Journal of Cystic Fibrosis Volume 10 Suppl 2 (2011) S86–S102

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Recommendations for the classification of diseases as CFTR-related disorders

C. Bombieri [a], M. Claustres [b], K. De Boeck [c], N. Derichs [d] J. Dodge [e], E. Girodon [f], I. Sermet [g], M. Schwarz [h], M. Tzetis [i], M. Wilschanski [j], C. Bareil [b], D. Bilton [k], C. Castellani [l], H. Cuppens [m], G.R. Cutting [n], P. Dřevínek [o], P. Farrell [p], J.S. Elborn [q], K. Jarvi [r], B. Kerem [s], E. Kerem [t], M. Knowles [u], M. Macek Jr [v], A. Munck [w], D. Radojkovic [x], M. Seia [y], D.N. Sheppard [z], K.W. Southern [aa], M. Stuhrmann [ab], E. Tullis [ac], J. Zielenski [ad], P.F. Pignatti [a], C. Ferec [ae,*]

[a] *Department of Life and Reproduction Sciences, Section of Biology and Genetics, University of Verona, Verona 37134, Italy*
[b] *Laboratoire de Génétique Moléculaire, CHU Montpellier and INSERM U827, IURC Montpellier, France*
[c] *Department of Pediatrics – Pediatric Pulmonology, University Hospital, Leuven, Belgium*
[d] *CF Centre, Medizinische Hochschule Hannover, Germany*
[e] *Department of Child Health, University of Wales Swansea, Singleton Hospital, Swansea SA2 8QA, UK*
[f] *APHP, Groupe Hospitalier Henri Mondor, Service de Biochimie-Génétique and INSERM U955 Equipe 11, Créteil, France*
[g] *Pole de Pédiatrie Multidisciplinaire, Hôpital Necker, Université René Descartes, 75015 Paris, France*
[h] *Regional Molecular Genetics Service – Genetic Medicine, St. Mary's Hospital, Manchester, UK*
[i] *Department of Medical Genetics, Athens University, St Sophia's Children's Hospital, Athens, Greece*
[j] *Pediatric Gastroenterology Unit and Cystic Fibrosis Center, Hadassah University Hospital, Jerusalem, Israel*
[k] *Royal Brompton Hospital, Sydney Street, London, UK*
[l] *Verona Cystic Fibrosis Centre, Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italy*
[m] *Department of Human Genetics, Katholieke Universiteit Leuven, Gasthuisberg O&N1 (602), B-3000, Leuven, Belgium*
[n] *McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA*
[o] *Department of Pediatrics, Charles University, Second Faculty of Medicine and University Hospital Motol, Prague, CZ 150 06, Czech Republic*
[p] *Department of Pediatrics and Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA*
[q] *School of Medicine, Dentistry and Biomedical Sciences, Queen's University of Belfast, Belfast, BT7 1NN, Northern Ireland, UK*
[r] *Divisions of Urology, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada*
[s] *Department of Genetics, The Life Sciences Institute, Givat Ram Campus, The Hebrew University, Jerusalem 91904, Israel*
[t] *Department of Pediatrics and Pediatric Pulmonology, Hadassah-Hebrew University Medical Center, Mount Scopus, Jerusalem, Israel*
[u] *Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA*
[v] *Department of Biology and Medical Genetics, Charles University, Second Faculty of Medicine and University Hospital Motol, Prague, CZ 150 06, Czech Republic*
[w] *CF Center, Department of Pediatric Gastroenterology and Nutrition, University Hospital Robert Debré, AP-HP 48, 75019 Paris, France*
[x] *Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia*
[y] *Laboratorio di Genetica Medica, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milano, Italy*
[z] *School of Physiology and Pharmacology, Medical Sciences Building, University of Bristol, Bristol BS8 1TD, UK*
[aa] *Institute of Child Health, University of Liverpool, Alder Hey Children's Hospital, Liverpool, L12 2AP, UK*
[ab] *Institute of Human Genetics, Medical School Hannover, Hannover, Germany*
[ac] *Adult Cystic Fibrosis Centre, St Michael's Hospital, and Division of Respirology, Department of Medicine, University of Toronto, Ontario, Canada*
[ad] *Genetics and Genomics Biology Program, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada*
[ae] *Génétique Moléculaire et Génétique Epidémiologique INSERM U613 Laboratoire de Génétique Moléculaire et d'Histocompatibilité CHU de Brest, F-29609, Brest, France*

* Corresponding author: Claude Ferec, MD, PhD, Professor of Molecular Genetics, Laboratory of Molecular Genetics, 46 rue Felix Le Dantec, 29200 Brest, France. Tel.: +33 398444138; fax: +33 298467910.
E-mail address: claude.ferec@univ-brest.fr (C. Ferec).

Abstract

Several diseases have been clinically or genetically related to cystic fibrosis (CF), but a consensus definition is lacking. Here, we present a proposal for consensus guidelines on cystic fibrosis transmembrane conductance regulator (CFTR)-related disorders (CFTR-RDs), reached after expert discussion and two dedicated workshops.

A CFTR-RD may be defined as "a clinical entity associated with CFTR dysfunction that does not fulfil diagnostic criteria for CF".

The utility of sweat testing, mutation analysis, nasal potential difference, and/or intestinal current measurement for the differential diagnosis of CF and CFTR-RD is discussed. Algorithms which use genetic and functional diagnostic tests to distinguish CF and CFTR-RDs are presented.

According to present knowledge, congenital bilateral absence of vas deferens (CBAVD), acute recurrent or chronic pancreatitis and disseminated bronchiectasis, all with CFTR dysfunction, are CFTR-RDs.

Keywords: CFTR-related disorders; CBAVD (Congenital Bilateral Absence of Vas Deferens); Pancreatitis; Bronchiectasis; Functional tests; NPD (Nasal Potential Difference); ICM (Intestinal Current Measurement)

Although the gene responsible for cystic fibrosis (CF) was identified more than twenty years ago, the relationship between genotype and phenotype in CF is still challenging and a matter of debate. CF is characterized by wide variability of clinical expression with regard to disease severity and rate of progression. This is caused, at least in part, by (i) the large number of different mutations affecting the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene, (ii) the existence of modifier genes and (iii) environmental factors, such as viral or bacterial pathogens, that influence disease phenotype [1,2].

Soon after the *CFTR* gene was discovered, it became clear that dysfunction of CFTR in a single organ was associated with clinical phenotypes distinct from CF. For example, *CFTR* mutations have been identified in infertile males with no evidence of CF lung disease [3–5]. To seek clarification of this issue, workshops were organized by a EuroCareCF Working Group in Garda, Italy (22–23 March 2007) and Prague, Czech Republic (15–16 June 2008). Thirty five experts from Europe and North America participated in these meetings. Here, we present the consensus recommendations, resulting from discussions at these meetings.

1. What is CF?

Even in 2010, CF remains in essence a clinical diagnosis [1,2,6]. It may also be defined either in molecular genetic terms as a disease caused by the presence of two CF-causing mutations one in each parental *CFTR* gene or in physiological terms as a disorder of electrolyte transport across epithelial membranes resulting from absence or abnormality of the CFTR protein. However, the wide variation in range and severity of symptoms and organs involved between and within individuals makes it a clinical decision as to whether or not a person should be managed as a CF patient. This is especially the case in a small number of difficult or ambiguous cases. Furthermore, in many countries proper classification is important for health insurance reimbursement purposes and for provision of social services to patients with CF.

For the majority of affected individuals, there is little or no difficulty in diagnosing their condition as CF. The classical clinical syndrome is well known and easily recognised when an individual's signs and symptoms are being diagnosed. Moreover, even before the molecular basis of the disease was understood patients were readily identified by their clinical presentation and a confirmatory sweat test documenting a sweat chloride concentration above 60 mmol/L. Eighty five percent of CF patients require pancreatic enzyme supplementation to digest food. These CF patients are termed "pancreatic insufficient" (CF-PI). Fifteen percent of CF patients do not require pancreatic enzyme supplementation and hence, are termed "pancreatic sufficient" (CF-PS). The distribution of causative mutations differs substantially across Europe with a marked decrease of the commonest CF mutation, p.F508del from NW to SE Europe. Nevertheless, the diagnosis of most CF patients with Northern European descent as CF-PI is generally straightforward [7,8]. Similarly, the diagnosis of many patients of Southern European descent, who tend to be CF-PS, is relatively clear-cut [9,10].

Most atypical CF patients can be confidently diagnosed with the help of reliable sweat tests and/or genetic analysis. These individuals usually present later in their lives with pancreatic sufficiency and milder respiratory disease. The remaining cases, termed "atypical", "possible" or "borderline", are difficult to diagnose and manage because there is poor agreement between sweat test results and respective clinical signs and symptoms. Currently, over 1700 mutations and polymorphisms have been identified in the *CFTR* gene (see The Cystic Fibrosis Mutation Database; http://www.genet.sickkids.on.ca/cftr/). Because different mutations or variations in the *CFTR* gene are associated with a wide spectrum of clinical phenotypes or even associated with no disease at all, the demonstration of mutated *CFTR* genes in an individual does not predict with certainty, only at best with probability, this person's prognosis [9,10]. No doubt interactions with other genes and environmental factors substantially modify the clinical picture in each individual [8]. Similarly, demonstration of an abnormal sweat test or abnormal potential difference across epithelial membranes might be helpful in assigning an individual to a CF or non-CF category. However,

these functional tests will not predict an individual's clinical syndrome or the range of disease severity.

1.1. Diagnostic criteria

In 1998, a first consensus statement [11] listing criteria for the diagnosis of CF was issued by the US Cystic Fibrosis Foundation (Bethesda, USA). The listed criteria were: (i) one or more of the phenotypic features of the disease or (ii) CF in a sibling or (iii) a positive immunoreactive trypsin (IRT, a neonatal screening test), in association with at least one other feature. The additional features included a positive sweat test result on two occasions, a CF-causing mutation in each *CFTR* gene or an abnormal nasal potential difference (NPD) [12,13]. The consensus statement of the US Cystic Fibrosis Foundation was later modified slightly by a European working group [6].

Clinical manifestations suggestive of CF have been described in detail by De Boeck et al. [6]. A sweat chloride concentration above 60 mmol/L and/or the presence of 2 clinically relevant CF-causing mutations is uniformly accepted as diagnostic for the classical form of the disease [2,6,11,12]. However, this strict definition has obvious flaws. Patients with particular genotypes combining two CF-causing mutations may have a sweat chloride value in the intermediate range (30–60 mmol/L). Conversely, it is too limiting to consider a list of only 23 CF-causing mutations as defined by an American College of Medical Genetics panel when more than 1700 *CFTR* mutations have been identified in CF patients, many of which are included in registries of CF patients. Moreover, this list is too loose, because it includes the p.R117H mutation that is most often associated with no disease when identified by newborn screening [14]. The difficulty occurs when patients present with clinical symptoms suggestive of CF and a sweat chloride value in the intermediate range. Among these subjects, the subset with abnormalities in NPD measurement or 2 identified *CFTR* mutations has, on average, more severe lung disease [15]. However, their disease symptoms are milder than those in subjects with a sweat chloride concentration above 60 mmol/L. Therefore, it is appropriate from a physician's and also from a patient's perspective to categorise these individuals differently from subjects with the classical life-shortening form of CF. The advent of neonatal screening has many advantages for patient outcome, but it underscores the discussion about how to interpret test results and what may be considered "diagnostic" for CF. Indeed, with the wide adoption of newborn screening extreme caution should be taken not to erroneously label a subject as CF [7]. Neonatal screening only identifies subjects at risk of being CF in whom the diagnosis needs to be further substantiated by a positive sweat test or by other physiological tests of CFTR function (e.g. NPD or intestinal current measurement (ICM)). The example of the p.R117H mutation demonstrates clearly that it is inappropriate to rely solely on IRT results and mutation analysis [14]. On the other hand, when subjects have a clear track record of CF-like lung disease plus several "markers" consistent with a CF diagnosis (e.g. an intermediate sweat chloride concentration and an abnormal NPD or 2 identified *CFTR* mutations), it would be overly cautious not to consider this person a CF patient, even in an atypical form [15]. Thus, we stress the importance of the clinical picture in addition to test results to interpret fully a patient's condition. In infants identified by newborn screening, it should be emphasized that clinical history is very limited. All parties concerned should accept that occasionally a clinical assignment might need to be revised because an individual's clinical presentation has changed.

There is a need to qualify patients who do not meet the diagnostic criteria of CF, but for whom there is evidence of CFTR dysfunction. However, even among these individuals, there is a broad range of clinical phenotypes and disease severity. Clinicians have for decades acknowledged the wide difference in disease severity and studied the factors most likely responsible for this variation: class of mutation, gene modifiers, age at diagnosis, quality and intensity of treatment and adverse environmental stress [16]. Although the concept of "non-classical" or "atypical" CF [6] might apply clinically especially to children with multi-system disease and borderline sweat chloride values or the presence of at least one *CFTR* mutation of uncertain clinical relevance, the term "CFTR-related disorders" (CFTR-RDs) has gained wide acceptance to designate these varied conditions, which also include monosymptomatic disorders in adults [13,17].

