



# Holt-Oram syndrome: clinical and molecular description of 78 patients with *TBX5* variants

Clémence Vanlerberghe<sup>1,2</sup> · Anne-Sophie Jourdain<sup>2,3</sup> · Jamal Ghoumid<sup>1,2</sup> · Frédéric Frenois<sup>2</sup> · Aurélie Mezel<sup>4</sup> · Guy Vaksman<sup>5</sup> · Bruno Lenne<sup>6</sup> · Bruno Delobel<sup>6</sup> · Nicole Porchet<sup>3</sup> · Valérie Cormier-Daire<sup>7</sup> · Thomas Smol<sup>1,2,8</sup> · Fabienne Escande<sup>2,3</sup> · Sylvie Manouvrier-Hanu<sup>1,2</sup> · Florence Petit<sup>1</sup>

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## Abstract

Holt-Oram syndrome (HOS) is an autosomal dominant condition characterised by the association of congenital heart defect (CHD), with or without rhythm disturbances and radial defects, due to *TBX5* variants. The diagnosis is challenged by the variability of expression and the large phenotypic overlap with other conditions, like Okihiro syndrome, TAR syndrome or Fanconi disease. We retrospectively reviewed 212 patients referred for suspicion of HOS between 2002 and 2014, who underwent *TBX5* screening. A *TBX5* variant has been identified in 78 patients, representing the largest molecular series ever described. In the cohort, 61 met the previously described diagnostic criteria and 17 have been considered with an uncertain HOS diagnosis. A CHD was present in 91% of the patients with a *TBX5* variant, atrial septal defects being the most common (61.5%). The genotype–phenotype study highlights the importance of some critical features in HOS: the septal characteristic of the CHD, the bilateral and asymmetric characteristics of the radial defect and the presence of shoulder or elbow mobility defect. Besides, 21 patients presented with an overlapping condition. Among them, 13 had a typical HOS presentation. We discuss the strategies that could be adopted to improve the molecular delineation of the remaining typical patients.

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✉ Clémence Vanlerberghe  
clemence.vanlerberghe@chru-lille.fr

- <sup>1</sup> CHU Lille, Clinique de Génétique, 59000 Lille, France
- <sup>2</sup> Univ. Lille, RADEME, EA 7364, 59000 Lille, France
- <sup>3</sup> CHU Lille, Institut de Biochimie et Génétique Moléculaire, 59000 Lille, France
- <sup>4</sup> CHU Lille, Unité de chirurgie orthopédique infantile, 59000 Lille, France
- <sup>5</sup> Hôpital privé de La Louvière, unité de cardiologie pédiatrique, Lille, France
- <sup>6</sup> Centre de Génétique Chromosomique, Hôpital Saint Vincent de Paul, Lille, France
- <sup>7</sup> Service de Génétique, U781, Fondation Imagine, Hôpital Necker-Enfants malades AP-HP, Paris, France
- <sup>8</sup> CHU Lille, Institut de Génétique Médicale, 59000 Lille, France

## Introduction

Congenital heart disease (CHD) is the most common congenital anomaly with an estimated prevalence of 9 per 1000 at birth [1]. Septal defects represent 47% of CHD [1]. Several genes contribute to heart development. Although *NKX2-5* and *GATA4* have been associated with isolated CHD, their partner *TBX5* is implicated in Holt-Oram syndrome (HOS) (MIM #142900). This autosomal dominant condition is characterised by the association of CHD and anterior upper limb defect, with a high penetrance and a wide variability of expression [2]. Its prevalence has been estimated at 1 in 100,000 births [3]. A CHD is present in 70–95% of the individuals according to the literature [4–10]. The most common are septal defects: *ostium secundum* atrial septal defect (44.4%) and ventricular septal defect (29.4%). More severe CHD have also been reported: atrioventricular septal defect, Fallot tetralogy, left ventricle hypoplasia, and aortic coarctation. Rhythm disturbances (39%) can be found isolated or associated with a CHD, and may appear over the course of life. The most common are sinus bradycardia and right bundle branch block [4, 6, 7, 9, 11]. Upper limb involvement is constant but very

variable. Its spectrum extends from an isolated thenar hypoplasia, a mobility defect affecting the thumb, elbow, or shoulder, up to phocomelia. The most common defects are thumb hypoplasia at various degrees or a triphalangeal/fingerlike thumb, associated or not with radial hypoplasia. A left-right asymmetry may be observed, with usually more severe defects on the left-hand side [4, 6]. No correlation between the severity of the skeletal and the cardiac defects has been observed [6].

