Cystic fibrosis as a cause of infertility

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SUMMARY

Cystic fibrosis (CF) is one of the autosomal recessive diseases, caused by mutations in a gene known as cystic fibrosis transmembrane regulator (CFTR). The majority of adult males with CF (99%) is characterized by congenital bilateral absence of vas deferens (CBAVD). CBAVD is encountered in 1-2% of infertile males without CF. Females with CF are found to be less fertile than normal healthy women. In females with CF, delayed puberty and amenorrhea are common due to malnutrition. CFTR mutations are also associated with congenital absence of the uterus and vagina (CAUV). The National Institutes of Health recommend genetic counseling for any couple seeking assisted reproductive techniques with a CF male or obstructive azoospermia which is positive for a CF mutation. Reproductive Biology 2004 4 (2): 119-129.
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INTRODUCTION

A qualitative diagnosis of infertility requires attention to female and male physical abnormalities, endocrine anomalies (their hormonal and genetic background) and genetic conditions of both partners that interfere with reproduction. Many genes are likely to be involved in the complex process of reproduction [20]. Genetic and non-genetic causes of infertility are increasingly being identified.

The main genetic causes of male infertility diagnosed are microdeletions of the Y chromosome (AZF region) connected with oligoasthenospermia or aspermia as well as mutation of cystic fibrosis transmembrane conductance regulator (CFTR) gene which is connected with aspermia and congenital bilateral absence of vas deferens (CBAVD; [7]).

The cystic fibrosis gene encodes a cell membrane protein

The CFTR gene, located at chromosome 7, spans about 250 kb of genomic DNA and contains 27 exons [34]. In 1989 it was the first anion channel to be identified by positional cloning and sequencing [24]. The product of CFTR gene regulates airway chloride transport. It is also a regulator of sodium and water transport across the epithelium and perhaps acts as a transporter of a still undefined substrate, or substrates [26, 30].

CFTR gene is a unique member of a large ATP binding cassette (ABC) family of proteins known either as “ABC transporters” or “Traffic ATPases” [8, 10]. CFTR protein is a single polypeptide chain of 1480 amino acids that supposedly folds into five domains: two transmembrane-spanning domains (TMS1 and TMS2) with tandem repetition of six putative transmembrane helices also called membrane-spanning domains (MSD1 and MSD2), two nucleotide-binding domains (NBD1 and NBD2), linked by regulatory domain (R) containing numerous phosphorylation sites (fig.1; [24]).
Activation of CFTR is a multistep process that requires calcium and phospholipid-dependent protein kinase C (PKC) and cAMP-dependent protein kinase A (PKA) and a high ATP/ADP ratio. Phosphorylations by kinases and ATP hydrolysis or binding are crucial steps for activating the channel [12]. Phosphorylation of CFTR protein occurs only in cytoplasmic R domain, whereas ATP binding or hydrolysis occurs in one of either NBD. ATP was found to be hydrolyzed during gating activity of CFTR, but a mechanistic link between ATP hydrolysis and specific step in CFTR gating is still unexplained.

Cheung et al. have reported an interaction between CFTR and aquaporin-9 (AQP-9). The CFTR and AQP-9 were found in the luminal membrane of the epididymis, where they play a crucial role in the formation of the epididymal fluid and controlling water permeability. AQP-9 alone causes an increase of water permeability in the epididymis, which is further potentiated by CFTR [4].

Fig. 1. The structure of cystic fibrosis transmembrane regulator (CFTR). This membrane protein is composed of membrane-spanning domains (MSD1 and MSD2), nucleotide-binding domains (NBD1 and NBD2) and regulatory domain (R) containing numerous phosphorylation sites.
CFTR mutations

Over 1000 mutations reflecting all classes of genetic mutations have been identified in CFTR gene and these mutations are located in all domains of the protein. The result of mutations of the CFTR gene is multi-organ disease - cystic fibrosis (CF). CF is the most common autosomal recessive disorder in which abnormal regulation of epithelial Cl channels is associated with the pathophysiology of the disease. It is apparent that CFTR dysfunction results in a range of effects which may contribute to clinical phenotype. The hallmarks of CF include thick and dehydrated airway mucus, pancreatic insufficiency, bile duct obstruction, infertility in males, reduced fertility in females, high sweat Cl, intestinal obstruction, nasal polyp formation, and chronic sinusitis [6].

