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ORIGINAL INVESTIGATION

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A quarter of men with idiopathic oligo-azoospermia display chromosomal abnormalities and microdeletions of different types in interval 6 of Yq11

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Abstract Cytogenetic investigations and molecular analysis of the Y chromosome by the polymerase chain reaction amplification of sequence-tagged sites (STS-PCR) technique were performed in 126 patients affected by idiopathic oligo-azoospermia following accurate selection of cases. Seventeen patients evidenced an abnormal karyotype. Fourteen patients with a normal karyotype had microdeletions of the Y chromosome within interval 6. In azoospermic patients microdeletions were scattered along different subintervals, while in oligozoospermic patients they were clustered in subinterval 6E. The size of the deletion was not apparently related to the severity of the disease. These results suggest that cytogenetic analysis and the STS-PCR technique can detect a genetic cause of infertility in about one-quarter of patients with idiopathic oligo-azoospermia.

Introduction

Infertility affects about 15% of the general population, and in almost half of the cases is ascribable to the male partner (Hargreave 1990; Skakkebaek et al. 1994). Different causes of male infertility are known, including congenital anatomic alterations, infections, drug abuse, environmental agents, abnormal karyotype and Mendelian disorders. The role of other pathological conditions, including varicocele and cryptorchidism, is still debated (Skakkebaek et al. 1994; Mak and Jarvi 1996; de Krestser 1997). During recent years, great efforts have been made to identify a locus specifically involved in the failure of spermatogenesis (AZF, azoospermia factor) whose localization on the long arm of the Y chromosome (band Yq11 or deletion interval 6) had been suggested on the basis of cytogenetic and molecular findings (Tiepolo and Zuffardi 1977; Vergnaud et al. 1986; Vogt et al. 1992; Nagafuchi et al. 1993). A detailed molecular analysis of the Y chromosome revealed that interval 6 includes six subintervals (A to F), providing a sequence-tagged site (STS)-based map of this region (Foote et al. 1992; Vollrath et al. 1992). Polymerase chain reaction (PCR) amplification of these STSs (STS-PCR) allowed the detection of microdeletions within interval 6 in infertile patients with a normal karyotype, with an incidence ranging from 3 to 30% (Kobayashi et al. 1994; Kent-First 1996; Najmabadi et al. 1996; Stuppia et al. 1996a; Foresta et al. 1997; Pryor et al. 1997; Simoni et al. 1997). So far, two gene families (RBM and DAZ) have been isolated from interval 6 and proposed as candidates for AZF (Ma et al. 1993; Reijo et al. 1995). However, additional results support the idea that AZF consists of more loci and different genes (Najmabadi et al. 1996; Stuppia et al. 1996b, 1997; Vogt et al. 1996). In particular, Vogt et al. (1996) hypothesized the existence of three AZF loci, AZFa, AZFb and AZFc, the first two of which are implicated in azoospermia, while the third, including the DAZ gene, is also deleted in patients with oligozoospermia. More recently, the existence of an oligozoospermia critical region in distal interval 6, outside the DAZ region, has been postulated (Stuppia et al. 1997).

The identification of males in which disruption of spermatogenesis derives from microdeletion of the Y chromosome is of great importance. In fact, the application of intracytoplasmic sperm injection (ICSI) for treating infertili-

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ty could lead to the transmission of mutations that could either perpetuate infertility or result in complications with a related genetic background (Aitken and Irvine 1996; Kent-First et al. 1996; Mulhall et al. 1997).

Here we report our experience on 126 cases with idiopathic oligo-azoospermia, investigated by means of cytogenetic analysis and STS-PCR of 27 loci, which contribute to a definition of the incidence of Y chromosome deletions related to male infertility and genotype-phenotype correlation.

