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Review

CFTR complex alleles and phenotypic variability in cystic fibrosis disease

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Abstract

Cystic fibrosis (CF) is inherited by CFTR (cystic fibrosis transmembrane conductance regulator) gene mutations. A variety of mutations have been identified in the CFTR gene that may be associated with cystic fibrosis, and these mutations demonstrate extensive molecular genetic heterogeneity in this disease. Little is known about the molecular mechanism by which mutations affect CFTR function, and only a minority of mutations have been characterized by functional studies. There has been an increase in the number of complex alleles. This may partly explain the difficulty in establishing genotype-phenotype correlations and complicate genetic counseling and diagnosis in some cases. Therefore, the identification of complex alleles has several important implications for recessive disorders. This will facilitate diagnosis; improve judgments concerning prognosis, and enable appropriate genetic counselling for affected families. This review describes the complex cystic fibrosis allele to better understand the contribution of this allele in the wide phenotypic variability of cystic fibrosis disease. It occurs in the complex allele that the second cis mutation can modulate the effects of the first mutation or vice versa. The phenotypic variability between CF or CFTR-RD (CFTR-related disease) patients may be due to several factors, including different genetic and environmental backgrounds. It is important to determine the allele complex so that optimal treatment can be established.

Keywords: CFTR, Complex alleles, Phenotypic variability, Implication for diagnosis and treatment.

1. Cystic fibrosis

Cystic fibrosis (CF) is a common and life-threatening genetic disease with an incidence of 1 in 3,000 Caucasians, 1 in 4,000-10,000 for Latin Americans, and 1 in 15,000-20,000 African Americans [1,2]. It is inherited by cystic fibrosis transmembrane conductance regulator) (CFTR) gene mutations. This gene located on chromosome 7q inhibits folding of the protein or its channel activity [3], resulting in a chloride ion channel defect [4]. Defects in CFTR biosynthesis or channel activity cause accumulation of thick dehydrated mucus in the airways that ultimately lead to deterioration and eventual failure of the lungs due to chronic lung infections. This defect affects mucus-producing organs including the lungs, pancreas, sweat glands, and reproductive organs [5]. Classical cystic fibrosis is characterized by chronic obstructive pulmonary disease, pancreatic dysfunction, and elevated sweat electrolyte levels. The clinical review of cystic fibrosis is dominated by progressive lung disease, with many infections and reduced lung function [6]. Associated pancreatic insufficiency (PI), which characterizes the most severe form of cystic fibrosis (PI-CF), presents early and leads to difficulty digesting and assimilating fat. In addition to classic cystic fibrosis, several "atypical" or "benign" forms exist. These forms are characterized by a less severe phenotype, ranging from diseases alone (eg, idiopathic pancreatitis or diffuse bronchiectasis) to cystic fibrosis with pancreatic insufficiency [7]. The phenotype variability is due to the presence of different CFTR mutations, which can be classified as 'severe' (CF) or 'mild' (CFm) mutations [8]. Classic PI-CF is associated with the presence of two severe mutations (the 'CF/CF' genotype), whereas homozygous CFm/CFm or heterozygous CF/CFm genotypes produce atypical CF [7].

Although CF is a monogenic disease, there is a wide variability in clinical phenotype expression among patients with the same mutation due to mutant CFTR-related functions, modifier genes, excessive inflammatory response, dysregulation of cytokines secretion, and impaired innate immunity as well as interaction of these factors and the environment [9]. The advances in CF therapy extended the mean length of life in these patients up to 36 years and improved their life quality [10]. A wide range of muta-

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tions identified in the CFTR gene may be associated with cystic fibrosis disease and these mutations demonstrate the extensive molecular genetic heterogeneity in this disease. The identification of the CFTR gene in 1989, has led to many new discoveries. Even after extensive investigations of the CFTR gene, a significant percentage of the CF alleles remain unknown in most of the studied populations across the world. Some of the disease-related alleles that remain unidentified could be accounted for by the presence of large genomic rearrangements in this gene.

The study of CFTR mutations has provided important insights into the regulation of almost all aspects of CFTR biology including biosynthesis [11,12], gating [13,14], endocytosis [15,16], and degradation [17,18]. The first therapeutic strategy is targeting the basic defects associated with mutant CFTR proteins to correct these defects in vivo [19,20]. Consequently, the characterization of the processes by which CFTR is regulated in normal and disease states will provide critical insights into normal CFTR regulation as well as aid in the development of new treatment approaches.

The most common CF mutation is the deletion of 3 nucleotides, resulting in the deletion of a single phenylalanine (F) residue at position 508 (F508del) [21,22]. This mutation is associated with a severe form of the disease, with more than 90% of CF patients having at least this allele. Almost half of the CF patients are homozygous for this mutation. This allele encodes an unstable and inefficiently folded CFTR protein, the major consequence being the failure of the mutant protein to be correctly processed and delivered to its proper cellular location in the plasma membrane [23,24]. Therefore, the mutant protein is retained in the endoplasmic reticulum (ER) and rapidly targeted for degradation [17,23].

2. CFTR-related Disorders (CFTR-RD)

Mutations in the CFTR gene are also involved in diseases that share a part of the CF symptoms (CFTR-RD), such as congenital bilateral absence or atrophy of the vas deferens (CBAVD), obstructive azoospermia, disseminated bronchiectasis, diffuse panbronchiolitis, pulmonary emphysema, allergic bronchopulmonary aspergillosis, asthma, chronic pancreatitis and neonatal hypertrypsinaemia. Mutated CFTR may play a role in their pathogenesis, but more likely, these conditions result from multifactorial non-CFTR genetic and nongenetic environmental influences [25]. For some diseases, it should be noted that the discovery of involvement of CFTR in disease is based on limited or even single studies (http://www.cfww.org/docs/who/2002). There is increasing appreciation, however, for the presence of diseases, which while they do not fit the criteria for classic cystic fibrosis, are caused by dysfunction of CFTR [26,27,28,29]. Therefore, the spectrum of CF or CFTR-RD diseases could be seen with CFTR dysfunction, due to mutations in the CFTR gene as follows:

2.1. CBAVD

CBAVD patients semen analysis shows azoospermia [30,31]. They frequently carry mutations in the CFTR gene: around 70% of CBAVD patients have one known CFTR mutation and 10% have two. The T5 allele in CFTR intron 9 inducing skipping exon 10 is 4 to 6-fold higher than among in the normal or CF population [32]. CBAVD patients are frequently heterozygous for a known disease-associated CFTR mutation and this T5 allele. In some cases, the proportion of full-length CFTR transcripts is increased in the adult vas deferens compared to nasal epithelial cells of the same individual [33]. As CBAVD patients have no pulmonary disease, one hypothesis is that the amounts of functional mRNA exceed the necessary 'threshold' transcript level for a non-CF phenotype in these tissues. Moreover, the prevalence of natural polymorphisms in the CFTR gene in combination with the T5 allele affects the quantity/quality of CFTR, resulting in a partial CF phenotype [34]. Moreover, in a recent study [35] six genotypes were detected in CBAVD patients and only the correlation between TG12-13-5T haplotype with CBAVD was high, suggesting that this haplotype increases the risk of this disease.

2.2. Disseminated bronchiectasis

Bronchiectasis is a lung disease that causes abnormal stretching and enlargement of the bronchi and is often found to be idiopathic. Advances in lung imaging facilitate the diagnosis of bronchiectasis, however, this did not lead to the expected increase in the number of patients. On the contrary, it was found that the occurrence of bronchiectasis has dropped significantly in the last decades. The decrease might be due to the successful treatment of lung infections with antibiotics and current vaccination programs preventing such infections. Furthermore, the etiology of the disease in the remaining patients is believed to be congenital [36]. Mutations in the CFTR gene have also been implicated in obstructive lung diseases other than cystic fibrosis. For example, the frequency of the T5 allele was significantly higher in 16 patients with disseminated bronchiectasis than in the control [37]. Besides, 32 patients with disseminated bronchiectasis were analyzed and 13 CFTR mutations were detected [38].

2.3. Pancreatitis

This disorder involves atrophy of pancreatic acinar tissue, fibrosis and inflammation. In several studies, an increased incidence of CFTR mutations was observed in patients with pancreatitis. For example, 14% carried CFTR mutations on one allele and 10% had the T5 allele (twice the expected frequency) [39]. Another study, however, indicated that the frequency of the T5 allele was not higher in patients with pancreatitis than in the general population [40]. Therefore, the involvement of the T5 polymorphism in this disorder remains unclear and environmental and genetic factors are likely implicated. Nevertheless, CFTR mutations are associated with idiopathic chronic pancreatitis. Indeed, 37% of 27 patients with idiopathic chronic pancreatitis (ICP), carried at least one abnormal CFTR allele [27]. Furthermore, in two other studies [41,42] at least 30% of patients had CFTR mutations. In a recent American study [43], between 28% to 80% of patients are classified as having "idiopathic Chronic Pancreatitis." Up to 50% of these individuals have mutations of the trypsin inhibitor gene (SPINK1) or CFTR gene.

2.4. Other disorders

Other sino-pulmonary syndromes have been studied to see if there is an association with mutant CFTR such as sinusitis, allergic bronchopulmonary aspergillosis, and asthma. They could mostly reflect non-CFTR gene modifiers and environmental influences [44].

2.4.1. Sinusitis

Nearly all people with CF will develop chronic rhinosinusitis. It is characterised by viscous mucus, impaired mucociliary clearance and chronic inflammation/infection of the sinonasal cavity. Current treatment for CF sinusitis includes the use of hypertonic saline, topical and systemic steroids, antibiotics and endoscopic surgery. Research investigating novel therapies designed to target the primary defect of CF is showing promise for reversal of CF sinus disease, in addition to potential for disease prevention [45]. The combination of a severe mutation on one allele with a mild sequence variant on the opposite allele may be responsible for this disease [46]. By analogy, enough residual CFTR function is necessary to protect against early classic sino-pulmonary disease and a pancreatic phenotype, but clearly, other non-CFTR factors may also be at play.

