Cystic fibrosis and infertility caused by congenital bilateral absence of the vas deferens and related clinical entities

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The condition of congenital bilateral absence of the vas deferens (CBAVD) is, in the majority of patients, related to defects in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CBAVD patients either are compound heterozygotes (carrying different mutations in their two CFTR genes) or carry a mutation in one of their CFTR genes and an intron 8 5T splice variant, associated with low levels of functional CFTR protein, in their second gene. The relationship between cystic fibrosis (CF) and CBAVD requires a proper clinical examination of the patient, a CFTR mutation analysis for himself and his family and genetic counselling. A mutation analysis should also be performed for the wives of CBAVD males because such couples now have the possibility of having their own genetic children but are at increased risk of having children with CF and/or CBAVD. The aetiology of some conditions of CBAVD is not related to CF, especially when CBAVD is associated with urinary tract malformations (up to 20% of cases). In couples with CBAVD not related to CF there is no increased risk of CF children, but it is not known whether they have an increased risk of having sons with CBAVD. In some of the patients with congenital unilateral absence of the vas deferens (CUAVD) the condition is also related to CF, especially in cases where there is an occlusion of the palpable vas. The CFTR gene is probably not involved in the aetiology of Young's syndrome. Follow-up studies of children born to couples where the males have CBAVD, CUAVD or Young's syndrome are mandatory and will help to better define the risk to their offspring of CF and/or of inheriting their paternal infertility condition.

Key words: congenital bilateral absence of the vas deferens/cystic fibrosis/genetic counselling/male infertility/obstructive azoospermia

Introduction

Cystic fibrosis (CF) is one of the most common genetic disorders among Caucasians (Welsh *et al.*, 1995; Zielenski and Tsui, 1995). It is inherited as an autosomal recessive disease affecting ~1 in 2500 children (carrier frequency ~1 in 25). The major clinical manifestations of CF are chronic obstruction and infection of the respiratory tract and, in most cases, exocrine pancreatic insufficiency. Pulmonary obstruction by viscous mucus and infection often lead to respiratory failure and death. In recent years, the prognosis for survival has improved considerably as a result of symptomatic therapy, and 50% of patients will survive to 29 years of age. Another typical manifestation of the disease is an increased electrolyte (sodium and chloride) level in sweat, which is used to confirm the clinical diagnosis of CF.

More than 95% of males with CF are also infertile (Welsh *et al.*, 1995). This infertility is the result of obstructive azoospermia caused by maldevelopment of the mesonephric ducts, resulting in either agenesis or atresia of the epididymis, vas deferens or seminal vesicles. Whether maldevelopment is a primary defect related to the disease or a secondary degenerative change resulting from obstruction by mucus is not known (Landing *et al.*, 1969; Heaton and Pryor, 1990). Reduced fertility is also present in females with CF, although no equivalent anomalies in the female reproductive tract exist. Infertility in females is possibly a consequence of the presence of thick mucus in the genital tract which forms a barrier to sperm penetration (Welsh *et al.*, 1995).

Congenital bilateral absence of the vas deferens (CBAVD) leading to obstructive azoospermia in otherwise healthy males is responsible for up to 2% of male infertility (Dubin and Amelar, 1971). CBAVD usually occurs sporadically, but several familial cases have been described (McKusick, 1994). Because absence of the vas deferens is also present in the majority of CF males, the hypothesis that CBAVD males represent an 'incomplete' form of CF, without the characteristic lung and pancreatic symptoms, was proposed as early as 1971 (Holsclaw *et al.*, 1971). However, as long as the CF gene was unknown, it was impossible to test this hypothesis. Soon after the identification of the CF gene and its commonest mutation, ΔF508, leading to CF, it was shown that the frequency of this mutation in CBAVD patients was significantly higher than expected on the basis of CF carrier frequency in the general population under consideration (Dumur *et al.*, 1990). Since then, many studies using more extensive analyses of the CF gene in CBAVD patients have confirmed the role of defects in this gene in CBAVD. Nevertheless, there is evidence that not all cases of CBAVD are related to CF.

This report deals with the current knowledge of the genetic relationship between CBAVD and related clinical entities, such as congenital unilateral absence of the vas deferens (CUAVD) and Young's syndrome, and CF. Another part of this report deals with the consequences of this relationship in terms of reproductive options for these patients and their families. Particularly as spermatogenesis is known to be normal in these patients, microsurgical sperm aspiration (MESA) and in-vitro fertilization (IVF) or, more recently, intracyto-

plasmic sperm injection (ICSI), can be used for the treatment of obstructive azoospermia. Couples where the men have CBAVD or related conditions associated with CF and the women carry a CF mutation have an increased risk of having children presenting with CF or CBAVD (Rigot *et al.*, 1991). A mutation analysis in both partners, genetic counselling and the option of prenatal diagnosis or preimplantation diagnosis should be offered and discussed with these couples. Moreover, genetic counselling should be offered to male siblings of CBAVD patients, who may similarly be affected, and to other family members who may be carriers of a CF mutation. In couples with CBAVD not related to CF, the risk of CF children is no different from the risk for couples in the general population. Whether these couples have an increased risk of CBAVD in their children is not known so far.

The CF gene: mutation analysis

The CF gene was first localized in 1985, in the vicinity of PON, a genetic determinant for serum paraoxonase activity (Eiberg *et al.*, 1985). Soon after, a genetic linkage between CF and polymorphic DNA markers previously localized to the long arm of chromosome 7 was demonstrated (Knowlton *et al.*, 1985; Tsui *et al.*, 1985; Wainwright *et al.*, 1985; White *et al.*, 1985). The gene was finally identified in 1989 by chromosome walking and jumping (Kerem *et al.*, 1989; Riordan *et al.*, 1989; Rommens *et al.*, 1989). The gene consists of 27 protein coding regions (exons) spread over almost 230 kb of genomic DNA. The exons are numbered 1–24, with subdivisions a and b for exons 6, 14 and 17 because these exons were not recognized as separate units in the initial publication. The gene is transcribed in a major mRNA of ~6100 nucleotides and is expressed in many tissues, with higher levels in tissues severely affected in CF patients. The transcript is translated in a 1480 amino acid transmembrane protein, the CF transmembrane conductance regulator or CFTR. The CFTR protein consists of two similar motifs, each comprising six membrane-spanning regions and one nucleotide (ATP)-binding fold (NBF); these are linked by a unique highly polar domain that is involved in regulation of the protein (R or regulatory domain). The protein has the typical features of a group of ATP-binding cassette (ABC) membrane transporters and therefore belongs to this family (Hyde *et al.*, 1990).

Genetic evidence that this gene is indeed defective in CF came from the initial observation that 68% of 214 CF chromosomes were carrying a 3 bp deletion (CTT) in exon 10, leading to the deletion of a phenylalanine at amino acid position 508 (ΔF508 mutation). None of the 198 normal chromosomes of CF parents had this deletion. These results were confirmed by the study of a large number of CF chromosomes worldwide: 66% of almost 44 000 chromosomes studied were found to carry this mutation [Cystic Fibrosis Genetic Analysis Consortium (CFGAC), 1994]. However, the frequency of this major CF mutation is not equally distributed across different populations. For instance, in Denmark this mutation represents almost 90% of CF chromosomes, but is only present in

40–50% of chromosomes in Southern Europe (Italy, Spain and Portugal). Soon after the identification of the gene, CFGAC was founded and one of the main objectives was to increase and facilitate communication among CF researchers working on mutation identification and population screening. To date, >500 mutations, spread over the whole gene, have been reported through CFGAC.