Materials and methods

Patients

One hundred and twenty-six consecutive males, averaging 33 years (range 19–55 years) in age, with diagnosis of oligozoospermia or azoospermia of unknown origin entered this study (patients 1–126). On the basis of semen analysis (WHO criteria 1992), 58 patients had a diagnosis of azoospermia (patients 1–58), and 68 of oligozoospermia (<20 million sperm per milliliter of ejaculate) (patients 59–126). All cases were carefully evaluated for the presence of an obstruction of the spermatic duct, and patients with obstructive azoospermia were excluded from the study, except one (patient 34). In this patient a testis biopsy disclosed the presence of a block of spermatogenesis, with absence of mature spermatozoa in all tubules observed, thus demonstrating that the obstruction was not the only cause of the disease. Patients were asked to fill in a questionnaire on their medical history (varicocele, cryptorchidism, infections, drug use, traumas), family history, and sexual history. Ten patients referred to a previous history of varicocele (patients 16, 79, 91–92, 94, 96, 107, 113, 114, 115) and 6 had cryptorchidism (32, 39, 46, 54, 99, 110). The remaining 110 patients had an unremarkable clinical history. The follicle-stimulating hormone and luteinizing hormone values were available for all patients.

Karyotype and fluorescence in situ hybridization

Cytogenetic analysis was performed in all patients on peripheral blood cells cultured for 72 h in the presence of phytohemagglutinin. Karyotypes were analyzed by GTG- and *Alu*I-banding (Seabright 1971; Mezzanotte et al. 1983). For each case, at least 20 metaphases were examined. Fluorescence in situ hybridization (FISH) was performed on three cases (patients 6, 13, 35) with a probe specific for the SRY gene (Sinclair et al. 1990).

Amplification of STSs by the PCR

PCR analysis was performed on 111 patients, of whom 109 had a normal karyotype (patients 2–5, 9–12, 14–16, 18–21, 23–27, 29, 32–34, 36, 39, 41, 43–52, 54–88, 90–126) and two had a del(Y)(q11) and an idic(Y)(q11), respectively (patients 8, 89). DNA was extracted from peripheral blood cells or from fixed cytogenetic samples, as previously described (Stuppia et al. 1993). For each patient, we analyzed 27 STSs mapped within interval 6 of the Y chromosome, namely sY129, sY130, sY131, sY134, sY164 (subinterval A); sY138, sY143, MK5 (subinterval B); sY139, sY153, sY150, sY152, sY220 (subinterval C); sY155, sY147, sY149, sY254, sY255, SPGY (subinterval D); sY272, sY273, sY269, sY243 (subinterval E); sY167, sY158, and sY166 (subinterval F), for a total of more than 3000 reactions. The STSs MK5, sY254 and sY255 were considered specific for the RBM1 (MK5) and DAZ (sY254 and sY255) genes (Reijo et al. 1995; Stuppia et al. 1996a). In order to compare the present results with the map of Vogt et al. (1996), which was based on a different set of STSs, subintervals 6A, 6B and 6C were considered to correspond to AZFb, and subintervals 6D, 6E and 6F to AZFc. As our study was concentrated on interval 6, AZFa was not investigated. An STS was scored as absent after at least three amplification failures. Twenty fertile men, including the fathers of seven patients (patients 2, 16, 19, 21, 70, 76, 89) were investigated as controls. Paternity was tested by PCR amplification of hypervariable DNA regions, which always showed compatibility between father and son.

Results

Karyotyping and FISH

Seventeen out of the 126 patients (13.4%) showed an abnormal karyotype: 47,XXY in 11 cases (patients 1, 7, 17, 22, 30–31, 37–38, 40, 42, 53); 46,XX in 3 (patients 6, 13, 35); idic(Y)(q11), del(Yq) and t(13;14) in 1 patient each (patients 8, 89, 28). All these patients had a diagnosis of azoospermia, except one who had oligozoospermia (patient 89). The remaining 109 patients displayed a normal diploid karyotype. In the three 46,XX patients, FISH analysis revealed an SRY gene signal on the short arm of one X chromosome.

