Original Article

Limitations of G-banding Karyotype Analysis with Peripheral Lymphocytes in Diagnosing Mixed Gonadal Dysgenesis

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Abstract. Mixed gonadal dysgenesis (MGD) is an abnormal sexual differentiation syndrome usually presenting with ambiguous genitalia. Karyotype analysis is one of the essential components in the diagnosis of MGD and is conventionally done with peripheral lymphocytes by the G-banding technique. It is speculated that this conventional karyotype analysis has limitations since there are often difference in gonadal tissue analysis. Here we present four cases of MGD, in which karyotype analysis were performed by peripheral lymphocytes fluorescence in situ hybridization (FISH), gonad fibroblasts FISH and gonad fibroblasts G-banding technique, in addition to the conventional peripheral lymphocytes G-banding technique. In Case 1, the percentage of the 45,X cell line in lymphocytes decreased after birth and detection of mosaicism could only be done by karyotype of gonads at 7 mo of age. In Case 2, FISH analysis with peripheral lymphocytes was more useful for detecting low frequency mosaicism. In all cases, phenotype of gonads and external genitalia were more consistent with karyotype of gonads than that of the peripheral lymphocytes G-banding technique. In conclusion, conventional G-banding karyotype analysis with peripheral lymphocytes has limitations in the diagnosis and evaluation of MGD. Karyotype analysis by FISH or by using gonads is useful for diagnosing MGD and understanding of the phenotype of gonadal tissue.

Key words: mixed gonadal dysgenesis, mosaicism in peripheral lymphocytes and gonads

Introduction

Mixed gonadal dysgenesis (MGD) is an abnormal sexual differentiation syndrome usually presenting with ambiguous genitalia, but the phenotype can vary from a normal male to female with or without Turner stigmata (1–3). Originally, MGD was defined on the basis of gonadal morphologic features, i.e., an abnormal testis on one side and a rudimentary gonad, streak gonad, or no gonad at all on the other (4). However, some investigators have come to consider MGD a broad syndrome that includes certain patients with bilateral testes or bilateral streak gonads because of the clinical variety in patients with 45,X/46,XY mosaicism (2, 5).

The most common gonads in MGD are dysgenetic testis on one side and a streak gonad...
on the other side, and the typical karyotype is 45,X/46,XY mosaicism (6, 7). Karyotype analysis is an essential component in the diagnosis of MGD, and is usually performed on peripheral lymphocytes using the G-banding technique. However, the information obtained from this conventional method has several limitations. For example, it is influenced by the number of cells examined or by different percentages of mosaicism in different tissues. Here we discuss the limitations of karyotype analysis of peripheral lymphocytes with the G-banding technique in four cases.

**Methods**

G-banding karyotype analysis was performed on peripheral lymphocytes and fibroblasts of gonads by standard methodologies. Fibroblasts or fibroblast-like cells were cultured for karyotype analysis from gonads after gonadectomy. In this paper, we refer to this as “karyotype of gonad”.

FISH analysis was also performed on peripheral lymphocytes and fibroblasts of gonad using three probes: the X centromeric satellite probe (DXZ1), the Y centromeric alpha satellite probe (DYZ3) and the telomeric Yq12 probe (DYZ1). The X chromosome was defined as one signal (DXZ1). The Y chromosome was defined as two signals (DYZ1 and DYZ3). One thousand interphase cells were analyzed by FISH in Case 2 and Case 4, and one hundred cells were analyzed in Case 3.

Informed consent was obtained from the parents of the patients.

