J Assist Reprod Genet (2011) 28:419–423
DOI 10.1007/s10815-011-9542-8

GENETICS

Primary male infertility in Izmir/Turkey: a cytogenetic and molecular study of 187 infertile Turkish patients

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Received: 6 August 2010 / Accepted: 2 February 2011 / Published online: 22 February 2011

Abstract
Purpose To detect somatic cytogenetic abnormalities and AZF microdeletions in a sample of 187 Turkish infertile men to determine the frequencies and the characteristics of our primary male infertility data in order to perform appropriate genetic counseling.
Methods This study included 187 infertile men. Chromosomal studies and screening of AZF deletions was performed by multiplex polymerase chain reaction (PCR) analysis using the Y Chromosome Deletion Detection System.
Results Cytogenetic study revealed chromosomal abnormality in 9 subjects (4.8%). In remaining 178 subjects, 7 subjects (3.93%) were detected to have Y chromosome microdeletions. The AZFc region was the most frequently involved region in microdeletion process in affected subjects. All subjects having microdeletion were azoospermic
Conclusions Cytogenetic and molecular study should be performed to obtain reliable genetic information for the genetic counseling of primary infertile man. Y chromosome microdeletion diagnosis is useful in decision making for assisted reproductive technics.

Keywords Primary male infertiliy · Genetic factors · Y chromosome · Microdeletions

Capsule Cytogenetic abnormalities and AZF microdeletions have been analyzed to determine the characteristics of regional primary male infertility data in order to perform appropriate genetic counseling.

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Introduction

Infertility is an important health problem affecting 10–15% of couples. The contribution of male factors to infertility is about 30–50%. Recent studies have indicated that both environmental and genetic factors are involved in the decline of reproductivity in males [1]. The main genetic factors playing a role in male infertility are somatic chromosomal abnormalities and Y chromosomal microdeletions within the Yq11 region. The genes which control spermatogenesis located in Yq11 region are known as azoospermia factor genes (AZF) [2]. After Klinefelter syndrome, Y chromosomal microdeletions are the most frequent cause of male infertility [3]. The AZF region has 3 non-overlapping loci-AZFa, AZFb, AZFc which are required for normal spermatogenesis [4].

AZFb contains eight protein-coding genes (CDY2, EIF1AY, HSFY, PRY, RBMYL1, RPS4YS, SMCY, and XKRY) and AZFc contains five similar genes (BPY2, CDY1, CSPG4LY, DAZ, and GOLGA2LY). All these genes are expressed in testicular tissue showing that they a play role in human spermatogenesis. Microdeletions of the AZF genes are caused by intrachromosomal recombinations between large homologous repetitive sequence blocks [5].

Here, we detected somatic cytogenetic abnormalities and AZF microdeletions in a sample of 187 Turkish infertile men to determine the frequencies and the characteristics of our primary male infertility data in order to perform suitable genetic counseling.

Materials and methods

This study included 187 infertile men aged 23–54 years, who were referred to Ege University Medical Genetics

Department between January 2005 and October 2007. All the patients had primary infertility and none had obstructive azoospermia. Chromosomal studies using peripheral blood and trypsin-G banding techniques following the International System for Human Cytogenetic Nomenclature [6] were performed on all patients. At least 20 metaphases were analyzed for each patient and in cases with mosaicism this number rose up to 100 metaphases.

High-molecular-weight genomic DNA was extracted from peripheral blood lymphocytes according to standard protocols [7]. After DNA extraction, screening of AZF deletions was performed by multiplex polymerase chain reaction (PCR) analysis using the Y Chromosome Deletion Detection System, version 2.0 (Promega), and the addition of Multiplex Master Mix E (Promega). In the current study, the system consisted of 24 primer pairs, of which 20 primer pairs are homologous to previously identified and mapped sequenced tag sites (STSs) within the AZF regions on the Y chromosome. All the loci analyzed in this study have been recommended by the European Quality Monitoring Network Group for detection of Yq11 deletions associated with male infertility [8].

