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Detection of Y chromosome microdeletions and mitochondrial DNA mutations in male infertility patients

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ABSTRACT. Infertility affects about 10-15% of all couples attempting pregnancy with infertility attributed to the male partner in approximately half of the cases. Proposed causes of male infertility include sperm motility disturbances, Y chromosome microdeletions, chromosomal abnormalities, single gene mutations, and sperm mitochondrial DNA (mtDNA) rearrangements. To investigate the etiology of decreased sperm fertility and motility of sperm and to develop an appropriate therapeutic strategy, the molecular basis of these defects must be elucidated. In this study, we aimed to reveal the relationships between the genetic factors including sperm mtDNA mutations, Y chromosome microdeletions, and sperm parameters that can be regarded as candidate factors for male infertility. Thirty men with a history of infertility and 30

fertile men were recruited to the study. Y chromosome microdeletions were analyzed by multiplex PCR. Mitochondrial genes *ATPase6*, *Cytb*, and *ND1*, were amplified by PCR and then analyzed by direct sequencing. No Y chromosome microdeletions were detected in either group. However, a total of 38 different nucleotide substitutions were identified in the examined mitochondrial genes in both groups, all of which are statistically non-significant. Fifteen substitutions caused an amino acid change and 12 were considered novel mutations. As a conclusion, mtDNA mutations and Y chromosome microdeletions in male infertility should be examined in larger numbers in order to clarify the effect of genetic factors.

Key words: Male infertility; Y chromosome microdeletion; mtDNA mutations

INTRODUCTION

Male infertility is a common condition affecting up to 50% of infertility cases, which comprise 10-15% of couples (Moore and Reijo-Pera, 2000). One of the major factors associated with male infertility is the quality and quantity of sperm produced and sperm function such as sperm motility (Selvi et al., 2006). The conventional causes of male infertility are varicocele, cystic fibrosis, trauma, tumors, and genetic factors such as chromosomal abnormalities, Y chromosome microdeletions, single gene mutations, and sperm mitochondrial DNA (mtDNA) rearrangements (Kao et al., 1998; Agarwal et al., 2008). The mutations occurring in mtDNA attract great interest in terms of some human diseases, either independent of or in association with nuclear DNA mutations (Taylor and Turnbull, 2005). These alterations especially affect organs that have a high demand for respiratory energy (Wallace, 1992). Because the mtDNA genes encode polypeptides for only the oxidative phosphorylation elements and enzymes (OXPHOS), mtDNA damages cause deficiency in ATP production (Solano et al., 2001).

The mature human sperm contains 70-80 mitochondria located in the midpiece segment. The flagellar propulsion of the sperm requires a large source of energy supplied by the mitochondria (Kao et al, 1998; Selvi et al., 2006). Mitochondria play a key role in energy metabolism by containing OXPHOS (Alcivar et al., 1989). Each mitochondrion has 2-10 DNA molecules (mtDNA) responsible for coding the few subunits of the OXPHOS enzymes. The *ND1*-*ND6* and the *ND4L* genes encode seven complex I subunits (NADH-ubiquinone oxidoreductase); the *Cytb* gene encodes one complex III subunit (ubiquinol-cytochrome *c* oxidase oxidoreductase), and the *ATPase6* and *ATPase8* genes encode complex V subunits (ATP synthase) (Spiropoulos et al., 1999).

mtDNA mutations affecting flagellar movement are a cause of sperm dysmotility. DNA rearrangements including point mutations and deletions of mtDNA have been reported in patients with low sperm motility who have asthenozoospermia and oligoasthenozoospermia (Folgero et al., 1993; Lestienne et al., 1997; Kao et al., 1998).

The Y chromosome is essential not only for human sex determination but also for maintenance of sperm cells and their development. The regions of the Y chromosome re-

sponsible for male infertility are located on the long arm of the chromosome and are termed AZFa, AZFb and AZFc (AZF: azoospermia factor) (Burgoyne, 1998). Microdeletions in AZF are associated with male infertility (Vogt et al., 1996). As the severity of the spermatogenesis increases, the frequency of the microdeletions also increases.

