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Genetic risk factors in infertile men with severe oligozoospermia and azoospermia

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BACKGROUND: Male infertility due to severe oligozoospermia and azoospermia has been associated with a number of genetic risk factors. METHODS: In this study 150 men from couples requesting ICSI were investigated for genetic abnormalities, such as constitutive chromosome abnormalities, microdeletions of the Y chromosome (*AZF* region) and mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. RESULTS: Genetic analysis identified 16/150 (10.6%) abnormal karyotypes, 8/150 (5.3%) *AZFc* deletions and 14/150 (9.3%) *CFTR* gene mutations. An abnormal karyotype was found both in men with oligozoospermia and azoospermia: 9 men had a sex-chromosomal aneuploidy, 6 translocations were identified and one marker chromosome was found. Y chromosomal microdeletions were mainly associated with male infertility, due to testicular insufficiency. All deletions identified comprised the *AZFc* region, containing the Deleted in Azoospermia (*DAZ*) gene. *CFTR* gene mutations were commonly seen in men with congenital absence of the vas deferens, but also in 16% of men with azoospermia without any apparent abnormality of the vas deferens. CONCLUSIONS: A genetic abnormality was identified in 36/150 (24%) men with extreme oligozoospermia and azoospermia. Application of ICSI in these couples can result in offspring with an enhanced risk of unbalanced chromosome complement, male infertility due to the transmission of a Y-chromosomal microdeletion, and cystic fibrosis if both partners are *CFTR* gene mutation carriers. Genetic testing and counselling is clearly indicated for these couples before ICSI is considered.

Key words: CFTR gene mutations/chromosomal abnormalities/ICSI/male infertility/Y chromosome microdeletions

Introduction

Male infertility has been associated with several genetic and non-genetic conditions, such as hypogonadotrophic hypogonadism, testicular maldescence, structural abnormalities of the male genital tract, genital infections, previous scrotal or inguinal surgery, varicoceles, chronic illness, medication and exposure to chemicals. In at least 40% of men no cause of the infertility was found (Crosignani *et al.*, 1992). Genetic abnormalities were identified in men with unexplained oligozoospermia and azoospermia, including numerical and structural chromosomal abnormalities (Chandley, 1998), deletions of the azoospermia factor region (*AZF*) of the Y chromosome (Reijo *et al.*, 1995; Vogt *et al.*, 1996) and mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, commonly associated with congenital vas deferens abnormalities (Jaffe and Oates, 1994; Dohle *et al.*, 1999). Most numerical chromosome abnormalities and *AZF* deletions are de-novo events in the parental germ cells. Some abnormalities associated with infertility are inherited, like reciprocal and Robertsonian translocations and *CFTR* mutations (Mak and Jarvi, 1996).

ICSI is the most significant recent development in the treatment of male infertility, enabling couples who were previously deemed infertile to produce offspring, however with the risk of passing on genetic abnormalities, and possibly decreased fertility. To assess the couple's risk of transmitting a genetic abnormality we analysed the results of a combined andrological, cytogenetic and molecular genetic screening of 150 men with oligozoospermia and azoospermia. In addition, we discuss the necessity of genetic counselling for these infertile couples.

Materials and methods

For this study 150 men were selected from couples requesting ICSI who had an infertility duration of at least one year and a semen analysis showing $<1\times10^6$/ml motile sperm. History taking focused on urogenital development, chronic illness, pulmonary diseases, growth disturbances, medication, male accessory gland infections (MAGI), previous inguinal and scrotal surgery and occupational exposure to heat or chemicals. All men were investigated for genital malformations and ultrasound investigation of the scrotal content was

Table I. Male infertility associated factors in 150 men with oligozoospermia and azoospermia

Male infertility associated factor	*n* (%)	Genetic abnormality (%)
Cryptorchidism	23 (13.3%)	5 (21.7%)
Varicocele	15 (10.0%)	3 (20.0%)
Congenital abnormality of the vas deferens	6 (4.0%)	4 (66.6%)
Male accessory gland infection	8 (5.3%)	0
Endocrine abnormality	3 (2%)	0
Chronic illness	4 (2.6%)	0
Malignancy	2 (1.3%)	0
Previous inguinal and scrotal surgery	4 (2.6%)	0
Unexplained male infertility	85 (56.6%)	27 (31.7%)

performed. Written, informed consent was obtained from each patient prior to the study commencing.

