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Original Article

Detection of the SRY gene in patients with Turner Syndrome

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ABSTRACT

Background: If turner syndrome (TS) patients have a Y-containing cell line, they have an increased risk for gonadal tumors. TS patients are therefore screened for Y-chromosome and Y-specific sequences, such as *SRY, DYZ1, DYZ3, DYS132, ZFY, TSPY,* etc. In addition, since the dysgenetic gonad may include the stroma and granulosa/sertoli cells, which produce androgens, virilization can seen in girls with Y-chromosomal material. Prophylactic gonadectomy may therefore be required for optimal management in such patients. Our aim is to discuss our observations in the follow-up of TS patients.
Methods: SRY was investigated in 71 out of 85 TS cases (aged 3 months-27 years) between 2005 and 2017. Fluorescent in situ hybridization (FISH) was used until 2014, after which *SRY* analysis was performed using the polymerase chain reaction (PCR) method. *SRY* analysis was performed a second time using PCR in 25 cases previously investigated with FISH.
Results: We identified no positive cases. No pathological findings in terms of virilization, clitoromegaly, or posterior labial adhesions were also determined in our TS cases. Further studies were not required since no pathological findings also were detected at ultrasonography.
Conclusion: If Y-chromosome material has not been detected by conventional cytogenetic methods in TS patients with masculine features, further techniques should be applied to prevent the risk of invasive tumors, such as multiple sequences beside the Y centromere. This approach will prevent overtreatment.

Introduction

Turner syndrome (TS) is a frequent chromosome abnormality. The incidence of TS in female births is 1:2500. The clinical features may vary extensively, but associated problems manifest as developmental, endocrine, cardiovascular, psychosocial and reproductive disorders. These issues become more pronounced over time, and a systemic approach is therefore required [1].

Since pure monosomy is fatal, patients with 45,X0 karyotypes should be screened for hidden Y-chromosomal material. It has been hypothesized that live-born patients with a 45,X0 karyotype may exhibit some degree of cryptic mosaicism. In addition, forms of mosaicism apart from monosomy can include hidden Y chromosome, increasing the risk of gonadoblastoma [1,2].

If patients have a Y-containing cell line, they have an increased risk for gonadal tumors. For this reason, TS patients are screened for Y-chromosome and Y-specific sequences, such as SRY, *DYZ1, DYZ3*,*DYS132*, ZFY, TSPY, etc. However, further examinations in addition to standard cytogenetic for all TS patients are still controversial due to overtreatment. However, the real concern in this regard is that most gonadoblastomas may progress to invasive germ cell tumors, such as dysgerminoma [3,4]. Little information is available concerning the use of imaging or serum markers for surveillance of potential gonadal tumors in TS patients [5]. In addition, since the dysgenetic gonad may include the stroma and granulosa/Sertoli cells, which produce androgens, virilization can be seen in girls with Y-chromosomal material [5]. Therefore, prophylactic gonadectomy may be required for optimal management in such patients.

In this study, we discuss our observations in the follow-up TS patients diagnosed by karyotype analysis in terms of blood *SRY* results and clinical findings.

Materials and methods

Between 2005 and 2017, we evaluated 85 patients with TS diagnosed by karyotype analysis in the Dr. Sami Ulus Obstetrics and Gynecology and Pediatrics Training and Research Hospital Department of Pediatric Endocrinology, Ankara, Turkey. Of these 85 cases, 71 were finally included in the study. The remaining 14 subjects could not be contacted, since they had grown older or migrated to other cities. *SRY* analysis was

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performed in serum following receipt of informed consent from the patients. Systemic and genital examinations were performed on all patients (especially for virilization). Systemic virilization findings such as hirsutism and acne were evaluated in all patients. The uterus and ovaries were also scanned by ultrasound (USG) at follow-up.

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Local Ethics Committee.

Mutation analysis

Conventional cytogenetic analysis was performed (20–100 metaphase plates) for all 85 girls. TS patients were subsequently visualized using specific probes with the fluorescence in situ hybridization (FISH) technique. Suitable probes recommended by the manufacturer were employed (Vysis SRY Probe LSI SRY Spectrum Orange/CEP X Spectrum Green, Abbott Molecular). The peripheral stem was completed using *SRY* specific primers from the resulting DNA sample.

Results

The ages of the 85 TS patients followed-up in our clinic between 2005 and 2017 ranged from 3 months to 27 years. Forty-nine (57.6%) cases were 45,X0 and 36 (42.4%) were mosaic type TS. Table 1 shows the distribution of karyotypes in our TS patients. Physical examination revealed a Turner phenotype and 45, X0/46, XY at cytogenetic analysis in one patient, and this case was excluded from the study because it was included in the mixed gonadal dysgenesis (MGD) group. As anticipated, gonadectomy was performed on this *SRY*-positive patient with inadequate gonadal functions. Pathological examination was reported as immature ovarian tissue.

No patients with TS exhibited clitoromegaly. There was also no evidence of acne, hirsutism, or voice thickening, which might suggest virilization, at systemic examinations. USG revealed no pathological findings except for dysgenetic/small ovaries. *SRY* analysis was performed in 71 cases. Before 2014, *SRY* was investigated using the FISH method in our clinic, followed by polymerase chain reaction (PCR). Until 2014, *SRY* was determined as negative in 41 TS patients with the FISH method. *SRY* by PCR was amplified in 55 patients after 2014. *SRY* was investigated using PCR in 25 patients with negative FISH analysis results and in 30 TS patients diagnosed after 2014, and was negative in all cases. Twenty-five patients were *SRY*-negative at both FISH and PCR. *SRY* was not detected by PCR in one patient with 45,X0/46,X,+ mar.

