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Phenotypic Characteristics of Male Subfertility and Its Familial Occurrence

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ABSTRACT: Genetic factors can attribute to male subfertility. A case-control study was carried out to investigate familial occurrence of male subfertility and the phenotypic characteristics of familial male subfertility. The medical data and family histories of 253 severely subfertile men who were candidates for intracytoplasmic sperm injection were compared to the data from 243 randomly selected men. The prevalence of male fertility problems among brothers and maternal uncles of subfertile men was significantly higher than among controls (brothers 10.4% vs 0.5% and maternal uncles 1.7% vs 0.2%). The phenotypes of subfertile men with a positive family history more often showed normal levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) compared to the phenotypes of subfertile men with a negative family history. In addition, subfertile men with a positive family history had a lower percentage of motile sperm. Genetic aberrations, including a chromosomal abnormality or a microdeletion of the Y chromosome, were present in 13.8% of the severely subfertile men. Male subfertility appears to have a familial occurrence, especially among brothers and maternal uncles. Furthermore, examinatoin of the data suggests that subfertile men with a familial occurrence of male subfertility more often have normal levels of FSH and LH and a lower percentage of motile sperm.

Key words: Male infertility, intracytoplasmic sperm injection, genetics, family history.

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Male subfertility has a wide variety of causes. Some of these involve genetic abnormalities such as chromosome abnormalities (Tuerlings et al, 1998), microdeletions of the Y chromosome (Tiepolo and Zuffardi, 1976; Reijo et al, 1995; Kremer et al, 1997), and diverse monogenetic factors such as cystic fibrosis transmembrane conductance regulator (CFTR) mutations in men with congenital bilateral absence of the vas deferens (CBAVD) (Dodge, 1995). However, in the majority of subfertile men the etiology remains unknown and the subfertility must to be classified as idiopathic (Dubin and Amelar, 1971; De Kretser, 1997). This idiopathic male subfertility could have a genetic origin, considering the observed familial occurrence of male subfertility (Budde et al, 1984; Lilford et al, 1994; Meschede et al, 2000; Gianotten et al, 2004).

The family history may provide an important clue for genetic causes of male subfertility and its possible pattern(s) of inheritance. When known, patterns of inheritance can contribute to both genetic research and clinical management of male subfertility.

We studied whether subfertility occurs more often among relatives of severely subfertile men than among relatives of randomly selected men and examined the possible phenotypic differences between subfertile men with and without a positive family history.

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Materials and Methods

Patients

We prospectively collected data from subfertile men visiting our fertility clinic from April 1998 to April 2000. Subfertile men are candidates for intracytoplasmic sperm injection (ICSI) in our clinic if their ejaculate contains less than 1.0×10^6 spermatozoa with propulsive motility (World Health Organization, 1992). We included 253 men with an azoospermia or a severe oligoasthenoteratozoospermia (OAT) with semen parameters that met our ICSI criteria. Patients with a previous sterilization or a testicular malignancy in their medical history were excluded from this study.

Before the visit to our outpatient clinic, a questionnaire was sent to the subfertile men to collect information on their family history (up to second-degree relatives), focusing on fertility problems. At the clinic, we performed a physical examination and took blood samples for measurements of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone; chromosome analyses; and screening for microdeletions of the azoospermia factor (AZF) a, b, and c region of the Y chromosome (Hoefsloot et al, 1997). In cases of obstructive azoospermia, the patient was directed to the Department of Urology for

further investigation. When a patient was diagnosed with a congenital unilateral absence of the vas deferens (CUAVD) or CBAVD, screening for CFTR mutations was performed.

During the visit we specifically asked the patient about involuntary childlessness, fertility problems in the family, or both with the provided family history as guidance. The family members were classified into 1 of 4 categories, that is, *fertile* if they had established a pregnancy or had children without reporting subfertility; *subfertile* if they reported subfertility; *not at risk* if they did not report subfertility and had no children, when there was no heterosexual relationship, when the childlessness was reported to be voluntary, when the relative had died before 25 years of age, or when the relative was mentally retarded; and *unknown* if the former categories did not apply.

Subfertility was defined as a lack of conception after at least 12 months of unprotected intercourse. For the subfertile subgroup, we recorded whether the subfertility was due to a male, female, combined, or unknown factor. We considered fertile and subfertile people to be ''at risk'' for fertility problems. In the analysis, we excluded people classified as ''not at risk.''

