Cystic fibrosis gene mutations and infertile men with primary testicular failure

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It has been proposed that the gene responsible for cystic fibrosis, called the cystic fibrosis transmembrane conductance regulator (CFTR) gene, may play an important role in the process of spermatogenesis. A group of azoospermic men with primary testicular failure underwent CFTR mutation analysis, including assessment of the intron 8 polythymidine tract (IVS8-T tract). An association was not found between CFTR mutations or the 5T variant of the IVS8-T tract and the primary testicular failure phenotype. This finding suggests that CFTR does not play a significant role in the aetiopathogenesis of primary spermatogenic dysfunction. Therefore, the abnormal testicular histological findings in some post-pubertal men with cystic fibrosis may be a result of nutritional deficiency or testicular obstruction rather than a primary defect in spermatogenesis. In addition, the decreased sperm count in oligozoospermic men with CFTR mutations may be secondary to partial reproductive tract obstruction and not abnormal spermatogenesis. Lastly, routine screening of men with primary testicular failure for CFTR gene mutations is not warranted.

Key words: azoospermia/CFTR mutations/cystic fibrosis/male infertility/testicular failure

Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasians, with an incidence of approximately 1 in 2500 and a carrier frequency of 1 in 25 (Welsh et al., 1995). The gene responsible for CF, named the cystic fibrosis transmembrane conductance regulator (CFTR), encodes the cyclic adenosine monophosphate (cAMP) -dependent chloride channel found in the apical membrane of secretory epithelial cells (Welsh et al., 1995). Clinical features of CF include chronic pulmonary obstruction and infections, exocrine pancreatic insufficiency, elevated sweat electrolytes and male infertility (Welsh et al., 1995). There is increasing evidence that mutations in the CFTR gene may contribute aetiologically to certain monosymptomatic disorders. For instance, individuals afflicted with isolated nasal polyposis (Burger et al., 1991), disseminated bronchiectasis (Pignatti et al., 1995, 1996), allergic bronchopulmonary aspergillosis (Miller et al., 1996) or chronic pancreatitis (Cohn et al., 1998; Sharer et al., 1998) have been found to harbour a higher than expected frequency of mutations in the CFTR gene. Some forms of infertility found in otherwise healthy men have also been reported to associate with CFTR gene mutations, especially obstructive azoospermic conditions such as congenital bilateral absence of the vas deferens (CBAVD) (Chillon et al., 1995), unilateral absence of the vas deferens (Mickle et al., 1995), epididymal obstruction (Jarvi et al., 1995) and bilateral ejaculatory duct obstruction with concomitant seminal vesicle anomalies (Meschede et al., 1997).

Three alleles have been identified within the polypyrimidine tract of the CFTR intron 8 splice acceptor site (IVS8-T tract), consisting of nine, seven and five thymidines (9T, 7T and 5T) (Chu et al., 1993). The 5T variant produces a lower level of normal CFTR mRNA transcripts than the 9T and 7T alleles (Chu et al., 1993; Mak et al., 1997) and has been demonstrated to be significantly associated with some of the above-mentioned conditions, including disseminated bronchiectasis (Pignatti et al., 1996), CBAVD (Chillon et al., 1995) and epididymal obstruction (Jarvi et al., 1995). A number of studies have suggested that the CFTR gene may play a direct role in spermatogenesis (Trezise and Buchwald, 1991; Trezise et al., 1993), and that mutations in the gene, including the 5T variant, may have an adverse effect on this process (van der Ven et al., 1996; Larriba et al., 1998). We examined the role of CFTR in spermatogenesis by assessing the frequency of CFTR gene mutations and the 5T variant in infertile males with non-obstructive azoospermia due to primary spermatogenic failure.