Semen analysis was performed twice according to the World Health Organization (WHO) guidelines for semen analysis (WHO, 1992) with an interval of at least one month. Laboratory investigations included serum gonadotrophins, testosterone, prolactin and sex hormone binding globulin concentrations.

Cytogenetic analysis was performed on metaphase spreads of cultured lymphocytes. Fluorescence in-situ hybridization (FISH) was applied in case 10 (Kievits *et al.*, 1990) with the probes cp9-23.1 and pDP105, specific for the X and Y chromosome short arm telomere regions and the testis determining region of the Y chromosome respectively.

DNA was extracted according to standard procedure and the presence of sub-microscopic deletions of the *AZF* region on the Y chromosome long arm was analysed using a multiplex PCR for Y-specific markers, including DYS148 and DYS273 (*AZFa*), DYS218 and DYS224 (*AZFb*), SY245 and SY255 (*DAZ* gene, *AZFc* region). These sequence-tagged sites (STS) were chosen based on the laboratory guidelines described by Simoni *et al.* (Simoni *et al.*, 1999).

Twelve common mutations of the *CFTR* gene were tested (ΔF508, A445E, G542X, 1717–1G→A, R553X, R117H, R1162X, N1303K, W1282X, 3659delC, E60X and S1251N). The mutations tested comprise about 85% of the mutations identified in the Dutch Caucasian cystic fibrosis population. The length of the T-stretch in intron 8 was tested as previously described (Dohle *et al.*, 1999).

Results

Genetic testing was performed on 37 men with azoospermia (24.7%) and 113 with severe oligozoospermia (75.3%). Table I shows the results of the andrological evaluation. A testicular tumour was found in 2 cases. In 85 (56.6%) of the andrological evaluations no male infertility associated factor was identified.

Table II shows 38 genetic abnormalities identified in 36 (24%) patients**.** Cytogenetic analysis showed an abnormal result in 16 (10.6 %) patients. A sex-chromosomal aneuploidy was identified in 9 men. The Klinefelter syndrome (47,XXY) was present in 6 patients, of which 3 had azoospermia and 3 had an occasional non-motile sperm in the ejaculate (cryptozoospermia). One azoospermic patient presented a 46,XX karyotype, with Y-short arm DNA sequences present in one of the X chromosome short arms. FSH was high (>7 IU/ml) in all men with a 47,XXY karyotype and in the 46,XX male (33 IU/ml). One Klinefelter patient (case no. 6) also carried a *CFTR* gene mutation (R117H). Six balanced translocations and one marker chromosome were found in men with oligozoospermia and a normal phenotype. FSH was either normal or slightly elevated, and oligozoospermia was present in all translocations.

Analysis of the *AZF-a*, *-b* and *-c* regions of the Y chromosome identified 8 (5.3%) men with an *AZFc* (*DAZ*) deletion, 5 with severe oligozoospermia and 3 with azoospermia. The deleted markers were sY254 and sY255. A normal testicular volume was present in 5 men and FSH was increased in 3. Six men with a Yq11 microdeletion had unexplained male infertility, one man (case no. 2) also carried a *CFTR* gene mutation (ΔF508).