To investigate the etiology of decreased fertility and motility of sperm and to develop an appropriate therapeutic strategy, the molecular basis of these defects must be ascertained. Therefore, we aimed to reveal the relationships between genetic factors including sperm mtDNA mutations in the *ATPase6*, *Cytb* and *ND1* gene regions, Y chromosome microdeletions and sperm parameters that could be regarded as candidate factors for male infertility in the Turkish population.

MATERIAL AND METHODS

Study and control groups

Thirty infertile men were recruited to the study. Of them, 19 were normospermic and the rest were oligospermic, according to the semen analysis results implemented by Marmara University, Faculty of Medicine, Department of Urology. The control group consisted of 30 fertile and normospermic men who fathered at least one child. The experimental protocol was approved by the Ethics Committee of Marmara University.

Karyotyping

A standard protocol was used for chromosomal analysis. Lymphocyte cultures were established from all participants, and GTG-banded chromosomes were prepared in mitotic cells; chromosome analysis was performed under light microscopy.

DNA extraction

DNA samples were extracted from peripheral blood using the High Pure PCR Template Preparation kit (Roche, Germany) for the detection of Y chromosome microdeletions. For analyzing mtDNA mutations, sperm were purified from semen using the Invisorb Spin Tissue Mini Kit (Invitek, Germany) according to the manufacturer protocol.

Multiplex polymerase chain reaction (multiplex PCR)

For detection of Y chromosome microdeletions, isolated DNA was amplified by multiplex PCR. The AZF Extension kit (AB Analitica), which is recommended by the European Andrology Association, was used in multiplex PCR. By using this kit, 13 different regions could be investigated at the same time by performing 3 multiplex PCRs for each sample. Three primer sets, each containing primers that are unique to the ZFX/Y locus, which also exist on the X chromosome, are shown in Table 1.

In addition to the mixtures in the AZF Extension kit, 0.3 µL Taq DNA polymerase and 8 µL DNA sample were added to each tube during multiplex PCR. The conditions of PCR amplification were as follows: a denaturation step at 94°C for 5 min followed by 35 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and then a final extension at

72°C for 7 min and stop at 4°C. After multiplex PCR, products were electrophoresed on a 2% agarose gel.

Table 1. Primers used for multiplex PCR and the length of amplicons.

MIX1	Amplicon length (bp)	MIX2	Amplicon length (bp)	MIX3	Amplicon length (bp)
ZFX/Y	495	ZFX/Y	495	DBY	689
SRY	472	SRY	472	ZFX/Y	495
sY 254	380	sY 95	303	SRY	472
sY 86	320	sY 117	262	sY 84	326
sY 127	274	sY 125	200	sY 134	301
sY 255	120			DFFRY	155

Amplification, purification and sequencing of mtDNA

The *ND1*, *ATPase6* and *Cytb* genes were amplified in a total volume of 50 µL containing 50-100 ng DNA template in 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 1 mM of each dNTP, 1.0 U Taq DNA polymerase and 1.0 mM of each primer. The conditions of PCR amplification were as follows: a denaturation step at 94°C for 5 min followed by 35 cycles at 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min, and then a final extension at 72°C for 5 min and stop at 4°C (Chen et al., 2004). The sizes of the fragments obtained after amplification were 934, 675 and 1064 bp for the *ND1*, *ATPase6* and *Cytb* genes, respectively. All PCR products were fractionated by electrophoresis on 2% agarose gel and sequenced with the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA) using the DYEnamic ET Terminator Cycle Sequencing kit (Amersham, England). The primers used for PCR and direct sequencing of the *ND1*, *ATPase6* and *Cytb* genes are shown in Table 2.