Undoubtedly, there are positive and negative aspects of performing investigations to make a definitive diagnosis and the outcome is not always necessarily to the patient's benefit. The implications of labelling patients with mild manifestations with a CF diagnosis include an implied clinical course and prognosis, which might well be unduly adverse for, say, an adult with agenesis of the vas deferens and the presence of nasal polyps or another with recurrent pancreatitis, one mutation and a sibling with CF. The negative implications for such individuals and their relatives include psychological, reproductive, social, employment, and insurance issues, regardless of the fact that they will not bear the whole burden of treatment modalities required for "classical" CF patients. To accommodate the needs of these patients whose mild symptoms or single-organ disease do not, at least in the short term, justify including them together with the majority, it might be preferable to use a wider diagnostic vocabulary and the term CFTR-RD is a reasonable choice. In addition, when studying improvement in survival or disease severity outcome over time, it is misleading to enrich the study population with patients with milder disease phenotypes identified using more sophisticated diagnostic tests (e.g. NPD and/or ICM). In this respect, comparisons of birth cohorts of p.F508del homozygous subjects or CF-PI subjects are scientifically much more correct. (Guidelines for performing and interpreting NPD and/or ICM are discussed in the accompanying EuroCareCF guidelines on diagnostic tests [18]).

2. CFTR-related disorders

A CFTR-related Disorder (CFTR-RD) is defined as: *a clinical entity associated with CFTR dysfunction that does not*

fulfil the diagnostic criteria for CF. Three main clinical entities illustrate these phenotypes: CBAVD (congenital bilateral absence of the vas deferens) with CFTR dysfunction, acute recurrent or chronic pancreatitis with CFTR dysfunction and disseminated bronchiectasis with CFTR dysfunction. Careful attention should be paid to exclude other known aetiologies, to the degree of screening for *CFTR* mutations and to the evaluation of CFTR function in these patients.

2.1. CBAVD

Congenital bilateral absence of the vas deferens (CBAVD) in otherwise healthy males (also named "isolated CBAVD") accounts for approximately 3% of cases of infertility. The incidence of CBAVD, based on estimations, is approximately 1:1000 males [19–21]. While the prevalence of CF is very low in non-Caucasian countries, the prevalence of CBAVD does not seem to differ between populations, as reported for example in Japanese and Taiwanese males [22,23].

In the majority of cases, isolated CBAVD is recognized as an autosomal recessive genetic disorder (MIM#277180) associated with anomalies of the *CFTR* gene: alterations that retain enough residual CFTR function might result in milder phenotypes such as CBAVD. Initially, it was found that a significant proportion of males with isolated CBAVD had inherited one *CFTR* mutation [3–5,24]. A crucial step in the assessment of a common aetiology for CF and CBAVD was the report from Chillon et al. [25] that the so-called "normal allele" of many heterozygotes carried the same mild modification in a non-coding DNA sequence, the splice variant "IVS8-5T allele" [25,26]. Extensive investigations of the *CFTR* gene using powerful technologies have demonstrated that CBAVD is caused by mutations in the two copies of the *CFTR* gene in 70–90% of cases depending on ethnic/geographic populations (reviewed in [27–29]) and, as such, it was proposed initially to consider CBAVD as a primary "genital form of CF". However, CBAVD and CF, although constituting different ends of a spectrum, have completely separate clinical and prognostic characteristics [30]. Consequently, isolated CBAVD should be considered a "CFTR-RD", which seems a more appropriate term than the usual terminologies ("mild", "atypical" or "non-classic" CF).

Diagnosis of CBAVD is based on impalpable vas deferens on scrotal examination undertaken by an andrologist. The testes are of normal or subnormal size. In a proportion of men scrotal palpable vas deferens are present, however surgical exploration reveals a fibrous cord or a non-permeable duct (non continuous lumen as indicated by deferentography). Semen analysis reveals azoospermia (no sperm seen in the ejaculate) with low seminal volume (<1.0 ml), low pH (average <6.8) and low or absent fructose [19,31]. Although it has been reported that these abnormal findings are good predictors of the presence of *CFTR* mutations [32,33], there is no clear correlation with the biochemical variables studied. Transrectal ultrasonography is used to examine the morphology and size of the seminal vesicles, prostate and ejaculatory ducts. Typically, the body and tail of the epididymis are atrophic, absent or the epididymal remnants are distended, whereas the caput of the epididymis is present [34]. Abdominal ultrasonography is performed to evaluate the upper urinary tract.

Extensive genotype-phenotype studies have identified two categories of *CFTR* mutations, "severe" (virtually no functional CFTR protein or not enough to prevent pancreatic dysfunction, i.e. mutations belonging to *classes 1–3*) and "mild" (more likely to have enough residual CFTR activity to sustain pancreatic function, i.e. mutations belonging to *classes 4 or 5*) [35,36]. CBAVD is caused by compound heterozygosity for either one severe and one mild mutation or two mild mutations (reviewed in [28,29]). Thereby, the distribution of *CFTR* mutations and genotypes in CBAVD differs substantially from classical CF: among males with two identified *CFTR* mutations, CF patients have either two severe (88%) or one severe and one mild/variable *CFTR* mutations (12%), whereas CBAVD males have either a severe and a mild/variable (88%) or two mild/variable (12%) *CFTR* mutations [37,38]. No CBAVD patient without CF carries two severe *CFTR* mutations. The two most common compound heterozygous genotypes found in European males with CBAVD are p.F508del *in trans* with IVS8-5T (28%) and p.F508del *in trans* with p.R117H (6%).

The frequency of p.F508del, the most common CF-causing mutation, in CBAVD varies from 21–33% in USA [20], Canada [39], and Northern Europe [37,38,40,41] to 12–18% in Southern Europe [41–43] and India [44]. In contrast to p.F508del, whose frequency in CBAVD is lower in non-European populations, the IVS8-5T allele is found at either the same (Indians, 25% [44]; Japanese, 30% [22]) or higher frequencies (Egyptians, 44% [45]; Taiwanese, 44% [23]), while its frequency in the general population is often similar (5%). Thus, the IVS8-5T variant is involved in many cases of CBAVD even in populations where CF is rare, with homozygosity for this allele being very common; many patients from Asia remain negative for other *CFTR* mutations.

The IVS8-5T allele, which is present on at least 5% of *CFTR* genes, is the most common "mild" *CFTR* allele worldwide [46]. Its frequency in CBAVD males (25–40%) is 5–8 times higher than in the general population [25,26,46–48]. Approximately 34% of men with CBAVD of European descent have inherited a *CFTR* mutation in one gene and the IVS8-5T allele on the other, a combination that does not result in CF, but does reduce levels of functional CFTR protein in the vas deferens to produce isolated CBAVD. A complex network of DNA sequences within the *CFTR* gene and cellular splicing factors modulate alternative splicing of *CFTR* exon 9 [49–54]. The efficiency of exon 9 splicing is lower in Wolffian tissues, which constitutively produce less full-length *CFTR* mRNAs than other tissues. The presence of a splicing variant, such as the IVS8-5T allele, reduces further the level of full-length transcripts below the threshold transcript level necessary to maintain a normal phenotype in Wolffian tissues, while exceeding that required in other organs [55–57]. Thus, the vas deferens appears to be the tissue most sensitive to reduction of functional CFTR protein.

The IVS8-5T allele is also found on 2–3% of non-CF alleles of fathers from CF patients [25,46] and in CF-PS patients. Hence, inheritance of a IVS8-5T allele *in trans* with a severe *CFTR* mutation might not be sufficient to produce CBAVD in every case. By comparing the frequency of the IVS8-5T allele in fathers (2.07) and mothers (4.67) of CF patients, the degree of penetrance of the IVS8-5T variant as a CBAVD-causing allele (in combination with a CF mutation) has been estimated to be 56% (1–2.07/4.67) [25] similar to that calculated for other populations [46]. The IVS8-5T allele is also a genetic modifier of the mild mutation p.R117H when they are associated *in cis*. Thus, the combination p.R117H-7T is often found in CBAVD, p.R117H-5T is frequently found in CF-PS patients, whereas p.R117H-9T is generally not associated with disease [58].

The IVS8-5T allele is considered a CBAVD mutation with incomplete penetrance. The polymorphic dinucleotide repeat, IVS8-TG, lying immediately upstream of the IVS8-Tn tract also influences the efficiency of exon 9 splicing [59]. Thus, longer IVS8-TGm and shorter IVS8-Tn repeats increase exon 9 skipping and lead to the production of misfolded and nonfunctional CFTR protein [60,61]. IVS8-5T alleles derived from CBAVD males were found to carry a high number of IVS8-TG repeats (e.g. 12 or 13), whereas those derived from healthy fathers with similar genotype harboured a low number of IVS8-TG repeats (e.g. 10 or 11) [59]. Longer IVS8-TG repeats (IVS8-TG12 or TG13) *in cis* with IVS8-5T were also found to correlate with disease status (CBAVD or CFTR-RD). Therefore, the IVS8-TG repeat number is a reliable predictor for the penetrance of IVS8-5T as a disease-causing allele because the odds of pathogenicity are 28 and 34 times greater for the haplotypes TG12-T5 and TG13-T5, respectively, than for the TG11-T5 allele [62]. The TGmTn allele represents a model of CBAVD "polyvariant mutant CFTR".

Occasionally, rare variants of IVS8-Tn alleles have been identified in CBAVD males, including for example cases of IVS8-T3-TG12 *in trans* with F508C [54], IVS8-T2-TG13 *in trans* with R117H-TG11T9 [63] and IVS8-T6 [64,65]. Analysis of splicing patterns derived from minigenes expressing T2 or T3 repeats in epithelial cells demonstrates that these alleles dramatically increase exon 9 skipping and should be considered as "CF" (severe) and probably fully penetrant mutations, in contrast to the IVS8-5T allele [52,54,63].

CFTR gene defects in CBAVD are essentially point mutations. However, in a very small number of cases large rearrangements (deletions or duplications) within the *CFTR* locus are identified. In contrast to CF patients, where 15–25% of unidentified alleles are discovered to be large rearrangements, in CBAVD this proportion is estimated to be 6–10% [66,67]. Overall, large rearrangements (null mutations, classified as "severe") represent <1% of CBAVD alleles, a lower proportion than in CF, which reflects the higher contribution of severe alleles to the pathogenesis of CF.

Complex alleles (more than one mutation on a single *CFTR* gene) are found in CBAVD patients whose DNA is extensively scanned or sequenced across all CFTR coding/flanking sequences. The most common complex alleles are p.[G576A;R668C], p.[D443Y;G576A;R668C], p.[R74W;V201M;D1270N] and S1235R-IVS8-5T [37,38,66,68].

Polymorphisms, resulting in decreased functional CFTR protein might have functional consequences. One example is p.M470V [59], which is strongly associated with the IVS8-5T allele in CBAVD [41,62,69]. Cuppens et al. [59] proposed to name *CFTR* genes that harbour a particular combination of alleles and variants at polymorphic sites "*polyvariant mutant CFTR genes*". Other coding SNPs (single nucleotide polymorphisms) within the *CFTR* gene, which modulate splicing efficiency of exons 9 or 12 [70] might be mild CBAVD mutations with incomplete penetrance [71].

Commercially available mutation-screening kits only identify the most common CF-causing *CFTR* mutations and the IVS8-Tn variants. The vast majority of mutations found in CBAVD cannot be detected by routine tests, requiring full exploration of coding/flanking CFTR sequences by scanning and sequencing methods [38,72]. Optimized methods for detecting mutations and IVS8-TnTGm variants are now available, including rapid sequencing assays useful for diagnostic laboratories [68,73,74]. Complete exploration of coding/flanking CFTR sequences for point mutations and large rearrangements detects at least one CFTR alteration in 90–92% of CBAVD patients from Caucasian descent without associated renal anomaly with up to 85% of patients harbouring two mutations [66,67]. One study reported 100% detection of *CFTR* mutations in 45 patients carefully selected using strict clinical criteria [75]. Identification of mutations *in cis* and *trans* configurations as well as IVS8-TnTGm variants requires analysis of parents and/or relatives of the proband to establish the haplotypic phase.

The mechanisms by which *CFTR* mutations and polyvariants contribute to the pathogenesis of CBAVD are still not fully understood. One report described a CF fetus with absence of the epididymis [76], whereas in two other fetuses aborted at 12 and 18 weeks, respectively, vas deferens were normal [77]. Consistent with the latter data, a high proportion of prepubertal CF boys have normal ducts [78], while in a CFTR knockout pig model of CF, the vas deferens appeared intact at birth [79]. These observations favour the hypothesis that *CFTR* alterations cause progressive post-natal regression of distal epididymis and vas deferens in CBAVD rather than a defect during organogenesis. Reduction of CFTR function might make this long (7 m), thin (<0.5 mm) and tortuous ductal system very vulnerable to luminal dehydration, especially in the distal portion of the vas deferens where CFTR expression is normally low [80,81]. The term "*atresia*" has been proposed in cases of CBAVD associated with *CFTR* mutations. By contrast, the term "*agenesis*" should be used for cases of CBAVD associated with urogenital abnormalities that occur during organogenesis of the Wolffian duct [77].

