Case Report

DNA ANALYSIS OF TWO PATIENTS WITH A NON-FLUORESCENT Y CHROMOSOME

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Summary Results of DNA study on two patients of gonadal dysgenesis with a 45,X/46,X,Ynf (non-fluorescent Y chromosome) karyotype are described. In one patient who developed gonadoblastoma, all 12 loci on the non-fluorescent part of Yq were detected. Another patient did not have gonadoblastoma at 20 years, and only the proximal 6 loci out of 12 were detected.

Key Words gonadoblastoma, gonadal dysgenesis, DNA analysis, Y chromosome

INTRODUCTION

Increased incidence of gonadoblastoma in patients with both dysgenetic gonads and a Y chromosome has been reported (Scully, 1970; Shellhas, 1974; Manuel *et al.*, 1976). This tumor also occurs in patients where dysgenetic gonads are associated with the presence of a part of the Y chromosome. From these facts, the role of Y chromosome in gonadoblastoma development in a dysgenetic gonad has been discussed (Page, 1986, 1987; Lukusa *et al.*, 1986).

In this report, two patients of gonadal dysgenesis with a karyotype 45,X/46,X,Ynf (non-fluorescent Y chromosome) are described. The one showed gonadoblastoma and the other did not. A detailed analysis of Y chromosome including 25 loci revealed that the first patient retained all of the 12 loci on the non-fluorescent part of the long arm of Y, while the second patient lost six of the 12 loci.

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CASE REPORTS

Patient 1 (case 877) is a 10-year-old girl. During neonatal period, a cytogenetic study was carried out because of low birth weight (2,180 g at 40 weeks) and short stature. The karyotype was apparently 45,X/46,X,Y in a non-banded preparation. However, the "Y" chromosome showed a very unusual G-banding pattern and the origin of the "Y" chromosome couldn't be identified. She was followed up because of the possible risk of gonadoblastoma.

At 10 years of age, detailed examinations were carried out. She was short (119 cm, -2.8 standard deviations) and had a shield chest. External genitalia was those of a normal female. Plasma levels of FSH and LH (follicle stimulating hormone, luteinizing hormone) were elevated and markedly increased after the administration of LH-RH (LH-releasing hormone). Both plasma testosterone and estradiol were low and did not respond to hCG (human chorionic gonadotropine). Intravenous pyelography (IVP) showed a horse-shoe kidney and duplicated right ureters. Exploratory laparotomy revealed small uterus and Fallopian tubes. Neither testis nor ovary was detected. Adnexal structure of both sides were removed. Microscopic examination revealed bilateral streak gonads with a small gonadoblastoma (3 mm in diameter) on the left.

Patient 2 (case 878), a 20-year-old female, was referred to us because of primary amenorrhea and short stature (152 cm) at 20 years old. She had infantile genitalia. Pubic hair was sparse and breasts were poorly developed. Plasma levels of LH and FSH were high and further increased by LH-RH administration. Plasma testosterone and estradiol levels were low and did not respond to hCG. IVP revealed a horse-shoe kidney. Exploratory laparotomy showed a uterus and poorly developed Fallopian tubes. Microscopic observation revealed streak gonads. No gonadoblastoma was detected.

CYTOGENETIC ANALYSIS

Chromosome analysis was carried out using standard leukocyte culture technique. The patient 1 showed an apparent 45,X/46,XY karyotype in a non-banded preparation, the proportion of cells being 20:80. The "Y" chromosome was an acrocentric chromosome slightly larger than chromosome 22, not involved in satellite associations and was Q (quinacrine)-negative. Trypsin G-banding revealed a homologically stained long arm with two slightly darker bands, presumably duplicated Yq11.22 bands, in the middle of the long arm (Fig. 1). Both Q- and C-banding revealed no positive segment on the "Y." One possible interpretation would be the "Y" is in fact *psu dic (Y) (pter→cen→q11.23::q11.23→pter)*.

The patient 2 had a 45,X/46,X,+mar karyotype, the proportion being 43:57. The marker chromosome was very small, Q-negative and not involved in satellite associations.

Fig. 1. Partial karyotype with G- and Q-banding of patient 1.

