Aortic tissue analysis in Turner syndrome

Bruno Donadille, Alexander Valent, Kisaki Amemiya, Nicolas Rive Le Gouard, Laurence Iserin, Paul Achouh, Tatiana Lecot-Connan, Patrick Bruneval, Jean-Pierre Siffroi, Sophie Christin-Maitre

Title: Aortic tissue analysis in Turner syndrome

Authors: Bruno Donadille, MD[a]; Alexander Valent, MD, PhD[b]; Kisaki Amemiya, MD, PhD[c,d]; Nicolas Rive le Gouard, MD[e]; Laurence Iserin, MD[f]; Paul Achouh, MD, PhD[g,h]; Tatiana Lecot-Connan, MD[a]; Patrick Bruneval, MD[c,h]; Jean-Pierre Siffroi, MD, PhD[e,i] ; Sophie Christin-Maitre, MD, PhD[a,i]

Total word count: 800

Affiliations:

a) Department of Endocrinology, Saint Antoine Hospital, Assistance Publique-Hôpitaux de Paris, Sorbonne Université. *Centre de Référence des Maladies Endocriniennes Rares de la Croissance et du Développement* (CRMERCD), Filière FIRENDO, Endo-ERN [739527], Paris, France.
b) Department of Molecular Pathology, Cytogenetics and Medical Biology, Institut Gustave Roussy, Villejuif, France.
c) Department of Pathology, European Georges Pompidou Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France.
d) Department of Pathology, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan.
e) Departement of Cytogenetics, Armand Trousseau Hospital, Assistance Publique-Hôpitaux de Paris, Sorbonne Université, Paris, France.
f) Department of Adult Congenital Cardiopathies, European Hospital Georges Pompidou, Assistance Publique-Hôpitaux de Paris, Paris, France.
g) Department of Cardiovascular Surgery, European Hospital Georges Pompidou, Assistance Publique-Hôpitaux de Paris, Paris, France.
h) Université Paris Cité, INSERM U970 PARCC, Paris, France.
i) Sorbonne Université, INSERM U933, Paris, France

Sources of Funding: Dr Kisaki Amemiya was supported in part by the «Programme pour la formation des résidents étrangers des hôpitaux de Paris à l'Assistance Publique-Hôpitaux de Paris».

Disclosures: None.

Corresponding author: (E-mail): bruno.donadille@aphp.fr; Reference Center for Rare Endocrine Growth Diseases; Saint Antoine Hospital, Sorbonne Université; AP-HP; 187 rue du Faubourg St Antoine, 75012 PARIS, France. (Phone): +331 49 28 24 00

Keywords: Turner syndrome; Aortic dilatation; Aortic Aneurysm; FISH analysis; Karyotype.

Non-standard Abbreviations and Acronyms:

AD: Aortic dilatation;
ASI: Aortic size index;
BAV: Bicuspid aortic valve;
ERCB: Ethics Review Committee for Biomedical research
FFPE: Formalin-fixed and paraffin-embedded;
MDC: Media degenerative changes;
MEMA: Mucoid extracellular matrix accumulation (-I: Intralamellar and –T: Translamellar);
TAA: Thoracic aorta aneurysm.

Prevention of aortic dissection is difficult in patients with Turner syndrome (TS). The aim of our study was to describe aortic walls' cytogenetics and histology in patients having a prophylactic surgery for a thoracic aorta aneurysm (TAA).

TS affects 1/2500 female at birth [1]. In half of cases, a complete loss of one X-chromosome is found in blood lymphocyte karyotype. A 45,X/ 46,XX mosaicism is present in 15-20% of patients. A structural anomaly of the X chromosome (isochromosome, ring) is present in the remaining patients.

Congenital heart abnormalities in TS include bicuspid aortic valve (BAV) and aortic coarctation, in 20-30% and 15-20% of patients, respectively [2]. Aortic dilatation (AD) is common in TS [3]. The risk factors for aortic dissection are BAV, aortic coarctation, AD, hypertension, a 45,X karyotype and pregnancy. According to the current guidelines [1,2], prophylactic surgery is recommended to prevent any dissection event, when the ascending aortic size index (ASI) is higher than 25 mm/m².

TS patients were recruited from our Reference Center for Rare Diseases (Endo-ERN). This study was approved by the Paris North ERCB (N°12-029). Aortic histology and media degenerative changes (MDC) score were assessed using a semi quantitative score [4]. The X monosomy was searched in blood karyotype, buccal smear and aortic media using Fluorescence *in Situ* Hybridization (FISH). For each aortic tissue, one hundred cells (media/adventice) were analyzed. These results were compared to data obtained from the blood karyotype and buccal smears FISH results, when available. For one mosaic patient, an additional FISH with TFE3 probe (Xp11.23) was performed on 50 nuclei to confirm the aortic mosaicism.