CBAVD is not always caused by mutations in the *CFTR* gene. A proportion of CBAVD males (11–20%) suffer from concomitant urogenital abnormalities, such as unilateral renal aplasia. There is usually no difference in physical exam-

ination, laboratory assays and ultrasonographic assessment with CBAVD without urogenital associated abnormality [82]. However, the association of *CFTR* mutations remains controversial, as almost all cases published over a period of 15 years are seen in the group without *CFTR* mutations [37,48,83,84], and only rarely are patients detected with one or even more unusually two *CFTR* mutations [32,41,45]. It is plausible that these observations are pure coincidence, because of the frequency of p.F508del (4%) and IVS8-5T (5%) alleles in the general population. Because *CFTR* mutations are observed in CBAVD males with normal renal systems, the simplest explanation of the data is that *CFTR* dysfunction alters the vas deferens after its separation from the renal system. Consistent with this hypothesis, fertile males with CBAVD (i.e. those with a patent contralateral vas) have a higher frequency (up to 80%) of renal agenesis [85,86]. Thus, most cases of CBAVD with associated renal abnormalities represent a distinct clinical entity, presumably due to other genetic causes than *CFTR* mutations.

Several studies provide evidence for genetic heterogeneity in CBAVD. First, a proportion of extensively studied men with CBAVD, variable depending on country of origin (6–15% in Europe [66,67]), do not display any abnormalities in the *CFTR* gene. Although it is always possible that still unknown *CFTR* defects might be discovered with future technology, these cases are most probably not related to *CFTR* mutation, as suggested by cases with discordant familial segregation analysis [87,88].

A small subgroup of CBAVD males has impaired spermatogenesis (hypospermatogenesis and dysmorphogenesis) [89]. The underlying cause can be *CFTR* mutations, other genetic or non-genetic conditions and the impact of chronic obstruction [90]. This observation led to the hypothesis that the *CFTR* gene might be responsible for reduced sperm quality in otherwise healthy men with non-obstructive azoospermia or oligozoospermia [91–93]. However, these findings were not confirmed in other studies [94–97]. CFTR plays an important role in HCO_3^- transport [98–100]. In sperm, HCO_3^- transport by CFTR or a transporter with which CFTR interacts is critical for sperm capacitation [101]. This suggests that CFTR dysfunction might lead to male infertility, at least in part, by attenuating sperm fertilizing capacity [101,102].

Isolated CBAVD is diagnosed primarily in asymptomatic adult males consulting for sterility. Most do not have pulmonary or gastrointestinal manifestations of CF at the time of diagnosis, although some mild manifestations may occur later in life. A proportion of CBAVD men show elevated sweat chloride concentrations, polyps or episodes of rhinosinusitis, bronchitis or sinusitis [41,103]. Some overlap exists between the CBAVD phenotype and a very mild CF phenotype as some individuals with CBAVD also report respiratory or pancreatic problems [104]. Thus, males with CBAVD should be followed up long-term for respiratory and gastrointestinal involvement. The sweat test is the gold standard for the CF phenotype. However, the absence of groups of individuals with CFTR-RDs from previous studies designed to validate the sweat test argues that this test might be of limited value in the case of individuals with mild phenotypes carrying *CFTR* mutations that result in sweat chloride concentrations in the low or intermediate range [105,106].

CFTR mutations have also been reported to be associated with congenital unilateral absence of the vas deferens (CUAVD), but the number of such patients so far reported is limited and the frequency of *CFTR* mutations is considerably less than in CBAVD [93,96]. Obstruction of the epididymis is yet another Wollfian duct anomaly associated with CFTR mutations, but there are only a limited number of patients reported with this condition [21]. The frequency of CFTR mutations in these men is about one-third that of men with CBAVD.

Most patients with CBAVD have normal spermatogenesis. With advances in assisted reproductive technologies (ART), it is possible for them to father their own biological children and many cases of successful pregnancy and birth have now been reported [107]. As there is the risk of transmitting a mutant CF allele, it is mandatory to offer genetic counselling to both partners before performing ART. Molecular genetic testing is most informative when CBAVD-causing *CFTR* alleles have been identified in the proband. The CBAVD male should be screened first for the most common CF mutations using commercial mutation-screening kits and for the IVS8-TnTGm tract [68], followed by whole gene exploration as most point mutations are private and mild and will not be detected using commercial kits [38]. If one or two *CFTR* mutations are not detected, the *CFTR* gene should finally be screened for large rearrangements [66,67]. Although not always feasible, the only way to confirm compound heterozygote status is to test the parents of the CBAVD male. Finally, it is critical to screen the female partner of a CBAVD patient for the most common CF mutations followed by further investigation depending on the context and ethnic origin.

2.2. Idiopathic chronic pancreatitis

CF-causing mutations, p.F508del being the most common, that generally have <2% of normal CFTR function lead typically to pancreatic insufficiency in homozygotes. In contrast, CF patients with genotypes producing ~5% of normal CFTR function often have pancreatic sufficiency [108].

In the human exocrine pancreas, CFTR is predominantly expressed at the apical membrane of the ductal and centroacinar cells that line small pancreatic ducts where it controls cAMP-stimulated HCO_3^- secretion into the duct lumen [98–100]. The major role of CFTR in pancreatic ducts is to dilute and alkalinize the protein-rich acinar secretions, thereby preventing the formation of protein plugs that predispose to pancreatic injury [108]. Stimulated by findings that (i) both idiopathic chronic pancreatitis (ICP) and CF pancreatic disease show early ductal plugging resulting from inspissated secretions, (ii) chronic pancreatitis is a known cause of false-positive sweat tests [2] and (iii) CF patients occasionally suffer from pancreatitis, in 1998 two groups simultaneously reported an association between *CFTR* mutations and ICP [109,110].

About 30% of patients with ICP or recurrent acute pancreatitis are found to carry *CFTR* mutations. No specific *CFTR* mutations are associated with ICP, but rare or private class 4 or class 5 mutations [35,36] are generally found in these patients. Until 2000, most studies had analyzed only the most common CF-causing mutations and the most common milder variations associated with CBAVD. Combined data from these earlier studies indicated that ∼18% of subjects with ICP had common CF-causing mutations, whereas ∼2% were compound heterozygotes who had a CF-causing mutation plus a milder *CFTR* allele [111]. Two studies analyzed all *CFTR* exons and flanking regions in 78 well-defined ICP patients. These studies demonstrated that the risk of ICP increases to 6.3, 2.4, and 37 times that of normal with a CF-causing mutation, the IVS8-5T allele, and a CF-causing mutation plus a milder allele in *trans*, respectively [112,113]. More recent studies corroborating these findings suggest that CF carriers exhibit slight CFTR dysfunction (i.e. individuals with 50% of normal CFTR function account for most *CFTR*-related attributable risk, because they represent 3% of the population in many countries [114–119]).

SPINK1 (encoding serine peptidase inhibitor, Kazal type 1, a trypsin inhibitor secreted by the pancreas) is one of the three ICP susceptibility genes involved in the pathway of premature trypsinogen activation and inactivation [108]. Gene-gene interactions have been documented in individuals who inherit both low-penetrance *SPINK1* variants and *CFTR* mutations in ICP. Coinheritance of the common *SPINK1* N34S allele [120] and at least one abnormal *CFTR* allele accounts for 1.5% (1/67), 4% (1/25), 5.1% (2/39) and 7.7% (3/39) of the total patients analyzed, respectively, in four studies in which all the *CFTR* and *SPINK1* exons were analyzed and the diagnosis of ICP was unambiguous [111,112,116,118]; a total of ∼4.1% of ICP patients were double heterozygotes of *SPINK1/CFTR* variations. In particular, a synergistic effect was observed between a *CFTR* compound heterozygote genotype and the *SPINK1* p.N34S allele. Pancreatitis risk is increased ∼40-fold with two *CFTR* mutations, 20-fold with p.N34S, and 900-fold with both *CFTR* and *SPINK1* mutations [111].

Whether the co-inheritance of *SPINK1* and *CFTR* variants/mutations is *bona fide* digenic inheritance or, perhaps more likely, evidence of the action of a genetic modifier, is unclear in most cases. In this regard, using quantitative fluorescent multiplex-PCR, Masson et al. [121] found a novel heterozygous deletion encompassing the entire *SPINK1* gene in the index patient, her affected father and paternal uncle in one family with chronic pancreatitis, but not in 50 healthy French Caucasians. Remarkably, in all three affected individuals, the *SPINK1* deletion was found to be co-inherited with a heterozygous p.L997F missense mutation in the unlinked *CFTR* gene [121]. Heterozygosity for p.L997F had been previously reported in association with a variety of different conditions including ICP, disseminated bronchiectasis, primary sclerosing cholangitis, and hypertrypsinemia, but there is evidence that p.L997F is not a CF causing mutation [122]. Given that deletion of the entire *SPINK1* gene is disease-causing in its own right, the *CFTR* p.L997F missense mutation (which has a frequency of <1% in the French population) might simply be acting as a disease modifier, at least in the context of this particular family [121].

CFTR might also play a role in alcoholic chronic pancreatitis (ACP). In one study [123], three *CFTR* mutations (p.F508del, p.G542X and c.579+1G>T (previously named 711+1G>T)) were detected in 8.9% of 449 ACP patients, although the mutation detection rate was not significantly different from that observed in patients with alcoholic liver disease (3.0%) nor that expected in the geographical area under investigation (3.2%). More recently, da Costa et al. [124] investigated the frequency of polymorphisms in intron 8 of the *CFTR* gene in three groups of subjects: group A – 68 adult patients with ACP; group B – 68 adult alcoholics without pancreatic disease or liver cirrhosis; and group C – 104 healthy nonalcoholic adults. The authors found that (i) the T5/T7 genotype was more frequent in group A (11.8%) than in group B (2.9%) ($P = 0.048$), (ii) there was no statistical difference between groups A and C (5.8%) ($P = 0.132$) and (iii) the haplotype combination IVS8-(TG)10-T7/(TG)11-T7 was more frequent in groups B (23.5%) and C (20.2%) than in group A (7.3%) ($P = 0.008$ and 0.016, respectively). The authors interpreted these data to suggest that individuals with the IVS8-T5/T7 genotype might have a greater risk of developing chronic pancreatitis when they become chronic alcoholics [124].

Tropical chronic pancreatitis (TCP) is found only in developing countries located in tropical regions. Although the exact environmental factor(s) predisposing to TCP remain controversial, predisposing genetic factors are well documented. Variations in both the *SPINK1* and *CTRC* (encoding chymotrypsinogen) genes have been reported to be significantly overrepresented in TCP patients compared with controls [108]. *CFTR* variations were also analyzed in TCP patients, but the very limited sample size prevents any definitive conclusions to be drawn ($n = 20$ in ref. [125]; $n = 9$ in ref. [126]). To circumvent this problem, assays of CFTR function might be applied to patients with recurrent pancreatitis. Interestingly, in a group of 33 patients with recurrent pancreatitis, Segal et al. [127] found that 7 (21%) had an abnormal NPD even though their sweat chloride concentration and mutation profile did not differ from control subjects.

2.3. Disseminated bronchiectasis

Bronchiectasis is a pathological description of lung damage characterised by an abnormal and irreversible dilatation of thick-walled bronchi. Affected areas are inflamed and easily collapsible, resulting in airflow obstruction and impaired clearance of secretions. Symptoms include recurrent lower respiratory tract infections, chronic cough and mucopurulent sputum production. In approximately 50% of cases, bronchiectasis is associated with underlying conditions such as CF, childhood infections, allergic broncho-pulmonary aspergillosis, immune defects, primary ciliary dyskinesia, aspiration of irritants, ulcerative colitis, rheumatoid arthritis and other connective tissue disorders. In the remainder of cases, causative factors cannot be identified (idiopathic bronchiecta-

sis; [128]). Bronchiectasis can present in either of two forms: a local or focal obstructive process of a lobe or segment of a lung, or a diffuse process involving much of both lungs and often accompanied by other sinopulmonary diseases [129].

An increased incidence of *CFTR* gene mutations has been found in bronchiectasis. At least 1 *CFTR* mutation was reported in 10–50% of a series of patients in different studies [130–135]. Two mutations were found in 5–20% of cases, but not all studies specified whether a segregation analysis had been performed to establish if those subjects carried the 2 mutations *in cis* or *in trans*. Often, in these patients only one mutation is CF-causing. No specific *CFTR* mutation is associated directly with bronchiectasis. Instead, a wide spectrum of *CFTR* mutations have been identified, most being uncommon and likely to result in residual CFTR function. A high incidence of the IVS8-5T allele is generally reported in bronchiectasis, even if not as high as occurs in CBAVD [131,133,134,136–138]. The variety of *CFTR* mutations associated with bronchiectasis likely reflects the heterogeneous nature of this condition and possibly also how exhaustively other aetiologies were investigated. Moreover, differences in the frequencies of reported mutations might be caused by the enrolment of insufficient numbers of patients as well as the methods used to detect *CFTR* mutations, which are not always based on whole *CFTR* gene sequencing. Few studies have been performed to investigate CFTR dysfunction in bronchiectasis. As a result, comparison between CF and other CFTR-RDs is not feasible at the present time.