Polymerase chain reaction

Sixteen patients showed microdeletions of one or more STSs (patients 2, 8, 16, 19, 21, 34, 59, 69, 70, 76, 89, 93, 107, 123, 124, 125). Six of these had azoospermia (patients 2, 8, 16, 19, 21, 34), six mild oligozoospermia (patients 69, 76, 93, 107, 123, 124) and four severe oligozoospermia (<2 million sperm per milliliter ejaculate) (patients 59, 70, 89, 125). Three of six patients with azoospermia had deletion of a single STS, namely sY134 (patient 2), sY139 (patient 21) and sY164 (patient 34). The remaining three showed deletion of multiple STSs: patient 8, with an idic(Yq), had a terminal deletion, with loss of all the STSs except sY129, sY130, sY131, sY134; patient 16 had deletion of sY138, sY152, sY254, sY255 and sY158; patient 19 had lost sY155, sY254, sY255 and SPGY. Of the six patients with mild oligozoospermia, one had deletion of a single STS (sY273) (patient 123), while the five others had loss of more STSs, namely sY272, sY273, sY269, sY243 (patient 69); sY269, sY243, sY167 (patient 76); sY272 and sY273 (patients 93 and 124); sY147 and sY273 (patient 107). The four patients with severe oligozoospermia were deleted for a single STS (sY147) in one case (patient 70), for two STSs (sY273 and sY269) in another case (patient 59), for a large deletion including sY153, sY220, sY254, sY255, SPGY, sY272, sY273, sY269 and sY243 in the third case (patient 125), and for a terminal deletion with loss of sY269, sY243, sY158, sY166, sY167 in the last case (patient 89). In all cases controls displayed amplification of all the STSs, except for the father of patient 89, described elsewhere (Stuppia et al. 1996b). A complete summary of microdeletions is reported in Fig. 1.

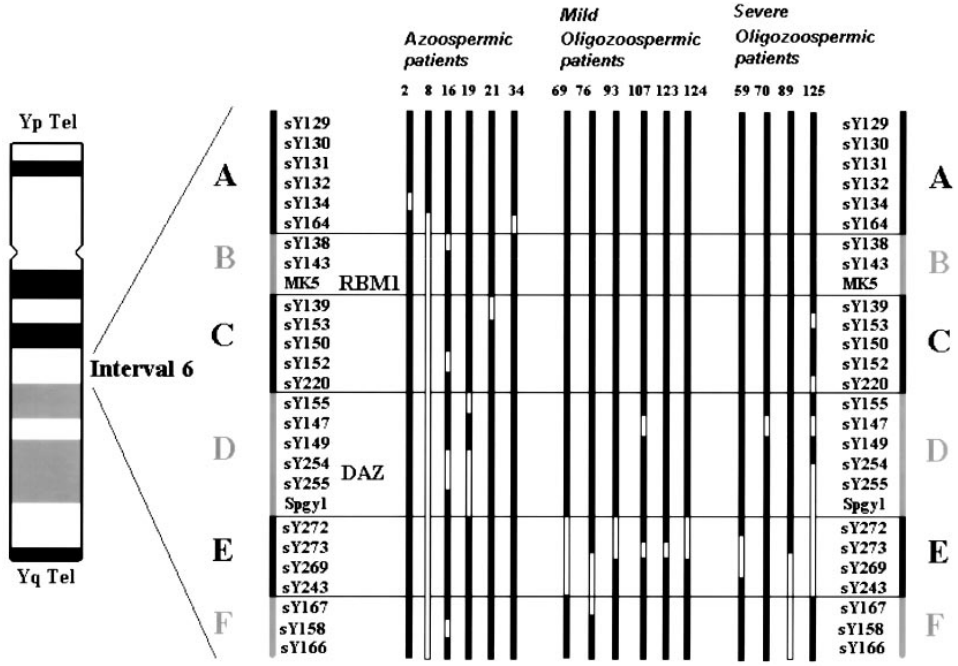

Fig. 1 Diagram showing microdeletions of interval 6 in 16 patients with idiopathic infertility. *Filled bars* presence of the sequence-tagged site (STS), *empty bars* absence of the STS