When the patient could not give the actual cause of childlessness of a family member, we asked him to contact that family member to obtain the information. When the cause was subfertility due to a male factor, we asked the patient to inform his relative about the study and to request his permission for a telephone call by one of the researchers. When the family member gave permission, we asked him to undergo the same investigations as we performed on our patient group to describe the phenotype.

Controls

We sent the same questionnaire we used for the patients to 474 randomly selected men, aged 25–40 years, living in the city of Boxmeer, The Netherlands. In our study, we included all men who completed the questionnaire giving details on at least their first-degree relatives (243/474; 51.3%). Details of this part of the study are described in our article on using family history to collect data about male subfertility among relatives (Van der Avoort et al, 2003).

Statistics

For both groups, we only included full siblings and relatives in the analyses. Differences between patients and controls were calculated with odds ratios (ORs) and their 95% confidence intervals (95% CIs). The statistical software package SAS version 6.12 (SAS, Cary, NC) was used for analyses. The study was approved by the local Institutional Review Board.

Results

Patients' Clinical Characteristics

The patient group consisted of 46 men with an azoospermia, 10 men with an asthenozoospermia, and 197 men with an OAT. The most frequently encountered clinical abnormality was maldescended testes (n = 69), followed by varicocele (n = 34), surgical correction of an inguinal hernia (n = 32), and a history of male adnexitis (n = 26). The mean ages of the patients and the controls were similar: 33.0 years ± 4.5 SD and 33.3 ± 4.4 years.

Table 1. *Genetic aetiology of the fertility problem*

Type of Genetic Abnormality	Number of Patients
Autosomal structural chromosome abnormaility*	6
Sex chromosome abnormality†	6
Microdeletion of Y chromosome	2
Syndromal disorder with male subertility‡	3
CFTR mutations in CBAVD§	14
CFTR mutations in CUAVD‖	2
Globozoospermia	3

* Robertsonian translocations (n = 2), reciprocal translocation (n = 3), and inversion (n = 1).

† 47, XXY (n = 3); 47, XY (n = 1); 45,X/46,XY (n = 1); and 46,Xinv(Y)(p11.2; q11.23) (n = 1).

‡ Kartgeners syndrome (n = 2) and Klippel-Feil syndrome (n = 1).

§ CFTR indicates cystic fibrosis transmembrane conductance regulator; CBAVD, congenital bilateral absence of the vas deferens.

‖ CUAVD indicates congenital unilateral absence of the vas deferens.

Patients' Genetic Abnormalities

Among the patient group, 35 (13.8%) of 253 patients had a total of 36 genetic abnormalities (for details, see Table 1). One man had 2 genetic defects. He suffered from Kartagener syndrome and he had a sex chromosomal abnormality, 46, X inv(Y) (p11.2; q11.23). The frequency of microdeletions of the Y chromosome in the patient group was 2 (0.8%) of 253.

Family History

The prevalence of male fertility problems is statistically significantly higher among brothers and maternal uncles of subfertile men than among brothers and maternal uncles of controls. The prevalence of male fertility problems is 18 (10.4%) of 173 among brothers of subfertile men versus 1 (0.5%) of 222 among brothers of controls (OR = 26.0; 95% CI: 3.4–196.8). Among maternal uncles, the prevalence of male fertility problems is 9 (1.7%) of 523 and 1 (0.2%) of 534, respectively (OR = 9.3; 95% CI: 1.2–73.9). A statistically significant difference also was found between the prevalence of male fertility problems among maternal and paternal uncles of subfertile men (9/523 [1.7%] vs 2/582 [0.3%]; OR = 5.0; 95% CI: 1.0–33.7).

No statistically significant differences were found between the other family members of patients and controls. Our study design did not focus on the fertility status of cousins. However, 10 patients reported that they had 1 or more cousins with a male subfertility problem. These 16 cousins were 15 sons of maternal aunts and only 1 son of a paternal aunt. In the control group, no cousins were reported to have a fertility problem.