At the moment, *CFTR* mutation screening is mostly performed for research purposes. It is not advised for identifying bronchiectasis, but rather as one step in the exclusion of CF. In all cases of bronchiectasis, patients should be referred to a CF clinic for sweat test, *CFTR* mutation analysis and other diagnostic tests for CF. Because of the heterogeneous nature of bronchiectasis, it is important to rule out all other aetiologies first, to make a careful differential diagnosis with CF and then follow up the most suspicious cases. Older patients with mild pulmonary disease, including bronchiectasis, may not present with symptoms until later in life, but they are often found to have atypical CF when appropriate investigations are performed [139–142]. A thorough clinical examination at a specialised CF centre and follow up of the most suspicious cases should be recommended, as these symptoms may suggest undiagnosed CF [17].

3. Functional tests

Functional tests that quantify CFTR-mediated transepithelial ion transport have contributed greatly to understanding of disease mechanisms (for review, see [18]). CFTR-RDs are clinical entities that display a wide spectrum of disease and occur within a continuous gradient of CFTR dysfunction. In such cases, CFTR function may serve as a surrogate marker for CF diagnosis. CFTR-dependent chloride secretion is absent or residual in classic or non-classic CF disease, but is normal or only minimally reduced among heterozygotes [143,144]. CFTR-dependent chloride secretion can be assessed indirectly in the nasal epithelium by measuring transepithelial NPD and in colonic epithelia by performing ICM. These functional tests provide a tool to distinguish individuals with non-classic forms of CF with evidence of CFTR dysfunction from subjects whose normal CFTR function indicates that they are unlikely to have CF. Below, the current experience and utility of NPD and ICM for the differential diagnosis of CFTR-RD is discussed.

3.1. Functional tests for CFTR analysis

3.1.1. Nasal potential difference

The method is described in more detail in the consensus statement of the ECFS Diagnostic Network Working Group (EuroCareCF Deliverable 4a) [18]. Briefly, NPD is based on the difference of electric potential measured between a reference electrode placed subcutaneously to create a suitable site of zero potential and an exploring silver/silver chloride electrode connected to a nasal catheter. The probe is placed under the inferior turbinate at the point of maximal negative voltage and maintained in this position for the duration of the recording. Recordings are made during continuous flow of salt solutions at a rate of 5 ml/min. After a consistent baseline NPD has been measured in saline, a standard protocol is followed to investigate CFTR function: (i) amiloride (100 μM) in the saline solution to block the epithelial sodium channel (ENaC); (ii) chloride-free saline solution in the continuous presence of amiloride (100 μM) to drive chloride secretion and (iii) isoproterenol (10 μM) in a chloride-free saline solution containing amiloride (100 μM) to activate CFTR. The sum of the NPD responses in the chloride-free saline solution and the chloride-free saline solution containing isoproterenol serves as an index of CFTR function. It is assumed that a hyperpolarisation of more than −5 mV is indicative of the function of wild-type CFTR. However, despite numerous studies that have sought to assess the role of NPD as a diagnostic tool for CF disease [145–148], the test has neither been standardised nor validated for diagnostic accuracy [149]. We still lack rigorous case-control studies to define the best cut-off point for differentiating CF patients from normal subjects. Thus, at the present time, no clear NPD criteria exist to discriminate CFTR-RDs from other conditions.

3.1.2. Intestinal current measurement

Ex vivo ICM has been used to study CFTR function in human colonic epithelia [150,151]. Micro-Ussing chambers are used to record either the transepithelial short-circuit current (I_{sc}) [152] or the transepithelial voltage (V_{te}) [153] in freshly obtained human rectal biopsies as a measure of ion transport after stimulation with chloride secretagogues (e.g. the cAMP agonist forskolin and the Ca^{2+} agonist carbachol). In this way, the magnitude of CFTR function and the activity of alternative chloride channels is investigated [152–155]. Because the CFTR defect is manifest in both respiratory and intestinal epithelia, the ICM and NPD assays have been evaluated in cohorts of CF patients and control

subjects and good correlation demonstrated between the two methods [156,157]. These studies provided evidence for a consistent degree of CFTR dysfunction measured by NPD and ICM. Advantages of ICM are that it is a minimally invasive, safe procedure applicable to all ages starting from newborns. Suction biopsies are made without sedation or special treatment and limitations are rare [152]. Because ICM has mainly been employed as a research tool, to date the technique has only been included in some diagnostic algorithms and consensus guidelines [6,12,158]. Nevertheless, Veeze [144] observed no difference between ICM values in healthy controls and obligate heterozygotes. Moreover, ICM was prospectively validated as a diagnostic test for the equivocal diagnosis of CF in large groups of control subjects, CF-PI, CF-PS and questionable CF patients [159]. Using a standardised protocol [152], Derichs et al. [159] identified clear-cut reference/threshold values to distinguish between CF-PS and control subjects, supporting the role of ICM in the diagnosis of CF. In general, ICM should be performed for diagnostic purposes only when the sweat test and *CFTR* mutation analysis are equivocal and further evidence of CFTR dysfunction or normality is required to establish a comprehensive clinical description. (Further details of the ICM method are discussed in the consensus statement of the ECFS Diagnostic Network Working Group (EuroCareCF Deliverable 4a) [18]).

3.2. Experiences with functional tests in CFTR-RDs

3.2.1. Nasal potential difference

Most of the studies evaluating patients with possible mild CF or CFTR-RD using NPD have only enrolled adults. They demonstrate that patients with abnormal NPD tend to develop more severe pulmonary disease and more frequently carry CF-causing mutations. For example, Wilschanski et al. [145] identified in a group of 70 adults with normal clinical phenotype 24 patients with NPD results in the CF range. Re-evaluation of these same 24 patients years later revealed that 16 of them were now followed in CF centres [160]. Genetic analysis indicated that none of the patients with normal NPD results carried 2 *CFTR* mutations, whereas 5 patients in the group with abnormal NPD results harboured *CFTR* mutations [160]. Delmarco et al. [148] demonstrated that CF patients with a borderline sweat test and 2 CF-causing mutations all had a NPD test in the abnormal range. Conversely, individuals with respiratory diseases distinct from CF, such as idiopathic asthma, all had normal NPD results. Finally, Groman et al. [161] reported that individuals with a CF phenotype in the absence of *CFTR* mutations had NPD values distinct from CF patients with 2 *CFTR* mutations.

NPD studies are lacking in children. In one unpublished study, I Sermet and colleagues (Université René Descartes) evaluated 50 children (3 months–18 years) with equivocal diagnosis, borderline (30–60 mEq/L) sweat test and no conclusive genetic screening using the NPD assay. In this study, children with NPD results in the CF range more frequently carried two *CFTR* mutations and had chronic cough, sputum production, recurrent lower respiratory tract infection and chronic colonisation by *S. aureus* (I Sermet, unpublished data). To summarise, only limited experience with NPD in CFTR-RD is available. Further investigation in patients with different features of CFTR-RD is required to better define criteria of CFTR dysfunction.

3.2.2. Intestinal current measurement

Besides using ICM for classification of patients with questionable CF, the method has been evaluated in a limited number of CFTR-RD cases. Dohle et al. [162] studied a group of 21 patients with CBAVD, while Ockenga et al. [163] tested single patients with recurrent or chronic pancreatitis. Data from these preliminary studies are insufficient to determine the diagnostic value of ICM for CFTR-RDs. However, clear differentiation between CF-PS and control subjects by ICM appears feasible [159].

3.3. Further information required

The NPD assay has been performed in a wide range of individuals from CF patients to individuals heterozygous for *CFTR* mutations and normal subjects. Although there is clear separation between CF-PI patients and controls, there is overlap between heterozygotes, CF-PS and patients with CFTR-RDs [164] impairing diagnostic value in individual cases with borderline CFTR function. This demonstrates that evaluation of ion transport across the nasal epithelium is a (bio)-marker of CF disease, but a definitive diagnosis must employ a battery of tests, including clinical follow-up, repeated sweat tests, ICM and genetic screening.

For ICM, the test has been proven to discriminate clearly between CF-PS and control subjects [159]. The next efforts should be to describe ICM values in larger groups of obligate heterozygotes and patients with CFTR-RDs, who do not fit the CF diagnostic ICM cut-off. These reference data have to be precisely determined for specific protocols and measurement methods (I_{sc} vs. V_{te}) and correlated with comprehensive analyses of *CFTR* genotype.

To summarise, both NPD and ICM contribute to the analysis of the functional consequences of *CFTR* mutations and hence, to better understanding of clinical interpretations. However, additional studies are required to validate these tests as diagnostic tools. This applies not only to *CFTR* mutations described in recent consensus statements [13,17] as CF disease-causing or CFTR-RD-associated, but also to the large majority of *CFTR* mutations with unknown clinical relevance.

4. Molecular investigations of *CFTR* mutations associated with CFTR-RDs

Biochemical and function studies of *CFTR* mutations have provided great insight into the molecular basis of CF. Drumm et al. [165] first demonstrated that when *CFTR* mutations associated with CF are expressed in heterologous cells the amount of CFTR-mediated Cl^- current is reduced in the rank

order: wild-type CFTR > CF-PS mutants > CF-PI mutants. These results suggested a relationship between genotype, clinical phenotype and Cl^- channel function. To understand this relationship, the effects of CF-associated *CFTR* mutations on (i) the processing and intracellular trafficking of CFTR protein and (ii) the biophysical properties and regulation of CFTR Cl^- channels were investigated. These studies identified five general mechanisms of CFTR dysfunction: defective protein production, defective protein processing, defective channel regulation, defective channel conduction and reduced protein synthesis [35,36].

Importantly, studies of the biosynthesis and single-channel behaviour of CFTR mutations enable the attenuation of CFTR-mediated Cl^- currents in the apical membrane of epithelia by CF-associated *CFTR* mutations to be quantified. Apical CFTR Cl^- current (I^{CFTR}(apical)) is determined by the product of the number of CFTR Cl^- channels in the apical membrane (N), the amount of current flowing through an open CFTR Cl^- channel (i) and the probability (P_o) that a single CFTR Cl^- channel is open: $I^{CFTR}(\text{apical}) = N \times i \times P_o$. Using biochemical (N) and functional (i and P_o) data, the apical CFTR Cl^- current generated by the CF-PI mutant p.F508del-CFTR and the CF-PS mutants p.R117H-, p.R334W-, p.R347P-, p.A455E- and p.P574H-CFTR were predicted [166,167]. Despite possible errors resulting from (i) the assumption that N is equivalent to the amount of fully glycosylated CFTR protein (band C) and (ii) the supposition that the P_o of CFTR Cl^- channels in the apical membrane of heterologous epithelia is equivalent to values measured in excised membrane patches from non-polarised cells, the predicted values agreed well with the measured data [166–168]. These studies suggest that biochemical and functional studies might be used to explain how *CFTR* mutations associated with CFTR-RDs disrupt CFTR function. They also raise the possibility that CFTR-RD-associated *CFTR* mutations might exert complex effects on CFTR expression and function. For example, the CF-PS mutant p.P574H-CFTR disrupts CFTR processing, albeit not as severely as p.F508del-CFTR, but generates a CFTR Cl^- channel with a P_o value greater than that of wild-type CFTR [167].

To understand the molecular mechanisms of CFTR dysfunction caused by CFTR-RD-associated *CFTR* mutations, heterologous epithelial cells expressing identical amounts of CFTR mutants, such as those developed by Krasnov et al. [169] will be required. However, it might also be necessary to study primary cultures of epithelial cells isolated from CFTR-RD patients to understand fully the mechanisms of CFTR dysfunction. Although technically-challenging, such studies will prove very rewarding, providing deep insight into the molecular mechanisms of CFTR dysfunction in CFTR-RD and hence, new approaches to therapy.

