

Frequent Detection of Y Chromosome Sequences in Japanese Turner's Syndrome by Southern Blot Analysis of Amplified DNA

Yuichi Nakagawa, Hiromune Natsume, Akira Kubota, Hiromi Takeuchi, Kaoru Nasuda, Kazuhiko Toya, Akira Endoh, Hirokazu Saegusa, Rika Takeuchi, Haruo Ogawa and Yoshio Igarashi

Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu 431-31, Japan

Abstract In Turner's syndrome, the detection of Y chromosome sequences is very important because the presence of the Y chromosome increases the risk of gonadoblastoma induction. Some recent studies have indicated the presence of the Y chromosome in patients with Turner's syndrome, by methods in which the Y chromosome was not detected by a standard cytogenetic method, but these studies concentrated on a part of the Y chromosome or used an insensitive methods. In this study, we approached the detection of Y chromosome sequences by a sensitive method, polymerase chain reaction (PCR) with seven pairs of primers that span the Y chromosome, followed by Southern blot analysis, in eleven Japanese patients with Turner's syndrome. Ten of the eleven patients had some Y chromosome sequences determined by PCR followed by Southern blot. This suggests that most patients with Turner's syndrome have cryptic Y chromosomes, but further studies and long-term follow-up of patients with the Y chromosome are needed to support our data and to clarify the significance of a small amount of Y chromosome.

Key words: Turner syndrome, Y chromosome, PCR, Southern blot

Introduction

It is now believed that approximately half of all patients with Turner's syndrome are monosomic for the X chromosome, while the remaining patients show mosaics with various karyotypes (1, 2).

The detection of the Y chromosome is very important, because female individuals with a Y

chromosome and gonadal dysgenesis have about a 20% risk of gonadoblastoma induction (3, 4).

Some authors (5, 6) have recently reported the presence of Y chromosome sequences in patients who showed no sign of the Y chromosome by the classical cytogenetic method, but these studies were done by using an insensitive method or concentrating on a part of the Y chromosome.

In this study involving eleven Japanese patients with Turner's syndrome, we approached the detection of Y chromosome sequences by Southern blot analysis of amplified DNA with primers that span the Y chromosome.

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Correspondence: Dr. Yuichi Nakagawa, Department of Pediatrics, Hamamatsu University School of Medicine, Handa-cho 3600, Hamamatsu 431-31, Japan

Table 1 Karyotypes and PCR results for patients

Patients	Karyotype	SRY	AMGL	DYZ3	DYS139	DYS132	DYS1	DYZ1
1	45, X/46, X, i(Xq)	±	—	±	—	—	±	—
2	45, X/46, X, +mar	±	—	—	—	—	—	—
3	45, X/46, X, r(X)	±	—	±	—	—	—	—
4	45, X/46, X, i(Xq)	—	—	—	—	—	—	—
5	45X	±	—	±	—	—	—	—
6	45, X/47, X, X, X	±	—	+	—	—	—	±
7	45, X/47, X, X, X	—	—	—	—	—	—	—
8	45, X	—	—	±	—	—	—	—
9	45, X/46, X, i(Xq)	—	—	+	—	—	—	±
10	45, X	±	—	+	—	±	—	—
11	45, X/46, X, r(minute)	+	—	±	±	—	—	—

— : no signal, ±: faint signal, +: definite signal.

Materials and Methods

Patients

The cases of eleven Japanese patients with Turner's syndrome were studied. These patients had short statures and various typical features characterizing the Turner phenotype.

Cytogenetic analysis

Karyotypes from peripheral blood leucocytes were analyzed by standard techniques. At least 30 cells per patient were scored for chromosomal abnormalities.

Polymerase chain reaction (PCR) and Southern blot

Seven loci that span the Y chromosome were selected for this study: SRY and AMGL located on the short arm of the Y chromosome, and DYZ3 (Y centromeric sequence), DYS139, DYS132, DYS1 and DYZ1 located on the long arm. PCRs for DYS139, DYS132, and DYS1 were performed according to the method of Nagafuchi *et al.* (7). PCRs for SRY and DYZ1 were performed according to the method of Nakagome *et al.* (8, 9). PCR for AMGL was performed according to the method of Nakahori *et al.* (10). PCR for DYZ3

was performed according to the method of Witt *et al.* (11).