The group of subfertile men had fewer siblings than

the controls (2.4 and 3.0, respectively; mean difference: 0.6; 95% CI: 0.1–0.9). The difference in numbers of siblings could be explained by the difference in brothers: subfertile men had fewer brothers (mean difference: 0.3; 95% CI: 0.1–0.6). No statistically significant difference was found in the numbers of sisters.

Phenotypic Characteristics of Subfertile Men

The main clinical characteristics of the 253 subfertile men, such as testis volume, semen analysis, and hormone measurements are given in Table 2. The group has been divided into men with and men without a positive family history for male subfertility. Family history was considered to be positive when a patient reported having 1 or more brother(s) or maternal uncle(s) with male subfertility.

We found statistical significantly lower FSH and LH levels among men with a positive family history. The difference in FSH levels was 2.5 IU/L (95% CI: 0.3–4.7) and the difference in LH levels was 1.6 IU/L (95% CI: 0.9–2.2). Subfertile men with a positive family history more often showed normal levels of FSH and LH. Furthermore, subfertile men with a positive family history had a statistically significant lower percentage of motile sperm, with a difference of 6.3% (95% CI: 0.9–11.6).

As shown in Table 3, we were able to obtain the phenotype of 12 male relatives of 12 patients with a positive family history. In the other 15 patients with a positive family history, we were not able to obtain direct information from the family member. The reasons for the patients not to contact their family members were not wanting to inform family members about their own fertility problem and not wanting to confront their relatives with questions concerning fertility. These reasons for noncooperation applied more often to patients with subfertile maternal uncles than to patients with a subfertile brother.

For some of the genetic aberrations we found, a brother or uncle of the patient of concern had the same aberration (see families 1 and 2). Other than the cases of globozoospermia and CBAVD, no specific phenotype could be recognized. Two patients and their maternal uncles had high FSH levels.

Discussion

Male subfertility might have a familial occurrence. The statistically significant higher prevalence of male subfertility we found among brothers and maternal uncles of subfertile men suggests this. The higher prevalence of male subfertility among brothers of subfertile men might be caused by several modes of inheritance. There could be an autosomal recessive inheritance, as has been proposed by Lilford et al (1994). Alternatively, sex-limited

Table 2. *Testis volume, semen analysis, and hormone measurements of the cases wiht a positive and a negative family history (values are presented as mean ± SD; values in parentheses are medians)**

	Testis Volume Right, mL	Testis Volume Left, mL	Sperm Concentration, 10^6/mL	Motility, % propulsive	FSH, IU/L	LH, IU/L	Testosterone, nmol/L
Reference range	>15	>15	≥20	≥50	2.0–7.5	1.8–9.5	11–45
Positive family history (n = 26)	16.19 ± 5.59 (18.0)	17.08 ± 4.96 (15.0)	5.00 ± 9.54 (1.6)	15.06 ± 9.76† (15.0)	7.21 ± 4.88† (5.5)	3.54 ± 1.01† (3.5)	16.67 ± 6.29 (16.0)
Negative family history (n = 227)	16.15 ± 4.55 (15.0)	15.47 ± 4.73 (15.0)	4.00 ± 11.40 (1.0)	21.35 ± 16.56† (20.0)	9.68 ± 7.53† (7.1)	5.09 ± 3.31† (4.2)	16.46 ± 6.93 (15.0)

† Statistically significant difference between subfertile men with or without a positive family history ($P < .05$).
* FSH indicates follicle-stimulating hormone; IU, international units; and LH, luteinizing hormone.