Table 2. The primer sequences used for PCR and direct sequencing of human mtDNA genes.

mtDNA gene	Primer sequence (5'→3') of PCR
ND1	5'-primer: 5'-CCA ACC TCC TAC TCC TCA TTG T-3' (3318-3339) 3'-primer: 5'-GGG AAT GCT GGA GAT TGT AAT G-3' (4231-4252)
ATPase6	5'-primer: 5'-AAC GAA AAT CTG TTC GCT TCA T-3' (8531-8552) 3'-primer: 5'-ATG TGT TGT CGT GCA GGT AGA G-3' (9185-9206)
Cytb	5'-primer: 5'-ACC CCA ATA CGC AAA ATT AAC C-3' (14751-14772) 3'-primer: 5'-TAC GGA TGC TAC TTG TCC AAT G-3' (15794-15815)
mtDNA gene	Primer sequence (5'→3') of direct sequencing
ND1	5'-primer: 5'-CCA ACC TCC TAC TCC TCA TTG T-3' 5'-primer: 5'-TGA TCA GGG TGA GCA TCA AA-3'
ATPase6	5'-primer: 5'-AAC GAA AAT CTG TTC GCT TCA T-3' 3'-primer: 5'-ATG TGT TGT CGT GCA GGT AGA G-3'
Cytb	5'-primer: 5'-TAT CCG CCA TCC CAT ACA TT-3' 3'-primer: 5'-GGT GAT TCC TAG GGG GTT GT-3'

Sequencing reactions were performed on both strands of amplicons. The results of sequenced fragments were compared to human mtDNA revised Cambridge reference sequence (Ruiz-Pesini et al., 2007). Sequence variations of mtDNA found in both groups were recorded

as germline polymorphisms, whereas those not found in controls and in the database were categorized as novel mtDNA polymorphisms.

Statistical analysis

Statistical analysis was performed with the chi-square test using SPSS-18.0.

RESULTS

All patients and controls had a normal 46,XY male karyotype. None had a Y chromosome microdeletion. PCR products of one patient, which includes Y chromosome loci, are shown in Figure 1.

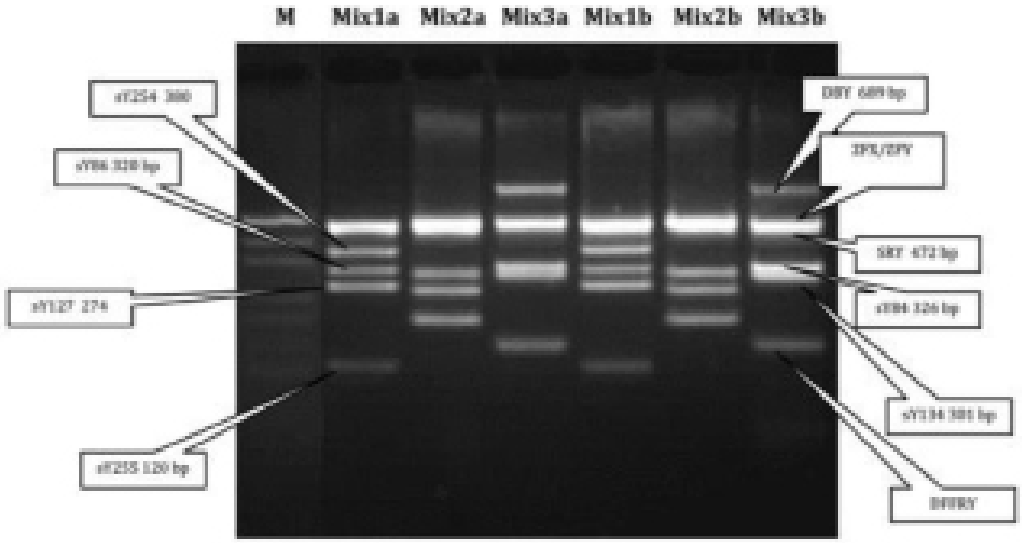

Figure 1. Multiplex PCR analyses of Y chromosome microdeletions. *Lane M* = 50-bp ladder (Fermentas, Germany); Mix1a, Mix2a and Mix3a: 3 sets of PCR that amplify different loci on the Y chromosome for sample a; Mix1b, Mix2b, Mix3b for sample b; boxes indicate the region and the length of the amplicons.

All polymorphisms found in the sequenced mtDNA regions are summarized in Table 3. Briefly, 38 different nucleotide substitutions were identified, of which 15 caused an amino acid change. In addition, 12 were considered novel mutations. C8927G, A9041G, C9105G novel polymorphisms were found in the *ATPase6* gene, and T14969C, C15143T, T15282G, A15296C, T15804A, and G15806C novel polymorphisms were found in the *Cytb* gene. Also T4114G, G4153A and C4159A novel polymorphisms were found in the *ND1* gene. All these novel mutations cause amino acid changes. DNA sequencing representative chromatograms of novel mutations are shown in Figure 2. No significant associations between control and patient groups were detected according to the chi-square test ($P > 0.05$).