Variants in the *TBX5* gene (chromosome 12q24) have been found to cause HOS in 1997 [12, 13]. *TBX5* encodes a transcription factor belonging to the T-box family, regulating a large variety of developmental processes in vertebrates [14]. During heart development, *Tbx5* expression appears uniformly at the early stages, then is left-biased as the heart tube loops, being totally absent from the right ventricle [15]. Its role is crucial for cardiomyocytes proliferation and differentiation, septum formation and the establishment of the conduction system (reviewed in the ref. [16]). During limb development, *Tbx5* is expressed in forelimbs and not in hindlimbs. Its action is essential at early stages of limb initiation (E9.5–E10.5) to trigger the epithelio-mesenchymal growth loop between *Fgf10* in the mesenchyme and *Fgf8* in the apical ectodermal ridge. At later stages (E11.2–E12.5), *Tbx5* is involved in muscle and tendon patterning and no longer needed for limb outgrowth [17].

About 300 HOS patients have been described in the literature. However, for most of them, the diagnosis was only established on clinical features [4–11]. The molecular confirmation of HOS diagnosis is crucial because of the cardiac monitoring required by this condition, and its impact on the genetic counselling. The detection yield of *TBX5* variants in HOS patients ranges from 22 to 74% depending on the studies [8, 11, 18–20]. The diagnostic criteria were consistent in most studies (uni or bilateral anterior upper limb defect, associated with a personal or familial history of CHD or rhythm disturbances). Mc Dermott et al. showed that the variant detection rate increases to 74% when the absence of associated features (except for spinal deformities) was added to the previous criteria. Of note, the molecular analysis methods were heterogeneous in the reported series of the literature and none of them included intragenic rearrangements screening.

Here, we report on the clinical and molecular characterisation of 78 unrelated HOS individuals harbouring a *TBX5* variant, allowing the description of the largest molecular series so far. These data allow detailed genotype–phenotype correlations and support the idea that molecular analyses are essential to distinguish HOS from overlapping conditions, which could be clinically confused.

## Patients and methods

### Patients

A multicentre, retrospective study was set up to collect data on 228 patients addressed to our laboratory for suspicion of HOS, between 2002 and 2014. This collaboration involved 56 departments of clinical genetics. Sixteen patients were excluded because of a non-appropriate diagnosis of HOS, in presence of postaxial upper limb involvement, lower limb defect or multiple associated malformations. Patient phenotypes were divided into three groups: typical HOS with heart defect, typical HOS without heart defect, uncertain HOS. Typical HOS patients met the diagnostic criteria established by Mc Dermott et al. HOS was considered as uncertain, if the preaxial defect, associated with a heart defect, was unilateral and/or in presence of one or two minor additional features. The associated features were considered as minor if they were previously described in a patient with a *TBX5* variant or if they are common in general population (growth retardation, urogenital malformations for example). Data from 212 patients were recorded. Clinical and radiological information were collected at the time of diagnosis and updated thanks to a clinical questionnaire as part of the study (available on request).

### Ethics statement

Our study was performed using the Declaration of Helsinki protocol. Written informed consents were obtained from the patients or their parents prior to the molecular diagnostic analysis.

### Genotyping

Peripheral blood was collected from patients and relatives. We performed Sanger sequencing of all *TBX5* coding exons and their flanking intronic regions. Primers sequences are available on request. Variants are given according to reference sequence NM\_000192.3 (Hg19). Large intragenic *TBX5* rearrangements were tested by a multiplex ligation-dependent probe amplification (MLPA) kit (PE180 Limb malformations probemix, MRC-Holland). *TBX5* variants were submitted to ClinVar database, available at the following link: <http://www.ncbi.nlm.nih.gov/clinvar/?term=TBX5> [gene]. For the interpretation of missense variants, pathogenicity prediction software (SIFT, Polyphen) and proteic structure prediction software (PHYRE2, SuperPose) were used.

## Statistical analysis

Comparisons between the different groups of patients have been assessed by the Chi-square test, with Yates correction when the application conditions were not respected.