2.4.2. Allergic bronchopulmonary aspergillosis

Genetic factors may play a role in the pathogenesis of allergic bronchopulmonary aspergillosis (ABPA) in some patients suffering from asthma and CF. In a small number of patients who met criteria for ABPA, there was a higher frequency of abnormal CFTR alleles than expected. Mutations in the CFTR gene may play a role in ABPA, either as a result of heterozygosity alone (and 50% CFTR function), or heterozygosity plus other genetic factors that were not detected [26].

2.4.3. Asthma

There are conflicting data on whether patients with asthma have more or less of the CFTR gene [47-56]. No evidence supports a link between asthma and an abnormality in the CFTR, suggesting a small role in the overall pathogenesis of this disease, with a much larger role played by genetic factors and environmental influences as probably in ABPA [26].

2.4.4 Diabetes

The cystic fibrosis-related diabetes (CFRD) affects 40-50% of CF adults. This comorbidity is an endocrine disorder and significantly influences the pulmonary function and longevity of CF patients, yet a lack of consensus on the best methods to diagnose and treat CFRD remains [57,58].

3. The CFTR gene structure

In 1985, the CFTR gene was localized to 7q31.2 on the long arm of chromosome 7 [59]. The gene sequence was identified in 1989 [3,4,21] and its 27 exons encode a protein of 1480 amino acids, known as CFTR (Figure 1). To date, more than 2,000 mutations have been reported, many of which are rare and some may not cause clinical signs or symptoms. Different mutations affect CFTR function in different ways. Some lead to abnormal CFTR production and others affect intracellular CFTR processing, channel function, or a combination of these [32].

The most common mutation in the world is F508del. Deletion of three base pairs in exon 10 of the CFTR gene results in a lack of phenylalanine at position 508. A mutant CFTR protein, which cannot fold into the proper conformation, is produced. Quality control mechanisms inside the cell destroy this abnormal protein before it can reach the cell surface, where its main normal function is to act as a channel through which chloride ions can enter and leave cells [23].

CFTR gene is a member of the ABC Transporter Family (for ATP Binding Cassette Transporters); called this because they all bind and hydrolyze ATP. While most seem to function as transporters, some such as CFTR appear to function mainly as ion channels and/or regulators of other ion channels. There are over 30 known ABC transporters; some of which are found in prokaryotes as well as in eukaryotes. There is often in excess of 30% sequence similarity between the ABC family members in prokaryotes and eukaryotes. Humans have approximately 12 different ABC Transporter genes including the one for CFTR [26].

4. Inheritance of CFTR gene and possible genetic risks

Cystic fibrosis has a simple Mendelian autosomal recessive inheritance. This means that people with cystic fibrosis have two copies of the mutated CFTR gene, one inherited from both parents. Carriers have one normal CFTR gene and one mutant gene and their health is not affected because the normal CFTR gene ensures sufficient protein production to support normal cellular function. Carriers have a 50% chance of transmitting the mutated CFTR gene to their offspring. When both parents are carriers, there is a 25% chance that the child will have CF in each pregnancy, a 25% chance that the child will have two normal CFTR genes and a 50% chance that the child will have CFTR are carriers of the disease.

5. Protein structure

The cystic fibrosis transmembrane conductance regulator (CFTR) is a membrane-bound glycoprotein with a molecular mass of 170,000. It is a member of the ATP binding cassette (ABC) superfamily of proteins which includes several clinically important proteins such as P-glycoprotein (P-gp), multidrug resistance-associated protein and the TAP transporters. The protein is made of five domains (Figures 1 and 2). These include two transmembrane do-

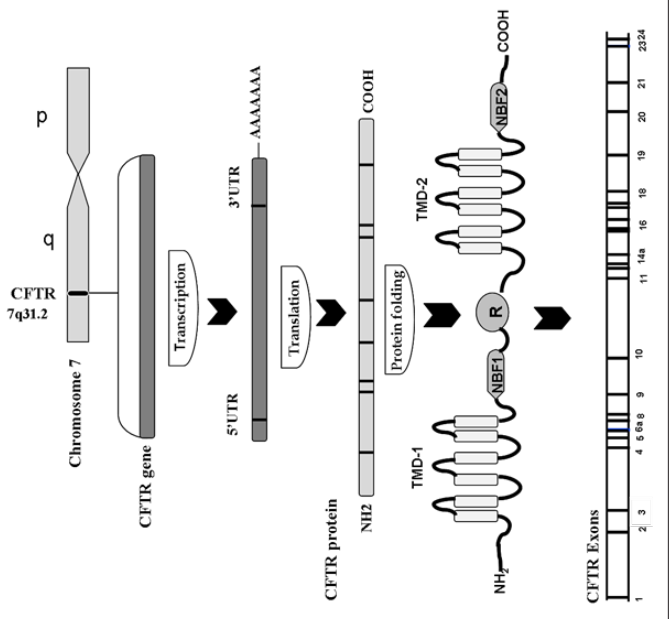

Fig. 1. Diagram showing CFTR and resulting protein. The CFTR protein is comprised of five domains: (2) TMD, transmembrane membrane domain; (2) NBD, nucleotide-binding domain, and R, regulatory domain.

mains (TMDs), that, as their name suggests, reside in the membrane of cell, two nucleotide-binding domains (NBDs) that are crucial to ATP binding, and are regulatory domains [60]. The R-domain is a specific sequence of CFTR within the ABC superfamily, which is comprised of many charged amino acids.

5.1 Nucleotide Binding Domains (NBD)

NBD1 and NBD2 are arranged in a head-to- tail orientation and contain two ATP-binding sites each composed of the Walker A and B motifs. The Walker A sequences are GSTGAGKT and GRTGSGKST respectively in NBD1 and NBD2. The Walker B sequences are LYLLDS and ILLLD respectively in NBD1 and NBD2. The Q loop in NBD1 is QFSWIMPG and QKVFIFSG in NBD2. The H loop in NBD2 role is required for efficient chloride channel closing [61].

5.2. The Regulatory domain (R).

CFTR encodes a unique ABC transporter protein, which contains specifically another domain R (regulatory domain). This intrinsically unstructured domain controls channel activity via PKA-mediated phosphorylation. This divergence appears recently and induces an insertion of around 240 amino acids in the middle of protein [62,63, CFMD: http://www.genet.sickkids.on.ca]. The R domain, encoded by exon 14, spans the region between NBD1 and the second transmembrane region. It includes some potential sites for phosphorylation by cAMP-dependent PKA or PKC. The activity of CFTR as an ion channel depends upon phosphorylation of the R domain and binding of ATP to the nuclear binding domains. The N terminal portion of the R domain (RD1) is highly conserved between species.

5.3. Extracellular domains.

More than 4% of the CFTR protein is found in the extracellular loops (see the gene sequence and structure section). The loops namely M1-M2, M3-M4, M5-M6, M7-M8, M9-M10 and M11-M12 are designated according to the membrane-spanning regions they connect. These extracellular domains are very short, except for the first extracellular loop of each TMD (M1-M2 and M7-M8). Two glycosylation sites were described by [64]: Asn 894 and Asn 900 on the first extra loop of TMD2. These sites are essential for the maturation of CFTR protein.

5.4. Transmembrane domains.

19 % of the CFTR protein constitutes the twelve transmembrane domains (M1 - M12). Higgins [65] described six helix-alpha per TMD. A high number of their residues play an efficient role in regulating pore function: in particular, six positively charged residues well conserved across species [K95 (M1), R134 (M2), R334 (M6), K335 (M6), R347 (M6) and R1030 (M10]. R334Q/W and R347C/H/L/P are CF mutations [66].

5.5. The intracellular domains.

The intracellular loops (ICL1 - 4) are very important for the processing of CFTR and correct delivery to the cell membrane. In vitro studies using site-directed mutagenesis methods have shown that ICL2 and ICL3 may be closed to the intracellular opening of the CFTR pore. Indeed, the conductance state of the channel will be altered with variations in these regions (http://users.ox.ac.uk/~genemed/cysticfibrosis/protein.html).

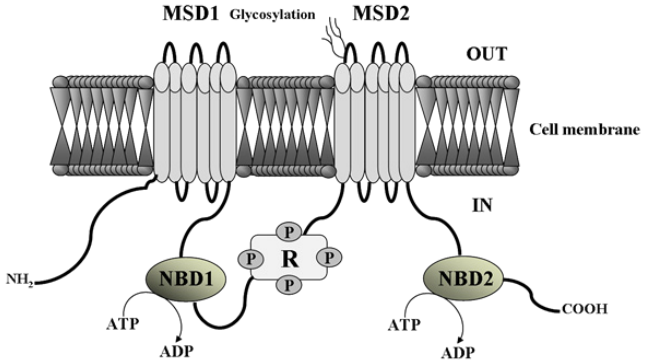

Fig. 2. The proposed domain structure of the CFTR protein within the cell membrane shows five distinct domains: two nucleotide-binding domains (NBD1 & NBD2), two membrane-spanning domains (MSD1 & MSD2), and a regulatory region (R domain).

6. Mutations in the CFTR gene and its consequences

Phenotype heterogeneity is partly due to CFTR mutational and genotype heterogeneity, interplaying with other genetic factors/modifiers and environmental factors. CFTR mutations induce cystic fibrosis disease. It is the most common fatal disease in the Caucasian population. Milder forms of CFTR-related disorders are called the CFTR-RD [67]. Patients with the severe form of the disease have different types of CF-causing mutations in each copy of the gene, whereas patients with a CFTR-RD are heterozygous with a CF mutation in one copy and a mild mutation in the other allele, or carry mild mutations in both copies.