5. Algorithms for the diagnostics of CFTR-RD: a European perspective

Here, we propose a series of flow charts that summarize clinical and molecular investigations in patients with a clinical presentation suggestive of a CFTR-RD (Figs. 1–

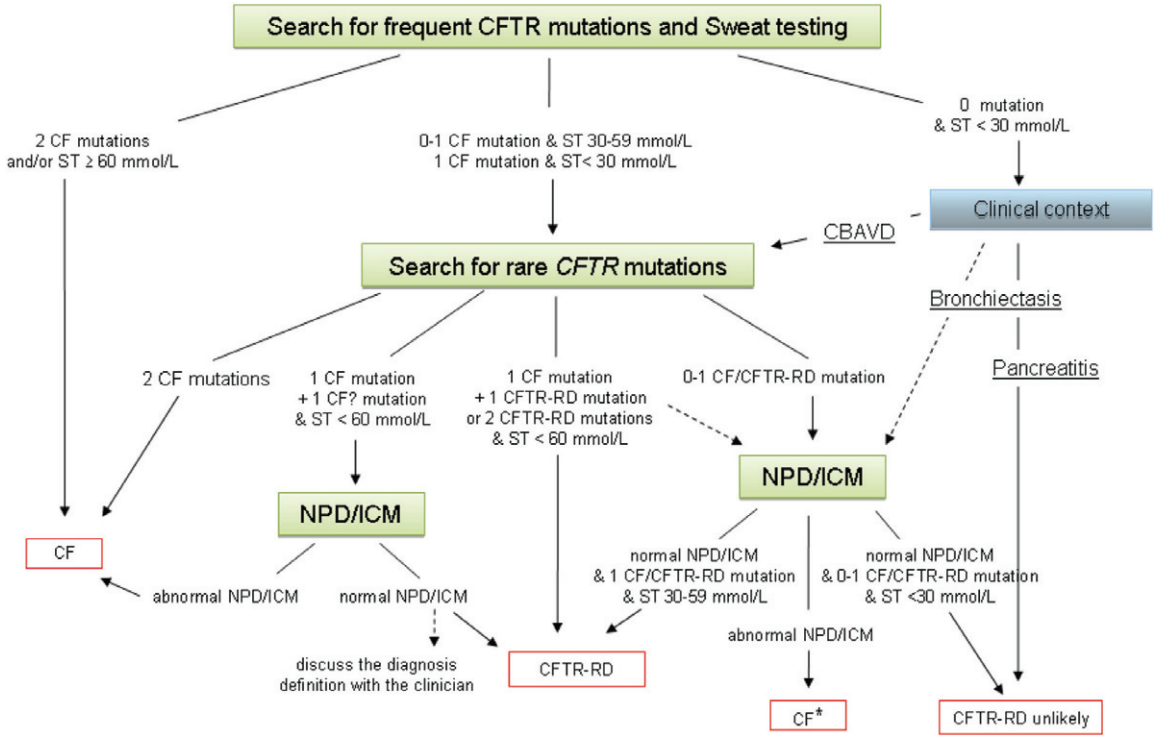

Fig. 1. Global diagnostic algorithm for CF and CFTR-RD. A global flow-chart of genetic and functional diagnostic testing in CF and CFTR-RD is presented. Abbreviations: CF? mutation, mutation of unproven or uncertain clinical significance; CF*, diagnosis of CF or consider this diagnosis; CFTR-RD, CFTR-related disorders; ICM, intestinal current measurement; NPD, nasal potential difference; ST, sweat test (repeated; false positive should be excluded/sought in a specialized centre).

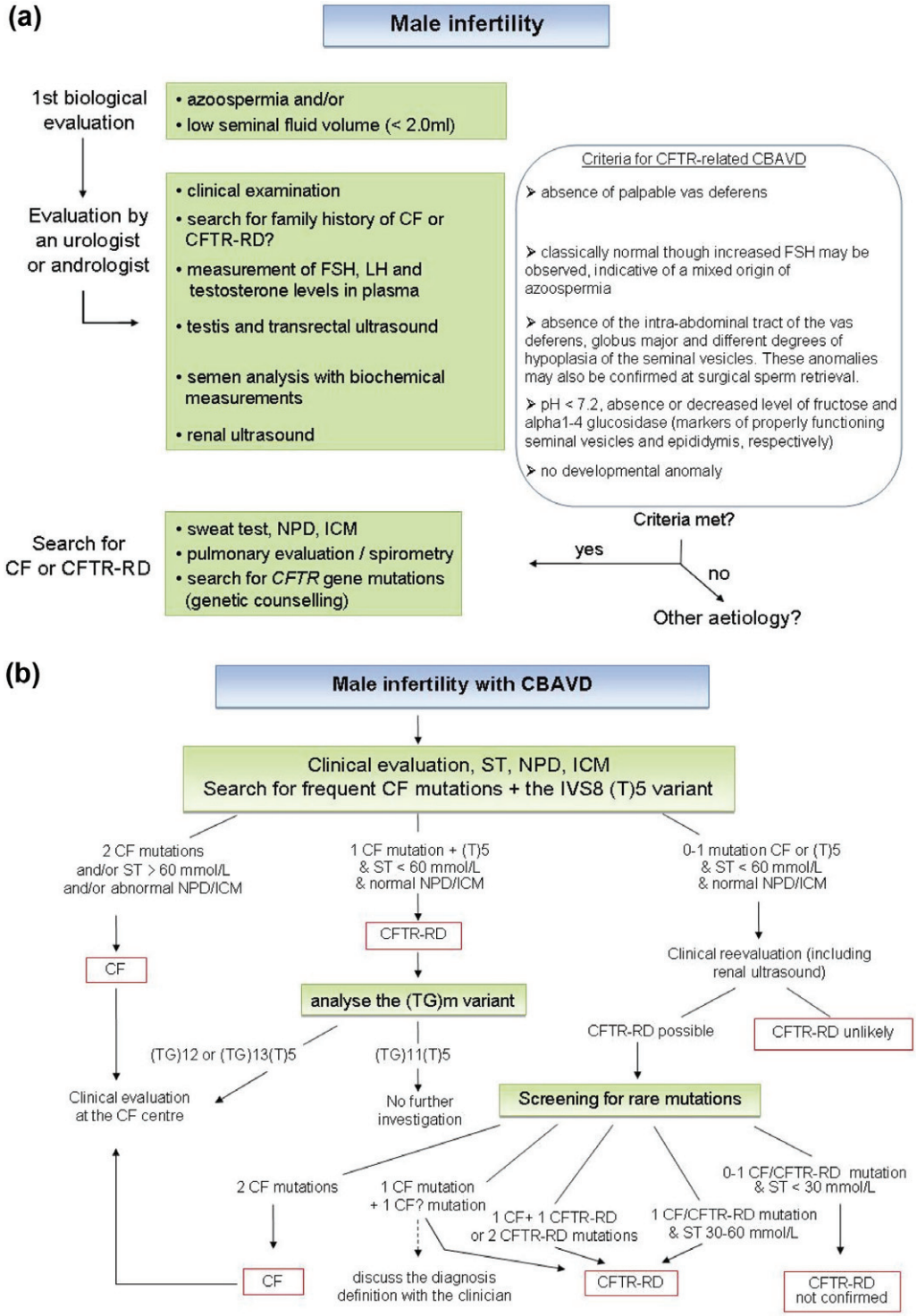

Fig. 2. Diagnostic algorithm for male infertility and CBAVD. The flow charts summarize clinical, functional and genetic diagnostic testing in patients presenting with male infertility (a) and male infertility with CBAVD (b). Abbreviations: CF? mutation, mutation of unproven or uncertain clinical significance; CFTR-RD, CFTR-related disorders; FSH, follicle-stimulating hormone; ICM, intestinal current measurement; LH, luteinising hormone; NPD, nasal potential difference; ST, sweat test (repeated; false positive should be excluded/sought in a specialized centre).

4). A thorough clinical examination at a specialised CF centre and follow-up of the most suspicious cases is recommended, as these symptoms may suggest undiagnosed CF.

Careful attention should be paid to exclude other known aetiologies, to the degree of screening for *CFTR* mutations and to the evaluation of CFTR function in these patients. A continuous dialogue between clinicians, geneticists and physiologists is of the upmost importance for the diagnosis and follow up of patients with a clinical presentation suggestive of a CFTR-RD. While comprehensive *CFTR* gene analysis may not be widely advised in CFTR-RD, genetic counselling for the patients or their relatives is recommended.

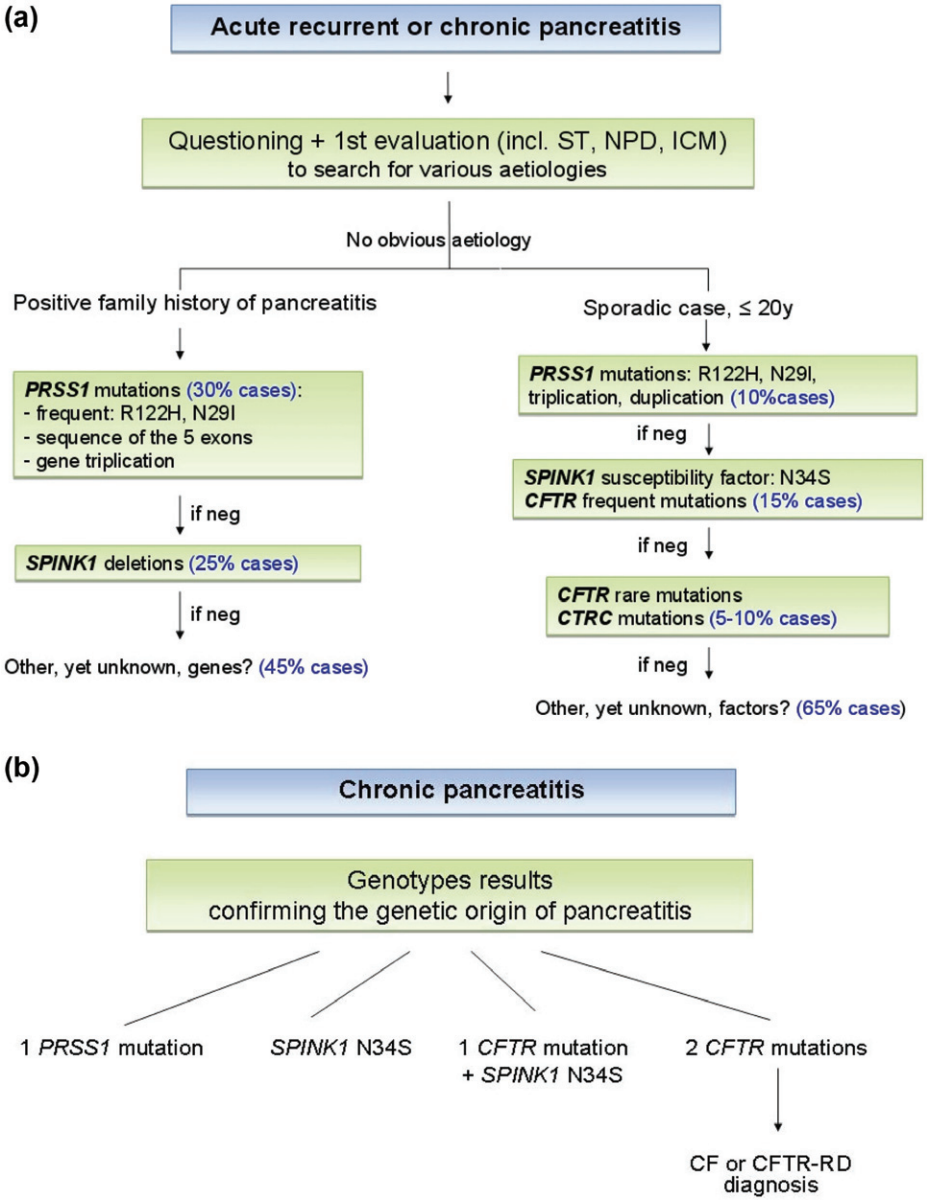

Fig. 3. Diagnostic algorithm for chronic pancreatitis. The flow charts summarize clinical, functional and genetic diagnostic testing in patients presenting with acute recurrent or chronic pancreatitis (a) and chronic pancreatitis (b). Abbreviations: PRSS1, cationic trypsinogen; SPINK1, serine protease inhibitor; CTRC chymotryspinogen C.

We emphasize that these flow charts should not be considered "Tables of Law" nor should they be interpreted as dogma. Instead, these flow charts are simply guidelines, which we hope will prove helpful and practical when clarifying the diagnosis of CFTR-RD patients.

6. Conclusions

During the past 20 years our knowledge of CF has changed dramatically. Understanding of the *CFTR* gene and protein has permitted relationships between genotype and clinical phenotype to be made, which have revealed a continuum of clinical presentation from severe disease to milder forms. Moreover, in the 1990s the discovery that mutations in the *CFTR* gene or mild dysfunction of the CFTR Cl^- channel are associated with particular clinical phenotypes (the best example being CBAVD in males) led to a variety of names being proposed to define borderline cases associated with dysfunction of the CFTR protein (e.g. atypical CF, borderline CF and genital form of CF). The objectives of the EuroCareCF Working Group on CFTR-RD that we summarize here were to try to clarify the description of CFTR-RD, to bring a consensual definition and approach to the concept of CFTR-RD even if the positions of different specialists were sometimes difficult to unite.

