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Association Between Fetal Lymphedema and Congenital Cardiovascular Defects in Turner Syndrome

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ABSTRACT. *Objectives.* **Turner syndrome (TS) is associated with congenital cardiovascular defects (CCVDs), most commonly bicuspid aortic valve (BAV) and aortic coarctation (COARC), congenital renal anomalies, and fetal lymphedema. It has been theorized that compressive or obstructive effects of fetal lymphedema may actually cause cardiovascular and renal dysmorphogenesis in TS. The objective of this study was to determine whether there is a specific association between a history of fetal lymphedema and CCVDs in monosomy X, or TS, independent of karyotype or general severity of the phenotype.**

Methods. **This was a prospective study of 134 girls and women who have TS (mean age: 30 years) and were clinically evaluated for evidence of fetal lymphedema, classified as central (signified by the presence of neck webbing) or peripheral (current or perinatal, or dysplastic fingernails). The presence of BAV and/or COARC was detected by magnetic resonance imaging combined with echocardiography, and renal anomalies were determined by ultrasound.**

Results. **There is a strong association between developmental central lymphedema, signified by neck webbing, and the presence of BAV ($\chi^2 = 10$) and COARC ($\chi^2 = 8$). The association between webbed neck and CCVDs was independent of karyotype. There was, in contrast, no significant association between renal anomalies and webbed neck or CCVDs.**

Conclusions. **The strong, statistically significant association between neck webbing and the presence of BAV and COARC in TS suggests a pathogenetic connection between fetal lymphatic obstruction and defective aortic development. The presence of neck webbing in TS should alert the clinician to the possibility of congenital cardiovascular defects.** ***Pediatrics*** **2005;115:732–735;** ***X-chromosome, aortic coarctation, bicuspid aortic valve, lymphedema.***

ABBREVIATIONS. CCVD, congenital cardiovascular defect; BAV, bicuspid aortic valve; COARC, coarctation; TS, Turner syndrome.

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Congenital cardiovascular defects (CCVDs), including most commonly bicuspid aortic valve (BAV) and coarctation of the aorta (COARC), are well-known features of Turner syndrome (TS).[1–3] It is unknown whether these defects are attributable to haploinsufficiency for X-chromosome gene(s) involved in cardiovascular development or secondary to other features of the syndrome, such as massive fetal lymphedema interfering with heart and major vessel formation. This latter possibility was suggested by observations of an apparent increased prevalence of CCVD in individuals with TS and neck webbing[4]—the postnatal residua of nuchal cystic hygromas caused by obstructed jugular lymphatics in utero. On the basis of this observation, Clark[4] proposed that jugular lymphatic obstruction led to compression of the ascending aorta, resulting in reduced flow and left-sided cardiovascular outflow tract anomalies. This view was supported by further epidemiologic observations in a study of 120 infants with neck webbing reported in the Iowa Birth Defects registry, among which 66% were found to have flow-related defects.[5] This hypothesis obtained additional support from studies of 45,X embryos ascertained because of cystic hygromas, with the majority demonstrating left-sided defects, from left heart hypoplasia to aortic valve defects, aortic hypoplasia, and/or coarctation.[6,7] Moreover, these studies documented the presence of dilated lymphatics in the vicinity of the developing heart and great vessels.

The original observations associating neck webbing with CCVDs came from a retrospective review of published cardiology clinic cases,[4] suggesting a likely bias toward the most severely affected individuals. Likewise, the pathologic studies certainly focused on the most severely affected fetuses, raising the possibility that the association between CCVDs and neck webbing simply reflects the most severe phenotype in 45,X individuals rather than a specific connection between these 2 phenotypic features of X-chromosome deletion. In the present study, we revisit the issue of association between signs of fetal lymphedema and the presence of CCVDs in a prospective study of 134 volunteers with TS, not selected for cardiovascular disease, who underwent physical examination and cardiovascular evaluation by MRI and echocardiography in a tertiary clinical research center.