Table 1
Distribution of karyotypes in the 85 TS patients.

Karyotype	Number (%)
45,X0*	49 (57.6)
45,X0/46,XX**	15 (17.6)
45,X0/46,X,i(Xq)	8 (9.4)
45,X0/46,X, del (Xq)	4 (4.7)
45,X0/46,X, der (Xq)	2 (2.4)
46,X,del(Xq)	2 (2.4)
45,X0/46,X,+mar	1 (1.1)
45,X0/46,X,r (X)	1 (1.1)
46,X,i(Xq)	1 (1.1)
45,X0/46,XX/47,XXX	1 (1.1)
45,X0/46,X, i(Xq)/47X,i(Xq)	1 (1.1)

* 41 patients investigated for SRY.
** 9 patients investigated for SRY.

Discussion

Routine cytogenetic analysis does not always reveal cell lines including the hidden Y-chromosome. Therefore, as part of the diagnostic process in TS patients, further analysis may be required to evaluate neoplasm risks. These sophisticated techniques consist of PCR, and FISH analysis of peripheral blood and/or tissues, such as gonads and buccal cells. Hidden Y chromosome mosaicism can be detected this approach [6,7]. Previous studies have demonstrated different rates of occult Y-chromosomal material in TS patients, varying between 0% and 61.1% [7–24]. In addition, the rate of occult Y-chromosome ranging from 6.9% to 60% have been reported in TS patients with marker chromosomes [6,7,18,24–28]. We identified no positive cases in this study. One patient with marker chromosome, with a higher rate of positivity for Y-chromosome material, was detected among the 85 cases. That case was investigated using the PCR method and was found to be negative for *SRY*. Patient selection criteria, number of metaphases analyzed, samples taken, methodology employed, and the Y-chromosome sequence markers used may account for these differences. In addition, data for the long-term outcomes of those cohorts are incomplete.

Although the rates of malignity risks in previous studies differ, probably in association with the study designs and potential selection bias, gonadoblastoma occurs in approximately 10% of TS cases with Y-chromosomes. If TS patients without Y-chromosome sequences are included, the rate may be approximately 1% [29]. Similarly, although we used only SRY gene Y-specific sequences, we observed no virilization, clitoromegaly, or gonadal lesions at pelvic USG in any of our cases at long-term follow-up. Although some authors classify 45,X0/46,XY karyotype as TS based on phenotypic properties, including short stature, and primary ovarian deficiency [30], we included one similar case in the MGD group. We therefore believe that the Y-cell line rate in our cases is accurate. In contrast, spontaneous puberty and a well-supervised successful pregnancy have been reported to be possible in individuals with TS and Y-chromosome material [31–33]. All clinical experience with gonadoblastoma/dysgerminoma in girls with TS derives from cases in which the karyotype included visible Y-chromosome material or in which the patient presented with clinical evidence of virilization. The recommendation for gonadectomy is therefore applicable only to individuals with either visible Y-chromosome material or virilization. PCR amplification may provide false positive results, and immature ovaries may consist of nests of germinal cells resembling benign gonadoblastoma, so the major concern in this regard is overtreatment [5].

In general, as in our study, *SRY* sequences are used, because this gene plays a central role in the process of sex determination [5,6,29]. Although *SRY* plays an important role during this process, the mapped gonadoblastoma locus does not contain this gene. Whereas the chromosomal location of *SRY* is the short arm of the Y-chromosome, the locus of gonadoblastoma is beside the centromere region of the chromosome. For these reasons, the current approach recommends the restriction of the use of FISH with the *SRY* probe. In order to identify the origin of ring or small marker chromosomes, FISH with X and Y probes can be used [29]. Many patients with gonadoblastoma have no *SRY* region, and these findings support the idea that FISH with the *SRY* probe should be restricted in order to eliminate occult Y material. One recently published comprehensive guide recommends that when virilization is present, multiple tissue testing is required to reveal occult Y-chromosomal material. If Y mosaicism is not observed in patients with virilization, it may be possible to detect this in buccal cells [29]. Our cohort of TS patients supports these findings, because no additional pathological findings were found in these subjects without virilization.

Little information is available concerning the use of illustrating or serum markers for following-up hidden gonadal tumors in TS. Although *SRY* was detected in the peripheral sample in one patient with MGD (45,X0/46,XY), *SRY* was not detected in streak gonads [34]. In contrast, Y-chromosome material was observed in the gonad, but not in the blood sample from one virilized TS patient [35]. Y-chromosomal determination in blood samples does not always mean that it exists in gonadal tissue, and this is not therefore a good surrogate marker of gonadal prevalence. However, the most cautious approach for preventing gonadal tumors involves prophylactic gonadectomy due to the correlation between existing Y-chromosome material and malignancy.

In conclusion, if Y-chromosome material has not been detected by conventional cytogenetic methods in TS patients with masculine features, further techniques should be applied to prevent the risk of invasive tumors, such as multiple sequences beside the Y centromere. This approach will prevent overtreatment.

Disclosure statement

The authors have no conflicts of interest to disclose in relation to this paper.

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