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ARTICLE

X-linked transposition of the great arteries and incomplete penetrance among males with a nonsense mutation in *ZIC3*

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We report on a Lebanese family in which two maternal cousins suffered and died very early in life from cardiac malformations. Both presented with a transposition of the great arteries associated with one or several other cardiac defects. Various minor midline defects were also observed, but there were no situs abnormalities other than a persistent left superior vena cava in one. A maternal uncle of these two babies was born cyanotic and died on the third post-natal day. Analysis of the *ZIC3* gene, revealed the presence of a mutation in the second exon leading to a truncation of the protein. Surprisingly, another maternal uncle of the two affected cousins also had the mutation but was not clinically affected. To our knowledge, this is the first instance of incomplete penetrance in a male for a mutation in a chromosome X gene.
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Introduction

The transposition of the great arteries (TGA) is a cardiac malformation in which the right ventricle is connected to the aorta, whereas the left ventricle is connected to the pulmonary artery. It accounts for about 4–10% of all cyanotic heart defects, and is observed about twice as frequently in males as in females.[1,2] It usually occurs sporadically and is either isolated or associated with various defects, cardiac and non-cardiac.[3] The incidence of congenital heart defect in relatives of children wit TGA is in the range 0.27–2% depending on the type of TGA.[4] In TGA, a patient's life is possible only if there is at least one communication between the two vascular systems that allows mixing between oxygenated and non-oxygenated blood. Beside putative multifactorial origins, a deletion in the 22q11.2 locus[5,6] and a mutation in the *ZIC3* gene,[7] a zinc-finger transcription factor, have been found to be associated with TGA. This gene (OMIM 306955) was identified in a family with lateralisation defects (or heterotaxy) and midline development anomalies.[7]

Here, in a Lebanese family where males were affected by TGA, we have identified a novel mutation in the *ZIC3* gene that causes a truncation of the protein. Surprisingly, a maternal uncle of the affected males also had the mutation but was not clinically affected. To our knowledge, this is the first instance of incomplete penetrance in a male for a mutation in a chromosome X gene.

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Subject, material and methods

Clinical investigations

In the Lebanese family presented in Figure 1, each member was clinically examined by a cardiologist and a geneticist. Electrocardiogram, chest X-ray, cardiac and abdominal echographies were obtained from the surviving founder (I-2, Figure 1) of the family and her descendants. Clinical features of the two affected cousins are summarised in Table 1.

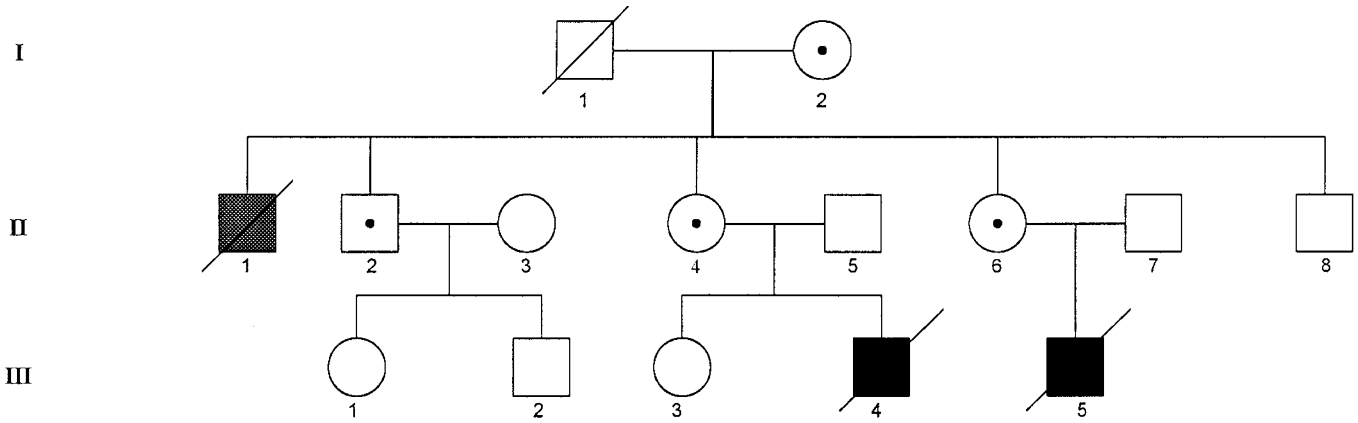

Figure 1 Pedigree of the family. Solid symbols represent affected patients, shaded symbol the unspecific congenital heart defect; dotted symbols the mutation carrier cases.