## Results

Among the 212 patients, 105 met the established diagnostic criteria (79 with heart defect, 26 without heart defect) and 107 were considered with an uncertain HOS diagnosis. *TBX5* molecular analysis identified 78 probands with *TBX5* variants: 61 who meet the diagnostic criteria and 17 who were clinically considered as uncertain. According to the diagnostic criteria established by Mc Dermott et al., the detection rate of *TBX5* variant was 58%. This detection rate was better in the subgroup associating bilateral anterior upper limb defect and CHD (70%).

### Description of the patients with *TBX5* variants

Within the 78 probands with *TBX5* variants, 47 were sporadic cases. The clinical and radiological features observed in the patients and their 16 affected relatives are detailed in Tables 1 and 2. A cardiac defect was observed in 91% (71/78) of the patients. ASD was the most frequent (61.5% of the cases). Rhythm disturbances were observed in 30% of the living patients. The range of the limb phenotypes is depicted in Fig. 1. The upper limb defects were bilateral in 96% (75/78) of the patients. A left/right asymmetry was observed in 91% (31/34) of the patients when this information was available. The observed malformations were most often reduction defects (79.5%). None of the patients presented with preaxial polydactyly. Fourteen patients did not fit into the strict diagnostic criteria: 7 presented with the association of one or two skeletal features (scoliosis [4], *pectus excavatum* [3], costal deformities [1]), or one or two non-skeletal features (growth retardation [2], bilateral pyelic dilatation [1], familial form of Kallmann syndrome [1], laryngomalacia [1], pulmonary malformation [2], micropenis [1]). We describe 11 prenatal cases: 4 were revealed by an isolated upper limb defect (thumb agenesis, radial hypoplasia, phocomelia), 2 by an isolated CHD (VSD, AVD), 5 by the association of upper limb defect and CHD. The severity of the upper limb defects led to pregnancy termination for 6 fetuses. Data regarding the management and the evolution of the patients were often missing in the questionnaire filled in mainly by clinical geneticists. Orthopaedic surgery was reported for 25% (18/71) of the patients and consisted for 72% of them in

pollicisation of the index finger. Cardiac surgery was performed in 47% (31/66) of the living patients with CHD and a pacemaker was placed for 38% (8/21) of the patients with rhythm disturbances. Two deaths occurred, including one during a cardiac surgery, due to the severity of the CHD consisting of atrioventricular septal defect either isolated or associated with a tight isthmic coarctation.

Among the observed *TBX5* variants, 87% were point changes. Most of them were truncating (37% nonsense, 26% frameshift, 10% splice site), while 14% were missense variants. MLPA analysis identified *TBX5* intragenic deletions in 8% and intragenic duplications in 4%. Finally, an apparently balanced translocation t(2;12)(q31;q24.3) implicating the *TBX5* locus did segregate in 3 affected cases of the same family. All described variants are illustrated in the Fig. 2. We identified 49 novel variants. Two novel variants responsible for an extended *TBX5* protein were found: one familial case (c.1303delC, p.(Leu435Trpfs\*147), triphalangeal thumbs, radial hypoplasia, single auricle with sinus bradycardia) and one sporadic foetus (c.1346delA, p.(Gln449Argfs\*70) de novo, bilateral thumbs and radial hypoplasia, complex CHD and a micropenis). Eight distinct missense variants have been identified in 11 unrelated patients. Their pathogenicity was appreciated according to criteria developed in Supp table 1. Two of them occurred de novo in sporadic cases, therefore considered as likely pathogenic. Two of them concerned the amino acid 237, crucial for DNA interaction. Functional tests for these 2 variants have been previously published, allowing us to consider them as pathogenic. The remaining 4 variations have been considered to be of uncertain significance.

### Lack of penetrance—Mosaicism

In our series, 4 non-related parents of an affected child harboured the familial variant but no clinical HOS features. Two of them had normal skeletal X rays, cardiac ultrasound and electrocardiogram, whereas their children had typical features of HOS, up to phocomelia. This lack of penetrance was observed for 4 different variants: c.713dupG (p.(Ser239Glnfs\*2)), duplication of exons 1 to 7 (c.(?-667)\_ (755+1\_756-1)dup), deletion of exon 7 (c.(663+1\_664-1)\_ (755+1\_756-1)del) and c.710G>A (p.(Arg237Gln)). Furthermore, one asymptomatic father of a proband presented with a somatic mosaicism (around 10% of the alleles in blood sample) for the variant identified in his son c.537C>A (p.(Tyr179\*)).