We must keep in mind that most of the time the diagnosis of CF does not raise any difficulty. It is clear that in the vast majority of cases, a patient's clinical symptoms, positive sweat test and mutations in the *CFTR* gene provide convincing evidence of the diagnosis. The situation is similar with many

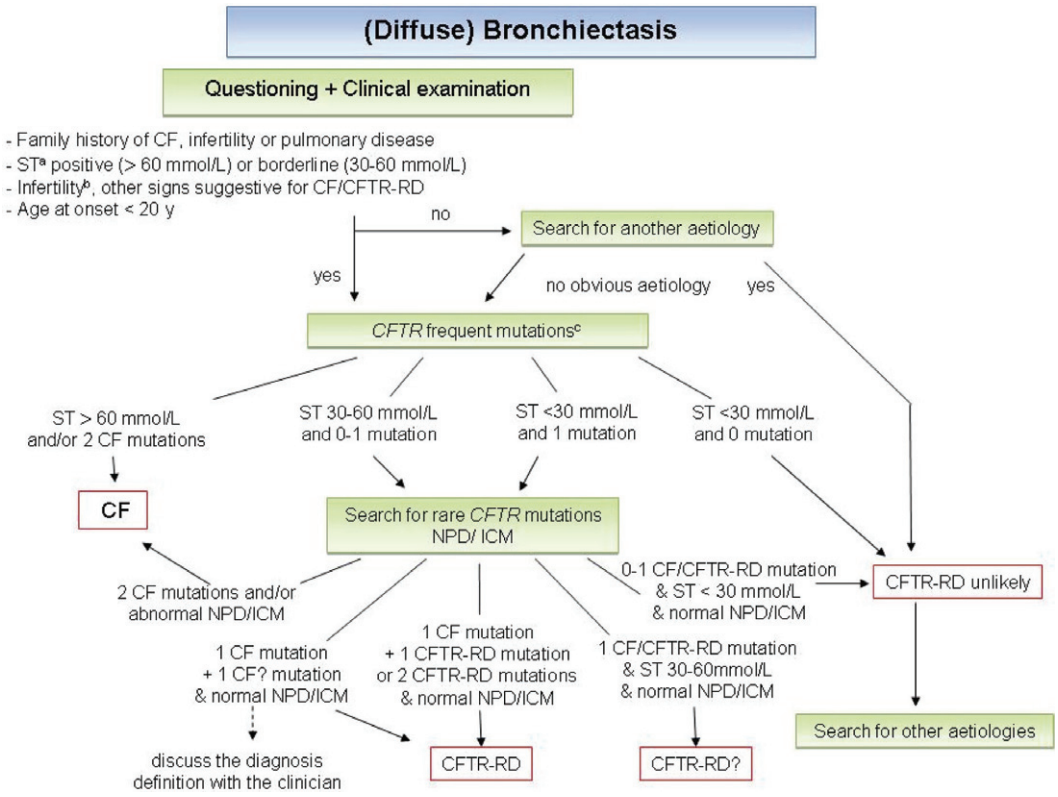

Fig. 4. Diagnostic algorithm for disseminated bronchiectasis. The flow chart summarizes clinical, functional and genetic diagnostic testing in patients presenting with disseminated bronchiectasis. Footnotes: (a) sweat test repeated; false positive should be excluded/sought in a specialized centre; (b) CBAVD suggestive for CFTR-RD; immobile cilia suggestive for primary ciliary dyskinesia; (c) search for frequent CFTR mutations should consider the patient's geographic/ethnic origins. Abbreviations: CF? mutation, mutation of unproven or uncertain clinical significance; CFTR-RD, CFTR-related disorders; ICM, intestinal current measurement; NPD, nasal potential difference; ST, sweat test.

CFTR-RD patients. For example, although the vast majority of CBAVD men are infertile, they have good health. Similarly, patients with recurrent acute or chronic pancreatitis present with no other symptoms. When patients present with these conditions, there is no discussion that these individuals might have a mild form of CF.

However, the diagnosis of a limited number of CFTR-RD patients is not clear-cut. A grey zone exists between CFTR-RD and CF. If we are very close to the criteria required to identify these individuals as CF patients, the preferred option is to designate them as CFTR-RD and to follow them at least once a year. Some of these CFTR-RD patients will never meet the criteria to be classified as CF. In the case of other CFTR-RD patients, some years later these individuals will progress to a diagnosis of CF and will require follow-up in CF centres. As our knowledge and understanding of the dysfunction of CFTR in CF and CFTR-RD progresses, we are confident that the diagnosis and management of individuals with these disorders will continue to improve.

Acknowledgements

We would like to particularly thank, Dr Dominique Hubert (Paris), Dr Dominique Grenet (Suresnes), Dr Laurence Bassinet (Créteil), Dr Vincent Izard (Clamart), Dr Jean-Claude Soufir (Paris) and Dr Jean-Marc Rigot (Lille) for their excellent assistance with the diagnostic algorithms and Drs Peter Durie and Tanja Gonska (Toronto) for stimulating discussions. This work was supported by the European Union Sixth Framework Programme (contract no. LSHM-CT-2005-018932, EuroCareCF) and the Czech Ministry of Health (MZ0FNM2005 to Milan Macek Jr).

Conflict of interest

All authors state that there is no conflict of interest.

References

[1] WHO, World Health Organization 2001. Classification of Cystic Fibrosis and Related Disorders. Report of a joint WHO/ICF(M)A-ECFTN meeting. Reprinted in J Cyst Fibros 2002;1:5–8.
[2] Welsh MJ, Ramsey BW, Accurso F, Cutting GR. Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic and Molecular Basis of Inherited Disease. New York: McGraw-Hill Inc., 2001; pp. 5121–88.
[3] Dumur V, Gervais R, Rigot JM, et al. Abnormal distribution of CF delta F508 allele in azoospermic men with congenital aplasia of epididymis and vas deferens. Lancet 1990;336:512.
[4] Anguiano A, Oates RD, Amos JA, et al. Congenital bilateral absence of the vas deferens: a primarily genital form of cystic fibrosis. JAMA 1992;267:1794–7.
[5] Patrizio P, Asch RH, Handelin B, Silber SJ. Aetiology of congenital absence of vas deferens: genetic study of three generations. Hum Reprod 1993;8:215–20.

[6] De Boeck K, Wilschanski M, Castellani C, et al. Cystic fibrosis: terminology and diagnostic algorithms. Thorax 2006;61:627–35.
[7] Castellani C, Southern KW, Brownlee K, et al. European best practice guidelines for cystic fibrosis neonatal screening. J Cyst Fibros 2009;8:153–73.
[8] Accurso FJ, Sontag MK. Gene modifiers in cystic fibrosis. J Clin Invest 2008;118:839–41.
[9] ECFS. European Registry for Cystic Fibrosis, Report 2006. Available from: http://www.ecfs.eu/files/webfm/webfiles/File/ecfs_registry/ECFRreport2006.pdf
[10] CFF patient Registry annual data report 2007. Available from: http://www.cff.org/UploadedFiles/research/ClinicalResearch/2007-Patient-Registry-Report.pdf
[11] Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. J Pediatr 1998;132:589–95.
[12] Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. J Pediatr 2008. 153:S4–S14.
[13] Castellani C, Cuppens H, Macek M Jr, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros 2008;7:179–96.
[14] Thauvin-Robinet C, Munck A, Huet F, et al. The very low penetrance of cystic fibrosis for the R117H mutation: a reappraisal for genetic counselling and newborn screening. J Med Genet 2009;46:752–8.
[15] Goubau C, Wilschanski M, Skalicka V, et al. Phenotypic characterisation of patients with intermediate sweat chloride values: towards validation of the European diagnostic algorithm for cystic fibrosis. Thorax 2009;64:683–91.
[16] Wolfenden LL, Schechter MS. Genetic and non-genetic determinants of outcomes in cystic fibrosis. Paediatr Respir Rev 2009;10:32–6.
[17] Dequeker E, Stuhrmann M, Morris MA, et al. Best practice guidelines for molecular genetic diagnosis of cystic fibrosis and CFTR-related disorders – updated European recommendations. Eur J Hum Genet. 2009;17:51–65.
[18] De Boeck K, Derichs N, Fajac I, et al. New clinical diagnostic procedures for cystic fibrosis in Europe. J Cyst Fibros 2011;10(Suppl 2):53–66.
[19] Holsclaw DS, Perlmutter AD, Jockin H, Shwachman H. Genital abnormalities in male patients with cystic fibrosis. J Urol 1971;106:568–74.
[20] Oates RD, Amos JA. The genetic basis of congenital bilateral absence of the vas deferens and cystic fibrosis. J Androl 1994;15:1–8.
[21] Mak V, Jarvi KA. The genetics of male infertility. J Urol 1996;156:1245–56; discussion 1256–7.
[22] Anzai C, Morokawa N, Okada H, Kamidono S, Eto Y, Yoshimura K. CFTR gene mutations in Japanese individuals with congenital bilateral absence of the vas deferens. J Cyst Fibros 2003;2:14–8.
[23] Wu CC, Hsieh-Li HM, Lin YM, Chiang HS. Cystic fibrosis transmembrane conductance regulator gene screening and clinical correlation in Taiwanese males with congenital bilateral absence of the vas deferens. Hum Reprod 2004;19:250–3.
[24] Culard JF, Desgeorges M, Costa P, et al. Analysis of the whole CFTR coding regions and splice junctions in azoospermic men with congenital bilateral aplasia of epididymis or vas deferens. Hum Genet 1994;93:467–70.
[25] Chillon M, Casals T, Mercier B, et al. Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. N Engl J Med 1995;332:1475–80.
[26] Costes B, Girodon E, Ghanem N, et al. Frequent occurrence of the CFTR intron 8 (TG)n 5T allele in men with congenital bilateral absence of the vas deferens. Eur J Hum Genet 1995;3:285–93.
[27] De Braekeleer M, Ferec C. Mutations in the cystic fibrosis gene in men with congenital bilateral absence of the vas deferens. Mol Hum Reprod 1996;2:669–77.
[28] Cuppens H, Cassiman JJ. CFTR mutations and polymorphisms in male infertility. Int J Androl 2004;27:251–6.
[29] Claustres M. Molecular pathology of the CFTR locus in male infertility. Reprod Biomed Online 2005;10:14–41.
[30] Colin AA, Sawyer SM, Mickle JE, Oates RD, Milunsky A, Amos JA. Pulmonary function and clinical observations in men with congenital bilateral absence of the vas deferens. Chest 1996;110:440–5.
[31] Casals T, Bassas L, Ruiz-Romero J, et al. Extensive analysis of 40 infertile patients with congenital absence of the vas deferens: in 50% of cases only one CFTR allele could be detected. Hum Genet 1995;95:205–11.
[32] Daudin M, Bieth E, Bujan L, Massat G, Pontonnier F, Mieusset R. Congenital bilateral absence of the vas deferens: clinical characteristics, biological parameters, cystic fibrosis transmembrane conductance regulator gene mutations, and implications for genetic counseling. Fertil Steril 2000;74:1164–74.
[33] von Eckardstein S, Cooper TG, Rutscha K, Meschede D, Horst J, Nieschlag E. Seminal plasma characteristics as indicators of cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in men with obstructive azoospermia. Fertil Steril 2000;73:1226–31.
[34] McCallum TJ, Milunsky JM, Cunningham DL, Harris DH, Maher TA, Oates RD. Fertility in men with cystic fibrosis: an update on current surgical practices and outcomes. Chest 2000;118:1059–62.
[35] Welsh MJ, Smith AE. Molecular mechanism of CFTR chloride channel dysfunction in cystic fibrosis. Cell. 1993;73:1251–4.
[36] Zielenski J, Tsui LC, Cystic fibrosis: genotypic and phenotypic variations. Annu Rev Genet. 1995;29:777–807.
[37] Dork T, Dworniczak B, Aulehla-Scholz C, et al. Distinct spectrum of CFTR gene mutations in congenital absence of vas deferens. Hum Genet 1997;100:365–77.
[38] Claustres M, Guittard C, Bozon D, et al. Spectrum of CFTR mutations in cystic fibrosis and in congenital absence of the vas deferens in France. Hum Mutat 2000;16:143–56.
[39] Jarvi K, McCallum S, Zielenski J, et al. Heterogeneity of reproductive tract abnormalities in men with absence of the vas deferens: role of cystic fibrosis transmembrane conductance regulator gene mutations. Fertil Steril 1998;70:724–8.
[40] Stuhrmann M, Dork T. CFTR gene mutations and male infertility. Andrologia 2000;32:71–83.
[41] Casals T, Bassas L, Egozcue S, et al. Heterogeneity for mutations in the CFTR gene and clinical correlations in patients with congenital absence of the vas deferens. Hum Reprod 2000;15:1476–83.
[42] Kanavakis E, Tzetis M, Antoniadi T, Pistofidis G, Milligos S, Kattamis C. Cystic fibrosis mutation screening in CBAVD patients and men with obstructive azoospermia or severe oligozoospermia. Mol Hum Reprod 1998;4:333–7.
[43] Grangeia A, Niel F, Carvalho F, et al. Characterization of cystic fibrosis conductance transmembrane regulator gene mutations and IVS8 poly(T) variants in Portuguese patients with congenital absence of the vas deferens. Hum Reprod 2004;19:2502–8.
[44] Sharma N, Acharya N, Singh SK, Singh M, Sharma U, Prasad R. Heterogenous spectrum of CFTR gene mutations in Indian patients with congenital absence of vas deferens. Hum Reprod 2009;24:1229–36.
[45] Lissens W, Mahmoud KZ, El-Gindi E, et al. Molecular analysis of the cystic fibrosis gene reveals a high frequency of the intron 8 splice variant 5T in Egyptian males with congenital bilateral absence of the vas deferens. Mol Hum Reprod 1999;5:10–13.
[46] Zielenski J, Patrizio P, Corey M, et al. CFTR gene variant for patients with congenital absence of vas deferens. Am J Hum Genet 1995;57:958–60.
[47] Jarvi K, Zielenski J, Wilschanski M, et al. Cystic fibrosis transmembrane conductance regulator and obstructive azoospermia. Lancet 1995;345:1578.
[48] Dumur V, Gervais R, Rigot JM, et al. Congenital bilateral absence of the vas deferens (CBAVD) and cystic fibrosis transmembrane regulator (CFTR): correlation between genotype and phenotype. Hum Genet 1996;97:7–10.
[49] Niksic M, Romano M, Buratti E, Pagani F, Baralle FE. Functional