Table 1 Clinical summary of the two affected cousins

	Patient 1	*Patient 2*
Sex	M	M
Birth weight (centile)	3650 (75)	3150 (40)
Birth length (centile)	49 (50)	49 (50)
Head circumference at birth (centile)	36 (50)	?
Cyanosis at birth	+	+
Mild hypertelorism	+	+
Broad nasal base	+	+
Craniosynostosis	+	–
Transposition of the great arteries	+	+
Other cardiac defects	–	+
Respiratory distress	+	+
Early death	+	+
Normal karyotype	+	+

The propositus (case III-4) was born at term after normal gestation and delivery. Birth weight was 3650 g (75th centile), length 49 cm (50th centile), and occipitofrontal circumference 36 cm (50th centile). He was cyanotic at birth and had severe respiratory distress. There was moderate hypertelorism, broad nasal base, and fusion of the sagittal sutures (Figure 2a). Heart investigations revealed a TGA with side-to-side aorta and pulmonary arteries. The right coronary artery was coming out of the left coronary system, running across the outflow tract of the right ventricle. He was operated on the 5th post-natal day, by a Senning procedure. No undiagnosed abnormalities were detected during the operation. In particular, the diaphragm and lungs were normal. A post-operative abdominal echography was unremarkable. The post-operative course up to the 5th month was dramatic with repeated episodes of bronchopulmonary infections, pleural effusions, and heart failure. A karyotype was performed and was found to be a normal 46,XY, with no microdeletion of the CATCH 22 region after FISH analysis with specific probes. The infant died at 5 months from severe bronchiolitis.

A maternal cousin (case III-5) was delivered a few months later by cephalic presentation at 40 weeks. Gestation was unremarkable and there was no exposure to toxins or known teratogens. Birth weight was 3150 g (40th centile), and length 49 cm (50th centile). At birth he presented like his affected cousin, wholly cyanotic, and with severe respiratory distress.

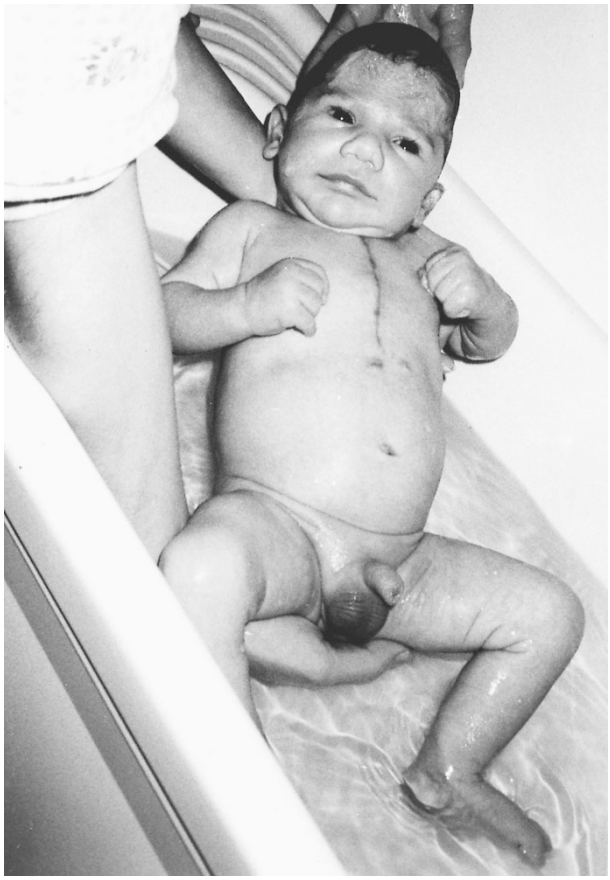

Figure 2a Patient III-4 at age 2 months. Note the large nasal base.