METHODS

Individuals with TS were recruited for this clinical study mainly through notices posted on Internet web sites (eg, http://turners.nichd.nih.gov/). Study participants signed informed assents/consents that were approved by the National Institute of Child Health and Human Development Institutional Review Board. Karyotype analysis of G-banded chromosomes in 50 lymphocytes was performed for all study participants. Diagnosis of TS was based on X-monosomy or X- or Y-deletion/rearrangement affecting ≥70% of cells. Participants with ≥30% normal cells were excluded from the study. A total of 134 girls and women with TS (mean age: 30; range: of 7–60 years) were studied. Adult participants underwent MRI that included contrast-enhanced, 3-dimensional MR angiography[8]; girls who were younger than 18 years had noncontrast MRI. All participants also had 2-D echocardiography with Doppler and renal ultrasound examination. MR images were evaluated by a single cardiovascular radiologist (V.B.H.), and echocardiograms were evaluated by a single cardiologist (D.R.R.). There was complete concordance for the 2 imaging methods for diagnosis of BAV, but echo was unable to define the aortic valve in 34 cases. MRI, however, was able to define the valve in all but 9 of these 34 patients, so the total for evaluation of BAV was 125. The diagnosis of COARC was by MRI or by history of surgical COARC repair.

Each study participant underwent a full physical examination and clinical cardiac evaluation. The presence of neck webbing was distinguished from a simply short neck by the finding of skin folds flaring from the lateral base of the skull to the mid- or lateral shoulder. The findings reported on physical examination were confirmed by review of neck photographs in anterior, lateral, and posterior views. A few participants had a history of surgical correction of webbing, verified by residual webbing and surgical scars.

Associations between phenotypic features were evaluated by χ^2 analysis with Yate's correction and forward stepwise regression in a linear model (SigmaStat, version 2.0; SPSS Inc, Chicago, IL).

RESULTS

We found that 26 (21%) of 125 of our study participants had a BAV, and 16 (12%) of 134 had COARC, with 9 individuals having both. Forty-seven (35%) of 134 had a webbed neck. The association of webbed neck and these specific CCVDs was assessed by χ^2 analyses (Table 1). BAV was significantly associated with neck webbing, with a $P < .002$. COARC was also significantly associated with neck webbing, as was the presence of either COARC or BAV (Table 1). Of note, the presence of COARC was highly associated with BAV ($\chi^2 = 15.8$, $P = .0001$). Nine participants had both COARC and BAV, and 5 had COARC with a normal, tricuspid valve (1 had an aortic valve that was not defined by MR angiography or echocardiography).

It is often assumed that the more severe phenotypic features of TS may be associated simply because they are more frequently found in individuals with a 45,X karyotype. We found a strong statistical association between the presence of a webbed neck and the 45,X karyotype ($P < .001$) and a weaker but significant association between BAV and the 45,X karyotype ($P = .014$). However, in a forward stepwise regression in a linear model with independent variables karyotype and webbed neck, only a webbed neck was a significant predictor of BAV ($P < .0001$). Likewise, in the same type of regression analysis, webbed neck but not karyotype was significantly associated with COARC ($P < .002$). We also investigated the relationship between CCVDs and peripheral lymphedema, which was identified in 2 ways: by a history of perinatal peripheral lymphedema and/or current peripheral lymphedema and by the presence of dysplastic fingernails. These diagnoses, each including 30% to 35% of study participants, were not associated with BAV, COARC, or webbed neck.

To clarify the specificity of the association between webbed neck and cardiovascular anomalies, we investigated the association between webbed neck and renal anomalies. Renal anomalies, including horseshoe kidney, single kidney, and duplicated collecting systems, were found in 28 (21%) of 134; 11 of these had a webbed neck, and 17 did not ($\chi^2 = 0.385$, $P = .65$). There was no association between the presence of BAV or COARC and renal anomalies (BAV: $\chi^2 = 0.24$, $P = .99$; COARC: $\chi^2 = 1.8$, $P = .2$). The presence of renal anomaly, however, was significantly associated with the 45,X karyotype ($P < .02$).

