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Clinical and prognostic importance of chromosomal abnormalities, Y chromosome microdeletions, and CFTR gene mutations in individuals with azoospermia or severe oligospermia

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Aim: To illustrate the importance of genetic screening in the assessment of fertility and the correct diagnosis in patients with azoospermia or severe oligospermia.

Materials and methods: This study examined 500 patients with reproductive failure, having fewer than 5 million sperm/mL detected in at least 2 consecutive spermiograms, who presented at a medical genetics polyclinic between 2008 and 2012. Metaphase preparations obtained from cell cultures were stained by trypsin-Giemsa banding. After DNA isolation, Y chromosome loci, including AZFa (SY84, SY86), AZFb (SY127, SY134), AZFc (SY254 SY255), and AZFd, were amplified by polymerase chain reaction using specific primers. Thirty-five patients with congenital unilateral absence of the vas deferens or congenital bilateral absence of the vas deferens (CBAVD) and a positive cystic fibrosis family history were evaluated for cystic fibrosis transmembrane conductance regulator gene mutations.

Results: No chromosomal abnormalities were noted in 440 (88%) of the 500 patients, whereas structural or numerical chromosomal abnormalities were detected in 60 patients (12%). Individuals with Y deletions made up 5.6% (n = 28) of the study sample. Three patients with no AZF deletion or chromosomal abnormality, but with CBAVD, were heterozygous for I148T, G1130A, or IVS3 406-3T>C mutations.

Conclusion: This study shows that genetic testing can make an important contribution to the treatment of patients planning in vitro fertilization due to azoospermia or severe oligospermia.

Key words: Infertility, Y chromosome microdeletion, cystic fibrosis transmembrane conductance regulator

1. Introduction

Infertility affects 15% of couples worldwide, and about 50% of affected couples have male factor infertility (1,2). Despite the identification of many congenital and acquired factors in the etiology of male infertility, the frequency of unexplained cases has increased steadily. Data from the World Health Organization and from the European Association of Urology in 2009 indicated a rate of 75.1% in Europe (3). Genetic factors were found to have important roles in the etiology of idiopathic azoospermia and severe oligospermia, conditions that affect 30% of individuals seeking treatment at infertility clinics (4). Karyotypic abnormalities and Y chromosome microdeletions were present in 2%–16% and 1%–55% of subjects with azoospermia or severe oligospermia, respectively (4,5). Congenital bilateral absence of the vas deferens (CBAVD) is seen less frequently in infertility clinics. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations were identified in 85% of patients with CBAVD, representing 1.4% of all subjects with nonobstructive azoospermia (6). CBAVD was detected in 97%–98% of men with cystic fibrosis; only 2%–3% of these men were fertile (7).

In the present study, the results of karyotype, Y chromosome microdeletion, and CFTR mutation analyses were evaluated during the assessment of fertility or prior to in vitro fertilization (IVF)/intracytoplasmic sperm injection(ICSI)-embryo transfer procedures in patients who presented at our medical genetics polyclinic because of azoospermia, severe oligospermia, or CBAVD.

2. Materials and methods

A total of 500 patients who applied to the Süleymaniye Maternity Hospital and Abant İzzet Baysal University

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medical genetics polyclinic between 2008 and 2012 because of reproductive failure, having fewer than 5 million sperm/mL detected in at least 2 consecutive spermiograms, were included in this study. Peripheral blood lymphocytes were cultured for 72 h, and metaphase preparations were stained by trypsin-Giemsa banding (GTG) to identify each patient's karyotype. DNA extraction was performed using a peripheral blood DNA isolation kit (Gentra, QIAGEN, Germany) according to the manufacturer's protocol. After the isolation of DNA, Y chromosome loci AZFa (SY84, SY86), AZFb (SY127, SY134), AZFc (SY254, SY255), and AZFd were amplified by polymerase chain reaction (PCR) using specific primers. Thirty-five patients with unilateral or bilateral congenital absence of the vas deferens and a positive cystic fibrosis family history were evaluated for CFTR gene mutations. Primers for the amplification of the CFTR gene were prepared based on gene sequences from http://www.ncbi.nlm.nih.gov. All products derived from PCR with each of the primer pairs were analyzed on 1.5% agarose gels by comparison with DNA size markers. PCR products were purified (Bio Basic, Canada) and reamplified in cycle sequencing reactions with dideoxynucleotides, using 4 μL of terminator-ready reaction mix (BigDye v.3.1), 3.2 pmol of primer, and 2.5 μL of purified PCR product. Cycling conditions were 25 cycles of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min (Applied Biosystems, USA). Samples were purified again (NucleoSEQ, Macherey-Nagel, Germany) and sequenced (ABI PRISM 3130 Genetic Analyzer, USA). The results were analyzed using ABI DNA sequencing analysis software (v5.2 and 3130xl; Applied Biosystems).