More than 2000 sequence alterations have been detected in the CFTR gene, most of which are presumed to be pathologic mutations. About half of the reported CFTR mutations are amino acid substitutions (missense mutations) and about 20% are splicing mutations. The remainders are nonsense and frameshift mutations (Cystic Fibrosis Genetic Analysis Consortium, CFGAC). One single mutation, F508del accounts for about 70% of CF chromosomes worldwide. The profile of CFTR mutations is different between populations due to different factors, such as ethnic background and geographical location [68]. Generally, a higher frequency of this mutation is observed for northern Europeans in comparison to southern European populations [69]. F508del accounts for about 54% of CF chromosomes in Greece; therefore the percentage of non-F508del chromosomes associated with CF is high [70]. Most reported mutations, including those specific to the Greek population, have only been described at the DNA level and have not been studied at the functional and biochemical level. It has been shown that different mutations in CF can be classified on the basis of defects in protein production and function [69, 71]. Ultimately, however, all the disease-causing mutations result in defective cAMP-regulated Cl– secretion by epithelial cells, though for various reasons, namely defective protein production (class I), defective protein processing (class II), defective regulation (class III), defective conduction (class IV), or reduced synthesis via missense or splice junction mutations (class V) [24, 32; 72].

The cystic fibrosis transmembrane conductance regulator is a multidomain protein characterized by a complex

regulation. As a result, nucleotide alterations in the CFTR gene can disrupt CFTR function by different mechanisms depending on their nature, and on the domain in which these alterations occur. According to these observations, CFTR mutations were subdivided into 6 classes [73]. This subdivision is still basically correct and straightforward. However, insight into CF-related phenotypes associated with particular CFTR mutations [8] and into the regulatory properties of CFTR, on other ion channels [74], has made the extension of the number of classes to six (Figure 3). F508del, the most common CF mutation, is found on at least one chromosome in 90% of affected individuals and it is a processing defective mutation [75]. The F508del protein is defective in folding so it fails to reach the cell surface because it accumulates in the ER (endoplasmic reticulum) and is rapidly degraded [23, 76].

The mutations in the CFTR gene are grouped into six classes (Figure 3). These include class I (defective protein synthesis, where there is no CFTR protein at the apical membrane), class II (abnormal/ defective processing and trafficking, where there is no CFTR protein at the apical membrane), class III (defective regulation, where there is a normal amount of non-functional CFTR at the apical membrane), class IV (decreased conductance, where there is a normal amount of CFTR with some residual function at the apical membrane), class V (reduced or defective synthesis/trafficking, where there is a decreased amount of functional CFTR at the apical membrane), and class VI (decreased stability, where there is a functional but unstable CFTR at the apical membrane) [7, 77]. To simplify the description of mutations, the new nomenclature is used to name the number of exon and intron, except if it is mentioned legacy name for mutations in the promotor and 5'UTR, and the name of mutations are specified in 1 letter genetic code.

The most common mutation worldwide is the F508del, class II, with varying frequency among ethnic groups [78]. Mutation frequencies in Ashkenazi Jews are different e.g. F508del accounts for 27% and W1282X for 51% of their mutations [79]. It was important to notice that cystic fibrosis is rare in blacks and Asians, but is the most common lethal recessive genetic disease in the Caucasian populations. However, it was interesting to note that F508del mutation is the most frequent mutation but depends on the localization of population following a gradient increase from South to North. So rare mutations detected in European populations could be more frequent in Arabs, who are of Caucasian descent. For the same reason, new mutations could be also detected. For example, the mutation N1303K is sometimes more frequent than F508del in these populations[80]. New variations could be also discovered as the results obtained by [81, 82] in the Egyptian population.

6.1. Class I mutations

The first class of mutations includes nonsense mutations, frame shift mutations and splice site mutations. They give rise to the formation of premature STOP codon or alteration of critical RNA signal. Consequently, they fail to synthesize full-length transcripts and or aberrant proteins. Proteins containing deleted or novel amino acid sequences are often unstable and are degraded relatively rapidly. As protein synthesis defects, class I mutations have little or no functional CFTR.

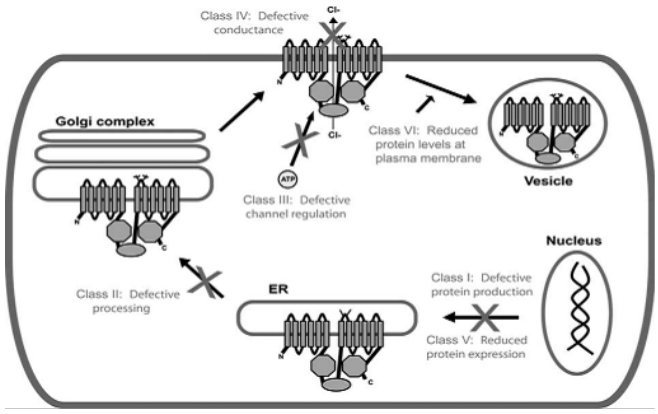

Fig. 3. Classification of CF Causing Mutations. Disease-causing mutations in the CFTR protein are grouped into 6 classes based on the affected stage of biogenesis. Adapted from [73]

6.2. Class II mutations

The second class of mutations contains the majority of the CF mutations, including the most frequent one F508del, and is associated with improper folding of the corresponding CFTR proteins, resulting in defective cellular processing and delivery of CFTR to the cell surface (trafficking). Wild-type CFTR translation products are inserted into the membrane of the endoplasmic reticulum and are core glycosylated through addition of two high mannose sugar groups on the fourth extracellular loop (Figure 2). The immature mutant proteins will remain associated with molecular chaperones, and ultimately be ubiquitinated and degraded by the 26S proteasome present in the cytosol [83]. The quantity of F508del-CFTR that reaches the cell membrane is tissue-specific [84]. These cell-specific differences in quality control might be explained by different amounts of the Hsc70 co-chaperone CHIP that targets immature CFTR proteins to the ubiquitin-proteasome pathway [85]. However, rescued F508del-CFTR proteins show 5–20-fold accelerated degradation, when compared to wild-type CFTR. Therefore, differences in both the folding defects in the ER and the instability of the rescued F508del-CFTR may contribute to the cell type-specific differences in accumulation of mutant CFTR at the cell membrane. To design new therapies for the trafficking defect of F508del CFTR, both factors will have to be taken into account [86,87]. Class II mutations are distributed throughout the CFTR protein [88-91]. However, a defect in maturation is more frequently observed when NBD1 amino acids are altered, suggesting that the folding pattern of NBD1 itself, or the surrounding sequences, is very sensitive to mutational changes [92]. Moreover, the C terminal tail contains amino acids that influence the stability of the mature protein and mutations of these amino acids could grouped as a new class of mutations [93].

6.3. Class III mutations

This class causes a defect in regulation that impairs opening of the CFTR chloride channel (gating), inducing very little Cl^- transport. Wild-type CFTR channel activity is regulated by phosphorylation of the R domain and binding and hydrolysis of ATP at both nucleotide-binding domains. Class III mutations interfere with these processes, and most of these mutations are found in the nucleotide-binding domains and interfere with the binding of ATP to

these domains or with the stimulation of the channel by ATP, resulting in a decrease in the net chloride transport activity of the channel.

6.4. Class IV mutations

The fourth class of mutations causes a structural defect in the CFTR channel that decreases the transport of ions through the channel-opening conductance. This class affects amino acids located in the pore of CFTR channel: R117H, R334W and R234P all give rise to a chloride channel with normal phosphorylation and ATP-dependent regulation, but with reduced single-channel currents. This class of mutations is in most cases associated with a milder clinical phenotype [94].

6.5. Class V mutations

Mutations of class V cannot only result in CF but are also observed in patients presenting only a partial CF phenotype like congenital bilateral absence of the vas deferens (CBAVD) [34], obstructive azoospermia [95], disseminated bronchiectasis [96; 97], allergic bronchopulmonary aspergillosis [26], hypertrypsinaemia [98] and chronic pancreatitis [27]. Mutations associated with these diseases mostly induce a decrease in the amount of CFTR proteins produced. As a result, only the most CFTR-needing organs will be affected [8]. These mutations are often due to errors in RNA splicing that lead to reduced (variable) quantity of functional CFTR. This is the case for the T5 polymorphism in intron 9 which affects the splice acceptor site [34] and 3849q10 kb which activates a cryptic exon [99]. To some extent, normal splicing can still occur in a small proportion of these transcripts and, as a consequence, a small amount of functional CFTR will be generated, inducing some Cl- transport, resulting in a mild phenotype. Mutations that cause a small defect in maturation but normal or increased chloride transport activity, like A455E and P574H tend to be classified in this fifth class of mutations [100].

6.6. Class VI mutations

This class decreases CFTR stability causing increased cell surface turnover and degradation of CFTR. Moreover, CFTR also exhibits regulatory properties towards other ion channels like ENaC, and ORCC, and this class harbors nucleotide alterations that affect the regulatory properties of the CFTR protein. Sugita et al. [101] highlighted that a CFTR protein with a functional R domain is required to activate an ATP channel associated with CFTR. Moreover, different regions of the CFTR protein are needed to function as a chloride transporter or as a regulator of other proteins: expression of a CFTR protein containing only the first transmembrane domain is already sufficient to produce a chloride channel (constitutively active) but the presence of the first nucleotide-binding domain and their regulatory-domain is necessary to regulate the activity of ORCC [102]. Moreover, two CFTR mutations of class III (A455E and G551D) have a different impact on the regulation of ORCC: A455E retains its ability to stimulate ORCC and G551D. Some mutations, however, will disrupt the normal functioning of the CFTR protein in more than one way, and will therefore have to be classified in different mutation classes.

7. Complex alleles

A comprehensive mutational analysis revealed multiple mutations in the same CFTR gene. If both mutations are on the same parental CFTR gene, they are said to be cis; if each mutation is on a different parental CFTR chromosome, they are said to be in the trans state. When two mutations are in the cis form, CF may not be confirmed and the search for another allele mutation, which is in the trans form of the other two mutations, needs further detection. CFTR genes that carry at least two functional cis-DNA variants are termed “complex alleles” [103]. This makes it more difficult to determine whether a certain CFTR mutation is causing disease, as researchers cannot determine which mutation in the same gene is CF or CFTR-RD. The relationship between mutation (genotype) and disease expression (phenotype) is not straightforward [104]. This association shows phenotypic differences in severity between genotyped patients due to the occurrence of the same putative genotype in healthy subjects and patients with cystic fibrosis. Furthermore, the genotype-phenotype relationship remains unresolved in the use of complex alleles as an additional source of CFTR genetic variation. However, genetic modifiers in the CFTR gene are also known to influence the expression of disease phenotypes by modulating the effects of mutations [105].

