Original articles

Clustering of Y chromosome deletions in subinterval E of interval 6 supports the existence of an oligozoospermia critical region outside the DAZ gene

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Abstract

Y chromosome molecular analysis was performed using the STS-PCR technique in 50 patients with oligozoospermia. Microdeletions of interval 6 of the Y chromosome were detected in seven patients, in six of whom subinterval E was affected. All patients retained the RBM1 and DAZ genes, while in one deletion involved the SPGY gene. The size of the deletion was not apparently related to the severity of the disease. These results suggest the presence of an oligozoospermia critical region on the Y chromosome within subinterval E of interval 6.

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microdeletions outside the DAZ gene.⁵ Vogt *et al*¹¹ have argued that no fewer than three AZF loci exist, AZFa, AZFb, and AZFc, the latter including the DAZ and SPGY genes. AZFa and AZFb deletions result in azoospermia, while AZFc deletions at subintervals D, E, and F can cause both azoospermia and oligozoospermia. We have reported an oligozoospermic patient and his father, both with a del(Yq11), where molecular analysis disclosed maintenance of the subinterval E in the father and its deletion in the son.¹² This has suggested to us a relationship between subinterval E deletion and oligozoospermia.¹² In order to test this hypothesis, a systematic molecular analysis using STS-PCR⁸ was performed in 50 patients with idiopathic oligozoospermia.

Materials and methods

PATIENTS

Fifty consecutive subjects with idiopathic oligozoospermia, including a previously reported case,¹² with a mean age of 34 years (range 23-48) were entered into this study. The diagnosis was made on the basis of semen analysis according to the WHO guidelines.¹³ All subjects had a sperm count below 20 million per ml ejaculate, with or without additional abnormalities of sperm motility or head and tail morphology. Each patient was carefully examined to rule out other causes of infertility. FSH, LH, and TSH values were available for all patients. A normal male karyotype was detected in 50 metaphases from each patient except one, who showed a del(Yq11), as previously reported.¹²

STS-PCR ANALYSIS

Each patient was examined for 27 loci mapped to interval 6 of the Y chromosome using the STS-PCR approach. These loci represent a selection of STS primers from subintervals A-F. In detail, we analysed sY129, sY130, sY131, sY132, sY134, and sY164 (subinterval A); sY138, sY143, and MK5 (subinterval B); sY139, sY153, sY150, sY152, and sY220

Male spermatogenesis involves different genes regulating mitotic and meiotic cell divisions, as well as subsequent differentiation to mature spermatozoa.¹ Several reports, including consistent observation of microdeletions in infertile patients, have located a critical region on Yq.²⁻⁶ Based on molecular results, the Y chromosome has been subdivided into seven deletion intervals and the spermatogenesis failure (azoospermia factor, AZF) locus assigned to interval 6 at band Yq11.⁷ This latter region has been further subdivided into six subintervals (A, B, C, D, E, and F).⁸ Three genes have been cloned from interval 6, RBM,⁹ DAZ,¹⁰ and SPGY,¹¹ and are all thought to be related to male infertility. The existence of additional spermatogenesis genes within this interval cannot be excluded. In fact, deletion in interval 6 produces distinct phenotypes of the number and stage of germ cells in testis tissue sections. Most tubules had a complete absence of germ cells. In some tubules, germ cells at different developmental stages (spermatogonia, spermatocytes, spermatids) were recognised. Some men were diagnosed as having oligozoospermia. In addition, some infertile men show

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Table 1 Clinical, cytogenetic, and molecular data of the patients with Y chromosome microdeletions

Patient	Age	Sperm count	FSH	LH	TSH	Karyotype	Deleted STSs
1	39	<2M/ml	9 mIU/ml (NR <15)	4.2 mIU/ml (NR <20)	7 ng/ml (NR 3–10)	46,XY	sY273, sY269
2	26	5M/ml	12 mIU/ml (NR <15)	3 mIU/ml (NR <20)	5 ng/ml (NR 3–10)	46,XY	SPGY, sY272, sY273, sY269, sY243
3	23	<2M/ml	8 mIU/ml (NR 1–14)	2.6 mIU/ml (NR 1.5–9.2)	4 ng/ml (NR 3–10)	46,XY	sY147
4	28	7M/ml	16 mIU/ml (NR <15)	6.3 mIU/ml (NR <20)	5 ng/ml (NR 3–10)	46,XY	sY269, sY243, sY167
5	32	<2M/ml	5.4mIU/ml (NR 1–9)	3.22 mIU/ml (NR 1–5)	5 ng/ml (NR 3–10)	46,X,del(Y)(q11)	sY269, sY243, sY167, sY166, sY158
6	30	9M/ml	10 mIU/ml (NR <15)	3.8 mIU/ml (NR <20)	7 ng/ml (NR 3–10)	46,XY	sY272, sY273
7	27	8M/ml	45 mIU/ml (NR 2–20)	17 mIU/ml (NR 2–10)	6 ng/ml (NR 3–10)	46,XY	sY147, sY273