Southern blot analysis was carried out according to the method of Kocova *et al.* (6). The probes to detect the seven loci were obtained by amplification of healthy male DNA with each pair of primers and purification with Gene-Clean (Bio 101).

Results

The karyotypes of the patients are shown in Tables 1 and 2. Three patients had 45, X and eight patients had mosaics for 45, X.

Seven patients showed a positive signal on agarose gel after PCR for SRY. The status of other positive signals on agarose gel after PCR was none for AMGL, nine for DYZ3, one for DYS139, three for DYS132, one for DYS1, and two for DYZ1 (Table 1).

By Southern blot of the PCR products, eight patients showed a positive signal in the analysis of SRY, eight in analysis of AMGL, nine in analysis of DYZ3, four in analysis of DYS139, six in analysis of DYS132, three in DYS1, and four in analysis of DYZ1. Patients who showed a positive signal by Southern blot included PCR positive individuals (Fig. 1 Table 2).

Table 2 Summary of Southern blot analysis

Patients	Karyotype	SRY	AMGL	DYZ3	DYS139	DYS132	DYS1	DYZ1
1	45, X/46, X, i(Xq)	+	+	±	-	±	±	-
2	45, X/46, X, +mar	+	+	±	-	±	±	-
3	45, X/46, X, r(X)	+	+	±	±	-	-	-
4	45, X/46, X, i(Xq)	-	-	-	-	-	-	-
5	45X	+	+	±	-	+	±	-
6	45, X/47, X, X, X	+	+	+	+	-	-	+
7	45, X/47, X, X, X	+	-	-	-	±	-	-
8	45, X	-	-	+	-	-	-	-
9	45, X/46, X, i(Xq)	-	±	+	-	±	-	±
10	45, X	+	+	+	+	±	-	±
11	45, X/46, X, r(minute)	+	+	±	+	-	-	±

- : no signal, ±: faint signal, +: definite signal.