There was moderate hypertelorism, large nasal base, definite right peripheral facial palsy, and a polyp on the right ear. A cardiac echography showed a left superior vena cava, atrial septal defect of the sinus venosus type, a large atrioventricular canal, a hypoplastic right ventricle, severe subvalvar and valvar pulmonary stenosis, and complete d-TGA (Figure 2b). These findings were confirmed by angiocardiography. Convulsive seizures occurred on the 4th and 5th day and were found to be related to severe hypocalcaemia (1.5 mmol/l). Further search for hypoparathyroidism, and abnormal T and B cells was not performed. MRI of the brain and abdominal ultrasounds were normal. The baby died in respiratory distress, with heart failure on the 15th post-natal day. Autopsy was not accepted by the parents. Chromosome study of lymphocytes with high resolution G and R-banding showed a normal 46,XY karyotype. Fluorescent hybridisation with probes KI506 (*D22S139*), and c48F8 (*HIRA*) failed to detect a microdeletion in the 22q11.2 locus which ruled out a diagnosis of DiGeorge syndrome.

Family history disclosed a maternal uncle (II-1) who was also cyanotic at birth, and had died on the 3rd postnatal day. Clinical investigations of all the other members of the family, in addition to systematic chest X-rays, electrocardiogram, cardiac and abdominal echographies were found to be normal, except for case II-4 who has a floppy mitral valve and case II-6 who had a mild mitral valve regurgitation.

Mutational analysis

After familial investigations, EDTA blood samples were collected for genetic studies. Informed consent was obtained from each family member before blood sampling and DNA analyses.

DNA was extracted from lymphocytes by standard methods,[8] and amplified according to primers in Gebbia *et al*.[7] Amplicons were sequenced by dye terminator (FS) according to the manufacturer's instruction (Perkin Elmer, Norwalk, CT, USA). All amplicons were sequenced in both forward and reverse directions.

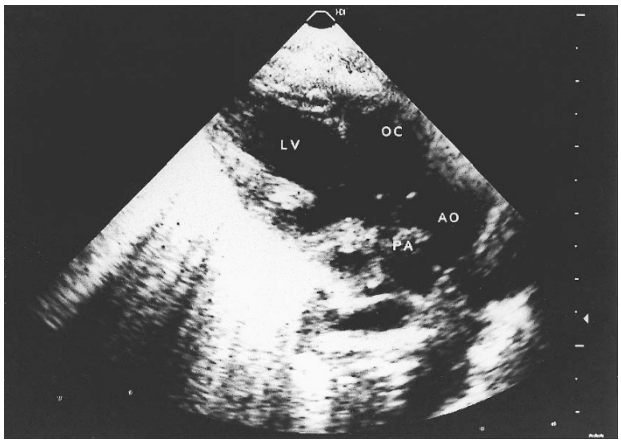

Figure 2b Cardiac echography of case III-5 showing a TGA: the pulmonary artery (PA) which emerges from the left ventricule (LV), lies posteriorly and parallel to the aorta (AO) which emerges from the right ventricle (OC: outflow chamber).

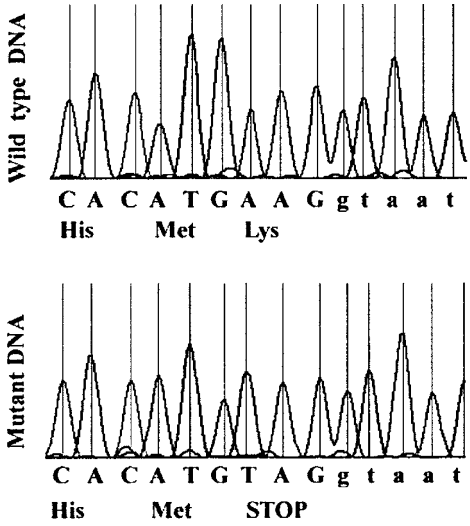

Figure 3a DNA sequence analysis at the end of exon 2 and beginning of flanking intron of a normal individual (top) and patient II-5 (bottom) who carries the A1741T transversion resulting in a lysine to amber mutation. Exonic sequences are in capital letters. Nucleotide number refers to the cDNA sequence of *ZIC3* in AF028706.