The prevalence of congenital BAV or COARC by TS karyotype is shown in Table 2. This table is provided primarily as a reference for meta-analyses to accumulate data on genotype-phenotype correlations in larger numbers of subjects, because there are clearly too few individuals in genotype groups other than 45,X to allow any statistically meaningful inferences from this study alone. It is worth noting, however, that a relatively large percentage of 45,X/46,XY individuals have CCVDs. Adding our data to that previously reported, summarized by Sybert,[3] the prevalence of CCVDs in 45,X/46,XY is 14 (50%) of 28, substantially higher than the 30% to 40% typically noted in 45,X. To facilitate the localization of the CCVD locus on the X chromosome, we listed separately individuals with virtually complete deletions of Xp or Xq. We found BAV in 1 of 7 participants with 46,XiXq—which, combined with the 3 of 27 reported by Sybert for a prevalence of 12%, is significantly above that of the normal population, whereas to our knowledge, there are no reported congenital heart defects in subjects with 46,XXq-.

TABLE 1. Association Between Webbed Neck and Cardiac Anomalies in TS

Webbed Neck	Aortic Valve			COARC			BAV or COARC		
	BAV	TAV	Total	COARC+	COARC−	Total	Either	Neither	Total
Web+	16	28	44	11	36	47	18	26	44
Web−	10	71	81	5	82	87	15	67	82
Total	26	99	125	16	118	134	33	93	126
χ^2		10.0			9.05			8.5	
P		.002			.003			.004	
OR (95% CI)		4.1 (1.6–10.0)			5.0 (1.6–15.5)			3.3 (1.5–7.4)	

OR indicates odds ratio; CI, confidence interval.

TABLE 2. Genotype and Congenital Cardiovascular Defects (BAV or COARC)

	45X	45X/46XX*	45X/46XXp_{del}	46X,Xp_{del}	45X/46XY	46X,Xq_{del}	45X/46XXq_{del}	Total
N	83	21	14	8	5	2	2	134
No with cardiac defects	27	0	2	1	3	0	1	34
Incidence of cardiac defects, %	33	0	14	13	60	0	50	25

X* indicates a normal X, ring X, or X with partial q or p deletions.
Xp_{del}-includes only the most proximal small arm deletions (<11.3) and iXq.

DISCUSSION

The present observations in a large group of participants with TS not selected for cardiovascular disease confirms a significant association between central fetal lymphedema, signaled by neck webbing, BAV, and COARC. We have also shown that the association between neck webbing and CCVDs is not merely a reflection of a more severe phenotype in 45,X individuals but is a statistically significant connection independent of karyotype and parental imprinting and specific for cardiovascular but not renal anomalies. Our study confirms the modern observation of Mazzanti et al[2] that COARC is significantly associated with neck webbing and extends the finding to BAV as well. This highly significant association between central lymphedema during fetal development and CCVDs could reflect a cause and effect relation between these 2 features.

One study suggested that defective cardiovascular development may be a primary feature in TS, with impaired lymphatic development and generalized hydrops as consequences.[9] This proposition seems unlikely for a number of reasons. The anatomic defects associated with fetal lymphedema in TS are decreased numbers of lymphatics and dilated lymphatic channels that end in distended sacs, lacking connections with the venous system.[4,6,10,11] The developmental delay is particularly pronounced in the axillojugular lymphatics,[10] which drain the upper half of the body. The nuchal cystic hygromas are loculated collections of lymph associated with blind-ended jugular lymphatics. Central lymphatic obstruction is apparent in fetuses with TS as early as 10 to 12 weeks but resolves during the latter half of gestation and is detected at birth only by the redundant skin folds of the neck. It is difficult to imagine how delayed or defective lymphatic development could be caused by heart failure as shown in the Barr and Oman-Ganes study.[9] It is equally difficult to reconcile this notion with the observation of many individuals who have TS with neck webbing, with or without CCVDs, but no sign of heart failure. It seems more likely that severe lymphatic obstruction early in fetal development may cause heart failure from compression and/or impaired filling of developing cardiovascular structures, leading to fetal hydrops and demise, as was the case for most of the cases examined in the Barr and Oman-Ganes study.[9] Thus, if there were a cause-and-effect relation between fetal lymphedema and CCVDs in TS, then it would seem most likely that the lymphedema is primary.

