tion of CBS (an expected incidence of ~1% in our population) might further hamper the homocysteine metabolism.

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Widening of a Y-Chromosome Interval-6 Deletion Transmitted from a Father to His Infertile Son Accounts for an Oligozoospermia Critical Region Distal to the RBM1 and DAZ Genes

To the Editor:

Great interest has been devoted in the last several years to the identification within the Y chromosome of the gene or genes involved in the etiology of idiopathic male infertility (azoospermia factor [AZF]; Vogt et al. 1992; Nagafuchi et al. 1993). Two genes, Y-chromosome RNA-recognition motif (YRRM; Genome Data Base symbol RBM; Ma et al. 1993) and deleted-in-azoospermia (DAZ; Reijo et al. 1995), have been mapped to Yq11 (interval 6). Both genes are considered to be candidates for AZF and have proved to be deleted in a number of infertile patients. Some observations suggest that RBM and DAZ are not the only genes responsible for idiopathic infertility. In fact, deletions of interval 6 also have been detected in infertile subjects retaining RBM (Kobayashi et al. 1994; Reijo et al. 1995; Stuppia et al. 1996). Moreover, deletions of DAZ have been identified in patients with different spermatogenic disorders, which suggests that AZF could be owing to more genes (Reijo et al. 1995, 1996).

We are reporting on the molecular analysis of sequence-tagged sites (STSs) in an oligozoospermic male and his father, presenting with a morphologically identical Y-chromosome deletion. R. A., a 32-year-old male,

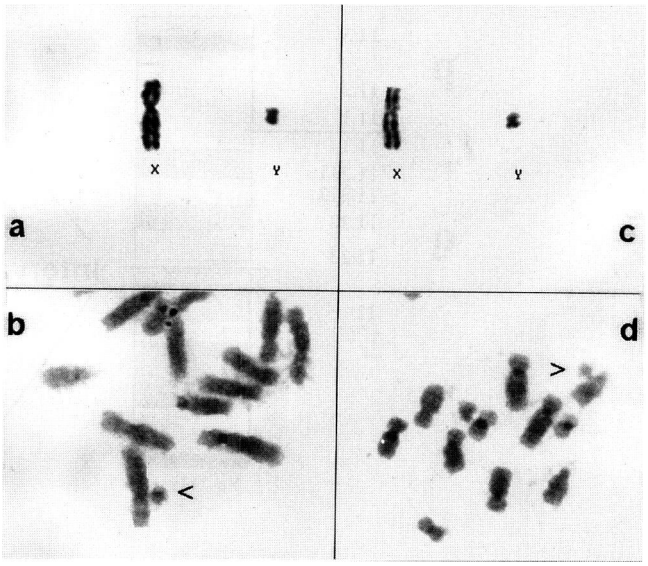

Figure 1 Cytogenetic analysis of the proband and his father. *a*, Proband, GTG bands. *b*, Proband, *AluI* bands. *c*, Father, GTG bands. *d*, Father, *AluI* bands. Arrows indicate the deleted Y chromosome.

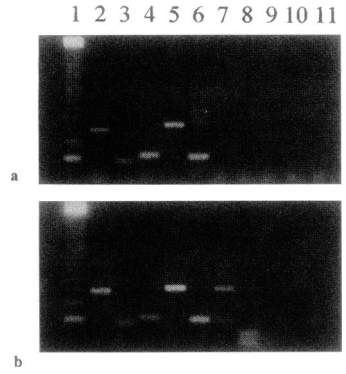

Figure 2 PCR analysis of the subintervals D–F of interval 6 in the proband (*a*) and his father (*b*). Lane 1, Molecular-weight-marker 123-bp ladder. Lane 2, sY155 (349 bp). Lane 3, sY147 (100 bp). Lane 4, sY149 (132 bp). Lane 5, sY254 (350 bp). Lane 6, sY255 (126 bp). Lane 7, sY243 (118 bp). Lane 8, sY269 (94 bp). Lane 9, sY158. Lane 10, sY166. Lane 11, sY167. The presence, in the amplification product of sY243, of an extra band of ~350 bp has been observed in all the investigated samples.

was referred to our laboratory because of infertility. Repeated semen analyses showed sperm concentrations in a range of 1.7×10^6/ml to 1×10^7/ml, with a 70% incidence of atypical forms (abnormal morphology of the head and the tail) and with a 30% incidence of reduced motility, at 2 h. Ultrasound examination displayed normal testes without any appearance of obstruction. The levels of FSH, luteinizing hormone, prolactin, and TSH were within the normal range. On the basis of these data, the diagnosis was oligozoospermia of unknown origin. The father of the patient was 65 years old and had no evident history of subfertility. He has an only child because a family doctor had counseled him that his wife should avoid further pregnancies, since he and his son have Rh+ blood and his wife has Rh− blood. Cytogenetic studies, performed on peripheral blood lymphocytes by means of GTG (Seabright 1971) and *Alu*I (Mezzanotte et al. 1983) banding, showed in the proband and his father a del(Y) with a break at band q11 (fig. 1, *a–d*). FISH analysis, performed with a probe specific for all telomeric regions, demonstrated in both cases the presence of telomeres on the del(Y). To better define the breakpoints in the Y chromosome, a PCR analysis was performed as described elsewhere (Stuppia et al. 1996), with primers MK5 1372U25 and MK5 1535L20, specific for RBM1, and with primers for 23 STSs mapping within interval 6. Six STSs were from subinterval A (sY129, sY130, sY131, sY132, sY134, and sY164), two were from subinterval B (sY138 and sY143), five were from subinterval C (sY139, sY153, sY150, sY152, and sY220), five were from subinterval D (sY155, sY147, sY149, sY254, and sY255), two were from subinterval E (sY243 and sY269), and three were from subinterval F (sY158, sY166, and sY167) (Vollrath et al. 1992; Reijo et al. 1995). Amplification products were analyzed on a 2% agarose gel. An STS was recorded as absent after at least three amplification failures. This approach showed, in proband, deletion of sY243 and sY269 (subinterval E) and sY158, sY166, and sY167 (subinterval F), whereas his father showed