Besides the ΔF508 mutation, only ~20 mutations have been found on 50 or more chromosomes (CFGAC, 1994). The remaining mutations occur with a very low frequency or are even unique to single CF families. As for the ΔF508 mutation, some mutations occur more frequently in different populations. An extreme example is the W1282X mutation among Ashkenazi Jews, where it accounts for almost 50% of CF chromosomes (ΔF508 27%), while in non-Ashkenazi Jews (ΔF508 43%) and other populations it is present in ≤3% of the chromosomes (CFGAC, 1994). In some genetically homogeneous populations a limited number of mutations account for the majority of CF chromosomes. For instance, screening for five mutations results in the detection of 97% of the CF chromosomes among Ashkenazi Jews, and 19 mutations located in nine exons account for >98% of mutant alleles in a Celtic population in France (Abeliovich *et al.*, 1992; Férec *et al.*, 1992). In contrast, the number of mutations may be very large in genetically heterogeneous populations. In Spain, for instance, 43 different mutations identify only 78% of CF chromosomes (Chillon *et al.*, 1994). From these data it is obvious that for some populations CF carrier screening can be performed with a reasonable detection rate, while for others it would be almost impossible in terms of financial and technical considerations. The issue of carrier screening in the general population has been the subject of much controversy (Scriver and Fujiwara, 1992; Williamson, 1993; Brock, 1995). Nevertheless, an individual from the general population screened for a fraction (a) of the CF mutations found in that population, in which the CF carrier frequency is q, will have, in cases of negative screening, a residual risk of being a carrier of $Z = q(1 - a)/(1 - aq)$. Considering a carrier frequency of 1/25, then this prior risk will be lowered to 1/81, 1/121 and 1/241 when 70, 80 and 90% of the mutations in the given population are screened for (ten Kate, 1990).

The technical possibilities for tracing mutations already described are numerous. In general, the DNA fragment of interest will be amplified from genomic DNA by the polymerase chain reaction (PCR) (Saiki *et al.*, 1985). This fragment can then be analysed for the presence or absence of a well-defined mutation, e.g. by determination of the fragment length (ΔF508 mutation), by digestion of the fragment with a restriction enzyme where the mutation destroys or creates a site, or by hybridization with oligonucleotides specific for the normal and mutated sequences.

Analyses for the identification of unknown mutations in CF chromosomes have focused mainly on the study of the 27 exons and exon–intron boundaries generally, starting from genomic DNA isolated from white blood cells. After amplification by PCR, the exon-specific DNA fragments are analysed by using denaturing gradient gel electrophoresis, single-strand conformation polymorphism (SSCP) analysis, chemical cleavage or related techniques, followed by DNA

sequencing for the exact identification of nucleic acid changes, or DNA sequencing directly (Grompe, 1993). The identification of all mutations in a given CF population has so far been exceptional and has been limited to relatively small ethnic groups (Zielenski *et al.*, 1993; Mercier *et al.*, 1994).

Failure to identify mutations could be the result of several causes, the most important of which are listed.

(i) The sensitivity of the technology used is not absolute and some mutations will not be detected.

(ii) The mutation is not present in the gene fragments analysed. Not as many mutation studies of the CF gene have included the region 5′ to the translation initiation codon. A putative defect in this region has been described recently by Bienvenu *et al.* (1995). Alternatively, mutations are present in the large intronic regions (non-coding regions) of the gene that are not studied. These mutations could lead to the creation of a new exon that is incorporated into the mature mRNA and distorts the production of normal CFTR. Examples are the mutations 1811+1.6kbA → G in intron 11 and 3849+10kbC → T in intron 19 (Highsmith *et al.*, 1994; Chillon *et al.*, 1995b). These two mutations would not have been detected with the approaches described above, and were found by analysing CFTR cDNA transcribed *in vitro* from mRNA.

(iii) The mutation is a deletion encompassing one or more exons such that genomic analysis of these exons represents only one allele (the second one is not amplified by PCR because of the deletion). Most of the mutations found so far in the CF gene are small nucleic acid changes. However, three independent CF families with a 50 kb deletion have been described (Morral *et al.*, 1993). These three families were detected by studying intragenic microsatellite polymorphisms in the CF patients and their parents.

Molecular mechanisms of CFTR protein dysfunction

There is overwhelming evidence now that the CFTR protein forms a cAMP-regulated Cl channel located in apical membranes (reviewed in Welsh *et al.*, 1995). Dysfunction of the protein leads to a reduction or lack of ion transport resulting in an imbalance of salt and water and dehydration of mucus secretions. The protein also regulates the activity of several other ion channels, and deregulation of these channels by CFTR dysfunction may also contribute to the pathophysiology of CF (Higgins, 1995). Here we will focus on the way specific changes in the CFTR protein (caused by mutations in the CF gene) alter the function of the Cl channel. The effects of most mutations found so far have not been studied at the functional level. However, from the available data, four mechanisms that disrupt CFTR function have been proposed (Welsh and Smith, 1993).

Class I mutations result in defective CFTR protein production. Premature translation termination signals are produced because of frameshift mutations (small insertions or deletions, e.g. 3905insT and 394delTT), splice site

abnormalities that shift the open reading frame (e.g. 621+1G → T) or missense mutations (G542X replacing glycine at amino acid position 542 with a stop codon). In general, little or no CFTR protein is formed.

Class II mutations result in defective CFTR protein processing. These mutations, including the ΔF508 mutation, result in the production of a protein that fails to traffic to the correct cellular location, the apical membrane. The mechanism for this failure is unknown, but it has been suggested that these mutations prevent CFTR from adopting its correct conformation, necessary for transport to the membrane. As a result, the protein is probably degraded, resulting in the absence of, or strongly reduced amounts of CFTR protein in the apical membrane.

Class III mutations result in CFTR protein with defective regulation. These CFTR proteins normally reach the apical membrane but have defective regulation with a resulting decrease in net Cl channel activity. Most of these mutations are in the NBF and probably also in the R domain.

Class IV mutations alter the Cl conduction of CFTR protein. These proteins are correctly processed, are present in the apical membrane and do generate cAMP-regulated Cl currents similar to wild-type CFTR. However, the rate of ion flow through the channel is reduced. Missense mutations located in the membrane-spanning domains (such as R117H, R334W and R347P) belong to this class.

It must be stressed that a single mutation might cause more than one type of abnormality in the CFTR and hence fall into more than one class of mutations. For instance, the ΔF508 mutation disrupts CFTR function primarily by mislocalization (Class II). However, defective regulation of CFTR function has also been demonstrated (Class III).