Clarke[4] suggested that centrally localized distended lymphatics compress the developing aortic root, resulting in specific left-sided defects, including hypoplastic left heart, BAV, and COARC as a result of low flow, and specific right-sided defects such as persistent left superior vena cava, anomalous pulmonary venous return, and dilated right atrium as a result of back-pressure from obstruction to forward flow. The Clarke hypothesis seems plausible and does include the constellation of CCVDs most commonly found in TS, but after 20 years, it remains hypothetical, without direct proof. Although anatomic evidence shows that dilated lymphatics are observed in the vicinity of developing aorta, no correlation has been observed between the presence of dilated lymphatic and aortic defects.[4,6,7] The present study provides a significant correlation between the presence of CCVDs and centralized fetal lymphedema but obviously cannot establish cause and effect.

An alternative explanation for the association between neck webbing and CCVDs in TS is that haploinsufficiency for an X-chromosome gene independently causes fetal lymphedema (webbed neck) and congenital heart defects. Haploinsufficiency for an autosomal gene (*FOXC2;*16q) causes widespread lymphedema and occasional cardiac defects in the lymphedema-distichiasis syndrome,[12] but targeted deletion of this gene in mice results in abnormal aortic arch development in the absence of obvious lymphedema.[13] These observations suggest that the heart defects and lymphedema found in the human disorder may be independent effects of the same gene. Noonan syndrome may be another instance in which lymphedema and cardiac defects occur as independent effects of the same gene mutation/deletion. Heart defects are more common and include predominantly right-sided lesions in Noonan syndrome compared with TS.[14] Also in contrast to TS, Noonan syndrome manifests few abnormal features during early fetal development, with cystic hygroma being a rare finding and residual stigmata of fetal lymphedema are less common in Noonan syndrome.[15–17] Attempts to correlate webbed neck and cardiovascular lesions in this syndrome have been conflicting.[5,18] A specific gene mutation that is responsible for ~50% of Noonan syndrome cases has only recently been identified[19]; thus, the earlier studies that attempted to associate CCVDs and lymphedema may have included genetically heterogeneous populations.

The role of fetal lymphedema in the pathogenesis of CCVDs in TS may be resolved when the genes that are responsible for lymphedema are identified, allowing experimental studies to elucidate its function in developing lymphatic and cardiovascular systems. With this goal, a recent study has suggested that the "critical region" for the TS lymphedema gene lies at Xp11.4.[20] The careful phenotypic characterization of additional patients with TS and infor-

mative X-chromosome deletions will further advance this effort, aided by newly available X-chromosome sequence information.

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THE COST OF MEDICAL EDUCATION

"The cost of obtaining a medical education has been spiraling upward for the past 20 years. . . . The average tuition and fees at public medical schools during the 2003–2004 academic year amounted to $16 153, and the corresponding figure for private schools was $32 588. Adding $20 000 to $25 000 for living expenses, books, and equipment brings the estimated cost of 4 years of attendance to about $140 000 for public schools and $225 000 for private schools. . . . The average debt carried by 1984 graduates was $22 000 for public school and $26 500 for private school. By 2004, the debt had increased to $105 000 for public school and $140 000 for private school, and only about 20% of medical students graduated with no debt."

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