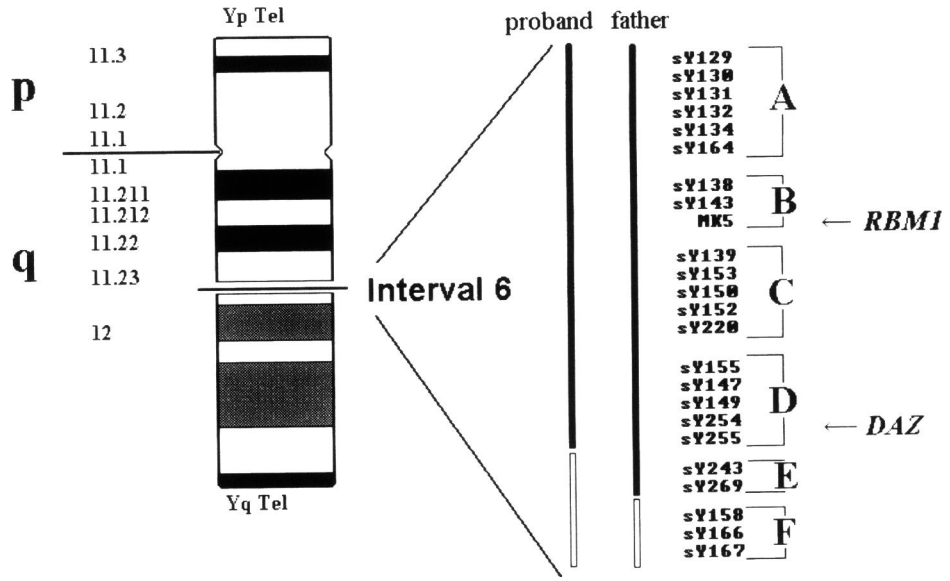

Figure 3 Diagram showing deletion at Yq11 (*left*) and molecular breakpoints within interval 6 of the Y chromosome in the proband and his father (*right*). Vertical bars indicate retained STSs (black) and deleted STSs (white). The proband's deletion includes interval E in addition to interval F, which is the only region lost in his father.

deletion of only sY158, sY166, and sY167 (subinterval F) (fig. 2, *a–b*). In both subjects, sY254 and sY255, mapping within the DAZ gene, and MK5, mapping within the RBM1 gene, were maintained (fig. 3).

In general, a Y-chromosome deletion that is associated with male infertility occurs as a de novo deletion (Reijo et al. 1995; Stuppia et al. 1996). In this study, both the proband and his father showed a del(Y)(q11), but the deletion in the proband was actually larger compared with that in his father, since the proband's deletion also involved STSs sY243 and sY269, which are located within the more proximal subinterval E of interval 6 (fig. 3, *right*). This result provides a biological support to the infertility found in the proband. This observation also confirms the critical role of interval 6 in the spermatogenetic process, which suggests that deletion of subinterval F, per se, is not associated with infertility. An additional point of interest is that both the proband and his father retained RBM1 and STSs sY254 and sY255, which are mapped within DAZ, in subinterval D. The segment deleted in the proband only, which likely is related to infertility, lies within subinterval E, which is outside the DAZ region. This implies that, in oligozoospermic patients at least, other genes in the region distal to DAZ and RBM1 may be involved in the spermatogenetic process. A matter of speculation is the role of the paternal deletion in the germ-cell mutation, which leads to the infertility in the proband. Deletions related to the spermatogenesis failure are regarded as de novo mutations. On the basis of the infertility rate among men and the percentage of infertile patients showing deletions of the Y chromosome, Reijo et al. (1995) have suggested that these mutations should occur in ~ 1 in 10^4 male newborns and that this high figure could arise by a mechanism involving repeated sequences flanking the gene. The present observation argues that some deletions should not lead necessarily to infertility, but these deletions make the Y chromosome more liable to a second mutation resulting in the spermatogenesis failure, as a consequence of DNA instability.

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Premeiotic Trisomy 21 in Oocytes and Down Syndrome: A Reply to Zheng and Byers's Hypothesis

To the Editor:

The parental and meiotic or mitotic origin of supernumerary chromosome 21 (HC21) in trisomy 21 has been extensively studied in recent years by use of DNA polymorphisms. According to the segregation of these DNA markers in nuclear families with trisomy 21 (father, mother, and trisomy 21 offspring), the origin of supernumerary HC21 has been assigned to maternal or paternal meiosis I or meiosis II errors or to postzygotic mitotic