Genotype–phenotype correlations in CF

Clinical heterogeneity among patients with CF has long been recognized. Since the identification of the CF gene, many reports have been published dealing with the correlation of phenotype and the mutations present in the patients. Besides the major phenotypic diagnostic criteria of CF, pulmonary disease, pancreatic sufficiency or insufficiency and elevated sweat electrolyte levels, many rarer clinical features have also been studied (Cystic Fibrosis Genotype–Phenotype Consortium, 1993; Dean and Santis, 1994). Although studies looking at genotype–phenotype correlations have in a way been disappointing, the phenotypic symptoms of CF in relation to genotype can, according to Welsh *et al.* (1995), be divided into three categories.

The first category includes the phenotypic symptoms common to the great majority of CF patients, regardless of the type of mutation. The major, and probably only, symptom in this category is an elevated sweat chloride level.

The second category includes symptoms that show a good correlation with the genotype. The only symptom that correlates well with the genotype of CF patients is pancreatic function. In CF patients from the same family, pancreatic

function, whether sufficient or insufficient, is almost the same, indicating that pancreatic status is determined primarily by the genotype at the CFTR locus. Other factors, such as lung disease, may vary considerably among these sibships.

The third category includes symptoms that do not show much correlation with the type of mutation or vary considerably within families. Pulmonary disease, liver disease and meconium ileus, among others, belong to this category. It is probable that both genetic and non-genetic (e.g. environmental) factors affect these symptoms.

The status of pancreatic function in CF patients allows us to draw an important conclusion in relation to the genotype. Class I and II mutations are predicted to result in the absence of CFTR product at the apical membranes and consequently no CFTR-driven Cl transport is possible. Therefore, these classes of mutation can be predicted to have a severe phenotypic effect (severe mutations). These mutations have indeed been found to be largely associated with pancreatic insufficiency. Typical examples are ΔF508 homozygous patients and compound heterozygotes (ΔF508/Class I or II). In Class III and IV mutations some mutant proteins retain significant residual Cl transport activity at the apical membranes, while others are severely deficient. The residual activity of some CFTR mutant proteins may be sufficient to lead to a pancreatic sufficiency phenotype (mild mutation), but for others it may be inappropriate and lead to pancreatic insufficiency (severe mutation). Although a correlation with lung disease and other symptoms of CF is not clear cut, mutations retaining some residual activity are generally considered to result in a milder phenotype with pancreatic sufficiency. Compound heterozygotes of a severe mutation (Class I and II mutations, and part of Class III and IV mutations) and a mild mutation will, in general, be pancreatic sufficient. Therefore mild mutations seem to be dominant over severe mutations probably because the former have some residual CFTR activity.

Genetics of CBAVD

Mutation analysis in the CFTR gene

The presence of obstructive azoospermia in both CF and CBAVD patients led Holsclaw *et al.* (1971) to suggest that obstructive azoospermia caused by CBAVD could be an incomplete or mild form of CF with absence of the pulmonary and digestive clinical signs of CF. However, it was not possible to verify this hypothesis until cloning of the CF gene. Indeed, if a genetic link between CBAVD and CF existed, mutations would be expected in the CFTR genes of both CBAVD and CF patients. A first report dealing with this issue was published in 1990 (Dumur *et al.*, 1990). These authors studied 17 CBAVD patients for the presence of the ΔF508 mutation. Seven of the patients were found to be carriers of this mutation, and six of these, as well as two of the ΔF508 non-carriers, were also found to have sweat chloride concentrations in the pathological range for

Table I. Cystic fibrosis transmembrane conductance regulator (CFTR) mutation detection in patients with congenital bilateral absence of the vas deferens

	CFTR genotype (% of patients studied)			
No. of patients	Two mutations	One mutation	No mutation	Reference
70	13	57	30	Zielenski *et al.* (1995)
67	24	42	34	Mercier *et al.* (1995)
49	18	64	18	Anguiano *et al.* (1992), Oates and Amos (1994)
45	33	56	11	Costes *et al.* (1995)
38	16	39	45	Dumur *et al.* (1996)
36	14	36	50	Rave-Harel *et al.* (1995)
30	10	57	33	Casals *et al.* (1995)
26	23	54	23	Jézéquel *et al.* (1995), Le Lannou *et al.* (1995)
26	12	27	61	Osborne *et al.* (1993)
25	16	20	64	Jarvi *et al.* (1995)
8	25	50	25	Culard *et al.* (1994)
Total 420	19	47	34	

CF. None of the patients had clinical signs of CF, but some had chronic sinusitis ($n = 4$), diabetes ($n = 1$) and chronic bronchial hypersecretion ($n = 1$). Therefore the high frequency of the ΔF508 mutation in these CBAVD patients (7/17 or 41% carrier frequency in comparison with the expected 4% in the general population) strongly supported the hypothesis of a genetic link between CBAVD and CF.

These results were confirmed in another cohort of 25 CBAVD patients in a more extensive study of the CFTR gene (Anguiano *et al.*, 1992). Here, 13 of the patients were found to carry one mutation, and three patients were compound heterozygotes. Of the 13 patients studied, three had elevated sweat chloride concentrations. The authors concluded that some, or perhaps all, instances of CBAVD in otherwise healthy men present with a 'genital' phenotype of CF. Gervais *et al.* (1993) found an increased frequency of the R117H mutation in CBAVD patients, a mutation also found in CF patients with a rather mild phenotype. A few CBAVD patients homozygous for this mutation have also been described (Bienvenu *et al.*, 1993; Patrizio *et al.*, 1993a,b).

Many more studies have since been conducted and all confirm the role of a defect in the CFTR gene in CBAVD. In most of these studies, the CFTR genes of these patients have been analysed extensively for the presence of mutations. The results are summarized in Table I. In a total of 420 patients, 19% were found to be compound heterozygotes, 47% were carriers of one mutation and in 34% of patients no mutation could be detected. Most of the mutations found have been described previously in patients with CF, but some have only been found in CBAVD. In the compound heterozygotes, none of the patients had two severe mutations, but rather a severe and a mild one or two mild ones.

The CF mutation frequency is thus clearly increased in these CBAVD males.

However, if a defective CFTR protein is responsible for CBAVD, why then do all of these patients not have a mutation in both of their CF genes? In fact, two mutations are found in <20% of the patients. The inability to identify two mutations in >80% of the patients therefore led to the conclusion that mutations in other parts of the gene, which had not been studied, might be responsible for CBAVD. This idea was also supported by one study in which it was demonstrated that in CBAVD males carrying one CFTR mutation a defect in chloride conductance across the nasal epithelium exists which is dissimilar to that found in CF patients (Osborne *et al.*, 1993). In addition, normal sodium transport was found. An alternative explanation would be that some conditions of CBAVD may not be related to CF or only partially. In other words, the causes leading to CBAVD might be heterogeneous. In the following sections we will deal with the variable number of thymidine (T) nucleotides at the intron 8–exon 9 acceptor splice site of the CFTR gene, which is involved in CBAVD, and then with (possible) indications for other causes of CBAVD.

