Electronic letter

Analysis of the *SRY* gene in Turner syndrome patients with Y chromosomal material

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EDITOR—Turner syndrome is one of the most common chromosomal abnormality syndromes affecting 1 in 2500 liveborn females. The syndrome is characterised by short stature, gonadal dysgenesis, congenital heart disease, renal anomalies, and a variety of somatic features including neck webbing, cubitus valgus, short neck, and widely set nipples. Nearly half of the patients have a classical 45,X karyotype while others have structurally abnormal sex chromosomes (for example, 46,X,i(Xq)) or are mosaics with other cell lines with normal (46,XX) or abnormal sex chromosomes.¹⁻⁴ Among these, patients with Y chromosomal material require specific attention since many of these 45,X/46,XY Turner syndrome patients develop gonadoblastoma or dysgerminoma later in life.^{5,6}

Conventional chromosomal analysis indicates that 4-20% of patients with Turner syndrome have a Y chromosome or its derivatives.¹⁻⁴ These figures could be even higher, since the more sensitive PCR based analysis has shown that 15-60% of cytogenetically 45,X females have Y chromosomal material.⁷⁻⁹ These findings mean that 10-50% of all Turner syndrome patients have Y chromosomal material and therefore are, to some extent, at risk of developing gonadoblastoma. A more precise understanding of the mechanism leading to generation of a 45,X/46,XY karyotype is therefore important for providing better care for these patients.

Karyotypes such as 45,X/46,XY are presumably caused by mitotic loss of the Y chromosome from the originally 46,XY fetus. It is not known, however, whether there is a predisposition towards the loss of the Y chromosome in these subjects or whether it is merely a random event caused by the inherent instability of XY chromosomal pairing. With regard to this question, Canto *et al*¹⁰ recently reported the surprising finding that mutations in the *SRY* gene occurred in two out of three subjects with a 45,X/46,XY karyotype, one with a cytogenetically normal Y chromosome and the other with a Y derived marker chromosome. In these two patients, they identified an identical missense mutation (G2128A, Ser18Asn) upstream of the high mobility group (HMG) box of the gene. This not only implies that 45,X/46,XY Turner syndrome comprises a

phenotypic spectrum with 46,XY females in which 10-15% have *SRY* mutations,^{11,12} but also suggests that single point mutations in the sex determining gene may cause gross structural chromosomal abnormalities, for example, formation of a marker chromosome or even the loss of the whole Y chromosome.

Methods

In order to determine the actual frequency of *SRY* mutations in 45,X/46,XY patients, we systematically analysed the Y chromosome and the *SRY* gene in 90 consecutive Turner syndrome patients seen at one of our institutions and sequenced the *SRY* gene in 11 patients with Y chromosomal material. Blood samples were drawn after informed consent. Genomic DNA was then isolated from peripheral blood leucocytes using a QIAmp Blood Kit (QIAGEN, Germany) according to the supplier's instructions. A highly repetitive sequence in the Y centromere (DYZ3) was amplified by using a primer set 5'-TGAAACTGCACAGAAGCTG-3'/5'-ACACATCACAAAGAACTATG-3'. The amplification procedure consisted of initial denaturation at 94°C for 10 minutes, then 30 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for two minutes in 25 µl reactions containing 50 ng of genomic DNA, 10 mmol/l Tris HCl (pH 8.3), 1.5 mmol/l MgCl₂, 50 mmol/l KCl, 0.2 mmol/l of dNTPs, 0.001% (w/v) gelatin, 25 pmol of each primer, and 1 unit of *Taq*Gold DNA polymerase (PE-Applied Biosystems, CA). The whole coding region of the *SRY* gene was amplified as two overlapping fragments using two primer sets (5'-TTGAGAATGAATACATGTGCAGGG-3'/5'-GGGTCGCTTCACTCTATCCTG-3' and 5'-CAGTGTGAAACGGAGAGAAAACAGT-3'/5'-CTTTGCATGTTAATCGTGTGACAC-3') (fig 1). The amplification was performed under the same conditions as those for DYZ3. PCR products were purified with a Wizard PCR Preps DNA

Figure 1 Locations of the primers used to amplify and sequence the *SRY* gene (arrowheads). The rectangle represents the coding region of the gene.