**Case Reports and Results of Cytogenetic Analysis**

**Case 1**

Case 1, a female, was referred to our hospital for ambiguous genitalia at 5 mo of age. She was born at 38 wk gestation, birth weight 2520 g, by vaginal delivery. G-banding karyotype analysis of peripheral lymphocytes from umbilical cord blood was 45,X[7]/46,XY[50]. The patient was already legally registered as a female. She had clitoromegaly (2 cm of length), scrotum-like labia major and urogenital sinus. The right gonad was palpable in the inguinal region; the left gonad was not palpable. Considering the patient’s asymmetrical external genitalia and karyotype at birth, MGD was suspected. She received laparoscopy and gonadectomy at seven months of age. The macroscopic findings showed uterus, bilateral testis-like gonads, ductus deference and epididymis. Fallopian tubes were not detected. Histological findings of the gonads were dysgenetic testes with immature seminiferous tubules and hyperplasia of Leydig cells. No ovarian tissues were found. Although the histological findings suggested dysgenetic male pseudohermaphroditism, we evaluated this patient’s condition as a MGD variant. Although G-banding karyotype analysis with peripheral lymphocytes at seven months of age showed 46,XY[100], chromosome analysis with gonads at the same time showed mosaicism as follows: left gonad 45,X[10]/46,X+mar[10]; and right gonad 45,X[16]/46,X+mar[3]/47,X+2mar[1]. This marker chromosome is presumably idic(Y)(q11), based on the result of fluorescence in situ hybridization (FISH) analysis with peripheral lymphocytes at seven years of age, 46,X,idic(Y)(q11),ish idic(Y).

The points to note about this case regarding the karyotype analysis are as follows: 1) the percentage of the 45,X cell line decreased in peripheral lymphocytes after birth; and 2) karyotype analysis with gonads provided more information than that of peripheral lymphocytes for clinical evaluation.

**Case 2**

Case 2 was a legally registered male who was referred to our hospital at 9 mo of age for evaluation of ambiguous genitalia. He was born at 40 wk gestation and his birth weight was 2788 g. On examination, penile hypospadias and bifid
scrotum were noted. The right testis was palpable in the scrotum, but the left was impalpable. Voiding cystourethrogram showed an enlarged (27 mm of length) prostatic utricle. Uterus was not detected by genitogram. Laparoscopic findings showed a left streak gonad and the patient received left gonadectomy. Although G-banding karyotype analysis with peripheral lymphocytes was 46,XY [50 cells] with metaphase cells, the peripheral lymphocyte FISH technique using interphase cells showed 45,X[15]/46,XY[916]. The left gonad was consistent with a streak gonad and its G-banding karyotype analysis showed mosaicism, 45,X[15]/46,XY[15].

The points to note about this case regarding the karyotype analysis are as follows: 1) FISH analysis with peripheral lymphocytes was useful for detecting low frequency mosaicism; and 2) G-banding karyotype analysis of the gonad was also useful in the evaluation of MGD.

Case 3

Case 3 was legally registered female who was pointed out to have mild clitoromegaly at 6 mo of age. She was referred to our hospital at 1 yr of age. She had coarctation of the aorta and bicuspid aortic valve. G-banding karyotype analysis of peripheral lymphocytes at six months of age showed 45,X[12]/46,XY[18] and 45,X[21]/46,XY[79] at one year of age. The patient showed clitoromegaly (3 cm) and scrotum- like labia major. The left gonad was palpable in the inguinal region, but the right gonad was not palpable. Uterus and vagina were detected by ultrasonography. The patient received bilateral gonadectomy and karyotype analysis from both gonads was performed at the age of 1 yr. The left gonad was an atrophic or hypoplastic testis, 45,X[67]/46,XY[33], and the right one was a streak gonad, 45,X[97]/46,XY[3] by FISH analysis. On histological examination, the left gonad contained seminiferous tubules and a small number of spermatogonia; hyperplasia of Leydig cells was not detected. The right gonad was comprised of fibrous tissue and contained ovariogenic stroma. There were tissues like gonadoblastoma in a section of the right gonad. Dysgenetic fallopian tube and dysgenetic vas deferens were shown bilaterally.

The point to note about this case regarding the karyotype analysis is that the difference in the ratio of mosaicism in the gonads of both sides was consistent with the phenotypes of the gonads and external genitalia.