analysis of cis-acting elements regulating the alternative splicing of human CFTR exon 9. Hum Mol Genet 1999;8:2339–49.
[50] Nissim-Rafinia M, Chiba-Falek O, Sharon G, Boss A, Kerem B. Cellular and viral splicing factors can modify the splicing pattern of CFTR transcripts carrying splicing mutations. Hum Mol Genet 2000;9:1771–8.
[51] Pagani F, Buratti E, Stuani C, et al. Splicing factors induce cystic fibrosis transmembrane regulator exon 9 skipping through a nonevolutionary conserved intronic element. J Biol Chem 2000;275:21041–7.
[52] Buratti E, Dork T, Zuccato E, Pagani F, Romano M, Baralle FE. Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. EMBO J 2001;20:1774–84.
[53] Hefferon TW, Broackes-Carter FC, Harris A, Cutting GR. Atypical 5' splice sites cause CFTR exon 9 to be vulnerable to skipping. Am J Hum Genet 2002;71:294–303.
[54] Disset A, Michot C, Harris A, Buratti E, Claustres M, Tuffery-Giraud S. A T3 allele in the CFTR gene exacerbates exon 9 skipping in vas deferens and epididymal cell lines and is associated with congenital bilateral absence of vas deferens (CBAVD). Hum Mutat 2005;25:72–81.
[55] Mak V, Jarvi KA, Zielenski J, Durie P, Tsui LC. Higher proportion of intact exon 9 CFTR mRNA in nasal epithelium compared with vas deferens. Hum Mol Genet 1997;6:2099–2107.
[56] Rave-Harel N, Kerem E, Nissim-Rafinia M, et al. The molecular basis of partial penetrance of splicing mutations in cystic fibrosis. Am J Hum Genet 1997;60:87–94.
[57] Teng H, Jorissen M, Van Poppel H, Legius E, Cassiman JJ, Cuppens H. Increased proportion of exon 9 alternatively spliced CFTR transcripts in vas deferens compared with nasal epithelial cells. Hum Mol Genet 1997;6:85–90.
[58] Kiesewetter S, Macek M Jr, Davis C, et al. A mutation in CFTR produces different phenotypes depending on chromosomal background. Nat Genet 1993;5:274–8.
[59] Cuppens H, Lin W, Jaspers M, et al. Polyvariant mutant cystic fibrosis transmembrane conductance regulator genes. The polymorphic (Tg)m locus explains the partial penetrance of the T5 polymorphism as a disease mutation. J Clin Invest 1998;101:487–96.
[60] Delaney SJ, Rich DP, Thomson SA, et al. Cystic fibrosis transmembrane conductance regulator splice variants are not conserved and fail to produce chloride channels. Nat Genet 1993;4:426–31.
[61] Strong TV, Wilkinson DJ, Mansoura MK, et al. Expression of an abundant alternatively spliced form of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is not associated with a cAMP-activated chloride conductance. Hum Mol Genet 1993;2:225–30.
[62] Groman JD, Hefferon TW, Casals T, et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. Am J Hum Genet 2004;74:176–9.
[63] Radpour R, Gourabi H, Gilani MA, Dizaj AV. Correlation between CFTR gene mutations in Iranian men with congenital absence of the vas deferens and anatomical genital phenotype. J Androl 2008;29:35–40.
[64] Dayangac D, Erdem H, Yilmaz E, et al. Mutations of the CFTR gene in Turkish patients with congenital bilateral absence of the vas deferens. Hum Reprod 2004;19:1094–1100.
[65] Viel M, Leroy C, Des Georges M, Claustres M, Bienvenu T. Novel length variant of the polypyrimidine tract within the splice acceptor site in intron 8 of the CFTR gene: consequences for genetic testing using standard assays. Eur J Hum Genet 2005;13:136–8.
[66] Ratbi I, Legendre M, Niel F, et al. Detection of cystic fibrosis transmembrane conductance regulator (CFTR) gene rearrangements enriches the mutation spectrum in congenital bilateral absence of the vas deferens and impacts on genetic counselling. Hum Reprod 2007;22:1285–91.
[67] Taulan M, Girardet A, Guittard C, et al. Large genomic rearrangements in the CFTR gene contribute to CBAVD. BMC Med Genet 2007;8:22.
[68] Bareil C, Guittard C, Altieri JP, Templin C, Claustres M, des Georges M. Comprehensive and rapid genotyping of mutations and haplotypes in congenital bilateral absence of the vas deferens and other cystic fibrosis transmembrane conductance regulator-related disorders. J Mol Diagn 2007;9:582–8.
[69] de Meeus A, Guittard C, Desgeorges M, Carles S, Demaille J, Claustres M. Linkage disequilibrium between the M470V variant and the IVS8 polyT alleles of the CFTR gene in CBAVD. J Med Genet 1998;35:594–6.
[70] Pagani F, Buratti E, Stuani C, Baralle FE. Missense, nonsense, and neutral mutations define juxtaposed regulatory elements of splicing in cystic fibrosis transmembrane regulator exon 9. J Biol Chem 2003;278:26580–8.
[71] Steiner B, Truninger K, Sanz J, Schaller A, Gallati S. The role of common single-nucleotide polymorphisms on exon 9 and exon 12 skipping in nonmutated CFTR alleles. Hum Mutat 2004;24:120–9.
[72] Mak V, Zielenski J, Tsui LC, et al. Proportion of cystic fibrosis gene mutations not detected by routine testing in men with obstructive azoospermia. JAMA 1999;281:2217–24.
[73] Strom CM, Huang D, Chen C, et al. Extensive sequencing of the cystic fibrosis transmembrane regulator gene: assay validation and unexpected benefits of developing a comprehensive test. Genet Med 2003;5:9–14.
[74] Lucarelli M, Narzi L, Piergentili R, et al. A 96-well formatted method for exon and exon/intron boundary full sequencing of the CFTR gene. Anal Biochem 2006;353:226–35.
[75] Grangeia A, Sá R, Carvalho F, et al. Molecular characterization of the cystic fibrosis transmembrane conductance regulator gene in congenital absence of the vas deferens. Genet Med 2007;9:163–72.
[76] Harris A, Coleman L. Ductal epithelial cells cultured from human foetal epididymis and vas deferens: relevance to sterility in cystic fibrosis. J Cell Sci 1989;92:687–90.
[77] Gaillard DA, Carre-Pigeon F, Lallemand A. Normal vas deferens in fetuses with cystic fibrosis. J Urol 1997;158:1549–52.
[78] Valman HB, France NE. The vas deferens in cystic fibrosis. Lancet 1969;2:566–7.
[79] Rogers CS, Stoltz DA, Meyerholz DK, et al. Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. Science 2008;321:1837–41
[80] Trezise AE, Chambers JA, Wardle CJ, Gould S, Harris A. Expression of the cystic fibrosis gene in human foetal tissues. Hum Mol Genet 1993;2:213–8.
[81] Tizzano EF, Silver MM, Chitayat D, Benichou JC, Buchwald M. Differential cellular expression of cystic fibrosis transmembrane regulator in human reproductive tissues. Clues for the infertility in patients with cystic fibrosis. Am J Pathol 1994;144:906–14.
[82] McCallum T, Milunsky J, Munarriz R, Carson R, Sadeghi-Nejad H, Oates R. Unilateral renal agenesis associated with congenital bilateral absence of the vas deferens: phenotypic findings and genetic considerations. Hum Reprod 2001;16:282–8.
[83] Augarten A, Yahav Y, Kerem BS, et al. Congenital bilateral absence of vas deferens in the absence of cystic fibrosis. Lancet 1994;344:1473–4.
[84] Schlegel PN, Shin D, Goldstein M. Urogenital anomalies in men with congenital absence of the vas deferens. J Urol 1996;155:1644–8.
[85] Weiske WH, Salzler N, Schroeder-Printzen I, Weidner W. Clinical findings in congenital absence of the vasa deferentia. Andrologia 2000;32:13–8.
[86] Sakamoto H, Yajima T, Suzuki K, Ogawa Y. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation associated with a congenital bilateral absence of vas deferens. Int J Urol 2008;15:270–1.
[87] Mercier B, Verlingue C, Lissens W, et al. Is congenital bilateral absence of vas deferens a primary form of cystic fibrosis? Analyses of the CFTR gene in 67 patients. Am J Hum Genet 1995;56:272–7.
[88] Rave-Harel N, Madgar I, Goshen R, et al. CFTR haplotype analysis reveals genetic heterogeneity in the etiology of congenital bilateral aplasia of the vas deferens. Am J Hum Genet 1995;56:1359–66.