### Differential diagnosis

Among the 212 patients, 21 turned out to present a differential diagnosis (Supp table 2). Thirteen of them had

**Table 1** Frequency of cardiac phenotypes in the 78 probands and their affected relatives in our series, compared to the literature [4–11]

	Patients from our study		Literature [4–11]
	Probands (N = 78)	Probands and relatives (N = 94)	
<i>Congenital heart disease (CHD)</i>			
General			
Single septal defect	38/78 (48.7)	44/94 (46.8)	nd
Multiple septal defect	12/78 (15.4)	16/94 (17)	nd
Septal defect, associated with another CHD	15/78 (19.2)	17/94 (18)	nd
Isolated valvular defect	2/78 (2.6)	2/94 (2.1)	nd
Details			
Septal defects			
Atrial septal defect	48/78 (61.5)	58/94 (61.7)	44.4%
Ventricular septal defect	27/78 (34.6)	32/94 (34)	29.4%
Atrioventricular septal defect	4/78 (5.1)	4/94 (4.3)	3.3%
Other			
Mitral valve abnormalities	3/78 (3.8)	3/94 (3.2)	4.1%
Patent ductus arteriosus	3/78 (3.8)	3/94 (3.2)	3.6%
Pulmonary stenosis	2/78 (2.6)	3/94 (3.2)	1.8%
Dextrocardia	2/78 (2.6)	2/94 (2.1)	1.8%
Left ventricle hypoplasia	2/78 (2.6)	2/94 (2.1)	nd
Aortic coarctation	4/78 (5.1)	4/94 (4.3)	nd
Left superior vena cava	3/78 (3.8)	3/94 (3.2)	nd
Complex CHD	1/78 (1.3)	1/94 (1)	nd
<i>Conduction disturbances</i>			
General			
Isolated	3/71 (4.2)	5/87 (5.7)	nd
Associated with CHD	18/71 (25.3)	20/87 (23)	nd
Details			
Sinus bradycardia	6/71 (8.5)	7/87 (8)	nd
Atrial flutter	2/71 (2.8)	2/87 (2.3)	nd
Atrioventricular block	2/71 (2.8)	4/87 (4.6)	nd
Right bundle branch block	5/71 (7)	6/87 (6.9)	nd
Junctional tachycardia	5/71 (7)	5/87 (5.7)	nd
Long QT	1/71 (1.4)	1/87 (1.1)	nd

Numbers in brackets are expressed in percent

nd not determined

typical presentation of HOS. Okiihiro syndrome was confirmed molecularly for eight patients. Fanconi anemia was diagnosed in presence of increased chromosome breakage on karyotype with mitomycin C. TAR syndrome was confirmed in presence of a 1q21.1 microdeletion involving *RBM8A*, even if the second hit (hypomorphic SNP) was not observed.

### Genotype–phenotype correlations

First, we compared the phenotypes between variant-positive and variant-negative patients, in the 3 phenotypic groups of patients. Some items were significantly more common in the variant-positive patients: the presence of a familial history, the presence of a septal defect, the association of a rhythm disturbance with a CHD, the bilateral characteristics of the radial defect and the presence of a shoulder or elbow mobility defect ( $p < 0.05$ ) (Supp Table 3).

In addition, we compared the phenotypes according to the type of *TBX5* variant: truncating (nonsense, frameshift, splice site point variants, intragenic deletions or

duplications) versus missense variants. We observed that isolated septal CHD are more common in the truncating than in the missense variants ( $p = 0.02$ ). Besides, complex CHD associated to septal defect seems to be more common in case of missense variants ( $p = 0.053$ ). Thus, we observed a tendency of less severe CHD in patients with truncating variants compared to the patients with missense variants. No significant difference was observed in the type or the severity of upper limb defects between the two groups.

### Discussion

This description of the largest molecular HOS series ever reported allows a better delineation of the HOS phenotype and genotype, and helps to direct appropriate molecular analyses by using more accurate diagnostic criteria for HOS and overlapping conditions.