Table 3. The mtDNA mutations identified in the *ATPase6*, *Cytb*, and *ND1* regions.

Nucleotide position	Nucleotide change	Amino acid change	Mutation frequency in controls (%)	Mutation frequency in cases (%)	P	Reported in Mitomap
ATPase 6 gene						
8584	G→A	Ala→Thr	0/30 (0)	1/30 (3.3)	>0.05	X
8697	G→A	Silent	4/30 (13.3)	3/30 (10)	>0.05	X
8701	A→G	Thr→Ala	1/30 (3,3)	1/30 (3,3)	>0.05	X
8730	A→G	Silent	0/30 (0)	2/30 (6,7)	>0.05	X
8927	C→G	Pro→Arg	0/30 (0)	1/30 (3,3)	>0.05	Novel
9041	A→G	His→Arg	1/30 (3,3)	3/30 (10)	>0.05	Novel
9055	G→A	Ala→Thr	2/30 (6,7)	2/30 (6,7)	>0.05	X
9091	A→G	Thr→Ala	1/30 (3,3)	1/30 (3,3)	>0.05	X
9105	C→G	Phe→Leu	0/30 (0)	2/30 (6,7)	>0.05	Novel
9196	G→A	Asp→Asn	0/30 (0)	3/30 (10)	>0.05	X
Cytb gene						
14793	A→G	His→Arg	0/30 (0)	2/30 (6,7)	>0.05	X
14798	T→C	Phe→Leu	2/30 (6,7)	4/30 (13,3)	>0.05	X
14969	T→C	Tyr→His	1/30 (3,3)	1/30 (3,3)	>0.05	Novel
14905	G→A	Silent	3/30 (10)	3/30 (10)	>0.05	X
15043	G→A	Silent	1/30 (3,3)	2/30 (6,7)	>0.05	X
15115	T→C	Silent	3/30 (10)	1/30 (3,3)	>0.05	X
15143	C→T	Leu→Phe	0/30 (0)	1/30 (3,3)	>0.05	Novel
15148	G→A	Silent	0/30 (0)	2/30 (6,7)	>0.05	X
15204	T→C	Ile→Thr	0/30 (0)	1/30 (3,3)	>0.05	X
15217	G→A	Silent	0/30 (0)	1/30 (3,3)	>0.05	X
15218	A→G	Thr→Ala	1/30 (3,3)	2/30 (6,7)	>0.05	X
15282	T→G	Phe→Ser	5/30 (16,7)	5/30 (16,7)	>0.05	Novel
15296	A→C	Ile→Leu	5/30 (16,7)	3/30 (10)	>0.05	Novel
15301	G→A	Silent	2/30 (6,7)	2/30 (6,7)	>0.05	X
15452	C→A	Leu→Ile	5/30 (16,7)	7/30 (23,3)	>0.05	X
15454	T→C	Silent	0/30 (0)	1/30 (3,3)	>0.05	X
15607	A→G	Silent	1/30 (3,3)	3/30 (10)	>0.05	X
15773	G→C	Val→Leu	0/30 (0)	1/30 (3,3)	>0.05	X
15804	T→A	Val→Glu	0/30 (0)	1/30 (3,3)	>0.05	Novel
15806	G→C	Ala→Pro	0/30 (0)	1/30 (3,3)	>0.05	Novel
ND1 gene						
3906	T→C	Silent	0/30 (0)	1/30 (3,3)	>0.05	X
4114	T→G	Phe →Val	0/30 (0)	1/30 (3,3)	>0.05	Novel
4153	G→A	Asp→Asn	0/30 (0)	1/30 (3,3)	>0.05	Novel
4159	C→A	Leu→Ile	0/30 (0)	1/30 (3,3)	>0.05	Novel
4216	T→C	Tyr→His	8/30 (26)	2/30 (6,7)	>0.05	X
4240	T→C	Ser→Pro	2/30 (6,7)	1/30 (3,3)	>0.05	X
4243	A→G	Ser→Gly	6/30 (20)	2/30 (6,7)	>0.05	X
4244	G→A	Ser→Asn	6/30 (20)	3/30 (10)	>0.05	X

