High incidence of Y-chromosome microdeletions in gonadal tissues from patients with 45,X/46,XY gonadal dysgenesis

A higher incidence of Y-chromosome microdeletions was found on gonadal DNA than on peripheral blood lymphocyte DNA and on streak gonads than on dysgenetic testis in 11 patients with 45,X/46,XY gonadal dysgenesis. It is probable that an association between Y-chromosome microdeletions and severity of the phenotype in 45,X/46,XY patients exists. (Fertil Steril 2008;89:458-60. ©2008 by American Society for Reproductive Medicine.)

The 45,X/46,XY mosaicism is an intriguing topic because: 1) it is associated with a broad clinical spectrum; 2) no correlation between clinical phenotype and the percentage 45,X cell line has been found by some authors; 3) most of the prenatally diagnosed fetuses with 45,X/46,XY have a normal male sexual development; and 4) a normal male with this sexual chromosome mosaicism and proven fertility has been identified in a screening for bone marrow transplantation. Therefore, we have proposed that an additional genetic or epigenetic factor is necessary to give rise to altered phenotypes in 45,X/46,XY subjects. In the past, we have attempted to evaluate this hypothesis with negative findings (1). However, mutations on the SRY gene in 45,X/46,XY patients have been found (2). In addition, subjects with 45,X/46,XY mosaicism have been recently found to have Y-chromosome microdeletions (3, 4).

In this context, to study if a direct relationship between Y-chromosome microdeletions and 45,X/46,XY mosaicism exists, and whether microdeletions of AZF loci may affect gonadal phenotype in 45,X/46,XY patients, we have tested the presence of Y-chromosome microdeletions in the AZF regions both on peripheral blood lymphocyte (PBL) and gonadal DNA in 11 patients with 45,X/46,XY gonadal dysgenesis. The Institutional Review Board and Ethics Committee approved the study design. All subjects (or their parents) gave informed consent for molecular analysis of their blood and gonadal samples. No chromosomal structural aberrations including Y-chromosome were identified in all patients. Monocentric Y-chromosome was shown by fluorescence in situ hybridization analysis in our patients (>100 interphase nuclei). The DNA was amplified by five multiplex polymerase chain reaction (PCR) kits using primers specific for 20 informative nonpolymorphic Y chromosome–specific sequence tagged site markers (Fig. 1). When a Y-chromosome microdeletion was detected in one patient, an additional simplex PCR was carried out three times in the specific deleted locus. In addition, ten loci spanning out on both arms of the Y-chromosome were analyzed to rule out the presence of false positives.

We found 27.2% (3 out of 11) of our patients had Y-chromosome microdeletions of AZF regions on PBL DNA. In addition, a high occurrence of Y-chromosome microdeletions was found on gonadal DNA samples (7 out of 11: 63.63%). Five patients had Y-chromosome microdeletions on gonadal DNA, i.e., two subjects showed Y-chromosome microdeletions on gonadal DNA, but not on PBL DNA; one of them had pure gonadal dysgenesis (PGD) with Turner stigmata (patient 6) and the other one had mixed gonadal dysgenesis (patient 10). In contrast with the clinical phenotype, Y-chromosome microdeletions on gonadal DNA were more frequently associated with streak gonads than with dysgenetic testis. Out of seven Y-chromosome microdeletions-positive gonads tested, six were streak gonads (85.71%). All streak gonads screened presented Y-chromosome microdeletions of the AZF regions, except for one gonadal DNA sample. Only one of four dysgenetic testes showed these deletions on the Y chromosome. Two patients (subjects 6 and 9) with large deletions on gonadal DNA were found to have PGD. The molecular studies and the extent of deletions identified in Yq are illustrated in Figure 1.

It has been suggested that the break points at the Y chromosome occur on specific regions where blocks of the massive sequence repeat units (amplicons) exist (5). These multiple areas are susceptible to breakage and reunion or deletions with formation of Y-chromosome microdeletions of the AZF regions and/or 45,X cell lines. Consequently, it is possible to find a direct relationship between both events (Y-chromosome microdeletions and...
45,X/46,XY mosaicism), because the formation mechanism is similar, i.e., DNA constitution of regions on the Y chromosome. Thus, both events are consequences of the same mechanism, but one is detected at a molecular level and the other one is detected at a cytogenetic level.

When phenotype/genotype correlation is studied in gonadal dysgenesis, cytogenetic or molecular analysis must be carried out on gonadal tissues. Different chromosomal or genic constitutions affecting the gonadal development between PBL and gonadal tissues have been reported by several investigators (6, 7). A possible relationship between gonadal phenotype and the presence of Y-chromosome microdeletions on gonadal DNA has not been investigated by other authors (3, 4). Therefore, the influence of Y-chromosome microdeletions in the AZF regions on testicular development could not be ruled out, because these studies were limited to analyzing only PBL DNA. We found two patients (6 and 10) who did not have Y-chromosome microdeletions on PBL DNA but did on their gonadal DNA. In addition, of 11 samples of gonadal DNA tested, seven of them presented with Y-chromosome microdeletions. Of these seven gonadal DNAs with Y-chromosome microdeletions, six were associated with streak gonads. Phenotypic variability of the patients published with 45,X/46,XY, including our patients, is probably the result of the additional genetic (or epigenetic) factor which may, to a varying degree, affect the gonadal development and differentiation mechanisms (1). In this aspect, mutations in SRY gene have been found among 45,X/46,XY patients (2). Similarly, Y-chromosome microdeletions, mainly those present on gonadal DNA, could enhance the abnormal testicular differentiation caused by a variable proportion of 45,X cell lines among 45,X/46,XY patients. This assumption is based on the fact that a number of genes expressed specifically in the fetal and adult testes have been cloned on AZF regions (8, 9).

In conclusion, the present findings support the close association between Y-chromosome microdeletions and 45,X/46,XY mosaicism, and they are compatible with a model in which the primary alteration in 45,X/46,XY mosaicism is a molecular defect in the Y chromosome-specific
sequences that result in Y-chromosome microdeletions and mitotic loss of the Y chromosome. Therefore, the global genetic Y-chromosome instability may be detected at a DNA level as partial and interstitial Y-chromosome microdeletions and at a cytogenetic level as a 45,X/46,XY mosaicism. More and larger Y-microdeletions were found on gonadal DNA rather than on PBL DNA and on the streak gonads than on the dysgenetic testes among our patients. Therefore, it is probable that an association between Y-chromosome microdeletions and severity of the phenotype in 45,X/46,XY patients exists. However, the present sample was not large enough, and more extensive studies are needed to elucidate these intriguing findings.

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