67. A Preliminary Note on a Phenotypic Male with an XX Constitution*

By Isamu HAYATA,**) Sajiro MAKINO,**) and Susumu SUTOU***)

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Subsequent to surprising advances in cytogenetic techniques, a good many cases of sex anomalies have chromosomally been analysed. A phenotypic male complained of some defects of urethra and was diagnosed as penoscrotal hypospadias. The patient was 25 years old and had penis of normal size together with male-type external genitalia.

Material and methods. Chromatin was analysed from buccal smears stained with acetic orcein, while drumsticks were studied on squashed peripheral leucocytes stained with acetic dahlia. Cultures established from the testicular tissue and venous blood were used for chromosome studies. Gonad biopsies were cut with scissors into fragments, about 1 mm in diameter, and planted directly on the glass surface of 2 flasks with plasma clots. The culture medium used was TC-109 (Difco) with 10 to 20 per cent inactivated bovine serum to which penicillin had been added; the cultures were incubated at 37°C. Two cultures of peripheral leucocytes were set up according to the standard method with minor modifications. Chromosome slides were made following the air-drying method and stained with Giemsa. Histological structure of both right and left testes was examined with hematoxylin-eosin slides.

Clinical findings. The propositus had the following characteristics: height 167 cm, weight 63 kg, chest circumference 92 cm, head circumference 57 cm, arm span 169 cm, nipple distance 19.5 cm, upper segment 84 cm, lower segment 83 cm. He was well-developed and nourished. No mental retardation, no gynaecomastia, normal axillary hair and normal male-type pubic hair were noted. The penis 5.5 cm in length, and 7.0 cm in circumference showed a typical high grade deformity of hypospadias with the opening of the urethral meatus on the penoscrotal junction. The testes were small and soft, the right being 2.5×1.2×1.5 cm and the left 2.5×1.7×1.5 cm, in a well-developed

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**) Zoological Institute, Faculty of Science, Hokkaido University, Sapporo.

***) Department of Urology, School of Medicine, Hokkaido University Sapporo.
bifid scrotum. The prostate was slightly small at palpation. As seen on urethrography the posterior urethra was normal without vaginal opening. A slight swelling of verumontanum-like protrusion was noted near the urethral opening. The urinary 17-KS and 17-OHCS were in the lower limits of normal with values of 11.6 and 9.5 mg/day, respectively. At operation the testes were atrophic, of brown coloration in upper 2/3 and white in lower 1/3. The vasa deferens and

Fig. 1. External genitalia of the patient. Arrow indicates the presence of hypospadias.

Figs. 2-4. Histological features of the left gonad.
2: hyalinization of seminiferous tubules (A), hyperplasia of Leydig cells (B) and real ghost tubule (C). ×150.
3: seminiferous tubule, showing Sertoli cells only and hyalinization of basement membrane. ×650.
4: interstitial tissue with many Leydig cells. ×650.

Fig. 5. A karyotype from a cultured leucocyte of the patient.
epididymides appeared normal on both sides.

**Histological pictures.** Histologically two biopsy specimens taken from portions involving brown and white parts of both testes showed a similar picture. The seminiferous tubules were reduced in number and size. There were diverse degrees of hyalinization of their walls with some real “ghost tubules”. No germinal cell was recognized. The Sertoli cells, generally normal in appearance, were rather reduced in number. The interstitial space was free of fibrosis, but a slight hyperplasia of the Leydig cells was noted. The changes observed in the testes are quite characteristic of those seen in the Klinefelter's syndrome.

**Cytological findings.** In 200 cells examined, 59 nuclei, or 29.5 per cent, showed a peripheral Barr body. A drumstick was found in some polymorphonuclear leucocytes.

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>Days in vitro</th>
<th>Chromosome number distribution</th>
<th>Total number of cells counted</th>
<th>analysed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>14</td>
<td>≤43 44 45 46 46* ≥80</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>1 — 3(3) 18(7) 1(1) —</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Blood I</td>
<td>3</td>
<td>— 4(4) 95(77) 1(1) —</td>
<td>100</td>
<td>82</td>
</tr>
<tr>
<td>Blood II</td>
<td>3</td>
<td>— 101(79) 1(1) —</td>
<td>103</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>1(1) 9(9) 228(176) 2(2) 4(2)</td>
<td>245</td>
<td>190</td>
</tr>
</tbody>
</table>

* Pseudodiploid.

