

Clinical Report

Tetra-Amelia and Lung Hypo/Aplasia Syndrome: New Case Report and Review

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Tetra-amelia is a rare malformation that may be associated with other anomalies and is usually inherited in an autosomal recessive pattern. We describe a fetus, born to a non-consanguineous couple, with tetra-amelia, bilateral cleft lip and palate and bilateral lung agenesis, without other anomalies. Karyotype was normal (46,XX) and premature centromere separation was excluded. No mutation was identified upon molecular analysis of *WNT3*, *HS6ST1*, and *HS6ST3*. We reviewed the literature and the differential

diagnosis to clarify the clinical delineation of conditions associated with tetra-amelia. The present report describes the sixth family with this pattern of malformations and reinforces the evidence that the “tetra-amelia and lung hypo/aplasia syndrome” is a distinct autosomal recessive condition, with no identified gene thus far. © 2008 Wiley-Liss, Inc.

Key words: tetra-amelia; lung aplasia; cleft lip/palate

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INTRODUCTION

Congenital limb defects have a birth prevalence of 0.55 per 1,000 [Evans et al., 1994]. Amelia, the complete absence of one limb, has a birth prevalence of 0.01 per 1,000 births, in which 50% are syndromic [Evans et al., 1994]. Tetra-amelia, the complete absence of all four limbs, is even rarer, frequently inherited in an autosomal recessive pattern. It is usually described as part of a multiple congenital anomaly syndrome (MIM 273395, 301090 and 273390), associated with craniofacial, pulmonary, central nervous system, heart, skeletal, urogenital malformations, ectodermal dysplasia, and lacrimal duct anomalies. The clinical delineation of the different entities associated with tetra-amelia is complex and the molecular findings are limited.

We report an additional patient with tetra-amelia and lung hypo/aplasia syndrome and review the differential diagnosis and clinical delineation of the conditions associated with tetra-amelia.

CLINICAL REPORT

A 17-year-old healthy woman presented at 20 weeks of gestation in her first pregnancy. There was no known teratogen exposure. The father was a healthy 22-year-old man and there was no consanguinity or relevant family history.

The ultrasonographic evaluation at 20 weeks identified tetra-amelia, bilateral cleft lip and palate, and possible pulmonary hypoplasia. No other anomalies were detected and the amniotic fluid quantity was normal. Pregnancy was terminated at 22 weeks. Clinical examination of the fetus was consistent with the prenatal findings (Fig. 1) and, in addition, micrognathia and low-set ears were

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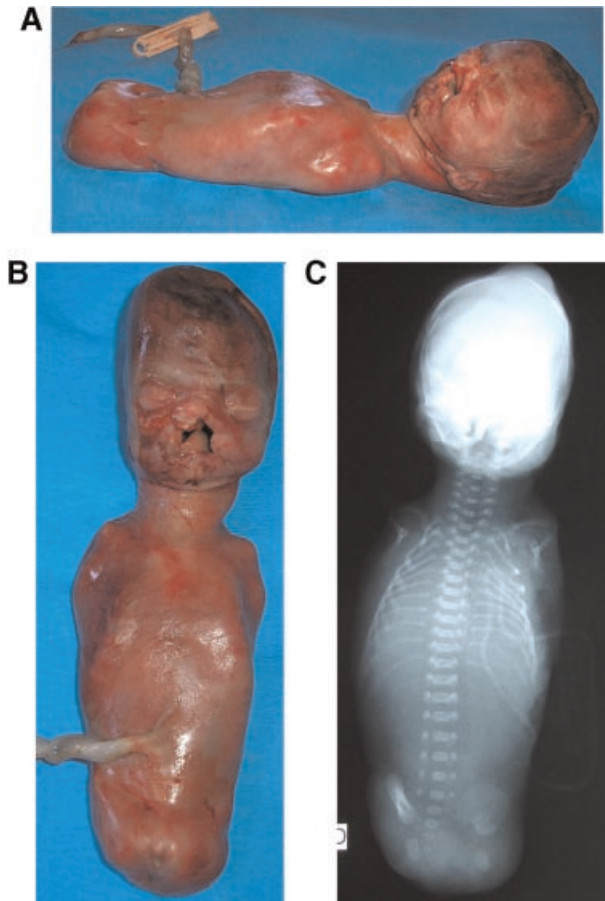