Eleven aortic tissues from 11 patients were included. The patients' median age at prophylactic aortic surgery was 39.0 years (IQR: 29.5-46.5). Their median age at TS diagnosis was 8.0 years (IQR: 2.8-13.0). BAV was present in 10/11 cases. According to Sievers' classification [5], 6/11 patients had a type 1 BAV, with one raphe and fusion of both coronary cusps (L-R).

The median ascending aortic diameter and ASI at surgery were 44 mm (IQR: 39.0-45.5) and 29.0 mm/m² (IQR: 26.7-30.1), respectively. The aortic surgical techniques were as follows: Yacoub (5/11), Bentall (5/11) and Tyron-David (1/11).

The blood karyotypes showed: an homogenous X monosomy in 7/11 cases, a 45,X/46,XX mosaicism in 1/11 and a structurally abnormal X chromosome in 3/11 cases (two X ring and one Xq isochromosome).

An early MDC (**Figure 1A**) was found in all patients. The MDC score was 9.0 (IQR: 7-10.5). It was characterized by a loss of smooth muscle cells, a mucoid extracellular matrix accumulation (MEMA-I or/and MEMA-T), as well as loss and/or fragmentation of elastic fibers.

In aortic media walls, the X monosomy was frequent, as it was present in 7/11 of cases. The level of 45, X mosaicism was otherwise higher than 60%.

Interestingly, one patient (**Figure 1B**) with a low 45,X mosaicism in blood (5%), had a higher rate of X monosomy (70%) in endothelial and muscle components of her ascending aortic media.

To our knowledge, this is the first aortic FISH description in patients with TS having prophylactic surgery. Histology revealed an early MDC in all cases, even in young TS patients. Our group previously identified similar levels of MDC in the aorta of non-TS patients with TAA, with or without BAV [4]. In TS patients, MDC was present, even with a low percentage of 45,X monosomy in blood. Therefore, a low level of 45,X monosomy in blood should not be reassuring concerning an aortic dissection risk.

References

1. Gravholt CH, Andersen NH, Conway GS, et al. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. *Eur. J. Endocrinol*. 2017; 177:G1–G70.

2. Silberbach M, Roos-Hesselink JW, Andersen NH, et al. Cardiovascular Health in Turner Syndrome: A Scientific Statement From the American Heart Association. *Circ. Genomic Precis. Med*. 2018; 11:e000048.

3. Donadille B, Tuffet S, Cholet C, et al. Prevalence and progression of aortic dilatation in adult patients with Turner syndrome: a cohort study. *Eur. J. Endocrinol*. 2020; 183:463–470.

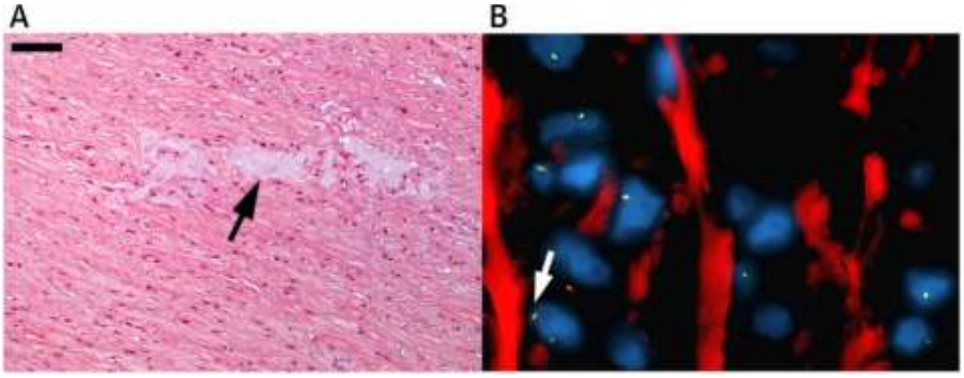
4. Amemiya K, Mousseaux E, Ishibashi-Ueda H, Achouh P, Ochiai M, Bruneval P. Impact of histopathological changes in ascending aortic diseases. *Int. J. Cardiol*. 2020; 311:91–96.

5. Sievers H-H, Stierle U, Mohamed SA, et al. Toward individualized management of the ascending aorta in bicuspid aortic valve surgery: the role of valve phenotype in 1362 patients. *J. Thorac. Cardiovasc. Surg*. 2014; 148:2072–2080.

Figure 1:

1A: Histology of an aortic wall showing a MEMA-T (arrow) and an aortic media multifocal extension. Bar = 200 micrometers.

1B: FISH analysis on the FFPE section of an aortic tissue: A 45,X monosomy is found in the majority of the nuclei of the aortic media (single green signal ; DXZ1 probe). In rare nuclei, 2 green signals are present (arrow).


A
B