3. Results

No chromosomal abnormalities were detected in 440 (88%) of the 500 patients examined. Structural or numerical chromosomal abnormalities were detected in 60 of the patients (12%). The most common chromosomal abnormality was 47,XXY (Klinefelter syndrome), with a prevalence of 71.6% (n = 43) (Table 1). Individuals with normal Y chromosomes constituted 94.4% (n = 472) of the study group; 5.6% (n = 28) had Y microdeletions. AZFc was the most common site of the Y microdeletions, in 4.2% of the subjects (n = 21) (Table 2). Three patients with no AZF deletion or other chromosomal abnormality,

Table 1. Numerical and structural chromosomal abnormalities in the patients.

	Karyotype	n
No chromosomal abnormalities	46,XY	440
Numerical chromosomal abnormality	47,XXY	43
Structural chromosomal abnormality	46,XY,t(4;18)(q22;p11.2)	1
	45,XY,rob(13;14)(q10;q10)	3
	mos45,X[8]/46,X,del(Y)(q12)[32]	1
	46,XY,t(11;22)(q25;q13)	1
	45,XY,rob(13;15)(q10;q10)	1
	46,XY,t(2;3)(q21;qter)	1
	46,XY,t(16;17)(q12.1;q23)	1
	46,XY,inv(8)(p22;q21)	1
	46,XY,t(2;8)(p22;q24.1)	1
	46,XY,t(3;5)(q12;p12)	1
	46,XY,t(13;21)(q21.1;q22.2)	1
	46,XY,t(11;14)(q13;q24)	1
	46,XY,t(1;3)(p22.3;p25),16qh+	1
	46,XY,t(10;13)(q11.2;q14.2)	1
	46,XY,t(4;18)(q22;p11.2)	1

Table 2. Y chromosome microdeletion loci in the patients.

Deletion type	n	%
No deletion	472	94.4
AZFa deletion	1	0.2
AZFb deletion	2	0.4
AZFb + AZFc deletions	2	0.4
AZFc deletion	21	4.2
AZFd deletion	1	0.2
AZFa + AZFb + AZFc deletions	1	0.2

but with CBAVD were heterozygous for I148T, G1130A, or IVS3 406-3T>C mutations in the CFTR gene. One patient analyzed for CBAVD was heterozygous for N894S (p.Asn894Ser, c.2681A>G) in exon 15 of the CFTR gene (Table 3). No mutation was detected in patients with unilateral absence of the vas deferens.

4. Discussion

Azoospermia is a common finding in men who suffer from infertility. Increasing numbers of patients with azoospermia, as well as advances in molecular genetic techniques, have increased the interest in etiological research on the subject (8). Azoospermia may arise from monogenic pathologies such as numerical or structural chromosomal abnormalities, Y microdeletions, or CFTR gene mutations (7). Previous studies have reported a wide incidence range (2%–16%) of chromosomal abnormalities in infertile patients (9,10). The most common karyotypic abnormality in men with severe male factor infertility is Klinefelter syndrome, affecting 7%–13% of azoospermic men. Other karyotypic abnormalities that have been identified include Robertsonian translocations, chromosomal inversions, and non-Klinefelter sex chromosome abnormalities. Bourrouillou et al. performed karyotyping for 952 infertile men, finding sex chromosome anomalies in 65 patients (6.8%) and autosomal chromosome anomalies in 33 (3.5%) (11). Similarly, we found autosomal chromosome anomalies at a rate of 3.4% and sex chromosome anomalies at a rate of 8.6% (Table 1). Translocation carrier subjects have an increased chance for creating unbalanced gametes and for abnormal sperm production. The frequency of

Table 3. Genotype of cystic fibrosis transmembrane conductance regulator gene mutations in 35 patients.

CFTR gene mutations	*CFTR* gene sequence variations	n
*N894S (p.Asn894Ser, c.2681A>G) heterozygous Exon 4: Heterozygous I148T	Exon 9: IVS81342-12(GT)10, Exon 10: M470V-, Exon 19: IVS18 3601-65 C/A	1
Exon18: Heterozygous G1130A	Exon 9: IVS81342-12(GT)10-, Exon 19: IVS18, 3601-65C/A	1
Exon4: Heterozygous IVS3 4063T>C	Exon 9: IVS8 1342-13G/T- IVS8 1342-12(GT)10-, Exon 10: M470V	1
	Exon 10: M470V	1
	Exon 10: 1540A/G	1
	Exon 9: IVS8,1342-13G/T,GT-, Exon 10: 1540A/G-, Exon 19: IVS18, 3601-65C/A	4
	Exon 9: IVS8,1342-13G/T-, Exon 10: 1540A/G	8
	7T polymorphism	11
	Exon 20: P1290P (4002A/G)	2

*Its pathological impact is not known.