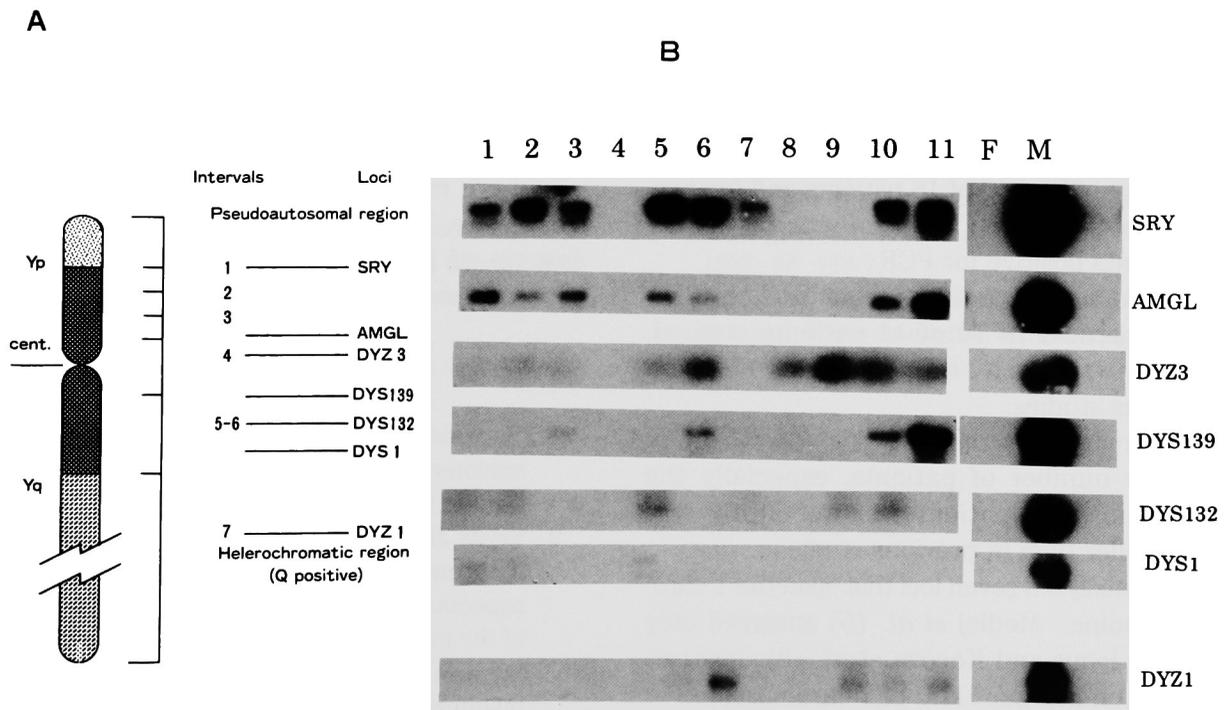


Fig. 1 (A) The location and order of the seven loci of the Y chromosome. (B) Southern blot of amplified DNA with the seven pairs of Y-specific primers. Numbers correspond to the numbers in the Tables. F: normal female, M: normal male, The signals of number 9 of AMGL; numbers 1, 2, 3, and 5 of DYZ3; number 3 of DYS139; numbers 1, 2, and 7 of DYS132; and numbers 1, 2, and 3 of DYS1 are very faint. - =no signal; ±=faint signal; +=definite signal.

Discussion

Turner's syndrome with 45,X/46,XY or 45,X/45,X derivative Y karyotypes have an increased risk (15-30%) of gonadoblastoma induction (12-14).

By standard cytogenetic methods, a Y chromosome or derivative Y is present in 2-6% of mosaic patients (15, 16) and an additional 3% have a marker chromosome derived from a Y chromosome or other chromosomes (16).

Recently, by molecular biological techniques, Medlej *et al.* (5) and Kocova *et al.* (6) detected Y chromosome sequences in patients with Turner's syndrome who showed no Y chromosomal materials by a standard cytogenetic method.

Medlej *et al.* (5) detected a Y chromosome sequence (SRY) in only one of 40 patients studied by PCR. On the other hand, by using PCR followed by Southern blot analysis, Kocova *et al.* (6) detected SRY in 6 of 18 patients and furthermore detected DYZ3 (Y centromeric sequence) in 5 patients positive for PCR.

In this study we detected some Y chromosome sequences in 10 of 11 patients studied. This ratio was much higher than that obtained in previous studies.

This may be explained by the following:

- 1) The number of patients, especially the monosomic patients, in our study was smaller than in previous studies.
- 2) We analyzed seven loci that span the Y chromosome. Medlej *et al.* (5) analyzed only one locus and Kocova *et al.* (6) only two loci on the short arm of Y chromosome.
- 3) Ethnic differences: Nagafuchi *et al.* (17) reported that the majority of the marker chromosomes in Japanese patients with stigmata of Turner syndrome were derived from Y chromosomes, although they employed only Southern blot and, in part, PCR.

Page (4) has postulated that the gonado-

blastoma locus on the Y chromosome (GBY) gene is located near the centromere or on the long arm of the Y chromosome. Nagafuchi *et al.* (17) also suggested that the GBY gene was mapped proximal to DYS132 located on the long arm of the Y chromosome. In this study six patients showed positive DYS132 sequences, and nine patients positive DYZ3.

We do not yet know the exact incidence of gonadoblastoma in Turner's syndrome or whether Japanese have gonadoblastoma more frequently than people in other countries. Generally pure gonadoblastoma is a clinically benign, in situ, germ-cell cancer (16, 18, 19). An exact diagnosis is therefore possible only by gonadectomy.

Lukusa *et al.* (20) reported that the proportion of Y-bearing cells in gonads in which gonadoblastomas developed was high. We do not yet understand the significance of the small amount of the Y chromosome sequence, and we also have not defined the exact position of the GBY gene. Further studies and long-term follow-up of patients with a small amount of Y chromosome are needed to clarify these issues.

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