Since the beginning of population screening for CF carriers, it has become apparent that complex CFTR alleles are not uncommon. Deciphering their impact on disease pathogenesis remains a challenge for both clinicians and researchers [106]. Genotype-phenotype relationship is probably to be affected by these complex alleles. When apparently identical mutated genotypes are detected in subjects with diverse phenotypes, complete CFTR mutational research is mandatory [107]. Although most cases of complex alleles (two CF-associated mutations carried on one chromosome) may represent association of a benign sequence variation with an actual disease-causing mutation in the same gene, there are examples where the second site mutation can modulate the effect of the principal mutation [108].

Our understanding of the molecular basis of CFTR complex allele will help in the interpretation of variable CF phenotype in CF or CF-RD patients. These data will provide insights into the pathogenesis of CF. The first complex allele to be described was in 1991 where R553Q was detected on the same allele as F508del of a CF patient also carrying the R553X mutation [109]. Several in vivo and in vitro investigations have also revealed cases in which there is one main mutation with the phenotypical effect that is worsened by a second mutation, which may even be a neutral variant when isolated, as occurs for F508C [110], R74W [111], S912L [112] and M470V [113].

The identification of complex alleles (Figure 4) has several important consequences for recessive gene disorders [112]: (1) Prognosis of a severe phenotype will lead to better patient follow-up; (2) Appropriate genetic counseling should be provided to relatives of affected children; (3) Knowledge of these complex alleles can contribute to a better understanding of genotype-phenotype relationships. In addition, the impact of these alleles on the CF population must be determined as their frequency remains unknown. Furthermore, the impact of these alleles on the CF population must be determined, their frequency is still

unknown [112]. Further studies have demonstrated the existence of complex genotypes and their involvement in the phenotypes of pathogenic mutations, providing a unique opportunity to investigate the impact of two genetic abnormalities gene interactions [114]. A major challenge in the description, interpretation and treatment of diseases of genetic origin is to identify the unique phenotypic features of diseases and to distinguish their underlying genotype [115]. With regard to the consequences of a revealed complex allele, it is necessary to distinguish between the discovery of mutations for two CFTRs known to cause disease in both alleles, which could lead to association of a clear genotype-phenotype system, and the discovery of mutations leads to misdiagnosis or misclassification of sequence variants.

Some complex alleles further complicate the establishment of genotype-phenotype correlations (http: //www.genet.sickkids.on.ca/cftr/). There are well-described examples of these alleles: the combination of two missense mutations in cis has been clinically described as decreased [R553Q; F508del], [R334W; R1158X] [109,116] or worsening [R74W; D1270N], [R347H; D979A] [117; 118] phenotypes of CF patients associated with the most frequent mutations (F508del, R1158X, D1270N, R347H); I148T and 3199del6; R74W and D1270N (sometimes with V201M); -102T>A and S549R (T>G); L997F and R117L; G576A and R668C; I1027T and F508del; and perhaps more. R117H, 5T and 12/13TG are all specific examples of complex alleles.

Different Classes of CFTR complex alleles expression of some of these complex alleles has been shown to alter Cl- channel activity in a different manner than their single mutant counterparts [111,113,119,120]. Double mutant alleles may be more common than expected and may influence the status of clinical phenotypes. Thus, such complex alleles could be classified into two main different groups according to their impact on CF phenotype and their role in the pathogenesis of CF.

7.1. Complex alleles associated with CF

This class contains complex alleles resulting from the combination of two mild mutations that, if isolated, cause CFTR-RD but if combined in the cis form, cause cystic fibrosis. In some cases, there is a primary mutation whose phenotypic effect is compounded by a second sequence variant that could even be a neutral variant if isolated, such as F508C, R74W, S912L or M470V [107]. The effect when it is in the cis form but at the base of the hyperactive CFTR when it is incorporated in the trans form, such as M470 and R1235 for example, has been described. There are many examples of this complex allele group as follows:

7.1.1. [H939R; H949L] complex alleles

Polizzi et al. [121] identified a novel complex CFTR allele in five patients. This complex allele is associated with two mutations namely, H939R and H949L, transmitted in cis in the exon 17 of CFTR gene. In addition, one different mutation per allele was inherited in trans in a population of 289 Caucasian CF subjects from South Italy. The genotype-phenotype relationship in patients carrying this complex allele was investigated. Two related mutations are associated with the classic phenotypes of severe cystic fibrosis. This allele is associated in trans with the severe mutations G542X, 1259insA, G1349D and F508del presenting the classic CF phenotype [121].

7.1.2. [I148T + 9T + 3199del6] and [G378X; I148T] complex alleles

Rolfs et al. [105] determined that the I148T allele occurs on at least three chromosomal backgrounds (I148T+7T, I148T+9T, and I148T+9T+3067del6) and that only the complex allele, I148T+9T+3067del6, appears to be associated with a classic CF phenotype. This allele is associated with a classic CF phenotype. The I148T allele alone, even when a severe allele is on the other chromosome, confers no apparent phenotype. The 3067del6 deletion in exon 19 is apparently a rare allele, in as much as it was identified in only 2 of 90 individuals who carried one copy of I148T and was not identified in 386 non-CF chromosomes. Screening 95 CF patients for 3067del6 did not identify the deletion, suggesting that it does not occur alone as a CF-causing allele. More recently, Terlizzi et al described the next complex allele [G378X; I148T] and focused on the fact that some patients with I138T combined with a CF-causing variant may develop CFTR-RD [122].

7.1.3. [R117L; L997F] complex alleles

This complex allele was found in one clinically classic CF patient with end-stage pancreatic insufficiency and had the highest sweat test values [107], while five other subjects had F508del/L997F and lower sweat test values. They have a milder form or CFTR-RD or have no disease. The combination of the presence of this complex allele and their clinical outcomes did not match when we compared two subjects carrying the same G85E mutation on one allele. One subject with the complex allele had the highest sweat test and the other without the complex allele had the lowest value. Moreover, in two other subjects carrying the W1282X mutation and this complex allele, the effect of simple compound heterozygosity could not be evaluated. A good correlation between genotype, sweat test, and clinical outcome was observed in both subjects. In fact, they had elevated sweat test values and moderate or severe CF. on clinical.

7.1.4. [G149R; G576A; R668C] complex alleles

Classical CF was only observed in patients carrying the complex allele [G149R; G576A; R668C] in trans with a severe CF mutation. Functional studies clearly showed that p.Gly149Arg confers the CF phenotype, with cellular mislocalization and misprocessing, as observed for F508del. By contrast, genotypes combining mutants other than G149R, namely R668C, [G576A; R668C], and [D443Y; G576A; R668C], in trans with a CF mutation, were not observed in patients with classical CF, although they were observed in patients with moderate phenotypes, in particular CBAVD. Furthermore, the observation of such compound heterozygous genotypes in seven healthy individuals argues strongly against the hypothesis that these mutants have severe deleterious effects. This observation is also consistent with the results of the functional studies, which demonstrated residual CFTR function, with D443Y affecting protein maturation, and G576A and R668C having an effect on Cl^- channel activity. Despite the observation of an association between a decrease in CFTR function and the triple mutant -which is likely to have been due to the combined effect of the mutations -

residual function was still present, which is compatible with a moderate or mild phenotype. The epidemiological data provided further evidence that [G576A; R668C] is a frequent variant in the general population. This was corroborated by the observation that additional, different mutations occurred on this haplotype (D443Y, G149R, and S519G, the latter being observed only once in the sample from the general population). These results have substantial implications for diagnostic and genetic counselling, as they classify [G149R; G576A; R668C] or G149R (even if no CF patient was detected with only this mutation) as a CF-causing mutation [123].

7.1.5. [R334W; R1158X/delta F508] complex alleles

The clinical picture of the patients with the genotype [R334W-R1158X/F508del] is characterized by pancreatic sufficiency and an atypical course of the disease [116].

7.1.6. [R347H; D979A] complex alleles

This complex allele was found in twins with CF. They are of mixed parentage: Japanese mother and German father. One case shows meconium ileus as a neonate. The other patient did relatively well until the age of 6 years when she was diagnosed with pulmonary aspergillosis in hospital. Standard therapies for CF including digestive enzymes, vitamins and periodic antibiotic therapy in the US were given. At 19 years of age, they were diagnosed with common mutations and one F508del CFTR allele was found. After genetic examination of their Japanese mother and grandmother, results revealed missense mutations in exon 8 (R347H) and exon 18 (D979A). Although the D979A mutant is very rare, this compound-complex allele could induce CF [118]. This complex allele induces a severe CF, whereas R347H causes a mild decrease of the channel activity, and the localization 979 in the third cytoplasmic loop is crucial for maturation. Both mutations in cis could act to alter CFTR function severely [119].

7.1.7. [R74W; R1070W; D1270N] complex alleles

This allele was found in a patient with clinical diagnosis of CF and elevated sweat conductivity measurements from a Moroccan family [106]. The allele [R74W; R1070W; D1270N] in trans with a type I CFTR mutation in a patient with clinical diagnosis of CF and elevated sweat conductivity measurements. R1070W is considered a mutation of "mild" pancreatic-sufficient CF or of CFTR-related disease including CBAVD. Functional studies have revealed abnormal localization of CFTR bearing R1070W [124].

7.1.8. [48C>G; 3532AC>GTA]+[F508del] complex alleles

This allele was identified in a CF patient harboring a 48C>G promoter sequence variation associated in cis of a 3532AC>GTA frameshift mutation and in trans with the F508del mutation. This patient has a positive sweat test, pulmonary symptoms, digestive manifestations and pancreatic insufficiency. The rare alterations 48C>G (legacy name) and 3532AC>GTA have been detected in only one patient. Functional analysis of a promoter variant associated in cis with a frameshift mutation [48C>G; 3532AC>GTA]+[F508del] showed that the 48C>G variant plays its effect at the transcriptional and mRNA levels. This outcome could be explained by the higher affinity of the altered sequence for the three transcription factors E2F, MZF1 and Sp1. The frameshift mutation has a deleterious effect at both the mRNA and protein levels, suggesting the involvement of NMD in the recognition and degradation of aberrant transcripts [125].