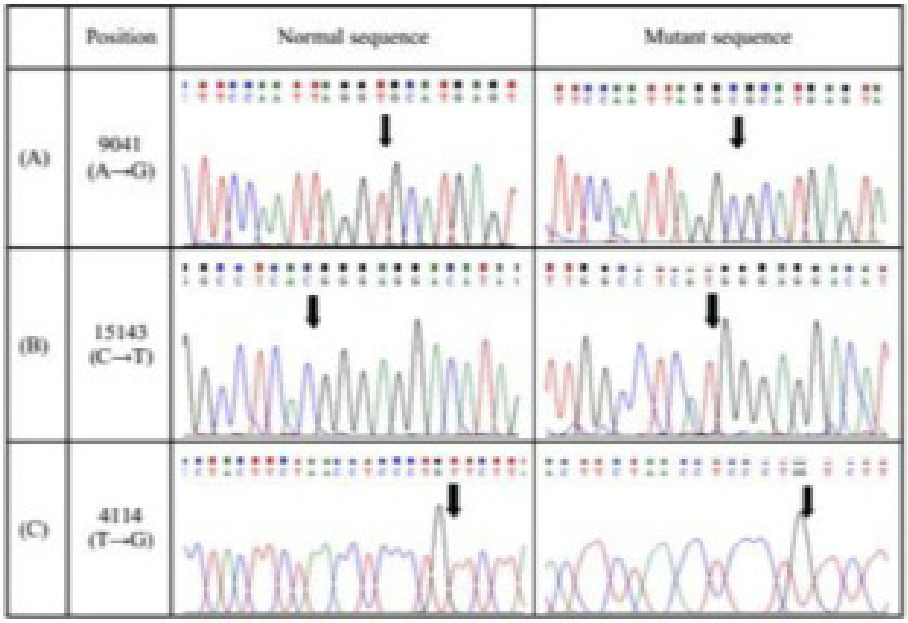

Figure 2. Representative chromatograms showing *ATPase*, *Cytb*, and *ND1* mutations. **A.** The point mutation at position 9041 within the *ATPase* gene. **B.** The point mutation at position 15143 within the *Cytb* gene. **C.** The point mutation at position 4114 within the *ND1* gene.

DISCUSSION

Male infertility accounts for half of the etiology of infertility in couples. Infertility itself affects about 10-15% of all couples attempting pregnancy. The suspected causes of male infertility include sperm motility disturbances, Y chromosome microdeletions, chromosomal abnormalities, hormonal and/or receptor disorders (Carra et al., 2004). mtDNA mutations affecting flagellar movement is also one of the causes of sperm dysmotility. DNA rearrangements including point mutations and deletions of mtDNA have been reported in patients with low sperm motility who have asthenozoospermia and oligoasthenozoospermia (Folgero et al., 1993; Lestienne et al., 1997; Kao et al., 1998). Therefore, in this study, the effects of Y chromosome microdeletions and mtDNA mutations on male infertility were examined.

The major determinant of sperm motility is strongly dependent on ATP biosynthesis, which is carried out by the mitochondrial OXPHOS (Ruiz-Pesini et al., 1998). Mitochondria play a major role in the energy metabolism of eukaryotic cells. In addition to serving as the major intracellular compartment of metabolic enzymes, mitochondria also contain their own genetic material (Clayton et al., 1974). The mutation rate of mtDNA is 10-20 times higher than that of nuclear DNA (Wallace, 1992). Lack of histone protection and efficient proofreading mechanism, high turnover rate, and exposure to ROS are the main reasons of high mtDNA mutation rates (Kumar et al., 2009). As the mitochondrial genome does not contain introns, any mutation occurring in mtDNA would affect the coding regions. Therefore, a variety of neutral and deleterious mutations are found in human populations.