CFTR gene mutations were identified in 14/150 (9.3.%) patients, associated with congenital abnormalities of the vas deferens in 4 cases. All congenital bilateral absence of the vas deferens (CBAVD) patients showed low volume (<1.0 ml) and low pH (<7.0) semen analysis and azoospermia and had normal abdominal ultrasound investigations. In 2 patients the scrotal vas deferens was present, but on vasography a blind ending was visualized in the distal part of the vas deferens. Both men also had low semen volume, low pH and azoospermia. A single *CFTR* gene mutation was identified in one. Six (16%) men with azoospermia but without an apparent congenital abnormality of the vas deferens showed a single *CFTR* mutation. In 5/113 (4.4%) patients with severe oligozoospermia a single *CFTR* mutation was identified.

A genetic abnormality was present in 36/150 (24.0%) men with severe oligozoospermia and azoospermia. Excluding those with a congenital abnormality of the vas deferens a genetic risk factor was present in 11/32 (34%) of men with azoospermia. In men with extreme oligozoospermia a genetic abnormality was identified in 20/113 (17.6%). Two patients carried two risk factors: 1 Klinefelter patient (47,XXY) also carried a R117H mutation in the *CFTR* gene; another man was found to have both an *AZFc* deletion of the Y chromosome and a ΔF508 *CFTR* gene mutation.

Discussion

Genetic counselling in reproductive medicine starts by obtaining an accurate diagnosis. The family history, physical examination (including evaluation of dysmorphology) and various laboratory tests on both partners are generally required (WHO, 1992). A substantial number of infertile men, however, present without a history associated with fertility problems and have normal findings on physical examination and endocrine laboratory testing. Only semen analysis is abnormal, often showing a decreased number of sperm cells (oligozoospermia), decreased sperm motility (asthenozoospermia) and many abnormal forms on morphological examination (teratozoospermia). Combinations of these abnormalities occur frequently and are described as the OAT(oligo-astheno-teratozoospermia)-syndrome. These idiopathic forms of male subfertility may be explained by factors like endocrine disruption due to environmental pollution, reactive oxygen species and genetic abnormalities (Crosignani *et al.*, 1992). Although infertility associated with chromosome abnormalities has long been

Table II. Genetic abnormalities in 36 men with severe oligozoospermia and azoospermia