7.1.9. [R1070Q; S466X] complex alleles

This complex allele composed of R1070Q mutation in cis with S466X mutation was identified in 11 patients with severe CF [124]. Another study stated that patients carrying R1070Q have CF; however, functional analysis revealed that CFTR R1070Q is normally expressed as wild-type CFTR. This data suggests that this complex induces the severe phenotype. Moreover, two CF Serbian patients carried the R1070Q mutation in cis with S466X, and both have the F508del mutation on the other allele [126]. Besides, they found 14 patients carrying R1070Q mutation with detailed clinical information. The sequencing of CFTR exon 20 showed that 11 carried S466X mutation in cis. Seven of 11 R1070Q-S466X patients had F508del in trans and the remaining had a variety of CF alleles in trans, one each of N1303K, 621+1G>T (c.489+1G>T), 711+3A>G (c.579+3A>G), and R1070Q-S466X. All 11 R1070Q-S466X patients had pancreatic insufficient CF. Of the five patients with R1070Q, only, 3 had CBAVD and 2 were diagnosed as CF. Of those with CBAVD, one patient carried a mutation known to cause pancreatic insufficient CF (S549N), a second patient carried a mutation associated with CBAVD (D1152H), and the third carried a mutation of unknown disease association (F1337V). Of the 2 patients diagnosed with CF, a female patient with the E822X mutation in her other CFTR gene had pancreatic insufficient CF and a patient carrying c.2657+5G>A had pancreatic sufficient CF. The E822X mutation has been associated with pancreatic insufficient CF while c.2657+5G>A is a pancreatic sufficient mutation (http://www.genet.sickkids.on.ca/cftr/). In brief, R1070Q alone appears to be able to confer mild disease (i.e., CBAVD) in some cases when paired with a known "severe" CF mutation, while the presence of the in cis S466X mutation was consistently associated with pancreatic insufficient CF [126].

7.1.10. [Leu467Phe; F508del] complex alleles

In Russian patients, [Leu467Phe; F508del] complex allele was detected [127]. These mutations are located in NBD1 domain and both lead to folding defects hampering protein maturation [128]. No significant differences in disease severity were detected between patients carrying complex alleles and patients homozygous for F508del [127]. However, mortality was higher in the group with genotype [F508del]; [F508del] than in group [L467F; F508del]; [F508del] [129]. Moreover, a weak impact of tezacaftor/ivacaftor on CFTR function was found in the model of intestinal organoids of the patient, in contrast to patients homozygous for the genetic variant F508del and without other mutations in the cis position [130].

7.1.11. [K464N; 5T] complex alleles

Farhat et al [131] showed multi-physiopathological consequences of the c.1392G>T CFTR mutation: firstly, this mutation causes aberrant splicing and exon 10 skipping (class V), secondly, this mutation (also named K464N) induces severe misprocessing as F508del, allow-

ing classification in class II, and thirdly in association with 5T increases the aberrant splicing suggesting a class I.

7.2. Complex alleles associated with CFTR-RD

Complex alleles belonging to this class represent the potential effect of the addition mutation in cis may even lead to a decrease in CF symptoms [132]. This influence has been demonstrated for -102T, R553Q, R553M and R334W. This potential mechanism is more complicated by the fact that some CFTR polymorphisms, combined in complex alleles, could show at least CFTR-RD [133,134].

7.2.1. [H939R; H949L] complex alleles

This complex allele was found in five unrelated patients, in whom the two CF-associated mutations, H939R and H949L, were both carried in the exon 17 on the same allele [121]. It seems that this complex allele greatly decreases the residual CFTR function. If a severe mutation, which produces a very low residual function, is also present in trans, the combined impact is an overall great reduction of CFTR functionality. On the other hand, when the other allele carries a mild mutation, the overall effect is a cumulative greater CFTR functionality.

7.2.2. [-102T>A; S549R] complex alleles

These alleles combine a sequence change in the minimal CFTR promoter (-102T>A, legacy name) and a missense mutation in exon 12: S549R. It was identified in two unrelated patients from southern France, both classified with milder forms of cystic fibrosis and pancreatic sufficient. [135]. As the S549R mutation has previously been described as a 'severe' allele, associated with pancreatic insufficiency, it appears that cis-mutations can modulate the clinical phenotype. In vitro analysis demonstrated that the -102T>A mutation resulted in an up-regulation of CFTR expression by the formation of a Yin Yang 1 (YY1) transcription factor-binding site in the CFTR promoter region [135]. This enhancement of CFTR promoter activity reduced the severity of the phenotype induced by S549R mutation.

7.2.3. [G576A; R668C] complex alleles

Polizzi et al. [121] found 2 sisters (7 and 9 years old respectively) carrying this complex allele in compound heterozygosity with F508del, showing a borderline sweat chloride test, recurrent asthmatic bronchitis and pancreatic sufficiency. Functional analysis showed normal effect on protein. However, G576A, a known splicing mutant [136], and R668C mutation mildly alter CFTR chloride conductance [123].

7.2.4. [D443Y; G576A; R668C] complex alleles

These alleles identified in maternal CFTR allele which carry three missense mutations, D443Y, G576A, and R668C, detected in males with CBAVD [137,138,139]. D443Y, G576A, and R668C have been observed independently or in combination. In patients with a CF-related syndrome, the whole CFTR coding sequence has been analysed: D443Y, G576A, R668C, [D443Y; G576A], [D443Y; R668C] were detected in CBAVD patients [137,138,139] and [G576A; R668C] in a patient with disseminated bronchiectasis, but with no other CF causing mutation found in trans [96]. The D443Y mutation was only observed in CBAVD patients and moderately alters CFTR maturation [138,139]. The G576A and R668C variations have both initially been described as polymorphisms since they were found on the non-CF chromosome of the mother of a CF child [140]. However, they were later considered as putative mild mutations associated with a CBAVD phenotype when combined in trans with F508del [132,141]. These genotypes may possibly not be disease-causing in women. Functional analysis showed normal effect on protein [123].

7.2.5. [R74W; V201M; D1270N] and [R74W; D1270N] complex alleles

The triple mutant allele is found in males with CBAVD and seems to have occurred on the same haplotype TG11-T7-V470 [142,143]. Addictive defects were shown for this complex allele. Indeed R74W displays partial exon 3 skipping [144], the both other mutations reduced function of protein his complex allele [111,142]. Moreover, the [R74W; D1270N] double-mutant is responsible for the CBAVD phenotype [111]. The contribution of each mutant to this phenotype may be as follows: R74W is a polymorphism that may slightly reduce the normal amount of CFTR protein (67% of responding cells versus 81-89%) in vivo and D1270N has a cAMP-responsive anion conductance with different ratios between fast and slow responder cells, as for the R117H mutation [111]. This finding suggests that the combination of R74W enhances the effect of D1270N by reducing the number of fast responder cells, as consequence of the defective regulation of the [R74W; D1270N] mutated protein or a different turn-over of the protein at the cell surface in vivo [111]. The [R74W; D1270N] double-mutant was present in the two unaffected individuals. The first case was a young boy who had been initially suspected of having CF at age 4 years because of allergic rhinitis but for whom the diagnosis of CF was later ruled out; no other CFTR sequence alteration could be identified and the sweat tests were negative (chloride values <40 mM). The second individual was the mother of a CF girl who was compound heterozygous for F508del and P67L. This woman, who was carrying P67L and [R74W; D1270N] in trans, was completely asymptomatic at age 45 years and displayed three negative sweat tests (chloride values <20 mM). The double mutant alleles seem to have occurred on the haplotype [TG11; T7; V470] [142]. Since the beginning of population screening for CF carriers, it has become apparent that complex alleles such as [R74W; D1270N] are not uncommon [145, http://www.hgvs.org/mutnomen].

7.2.6. [R334W; R1158X] complex alleles

These alleles contain the missense mutation R334W in exon 8 and the nonsense mutation R1158X in exon 22. Lymphocyte RNA analysis showed that (1) the mRNA corresponding to the complex allele is present although at markedly reduced levels, and (2) the nonsense mutation does not lead to detectable skipping of exon 22. The clinical picture of the patients with the genotype R334W-R1158X/F508del is characterized by pancreatic sufficiency and an atypical course of the disease. R334W mutation was found in two Spanish CF chromosomes. One of the patients has the F508 del mutation in the other chromosome and the other patient does not. The mutation destroys a *Map*I site and is easily identified by agarose gel electrophoresis after PCR with intron primers [146]. R1158X mutation was found in patients with pancreatic sufficient and carries an

unknown mutation on the other chromosome.

7.2.7. [TG13; T5], [TG12; T5] and [TG11;T5] complex alleles

A T(n) tract and TG(n) tract are located in front of exon 9. Three common alleles may be identified at the Tn locus namely: T5, T7 and T9, and a much rarer T3 [147] There is a stretch of 5, 7, 9 and 3 T-residues. A T5 haplotype can be identified in association with TG11, TG12, TG13 and especially with TG15 (11, 12, 13 and 15 TG repeats, respectively) [148]. The number of T and TG repeats within the polymorphic tract has a potential influence on splicing in CFTR exon 10: besides low numbers of T-residues and high numbers of TG repeats give rise to less efficient splicing [34,149,150]. Transcripts skipping exon 10 fail to mature [151,152]. In the Caucasian population, about 5% of the CFTR genes carry the T5 allele [153]. In most T5 tracts, the number of TG repeats in cis determines whether the amount of functional CFTR falls above or below the critical level for normal protein. A [TG12; T5] or [TG13; T5] CFTR gene found in compound heterozygosity with a CF-causing mutation, or possibly even in homozygosity, will in general result in a CFTR-RD, such as CBAVD or chronic idiopathic pancreatitis. Some CBAVD patients may develop mild lung symptoms. In exceptional cases, TG12-T5 and TG13-T5 may cause a mild form of CF [154]. A TG11-T5 CFTR gene is highly unlikely to cause disease. Approximately 90% of the T5 CFTR genes found in CBAVD patients associate with TG12 or TG13, while about 10% associate with TG11[34,155,156]. Hence, TG12-T5 variants may be considered pathogenic in the context of male infertility by the absence of vas deferens but not in the context of CF [157].