Results

Cytogenetic study revealed sex chromosomal abnormality in 9 subjects (9/187=4.8%) as shown in Table 1. The age range of these men with chromosomal abnormality was 29–43 years. Five of these patients had 47, XXY karyotype (5/9=55%), one patient had 47, XYY and one patient had 46, XX karyotypes. Two cases had structural abnormality as a cytogenetically detected deletion in Y chromosome [46, XY, del (Y) (q11.2)]. One of these Y chromosomal deletion was in the mosaic pattern. No cases had autosomal abnormalities. In the remaining 178 subjects, 7 subjects (7/178=3.93%) were detected as having Y chromosome microdeletions (Table 2). The AZFc was the region most frequently involved in the microdeletion process in the subjects affected. One of these affected subjects (patient 6) had microdeletion in 3 regions(AZFa, AZFb, AZFc) and one subject (patient 2) had microdeletions in both AZFb and AZFc regions. The remaining 5 subjects had microdeletions in only AZFc region. All patients having microdeletion were azoospermic.

Table 1 Distribution of chromosomal abnormalities among 187 infertile men

Karyotype	Number	(%)
Normal; 46,XY	175	93.5
Heteromorphism	3	1.7
46, XY, Yqh+	1	33.3
46, XY, 1qh+	1	33.3
46, XY, inv9 (p11; q13)	1	33.3
	178	95.2
Abnormal	9	4.8
47, XXY	5	55.5
47, XYY	1	11.1
46, XX (male)	1	11.1
46, XY, del Y	2	22.3

Discussion

Chromosomal abnormalities are more frequently observed in azoospermic and oligospermic patients than in the general population [9]. In the present study, the frequency of chromosomal aberrations (4.8%) among infertile men was found to lie within the previously reported range (1.9–16.9%) [10, 11]. AZF microdeletion frequency detected in our series of cytogenetically normal azoospermic men was 3.93% (7/178patients) being similar to that of previous reports [4, 12, 13]. Rolf et al. [12] detected a frequency of 7.3% from a sample of 3000 infertile males with a higher value in azoospermic men (15%) than that of oligospermic men. All of our patients with chromosomal abnormalities had sex chromosomal abnormalities, the majority of which were diagnosed as having Klinefelter syndrome (55.5%). This is in accordance with previously published studies, showing that both numerical sex chromosome aberrations and translocations are the most common chromosomal aberrations associated with male infertility [14]. Infertility in such patients may result from the interference of chromosomal aberrations with normal spermatogenesis [15]. We detected three chromosomal heteromorphisms in our study group. The frequencies of these polymorphisms were no different from the frequencies reported for the normal population.

The distribution of microdeletions detected in our sample showed the most frequently involved region was the AZFc region (71.4%). It was followed by the AZFb/AZFc group (14.3%), and by the AZFa/AZFb/AZFc (14.3%) group. These results resemble in part those reported by Simoni et al. [3]. However no subjects had microdeletions exclusively in the AZFb region in our study, even though they found this to be the second most frequently involved region in their sample of 34 patients (AZFc=79%; AZFb=9%; AZFb/AZFc=6%; AZFa=3%, and AZFa/AZFb/AZFc=3%). Pina-Neto et al. also observed similar results in their study [16].

The frequency of patients with AZF microdeletions in the present study (3.93%) was found to be respectively lower compared to the other reports (1–55%) [17, 18]. This

Table 2 Genotype-phenotype association in azoospermia factor gene (AZF) microdeletions

Case	A	RH	SA	TESE/IVF	BHP			Microdeletion				
								SRY		AZFa		
					$FSH_{mIU/mL}$	$LH_{mIU/mL}$	$PRL_{ng/mL}$	SY14	SY81	SY86	SY84	SY182
1 HO	41	PI	AIS	+/−	NA			+	+	+	+	+
2 CB	35	PI	AZ	−/−	3.74	0.1	12.6	+	+	+	+	+
3 I K	39	PI	AZ	−/−	NA			+	+	+	+	+
4 EK	31	PI	AZ	−/−	NA			+	+	+	+	+
5 MO	37	PI	AZ	+/−	NA			+	+	+	+	+
6 AG	30	PI	AZ	−/−	NA			−	−	−	−	−
7 KU	37	PI	AZ	+/−	8.79	6.45	2.14	+	+	+	+	+

Case	Microdeletion														
	AZFb								AZFb AZFc/d AZFc		AZFc				
	SY121	SYPR3	SY124	SY127	SY128	SY130	SY133	SY134	SY145	SY152	SY242	SY208	SY254	SY255	SY157
1 HO	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
2 CB	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
3 I K	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
4 EK	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
5 MO	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
6 AG	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7 KU	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−