abnormal sperm has been reported to be 3.4%–40% in Robertsonian translocation carrier men and 47.5%–81% in reciprocal translocation carrier men (12). Couples with chromosomal structural abnormalities should have genetic counseling, and the use of genetic preimplantation diagnosis is warranted for these couples. We could not compare abnormal sperm ratios between patients with detected chromosomal abnormalities and those without, as we examined only patients with azoospermia. This is an important limitation of our study. Increases in chromosome number anomalies and diploid sperm ratios in samples from infertile men with normal karyotypes and abnormal spermiograms were reported in a sperm FISH study (13). The lack of data regarding sperm aneuploidy rates is another limitation of our study. Several studies on the association of Y chromosome AZF microdeletion with male infertility detected microdeletions in 1% to 55% of infertile men with azoospermia or severe oligospermia (14–16). This large variation among reports may be attributable to ethnic differences or to the criteria applied for patient selection. We found Y chromosome microdeletion in 5.6% of our study subjects, and the AZFc deletion was the most common, at 4.2% (n = 21) (Table 2), as seen in the literature (14,15,17).

In the gonads of men with AZFc deletions, 45,X/46,XY mosaicism has been described. This condition may be associated with an unstable Y chromosome, which may result in an embryo monosomic for X in ICSI. It was also reported that a baby boy with mixed gonadal dysgenesis or with ambiguous external genitalia may be born as a result of mosaicism (18). The complete removal of the AZFa and AZFb regions are associated with severe testicular phenotype, Sertoli cell-only syndrome, and spermatogenic arrest. The specificity and the reported genotype/phenotype correlation confers to Y deletion analysis a diagnostic and a prognostic value for testicular sperm retrieval (3). Thus, we recommend that Y chromosome microdeletion tests, in addition to karyotype analysis, be performed before IVF/ICSI procedures in couples who are candidates for IVF.

Thirty-five patients with CBAVD and a cystic fibrosis-positive family history were evaluated for CFTR gene mutations. Recent studies have shown that the type and frequency of CFTR gene mutations in patients with CBAVD vary among populations (19). In the present study, no chromosomal anomalies or Y chromosome microdeletions were detected in the infertile patients in whom CFTR analysis was performed. Three of the patients were heterozygous for the I148T, G1130A, or IVS3 406-3T>C mutation (Table 3). I148T-CFTR has been associated with a severe cystic fibrosis phenotype, perhaps because of defects in CFTR regulation of bicarbonate transport, although this mutant transports chloride similarly to wild-type CFTR in model systems (20). Spermatogenesis has been reported to be normal in isolated CFTR carrier patients with reproductive tract abnormalities. The I148T-CFTR and 406 3T>C alleles were present in 2 patients, but we think that these might not have affected spermatogenesis; a coexistent M470V polymorphism can explain the azoospermia in these patients.

A common polymorphism of M470V appears to be associated with the intensity of the disease (21). The substitution 3041-15T>G disrupts the polypyrimidine tract of the intron 15 acceptor splice site and thus may result in aberrant splicing. G1130A leads to a subtle amino acid alteration in a less-conserved portion of the CFTR protein (6). The G1130A mutation might have been a secondary cause of azoospermia in a third patient, who was heterozygous for this allele. Dinić et al. investigated CFTR gene mutations and Y microdeletions in 33 infertile male patients and found CFTR mutations in 6 (18%) of the patients (22). They suggested that the prognostic value of AZF and CFTR mutations was important, but that they were not fully responsible for sperm quality (21). Dayangaç et al. identified 27 different CFTR mutations in 51 patients with CBAVD in a Turkish population, with IVS8-5T and D1152H being the predominant mutations (6).

CFTR mutations were less common in the present study than in previous studies, and all of our patients with mutated alleles were heterozygous. This may be related to the population or the selected patients studied. Although cystic fibrosis is rarely seen in Turkey, CFTR gene mutations are the major cause of CBAVD. Thus, mutation panels should be tailored to the specific population under study (6). In addition to known mutations in the CFTR gene, polymorphic changes can affect clinical status. At least 120 polymorphisms have been defined for the CFTR gene. The number of thymidine residues (5, 7, or 9) in a tract at the end of intron 8 is relevant for clinical outcomes. T5 is considered to be a mutation; T7 and T9 are accepted as polymorphisms. Additionally, M470V and (TG) n polymorphisms have been reported to affect clinical progress (23). We also detected polymorphisms, including M470V, which could explain our patients' clinical status (Table 3). One patient who underwent CFTR gene analysis was heterozygous for N894S (p.Asn894Ser, c.2681A>G) in exon 15. Its pathological impact is not known, as there are no data about this variant in the literature. To specify the appropriate therapy for patients presenting at fertility clinics, it is important to determine the etiology of the infertility. Routine genetic screening in patients with azoospermia, especially in potential IVF candidates, may help to avoid unnecessary surgical interventions and unsuccessful IVF procedures and may reduce the economic burden for patients.

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