7.2.8. [R117H-T5; R117H ; T7] complex alleles

R117H is a relatively common mutation in CF patients worldwide [158]. R117H can be cis with T5 or T7. R117H-T5 will produce less functional CFTR than R117H-T7. When found in a compound heterozygous with the cystic fibrosis mutation, or possibly, even homozygous, R117H-T5 usually leads to adequate cystic fibrosis, whereas R117H-T7 may lead to mild cystic fibrosis, obstructive azoospermia, or no disease at all. In newborn screening programs, up to 7% of infants have an elevated immune trypsinogen test and two mutations, the R117H-T7 heterozygous and the CF-causing CFTR mutation [103]. During the first years of life, these children show no obvious signs of cystic fibrosis, although it cannot be ruled out that they may develop manifestations of cystic fibrosis in adulthood [52].

7.2.9. [N1303K; 744-33 GATT (6); 869+11C>T] complex alleles

The N1303K was characterized by its severity on the pancreas and variability of the pulmonary status [159]. The presence of a complex allele could cause a worse outcome [112, 119]. The GATT polymorphic region, region was described in CFTR database (http://www.genet.sickkids.on.ca). It is located in the 5' flanking region of exon 7 and presents 5 to 7 GATT repeats [160]. The most frequent allele is the GATT (7) [161]. Associated with this haplotype, the polymorphism c.869+11C>T was detected in the 3' flank region of exon 7 in the CFTR gene (www.genet.sickkids.on.ca/cftr/.). At the mRNA level, no abnormal splicing with c.3909C>G was detected. However, a minor exon 7 was omitted in WT (c.[744-33GATT(7); 869+11C]); however if in the mutated complex allele (c.[744-33GATT(6); 869+11C> T]) the skipping is slightly higher, it could not explain the altered phenotype observed in patient N1303K [162].

7.2.10. [N1303K, TG12; T5; 2930C>T] complex alleles

This complex allele was associated with CFTR-RD as CBAVD in males and with recurrent pancreatic in women [163]. A study comparing patients from Lebanon, Egypt, and France with negative controls provided by their spouses revealed four complex alleles and three polymorphisms [164]. The two major haplotypes consistently showed the same association in cis, except in one Egyptian and one French patient with haplotype N1303 and two Lebanon patients with the corresponding K1303 haplotype. Two very rare polymorphisms have been observed. The effect of the haplotype causes weak alternative or abnormal splicing, which affects the quality and quantity of the CFTR proteins [162,165].

7.2.11. [R117L; L997F] complex alleles

This complex allele is associated with a mild CF phenotype whereas L997F alone could be associated with CFTR-RD [107].

8. New trends for therapies

The existence of CFTR complex alleles has an important role to play in diagnosis, genetic counselling and the choice of therapies even if the detection of a complex allele does not always affect the responsiveness to CFTR treatments [128]. However, additive defects or not have different consequences inducing different treatments such as trikafka (association of three CFTR modulators) [166,167,168,169), or more sophisticated with the CRISPR/Cas9 technology [170] or synthetic antisense oligonucleotide (ASOs) [171].

More recently, since the global pandemic COVID-19, a study performed on 2.585 individuals shows that the balance from COVID-19 mildness to severity is associated with the global CFTR activity from 110 to 50% [172], highlighting the role of the CFTR genotypes and especially the complex alleles to determine COVID-19 progression

9. Conclusion and perspectives

CFTR is a model for genetics with the quantity of mutations and polymorphisms, a model for the role of complex alleles, and more recently for splicing and protein folding. The most common mutation is F508del (in 90% of patients), which induces a misfolding and so firstly retention in the Reticulum Endoplasmic and secondly degradation by proteasome. The other mutations are rare. There has been an increase in the number of complex alleles. This complicates diagnosis and genetic counseling in some cases. Moreover, ranking CFTR mutations into different classes based on their functional effects has its limitations. Indeed, one mutation may exhibit multi-physiopathological consequences, and complex alleles sometimes increase the severity, and sometimes decrease the impact of severe mutation, complicating the diagnosis and the therapy.

Conflict of interests

The author has no conflicts with any step of the article

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Informed consent
The authors declare that no patients were used in this study.

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References

1. O'Sullivan BP, Freedman SD (2009) Cystic fibrosis. Lancet 373(9678): 1891–1904, doi: 10.1016/S0140-6736(09)60327-5
2. Sanders DB, Fink AK (2016) Background and Epidemiology. Pediatr Clin North Am 63(4):567-584. doi: 10.1016/j.pcl.2016.04.001
3. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, et al. (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 245(4922):1066-73. doi: 10.1126/science.2475911. Erratum in: Science 245(4925):1437
4. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N, et al. (1989) Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 245(4922):1059-1065. doi: 10.1126/science.2772657
5. Tsui LC (1995) The cystic fibrosis transmembrane conductance regulator gene. Am J Respir Crit Care Med 151(3 Pt 2):S47-53. doi: 10.1164/ajrccm/151.3_Pt_2.S47
6. Welsh MJ, Tsui L-C, Boat TF; Beaudet AL (1995) Cystic fibrosis. In Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D. (eds), The Metabolic and Molecular Bases of Inherited Disease. 7th edn. McGraw-Hill, New York, 3799–3876
7. Zielenski J (2000) Genotype and phenotype in cystic fibrosis. Respiration 67(2):117-133. doi: 10.1159/000029497
8. Estivill X (1996) Complexity in a monogenic disease. Nat Genet 12(4):348-350. doi: 10.1038/ng0496-348
9. Soferman R (2006) Immunopathophysiologic mechanisms of cystic fibrosis lung disease. Isr Med Assoc J 8(1):44-48
10. Minarowska A, Minarowski L, Karwowska A, Sands D, Dabrowska E (2007) The activity of cathepsin D in saliva of cystic fibrosis patients. Folia Histochem Cytobiol 45(3):165-168
11. Denning GM, Anderson MP, Amara JF, Marshall J, Smith AE, Welsh MJ (1992) Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive. Nature 358(6389):761-4. doi: 10.1038/358761a0
12. Du K, Sharma M, Lukacs GL (2005) The DeltaF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR. Nat Struct Mol Biol 12(1):17-25. doi: 10.1038/nsmb882
13. Drumm ML, Wilkinson DJ, Smit LS, Worrell RT, Strong TV, Frizzell RA, Dawson DC, Collins FS (1991) Chloride conductance expressed by delta F508 and other mutant CFTRs in Xenopus oocytes. Science 20;254(5039):1797-9. doi: 10.1126/science.1722350
14. Anderson MP, Welsh MJ (1992) Regulation by ATP and ADP of CFTR chloride channels that contain mutant nucleotide-binding domains. Science 257(5077):1701-1704. doi: 10.1126/science.1382316. Erratum in: Science 258(5089):1719
15. Silvis MR, Picciano JA, Bertrand C, Weixel K, Bridges RJ, Bradbury NA (2003) A mutation in the cystic fibrosis transmembrane conductance regulator generates a novel internalization sequence and enhances endocytic rates. J Biol Chem. 278(13):11554-11560. doi: 10.1074/jbc.M212843200
16. Jurkuvenaite A, Varga K, Nowotarski K, Kirk KL, Sorscher EJ, Li Y, Clancy JP, Bebok Z, Collawn JF (2006) Mutations in the amino terminus of the cystic fibrosis transmembrane conductance regulator enhance endocytosis. J Biol Chem 281(6):3329-34. doi: 10.1074/jbc.M508131200.
17. Ward CL, Omura S, Kopito RR (1995) Degradation of CFTR by the ubiquitin-proteasome pathway. Cell 83(1):121-7. doi: 10.1016/0092-8674(95)90240-6
18. Haardt M, Benharouga M, Lechardeur D, Kartner N, Lukacs GL (1999) C-terminal truncations destabilize the cystic fibrosis transmembrane conductance regulator without impairing its biogenesis. A novel class of mutation. J Biol Chem 274(31):21873-7. doi: 10.1074/jbc.274.31.21873
19. Pedemonte N, Lukacs GL, Du K, Caci E, Zegarra-Moran O, Galietta LJ, Verkman AS (2005) Small-molecule correctors of defective DeltaF508-CFTR cellular processing identified by high-throughput screening. J Clin Invest 115(9):2564-7251. doi: 10.1172/JCI24898.
20. Van Goor F, Straley KS, Cao D, González J, Hadida S, Hazlewood A, Joubran J, Knapp T, Makings LR, Miller M, Neuberger T, Olson E, Panchenko V, Rader J, Singh A, Stack JH, Tung R, Grootenhuis PD, Negulescu P (2006) Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. Am J Physiol Lung Cell Mol Physiol 290(6):L1117-30. doi: 10.1152/ajplung.00169
21. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC (1989) Identification of the cystic fibrosis gene: genetic analysis. Science 1989 245(4922):1073-80. doi: 10.1126/science.2570460
22. Davis PB, Drumm M, Konstan MW (1996) Cystic fibrosis. Am J Respir Crit Care Med 154(5):1229-1256. doi: 10.1164/ajrccm.154.5.8912731
23. Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, White GA, O'Riordan CR, Smith AE (1990) Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell 63(4):827-834. doi: 10.1016/0092-8674(90)90148-8
24. Welsh MJ, Smith AE (1993) Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell 73(7):1251-1254. doi: 10.1016/0092-8674(93)90353-r
25. Shruti S, Clem RL, Barth AL (2008) A seizure-induced gain-of-function in BK channels is associated with elevated firing activity in neocortical pyramidal neurons. Neurobiol Dis 30(3):323-330. doi: 10.1016/j.nbd.2008.02.002
26. Miller PW, Hamosh A, Macek M Jr, Greenberger PA, MacLean J, Walden SM, Slavin RG, Cutting GR (1996) Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. Am J Hum Genet 59(1):45-51
27. Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS (1998) Relation between mutations of the cystic fibro-

sis gene and idiopathic pancreatitis. N Engl J Med 339(10):653-8. doi: 10.1056/NEJM199809033391002