The intron 8 splice site variant 5T

In tissues from normal individuals, several studies looking at CFTR mRNA have identified, as well as a major full-length mRNA, various mRNA molecules that lack exon 4, 9 or 12 (Chu *et al.*, 1991; Bremer *et al.*, 1992; Slomski *et al.*, 1992). The reason for the loss or skipping of exons 4 and 12 has not yet been identified. However, the amount of CFTR mRNA without exon 9 (exon 9^-) depends on the number of T nucleotides in intron 8 just in front of exon 9 at the splice branch/acceptor site (Figure 1) (Chu *et al.*, 1992, 1993). In normal individuals five, seven or nine T have been found at this position. Individuals homozygous for the 5T allele have CFTR mRNA molecules with exon 9^- skipped up to 92% in respiratory epithelium. In contrast, individuals homozygous for 7T or 9T alleles have as a mean <25 and <15% of exon 9^- mRNA respectively. Heterozygous individuals have levels intermediate between the corresponding homozygous values. The exon 9^- mRNA produces non-functional CFTR protein (Delaney *et al.*, 1993; Strong *et al.*, 1993). A 5T allele homozygote must therefore have strongly reduced quantities of functional CFTR protein. Nevertheless, this quantity must still be sufficient, because in none of the four individuals originally studied (three females and one male) were clinical signs of CF observed. In the general population, the 7T variant is the most common (~85%), while the 5T (5%) and 9T (10%) variants occur at much lower frequencies (Kiesewetter *et al.*, 1993).

An important observation in relation to the intron 8 splice site variant has been made with regards the phenotype resulting from the R117H CFTR mutation (Kiesewetter *et al.*, 1993). The majority of CF chromosomes have genes carrying a 9T variant (9T background). This is especially the case because the ΔF508 mutation occurs exclusively on this background; even after exclusion of this mutation, a significant excess of CF mutations is found in genes carrying a 9T variant. Much fewer mutations are found on a 7T background, and only a very

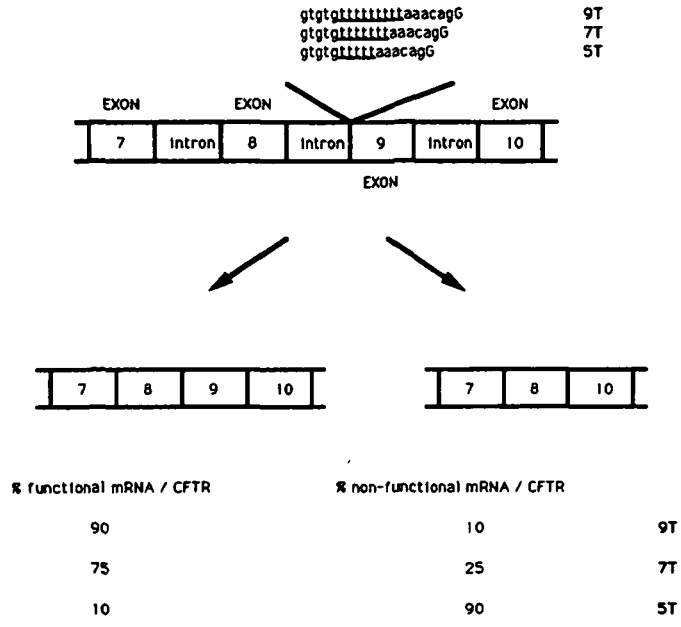

Figure 1. The intron 8 polypyrimidine variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene and their effects at the mRNA and protein levels. Part of the CFTR gene, including exons 7–10, is shown schematically at the top. The number of thymine (T) nucleotides in intron 8, just in front of exon 9, is variable. This sequence contains five, seven or nine T (underlined). The uppercase G indicates the first nucleotide of exon 9. During RNA processing, the introns are removed (spliced out) and the exons are joined together to form a mature CFTR mRNA (centre). In some fraction of the total mRNA produced, exon 9 is also spliced out depending on the number of T in intron 8. The 9T variant is the most efficient in producing CFTR mRNA containing exon 9, while the 5T variant produces almost exclusively mRNA lacking exon 9 (bottom). CFTR mRNA without exon 9 results in the synthesis of a non-functional CFTR protein.

few occur on a 5T background. A particular mutation usually occurs on the same background. In contrast, the R117H mutation was found to occur on two backgrounds (5T and 7T), resulting in different phenotypes. Patients carrying a severe mutation in one gene and a R117H mutation on a 5T background in the other gene had CF associated with pancreatic sufficiency. This was also true for one patient homozygous for R117H/5T. However, the combination of a severe mutation with R117H on a 7T background was found to be associated with three different phenotypes: CF with pancreatic sufficiency, CBAVD and a normal phenotype in one asymptomatic female. As mentioned above, the 7T variant results as a mean in 25% of non-functional (exon 9^-) CFTR protein. However, a considerable, but as yet unexplained, variation exists in this group: almost 0–50% of exon 9^- mRNA (Chu *et al.*, 1993). This variation in intron 8 splice site efficiency was considered to be the cause of the variation in phenotype. Because the R117H mutation produces partially functional CFTR protein, the combination of this mutation and the 7T splice variant would then result in the following situations. Individuals carrying the R117H mutation on a 7T background associated with inefficient splicing (higher range of the 0–50% variation in exon 9^- mRNA) would have low levels of full-length CFTR mRNA bearing the R117H mutation and consequently low levels of partially functional CFTR

Table II. Cystic fibrosis transmembrane conductance regulator (CFTR) mutation detection in patients with congenital bilateral absence of the vas deferens excluding (–) or including (+) the intron 8 5T variant

No. of patients	5T variant	CFTR genotype (% of patients studied)			Reference
		Two mutations	One mutation	No mutation	
102	–	19	53	28	Chillon *et al.* (1995a)
	+	53	25	22	
70	–	13	57	30	Zielenski *et al.* (1995)
	+	59	20	21	
45	–	33	56	11	Costes *et al.* (1995)
	+	80	9	5	
38	–	16	39	45	Dumur *et al.* (1996)
	+	40	26	34	
25	–	16	20	64	Jarvi *et al.* (1995)
	+	24	56	20	
Total 280	–	19	50	31	
	+	54	24	22	

protein. This condition is similar to a R117H mutation on a 5T background and will lead to a CF phenotype with pancreatic sufficiency. In contrast, individuals with low or almost normal levels of exon 9 skipping (lower range of the 0–50% variation in exon 9^- mRNA) would have higher or almost normal levels of partially functional R117H CFTR, and this condition would lead to a milder phenotype, such as CBAVD, or even to a normal phenotype.

The efficiency of splicing of exon 9 might therefore be involved in the expression of various phenotypes. The combination of a severe mutation on one chromosome and a 5T variant on the other might be involved in the pathogenesis of CBAVD. Inter-individual variations in the low level of normal exon 9^+ mRNA, and consequently normal CFTR protein, could then lead to either a CBAVD phenotype (almost no exon 9^+ mRNA) or a normal phenotype (low level of exon 9^+ mRNA surpassing a certain threshold value). A statistically significant higher frequency of the 5T allele was indeed found in a cohort of 102 CBAVD patients compared with normal controls (Chillon *et al.*, 1995a): 21.1 versus 5.2%. The 5T allele was not found in compound heterozygotes, where the CBAVD phenotype might already be explained by the presence of two CF mutations. However, many patients with one CF mutation detected had a 5T allele on the other chromosome. In a substantial fraction of patients with no CF mutations detected, one or even two (three individuals) 5T alleles were also found. Therefore, the 5T allele in combination with a CF mutation on the other chromosome seems to be one of the major causes of CBAVD. These results were confirmed in four further studies of a total of 178 CBAVD patients (Costes *et al.*, 1995; Jarvi *et al.*, 1995; Zielenski *et al.*, 1995; Dumur *et al.*, 1996). The results of these five studies are summarized in Table II.