A Age; *RH* Reproductive History; *PI* Primary Infertility; *SA* Semen Analysis; *AIS* Arrest in Spermatogenesis; *AZ* Azoospermia; *TESE* Testicular Sperm Extraction; *IVF* In vitro Fertilization; *BHP* Basal Hormone Profile

wide range of frequencies in different populations may be due to the ethnic differences and/or differences in their inclusion criteria. Recently occurrence of partial deletions in the AZFc region [19, 20] and other types of rearrangements [21, 22] have been reported more frequently. Some of these deletions and rearrangements might be of clinical significance, but the exact role of such findings in male infertility has not yet been elucidated [3]. In a previous study with a lower sample size which was conducted in our center showed a similar frequency (5,6%) [23].

Diagnosis of a microdeletion of the Y chromosome identifies the cause of azoospermia/oligozoospermia in the patient and aids clinicians decide further interventions. The AZFc deletion phenotype is less severe because, these deletions are compatible with residual spermatogenesis and are in rare cases transmitted naturally to the male offspring [12, 24, 25].

In cases of complete AZFa region deletion, testicular sperm retrieval for intracytoplasmic sperm injection (ICSI) is virtually impossible [3].

We determined 2 cytogenetically Y chromosomal deletions in this study, One of which was in mosaic pattern and we could not find any deletion in molecular study. In the mosaic patterns of chromosomal deletions, there may be a preferentially amplification from normal cells.

On the other hand, 46, XX male patients are in low prevalance in the population. Majority of XX males carry SRY gene translocated to the X chromosome, as in our case, due to a recombination between X and Y chromosome. These patients are sterile men but have normal genitalia [26].

Based on prevalence data and the lack of other phenotypic features, routine karyotyping of infertile men with unexplained spermatogenic failure and a sperm concentration less than 10 million/ml is widely recommended before ART [27].

AZF microdeletions are the second most frequent genetic cause of spermatogenic failure in male infertility after Klinefelter syndrome [3]. These microdeletions are the most common molecular genetic cause of human male infertility involving spermatogenic failure [28].The molecular diagnosis of Y chromosomal microdeletions is a mandatory issue in the diagnostic process of male infertility in men with azoospermia and severe oligozoospermia. Based on these reasons, Y chromosomal microdeletion detection has become routinely performed screening test worldwide in the workup of male infertility after routine karyotyping.

The marker sets used in the simple multiple PCR as described in the present study, may be used for initial screening to determine Y chromosome microdeletion. After this initial screening, the men with the deletion/deletions may optionally be evaluated with more STS markers to determine the extent of deletion. This approach may help decrease the cost and technical difficulty of the procedure and allow a more widespread use of Y chromosome microdeletion screening in infertility clinics [29].

The site and extent of Yq microdeletions affect the infertility phenotype. Sixty percent of deletions involve only the AZFc region, and in these men, a third have severe oligozoospermia, and in the majority who are azoospermic, more than half have sperm recoverable by TESE [30]. Larger deletions involving AZFa and/or b, as well as AZFb_c, are often associated with azoospermia and a poor prognosis for sperm recovery by TESE [1, 30, 31]. In agreement, the histological profile of men with AZFa deletions is usually that of Sertoli cell-only syndrome. Those with AZFb deletions tend to have germ cell arrest at the primary spermatocyte stage; however, patterns ranging from Sertoli cell-only syndrome to severe hypospermatogenesis have been reported [4]

Recognizable genetic disorders are the relatively rare causes of primary spermatogenetic failure or obstructive azoospermia, and routine karyotype and Y microdeletion analyses and *CFTR* screening are indicated, respectively. A wider range of genetic testing may have also an indication to perform within certain families and clinical settings. The genetic diagnosis is essential for appropriate genetic counseling and to perform effective and safe ART treatment and to determine accurate strategies including preimplantation genetic diagnosis [32]. By the time, excise genetic lesions will be identified, as a result of high throughput genetic data and more complete, rapid and cheaper testing strategies will become available covering the majority of the cases with male inferility.

In conclusion, cytogenetic and molecular study should be performed to obtain reliable genetic counseling in primary infertile man. Y chromosome microdeletion analysis is useful before the administration of assisted reproductive techniques.

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