In recent years, the effect of mtDNA mutations on male infertility has been studied. The mtDNA defects, which include point mutations or multiple deletions of mtDNA, have been found in patients with asthenozoospermia (Folgero et al., 1993) or oligoasthenozoospermia (Lestienne et al., 1997). In addition, in some studies, deletions in mtDNA tend to develop a decline in motility and fertility in human sperm (Cummins, 1998; Cummins et al., 1998; Kao et al., 1998; O'Connell et al., 2002a,b). Furthermore, it has been demonstrated that mtDNA base substitutions can greatly influence semen quality (Ruiz-Pesini et al., 2000; Holyoake et al., 1999, 2001). Therefore, we investigated the relationship between the mtDNA genes *ATPase6*, *Cytb* and *ND1* and male infertility.

In the patient group, 13 point mutations in the *ATPase6* gene were identified, of which 3 were novel and 8 caused amino acid changes (Table 3). Holyoake et al. (1999) found that severely oligospermic men with a T8821C point mutation in the *ATPase6* gene possess immature spermatids. This mutation changed the amino acid serine to proline at residue 99 of mitochondrial *ATPase6*. This mutation, therefore, may affect sperm motility (Holyoake et al., 1999). These authors also found a high incidence of nucleotide substitutions in the mitochondrial genome, which were associated with poor semen quality. They showed that 2.4% of normospermic men and 8.4% of men with poor semen quality had at least one nucleotide substitution. One of the most common substitutions associated with poor semen quality was found in the *ATPase6* gene at 9055. This substitution involves a G to A transition within the *ATPase6* gene, changing an alanine to threonine. About 10.7% of men with poor semen quality were shown to have the G9055A substitution, but only 1.3% of men with normal fertility had the same substitution (Holyoake et al., 2001). Thangaraj et al. (2003a) established that mitochondrial mutations in sperm can cause low sperm motility that is not related to infertility. They obtained 10 SNPs in the *ATPase6* gene, in the sperm DNA of an oligoasthenoteratozoospermic man. They found that these mutations were associated with low motility sperm but otherwise did not impair fertility (Thangaraj et al., 2003a). In another study, the T9098C transition was detected only in infertile cases, and the finding of this mutation was statistically significant when compared to controls (Kumar et al., 2009).

This is the first study that examined the association of *Cytb* mutations and infertility. We detected 20 point mutations in the *Cytb* gene, all of which were statistically non-significant. Of the 20 variations, 12 of them caused an amino acid change and 6 of them were considered novel mutations. Nonetheless, it is not clear whether these mutations caused infertility. The mutations, which were detected in this study, should also be further investigated.

In the present study, 8 point mutations were found in the *ND1* gene, 4 of which were found only in the patient group and the remainder in both groups (Table 3). Only the T3906C transition was silent, while the others alter the amino acid sequence of the protein. Three of the SNPs that were found in the patient group (T4114G, G4153A and C4159A) were classified as novel SNPs according to the www.mitomap.org as of March 23, 2011. A common 4216 SNP, which is found in the T haplogroup, was associated with poor semen quality, which can cause infertility (Ruiz-Pesini et al., 2000). However, Thangaraj et al. (2003a) reported that the SNPs found in the mitochondrial genes can effect sperm motility, not fertility. The SNPs that were found in our study population did not have any affect on human male fertility, neither in our study nor other published studies.

In our study, 11 of 30 patients were oligospermic and 19 were normospermic. Approximately 5-10% of oligospermic cases and 15-20% of azoospermic cases harbor genetic

abnormalities (Dada et al., 2006). Fattoruso et al. (2009) reported the frequency of Y chromosome microdeletions as 9% (12.7% in azoospermic and 4.5% in severe oligozoospermic patients). Balkan et al. (2008) detected an Yq microdeletion in 1 (1.9%) of 52 azoospermic cases, which has been supported by others (Qureshi et al., 1996; Sargin et al., 2004). In our study, no Y chromosome microdeletions that can cause male infertility were found, although most Y-linked mutations exert their effects on spermatogenesis (Hargreave, 2000; Singh et al., 2006). Furthermore, Y chromosome microdeletions were detected in patients with suboptimal semen parameters (7.4%) (Erasmuson et al., 2003). AZF consists of three regions: AZFa, AZFb and AZFc (Burgoyne, 1998). AZFc deletions, the most common form of Y chromosome microdeletions, are usually associated with decreased number of sperm in the ejaculate or in testis biopsies, whereas patients with AZFa or AZFb+c deletions usually do not produce testicular sperm (McLachlan and O'Bryan, 2010). In a study of 340 azoospermic patients, Y chromosome microdeletions were found in 29 patients (8.5%) and most of the deletions were in the AZFc region (82.8%), the AZFb region (55.2%) and the AZFa region (24.1%) (Thangaraj et al., 2003b).