As mentioned before, the 5T allele would not be expected to lead to CBAVD in all cases. The 5T allele was also found on the normal chromosomes of parents

of CF patients. In the mothers, the 5T allele would have no phenotypic effect, while in the fathers the presence of this allele could have led to CBAVD. While in the normal chromosomes of the mothers the 5T allele was present at a frequency comparable with that in the general population, its frequency among the fathers was considerably lower. This result could be expected because fathers of CF patients are normally fertile, and it also confirms the role of the 5T allele in CBAVD. By pooling the data of two studies the penetrance of the 5T allele in combination with a CF mutation can be estimated (Chillon *et al.*, 1995a; Zielenski *et al.*, 1995). In a total of 290 normal chromosomes, six CF fathers were found to carry the 5T allele (2.07%), while seven of 150 normal chromosomes of CF mothers (4.67%) carried this allele. The penetrance of the 5T allele is therefore estimated to be 0.56.

Further calculation, based on a CF carrier frequency of 4% (CF gene frequency 2%) and a 5T allele frequency of 5%, allows us to estimate that ~1 in 500 individuals in the Caucasian population will carry a CF mutation in one CFTR allele and a 5T in the other allele. Therefore, 1 in 500 Caucasian men would be at risk of having CBAVD. Taking into account a penetrance of 0.56 of the 5T allele, this risk would reduce to ~1/900, a figure approaching the previously estimated incidence of 1/1000 of CBAVD. Three CBAVD patients homozygous for a 5T genotype have been described so far (Chillon *et al.*, 1995a; Zielenski *et al.*, 1995; Dumur *et al.*, 1996). At present, it is unclear whether this genotype alone is sufficient to cause CBAVD in these patients or that an as yet undetected CFTR mutation is also involved. If homozygosity for 5T alleles can also lead to CBAVD, then ~1/222 (1/500 carriers of a CF mutation and a 5T allele + 1/400 5T homozygotes) or 1/396 (assuming a penetrance of 0.56) Caucasian men are at risk of having CBAVD.

Genetic heterogeneity in CBAVD

In a study of patients with obstructive azoospermia of unknown aetiology, a high incidence of CFTR mutations and the 5T variant was also found (Jarvi *et al.*, 1995). These results suggest that a broad range of male reproductive tract abnormalities may be associated with defects in the CFTR protein. Nevertheless, the inability to find CF mutations or the 5T variant in some patients might indicate that other factors are involved in the aetiology. With regards to CBAVD, it was first suggested by Augarten *et al.* (1994) that CBAVD associated with urinary tract malformations might not represent a mild form of CF but rather a distinct clinical entity. In a group of 47 CBAVD patients, these authors found that 10 (21%) had urinary tract malformations with agenesis of one kidney. In three of them the single kidney was ectopic. None of these 10 patients had abnormal sweat chloride concentrations, and no mutations in the CFTR gene could be detected. This observation of normal sweat test and no CFTR mutations was confirmed in another six CBAVD patients (16% of CBAVD patients studied) with urinary tract abnormalities (Dumur *et al.*, 1995). In two patients with renal agenesis, Casals *et al.* (1995) were also unable to find CFTR mutations. Together,

these data indicate that CBAVD with unilateral renal malformations might not be associated with mutations in the CFTR gene. However, the number of patients studied so far is relatively small and these results need to be confirmed in more patients. Nevertheless, this observation might have considerable implications for the CBAVD patient and his family (see below). On the other hand, at least part of the reason why no CFTR mutations are found in some CBAVD patients might be explained in this observation.

Another approach to studying the possibility of genetic heterogeneity in CBAVD was first used by Mercier *et al.* (1995). In families with one or more CBAVD patients and/or one or more fertile male siblings, it could be expected that all CBAVD patients in one family would inherit the same CFTR genes from their parents, while fertile male siblings should inherit at least one CFTR gene different from their CBAVD brothers. However, if CBAVD in the family under consideration is not linked to CFTR abnormalities, the inheritance of CFTR chromosomes should be random with respect to phenotype. By using polymorphisms intragenic and extragenic to the CFTR gene, three families have been identified so far among whom CBAVD brothers either inherit different CFTR genes from their parents or CBAVD and fertile male siblings inherit the same CFTR genes from their parents (Mercier *et al.*, 1995; Rave-Harel *et al.*, 1995). These results have been taken as evidence for genetic heterogeneity, at least in some cases of CBAVD. However, it must be mentioned that neither report studied the 5T variant allele of intron 8. That the study of the 5T variant might be important follows from the next example.

In one of our families with three brothers (two with CBAVD and one normally fertile) it was found that the two CBAVD brothers had both inherited the paternal ΔF508 mutation, but each had a different CFTR gene from their mother. The fertile brother was not a carrier of the ΔF508 mutation. An analysis of the T variant allele in this family indicated that the mother was homozygous for the 5T allele so that both CBAVD patients inherited a paternal ΔF508 mutation and a maternal 5T allele. The discordance between the CBAVD phenotype and the inheritance of a different maternal CFTR gene could thus be explained in this family by the presence of the 5T variant on both maternal chromosomes (Figure 2).

Genetic counselling in CBAVD

The CBAVD patient and his family

As shown above, the majority of cases with CBAVD are related to CF. The condition of CBAVD could therefore be described as a sex-limited autosomal recessive disease (Oates and Amos, 1994). However, part of the condition might not be associated with mutations in the CFTR gene. This is important for the patient, in terms of reproductive options (see below) and for his family (Meschede *et al.*, 1994; Durieu *et al.*, 1995; Patrizio and Zielenski, 1996). For this reason, each CBAVD male should be examined by a physician with experience in CF,