CFTR mutations are divided into 5 classes. Class I mutations (null mutations) do not produce CFTR protein because of premature stop signal in the CFTR DNA. Class II mutations lead to production of a CFTR protein, but this protein attains an unstable structure shortly after translation in the endoplasmic reticulum. Class III is based on production of a CFTR with reduced chloride transport because of abnormal regulation of the chloride channel. Class IV mutations partially reduce chloride conductance through CFTRs. Finally, class V mutations lead to a severe reduction in normal CFTR protein form [25].

The most common mutation associated with CFTR (responsible for 70% of abnormal CF gene) is a three base-paired deletion that produces an in-frame deletion of a phenyloalanine (F) at position 508 (NBD1 domain). Its frequency varies between ethnic groups (82% patients with CF in Denmark, 32% in Turkey; [22]). This mutant protein of CFTR is known as ΔF508 CFTR. The absence of phenyloalanine at position 508 appears to be associated with abnormal folding of CFTR protein during translation, an abnormality recognized by cellular chaperone proteins that mediate intracellular degradation by proteosomal and other pathways prior to transit of mutant protein to plasma membranes [3, 19, 32]. This mutation leads to a drastically reduced level of CFTR protein expressed on the plasma membrane of exocrine epithelia. If CFTRΔF508 protein reaches the cell surface without degradation, it exhibits a partial function as a cAMP-activated chloride channel.
Males with CF

Majority of adult males with CF (99%) has CBAVD. CBAVD is also encountered in 1-2% of infertile males without cystic fibrosis [2]. Men with CBAVD but without CF gene mutations have a high incidence of urinary tract malformations [9]. The group with urinary tract anomalies represents a separate clinical entity not related to CF and with different embryological pathogenesis. Some forms of infertility found in otherwise healthy men have also been reported to be associated with CFTR mutations, especially obstructive azoospermic conditions such as congenital bilateral absence of vas deferens (CBAVD), unilateral absence of vas deferens, epididymal obstruction and bilateral ejaculatory duct obstruction with concomitant seminal vesicle anomalies [33]. CBAVD is caused by a disruption in the vas deferens, a Wolffian duct derivative. Among 420 cases of men with CBAVD, 19% were carriers of two alleles mutations of CFTR, 47% of a single allele mutation and 34% of no identified mutation [18].

The diagnosis of CBAVD is based on the presence of azoospermia in subjects with normal or small size testes, non-palpable vas deferens, the characteristic ultrasonography view and changes in the physical and biochemical properties of ejaculate (small volume, low pH, and low fructose concentration; [17]). Genital abnormalities may occur early in CF, but are less common diagnosed than in adults. They are found more often in pancreatic insufficient than in pancreatic sufficient CF patients [2].

Mutations in the cystic fibrosis transmembrane conductance regulator gene are a relatively frequent cause of male infertility. Depending on their molecular consequences, CFTR mutations may either result in a typical cystic fibrosis, or in an atypical (often monosymptomatic) forms of CF such as congenital absence of the vas deferens (bi- or unilateral), bilateral ejaculatory duct obstruction or bilateral obstructions within the epididymides. Infertile men might have an elevated risk of being carriers of a mutation within the CFTR gene, in comparison with the general population. Therefore, it is recommended to conduct CFTR mutation tests in males with idiopathic infertility prior to reproduction techniques.
CFTR screening includes the most frequent CFTR mutations, for example in the German population: ΔF508, R347P, G542X, S549I, N, R (A→C), G551D, R553X, N1303K, 3849+10kbC→T [11]. In the French population high frequencies of the ΔF508 mutation (44.7%), the T5 allele (36.2%) and R117H mutation (19.1%) were observed [13]. The data above can be different for other ethnic groups.

Additionally, Chu et al. have identified the mutations within the pyrimidine tract of the CFTR intron 8-splice acceptor site (IVS8-T tract), consisting of five, seven, nine thymidines (5T, 7T, 9T). The 5T variant produces a lower level of normal CFTR mRNA transcripts than the 7T and 9T alleles and is associated with disseminated bronchiectasis, CBAVD and epididymal obstruction [5]. Arduino et al. have described a CBAVD patient with a compound heterozygosity in the CFTR gene for a stop mutation W1282X and a new missense mutation P499A. The P499A was found as a mild mutation, limited to the development of Wolffian duct derivatives and was revealed only in combination with a severe CFTR mutation [1].