FIG. 1. **A,B:** Fetal phenotype: complete absence of the four limbs (tetra-amelia) and facial cleft; **(C)** Fetal X-ray image showing a small thorax and the complete absence of limb bones with normal clavicles, scapulae and pelvis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

observed. The pathological examination further revealed bilateral lung agenesis (Fig. 2), with bilateral pulmonary artery agenesis and a small right heart, and excluded genito-urinary anomalies. The fetal X-ray confirmed the complete absence of limb bones and revealed normal clavicles, scapulae and pelvis (Fig. 1). Cytogenetic analysis of amniocytes and fetal

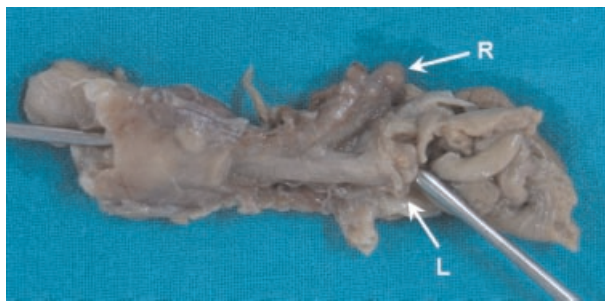


FIG. 2. Photograph of the posterior aspect of the tracheo-bronchial tree (esophagus sectioned) highlighting the bilateral lung agenesis with the right (R) and left (L) blind-ended main bronchi. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

blood was normal (46,XX), and premature centromere division was excluded.

Genomic DNA was extracted from cultured amniocytes. *WNT3* molecular analysis, being the only gene so far associated with tetra-amelia cases [Niemann et al., 2004], was performed by direct sequencing of the *WNT3* coding region. No mutations were identified. Subsequently, following a candidate gene approach based on the involvement of the heparan sulfatase *HS6ST* in both limb bud development and tracheal branching evidenced in different animal models [Kamimura et al., 2001; Izvolsky et al., 2003; Nogami et al., 2004], coding regions of the *HS6ST1* and *HS6ST3* genes were screened for mutations using direct sequencing as previously described [Krahn et al., 2005]. No sequence variation was found in *HS6ST1* gene. A homozygous SNP (rs9516771) was identified in exon 2 of the *HS6ST3* gene, which was predicted to be non-pathogenic using bioinformatics PolyPhen SNP-analysis [Ramensky et al., 2002].

DISCUSSION

The clinical delineation of the different entities associated with tetra-amelia is not yet clear. The present report supports the specific pattern of malformations (tetra-amelia, lung hypo/aplasia and, inconsistently, cleft lip/palate) of the five previously reported families (14 affected fetuses/infants) [Rosenak et al., 1991; Zlotogora et al., 1993; Basaran et al., 1994; Krahn et al., 2005] which are compared with the present patient and other conditions in the differential diagnosis in Table I. This condition, tetra-amelia and lung hypo/aplasia syndrome, has been classified as MIM273395.

The specific association of tetra-amelia, lung hypo/aplasia and cleft lip/palate, was first reported by Rosenak et al. [1991] (OMIM 273395) in two fetuses from an apparently nonconsanguineous Arab Muslim couple, including an 18-week female with tetra-amelia, bilateral lung agenesis, left bronchoesophageal fistula, low-set ears and micrognathia and an 14-week male with tetra-amelia, left cleft lip and severe lung hypoplasia. This couple also had one amelic female offspring that died soon after birth (not examined) and four normal children. Cytogenetic analysis was only performed on the last affected fetus. It was normal (46,XY) and no premature centromere separation was observed.

Later, Zlotogora et al., [1993] reported the incidence of tetra-amelia in two Muslim Palestinian Arab families. These families appeared to be unrelated but had the same geographical origin as the one described by Rosenak et al. [1991]. The first family described by Zlotogora et al., [1993] was a consanguineous couple that had seven children, two of which were affected females with enlarged head and tetra-phocomelia. In the second family, there were

TABLE I. Comparison of the Reported Cases With "Tetra-Amelia and Lung Hypo/Aplasia Syndrome" and the Main Differential Diagnosis