Mutation screening

Two-hundred ng genomic DNA were amplified with primers zic25F (5'-CTTGCCTCTGAGAAACTC-3') and zic26R (5'-GAAGACAGAGGGTTGGCA-3') according to Gebbia *et al*[7] in a 25 μL volume. A first stage at 95°C for 5 min was followed by 35 cycles of 94°C for 1 min, 63°C for 1 min, 72°C for 1 min. A final elongation stage was performed at 72°C for 10 min. To 10 μL of PCR, we added 5 U of Af1III, 1.5 μL of buffer and H2O to a total volume of 15 μL. After a 4 h incubation at 37°C, samples were run on a 0.8% agarose gel.

Results

Sequencing of *ZIC3* gene for case III-5, revealed the presence of a A1741T transversion resulting in a stop codon, 3 amino-acids before the end of the last zinc finger domain, leading to a truncation of the protein (Figure 3a). The mutation results in a new Af1III restriction site generating 223 and 102 fragments of the 325 nucleotide PCR amplification product upon digestion (Figure 3b).

Analyses of *ZIC3* gene for cases I-2, II-2, 4, 6, and 8, revealed that cases I-2, II-4 and II-6 were normal carrier women, whereas case II-8 was not a carrier (Figure 3b). Surprisingly, case II-2 was found to be a non-affected male carrier who had the same mutation as his affected nephew but no cardiac or extra-cardiac anomalies (Figure 3b). To confirm this result, the analysis was repeated on a new blood

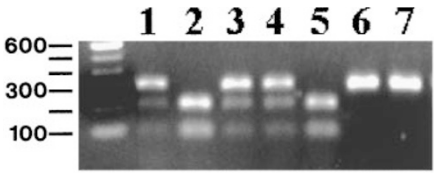

Figure 3b Digestion of PCR amplicons with Af1III. The mutation results in a new Af1III restriction site generating a 223 and a102 fragments of the 325 nucleotide fragment. Left lane: size marker (100 base-pair ladder), 1: I-2; 2: II-2; 3: II-4; 4: II-6; 5: III-5; 6: II-8; 7: control.

sample. The presence of 3 affected males and 3 unaffected carrier females in this family suggested an X-linked recessive inheritance but it was surprising to observe a case of incomplete penetrance in a male with a truncated ZIC3 protein resulting in an incomplete 5th zinc finger domain (Figure 3c). Random DNA samples from 100 males and 100 females (300 X chromosomes) were subsequently tested for this same mutation, and none was found.

Discussion

Recently, Gebbia *et al*[7] reported the positional cloning on Xq26.2 of a gene responsible for X-linked situs abnormalities.[7] This gene was referred to as *ZIC3*, a putative zinc-finger transcription factor, which shares 98% amino acid similarity with *Zic3* (Zinc-finger protein of the cerebellum) in mice,[9] that may act upstream of many known developmental regulatory genes.[10] It was cloned specially after the analysis of a family in which 11 males in two generations had complex cardiac defects, including TGA, lateralisation and midline defects,[7,11] which emphasises a possible role of *ZIC3* in left-right body axis formation of the embryo to establish normal left–right asymmetry during development.[7] Although a disruption in the molecular cascade that leads to normal situs may result in situs randomisation, this is not always the case. For example, a mouse knockout of the inversine gene[12] has nearly always a reversal of situs. Moreover, Gebbia *et al* reported that 'regardless of the specific ZIC3 mutation identified to date, all affected males are situs ambiguus'.[7] The only exception prior to this report is a 1.3 Mbp deletion in a male with features of VACTERL-H association but no situs anomaly.[13]