[89] Jarzabek K, Zbucka M, Pepinski W, et al. Cystic fibrosis as a cause of infertility. Reprod Biol 2004;4:119–29.
[90] Meng MV, Black LD, Cha I, Ljung BM, Pera RA, Turek PJ. Impaired spermatogenesis in men with congenital absence of the vas deferens. Hum Reprod 2001;16:529–33.
[91] van der Ven K, Messer L, van der Ven H, Jeyendran RS, Ober C. Cystic fibrosis mutation screening in healthy men with reduced sperm quality. Hum Reprod 1996;11:513–7.
[92] Dohle GR, Halley DJ, Van Hemel JO, et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. Hum Reprod 2002;17:13–6.
[93] Gallati S, Hess S, Galie-Wunder D, Berger-Menz E, Bohlen D. Cystic fibrosis transmembrane conductance regulator mutations in azoospermic and oligospermic men and their partners. Reprod Biomed Online 2009;19:685–94.
[94] Pallares-Ruiz N, Carles S, Des Georges M, et al. Complete mutational screening of the cystic fibrosis transmembrane conductance regulator gene: cystic fibrosis mutations are not involved in healthy men with reduced sperm quality. Hum Reprod 1999;14:3035–40.
[95] Ravnik-Glavac M, Svetina N, Zorn B, Peterlin B, Glavac D. Involvement of CFTR gene alterations in obstructive and nonobstructive infertility in men. Genet Test 2001;5:243–7.
[96] Larriba S, Bonache S, Sarquella J, et al. Molecular evaluation of CFTR sequence variants in male infertility of testicular origin. Int J Androl 2005;28:284–90.
[97] Tamburino L, Guglielmino A, Venti E, Chamayou S. Molecular analysis of mutations and polymorphisms in the CFTR gene in male infertility. Reprod Biomed Online 2008;17:27–35.
[98] Poulsen JH, Fischer H, Illek B, Machen TE. Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator. Proc Natl Acad Sci U S A 1994;91:5340–4.
[99] Ko SB, Zeng W, Dorwart MR, et al. Gating of CFTR by the STAS domain of SLC26 transporters. Nat Cell Biol 2004;6:343–50.
[100] Park HW, Nam JH, Kim JY, et al. Dynamic regulation of CFTR bicarbonate permeability by $[Cl^-]_i$ and its role in pancreatic bicarbonate secretion. Gastroenterology 2010;139:620–31.
[101] Xu WM, Shi QX, Chen WY, et al. Cystic fibrosis transmembrane conductance regulator is vital to sperm fertilizing capacity and male fertility. Proc Natl Acad Sci U S A 2007;104:9816–21.
[102] Chan HC, Ruan YC, He Q, et al. The cystic fibrosis transmembrane conductance regulator in reproductive health and disease. J Physiol 2009;587:2187–95.
[103] Josserand RN, Bey-Omar F, Rollet J, et al. Cystic fibrosis phenotype evaluation and paternity outcome in 50 males with congenital bilateral absence of vas deferens. Hum Reprod 2001;16:2093–7.
[104] Gilljam M, Moltyaner Y, Downey GP, et al. Airway inflammation and infection in congenital bilateral absence of the vas deferens. Am J Respir Crit Care Med 2004;169:174–9.
[105] Mackay RJ, Florkowski CM, George PM, Sies CW, Woods S. Uncertainty of sweat chloride testing: does the right hand know what the left hand is doing? Ann Clin Biochem 2008;45:535–8.
[106] Rose JB, Ellis L, John B, et al. Does the Macroduct collection system reliably define sweat chloride concentration in subjects with intermediate results? Clin Biochem 2009;42:1260–4.
[107] Meniru GI, Gorgy A, Podsiadly BT, Craft IL. Results of percutaneous epididymal sperm aspiration and intracytoplasmic sperm injection in two major groups of patients with obstructive azoospermia. Hum Reprod 1997;12:2443–6.
[108] Chen JM, Férec C. Chronic pancreatitis: genetics and pathogenesis. Annu Rev Genomics Hum Genet 2009;10:63–87.
[109] Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS. Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. N Engl J Med 1998;339:653–8.
[110] Sharer N, Schwarz M, Malone G, et al. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. N Engl J Med 1998;339:645–52.
[111] Noone PG, Zhou Z, Silverman LM, Jowell PS, Knowles MR, Cohn JA. Cystic fibrosis gene mutations and pancreatitis risk: relation to epithelial ion transport and trypsin inhibitor gene mutations. Gastroenterology 2001;121:1310–9.
[112] Audrézet MP, Chen JM, Le Maréchal C, et al. Determination of the relative contribution of three genes – the cystic fibrosis transmembrane conductance regulator gene, the cationic trypsinogen gene, and the pancreatic secretory trypsin inhibitor gene – to the etiology of idiopathic chronic pancreatitis. Eur J Hum Genet 2002;10:100–6.
[113] Cohn JA. Reduced CFTR function and the pathobiology of idiopathic pancreatitis. J Clin Gastroenterol 2005;39:S70–7.
[114] Bishop MD, Freedman SD, Zielenski J, et al. The cystic fibrosis transmembrane conductance regulator gene and ion channel function in patients with idiopathic pancreatitis. Hum Genet. 2005;118:372–81.
[115] Cohn JA, Neoptolemos JP, Feng J, et al. Increased risk of idiopathic chronic pancreatitis in cystic fibrosis carriers. Hum Mutat 2005;26:303–7.
[116] Weiss FU, Simon P, Bogdanova N, et al. Complete cystic fibrosis transmembrane conductance regulator gene sequencing in patients with idiopathic chronic pancreatitis and controls. Gut 2005;54:1456–60.
[117] Keiles S, Kammesheidt A. Identification of CFTR, PRSS1, and SPINK1 mutations in 381 patients with pancreatitis. Pancreas 2006;33:221–7.
[118] Tzetis M, Kaliakatsos M, Fotoulaki M, et al. Contribution of the CFTR gene, the pancreatic secretory trypsin inhibitor gene (SPINK1) and the cationic trypsinogen gene (PRSS1) to the etiology of recurrent pancreatitis. Clin Genet 2007;71:451–7.
[119] de Cid R, Ramos MD, Aparisi L, et al. Independent contribution of common CFTR variants to chronic pancreatitis. Pancreas. 2010;39:209–15.
[120] Chen JM, Férec C. The true culprit within the SPINK1 p.N34S-containing haplotype is still at large. Gut 2009;58:478–80.
[121] Masson E, Le Maréchal C, Levy P, et al. Co-inheritance of a novel deletion of the entire SPINK1 gene with a CFTR missense mutation (L997F) in a family with chronic pancreatitis. Mol Genet Metab 2007;92:168–75.
[122] Derichs N, Schuster A, Grund I, et al. Homozygosity for L997F in an individual with normal clinical and chloride secretory phenotype provides evidence that this cystic fibrosis transmembrane conductance regulator (CFTR) mutation does not cause cystic fibrosis. Clin Genet 2005;67:529–31.
[123] Perri F, Piepoli A, Stanziale P, Merla A, Zelante L, Andriulli A. Mutation analysis of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, the cationic trypsinogen (PRSS1) gene, and the serine protease inhibitor, Kazal type 1 (SPINK1) gene in patients with alcoholic chronic pancreatitis. Eur J Hum Genet 2003;11:687–92.
[124] da Costa MZ, Guarita DR, Ono-Nita SK, et al. CFTR polymorphisms in patients with alcoholic chronic pancreatitis. Pancreatology 2009;9:173–81.
[125] Bhatia E, Durie P, Zielenski J, et al. Mutations in the cystic fibrosis transmembrane regulator gene in patients with tropical calcific pancreatitis. Am J Gastroenterol 2000;95:3658–9.
[126] Rajesh G, Elango EM, Vidya V, Balakrishnan V. Genotype-phenotype correlation in 9 patients with tropical pancreatitis and identified gene mutations. Indian J Gastroenterol 2009;28:68–71.
[127] Segal I, Yaakov Y, Adler SN, et al. Cystic fibrosis transmembrane conductance regulator ion channel function testing in recurrent acute pancreatitis. J Clin Gastroenterol 2008;42:810–4.
[128] Pasteur MC, Helliwell SM, Houghton SJ, et al. An investigation into causative factors in patients with bronchiectasis. Am J Respir Crit Care Med 2000;162:1277–84.
[129] Barker AF. Bronchiectasis. N Engl J Med 2002;346:1383–93.
[130] Bombieri C, Benetazzo M, Saccomani A, et al. Complete mutational screening of the CFTR gene in 120 patients with pulmonary disease. Hum Genet 1998;103:718–22.
[131] Casals T, De-Garcia J, Gallego M, et al. Bronchiectasis in adult patients: an expression of heterozyosity for CFTR gene mutations? Clin Genet 2004;65:490–5.
[132] Coste A, Girodon E, Louis S, et al. Atypical sinusitis in adults must

lead to looking for cystic fibrosis and primary ciliary dyskinesia. Laryngoscope 2004;114:839–43.
[133] Divac A, Nikolic A, Mitic-Milikic M, et al. CFTR mutations and polymorphisms in adult with disseminated bronchiectasis: a controversial issue. Thorax 2005;60:85.
[134] Girodon E, Cazeneuve C, Lebargy F, et al. CFTR gene mutations in adults with disseminated bronchiectasis. Eur J Hum Genet 1997;5:149–55.
[135] Tzetis M, Efthymiadou A, Strofalis S, et al. CFTR gene mutations – including three novel nucleotide substitutions – and haplotype background in patients with asthma, disseminated bronchiectasis and chronic obstructive pulmonary disease. Hum Genet 2001;108:216–21.
[136] King PT, Freezer NJ, Holmes PW, Holdsworth SR, Saart DD. Role of CFTR mutations in adult bronchiectasis. Thorax 2004;59:357–8.
[137] Pignatti PF, Bombieri C, Benetazzo M, et al. CFTR gene variant IVS8-5T in disseminated bronchiectasis. Am J Hum Genet 1996;58:889–92.
[138] Ziedalski T, Kao P, Heing NR, Jacobs SS, Ruoss SJ. Prospective analysis of cystic fibrosis transmembrane regulator mutations in adults with bronchiectasis or pulmonary nontuberculous mycobacterial infections. Chest 2006;130:995–1002.
[139] Carter K, Currie GP, Devereux G. Patients with bronchiectasis: look for specific causes. QJM 2006;99:196–7.
[140] Mason AC, Nakielna BE. Newly diagnosed cystic fibrosis in adults: pattern and distribution of bronchiectasis in 12 cases. Clin Radiol 1999;54:507–12.
[141] Mott LS, Gangell CL, Murray CP, Stick SM, Sly PD; AREST CF. Bronchiectasis in an asymptomatic infant with cystic fibrosis diagnosed following newborn screening. J Cyst Fibros 2009;8:285–7.
[142] Noone PG Knowles MR. "CFTR-opathies": disease phenotypes associated with cystic fibrosis transmembrane regulator gene mutations. Respir Res 2001;2:328–32.
[143] Sermet-Gaudelus I, Dechaux M, Vallee B, et al. Chloride transport in nasal ciliated cells of cystic fibrosis heterozygotes. Am J Respir Crit Care Med 2005;171:1026–31.
[144] Veeze HJ. Pathophysiological aspects of cystic fibrosis: genotypes, phenotypes and intestinal current measurements. PhD thesis. Erasmus University, Rotterdam, 1995.
[145] Wilschanski M, Famini H, Strauss-Liviatan N, et al. Nasal potential difference measurements in patients with atypical cystic fibrosis. Eur Respir J 2001;17:1208–15.
[146] Southern KW, Noone PG, Bosworth DG, LeGrys VA, Knowles MR, Barker PM. A modified technique for measurement of nasal transepithelial potential difference in infants. J Pediatr 2001;139:353–8.
[147] Sermet-Gaudelus I, Girodon E, Huet F, et al. Nasal potential difference in cystic fibrosis diagnosis of very young children. J Pediatr 2007;150:e34–5.
[148] Delmarco A, Pradal U, Cabrini G, Bonizzato A, Mastella G. Nasal potential difference in cystic fibrosis patients presenting borderline sweat test. Eur Respir J 1997;10:1145–9.
[149] Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Standards for Reporting of Diagnostic Accuracy. Clin Chem 2003;49:1–6.
[150] Veeze HJ, Sinaasappel M, Bijman J, Bouquet J, de Jonge HR. Ion transport abnormalities in rectal suction biopsies from children with cystic fibrosis. Gastroenterology 1991;101:398–403.
[151] Veeze HJ, Halley DJ, Bijman J, de Jongste JC, de Jonge HR, Sinaasappel M. Determinants of mild clinical symptoms in cystic fibrosis patients: residual chloride secretion measured in rectal biopsies in relation to the genotype. J Clin Invest 1994;93:461–6.
[152] de Jonge HR, Ballmann M, Veeze HJ, et al. Ex vivo CF diagnosis by intestinal current measurements (ICM) in small aperture, circulating Ussing chambers. J Cyst Fibros 2004;3(Suppl 2):159–63.
[153] Mall M, Hirtz S, Gonska T, Kunzelmann K. Assessment of CFTR function in rectal biopsies for the diagnosis of cystic fibrosis. J Cyst Fibros 2004;3(Suppl 2):165–9.
[154] Bronsveld I, Mekus F, Bijman J, et al. and The European Twin and Sibling Study Consortium. Residual chloride secretion in intestinal tissue of ΔF508 homozygous twins and siblings with cystic fibrosis. Gastroenterology 2000;119:32–40.
[155] Derichs N, Mekus F, Bronsveld I, et al. Cystic fibrosis transmembrane conductance regulator (CFTR)-mediated residual chloride secretion does not protect against early chronic Pseudomonas aeruginosa infection in p.F508del homozygous cystic fibrosis patients. Pediatr Res 2004;55:69–75.
[156] Bronsveld I, Mekus F, Bijman J, et al. Chloride conductance and genetic background modulate the cystic fibrosis phenotype of ΔF508 homozygous twins and siblings. J Clin Invest 2001;108:1705–15.
[157] Stanke F, Ballmann M, Bronsveld I, et al. Diversity of the basic defect of homozygous CFTR mutation genotypes in humans. J Med Genet 2008;45:47–54.
[158] Mayell SJ, Munck A, Craig JV, et al. A European consensus for the evaluation and management of infants with an equivocal diagnosis following newborn screening for cystic fibrosis. J Cyst Fibros 2009;8:71–8.
[159] Derichs N, Sanz J, von Kaenel T, et al. Intestinal current measurement for diagnostic classification of patients with questionable cystic fibrosis: validation and reference data. Thorax 2010;65:594–9.
[160] Jaron R, Yaakov Y, Rivlin J, et al. Nasal potential difference in non-classic cystic fibrosis-long term follow up. Pediatr Pulmonol 2008;43:545–9.
[161] Groman JD, Meyer ME, Wilmott RW, Zeitlin PL, Cutting GR. Variant cystic fibrosis phenotypes in the absence of CFTR mutations. N Engl J Med 2002;347:401–7.
[162] Dohle GR, Veeze HJ, Overbeek SE, et al. The complex relationships between cystic fibrosis and congenital bilateral absence of the vas deferens: clinical, electrophysiological and genetic data. Hum Reprod 1999;14:371–4.
[163] Ockenga J, Stuhrmann M, Ballmann M, et al. Mutations of the cystic fibrosis gene but not cationic trypsinogen gene are associated with recurrent or chronic idiopathic pancreatitis. Am J Gastroenterol 2000;95:2061–7.
[164] Wilschanski M, Dupuis A, Ellis L, et al. Mutations in the cystic fibrosis transmembrane regulator gene and in vivo transepithelial potentials. Am J Respir Crit Care Med 2006;174:787–94.
[165] Drumm ML, Wilkinson DJ, Smit LS, et al. Chloride conductance expressed by ΔF508 and other mutant CFTRs in *Xenopus* oocytes. Science. 1991;254:1797–9.
[166] Sheppard DN, Rich DP, Ostedgaard LS, Gregory RJ, Smith AE, Welsh MJ. Mutations in CFTR associated with mild-disease-form Cl^- channels with altered pore properties. Nature. 1993;362:160–4.
[167] Sheppard DN, Ostedgaard LS, Winter MC, Welsh MJ. Mechanism of dysfunction of two nucleotide binding domain mutations in cystic fibrosis transmembrane conductance regulator that are associated with pancreatic sufficiency. EMBO J. 1995;14:876–83.
[168] Sheppard DN, Ostedgaard LS. Understanding how cystic fibrosis mutations cause a loss of Cl^- channel function. Mol Med Today. 1996;2:290–7.
[169] Krasnov KV, Tzetis M, Cheng J, Guggino WB, Cutting GR. Localization studies of rare missense mutations in cystic fibrosis transmembrane conductance regulator (CFTR) facilitate interpretation of genotype-phenotype relationships. Hum Mutat. 2008;29:1364–72.