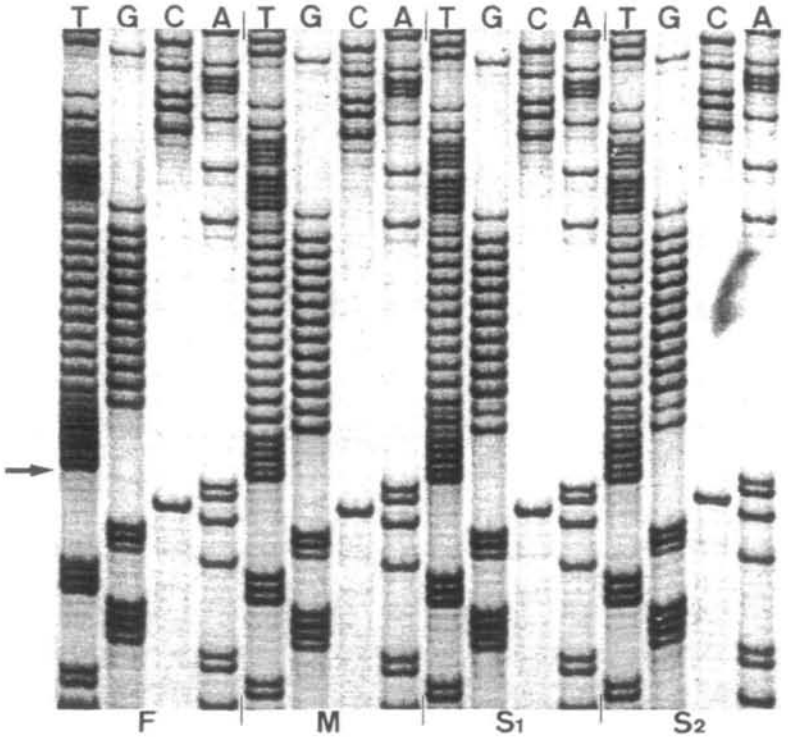

Figure 2. DNA sequence at the intron 8 polypyrimidine variant in a family with two congenital bilateral absence of the vas deferens (CBAVD) brothers inheriting a different cystic fibrosis transmembrane conductance regulator (CFTR) gene from their mother. The DNA sequencing results of the father (F), mother (M) and their two sons with CBAVD (S1 and S2) are shown. The father is a carrier of a ΔF508 mutation (on a 9T background), which is inherited by his two sons. By intragenic CFTR microsatellite analysis, the mother was shown to transmit a different CFTR gene to each of her sons. Homozygosity of the mother for 5T alleles explains the discordance between the transmission of different maternal CFTR genes and the CBAVD phenotype in her two sons. Sequencing was performed after polymerase chain reaction amplification of the region with primers CFI3 and CFI6 and direct sequencing with primer CF38 (Chu *et al.*, 1991). Exon 9 is at the bottom and intron 9 at the top of the figure. The arrow indicates the end of the polypyrimidine tract.

because minor symptoms of CF, such as sinusitis, might be present. A sweat test should be performed because a substantial fraction of patients have elevated or slightly abnormal chloride concentrations. Measurement of the bioelectric properties of nasal epithelium should also be included because abnormalities in chloride conductance across the nasal epithelium of CBAVD patients dissimilar to that found in normal individuals and in CF patients have also been found. An examination for renal malformations should be included although the combination of urinary tract abnormalities with CBAVD is probably not genetically linked to CF. Nevertheless, until the latter relationship has been studied in more patients, a CFTR mutation analysis is indicated in all CBAVD patients. A mutation analysis can be performed in two stages. First, the more frequent mutations present in the general population under consideration can be studied, together with the intron 8 splice site variant. If one or no mutations are found, a further screening of the entire coding region of the gene is indicated. Family studies

with intragenic and extragenic CFTR polymorphisms could also contribute to the discrimination of CFTR-linked and -unlinked forms of CBAVD. However, these studies will only be possible when the CBAVD patient has brothers with or without CBAVD.

If CFTR mutations are found, each sibling of the CBAVD male has a 50% risk of being a carrier. Male and female siblings have a 25% risk of inheriting the same genotype. The CBAVD male should therefore be informed about this condition and the possible consequences (see below). Where appropriate, his siblings should be informed about their possible risk of being carriers of a CFTR mutation and their increased risk of having children with CF if their partner is also a carrier.

The CBAVD couple

Patients with CBAVD have normal spermatogenesis. Since the introduction of MESA and IVF, it has become possible to successfully treat infertility in CBAVD (Silber *et al.*, 1987, 1988, 1990; Patrizio *et al.*, 1988). The success rates of the first attempts using conventional IVF were rather low (Silber *et al.*, 1988, 1990). However, since the development of ICSI (Palermo *et al.*, 1992; Van Steirteghem *et al.*, 1993a,b), success rates with MESA have been very high (Silber *et al.*, 1994, 1995; Tournaye *et al.*, 1994). The overall results have been demonstrated to be consistently better with ICSI than with conventional IVF.

It is now possible for men with CBAVD to father their own children. However, in these men with CBAVD linked to mutations in CFTR, the risk of having children with CF or CBAVD will be increased. As discussed above, examinations to confirm or exclude the genetic relationship between CBAVD and CF are therefore of major importance. In couples with CBAVD not linked to CF the risk of having a CF child will be the same as in the general population with the same ethnic background. So far, no information is available about the risk of having children with CBAVD. However, when CBAVD is linked to CF, the wife should also be tested for CF mutations, depending on her ethnic background. At present it is almost impossible, for practical and financial reasons, to screen the whole CFTR gene for individuals of the general population. The wife's risk of being a carrier after a negative screening for a fraction a of CFTR mutations in her ethnic background will therefore be $q(1 - a)/(1 - aq)$, with q the prior carrier risk (see above). If the CBAVD male is a carrier of one severe CFTR mutation, and 90% of mutations are studied in the female, then the risk to this couple of having a CF child will be 1/964. If no mutation is found in the male, the presence of a severe mutation cannot nevertheless be excluded, and the risk of a CF child remains unchanged. A mild mutation or a 5T allele in the male will most likely result in a CBAVD phenotype in any male offspring, with a risk in this example of 1/964 or 1/1721 (assuming a penetrance of 0.56) respectively.

If the wife is a carrier of a CF mutation, then the combined risk of a CF or CBAVD child approaches 1/2. When the mutations are also known in the male, prenatal diagnosis or better preimplantation diagnosis can be offered to the

Table III. Predicted phenotypes of offspring resulting from combinations of cystic fibrosis transmembrane conductance regulator (CFTR) genes from congenital bilateral absence of the vas deferens (CBAVD) males and their partners after micro-epididymal sperm aspiration/ intracytoplasmic sperm injection

CFTR genes in	Maternal CFTR genes				
CBAVD males	M_{severe}	M_{mild}	N	5T	Z
M_{severe}	CF	CF/CBAVD	N	CBAVD/N	Z (CF)/Z (CBAVD)/N
M_{mild}	CF/CBAVD	CF/CBAVD/N	N	CBAVD/N	Z (CF)/Z (CBAVD)/N
5T	CBAVD/N	CBAVD/N	N	CBAVD/N	Z (CBAVD)/N

M = CFTR mutation; N = normal CFTR gene; Z = residual risk of being a CF carrier defined as $q(1 - a)/(1 - aq)$, with q the CF carrier frequency and a the fraction of CFTR mutations studied in the population under consideration. Phenotypes: N = normal.