28. Sharer N, Schwarz M, Malone G, Howarth A, Painter J, Super M, Braganza J (1998) Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. N Engl J Med. 339(10):645-52. doi: 10.1056/NEJM199809033391001
29. Dohle GR, Veeze HJ, Overbeek SE, van den Ouweland AM, Halley DJ, Weber RF, Niermeijer MF (1999) The complex relationships between cystic fibrosis and congenital bilateral absence of the vas deferens: clinical, electrophysiological and genetic data. Hum Reprod 14(2):371-4. doi: 10.1093/humrep/14.2.371
30. Trezise AE, Linder CC, Grieger D, Thompson EW, Meunier H, Griswold MD, Buchwald M (1993) CFTR expression is regulated during both the cycle of the seminiferous epithelium and the oestrous cycle of rodents. Nat Genet 3(2):157-64. doi: 10.1038/ng0293-157
31. Dodge JA (1995) Male fertility in cystic fibrosis. Lancet 346(8975):587-8. doi: 10.1016/s0140-6736(95)91431-5
32. Zielenski J, Tsui LC (1995) Cystic fibrosis: genotypic and phenotypic variations. Annu Rev Genet 29:777-807. doi: 10.1146/annurev.ge.29.120195.004021
33. Teng H, Jorissen M, Van Poppel H, Legius E, Cassiman JJ, Cuppens H (1997) Increased proportion of exon 9 alternatively spliced CFTR transcripts in vas deferens compared with nasal epithelial cells. Hum Mol Genet 6(1):85-90. doi: 10.1093/hmg/6.1.85
34. Cuppens H, Lin W, Jaspers M, Costes B, Teng H, Vankeerberghen A, Jorissen M, Droogmans G, Reynaert I, Goossens M, Nilius B, Cassiman JJ (1998) 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 101(2):487-96. doi: 10.1172/JCI639
35. Zhao GG, Sun HB, Zhi HJ, Wang F, Wu QY, Xia XY, Xu XF (2019) [Correlation between CFTR 5T polymorphisms and the risk of congenital bilateral absence of the vas deferens]. Zhonghua Nan Ke Xue. 25(3):231-237
36. Guan WJ, Li JC, Liu F, Zhou J, Liu YP, Ling C, Gao YH, Li HM, Yuan JJ, Huang Y, Chen CL, Chen RC, Zhang X, Zhong NS (2018) Next-generation sequencing for identifying genetic mutations in adults with bronchiectasis. J Thorac Dis 10(5):2618-2630. doi: 10.21037/jtd.2018.04.134
37. Pignatti PF, Bombieri C, Benetazzo M, Casartelli A, Trabetti E, Gilè LS, Martinati LC, Boner AL, Luisetti M (1996) CFTR gene variant IVS8-5T in disseminated bronchiectasis. Am J Hum Genet 58(4):889-892
38. Girodon E, Cazeneuve C, Lebargy F, Chinet T, Costes B, Ghanem N, Martin J, Lemay S, Scheid P, Housset B, Bignon J, Goossens M (1997) CFTR gene mutations in adults with disseminated bronchiectasis. Eur J Hum Genet 5(3):149-55
39. Sharer N, Schwarz M, Malone G, Howarth A, Painter J, Super M, Braganza J (1998) Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. N Engl J Med 339(10):645-52. doi: 10.1056/NEJM199809033391001
40. Malats N, Casals T, Porta M, Guarner L, Estivill X, Real FX (2001) Cystic fibrosis transmembrane regulator (CFTR) DeltaF508 mutation and 5T allele in patients with chronic pancreatitis and exocrine pancreatic cancer. PANKRAS II Study Group. Gut. 48(1):70-74. doi: 10.1136/gut.48.1.70.
41. Ockenga J, Stuhrmann M, Ballmann M, Teich N, Keim V, Dörk T, Manns MP (2000) Mutations of the cystic fibrosis gene, but not cationic trypsinogen gene, are associated with recurrent or chronic idiopathic pancreatitis. Am J Gastroenterol 95(8):2061-7. doi: 10.1111/j.1572-0241.2000.02228.x
42. Audrézet MP, Chen JM, Le Maréchal C, Ruszniewski P, Robaszkiewicz M, Raguénès O, Quéré I, Scotet V, Férec C (2002) 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 10(2):100-6. doi: 10.1038/sj.ejhg.5200786
43. Singh VK, Yadav D, Garg PK (2019) Diagnosis and Management of Chronic Pancreatitis: A Review. JAMA 322(24):2422-2434. doi: 10.1001/jama.2019.19411
44. Noone PG, Knowles MR (2001) 'CFTR-opathies': disease phenotypes associated with cystic fibrosis transmembrane regulator gene mutations. Respir Res 2(6):328-32. doi: 10.1186/rr82
45. Le C, McCrary HC, Chang E (2016) Cystic Fibrosis Sinusitis. Adv Otorhinolaryngol. 79:29-37. doi: 10.1159/000444959
46. Wang X, Moylan B, Leopold DA, Kim J, Rubenstein RC, Togias A, Proud D, Zeitlin PL, Cutting GR (2000) Mutation in the gene responsible for cystic fibrosis and predisposition to chronic rhinosinusitis in the general population. JAMA 284(14):1814-1819. doi: 10.1001/jama.284.14.1814
47. Schroeder SA, Gaughan DM, Swift M (1995) Protection against bronchial asthma by CFTR delta F508 mutation: a heterozygote advantage in cystic fibrosis. Nat Med 1(7):703-5. doi: 10.1038/nm0795-703
48. Dahl M, Tybjaerg-Hansen A, Lange P, Nordestgaard BG (1998) DeltaF508 heterozygosity in cystic fibrosis and susceptibility to asthma. Lancet 351(9120):1911-3. doi: 10.1016/s0140-6736(97)11419-2. Erratum in: Lancet 1998 352(9135):1230
49. Lázaro C, de Cid R, Sunyer J, Soriano J, Giménez J, Alvarez M, Casals T, Antó JM, Estivill X (1999) Missense mutations in the cystic fibrosis gene in adult patients with asthma. Hum Mutat. 14(6):510-9. doi: 10.1002/(SICI)1098-1004(199912)14:6<510::AID-HUMU10>3.0.CO;2-O
50. Priel E, Adatia A, Kjarsgaard M, Nair P (2022) CFTR heterozygosity in severe asthma with recurrent airway infections: a retrospective review. Allergy Asthma Clin Immunol 18(1):46. doi: 10.1186/s13223-022-00684-0
51. Çolak Y, Nordestgaard BG, Afzal S (2020) Morbidity and mortality in carriers of the cystic fibrosis mutation *CFTR* Phe508del in the general population. Eur Respir J 56(3):2000558. doi: 10.1183/13993003.00558-2020
52. Miller AC, Comellas AP, Hornick DB, Stoltz DA, Cavanaugh JE, Gerke AK, Welsh MJ, Zabner J, Polgreen PM (2020) Cystic fibrosis carriers are at increased risk for a wide range of cystic fibrosis-related conditions. Proc Natl Acad Sci U S A. 117(3):1621-1627. doi: 10.1073/pnas.1914912117
53. Crespo-Lessmann A, Bernal S, Del Río E, Rojas E, Martínez-Rivera C, Marina N, Pallarés-Sanmartín A, Pascual S, García-Rivero JL, Padilla-Galo A, Curto E, Cisneros C, Serrano J, Baiget M, Plaza V (2021) Emerging Asthma Group. Association of the CFTR gene with asthma and airway mucus hypersecretion. PLoS One 16(6):e0251881. doi: 10.1371/journal.pone.0251881
54. Priel E, Adatia A, Kjarsgaard M, Nair P (2022) CFTR heterozygosity in severe asthma with recurrent airway infections: a retrospective review. Allergy Asthma Clin Immunol 18(1):46. doi: 10.1186/s13223-022-00684-0
55. Thilakaratne R, Graham S, Moua J, Jones CG, Collins C, Mann J, Sciortino S, Wong J, Kharrazi M (2022) CFTR gene variants, air pollution, and childhood asthma in a California Medicaid population. Pediatr Pulmonol 57(11):2798-2807. doi: 10.1002/ppul.26103
56. Koumpagioti D, Moriki D, Boutopoulou B, Matziou V, Loukou I, Priftis KN, Douros K (2023) The Association between CFTR Gene Mutation Heterozygosity and Asthma Development: A Systematic Review. J Clin Med 12(6):2403. doi: 10.3390/

