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

Tohru Yorifuji, Junko Muroi, Mitsukazu Mamada, Ayumi Uematsu, Masahiko Kawai, Toru Momoi, Masayuki Kaji, Chutaro Yamanaka, Tatsutoshi Nakahata

EDITOR—Turner syndrome is one of the most common chromosomal abnormalities in which 10-15% have SRY mutations, 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 QIAamp 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’-TGAAGAATGCGACAGAACGTG - 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 20 seconds, annealing at 60°C for 10 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, 100 bp markers, and 1 unit of TaqGold 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’-TTGAGAATGCGACAGAACGTG - 3’/5’ - GGAGAAAACAGT GGAGAAAACAGT - 3’/5’ - CTTTGCATGT GGAGAAAACAGT - 3’/5’ - TATTCGTGTTGACAC-3’) (fig 1). The amplification was performed under the same conditions as those for DY3. PCR products were purified with a Wizard PCR Preps DNA isolation kit (QIAGEN)

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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 (agonadal levels of FSH >60 mIU/ml). One patient had a cytologically obvious Y chromosome, while others had smaller 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 gonado-ablation (gonadoblastoma (+)).

Discussion
In contrast with the study by Canto et al., 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, where the whole 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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Table 1 Profiles of Turner syndrome patients with the SRY gene. Patients KK, AO, and NI were prepubertal, so that their puberal status could not be assessed.

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

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