Table 3. *Phenotypes of subfertile males in positive case families**

Right/Left Identification	Testes Volume	Spermatozoa, $\times 10^6$	Motility, %	FSH, IU/L	Karyotype	Clinical Remarks
1p	12/12	2.8	15	2.8	46, XY	Globozoospermia
1b	15/15	4.0	10	8.5	46, XY	Globozoospermia
2p	25/25	Azoospermia	—	2.5	46, XY	CBAVD
2b		Azoospermia	—	NA	46, XY	CBAVD
3p	15/15	1.3	20	4.7	46, XY	Inguinal hernia, epididymitis, maldescended testis
3b	12/10	0.1	—	11.0	46, XY	Maldescended testis
4p	15/15	4	30	7.9	46, XY	
4b	NA	Azoospermia	—	Normal	NA	Secondary subfertility, obstruction, CUAVD
5p	25/25	7.5	5	7.1	46, XY	Asthenozoospermia
5b	20/20	30	1	8.9	46, XY	
6p	15/15	4	30	8.8	46, XY	Hydrocele
6b	15/12	NA	NA	20.8	46, XY	Maldescended testis
7p	15/15	45	>1	10.3	46, XY	Varicocele, inversion of chromosome 7 in more
7b	15/15	OAT/azoospermia	—	2.3	46, XY	family members
8p	30/25	6	30	4.4	46,XY,inv(7) (q22.1q31.3)	
8b		Asthenospermia	NA	NA	NA	
9p	15/15	Azoospermia	—	3.8	46, XY	CBAVD
9b		Azoospermia	—	NA		
10p	15/15	Azoospermia	—	13.0	46, XY	Hypogonadism hypergonadotrope
10mu	15/15	Azoospermia	—	27.9	46, XY	Maldescended testis
11p	12/12	10	15	4.5	46, XY	Globozoospermia, maldescended testis
11mu	15/20	45	20	4.2	46, XY	Globozoospermia
12p	15/15	Azoospermia	—	24.7	46, XY	Maldescended testis
12mu	12/12	0.4	30	14.9		

* FSH indicates follicle-stimulating hormone; IU, international units; p, proband; b, brother; mu, maternal uncle; CBAVD, congenital bilateral absence of the ras deferencs; NA, not available; CUAVD, congenital unilateral absense of the vas deference; and OAT, oligosthenoteratozoospermia.

autosomal dominant or X-linked inheritance also could be involved in male subfertility and might just as well explain the increased frequency of male subfertility among maternal uncles. The finding that there is a higher prevalence of male factor subfertility among maternal uncles than among paternal uncles contributes to this hypothesis. This is not in line with the results of Lilford et al (1994), Meschede et al (2000), and Gianotten et al (2004), but is supported by the results of an abundance of X-linked expressed genes in mice spermatogonia (Wang et al, 2001). It should be noted here that during the study the subfertile men reported a total of 15 cousins, all sons of maternal aunts, with male fertility problems. Because there were no direct questions about subfertility among cousins, we do not know any numbers for the control group. Therefore, conclusions cannot be drawn at this moment.

Our results might have been influenced by diverse factors, which could contradict the hypothesis on the familial occurrence of male subfertility. First, subfertile men could have been better informed on fertility problems in their families than men from the control group, causing recall bias. This may explain the differences found in the prevalence of fertility problems between brothers and maternal uncles of subfertile men and their controls. Therefore, we also compared the prevalence of fertility problems among family members of the patients with the prevalence of fertility problems among responders of the control group, which are in the same range as earlier reports and indicate the population risk (Beurskens et al, 1995; Van der Avoort et al, 2003). No statistically significant differences could be found comparing the prevalence of fertility problems among brothers of subfertile men to the prevalence of fertility problems among responders of the control group, but there still is an OR of 2.1 (95% CI; 0.8–5.8).

To find possible new genetic causes of male subfertility, we should exclude all patients with known genetic causes for their male subfertility from our analysis. No significant differences can be found between relatives of subfertile men and controls when excluding these known genetic causes.

On the other hand, the familial clustering of male subfertility among patients' family members might even be more prominent than found in this study because of several possible factors. The calculated ORs among brothers and maternal uncles might even be higher when fertility problems among brothers and maternal uncles of subfertile men using a family history were underestimated (Van der Avoort et al, 2003).

Nonpaternity is another factor that might have led to underestimation of the calculated ORs. If male subfertility has a familial occurrence, then the opportunities for conception through another man are greatest among partners of subfertile men in these families.

In this study we also investigated the phenotypic characteristics of subfertile men. The clinical and genetic data revealed the normal frequencies as mentioned in the literature. A new finding is that subfertile men with a positive family history more often had normal serum concentrations of FSH and LH than subfertile men without a positive family history. This phenomenon is also observed in men with Y microdeletions in the AZF c region (Kremer et al, 1997). The difference in percentages of motile sperm is statistically significant (6.3%; 95% CI: 0.9–11.6).

From the current study, we conclude that male subfertility has a familial occurrence, although different kinds of bias may have influenced the results. Further basic and clinical research will contribute to our knowledge of genetic etiology and the clinical approach in cases of male subfertility.

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