Case 4

Case 4 was a legally registered female who was referred to our hospital because of short stature at 11 yr of age. Her growth followed the –2 standard deviation growth curve. She had an epicanthic fold, prominent ears and micrognathia. These minor anomalies are sometimes shown in Turner syndrome. She had normal female external genitalia. Uterus was detected by ultrasonography. G-banding karyotype analysis of peripheral lymphocytes was 45,X[4]/46,XY[26]. The patient received bilateral gonadectomy at 12 yr of age. Both gonads were streak gonads and gonadoblastoma was detected partially in the left gonad on histological examination. The karyotype analysis with FISH was 45,X[923]/46,XY[77] on the right side, and 45,X[974]/46,XY[26] on the left side.

The point to note about this case is that the difference in the ratio of mosaicism in the gonads is more consistent with the histological findings than that in peripheral lymphocytes.

Discussion

We presented four cases with MGD in order to show that the analysis of karyotype by FISH or by using gonads is useful for diagnosis of MGD and understanding the phenotype of gonadal tissues.

Originally, MGD was defined on the basis of gonadal morphological features, i.e., an abnormal testis on one side and a rudimentary gonad,
streak gonad, or no gonad at all on the other (4). MGD typically results from 45,X/46,XY mosaicism and presentation is usually with ambiguous genitalia, but the phenotype can vary from that of a normal male to a female with or without Turner syndrome stigmata and at a risk of gonadoblastoma (1–3, 6, 7). Previous reports showed that most subjects with 45,X/46,XY mosaicism have ambiguous genitalia or are phenotypically male, and that gonads were paired equally as bilateral testes, testis plus gonad, or bilateral streak gonads (8, 9). Consequently, some investigators have already come to consider MGD a broad syndrome that includes certain patients with bilateral testes or bilateral streak gonads [2, 5]. Whenever an interpretation of MGD is made, the result of karyotype analysis is an essential component of the diagnosis.

Conventional karyotype analysis of peripheral lymphocytes by the G-banding technique is supposed to have several limitations in the diagnosis of MGD. First, the detection of mosaicism in MGD depends on in vivo or in vitro selection against one of the cell lines; i.e., the number of the cells analyzed is one of the critical factors (10, 11). Second, karyotype analysis with peripheral lymphocytes may not reflect that in the gonad, which is theoretically more valid for interpreting phenotypes of gonads and external genitalia in patients with MGD. Finally, other newer techniques, such as FISH, may be more useful than G-banding analysis of peripheral lymphocytes.

The difference between G-banding karyotype analysis with peripheral lymphocytes and that with gonads at seven months of age in Case 1, namely disappearance of the 45,X cell line in peripheral lymphocytes (see Table 2, difference at birth and at 7 months), may have resulted from a difference in the turnover cycle of peripheral lymphocytes and gonadal fibroblasts. Held et al. suggest that there may be an in vivo selection against rearranged sex chromosomes, based on the continual decrease of the abnormal sex chromosome observed in long-term in vitro culture (12). The 46,XY (or its variant forms such as 46,X,idicY) cell line probably has a longer life than 45,X in the cell cycle. In fact, more than 90% of the 45,X/46,XY mosaics prenatally diagnosed have normal male genitalia (13,14). Chang et al. reported 92 prenatally diagnosed 45,X/46,XY mosaicism cases. About 60 percent of cases were re-evaluated after birth, and 80 percent of them showed decreased percentages

| Table 1 | Summary of phenotype and gonads of four patients with mixed gonadal dysgenesis |
|-----------------|------------------------------------------|
|                | Phenotype of external genitalia | gonads |
| Case 1 | ambiguous (clitoromegaly, urogenital sinus scrotum-like labia major, right inguinal-cryptorchidism, left unpalpable testis) | both: dysgenetic testis |
| Case 2 | ambiguous (penile hypospadias, bifid scrotum, left unpalpable testis) | left: streak right: testis |
| Case 3 | female (clitoromegaly, scrotum-like labia major, palpable gonad in the left inguinal region) | left: testis right: streak (with gonadoblastoma) |
| Case 4 | female | left: streak (with gonadoblastoma) right: streak |
Table 2  Results of cytogenetic analysis of four patients with mixed gonadal dysgenesis