No.	Genetic abnormality	Medical history	Physical examination	Semen analysis	FSH (IU/ml)	Remarks
Abnormal karyotype:						
1	47,XXY	No abnormalities	Hypogonadism	Cryptozoospermia	23	
2	47,XXY	Gynaecomastia	Hypogonadism	Cryptozoospermia	25	
3	47,XXY	Delayed puberty	Hypogonadism	Cryptozoospermia	25	
4	47,XXY	Cryptorchidism	Varicocele	Azoospermia	22	
5	47,XXY	Normal	Hypogonadism	Azoospermia	22	
6	47,XXY	Normal	Hypogonadism	Azoospermia	11	*CFTR*:R117H
7	47,XYY	Normal	Hypogonadism	OAT	12	
8	47,XYY	Normal	Hypogonadism	OAT		
9	46,XX ISH(cp9-23.1+; pDp105+)	Delayed puberty	Hypogonadism	Azoospermia	33	
10	46,XY,t(4;5)(q32;q14)	Normal	Normal	OAT	7.6	
11	45,XY,der(13;15)(q10;q10)	Diabetes mellitus	Normal	OAT	4.0	
12	45,XY,t(14;22)(q11;p?)	Cryptorchidism	Hypogonadism	OAT	9.6	
13	46,X,t(Y;2)(q11.22;q34)	Normal	Varicocele	OAT	1.9	
14	45,XY,der(13;14)(q10;q10)	Normal	Normal	OAT	7.7	
15	46,XY,t(4;15)(p16;q22.2)	Normal	Varicocele	OAT	6.0	
16	47,XY,+Mar(mar=iso(15p))	Urogenital abn.	Varicocele	OAT	6.4	
Deletion of the azoospermia factor (*AZF*) region:						
1	*AZFc*	Normal	Normal	OAT	2.8	
2	*AZFc*	Normal	Hypogonadism	OAT	7.3	*CFTR*:ΔF508/-
3	*AZFc*	Normal	Normal	OAT	1.0	
4	*AZFc*	Normal	Varicocele	OAT	6.0	
5	*AZFc*	Normal	Hypogonadism	Cryptozoospermia	8.0	
6	*AZFc*	Cryptorchidism	Normal	Azoospermia	6.1	
7	*AZFc*	Normal	Normal	Azoospermia	2.4	
8	*AZFc*	Normal	Hypogonadism	Azoospermia	14.8	
Cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutations (T-Stretch):						
1	R117H/- (7T/-)	Sinusitis	CBAVD	Azoospermia	2.3	
2	ΔF508/- (5T/9T)	Normal	CBAVD	Azoospermia	4.6	
3	ΔF508/R117H (7T/9T)	Normal	CBAVD	Azoospermia	4.9	
4	A445E/- (5T/9T)	Ejaculatory failure	Partial CBAVD	Azoospermia	3.3	
5	E60X/- (7T/7T)	MAGI	Normal	OAT	2.5	
6	R117H/- (7T/7T)	Urethral valves	Varicocele	OAT	4.6	
7	ΔF508/- (7T/9T)	Normal	Normal	OAT	2.8	
8	ΔF508/- (7T/9T)	Cryptorchidism	Normal	OAT	16.0	
9	ΔF508/- (7T/9T)	Normal	Hypogonadism	OAT	7.3	*AZFc* deletion
10	R117H/- (7T/9T)	Normal	Hypogonadism	Azoospermia	11.0	47,XXY karyotype
11	ΔF508/- (7T/9T)	Normal	Normal	Azoospermia	3.2	
12	ΔF508/- (9T/9T)	Cryptorchidism	Normal	Azoospermia	10.0	
13	ΔF508/- (7T/7T)	Normal	Normal	Azoospermia	20.0	
14	ΔF508/- (7T/7T)	Normal	Normal	Azoospermia	3.2	

CBAVD = congenital bilateral absence of the vas deferens; MAGI = male accessory gland infection; OAT = oligo-astheno-teratozoospermia; *AZF* = azoospermia factor (Yq11).

recognized, the introduction of ICSI as a method to overcome severe spermatogenic defects has stimulated many investigators to explore further the genetic basis of male infertility. In this study we confirmed that male infertility, both with and without a detectable cause, was associated with several genetic risk factors such as constitutional chromosome abnormalities, microdeletions of the Y chromosome and mutations in the *CFTR* gene.

An abnormal karyotype was found in 10.6% of the men with severe oligozoospermia or azoospermia. The frequency of karyotypic abnormalities in 1791 males with infertility was found to be as high as 12.67% in azoospermia and 4.6% in oligozoospermia (van Assche *et al.*, 1996; Nakamura *et al.*, 2001). Numerical sex chromosomal abnormalities, present in 63% of all cytogenetic abnormalities in infertile males, were commonly found together with azoospermia. Autosomal anomalies, like Robertsonian and reciprocal translocations and inversions were usually present in men with oligozoospermia. In the Klinefelter syndrome the extra X chromosome was associated with germ cell atresia, whereas in the other cytogenetic abnormalities no specific relation to meiotic disturbances was found as these abnormalities also occurred in men with normal spermatogenesis.

In this study DNA analysis of the *AZF-a*, *-b* and *-c* regions on the Y chromosome showed deletions in 5.3% of the patients. All deletions identified comprised the *AZFc* region, containing the Deleted in Azoospermia (*DAZ*) gene (Reijo *et al.*, 1995). The Y chromosomal microdeletions are the most common genetic causes of male infertility due to spermatogenic failure and have been reported in 3–21% of infertile men (van der Ven *et al.*, 1997; Simoni, 2001). These microdeletions were associated with severe oligozoospermia and non-obstructive azoospermia, normal physical findings and normal concentrations of gonadotrophins. Histological studies of testicular

biopsies from men carrying an *AZF* deletion exhibit a wide spectrum of spermatogenetic defects from complete absence of germ cells (Sertoli-cell-only syndrome) to maturation arrest with occasional production of mature, condensed spermatids (Reijo *et al.*, 1995).