Table 1 Clinical, cytogenetic and molecular data of patients with microdeletions of the Y chromosome. (*FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *M* million, *n.d.* not done, *SpcA* arrest at spermatocyte stage, *SpdA* arrest at spermatid stage)

Case	Age (years)	Semen analysis	FSH (U/l)	LH (U/l)	Testis histology	Karyotype	Deleted subinterval 6 (AZF locus)
2	26	Azoospermia	7	3.4	SpdA	46,XY	6A (AZFb)
8	40	Azoospermia	13	5	SpcA	46,X,idic(Y) (q11)	6B, 6C, 6D, 6E, 6F (AZFb, AZFc)
16	23	Azoospermia	6	2.5	SpdA	46,XY	6B, 6C, 6D (AZFb, AZFc)
19	28	Azoospermia	5.7	3.4	SpdA	46,XY	6D (AZFc)
21	30	Azoospermia	5	3.2	SpdA	46,XY	6A (AZFb)
34	30	Azoospermia	7.5	3	SpdA	46,XY	6C (AZFb)
59	39	<2 M	9	4.2	n.d.	46,XY	6E (AZFc)
69	26	5 M	12	3	n.d.	46,XY	6E (AZFc)
70	23	<2 M	8	2.6	n.d.	46,XY	6D (AZFc)
76	28	7 M	16	6.3	n.d.	46,XY	6E, 6F (AZFc)
89	32	<2 M	5.4	3.22	n.d.	46,X,delY (q11)	6E, 6F (AZFc)
93	30	9 M	10	3.8	n.d.	46,XY	6E (AZFc)
107	27	8 M	45	17	n.d.	46,XY	6D, 6E (AZFc)
123	21	7 M	9	6	n.d.	46,XY	6E (AZFc)
124	55	6 M	10	4	n.d.	46,XY	6E (AZFc)
125	32	<2 M	4.82	2.5	n.d.	46,XY	6D, 6E (AZFc)

Testis histology

Testis histology was analyzed in the six azoospermic deletion-bearing patients (patients 2, 8, 16, 19, 21, 34). In five of them testicular histology revealed spermatogenic arrest at the spermatid stage in all tubules observed (patients 2, 16, 19, 21, 34). In one patient, spermatocytes were blocked at the first meiosis, and no postmeiotic germ cells could be detected in any testis tubule analyzed (patient 8). In all patients spermatogonia were normal within all tubules.

A summary of the results in patients with microdeletions of the Y chromosome is reported in Table 1.