By contrast with patients who have been previously reported with a mutation in *ZIC3* gene,[7,13] our patients did not present situs abnormalities, nor spleen, lung or cerebromeningeal defects. They had TGA associated with other major cardiac defects, and non-cardiac defects such as hypertelorism, large nasal base, and craniosynostosis. The only anomaly that could be related to a lateralisation defect is the persistence of the left superior vena cava which normally becomes atretic. On the other hand, one might view TGA as a left–right body axis defect confined to the heart. In fact, intrafamilial variability of the defect of situs determination has already been observed. For example, in a family reported by Zlotogora *et al* the affected sibs presented situs inversus, situs inversus with heart malformations, or heart malformations without situs abnormalities.[14] Primary ciliary dyskinesia (PCD) (MIM 242650), an autosomal recessive disorder, is characterised by recurrent upper and lower respiratory tract infections referred to as Kartagener syndrome when associated with situs inversus (MIM 244400). Although PCD (also referred to as immotile cilia syndrome) is generally associated in about 50% of the cases with situs inversus,[15] it is not true for New Zealand Maoris and Samoan

MTMLLDGGPQFPGLGVGSFGAPRHHEMPNREPAGMGLNPFGDSTHAAAAAAAAAAFKLSPAAAHDLSSGQ

SSAFTPQGSGYANALGHHHHHHHHHHHTSQVPSYGGAASAAFNSTREFLFRQRSSGLSEAASGGGQHGLF

AGSASSLHAPAGIPEPPSYLLFPGLHEQGAGHPSPTGHVDNNQVHLGLRGELFGRADPYRPVASPRTDPY

AAGAQFPNYSPMNMNMGVNVAAHHGPGAFFRYMRQPIKQELSCKWIDEAQLSRPKKSCDRTFSTMHELVT

HVTMEHVGGPEQNNHVCYWEECPREGKSFKAKYKLVNHIRVHTGEKPFPCPFPGCGKIFARSENLKIHKR

THTGEKPFKCEFEGCDRRFANSSDRKKHMHVHTSDKPYI CKVCDKSYTHPSSLRKHMKVHESQGSDSSPA

ASSGYESSTPPAIASANSKDTTKTPSAVQTSTSHNPGLPPNFNEWYV

Figure 3c Human ZIC3 amino-acid sequence (access number: AAC05594). Zinc-finger domains are shaded. The previously reported mutations are indicated by the symbol ▲ (Gebbia[7]). The symbol ⇩ shows the beginning of the frameshift due to the 1507insTT (Gebbia[7]). The A1741T nonsense mutation reported in this study is indicated by an arrow ↑.

islanders who suffer from PCD but not from situs defect.[16] Thus, in the present report, the absence of situs abnormalities could be explained by the different expressivity of the syndrome related to a mutation in *ZIC3*, or by the particular mutation found. Indeed, this nonsense (amber) mutation, three amino-acids before the end of the last zinc finger domain (Figure 3c), leads to a truncated protein with probably some residual activity, whereas the previously reported mutations (Figure 3c) resulted in a protein truncation or alteration before the third zinc finger,[7] or in a complete deletion of the gene.[13] It can be speculated that these five zinc finger domains play a different role during development with the 5' ones being active during the lateralisation process and the 3' ones later in development. Thus, we suggest that a number of male patients with isolate or complex TGA might have a mutation in the *ZIC3* gene even if they present no signs of situs abnormalities, explaining in part the excess of males with TGA.

The penetrance of mutations in the *ZIC3* gene seems to be highly variable. In Gebbia's report, one family had three affected women who presented with situs inversus and ureteral or anal anomalies, out of six carrier females.[7] There is no doubt that their features are related to their carrier status. Relating the minor valve anomalies in carrier mothers II-4 and II-6 of the present report to the nonsense mutation is more difficult because these anomalies are not rare. Mitral valve prolapse, for instance, is observed in about 3% of the normal population.[17]

To our knowledge, case II-2 is the first case of incomplete penetrance in a male carrier of a chromosome X mutation ever reported. Male incomplete penetrance was suggested in fragile X syndrome until the elucidation of the underlying molecular mechanism. It is possible that the nonsense mutation in the last zinc finger domain leaves some activity to the protein and/or redundant protein(s) compensate(s) this 'mild' ZIC3 deficiency. Finally, craniosynostosis has never been reported before to be associated with TGA. It could be fortuitous, or part of a hitherto unreported X-linked TGA syndrome affecting this family. Reports on additional families or patients with *ZIC3* mutations would be helpful to better understand the action of this gene and expand its story.

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