Our clinical study highlights the importance of some critical features: the septal involvement for the CHD, the bilateral characteristics of the radial ray defects and the

**Table 2** Frequency of skeletal phenotypes in the 78 probands and their affected relatives in our series, compared to the literature [4–11]

	Patients from our study				Literature [4–11] Right and left
	Probands (N = 78)		Probands and relatives (N = 94)		
	Right	Left	Right	Left	
<i>Hand</i>					
Reduction defect of the thumbs					
Thenar hypoplasia	22/78 (28.2)	20/78 (25.6)	23/94 (24.4)	21/94 (22.3)	34.2%
Thumb hypoplasia	23/78 (29.5)	26/78 (33.3)	26/94 (27.7)	30/94 (31.9)	31.9%
Thumb agenesis	17/78 (21.8)	25/78 (32.1)	18/94 (19.1)	27/94 (28.7)	32.3%
I-II syndactyly	12/78 (15.4)	11/78 (14.1)	14/94 (14.9)	12/94 (12.8)	13.3%
First metacarpal agenesis	4/78 (5.1)	3/78 (3.8)	4/94 (4.3)	3/94 (3.2)	nd
Additional defect of the thumbs					
Triphalangeal thumb	21/78 (26.9)	19/78 (24.3)	21/94 (22.3)	20/94 (21.3)	23.6%
Digitalised thumb	5/78 (6.4)	2/78 (2.6)	5/94 (5.3)	4/94 (4.3)	31.9%
Bifid thumb	–	–	–	–	5%
II–III syndactyly	2/78 (2.6)	3/78 (3.8)	2/94 (2.1)	3/94 (3.2)	nd
Carpal bones malformation	3/78 (3.8)	3/78 (3.8)	5/94 (5.3)	5/94 (5.3)	35.3%
<i>Forearm</i>					
Radial defects					
Hypoplasia	17/78 (21.8)	17/78 (21.8)	19/94 (20.2)	20/94 (20.2)	34.2%
Agenesis	4/78 (5.1)	8/78 (10.3)	4/94 (4.3)	8/94 (8.5)	15.2%
Ulnar defects					
Radio-ulnar synostosis	3/78 (3.8)	5/78 (6.4)	3/94 (3.2)	5/94 (5.3)	nd
Limitation of pro-supination	14/78 (17.9)	16/78 (20.5)	19/94 (20.2)	20/94 (21.3)	25.8%
Limitation of elbow mobility	9/78 (11.5)	12/78 (15.4)	12/94 (12.8)	14/94 (14.9)	nd
<i>Arm</i>					
Humeral hypoplasia	–	1/78 (1.3)	–	1/94 (1)	32%
Phocomelia	3/78 (3.8)	4/78 (5.1)	4/94 (4.3)	5/94 (5.3)	8.4 %
<i>Shoulder</i>					
Limitation of elbow mobility	4/78 (5.1)	5/78 (6.4)	4/94 (4.3)	5/94 (5.3)	28.6%
Sloping shoulders	20/78 (25.6)	18/78 (23)	20/94 (20.2)	18/94 (19.1)	7.5%
Clavicle defects	8/78 (10.3)	8/78 (10.3)	10/94 (10.6)	10/94 (10.6)	31.6%
Muscular hypoplasia	5/78 (6.4)	5/78 (6.4)	5/94 (5.3)	5/94 (5.3)	38.2%

