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Perspectives and Editorials: Letter to the Editor

Molecular Barr Bodies: Methylation-Specific PCR of the Human X-Linked Gene FMR-1 for Diagnosis of Klinefelter Syndrome

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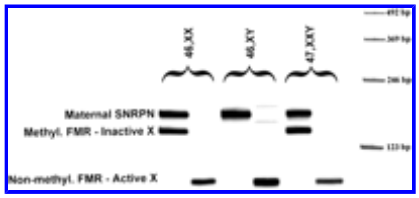
To the Editor:

Kamische et al (2003) should be congratulated for their thorough and informative article on Klinefelter syndrome, published in the January-February 2003 issue of the *Journal of Andrology.* However, we cannot agree with their favorable evaluation of Barr body analysis, because, in our experience, it is a test that does not have enough sensitivity to diagnose all cases of Klinefelter syndrome, especially in patients with mosaicism.

We wish to briefly describe a novel, simple, and highly sensitive molecular test based on methylation-specific PCR (MSP) of the human X-linked *FMR-1* gene, which can replace with enormous advantage the morphological Barr body analysis. The MSP test is done exactly as we described elsewhere for the diagnosis of Fragile X syndrome (Pena and Sturzeneker, 1999). Accordingly, DNA samples are first treated with sodium bisulfite to convert unmethylated, but not methylated, cytosines to uracil, followed by PCR amplification with oligonucleotide primers specific for methylated versus unmethylated DNA (Herman et al, 1996). We designed two primer pairs: one produces a 142-bp fragment from the bisulfite-treated methylated CpG island, and the other generates an 84-bp product from the treated non-methylated promoter (Figure). In normal males, only the 84-bp fragment is seen, but the diagnosis of Klinefelter syndrome is indicated by the appearance of a 142-bp methylated product (Figure). As an indispensable internal control for the efficiency of the sodium bisulfite treatment, we used a primer pair specific for the imprinted maternal methylated version of the CpG island of the *SNRPN* gene on human chromosome 15 (Figure). Using MSP, we identified, with 100% sensitivity and accuracy, 15 previously diagnosed male patients with Klinefelter syndrome mixed in with 40 normal control subjects. The test is simple, fast (it can be done in less than 48 hours), and does not depend on the use of expensive equipment.



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Polyacrylamide gel electrophoresis of the products of MSP of the human FMR1 gene on the X chromosome. Methyl. FMR indicates the 142-bp fragment from the bisulfite-treated methylated CpG island; Non-methyl. FMR, the 84-bp product from the treated nonmethylated promoter; Maternal SNRPN, the internal control 174-bp product of amplification of the imprinted maternal methylated version of the CpG island of the SNRPN gene on human chromosome 15 (Kubota et al, 1997). DNA samples from a normal woman (46,XX), a normal man (46,XY), and a patient with Klinefelter syndrome (47,XXY) previously diagnosed by chromosomal studies are shown. The diagnosis of Klinefelter syndrome is indicated by the presence in a male patient of the 142-bp product characteristic of the inactive (methylated) X chromosome. On the rightmost lane of the gel are molecular size standards.

The MSP test can detect the presence of the methylated X chromosome even when it is diluted 20-fold with normal male DNA (which does not contain the methylated X sequence). Thus, it should be sensitive enough to diagnose all patients with Klinefelter mosaicism. If needed, the test's sensitivity could be further increased by the use of fluorescently labeled primers and detection in an automatic DNA sequencer.

Because of its simplicity and high efficiency, MSP may become the method of choice for screening azoospermic males for Klinefelter syndrome. By its nature, the test can be aptly described as the "molecular Barr body test."

References

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