Results of chromosome counts made in 245 cells are presented in Table I. All cultures showed a modal chromosome number of 46. Out of the 245 cells, a detailed examination was carried on in 190 (see Table I). In the remaining 55 cells, the occurrence of at least 10 acrocentrics in D and G groups was ascertained, but detailed analysis of other groups was not done. Seven cells with the modal number, 2 from testis cultures and 5 from blood cultures, were karyotyped. There was no evidence for the occurrence of an XY sex-complex in any cell analyzed. Two abnormal cells, one from testicular cultures, and the other from leucocyte cultures, were karyotyped. In the former cell the occurrence of an extra, extremely small-sized, fragment was noted, in addition to a 46 female complement. The other cell had 46 chromosomes but one chromosome was seen to be quite similar to those of the D group, except for the absence of short arms, together with a somewhat larger size, suggesting a deletion of the short arm in one of the C group chromosomes. Cells with one to two chromosome breaks appeared with a frequency of approximately 5 per cent. The chromosome constitution of this
patient was judged as morphologically identical to a normal female complement of 46, XX.

Remarks. Gonad biopsies of the present case have revealed definitely a testicular tissue, though considerably atrophic. There was no evidence of ovarian stroma so far observed. Except for the occurrence of penoscrotal hypospadias, the clinical features of this patient seem to show no appreciable difference from those of previously reported XX males, by de la Chapelle et al. (1964, 1965), Therkelsen (1964), Court Brown et al. (1964), Lindsten et al. (1966), Furuyama et al. (1968), and some others. General histological pictures of the gonads described by them were hyalinization of the walls of seminiferous tubules containing Sertoli cells, and hyperplasia of Leydig cells. Therkelsen (1964) described in a sterile XX male that the testicular tissue was greatly degenerated with many unhyalinized tubules of "Sertoli-cell-only" type, while de la Chapelle et al. (1964) reported the occurrence of spermatogonia. No gynaecomastia, small soft testes and the presence of adult type penis were common clinical features occurring in those cases. Court Brown et al. (1964) described the presence of gynaecomastia, and Lindsten et al. (1966) reported harder testes. 17-ketosteroids excretion values being around the lower limits of normal were noted by de la Chapelle et al. (1964), Therkelsen (1964), and Lindsten et al. (1966). Our findings indicated that the phenotypically male patient had an XX sex-chromosome constitution without evidence for mosaicism in blood and testicular cells in culture.

It has generally been accepted, however, that the occurrence of the Y chromosome is essential for the development of the testis in man. It was suggested by de la Chapelle et al. (1964) that the XX male had originally had the karyotype 47, XXY which led to male sex differentiation, and that the Y chromosome had been lost during one of the first cleavage divisions. On the other hand, Ferguson-Smith (1966) suggested a possible interchange of a male determining gene (or genes) of the Y chromosome onto the paternally derived X chromosome in XX males. According to Hamerton (1968), both structural genes for testicular as well as ovarian determination are present on the euchromatic X chromosome, while the heterochromatic X or Y acts only as controlling center. At the present time, however, a single mechanism for the explanation of the difference between the gonadal and genetic sexes in XX males seems still premature. Further development of cytogenetical techniques and the assessment of gene expression mechanisms in sex determination are requested. Further detailed investigations are now in progress with additional material along with a familial survey.
Summary. A phenotypic 25-year-old male, diagnosed as penoscrotal hypospadias, was found to have an XX sex-chromosome constitution, being chromatin positive in buccal smears and showing drumsticks in some polymorphonuclear leucocytes. Biopsies from right and left testes showed histological pictures characteristic of Klinefelter's syndrome. Some clinical and cytogenetic features were considered in reported XX male cases in comparison with the present case.

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References