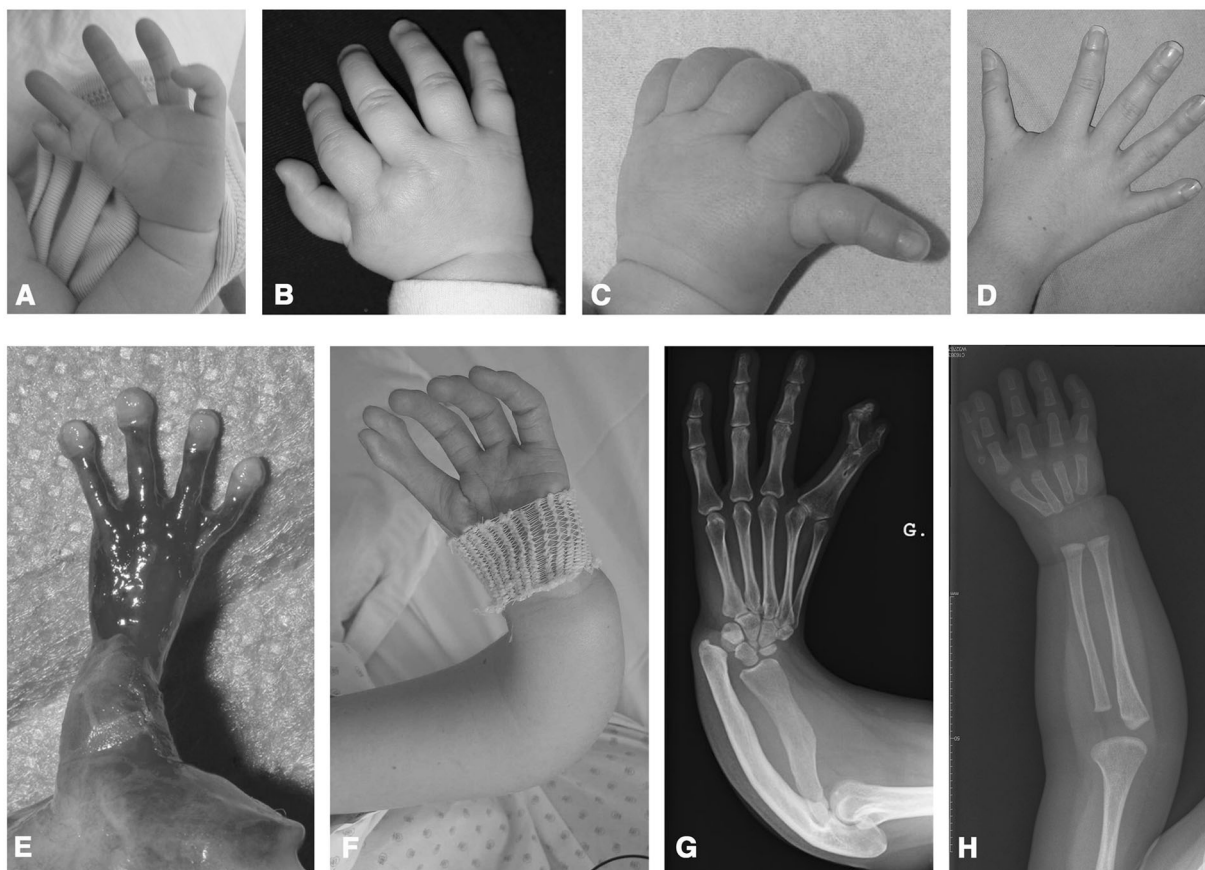
Numbers in brackets are expressed in percent

nd not determined

shoulder or elbow mobility defect (even in the absence of any skeletal involvement). Preaxial polydactyly, previously described in HOS in the literature [10], has not been observed in any of our patients with *TBX5* variants. We observed that the presence of pectus excavatum, spinal deformities or pulmonary malformations should not rule out the HOS hypothesis. The presence of *pectus excavatum* in HOS has been largely overestimated (30%) in the clinical series of the literature, probably because some of these patients had an overlapping condition [4, 6]. In our series, only 3 patients out of 78 (4%) presented with pectus excavatum, which is still higher than its frequency in the general population (0.3%) [21]. This association can be explained by the involvement of *Tbx5* in sternum development, demonstrated by the presence of abnormal sternal formation in a *Tbx5* conditional mutant [22]. Four patients out of 78 (5%) presented with *scoliosis*, which is not significantly different than its frequency in the general population (2–3%) [23], suggesting that this feature is

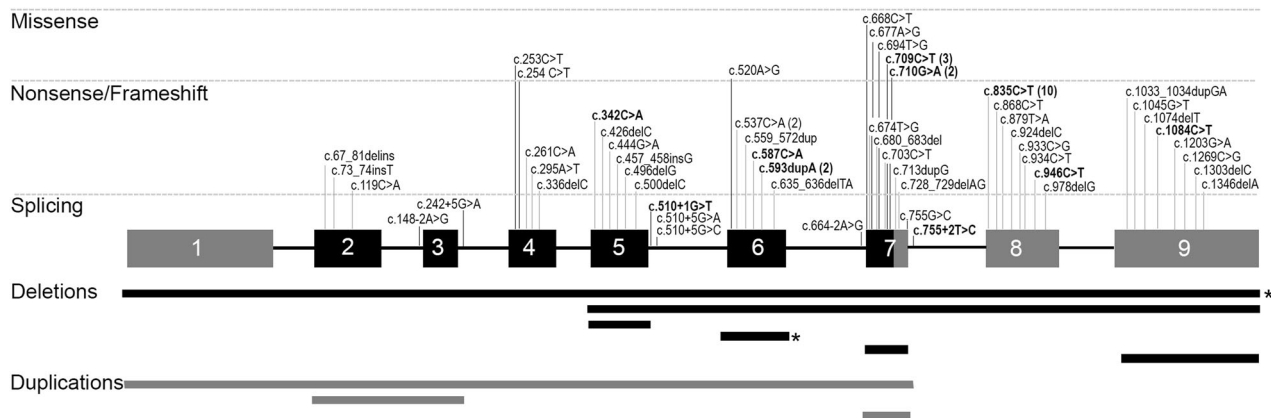
independent from HOS. Four patients from the literature presented with vertebral defects: fusion of cervical vertebrae, defect of anterior vertebral bodies, hemivertebra [4, 8, 10, 19], while *Tbx5* expression has never been reported in the spinal column. Two patients of our series presented with a *pulmonary malformation*. A right lung agenesis, a right lung hypoplasia and a horseshoe lung have been previously described in HOS [24–26]. *Tbx5* expression has been described in the mesenchyme of the developing lungs and trachea, and could participate, through Fgf10 pathway, to lung branching [27]. The other associated features were likely incidental.