| gonads                                      | karyotype | karyotype |
|---------------------------------------------|-----------|-----------|
|                                             | G-banding | FISH      |
| Case 1 both: dysgenetic testis              | Umbilical cord: 45,X[7]/46,XY[50] | 7M: 46,XY |
|                                             | 7Y: 46,X,idic(Y)(q11),ish idic(Y) | 9M: 46,XY[50] |
| Case 2 left: streak                         | 9M: 46,XY[50] | 9M: 45,X[15]/46,XY[916] |
| Case 3 left: testis                         | 6M: 45,X[12]/46,XY[18] | 9M: 45,X[15]/46,XY[79] |
| right: streak (with gonadoblastoma)         | 1Y: 45,X[21]/46,XY[79] | 11Y: 45,X[4]/46,XY[26] |
| Case 4 left: streak (with gonadoblastoma)   | 11Y: 45,X[4]/46,XY[26] | 11Y: 45,X[4]/46,XY[26] |
| right: streak                               | 11Y: 45,X[4]/46,XY[26] | 11Y: 45,X[4]/46,XY[26] |

*see Methods of cytogenetic section

of 45,X (14). In Case 1, the 45,X cell was not present at 7 mo of age although it was detected in the umbilical cord (14%). These results, in Case 1, indicate that natural selection of the normal cell line in lymphocytes may occur not only during the prenatal period but also after birth.

FISH analysis with interphase cells is more valuable for detecting mosaicism in this disorder as Case 2 (Case 2 was the only case in which both G-banding and FISH karyotype analysis were performed on peripheral lymphocytes). This may be due to the large number of cells analyzed in FISH, or due to the fact that the cells used in FISH analysis are in the interphase (not the metaphase as in conventional G-banding analysis of peripheral lymphocytes). Reddy et al. also reported the utility of FISH for detecting low frequency mosaicism in MGD (15). There have also been several studies of Turner syndrome reporting the detection of the Y chromosome by FISH (16–18).

Phenotypes of gonads (testis or streak gonad) and resultant external genitalia in MGD are presumably more influenced by karyotype of gonadal fibroblasts than that of peripheral lymphocytes. The turnover cycle of fibroblasts is slower than that of peripheral lymphocytes.
Although karyotype analysis of gonadal tissue-specific cell types such as Sertoli cells is difficult, gonadal fibroblasts have enough karyotype information. The predominance of 45,X cells or 46,XY cells in a developing gonad would promote the development of a streak gonad or a testis, respectively. In previous studies, no correlation has been reported between the percentage of 45,X and 46,XY cells in peripheral lymphocytes and the degree of phenotypic masculinization (8, 19). Moreover, monozygotic twins with different phenotypes (presumably due to different ratios of mosaicism of gonadal tissue) have been reported (20–22). Different levels of mosaicism of karyotype between lymphocytes and gonadal tissues have already been reported (23–25). The results of all four cases in this study confirmed that karyotype analysis of the gonad is more consistent with the phenotype of the gonad and external genitalia. We further speculate that natural selection, such as disappearance of 45,X cell line as described above, may occur less frequently in the gonad than in peripheral lymphocytes.

In conclusion, evaluation of the karyotype with peripheral lymphocytes by the G-banding technique has limitations in the diagnosis and evaluation of MGD. First, the percentage of the 45,X cell line in lymphocytes temporally decreases. Second, FISH analysis is more useful for detecting low frequency mosaicism through analysis of a large number of cells. Finally, the phenotype of gonads and external genitalia is more consistent with the karyotype of gonads than that of peripheral lymphocytes.

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