Journal of Andrology, Vol. 24, No. 6, November/December 2003

Perspectives and Editorials: Letters to the Editor

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

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.

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."

Respectfully,
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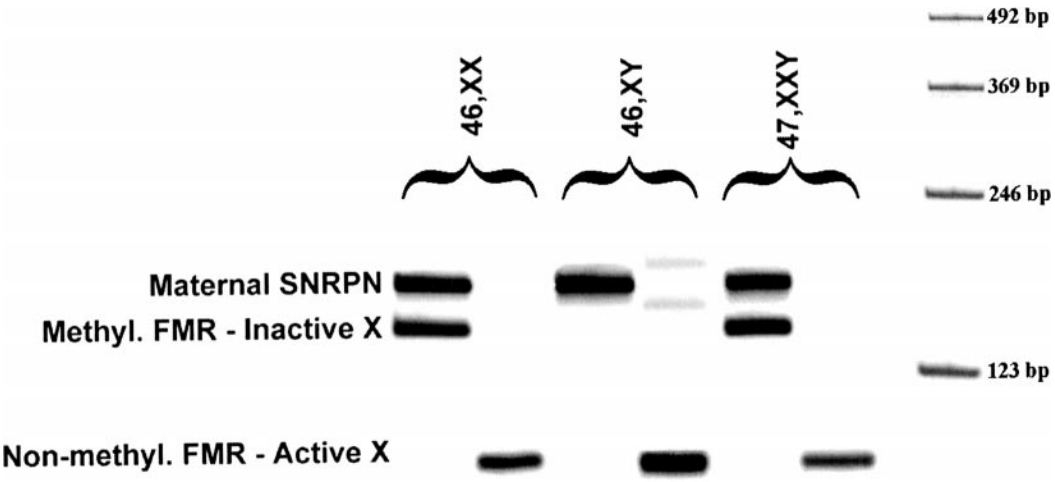

Polyacrylamide gel electrophoresis of the products of MSP of the human *FMR1* gene on the X chromosome. Methyl. FMR indicates the 142-bp fragment from the bisulfite-treated methylated CpG island; Non-methyl. FMR, the 84-bp product from the treated nonmethylated promoter; Maternal SNRPN, the internal 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.

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

To the Editor:

We appreciate the comments made concerning our article, ''Clinical and Diagnostic Features of Patients With Suspected Klinefelter Syndrome.'' As we repeatedly stated in our paper, we use the Barr body analysis only as a quick screening test on a routine clinical basis. We are aware that the Barr body analysis, at least in our hands, has a sensitivity of 82% and specificity of 95% for diagnosis of Klinefelter syndrome (see abstract, results, and discussion) and that for definite diagnosis, karyotype analysis is mandatory.

However, comments from Pena and Sturzeneker deal mainly with the description of a methylation-specific PCR (MSP test). Although on first sight the results appear interesting and applicable to the diagnosis of Klinefelter syndrome, the method has never been published for the diagnosis of Klinefelter syndrome in a peer-reviewed journal. The details of the methodology and description of a rather small number of Klinefelter syndrome patients given unsystematically in Pena and Sturzeneker's letter are not adequate for unequivocal statements on the suitability of the method. The statement that the method ''should be sensitive enough to diagnose all mosaic Klinefelter patients'' remains speculative as no karyotype of the 15 previously diagnosed Klinefelter patients is provided. In addition, the superiority of this method to karyotyping remains to be elucidated systematically and it is questionable whether a method that takes considerably longer (''can be done in less than 48 hours'') can replace the Barr body screenings, which can be performed within 1 hour.

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Re: Seasonal Variation and Age-Related Changes in Human Semen Parameters

To the Editor:

The study of seasonal variation in human semen parameters has, in our opinion, two different aspects—individual and demographic. The first refers to the consideration of each patient by an evaluation of male fertility status in the laboratory, and the second relies on basic epidemiological knowledge of how the male genital tract works. The present letter represents another milestone in the effort to better understanding these important issues.

We would like, first, to report our experience at the Andrology Laboratory of the University Clinical Hospital ''José de San Martín'' in Buenos Aires, Argentina. Our information was collected retrospectively on 904 semen analyses that were done in 2002. Semen samples were studied according World Health Organization (WHO) criteria (WHO, 1999); volume, pH, sperm concentration, motility, progressive motility, and morphology were assessed. Summer was defined as December, January, and February; fall as March, April, and May; winter as June, July, and August; and spring as September, October, and November. The mean temperatures in Buenos Aires are 22°C (range, 15°C to 28°C), 23°C (range, 17°C to 30°C), and 22°C (range, 16°C to 28°C) in December, January, and February, respectively. In the population we studied, the sperm concentration expressed per ejaculate was higher during winter, although statistically significant differences were not found (by analysis of variance). In our retrieved analysis records, we could not find differences in the other parameters—those reflecting either testicular or accessory gland function—considered.

Two issues sparked our interest in the Chen et al. article: 1) their data on sperm concentration and 2) their interpretation of the higher sperm count during winter. With reference to the first item, the authors reported an average sperm concentration of 136.1 million/mL (SD, 142.0), with a range of 2.2 to 847 million/mL, with only 10.5% of the sperm counts below WHO reference values. They assessed sperm concentration by computer aided sperm analysis (CASA). We have measured sperm counts smaller than those reported by Chen et al, probably because of the different methodology used to obtain the sperm count (WHO vs. CASA) biased the results (Curi et al, 2002).

With reference to the second item, the authors stated that 1) sperm production in humans is known to decrease when the testicular temperature is raised by experimental techniques, 2) normal spermatogenesis requires a temperature 2°C to 3°C lower than the rectal temperature, and 3) the effect of temperature is manifested approximately 90 days after exposure. We believe that the effect of experimental increases in temperature are not comparable to the effects of environmental increases in temperature, where homeostasis plays an important role. The summer temperature is not so extreme in either Buenos Aires or Boston as to affect spermatogenesis.

In the majority of species, the annual cycle of transitions between reproductive activity and quiescence are driven by environmental signals, mainly the photoperiod. These signals ensure the arrival of young at a time when conditions are optimal for their survival. This is not the case in humans, whose reproductive functions continue throughout the year, without any major or obvious changes in different seasons (Bartke, 1995).

The environment has a complex interaction in health and disease. In human semen parameters, factors other than temperature or photoperiod seem to have a greater significance when seasonal variation is considered. Environmental influences unique to our own species, such as occupational or accidental exposure to chemicals; the use of alcohol, psychotropic drugs, of anabolic steroids; stress; lifestyle; and abstinence time during each season should be thoroughly evaluated in relation to fertility status.

Respectfully,
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