We described a lack of penetrance in 4 different HOS families in our series, and a somatic mosaicism in the father of one proband. HOS has generally been described as a condition with complete penetrance, but 2 families presenting with missense variants and an incomplete penetrance have already been reported [8, 28]. Somatic mosaicism for a variant has previously been reported in an



**Fig. 1** Clinical and radiological phenotypes in HOS patients. **a** Left triphalangeal thumb, I–II syndactyly, elbow mobility defect; **b** Right thumb hypoplasia; **c** Left thumb hypoplasia; **d** Left triphalangeal

thumb; **e** Left thumb agenesis in a foetus; **f** Left digitalised thumb, I–II syndactyly, radial hypoplasia; **g** Radial and ulnar hypoplasia, I–II phalangeal syndactyly; **h** Right thumb hypoplasia



**Fig. 2** Representation of the *TBX5* variants identified in our series of 78 HOS patients. Dark exons represent the T-box domain. Point variants are represented at the superior part of the figure. Intragenic

deletions or duplications are represented at the inferior part. Variants in bold and deletions highlighted by a star have been described previously in the literature. The exons/introns are not at scale

asymptomatic parent in the literature [9]. This highlights the importance of the parental molecular analyses for all the probands with *TBX5* variants, even if the case seems to be sporadic, to be able to give an accurate genetic counselling to these families.

*TBX5* variants are distributed throughout the gene, but the majority are found within the T-box DNA binding domain. Nonsense variants are the most common in our series (37%), whereas missense variants are the most frequently reported *TBX5* variants in the literature (30%,

**Table 3** Summary and frequency of associated features that can be observed in the main differential diagnoses for HOS

	MIM#	Gene/Locus	Inheritance	Cardiac anomalies	Radial defects	Characteristic clinical features	References
Okhiro syndrome	607323	<i>SALL4</i>	AD	23%	80%	Duane anomaly, renal anomalies	[8, 31]
Fanconi pancytopenia	227650	FA core complex genes	AR, XL	6%	35%	Pancytopenia, growth retardation, microcephaly	[38]
Polydactyly, preaxial type II Syndactyly type IV	174500 and 186200	ZRS locus	AD	–	100%	Isolated pre-axial polydactyly with or without triphalangeal thumbs, mirror image polydactyly	[39]
Valproate embryopathy	–	–	sporadic	14–26%	30%	Developmental delay, neural tube defects, vertebral defects, hypospadias, cleft palate	[40, 41]
VACTERL association	192350	–	sporadic	40–80%	40–50%	Vertebral defects, anal atresia/stenosis, cardiac anomalies, tracheo-Esophageal fistula, renal anomalies, limb defects	[42]
Townes-Brockes syndrome	107480	<i>SALL1</i>	AD	9–40%	50–90%	Anal atresia, ears malformations, deafness, triphalangeal thumbs, renal anomalies	[43]
TAR syndrome	274000	<i>RBM8A</i>	AR	15–30%	100%	Thrombocytopenia, preserved thumbs, lower limbs defects	[44, 45]
Nager syndrome	154400	<i>SF3B4</i>	AD	15%	100%	Mandibulofacial dysostosis, deafness	[46]
LADD syndrome	149730	<i>FGF10, FGFR2, FGFR3</i>	AD	-	95%	Lacrymal ducts atresia, ears malformations, deafness, abnormal teeth, thumb anomalies	[47, 48]
Roberts syndrome	268300	<i>ESCO2</i>	AR	26%	100%	Growth retardation, craniofacial malformations, upper and lower limbs reduction defects	[49, 5, 50]