	Tetra-amelia and lung hypo/aplasia syndrome AR MIM273395										Zimmer type phocomelia AR (and XL2) MIM301090 WNT3 mut.	Roberts Syndrome AR MIM268300 ESCO2 mut.	AA/RR/SP Syndrome AR MIM276820 WNT7A mut.	Ohdo et al. [1987, 1994] AR MIM276820		
	Rosenak et al. [1991]					Basaran et al. [1994]					Zimmer et al. [1985],					
	F	F	M	F	M	F	M	F	M	F	Present case [2007]	Arab Moslem, Hispanic, Turkish				
Phenotypical sex	F	F	M	F	M	F	M	F	M	F	F	Arab Moslem, Hispanic, Turkish				
TOP (weeks gestation)	-	+	(18)	+	(14)	-	-	-	-	-	+	Arab Moslem, Hispanic, Turkish				
Neonatal death	+	-	-	+	+	+	+	+	+	+	+					
Post-mortem study	-	+	+	-	-	-	-	-	-	-	+					
Consanguinity	+	+	+	+	+	+	+	+	+	+	-	Arab Moslem, Hispanic, Turkish				
Ethnicity	Arab Muslim (Palestinian)	Arab Muslim (Palestinian)	Arab Muslim (Palestinian)	Arab Muslim (Palestinian)	Arab Muslim (Palestinian)	Arab Muslim (Palestinian)	Arab Muslim (Palestinian)	Arab Muslim (Palestinian)	Arab Muslim (Palestinian)	Arab Muslim (Palestinian)	Portuguese	Arab Moslem, Hispanic, Turkish				
Face	?	+	-	-	-	-	-	-	-	-	+					
Cleft lip/palate	-	-	-	-	-	-	-	-	-	-	+					
Eye malformation	-	-	-	-	-	-	-	-	-	-	-					
Nose malformation	-	-	-	-	-	-	-	-	-	-	-					
Lungs	?	?	?	?	?	?	?	?	?	?	+					
bilateral aplasia	+	-	-	-	-	-	-	-	-	-	+					
bilat. severe hypoplasia	-	+	-	-	-	-	-	-	-	-	-					
mild hypoplasia	-	-	-	-	-	-	-	-	-	-	-					
lobulation anomalies	-	-	-	-	-	-	-	-	-	-	-					
Abnormal genitalia	?	-	-	-	-	-	-	-	-	-	-					
Urinary malformations	?	-	-	-	-	-	-	-	-	-	-					
Pelvic hypoplasia	?	-	-	-	-	-	-	-	-	-	-					
Tetra-amelia	+	+	+	+	+	+	+	+	+	+	+					
Tetra-phocomelia	-	-	-	-	-	-	-	-	-	-	-					
Other features	-	Low set ears, Micro gnathia	-	Macrocephaly	-	-	-	-	-	-	-	Cranio-facial, abdominal and intestinal malformations				
				Absent nipple, Heart defect												
Karyotype	?	?	46,XY	?	?	?	?	?	?	?	46,XX	Normal				
Premature centromere division	?	?	No	?	?	?	?	?	?	?	No	No				
				46,XY	?	?	?	?	?	?	46,XX	Normal				
				No	?	?	?	?	?	?	No	No				

AA/RR/SP, Al-Awadi/Raas-Rothschild/Schinzel phocomelia syndrome; AR, autosomal recessive transmission; XL, X-linked transmission; F, female; M, male; TOP, termination of pregnancy; mut., mutations.

two consanguineous couples. The first one had 11 children, three of whom had tetra-amelia and one of those also had cleft lip. The second couple had two children with tetra-amelia and cleft lip. No cytogenetic analysis or autopsies were performed in these cases. All affected neonates from both families died soon after birth, which suggests that other malformations were present, namely pulmonary hypo/aplasia.

Basaran et al. [1994] described two patients with tetra-amelia from a Turkish consanguineous couple that had one healthy daughter. The first patient was a term male infant who died soon after birth with tetra-amelia and cleft lip-palate (no autopsy was performed). The second patient was a 26-week-old male fetus with tetra-amelia, bilateral complete cleft lip-palate, bilateral palpebral fusion, micrognathia, absence of nipples, small penis, intra-abdominal testes, lung agenesis, ventricular septal defect, and mitral valve aplasia. Chromosomes were normal (46,XY) without premature centromere division.

Krahn et al. [2005] reported two fetuses born to a consanguineous Moroccan couple who presented with tetra-amelia and bilateral pulmonary aplasia without other anomalies, also with normal cytogenetic studies. In that report, the existence of an abnormal FGFR2 isoform and mutations in *WNT3*, *FGF10*, *HS6ST1*, and *HS6ST3* genes were excluded.

Niemann et al. [2004] reported the only family thus far with tetra-amelia and positive molecular findings. A consanguineous couple had four affected fetuses with tetra-amelia and cleft lip/palate, microphthalmia, nose malformation, choanal atresia, gastroschisis, diaphragmatic hernia, lung lobulation abnormalities, renal agenesis, spleen agenesis, malformed uterus, persistence of cloaca, urethral atresia, anal atresia, and single umbilical artery but without pulmonary hypo/aplasia. Homozygosity mapping followed by mutation analysis revealed a pathogenic homozygous mutation in the *WNT3* gene. Although the clinical description of this family is also included under OMIM 273395, in our opinion and as previously suggested by Krahn et al. [2005], it fits better with the Zimmer type phocomelia phenotype (OMIM 301090).