Materials and methods

Patients and samples

Fifty-nine otherwise healthy male patients with non-obstructive azoospermia from primary testicular failure presenting to the Andrology Clinic at Mount Sinai Hospital in Toronto between 1995 to 1998, were approached for CFTR genotype analysis after having provided their informed consent and receiving appropriate genetic counselling.
Forty-five of these men (39 Caucasians, one black, five Asian) consented to participate in the study, which was approved by the hospital’s institutional review board and the Human Subjects Review Committee of the University of Toronto. The diagnosis of primary testicular failure was made based on a negative history of exogenous spermatotoxic insults (e.g., exposure to chemicals, radiation, chemotherapy, trauma, post-pubertal mumps orchitis), small testicular size (<12 ml) and an elevated serum follicle stimulating hormone (FSH) concentration (normal range 1–10 IU/l) (Sigman and Howards, 1998). In most cases, the diagnosis was confirmed by the absence of normal active spermatogenesis on testicular biopsy (Sigman and Howards, 1998). All histology slides from outside institutions were reviewed located in introns active spermatogenesis on testicular biopsy (Sigman and Howards, 1998). Second, analysis of the IVS8-T tract; exon 9 including the IVS8-T tract was PCR-amplified with primers located in introns flanking exon 9 (Zielenski et al., 1991), and evaluated by allele-specific oligonucleotide hybridization (Kiesewetter et al., 1993).

**Statistical analysis**
The χ² statistic, Fisher’s exact test, and unpaired t-test were used where appropriate. All P values were based on two-sided comparisons and those < 0.05 were considered to indicate statistical significance.

**Results**

**CFTR analysis**

Only one of the 45 subjects was found to have a CFTR gene mutation (ΔF508). This individual was Caucasian, had an elevated serum FSH concentration (16.0 IU/l), and was Sertoli cell-only on testicular biopsy. With respect to the IVS8-T tract, six of the subjects were 7T/9T heterozygotes, 34 were 7T/7T homozygotes, one was a 5T/9T heterozygote, and the remaining four were 5T/7T heterozygotes. As shown in Table I, the allelic frequencies of the 9T, 7T and 5T variants were not significantly different between subjects in the present study and the general population (Kiesewetter et al., 1993; Cuppens et al., 1994; Dork et al., 1994; Chillon et al., 1995) (9T: 7.8% versus 11.5%, P = 0.30; 7T: 86.7% versus 83.3%, P = 0.43; 5T: 5.6% versus 5.2%, P = 0.80).

**Table I. Frequencies of the IVS8-T alleles in subjects from present study and in general population**

<table>
<thead>
<tr>
<th>Group</th>
<th>IVS8-T allele frequencya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study (90 chromosomes)</td>
<td>79/90 (7.8)b 78/90 (86.7)c 5/90 (5.6)d</td>
</tr>
<tr>
<td>General population (498 chromosomes)</td>
<td>57/498 (11.5) 415/498 (83.3% 26/498 (5.2)</td>
</tr>
</tbody>
</table>

*aValues are number with allele/number studied. Values in parentheses are percentages.

**Table II. Patient characteristics**

<table>
<thead>
<tr>
<th>CFTR IVS8-T genotype</th>
<th>n</th>
<th>Age (years)</th>
<th>Right testis volume (ml)a</th>
<th>Left testis volume (ml)a</th>
<th>Serum FSH conc. (IU/l)</th>
<th>Testis biopsy (n)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-5T</td>
<td>40</td>
<td>33.3 ± 5.1</td>
<td>8.8 ± 3.8</td>
<td>8.5 ± 4.0</td>
<td>20.0 ± 10.8</td>
<td>Hypo (3)</td>
</tr>
<tr>
<td>7T9T</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MA (14)</td>
</tr>
<tr>
<td>7T/7T</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCO (17)</td>
</tr>
<tr>
<td>5T</td>
<td>5</td>
<td>34.6 ± 9.2a</td>
<td>9.1 ± 0.4d</td>
<td>7.9 ± 1.6c</td>
<td>13.0 ± 5.5d</td>
<td>N/A (6)</td>
</tr>
<tr>
<td>5T9T</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MA (1)</td>
</tr>
<tr>
<td>5T/7T</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCO (4)d</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

FSH = follicle stimulating hormone.

*a Testicular volume was evaluated by ultrasonography using the approximation for a prolate ellipsoid (volume = length × width × depth × 0.523 ml) (Forest et al., 1998).

bHypo = hypospermatogenesis; MA = maturation arrest; SCO = Sertoli cell-only; N/A = not available or not performed.

cP = 0.64 for comparison with mean in the non-5T group (unpaired t-test).

dP = 0.89 for comparison with mean right testicular volume in the non-5T group (unpaired t-test).

eP = 0.80 for comparison with mean left testicular volume in the non-5T group (unpaired t-test).

fP = 0.17 for comparison with mean serum FSH in the non-5T group (unpaired t-test).

gP = 0.43 for comparison with testis biopsy results in the non-5T group (Fisher’s exact test).
Comparison between study subjects with and without 5T

Although the number of subjects with 5T was small, no significant difference was found between these men and the others not carrying 5T with respect to age, testicular volume, serum FSH concentration and testicular histology (Table II).