couple. However, for some combinations of CFTR genes in these couples it will be very difficult to predict the phenotypic consequences for their children. For instance, if the male is a carrier of a rare CFTR mutation that has, until now, been detected only in CBAVD, and the female is a carrier of a severe mutation, it is impossible to predict whether the combination of these two genes will lead only to CBAVD in males, to a normal phenotype or possibly to CF in both males and females. In Table III we have indicated the most plausible phenotypes supposed to result from well-defined combinations of CFTR genes in CBAVD males and their partners. By combining two maternal and two paternal CFTR genes, depending on the mutation screening results in the couple, the highest risk for disease for the offspring of that couple can be calculated from the table. This table shows that in a couple with the male carrying a severe CFTR mutation (M_{severe}) and a 5T allele in the other CFTR gene (male genotype M_{severe} + 5T) and with the female a carrier of a severe CFTR mutation (female genotype M + N), 25% of their children will have CF (M_{severe} + M), 50% will be normal (M_{severe} + N and 5T + N) and 25% will also be normal in the case of females or normal or possibly have CBAVD (penetrance 0.56) in the case of males (M + 5T). The same table also shows that in a male with the same genotype as above (M_{severe} + 5T) and in a female with a risk Z of being a carrier after negative screening for CFTR mutations (genotype Z + N), this couple will have 50% of offspring with a normal phenotype (M_{severe} + N and 5T + N) due to at least one normal CFTR gene in the female. The highest risk of CF in the offspring of this couple comes from the combination of the severe mutation in the male and the residual risk of being a carrier in the female (25% of cases): Z/4. If 90% of mutations are studied in the female, the residual risk to the couple will be 1/964. Likewise, the risk of CBAVD in male offspring (genotype 5T + Z) will be Z/4 or 1/1721, taking into account a penetrance of 0.56 of the 5T allele. In couples with one or no mutations identified in the male, risk calculations are difficult to perform because the presence of a severe or mild and a severe

CFTR mutation cannot be excluded. In this case it is probably best to assume the presence of a severe and a mild mutation in the male and to calculate the risk likewise. If in these cases the female is a carrier, these couples might opt for the selection, after preimplantation diagnosis, of embryos not carrying the maternal CFTR mutation. This will reduce the risk of a CF child to almost zero. The risk of a CBAVD male child will also be negligible when the mother has a non-5T splice site variant on her normal CFTR allele.

Because these couples have to undergo assisted reproduction treatment with ICSI using epididymal or testicular spermatozoa, preimplantation diagnosis is preferable to prenatal diagnosis by chorionic villus sampling or amniotic fluid puncture for the genetic analysis of their embryos/fetuses. We have described a first preimplantation diagnosis in a CBAVD couple with the two partners being carriers of a ΔF508 mutation (Liu *et al.*, 1994). At the time of diagnosis, no second CFTR mutation had been found in the male. Of the five embryos obtained after MESA/ICSI, three were carriers of a ΔF508 mutation and two were affected (homozygous ΔF508). Because it could not be determined whether the ΔF508 mutation came from the father or the mother, they were told that the combination of the maternal ΔF508 allele with the unknown mutation of the father could give a risk of male children with CBAVD, but was less likely to produce children with CF because of the CBAVD phenotype of the father. The patients wished to take the risk and after replacement of the three carrier embryos a pregnancy ensued and a healthy boy without clinical signs of CF was born. A sweat test performed at 3.5 months was normal (sodium 18 mmol/l; chloride 30 mmol/l) and the presence of vasa deferentia was confirmed by physical examination and scrotal ultrasound. The father was subsequently found to carry a 5T allele on his non-ΔF508 allele and his son also had the same genotype. In the son, the 5T allele in combination with the ΔF508 mutation did not give rise to CBAVD. This couple returned for a second MESA/ICSI procedure but no pregnancy ensued after the replacement of three embryos not carrying the ΔF508 mutation. Another couple, the male carrying a 2183AA → G CFTR mutation and a 5T allele, the female the 1717-1G → A CFTR splice site mutation in front of exon 11, also underwent two cycles of MESA/ICSI and preimplantation diagnosis for the presence of the maternal mutation. In the first cycle only two embryos were available for preimplantation diagnosis and both were found to carry the maternal mutation. Consequently, no embryos could be replaced. In the second cycle, only two of seven oocytes became fertilized, one of which showed three pronuclei and the other fragmented after 1 day.

At present, a careful clinical examination of children born to couples with CBAVD in the male seems to be mandatory, especially in relation to CF and CBAVD. The major reasons for this have been mentioned above and are derived from the variable phenotypic expression of combinations of CFTR genes and the fact that CFTR mutations may have remained undetected in these couples. In CBAVD with an aetiology unrelated to CF, a clinical examination may teach us about the possible recurrence risk of CBAVD in the offspring of these couples.

Unilateral absence of the vas deferens

Males with CUAVD may be normally fertile. A substantial fraction of such patients may therefore remain undetected. Routine physical examination with palpation of the vas deferens, infertility or varied urological complaints may lead to the detection of CUAVD (Donohue and Fauver, 1989). In a study of 21 infertile men with CUAVD, it was observed that these patients could be subdivided into two groups depending on the status of the contralateral vas deferens and the CF mutation detection results (Mickle *et al.*, 1995). In 12 patients with anatomically complete and patent vasa deferentia on the side of the palpable vas no CFTR mutations could be found. In this group of patients, there was also a high incidence of a renal anomaly at the ipsilateral side of the absent vas (five of 12 patients). In contrast, the other nine patients had (non-iatrogenic) occlusion of the contralateral vas at either the inguinal or pelvic level, and in eight of them one CF mutation was found. No renal abnormalities were observed in these patients. The 5T intron 8 variant was not studied.

In another report, 10 patients with CUAVD were studied for CFTR mutations (Casals *et al.*, 1995). One patient was a carrier of a ΔF508 mutation, but no other mutations were detected in the remaining patients, four of whom had renal agenesis.

Chillon *et al.* (1995a) found an increased frequency of the 5T allele in 12 CUAVD patients compared with the general population. The frequency of this allele was lower than in CBAVD patients, although with no statistically significant difference. The renal status of the CUAVD patients was not described.

The aetiology of CUAVD is heterogeneous. In some of the patients the condition is associated with mutations in the CFTR gene, while in others CUAVD is probably caused by other factors. Renal agenesis and the condition of the palpable vas, together with a CFTR mutation analysis, including the intron 8 5T variant, are important factors by which to discriminate between these forms. As for genetic counselling, the same procedure holds as for men with CBAVD. It is as yet unknown whether couples with CUAVD in the males but unlinked to CF have an increased risk of having children with CUAVD or other reproductive tract abnormalities.

Young's syndrome

Young's syndrome presents as a combination of obstructive azoospermia and chronic sinopulmonary disease. The aetiology of the syndrome is unknown. Some authors believe that the respiratory disease and the vasal and epididymal obstruction are caused by inspissated secretions (Handelsman *et al.*, 1984), while others have suggested that ultrastructural abnormalities of the axoneme represent the primary defect (Wilton *et al.*, 1991). The similarity between the clinical features of Young's syndrome and CF, and the known association between CF and CBAVD, led Hirsh *et al.* (1993) to study the CFTR gene for mutations in

Young's syndrome patients. These authors detected, by a limited screening for four mutations, that two out of seven patients were carriers of the ΔF508 mutation. Although the number of patients was limited, this increased frequency of CFTR mutations (29%) supported the idea of a common aetiology of the syndrome and CF. However, Le Lannou *et al.* (1995) were not able to confirm these results in a more extensive mutation analysis of the CFTR gene in another 12 patients with Young's syndrome. They were unable to find any mutation in the CFTR gene of these patients, while in CBAVD patients a high frequency of mutations was found. Sweat tests, performed in 10 of the 12 patients, were all negative.