Although 30% of severe male infertility cases can be attributed to genetic defects, for a majority of patients, the cause of the dysfunction remains unknown. Nevertheless, infertility is not only associated with mtDNA mutations and Y chromosome microdeletions, and other genetic factors that affect male fertility should also be investigated in further studies. In conclusion, we believe that larger group studies are needed to understand the exact effects of related genes including the mtDNA mutations and Y chromosome microdeletions on spermatogenesis.

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REFERENCES

Agarwal A, Makker K and Sharma R (2008). Clinical relevance of oxidative stress in male factor infertility: an update. *Am. J. Reprod. Immunol.* 59: 2-11.

Alcivar AA, Hake LE, Millette CF, Trasler JM, et al. (1989). Mitochondrial gene expression in male germ cells of the mouse. *Dev. Biol.* 135: 263-271.

Balkan M, Tekes S and Gedik A (2008). Cytogenetic and Y chromosome microdeletion screening studies in infertile males with oligozoospermia and azoospermia in Southeast Turkey. *J. Assist. Reprod. Genet.* 25: 559-565.

Burgoyne PS (1998). The mammalian Y chromosome: a new perspective. *Bioessays* 20: 363-366.

Carra E, Sangiorgi D, Gattuccio F and Rinaldi AM (2004). Male infertility and mitochondrial DNA. *Biochem. Biophys. Res. Commun.* 322: 333-339.

Chen GF, Chan FL, Hong BF, Chan LW, et al. (2004). Mitochondrial DNA mutations in chemical carcinogen-induced rat bladder and human bladder cancer. *Oncol. Rep.* 12: 463-472.

Clayton DA, Doda JN and Friedberg EC (1974). The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria. *Proc. Natl. Acad. Sci. U. S. A.* 71: 2777-2781.

Cummins J (1998). Mitochondrial DNA in mammalian reproduction. *Rev. Reprod.* 3: 172-182.

Cummins JM, Jequier AM, Martin R, Mehmet D, et al. (1998). Semen levels of mitochondrial DNA deletions in men attending an infertility clinic do not correlate with phenotype. *Int. J. Androl.* 21: 47-52.

Dada R, Gupta NP and Kucheria K (2006). Cytogenetic and molecular analysis of male infertility: Y chromosome deletion during nonobstructive azoospermia and severe oligozoospermia. *Cell Biochem. Biophys.* 44: 171-177.

Erasmuson T, Sin IL and Sin FY (2003). Absence of association of androgen receptor trinucleotide expansion and poor semen quality. *Int. J. Androl.* 26: 46-51.

Fattoruso O, Zarrilli S, Coto I, De Rosa M, et al. (2009). Prevalence of Y microdeletions in azoospermic and severe oligozoospermic men in Southern Italy: application of a rapid capillary electrophoresis method. *J. Endocrinol. Invest.* 32: 223-227.

Folgero T, Bertheussen K, Lindal S, Torbergsen T, et al. (1993). Mitochondrial disease and reduced sperm motility. *Hum. Reprod.* 8: 1863-1868.

Hargreave TB (2000). Genetic basis of male infertility. *Brit. Med. Bull.* 56: 650-671.

Holyoake AJ, Sin IL, Benny PS and Sin FY (1999). Association of a novel human mtDNA ATPase6 mutation with immature sperm cells. *Andrologia* 31: 339-345.

Holyoake AJ, McHugh P, Wu M, O'Carroll S, et al. (2001). High incidence of single nucleotide substitutions in the mitochondrial genome is associated with poor semen parameters in men. *Int. J. Androl.* 24: 175-182.

Kao SH, Chao HT and Wei YH (1998). Multiple deletions of mitochondrial DNA are associated with the decline of motility and fertility of human spermatozoa. *Mol. Hum. Reprod.* 4: 657-666.