(subinterval C); sY155, sY147, sY149, sY254, sY255, and SPGY1 (subinterval D); sY272, sY273, sY269, and sY243 (subinterval E); and sY167, sY158, and sY166 (subinterval F). MK5, sY254–255, and SPGY1 are specific for the genes RBM1, DAZ, and SPGY, respectively. Amplifications were performed with 30 cycles of 94°C for one minute, 61°C for one minute, and 72°C for one minute. PCR products were analysed on a 2% agarose gel or on a 6% acrylamide gel, after staining with ethidium bromide. An STS was considered as absent only after at least three amplification failures, in the presence of a successful amplification of an internal control. To exclude the presence of genetic polymorphisms, STS-PCR analysis was also performed in the fathers of three patients (patients 3, 4, and 5) and in 10 fertile subjects, which were used as positive controls.

Results

PCR amplifications produced a band of the expected size for all the 27 loci investigated in the controls and in 43 patients. Seven patients (14%) showed a deletion of one or more STSs, namely sY273 and sY269 (patient 1), sY272, sY273, sY269, sY243, and SPGY1 (patient 2), sY147 (patient 3), sY269, sY243, and sY167

(patient 4), sY269, sY243, sY167, sY158, and sY166 (patient 5), sY272 and sY273 (patient 6), and sY147 and sY272 (patient 7). All deletions were interstitial, except in one patient (patient 5) where it was terminal. PCR amplification was also performed on the fathers of three of these seven patients (patients 3, 4, and 5). The fathers of two of these (patients 3 and 4) did not show microdeletions, while the father of patient 5 had a deletion of sY167, sY158, and sY166. The fathers of the other four patients with microdeletions (patients 1, 2, 6, and 7) were not available. A summary of data of the seven patients with microdeletions is shown in table 1, and a deletion map is shown in fig 1.

Discussion

Idiopathic oligozoospermia is the most common cause of male infertility, affecting 3–4% of men.¹⁴ Unlike azoospermic patients, subjects with oligozoospermia rarely show chromosome abnormalities. In recent years, several studies have reported microdeletions of interval 6 of the Y chromosome in both azoospermic and oligozoospermic patients.^{3–6} In particular, Reijo *et al*,¹⁵ using STS-PCR, described two subjects with microdeletions of interval 6, involving

Figure 1 Diagram showing microdeletions of interval 6 in seven patients with oligozoospermia. Filled bars=presence of the STS, empty bars=absence of the STS, *sY147 has also been mapped in subinterval E.

subintervals C, D, E, and F. The deletion of the DAZ gene in both cases supported a pathogenic role of this gene in oligozoospermia as well. Vogt *et al.*¹¹ using the same approach, found additional oligozoospermia patients with microdeletions in the distal interval 6, AZFc, a region of at least 500 kb, encompassing subintervals D, E, and F and including the DAZ and SPGY genes. We have argued that the oligozoospermia critical region is limited to subinterval E, outside the DAZ gene, based on the observation of an oligozoospermic patient with a microdeletion in this subinterval.¹² In the present study, we found microdeletions of subinterval E in six of 50 patients with oligozoospermia. The seventh patient was deleted for sY147, whose map position is still debated between subintervals D and E.^{8,16} The discovery of a second patient with deleted sY147, associated with loss of sY272 which maps within interval E, supports physical mapping of sY147 to the proximal subinterval E. All patients retained the DAZ and RBM1 genes and only one had a deletion of SPGY. These data confirm our previous claim about the presence of an oligozoospermia critical region within subinterval E¹² and further narrow this critical region. The study and characterisation of this region and the identification of one or more genes within subinterval E will improve current understanding of the biological basis of oligozoospermia. Our results indicate that 14% of oligozoospermic patients have Y chromosome de novo microdeletions which are probably related to the disease. At present no evidence has been found for a different genotype-phenotype correlation. We conclude that STS-PCR is valuable for screening the microdeletions in interval 6 of the Y chromosome in patients with infertility of unknown origin.

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