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Table 1 Profiles of Turner syndrome patients with the *SRY* gene. Patients KK, AO, and NI were prepubertal, so that their pubertal status could not be assessed

Patient	Karyotype (mosaic ratio)	Gonadal status
1	45,X/46,X,+mar(Y) (20:5)	LH 127.2 mIU/ml, FSH 99.2 mIU/ml, virilisation (+), gonadoblastoma (+)
2	45,X/47,XXY (36:64)	LH 31.6 mIU/ml, FSH 124.5 mIU/ml, virilisation (+), gonadoblastoma (+)
3	45,X/46,X,+mar(Y) (15:22)	LH 10.7 mIU/ml, FSH 72.4 mIU/ml
4	45,X/46,X,+mar(Y) (15:5)	NA
5	45,X/46,X,+mar(Y) (14:16)	LH 32.8 mIU/ml, FSH 147.5 mIU/ml
6	45,X/46,X,+mar(Y) (42:8)	NA
7	46,X,+mar(Y)	LH 19.6 mIU/ml, FSH 103.7 mIU/ml
8	45,X/46,X,+mar(Y) (1:9)	LH 44.4 mIU/ml, FSH 94 mIU/ml, gonadoblastoma (+)
9	45,X/46,X,+mar(Y) (300:2)	LH 15 mIU/ml, FSH 176.1 mIU/ml
10	46,X,+mar(Y)	NA
11	45,X/46,X,+mar(Y) (1:1)	LH 18 mIU/ml, FSH 51 mIU/ml, streak gonad

purification kit (Promega, WI) and directly sequenced with a BigDye Terminator Cycle Sequencing Kit (PE-Applied Biosystems) and an automatic sequencer (ABI-PRISM 310, PE-Applied Biosystems).

Results

PCR amplification of *DYZ3* showed the presence of Y chromosomal material in 12 out of 90 Turner syndrome patients (13.3%). Among these 12 patients, *SRY* was identified in 11 whose profiles are shown in table 1. All were phenotypically female, and except for three prepubertal patients, the basal levels of gonadotrophins suggested severe gonadal failure (gonadal levels of FSH >60 mIU/ml). One patient had a cytologically obvious Y chromosome, while others had small marker chromosomes whose origins were identified by PCR and FISH analysis using a Y centromere specific DNA probe (*DYZ3*). Four of the 11 patients underwent gonadectomy and gonadoblastoma was identified in three of them, while one patient had bilateral streak gonads. Two of the patients with gonadoblastoma showed signs of virilisation before gonadectomy. Patient 9 was initially believed to have a 45,X karyotype, but after detection of the presence of *DYZ3* by PCR, FISH analysis and karyotyping were repeated on 300 cells to show a low level mosaicism containing cells with Y chromosomal material. Sequencing analysis of the *SRY* gene showed no mutation in the coding region of the gene in any of these 11 patients.

Discussion

In contrast with the study by Canto *et al.*¹⁰ which identified two mutations in the *SRY* gene out of three patients, we could not find any mutation in the coding region of *SRY* in our 11 Turner syndrome patients with Y chromosomal material. The clinical phenotypes of our patients were, however, very similar to their subjects and we currently do not have an explanation for the discrepancies in our findings. Since our study population was an unbiased one, however, our results suggest that mutations in the *SRY* gene are not common in patients with 45,X/46,XY. Most 45,X/46,XY karyotypes are probably caused by different mechanisms from those leading to the generation of 46,XY females. When Y chromosomes

have gross structural abnormalities, it is quite conceivable that the instability of XY chromosomal pairing would lead to the loss of the Y chromosome. In contrast, it appears difficult to envisage a mechanism of point mutations in the *SRY* gene that would lead to loss of the whole Y chromosome. However, it is still possible that, in the case of 45,X/46,XY patients whose Y chromosome appears cytogenetically normal, mutations in other genes in the Y chromosome, which are more necessary for chromosomal integrity, would lead to eventual loss of the Y chromosome and generation of a 45,X cell line. Further efforts to identify Y chromosome abnormalities, especially in 45,X/46,XY subjects with a cytogenetically normal Y chromosome, are undoubtedly warranted.

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