Kumar R, Venkatesh S, Kumar M, Tanwar M, et al. (2009). Oxidative stress and sperm mitochondrial DNA mutation in idiopathic oligoasthenozoospermic men. *Indian J. Biochem. Biophys.* 46: 172-177.

Lestienne P, Reynier P, Chretien MF, Penisson-Besnier I, et al. (1997). Oligoasthenospermia associated with multiple mitochondrial DNA rearrangements. *Mol. Hum. Reprod.* 3: 811-814.

McLachlan RI and O'Bryan MK (2010). Clinical Review: State of the art for genetic testing of infertile men. *J. Clin. Endocrinol. Metab.* 95: 1013-1024.

Moore FL and Reijo-Pera RA (2000). Male sperm motility dictated by mother's mtDNA. *Am. J. Hum. Genet.* 67: 543-548.

O'Connell M, McClure N and Lewis SE (2002a). A comparison of mitochondrial and nuclear DNA status in testicular sperm from fertile men and those with obstructive azoospermia. *Hum. Reprod.* 17: 1571-1577.

O'Connell M, McClure N and Lewis SE (2002b). Mitochondrial DNA deletions and nuclear DNA fragmentation in testicular and epididymal human sperm. *Hum. Reprod.* 17: 1565-1570.

Qureshi SJ, Ross AR, Ma K, Cooke HJ, et al. (1996). Polymerase chain reaction screening for Y chromosome microdeletions: a first step towards the diagnosis of genetically-determined spermatogenic failure in men. *Mol. Hum. Reprod.* 2: 775-779.

Ruiz-Pesini E, Diez C, Lapena AC, Perez-Martos A, et al. (1998). Correlation of sperm motility with mitochondrial enzymatic activities. *Clin. Chem.* 44: 1616-1620.

Ruiz-Pesini E, Lapena AC, Diez-Sanchez C, Perez-Martos A, et al. (2000). Human mtDNA haplogroups associated with high or reduced spermatozoa motility. *Am. J. Hum. Genet.* 67: 682-696.

Ruiz-Pesini E, Lott MT, Procaccio V, Poole JC, et al. (2007). An enhanced MITOMAP with a global mtDNA mutational phylogeny. *Nucleic Acids Res.* 35: D823-D828.

Sargin CF, Berker-Karauzum S, Manguoglu E, Erdogru T, et al. (2004). AZF microdeletions on the Y chromosome of infertile men from Turkey. *Ann. Genet.* 47: 61-68.

Selvi RD, Vanniarajan A, Gupta NJ, Chakravarty B, et al. (2006). A novel missense mutation C11994T in the mitochondrial ND4 gene as a cause of low sperm motility in the Indian subcontinent. *Fertil. Steril.* 86: 1783-1785.

Singh AR, Vrtel R, Vodicka R and Dhaifalah I (2006). Genetic factors in male infertility and their implications. *Int. J. Hum. Genet.* 6: 163-169.

Solano A, Playán A, López-Pérez MJ and Montoya J (2001). Genetic diseases of human mitochondrial DNA. *Salud Pública México* 43: 1-11.

Spiropoulos J, Chinnery PF and Turnbull DM (1999). Pathogenic mitochondrial DNA mutations and human reproduction. *Hum. Fertil.* 2: 133-137.

Taylor RW and Turnbull DM (2005). Mitochondrial DNA mutations in human disease. *Nat. Rev. Genet.* 6: 389-402.

Thangaraj K, Joshi MB, Reddy AG, Rasalkar AA, et al. (2003a). Sperm mitochondrial mutations as a cause of low sperm motility. *J. Androl.* 24: 388-392.

Thangaraj K, Gupta NJ, Pavani K, Reddy AG, et al. (2003b). Y chromosome deletions in azoospermic men in India. *J. Androl.* 24: 588-597.

Vogt PH, Edelmann A, Kirsch S, Henegariu O, et al. (1996). Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum. Mol. Genet.* 5: 933-943.

Wallace DC (1992). Diseases of the mitochondrial DNA. *Annu. Rev. Biochem.* 61: 1175-1212.