Discussion

Many genes are likely to be involved in the complex process of spermatogenesis (Mak and Jarvi, 1996). Although several studies suggest that CFTR is directly involved in spermatogenesis, others do not seem to support this notion. Testicular biopsies of post-pubertal men with CF have shown abnormal histological findings, such as hypospermatogenesis and increased number of dysmorphic spermatozoa (Denning et al., 1968; Kaplan et al., 1968; Oppenheimer and Esterly, 1969; Holscaw et al., 1971; Gottlieb et al., 1991); however, testicular histology specimens obtained from men with isolated CBAVD have shown completely normal spermatogenesis (Goldstein and Schlossberg, 1988; Silber et al., 1990). Since the overall health and nutritional status in men with CF are poor compared with men with CBAVD, the abnormal testicular histological findings in some post-pubertal men with CF may be a result of nutritional deficiency, gonadotoxic drugs (e.g. antibiotics, corticosteroids) or systemic illness rather than a primary defect in spermatogenesis (Denning et al., 1968). Alternatively, the disrupted spermatogenesis may be due solely to long-standing testicular and/or epididymal obstruction, as evidenced in the acquired abnormal testicular findings in some post-vasectomized men (Jarow et al., 1985).

Expression studies of CFTR in rodent testes by in-situ hybridization demonstrated maximal expression in round spermatids (Trezise and Buchwall, 1991; Trezise et al., 1993), suggesting that dysfunctional CFTR may lead to decreased cytoplasmic volume reduction in early spermatids. Similar experiments in human males, however, failed to confirm these findings (Tizzano et al., 1994). The direct involvement of CFTR in spermatogenesis has also been suggested by the higher than expected frequency of CFTR mutations in men with azoospermia (van der Ven et al., 1996), but the decreased sperm count in these men with CFTR mutations may be secondary to partial reproduct ive tract obstruction and not to abnormal spermatogenesis (Silber and Rodriguez-Rigau, 1981). A description was reported recently (Larriba et al., 1998) of a paucity of mature spermatids in testicular biopsies of CBAVD men with 5T compared with those of CBAVD men with non-5T CFTR mutations, suggesting that the 5T allele may play a role in spermatogenesis. These investigators also recommended that all azoospermic men presenting with infertility undergo CFTR mutation analysis.

To conclude, we did not find an association between CFTR gene mutations and the non-obstructive azoospermia phenotype. However, it must be emphasized that screening for sequence alterations in all exons and their flanking intron sequences and promoter region, as in the present study, does not preclude the presence of mutations within introns of the CFTR gene. In addition, although our subjects did not undergo karyotypic or Y long-arm microdeletion analyses, it is likely that some of them would have chromosome abnormalities or azoospermia factor region deletions (Mak and Jarvi, 1996). We also did not find a difference between non-obstructive azoospermic men with and without 5T in terms of important clinical parameters that reflect adequacy of the seminiferous epithelium in supporting normal spermatogenesis such as testicular volume, serum FSH concentration and testicular histology. Therefore, our findings support the contention that CFTR does not contribute pathogenetically to the abnormal gametogenesis in primary testicular failure. Whether CFTR plays a role in the capacitation of spermatozoa, as implicated by the decreased fertilization rate using epididymal spermatozoa for in-vitro fertilization from CBAVD men with CFTR mutations compared with spermatozoa from CBAVD men without mutations (Patrizio et al., 1993), awaits further studies. Finally, although we concur with the general recommendation that all couples contemplating pregnancy should be offered CFTR mutation analysis (National Institutes of Health, 1997), based on the low frequency of CFTR gene sequence alterations in our study population, we suggest that routine screening of the male patient with non-obstructive azoospermia is not indicated.

Acknowledgements

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Financial disclosures

Professor Tsui founded Ellisis, Toronto, which is primarily concerned with cloning the genes associated with inflammatory bowel disease. Professor Tsui was on the scientific advisory board of Visible Genetics Inc., Toronto, which is concerned with DNA sequencing technology. These activities are not directly related to this study. Dr Durie was a medical advisor to Scandipharm, Birmingham, AL, USA, which manufactures pharmaceuticals for cystic fibrosis, but which does not manufacture diagnostic testing materials.

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