DNA ANALYSIS

DNA analysis was carried out using peripheral blood leukocytes. Extraction of DNA, electrophoresis, hybridization, and other techniques are those generally used (Maniatis *et al.*, 1982). In this study, 17 different probes were used. Ten probes including 87-4, 87-6, 87-7, 87-17, 87-19, 87-24, 87-26, 87-27, 87-28, and 87-31 were cloned and mapped on the non-fluorescent part of the Y chromosome by the authors (Nakahori *et al.*, 1991). An additional five probes were kindly supplied by Dr. J. Weissenbach (Paris). They were 12f3, 47z, 49f, 50f2, and 52d. The pDP1007 probe was supplied by Dr. D.C. Page (Cambridge, Massachusetts). The 50f2 detects five loci, A–E, and the 52d detects three loci, A–C, and 87-17 detects two loci, A and B. Each of the remaining probes detects single locus and these 16 probes detect a total of 23 single-copy sequences on the non-fluorescent part of the Y. Additional 2 loci on the short arm, PABY and SRY, were detected by PCR as described earlier (Nakagome *et al.*, 1991). Only the Y10 probe which detects repeated DNA family DYZ1 is mapped on the fluorescent part of the Y. DNA was digested with *EcoRI* for hybridization with the probes 50f2, 49f, 12f3, 87-6, 87-17, 87-24, 87-28, and 87-31. The rest of the probes except for Y10 were hybridized with *TaqI* digested DNA. Y10 was used with *StuI* (Nakahori *et al.*, 1989).

Results are summarized in Fig. 2. The patient 1 was positive for all the loci except for Y10. On the other hand, patient 2 was negative for loci Y10, 49f, 50f2C, 50f2E, 87-17, 52dA, and 87-6. Three of the 52f2 loci, A, B, and D and two of 52d loci, 52dB and 52dC, were positive. The 50f2D locus roughly corresponds to the centromere. Patient 1 retains most if not all the non-fluorescent part and patient 2 retains the short arm and the proximal six loci out of the 12 in the non-fluorescent part of the long arm. The marker chromosome of the patient 2 is very small and terminal deletion of the Y at about the middle of the non-fluorescent part, between loci 87-19 and 87-6, is a reasonable interpretation. On the other hand, the “Y” chromosome of the patient 1 is too large as a terminally deleted Yq-chro-

Fig. 2. Summary of DNA analysis, presence (+) and absence (-) of individual loci. "p" and "q" indicate the short arm and the long arm of Y chromosome respectively. "Cen" indicates the centromere.

mosome with a break point distal to 49f and proximal to DYZ1. Symmetric nature of G-band pattern and Q- and C-negative nature of the "Y" suggest that the "Y" is in fact a psu dic (Y) (q11.23) with break point just proximal to the Q- or C-positive segment.

DISCUSSION

Recently Y specific DNA probes have been used for analysis of Y chromosome, and using this technique, small deletions or abnormalities of the Y chromosome have become identifiable. At the moment, a total of 24 loci are detectable within the non-fluorescent part of the Y chromosome (Fig. 2). The fact that gonadoblastoma develops exclusively in patients with both dysgenetic gonads and a Y chromosome came to be approached using this technique.

Page proposed a hypothesis that the residual part of a deleted Y chromosome in a parient with both dysgenetic gonad and gonadoblastoma contains the portion responsible for gonadoblastoma (Page, 1987). He concluded that, if the gonadoblastoma locus exists, it is within the region defined by interval 4B-7 which extends from centromere to the distal end of long arm (Page, 1987; Vergnaud *et al.*, 1986). Lukusa *et al.* reported that except for a few cases among individuals with dysgenetic gonad, gonadoblastoma was not found in patients with a karyotype 45,X/46,X,Ynf (Lukusa *et al.*, 1986). They correlated the absence of the Q-bright region with the absence of gonadoblastoma in individuals carrying a non-fluorescent Y chromosome. Although Lukusa *et al.* discussed otherwise, this result indicates that gonadoblastoma locus could exist in either Q-positive region or around the borderline between Q-positive and Q-negative region.

In the present study, we examined 12 loci mapped within interval 5-6, and DYZ1 family which extends entire length of interval 7. Patient 1 lacked Q-positive segment and lacked DYZ1 locus. This patient retained all the remaining Yq loci and developed gonadoblastoma. Patient 2 lacked the distal six loci in the non-fluorescent part of the Yq. She was negative for gonadoblastoma at 20 years of age. One possible interpretation would be that gonadoblastoma gene is located within the segment present in case 1 and absent in case 2. According to this interpretation, a presumptive gonadoblastoma gene is located at or proximal to the locus detected by 49f probe. Another possibility is that patient 2 might develop gonadoblastoma later. It might be difficult to exclude this possibility at the moment. But the very small part of Yq that we proposed in the present report is consistent with most of the previous reports (Page, 1986, 1987; Lukusa *et al.*, 1986; Disteche *et al.*, 1986; Cantrell *et al.*, 1989; Magenis *et al.*, 1984). One of the author of this study (Y.N.) would like to initiate a collaborative effort to identify the segment related to the development of gonadoblastoma.

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