From this limited series of patients, it thus appears that Young's syndrome is not linked genetically to CF. However, it seems advisable that a clinical evaluation of these patients in relation to CF should be carried out, and that a mutation analysis of the CFTR gene be performed. Whether these patients have an increased risk of having sons with Young's syndrome is not known.

Conclusion

Absence, dysfunction or low levels of CFTR protein result in a broad range of clinical manifestations (reviewed in Pignatti, 1994; Welsh *et al.*, 1995). At the severe end of this phenotypic spectrum is CF with pancreatic insufficiency. At the other end are relatively mild conditions such as disseminated bronchiectasis (Pignatti *et al.*, 1995) and some forms of infertility in males (CBAVD, CUAVD and azoospermia of unknown aetiology). In the majority of cases, the condition of CBAVD results from compound heterozygosity for two CFTR mutations or a CFTR mutation in one gene and an intron 8 5T variant in the other gene. In some patients, only one CFTR mutation has been detected so far, but in these patients the presence of a second mutation can be expected. In all of these patients and in their families, proper management and counselling in relation to CF and CBAVD are mandatory. The development of MESA and ICSI has greatly improved the chances for CBAVD males to father their own children. However, the couples in which the male has CBAVD related to CF will also have an increased risk of having children with CF or CBAVD, particularly when the female is also a carrier of a CFTR mutation. They should be informed about this risk and the possibilities of disease prevention through prenatal diagnosis or, preferentially, preimplantation diagnosis. At present, one of the major problems may be the prediction of phenotypes resulting from combinations of parental CFTR genotypes.

Male patients with CF have normal ureters and kidneys (Landing *et al.*, 1969). However, in some of the patients with CBAVD the condition is associated with urinary tract malformations. These patients have normal CF sweat tests and no mutations are found in their CFTR genes. CBAVD associated with urinary tract malformations does not therefore seem to be related to CF. In males, both the ureter and ducts of the epididymis and the vas deferens derive from the Wolffian

or mesonephric duct. At around week 7 of gestation, the Wolffian duct splits into its reproductive and ureteral parts. As the ureters are normal in CF, the Wolffian duct must be present undamaged in early embryonic life. After splitting of the duct, defects in the genital ducts probably occur as a consequence of abnormal secretory processes resulting from defects in function of the CFTR protein (Trezise *et al.*, 1993; Tizzanno *et al.*, 1994). Examination of CBAVD patients for urinary tract or renal malformations is therefore important because it will help to define, together with a clinical evaluation of the patients with regard to CF and a CFTR mutation analysis, the risk to the patients of offspring with CF or CBAVD.

The aetiology of CUAVD is also associated with CF in some of the patients. The condition of CUAVD with renal abnormalities (at the ipsilateral side of the absent vas) is, as in CBAVD, not related to CF. Young's syndrome does not seem to be related to CF either. On the other hand, in patients with obstructive azoospermia of unknown aetiology, an increased frequency of CFTR mutations has been found.

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Discussion

Page: I was not quite clear about some of the details. What is the phenotype of a 5T-5T homozygote? Second, I understood that you make a distinction between CBAVD patients with two CFTR mutations and patients with a 5T variant and a mutation. Do you think a 5T should be labeled as a polymorphism, and not as a mutation? Clearly, it appears that the 5T has phenotypic consequences and so it is sort of blurring the distinctions not to call it a mutation in this context.
Lissens: Four individuals, homozygous for the 5T variant, have been described by Chu et al. (1992). These individuals had no clinical manifestations of CF and the fertility status of the male was not described. Three CBAVD patients, homozygous for 5T, have been described so far. At the moment it is not clear whether this homozygosity is the cause of the CBAVD phenotype or that as yet undetected mutations in the CFTR genes of these individuals are present. All evidence obtained so far indicates that the 5T variant is indeed involved and that the resulting phenotypes depend on the variability of levels of normal CFTR mRNA resulting from the presence of the 5T variant. Therefore, the phenotypes probably include CBAVD, mild to moderate CF and normal phenotypes without fertility problems.
The 5T variant indeed has phenotypic consequences and in that sense it should be called a mutation rather than a polymorphism. However, the penetrance of this mutation is incomplete. Based on figures in the literature, we have calculated that the penetrance of the 5T variant is only about 0.5. This figure was obtained by comparing the frequency of the 5T variant in normal alleles of parents of CF patients. In the fathers, we could expect to find the 5T variant at a lower frequency than in the mothers, if one assumes that a 5T in combination with a CF mutation on the other allele causes CBAVD, or in other words, infertility in the male. This was indeed found since the 5T allele occurs with a frequency of about 5% in the mothers and about 2.5% in the fathers.
Tarlatzis: Why is this phenotypic limitation found in the males and there is no phenotypic expression in the females?
Lissens: I do not know, but this has probably to do with the absence of comparable genital structures in the female. This difference of phenotypic expression is also seen in CF. The majority of male CF patients have obstructive azoospermia. Fertility in CF females is also reduced but to a much lesser extent.
Tarlatzis: Theoretically, some abnormalities would arise in females, eg. in the Fallopian tubes.
Silber: The comparison with the female would not be the Fallopian tubes. There is a pan-Wolfian duct defect. It involves not only an absence of the vas, it is an absence of the distal portion of the epididymis in most of the cases and also in the vast majority of cases an absence of the seminal vesicles. So it is a complete disruption of the reproductive portion of the Wolfian duct in the male. In the female, no one ever studied it, but it would be fascinating to use laparoscopy on women who had similar genetic findings to study perhaps the round ligament and observe what their Wolfian duct formation is like.

I saw a case 15 years ago of identical twin men and they were lost to follow up, but one of the men clearly had bilateral absence of the vas deferens and was sterile. The other had unilateral absence of the vas and was not sterile and had children. In fact, we used his spermatozoa to inseminate the wife of the identical twin brother who had no children, and so he had children also. Do you have an explanation for identical twins with the one having bilateral congenital absence of the vas deferens and the other with unilateral congenital absence of the vas deferens?

Lissens: It would be of interest to study these patients. There are not so many studies on CUAVD, but as I explained, the aetiology of the condition seems to be heterogenous with part of it associated with CF mutations, including the 5T variant. We have also seen that the phenotypic expression in compound heterozygotes carrying a CF mutation and a 5T allele is variable, possibly or probably due to subtle differences in the expression of the 5T allele. I could imagine that CUAVD without infertility is one of the intermediary phenotypes. Of course, these patients will normally not be detected, unless for other medical reasons than infertility. So, variable expression could explain the variable phenotype in your identical twin patients.

Merdad: My question is about counselling. When we have the wife who is a CF carrier, and the husband is a carrier, they are healthy. But what is the chance that the child will be affected? Is it one in four? One in two? How can we counsel those parents?

Lissens: When the female is a carrier, she has one chance in two to pass on the mutation to her offspring. The combined risk of a CF or CBAVD child for this couple then approaches at maximum 1/2. However, for some combinations of CFTR genes in these couples, the phenotypic consequences for their children will be very difficult to predict. For further details I refer you to Table 3 in the text (Lissens *et al.*).