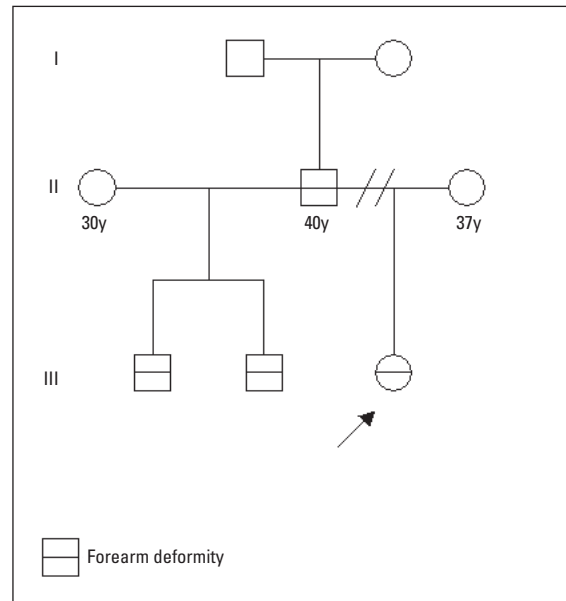


**Figure 1.** Frontal view of patient showing upper limbs and flexion deformity of the elbow.



**Figure 2.** Upper limbs of the father (top) and upper limb X-ray of the father (bottom) showing hypoplasia of the radius and flexion deformity of the elbow.

of the features led us to suspect a chromosomal aberration. The karyotype analysis of our patient (Figure 5), her father and two brothers revealed translocation  $t(9;15)(p12;q11.2)$ . The translocations were inherited



**Figure 3.** Family pedigree. The arrow indicates the index case.

from the father who carried the same chromosomal translocation as his children. The diagnosis of HOS was made on the basis of clinical findings consisting of cardiac and limb defects.

#### Cytogenetic studies

We studied 20 metaphases from peripheral blood lymphocytes from the proband, and all showed 46,XX der(9)  $t(9;15)$  (Figure 5), meaning that there was extra material on the p arm of chromosome 9 and a deletion on the q arm of chromosome 15. The karyotype of the father and the two brothers was performed on peripheral blood lymphocytes. A high resolution G-band showed a balanced translocation between chromosome 9 and 15 in all examined cells [46,XX der9  $t(9;15)(p12;q21.1)$ ] (Figure 5). The karyotype of the mother was normal.

#### DISCUSSION

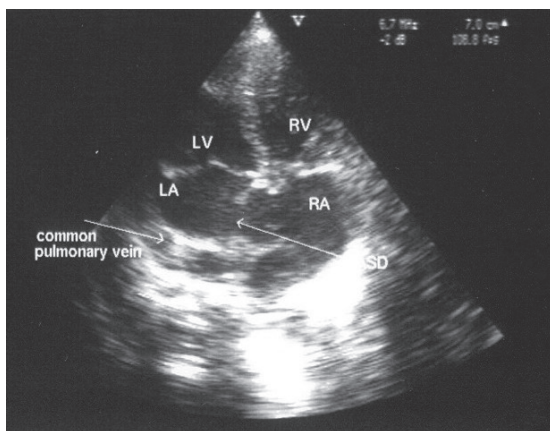
Limb and cardiac anomalies represent the main clinical features of some genetic conditions, such as Fanconi anemia syndrome, Okishiro syndrome, Tabatznik's syndrome, heart-hand syndrome type III, thrombocytopenia-absent radius syndrome and VACTERL (vertebral, anal, cardiac, tracheal, esophageal, renal, and limb) anomalies. Heart-hand syndromes are a broad category of disease, of which HOS is the most common form.

Congenital limb malformations occur in 1 in 500 to 1 in 1000 human live births and include both gross reduction defects and more subtle alterations in the number, length and anatomy of the digits. The major causes of limb malformations are abnormal genetic program-

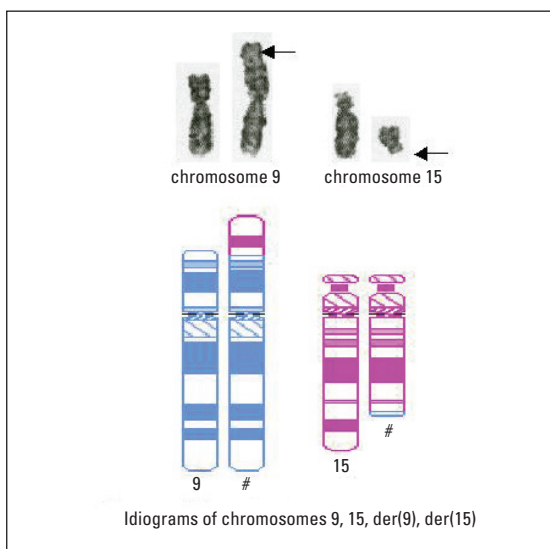
ming and intra-uterine disruption to development. Formation of the limbs was a relatively late refinement of vertebrate development and it is increasingly apparent that this has involved the co-option of existing molecular pathways.<sup>22,23</sup> It is not therefore surprising that many mutations that cause limb malformation also affect the development of other organ systems; this is termed pleiotropy.