Men with defects of the Wolffian duct, presenting as CBAVD, were found to carry different mutations of the *CFTR* gene (Jaffe and Oates, 1994; Oates and Amos, 1994). CBAVD, previously described as a genital form of cystic fibrosis, appears to be a heterogeneous clinical and genetic condition. We have shown that some patients with CBAVD also have non-genital symptoms of cystic fibrosis, like defective cellular chloride excretion and disturbed pancreatic function (Dohle *et al.*, 1999). It has been suggested that CBAVD patients are compound heterozygotes for a severe mutation in one allele in combination with a mild *CFTR* gene mutation in the other. Alterations in the non-coding regions of the gene, such as the polypyrimidine stretch in intron 8, in combination with a mutation in the other allele, were found to cause abnormal levels of CFTR protein, due to exon 9 skipping translation (Chu *et al.*, 1993). *CFTR* intron 8 DNA variants may alter the splicing efficiency of the CFTR mRNA in exon 9 (Kiesewetter *et al.*, 1993), thus causing reduced concentrations of CFTR protein (Chillon *et al.,* 1995). Impaired CFTR protein function may cause defective, but not absent chloride excretion resulting in absence of the vas deferens, but not in pulmonary or pancreatic insufficiency (Anguiano *et al.*, 1992). Mutations of the *CFTR* gene are commonly associated with obstructions of the male genital tract (Mak *et al.,* 2000), and not with spermatogenic failure (Pallares-Ruiz *et al.*, 1999). We detected at least one *CFTR* mutation in 4/6 men with CBAVD and in 10/144 men without a vas deferens-related problem. This seems to confirm the association of mutations in the *CFTR* gene with obstruction of the male genital tract rather than with primary testicular failure, although recently some men with CBAVD were found to have a defective spermatogenesis (Meng *et al.*, 2001). However, the rate of mutations detected in this study in the non-obstructed group does seem elevated compared with the carrier risk in the Dutch population of 1:30 (De Vries *et al.*, 1997). This prompted us to initiate an extended study with a larger cohort of patients. Since we tested previously for mutations that are common among cystic fibrosis patients, we are now using extensive screening methods to evaluate the true rate of *CFTR* mutations/variants in this group of infertile men.

In this study 38 genetic abnormalities were identified in 36 men from a population of 150 men with severe male infertilty. The application of ICSI in these couples may lead to offspring with an enhanced risk of unbalanced chromosome complement, male infertility due to transmission of an *AZF* deletion, and cystic fibrosis in the case of related genital abnormalities. We conclude that all men with severe oligozoospermia and azoospermia should be offered genetic testing and counselling before assisted reproduction is applied.

References

Anguiano, A., Oates, R.D., Amos, J.A. *et al.* (1992) Congenital bilateral absence of the vas deferens. A primarily genital form of cystic fibrosis. *J. Am. Med. Assoc.*, **267**, 1794–1797.

Chandley, A. (1998) Chromosome anomalies and Y chromosome microdeletions as causal factors in male infertility. *Hum. Reprod.*, **13**, 45–50.

Chillon, M., Casals, T., Mercier, B. *et al.* (1995) Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. *N. Engl. J. Med.*, **332**, 1475–1480.

Crosignani, P.G., Collins, J., Cooke, I.D. *et al.* (1972) Recommendations of the ESHRE workshop on unexplained infertility. *Hum. Reprod.*, **8**, 977–980.

Chu, C.-S., Trapnell, B.C., Curristin, S. *et al.* (1993) Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. *Nature Genet.*, **3**, 151–156.