versus 14% in our series), due to the larger propensity of publication of missense variant functional analyses (reviewed in the ref. [29]). Nonetheless, most of the reported *TBX5* variants (70%) lead to haploinsufficiency by synthesis of a non-functional truncated protein or by triggering the nonsense-mediated mRNA decay, by nonsense, frameshift or splice site variants [29]. Missense *TBX5* variants can cause loss-of-function or exceptionally gain-of-function. Sequence point variations leading to an extended protein and large intra and intergenic deletion/duplication have also been occasionally reported [29]. We observed a higher propensity of missense variants to be responsible for complex CHD associated to septal defect. In the literature, the few *TBX5* variants described in patients presenting with a non-septal CHD (Fallot tetralogy, cardiomyopathy) are also missense variants. Also, frameshift variants leading to an extended *TBX5* protein seem to be associated with more severe CHD and associated malformations [30]. This suggests a potential dominant-negative effect for these variants.

HOS is a challenging diagnosis by its variability of expression and the large phenotypic overlap with other conditions. Among the patients addressed to our laboratory for HOS suspicion, 21 turned out to present a differential diagnosis (Supp table 3). More than 50% of these patients met the HOS diagnostic criteria. The most common is Okhiro syndrome (MIM#607323), characterised by constant bilateral anterior upper limb defects, possibly associated with abnormality of the ocular motility named Duane anomaly (65% of the cases), morphological renal defects (28%), but also with cardiac (17%) or spinal defects and ear dysplasia or deafness [8, 31]. This autosomal dominant condition is due to variants affecting *SALL4*, which encodes a transcription factor acting synergistically with *TBX5* during heart and limb development [32, 33]. HOS can be mistaken with many other overlapping conditions (summarized in Table 3) and clinical assessment of patients should be standardized, as detailed in Fig. 3.

Finally, some patients with typical HOS features had no *TBX5* variant or identified overlapping condition after

<b>Assessment of patients suspected for HOS</b>	
<b>Detailed history :</b>	<ul style="list-style-type: none"> <li>- Familial history</li> <li>- Valproate exposure</li> </ul>
<b>Clinical examination :</b>	<ul style="list-style-type: none"> <li>- Growth evaluation (growth retardation, microcephaly)</li> <li>- Careful morphological examination (ear dysplasia, lower limb involvement)</li> </ul>
<b>Evaluations :</b>	<ul style="list-style-type: none"> <li>- Ophthalmologic (ocular mobility defect, lacrimal ducts)</li> <li>- ENT with hearing tests</li> <li>- Renal ultrasound</li> <li>- CBC, necessary if thumbs present (thrombocytopenia in TAR syndrome) or if growth retardation (pancytopenia of Fanconi disease)</li> </ul>

**Fig. 3** Assessment of patients suspected for HOS

additional screening by array-CGH and the targeted high-throughput sequencing of 124 genes involved in limb development (data not shown). Previously, Terrett et al. identified 2 families with a typical presentation of HOS that were not linked to the 12q24 locus [34], supporting the hypothesis of a genetic heterogeneity. However, we performed whole exome sequencing in 13 selected typical cases, revealing no candidate gene (data not shown). Recent data showed that regulatory variants may explain a large part of congenital malformations [35]. Three *TBX5* heart-specific enhancers have been described, and one homozygous rare variant in one of these sequences has been reported in a patient presenting with an isolated VSD [36]. A minimal regulatory sequence has been identified in intron 2, where Hox factors bind to activate *Tbx5* expression during mouse upper limb initiation [37]. No variant in this element has been reported so far. Whole genome sequencing will probably help to unravel the variant-negative cases by allowing the exploration of regulatory elements, and the detailed screening of balanced chromosomal rearrangements.

Our study illustrates the inconsistencies and issues of the molecular diagnosis of HOS, by both its clinical (variability of expression, lack of penetrance, numerous overlapping conditions) and molecular aspects (diversity of *TBX5* variants, potential genetic heterogeneity or regulatory variant). The diagnosis of HOS cannot be certainly affirmed or ruled out by strict diagnostic criteria and need to be confirmed by *TBX5* molecular analysis, since the typical presentation of HOS can be sometimes incomplete or associated with other features in patients with *TBX5* variants.

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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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