The gene locus for HOS was mapped to chromosome 12q24.1. Mutations of the *TBX5* gene were delineated as the underlying gene defect for HOS. Indeed, *TBX5* gene pleiotropic effects on multiple organ systems are responsible for HOS. Although mutations in the *TBX5* (MIM#601620) gene have been found in 30% to 35% of familial and sporadic cases with HOS.<sup>24,25</sup> Recently, a detection rate of 74% was reported following application of more stringent clinical criteria for HOS. Neither the type nor the location of a mutation in *TBX5* appeared to predict type and/or severity of malformations in individuals with HOS.<sup>25</sup> Another possible reason for the low detection rate of *TBX5* mutations is that HOS is genetically heterogeneous. However, with the exception of a single HOS pedigree in which linkage to chromosome 12q24 was excluded,<sup>26</sup> there is little evidence that genetic heterogeneity among HOS cases is common. Alternatively, the low detection rate could also reflect that most cases of HOS have not been screened for large deletions or mutations in *TBX5* regulatory regions.<sup>27</sup> To our knowledge, only two studies to date have screened *TBX5* for deletions in HOS cases without *TBX5* sequence variants.<sup>28</sup> In the first one, multiplex amplifiable probe hybridization was applied to 20 patient samples and one deletion was detected that spanned exons 3 to 9. The other study detected intronic and submicroscopic deletions within *TBX5* via real-time polymerase chain reaction with Syber Green. However, such deletions explain only about 2% of the *TBX5* mutational spectrum in HOS cases.

Cardiac defects range from atrial septal defects or ventricular septal defects in the majority of cases, to more complex cardiovascular abnormalities such as tetralogy of Fallot and isolated pulmonary arterial hypoplasia. In an earlier series of studies, heart defects in 189 patients were classified by severity. Of these patients, only 6% had severe combinations with life-threatening defects, including hypoplastic left heart, total anomalous pulmonary venous return and truncus arteriosus.<sup>4,5,20,21</sup> Our patient had a bilateral hypoplastic forearm and total anomalous pulmonary venous return with  $t(9;15)(p12;q11.2)$  due to paternal inherited translocation. The identification of this chromosomal translocation could



**Figure 4.** ECHO appearance of cardiac anomalies, showing total anomalous pulmonary venous return and atrial septal defect.



**Figure 5.** Partial karyotype of the proband showing normal chromosome 9 and derivative chromosome 9. The extra segment on the 9p arm distal to the arrow has translocated from the 15q and attached to the 9p arm. The arrow points to the breakpoints and reunions.

not by itself explain the clinical findings, especially the heart defects of the proband. We did perform molecular analysis of *TBX5* gene responsible for HOS. Alteration of a single gene simultaneously involved in heart and limb development could explain the phenotype. Indeed, the association of congenital cardiac and upper-limb malformations in several Mendelian disorders has suggested the existence of a cardiomeelic developmental field.<sup>29</sup> This hypothesis is supported by the existence of several genes, such as *TBX5* and *GPC3* (involved in Simpson-Golabi-Behmel syndrome), which control both cardiac and limb development.

Therefore, our observation addresses the question of whether there is a "heart-hand" locus on chromosome 9 or 15, or is the finding incidental. These hypotheses are applicable to our case: (1) The chromosomal rearrangement could separate the promoter from a distant regulatory element; (2) the chromosomal rearrangement might juxtapose a gene with a regulatory element from another gene; (3) the chromosomal rearrangement might bring a gene and its regulatory element closer to another gene, generating competition for the regulatory element between the two genes; or (4) the rearrangement could give rise to position effect variegation, a phenomenon first described in *Drosophila*<sup>36</sup> and later reported in mammalian systems.<sup>30,31</sup>

We conclude that the existence of a new "heart-hand" locus can be suspected on chromosome 9 or 15. Although further cases of chromosomal translocations including chromosome 15 and 9 are certainly needed to refine the critical region, linkage to this region should be considered in HOS-like families without detectable

TBX5 mutations.

Because of the phenotypic variability of HOS, genetic testing will also be useful in guiding the clinical management of mildly affected individuals since even those without structural heart disease are at risk for cardiac conduction disease.<sup>32</sup> Furthermore, families with an identified TBX5 mutation can be counseled about reproductive options, including preimplantation genetic diagnosis, for future pregnancies.<sup>33</sup> For individuals not meeting clinical criteria for HOS, more appropriate genetic testing should be considered. Other disorders to be considered in individuals with limb and cardiac malformation include Okihiro syndrome, Ulnar-mammary syndrome, thrombocytopenia absent radius syndrome, and VACTERL association. Appropriate definition and diagnosis of the clinical features of heart-hand syndromes like HOS will allow us to better understand the role of their causative genes in development and ultimately will foster improved molecularly based therapeutic approaches for affected individuals.

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