Zimmer type phocomelia, initially described as X-linked [Zimmer et al., 1985; Gershoni-Baruch et al., 1990; Kosaki et al., 1996], was further characterized by Kosaki et al. [1996] who reported a 46,XX fetus and suggested autosomal recessive inheritance. In the original family described by Zimmer there is no clear X-linked pattern of transmission. Genital anomalies precluded phenotypic sex determination in the fetuses and cytogenetic and pathological studies were only done in one of the seven affected fetuses. Features of this condition mainly include: tetra-phoco/amelia, facial clefts, nose and eye malformations, kidney agenesis, imperforate anus and vagina and abnormal genitalia. The main features that

distinguish Zimmer type phocomelia from tetra-amelia and lung hypo/aplasia syndrome are the absence of severe pulmonary hypoplasia or aplasia and the presence of internal, genital and craniofacial (in addition to cleft lip/palate) anomalies in Zimmer type phocomelia. Taking into account the family reported by Niemann et al. and the arguments listed above, we conclude that mutations in *WNT3* inherited in a recessive pattern are a cause of Zimmer type phocomelia. At present, there is not enough evidence to exclude the involvement of other genes and even an X-linked form, and further studies of other cases are required.

Several other authors have described cases with tetra-phoco/amelia as part of different patterns of malformation (Table I). One of the conditions in the differential diagnosis is Roberts syndrome—SC phocomelia (OMIM 268300), a well known disorder inherited in an autosomal recessive pattern characterized by a wide spectrum of limb deficiencies, severe craniofacial abnormalities, pre- and post-natal growth retardation, mental retardation and facial vascular malformations. It is associated with premature centromere separation and with homozygous mutations in *ESCO2* gene [McDaniel et al., 2005; Vega et al., 2005].

The Al-Awadi/Raas-Rothschild/Schinzel phocomelia (AA/RR/SP) syndrome (“absence of ulna and fibula with severe limb deficiency”, OMIM 276820) is characterized by limb defects, more severe in the lower limbs, pelvic hypoplasia, nail hypo/aplasia, and variable genitalia abnormalities. It is differentiated from the previous entities by the absence of pulmonary hypo/aplasia, absence of urinary and gastrointestinal malformations, normal facial structure and the presence of pelvic hypoplasia. Homozygous loss-of-function mutations in the *WNT7A* gene were identified in this condition, which is allelic to Fuhrmann syndrome (“fibular aplasia or hypoplasia, femoral bowing and poly-, syn- and oligodactyly”, OMIM 228930) associated with partial loss of *WNT7A* function [Woods et al., 2006]. There is still uncertainty in the understanding of this family of syndromes (OMIM 276820 and OMIM 228930), namely concerning the inclusion of the Schinzel phocomelia (SP) phenotype [Lonardo et al., 2007].

Another recognized entity which has been described in one family [Ohdo et al., 1987, 1994] is “tetra-amelia with ectodermal dysplasia and lacrimal duct abnormalities” (OMIM 273390) which has autosomal recessive inheritance. There is mental retardation in addition to the features mentioned in its description.

In conclusion, we propose that the family reported by Niemann et al. [2004] should be considered as part of the Zimmer type phocomelia spectrum (OMIM 301090); the Zimmer type phocomelia should be more accurately considered as being inherited in an autosomal recessive pattern, with the possibility of

genetic heterogeneity and a X-linked form, and the tetra-amelia and lung hypo/aplasia syndrome should be classified as a separate condition, as suggested [Kosaki et al., 1996; Krahn et al., 2005], associated with autosomal recessive transmission and with no identified gene. This hypothesis was supported by the exclusion of disease-causing mutations in *WNT3* gene, and also in candidate genes, including *HS6ST1* and *HS6ST3*, in the present and in the Krahn et al. [2005] cases. The modes of inheritance and MIM classification numbers of each condition are presented in Table I in accordance with these suggestions.

Additional molecular investigations are needed to further clarify the understanding of these rare conditions. Besides looking for new implicated genes, a more widespread study of the known genes (*WNT3*, namely in families with Zimmer type phocomelia, and *WNT7A*, especially in families with Shinzel phocomelia syndrome) would be useful.

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