In our investigations a sample patient is screened for the presence of 31 common CF mutations, including the 24 most common CF mutations worldwide, as identified by the CF consortium. This mutation detection system employs a rapid, single tube, multiplex DNA technology associated with CF incorporating polymerase chain reaction (PCR), oligonucleotide ligation assay (OLA) and sequence-coded separation (SCS) using the fluorescent technology. Products from the multiplex OLA are detected in a capillary electrophoresis, 4color fluorescent ABI 310 DNA Analyzer (Applera, USA). Sequence-Coded Separation identifies each ligation product as a unique combination of electrophoretic mobility and color.

Testicular biopsies of post-pubertal men with CF have shown abnormal histological findings, such as pathological spermatogenesis and increased number of dysmorphic spermatozoa, yet testicular histology specimens obtained from men with isolated CBAVD have shown completely normal spermatogenesis. This fact could be a result of nutritional deficiency, gonadotoxic drugs (e.g. antibiotics, corticosteroids) or systemic illnesses rather than a primary defect in spermatogenesis. There is no evidence of association between CFTR gene mutations and the non-obstructive azoospermia phenotype [21].
The use of percutaneous epididymal or testicular sperm aspiration (PESA, TESA) and intracytoplasmic sperm injection (ICSI) has allowed the attainment of pregnancies in this group of patients [27]. If any CF mutation is detected in a patient, CFTR screening should involve the female partner [11]. The female partner of a man with CFTR-mediated anomalies of the genital tract may be heterozygous purely by chance (4–5% in many countries).

Since infertility in many cases can be treated nowadays by assisted reproductive technology, there is an increased risk of having children with

Fig. 2. Screening of cystic fibrosis (CF mutation). Detection of mutation ΔF508 (peak within dotted ellipse) using CF Multiplex DNA Assay (Applera, USA).
CF if the male and female are carriers of CFTR mutations. Couples should be informed about the risk and risk prevention including pre-implantation diagnosis. Follow-up studies of children born to these couples are mandatory, whether male infertility is linked to CF or not [27].

**Females with CF**

Females with CF are found to be less fertile than normal healthy women. In CF females delayed puberty and amenorrhoea are common due to malnutrition as the main cause [29]. Delayed pubertal increments of serum gonadotrophin and sex steroids suggest late maturation of the reproductive endocrine system [23]. The patients who were homozygous for the most common mutation ∆F508 and those with pathological glucose tolerance test (OGTT) were significantly delayed in menarcheal age. The majority of the patients had essential fatty deficiency (EFAD) which may cause pubertal delay [15].

The low fertility in CF females is known to be caused mainly by tenacious impermeable cervical mucus, which does not undergo the typical changes during menstrual cycle, due to defective CFTR protein expressed in the cervix [16]. Some CF women do not ovulate. They have higher total serum testosterone concentration and signs of insulin resistance similar to those found in women with polycystic ovarian syndrome. Anovulation due to malnutrition and catabolism has been suggested as a secondary cause of infertility. CF patients might have inflammation in their tissues prior to infection with an imbalance of proinflammatory cytokines versus the anti-inflammatory ones.

Recently CFTR mRNA expression has been found in areas of rat hypothalamus which are involved in the regulation of sexual maturation and reproduction. These areas contain high concentrations of GnRH and estrogen-rich neurons. CFTR might increase acidification of synaptic vesicles and for that reason it plays an important role in central regulation of sexual maturation and fertility [14]. Ovulation in these chronically ill women may also be influenced by physiological and psychological stress. CFTR mutations are also associated with congenital absence of the uterus and vagina (CAUV; [31]). Since the embryologic development of the Mullerian ducts
directly depends on the prior normal development of the Wolffian ducts, the same gene products may be necessary for normal embryologic development of both duct’s systems.

The incidence of the 33 CFTR mutations found in the patients with CAUV (8%) was twice as high as that found in the general population (4%), but much less than the incidence of CFTR mutations in men with CBAVD (80%). This data suggests that it is unlikely for CFTR mutations to cause CAUV in females as they cause CBAVD in males. Furthermore, the data suggests that CAUV in females may be the same disorder as CBAVD in males who do not have CFTR mutations.

National Institutes of Health recommends genetic counseling for any couple attempting assisted reproductive techniques, when the man has CF or obstructive azoospermia and is positive for a CF mutation [28]. Clinical genetic conditions of a family considering assisted reproduction of infertility can be established by evaluating the full family history, documenting pregnancy, fetal, neonatal and pediatric loss of life as well as by cytogenetic studies of the couple and DNA mutation analysis for cystic fibrosis mutations.

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