De Vries, H.G., Colleé, J.M., De Walle, H.E.K. *et al.* (1997) Prevalence of deltaF508 cyctic fibrosis carriers in the Netherlands: logistic regression on sex, age, region of residence and number of offspring. *Hum. Genet.*, **99**, 74–79.

Dohle, G.R., Veeze, H.J., Overbeek, S.E. *et al.* (1999) The complex relationships between cystic fibrosis and congenital bilateral absence of the vas deferens: clinical, electrophysiological and genetic data. *Hum. Reprod.*, **14**, 371–374.

Jaffe, T. and Oates, R.D. (1994) Genetic abnormality and reproductive failure. *Urol. Clin. N. Am.*, **21**, 389–408.

Kiesewetter, S., Macek, M., Davis, C. *et al.* (1993) A mutation in *CFTR* produces different phenotypes depending on chromosomal background. *Nature Genet.*, **5**, 274–278.

Kievits, T., Dauwerse, J.G., Wiegant, J. *et al.* (1990) Rapid subchromosomal localization of cosmids by nonradioactive in-situ hybridization. *Cytogenet. Cell. Genet.*, **53**, 134–136.

Mak, V. and Jarvi, K.A. (1996) The genetics of male infertility. *J. Urol.*, **156**, 1245–1257.

Mak, V., Zielinsky, J., Tsui, L. *et al.* (2000) Cystic fibrosis gene mutations and infertile men with primary testicular failure. *Hum. Reprod.*, **15**, 436–439.

Meng, M., Black, L., Cha, I. *et al.* (2001) Impaired spermatogenesis in men with congenital absence of the vas deferens. *Hum. Reprod.*, **16**, 529–533.

Meschede, D. and Horst, J. (1997) Genetic counselling for infertile male patients. *Int. J. Androl.*, **20** (Suppl. 3), 20–30

Nakamura, Y., Kitamura, M., Nishimura, K. *et al.* (2001) Chromosomal variants among 1790 infertile men. *Int. J. Urol.*, **8**, 49–52.

Oates, R.D. and Amos, J.A. (1994) The genetic basis of congenital absence of the vas deferens and cystic fibrosis. *J. Androl.*, **15**, 1–8.

Pallares-Ruiz, N., Carles, S., Des Georges, M. *et al.* (1999) Complete mutational screening of the cystic fibrosis transmembrane conductance regulator gene: cystic fibrosis mutations are not involved in healthy men with reduced sperm quality. *Hum. Reprod.*, **14**, 3035–3040.

Reijo, R., Lee, T., Alagappan, R. *et al.* (1995) Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Genet.*, **10**, 383–393.

Rucker, G.B., Mielink, A., King, P. *et al.* (1998) Preoperative screening for genetic abnormalities in men with non-obstructive azoospermia before testicular sperm extraction. *J. Urol.*, **160**, 2068–2071.

Simoni, M. (2001) Molecular diagnosis of Y chromosome microdeletions in Europe: state-of-the-art and quality control. *Hum. Reprod.*, **16**, 402–409.

Simoni, M., Bakker, E., Eurlings, M.C.M. *et al.* (1999) Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. *J. Androl.*, **22**, 292–299.

van Assche, E., Bonduelle, M., Tournaye, H. *et al.* (1996) Cytogenetics of infertile men. *Hum. Reprod.*, **11** (Suppl. 4), 1–24.

Van der Ven, K., Montag, M., Peschka, B. *et al.* (1997) Combined cytogenetic and Y chromosome microdeletions screening in males undergoing intracytoplasmic sperm injection. *Hum. Reprod.*, **3**, 699–704.

Vogt, P.H., Edelman, A., Kirsch, S. *et al.* (1996) Human Y chromosome azoospermia factors (*AZF*) mapped to different subregions in Yq11. *Hum. Mol. Genet.*, **5**, 933–943.

WHO (1992) *World Health Organisation laboratory manual for the examination of human semen and sperm-cervical mucus interaction.* Cambridge University Press, Cambridge, UK.

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