Discussion

Microdeletions of interval 6 of the Y chromosome are associated with male infertility (Kent-First 1996; Najmabadi et al. 1996; Stuppia et al. 1996a; Foresta et al. 1997; Pryor et al. 1997; Simoni et al. 1997). Thus, search for Yq microdeletions in infertile patients by STS-PCR should be used as a routine test, although no guidelines for this approach have yet been established (Reijo et al. 1996; Pryor et al. 1997; Simoni et al. 1997). A matter for discussion is the number of STS to be investigated in each patient in order to detect as many microdeletions as possible. Data from the literature report the use of a number of STSs, ranging from 5 to 89 (Pryor et al. 1997; Simoni et al. 1997). In this study, we examined 126 patients with oligoazoospermia of nonobstructive origin. Of these, 109 patients with normal karyotype and two with rearrangements of the Y chromosome were investigated by STS-PCR analysis of 27 loci of the Y chromosome. In the group of patients with a normal karyotype, microdeletions were detected in 14 (13%), including 5 azoospermic and 9 oligozoospermic patients. Thus our STS-PCR protocol was highly effective in detecting microdeletions. In fact, the presented figure of 13% is consistently higher than that detected using 89 STSs on unselected patients (Pryor et al. 1997). Personal experience also shows that, after accurate selection of patients using clinical, laboratory and cytogenetic criteria, microdeletions can be detected using a set of about 30 STSs encompassing Yq interval 6. In some cases, the microdeletions involved multiple, noncontiguous STSs. This picture has already been reported by us and other authors, and could be explained by the presence of complex rearrangements as well as by the presence of repetitive loci (Najmabadi et al. 1996; Stuppia et al. 1996). Although there was a similar incidence of microdeletions in the oligozoospermic and azoospermic patients (5/42 and 9/68, respectively), the localization of these microdeletions within interval 6 of the Y chromosome was distinct in the two groups. In particular, in patients with azoospermia, microdeletions were scattered along the entire interval 6, two patients having microdeletions in 6A, one in 6C, one in 6D and one having multiple microdeletions involving 6B, 6C, and 6D. In these latter cases microdeletions occurred within AZFc, and involved the DAZ gene, while in the others they were proximal to DAZ, involving a region roughly corresponding to AZFb. These data support the involvement of the DAZ gene in azoospermia, but also the presence of more AZF loci in the long arm of the Y chromosome (Vogt et al. 1996). Since microdeletions outside the DAZ region do not involve any of the genes so far known to be involved in spermatogenesis, their role as a causative agent of sterility is still to be clarified. Testis histology in this series of azoospermic patients did not allow a correlation to be established between the localization of microdeletions and the phenotype of the patients, all cases showing an arrest at the spermatid stage, independently of the localization of the microdeletion. On the other hand, microdeletions in patients with mild or severe oligozoospermia were clustered in distal interval 6, particularly in subinterval 6E, and all patients except one retained the DAZ gene. Thus, as previously suggested (Stuppia et al. 1997), an oligozoospermia-specific locus should be located distal to DAZ. In agreement with published results, the size of the microdeletions was not related to the severity of the disease (Najmabadi et al. 1996; Foresta et al. 1997; Stuppia et al. 1997). In addition, their localization within interval 6E did not show consistent differences between the two groups, except in one patient with severe oligozoospermia who had a large deletion encompassing subintervals 6D and 6E, including DAZ. However, these results need to be corroborated by the analysis of a larger series of patients.

In two patients with an idic(Y)(q11) and a del(Y)(q11), respectively, STS-PCR analysis showed that the deletions involved interval 6. In the first patient, with azoospermia, the break occurred at 6A, while in the second, with severe oligozoospermia, it occurred in subinterval 6E. These observations confirm that different breakpoints within interval 6 result in different defects of spermatogenesis. The relationship between microdeletions and Y chromosome rearrangements is unclear. It has been suggested that somatic cell rearrangements could make the Y chromosome more liable to a second mutation in the germ cells (Stuppia et al. 1996b). Thus, children born by means of ICSI from fathers carrying microdeletions, could be at risk for Y chromosome rearrangements, including del(Yq) and idic(Y), with consequent instability of this chromosome and an increased risk of a pathological phenotype (Salo et al. 1995; Stuppia et al. 1996c). This recommends analysis of Y chromosome microdeletions in infertile patients admitted to ICSI protocol.

In conclusion, in the present series of patients, cytogenetic and molecular investigations allowed the detection of a Y chromosome abnormality in 32 out of 126 infertile patients (25.3%). This suggested that, following accurate selection of patients, in about one-quarter of them a genetic cause of infertility could be detected. In azoospermic patients, who have a higher incidence of chromosomal abnormalities, this figure increases to 38%. A genotype-phenotype correlation could be established based on the localization, but not on the size, of microdeletions. In particular, microdeletions in proximal interval 6 (AZFb) or in the DAZ gene are found in azoospermic patients, while microdeletions in distal interval 6 (subinterval E) are associated with oligozoospermia. With an improved knowledge of the molecular structure of different AZF loci, it will be possible to detect other Y chromosome anomalies, including point mutations within the genes involved in the spermatogenesis process. This will improve the efficiency of genetic testing in infertile patients and our understanding of the biological basis of idiopathic male infertility.

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