jcm12062403
57. Hwang TC (2014) CFTR: a missing link between exocrine and endocrine pancreas? Sci China Life Sci 57(10):1044-1045. doi: 10.1007/s11427-014-4740-0
58. Kayani K, Mohammed R, Mohiaddin H (2018) Cystic Fibrosis-Related Diabetes. Front Endocrinol (Lausanne) 9:20. doi: 10.3389/fendo.2018.00020
59. Wainwright BJ, Scambler PJ, Schmidtke J, Watson EA, Law HY, Farrall M, Cooke HJ, Eiberg H, Williamson R (1985) Localization of cystic fibrosis locus to human chromosome 7cen-q22. Nature. 4 318(6044):384-385. doi: 10.1038/318384a0
60. Hwang TC, Sheppard DN (2009) Gating of the CFTR Cl- channel by ATP-driven nucleotide-binding domain dimerisation. J Physiol 587(Pt 10):2151-61. doi: 10.1113/jphysiol.2009.171595
61. Kloch M, Milewski M, Nurowska E, Dworakowska B, Cutting GR, Dołowy K (2010) The H-loop in the second nucleotide-binding domain of the cystic fibrosis transmembrane conductance regulator is required for efficient chloride channel closing. Cell Physiol Biochem . 25(2-3):169-180. doi: 10.1159/000276549
62. Baker JM, Hudson RP, Kanelis V, Choy WY, Thibodeau PH, Thomas PJ, Forman-Kay JD (2007) CFTR regulatory region interacts with NBD1 predominantly via multiple transient helices. Nat Struct Mol Biol 14(8):738-45. doi: 10.1038/nsmb1278
63. Riordan JR (2008) CFTR function and prospects for therapy Annu Rev Biochem. 77:701-726. doi: 10.1146/annurev.biochem.75.103004.142532
64. Cholon DM, O'Neal WK, Randell SH, Riordan JR, Gentzsch M (2010) Modulation of endocytic trafficking and apical stability of CFTR in primary human airway epithelial cultures. Am J Physiol Lung Cell Mol Physiol 2010 298(3):L304-14. doi: 10.1152/ajplung.00016.2009
65. Higgins CF (2001) ABC transporters: physiology, structure and mechanism--an overview. Res Microbiol 152(3-4):205-10. doi: 10.1016/s0923-2508(01)01193-7
66. Sheppard DN, Welsh MJ (1999) Structure and function of the CFTR chloride channel. Physiol Rev 79(1 Suppl):S23-45. doi: 10.1152/physrev.1999.79.1.S23
67. Bombieri C, Claustres M, De Boeck K, Derichs N, Dodge J, Girodon E, Sermet I, Schwarz M, Tzetis M, Wilschanski M, Bareil C, Bilton D, Castellani C, Cuppens H, Cutting GR, Drevínek P, Farrell P, Elborn JS, Jarvi K, Kerem B, Kerem E, Knowles M, Macek M Jr, Munck A, Radojkovic D, Seia M, Sheppard DN, Southern KW, Stuhrmann M, Tullis E, Zielenski J, Pignatti PF, Ferec C (2011) Recommendations for the classification of diseases as CFTR-related disorders. J Cyst Fibros. Suppl 2:S86-102. doi: 10.1016/S1569-1993(11)60014-3
68. Strandvik B, Björck E, Fallström M, Gronowitz E, Thountzouris J, Lindblad A, Markiewicz D, Wahlström J, Tsui LC, Zielenski J (2001) Spectrum of mutations in the CFTR gene of patients with classical and atypical forms of cystic fibrosis from southwestern Sweden: identification of 12 novel mutations. Genet Test 5(3):235-242. doi: 10.1089/10906570152742290
69. Welsh MJ, Tsui L-C, Boat TF, Beaudet AL (1995) Cystic fibrosis. The metabolic and molecular bases of inherited disease. 17th ed. New York: McGraw-Hill. p 3799–3876
70. Tzetis M, Kanavakis E, Antoniadi T, Doudounakis S, Adam G, Kattamis C (1997) Characterization of more than 85% of cystic fibrosis alleles in the Greek population, including five novel mutations. Hum Genet Jan;99(1):121-125. doi: 10.1007/s004390050324
71. Kerem B, Kerem E (1996) The molecular basis for disease variability in cystic fibrosis. Eur J Hum Genet 4(2):65-73. doi: 10.1159/000472174
72. Kaffenberger TM, Eichar BW, McCoy JL, Yang TS, Shaffer AD, Dohar JE (2021) Cystic fibrosis mutation classes in pediatric otitis media - Fickle or faulty? Am J Otolaryngol 42(6):103067. doi: 10.1016/j.amjoto.2021.103067
73. Caldwell RA, Grove DE, Houck SA, Cyr DM (2011) Increased folding and channel activity of a rare cystic fibrosis mutant with CFTR modulators. Am J Physiol Lung Cell Mol Physiol 301(3):L346-52. doi: 10.1152/ajplung.00044.2011
74. Fulmer SB, Schwiebert EM, Morales MM, Guggino WB, Cutting GR (1995) Two cystic fibrosis transmembrane conductance regulator mutations have different effects on both pulmonary phenotype and regulation of outwardly rectified chloride currents. Proc Natl Acad Sci U S A. 92(15):6832-6. doi: 10.1073/pnas.92.15.6832
75. Bobadilla JL, Macek M Jr, Fine JP, Farrell PM (2002) Cystic fibrosis: a worldwide analysis of CFTR mutations--correlation with incidence data and application to screening. Hum Mutat. 19(6):575-606. doi: 10.1002/humu.10041
76. Ward CL, Kopito RR (1994) Intracellular turnover of cystic fibrosis transmembrane conductance regulator. Inefficient processing and rapid degradation of wild-type and mutant proteins. J Biol Chem 269(41):25710-8
77. Vankeerberghen A, Cuppens H, Cassiman JJ (2002) The cystic fibrosis transmembrane conductance regulator: an intriguing protein with pleiotropic functions. J Cyst Fibros. 1(1):13-29. doi: 10.1016/s1569-1993(01)00003-0
78. Morral N, Dörk T, Llevadot R, Dziadek V, Mercier B, Férec C, Costes B, Girodon E, Zielenski J, Tsui LC, Tümmler B, Estivill X (1996) Haplotype analysis of 94 cystic fibrosis mutations with seven polymorphic CFTR DNA markers. Hum Mutat 8(2):149-59. doi: 10.1002/(SICI)1098-1004(1996)8:2<149::AID-HUMU7>3.0.CO;2-6. Erratum in: Hum Mutat 1996;8(3):295-6
79. Kalman YM, Kerem E, Darvasi A, DeMarchi J, Kerem B (1996) Difference in frequencies of the cystic fibrosis alleles, delta F508 and W1282X, between carriers and patients. Eur J Hum Genet 2(2):77-82. doi: 10.1159/000472347
80. Desgeorges M, Mégarbané A, Guittard C, Carles S, Loiselet J, Demaille J, Claustres M (1997) Cystic fibrosis in Lebanon: distribution of CFTR mutations among Arab communities. Hum Genet 100(2):279-83. doi: 10.1007/s004390050505
81. El-Seedy A, Pasquet MC, Shafiek H, Morsi T, Kitzis A, Ladevèze V (2016) Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene mutations in North Egyptian population: implications for the genetic diagnosis in Egypt. Cell Mol Biol (Noisy-le-grand). 62(13):21-28. doi: 10.14715/cmb/2016.62.13.5
82. El-Seedy AS, Shafiek H, Kitzis A, Ladevèze V (2017) *CFTR* Gene Mutations in the Egyptian Population: Current and Future Insights for Genetic Screening Strategy. Front Genet 8:37. doi: 10.3389/fgene.2017.00037
83. Jilling T, Kirk KL (1997) The biogenesis, traffic, and function of the cystic fibrosis transmembrane conductance regulator. Int Rev Cytol 172:193-241. doi: 10.1016/s0074-7696(08)62361-x
84. Kälin N, Claass A, Sommer M, Puchelle E, Tümmler B (1999) DeltaF508 CFTR protein expression in tissues from patients with cystic fibrosis. J Clin Invest 103(10):1379-89. doi: 10.1172/JCI5731
85. Meacham GC, Patterson C, Zhang W, Younger JM, Cyr DM (2001) The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. Nat Cell Biol 3(1):100-5. doi: 10.1038/35050509
86. Sharma D, Xing S, Hung YT, Caskey RN, Dowell ML, Touchette DR (2018) Cost-effectiveness analysis of lumacaftor and ivacaftor combination for the treatment of patients with cystic fibrosis in the United States. Orphanet J Rare Dis 13(1):172. doi: 10.1186/s13023-018-0914-3

87. Mutyam V, Sharma J, Li Y, Peng N, Chen J, Tang LP, Falk Libby E, Singh AK, Conrath K, Rowe SM (2021) Novel Correctors and Potentiators Enhance Translational Readthrough in CFTR Nonsense Mutations. Am J Respir Cell Mol Biol 64(5):604-616. doi: 10.1165/rcmb.2019-0291OC
88. Seibert FS, Linsdell P, Loo TW, Hanrahan JW, Riordan JR, Clarke DM (1996) Cytoplasmic loop three of cystic fibrosis transmembrane conductance regulator contributes to regulation of chloride channel activity. J Biol Chem 271(44):27493-9. doi: 10.1074/jbc.271.44.27493
89. Seibert FS, Linsdell P, Loo TW, Hanrahan JW, Clarke DM, Riordan JR (1996) Disease-associated mutations in the fourth cytoplasmic loop of cystic fibrosis transmembrane conductance regulator compromise biosynthetic processing and chloride channel activity. J Biol Chem 271(25):15139-45. doi: 10.1074/jbc.271.25.15139
90. Cotten JF, Ostedgaard LS, Carson MR, Welsh MJ (1996) Effect of cystic fibrosis-associated mutations in the fourth intracellular loop of cystic fibrosis transmembrane conductance regulator. J Biol Chem 271(35):21279-84. doi: 10.1074/jbc.271.35.21279
91. Xiong X, Bragin A, Widdicombe JH, Cohn J, Skach WR (1997) Structural cues involved in endoplasmic reticulum degradation of G85E and G91R mutant cystic fibrosis transmembrane conductance regulator. J Clin Invest 100(5):1079-1088. doi: 10.1172/JCI119618
92. Gregory RJ, Rich DP, Cheng SH, Souza DW, Paul S, Manavalan P, Anderson MP, Welsh MJ, Smith AE (1991) Maturation and function of cystic fibrosis transmembrane conductance regulator variants bearing mutations in putative nucleotide-binding domains 1 and 2. Mol Cell Biol 11(8):3886-93. doi: 10.1128/mcb.11.8.3886-3893.1991
93. Haardt M, Benharouga M, Lechardeur D, Kartner N, Lukacs GL (1999) C-terminal truncations destabilize the cystic fibrosis transmembrane conductance regulator without impairing its biogenesis. A novel class of mutation. J Biol Chem 274(31):21873-21977. doi: 10.1074/jbc.274.31.21873
94. Koch C, Cuppens H, Rainisio M, Madessani U, Harms H, Hodson M, Mastella G, Navarro J, Strandvik B, McKenzie S, Investigators of the ERCF (2001) European Epidemiologic Registry of Cystic Fibrosis (ERCF): comparison of major disease manifestations between patients with different classes of mutations. Pediatr Pulmonol 31(1):1-12. doi: 10.1002/1099-0496(200101)31:1<1::aid-ppul1000>3.0.co;2-t.
95. Jarvi K, Zielenski J, Wilschanski M, Durie P, Buckspan M, Tullis E, Markiewicz D, Tsui LC (1995) Cystic fibrosis transmembrane conductance regulator and obstructive azoospermia. Lancet 345(8964):1578. doi: 10.1016/s0140-6736(95)91131-6
96. Pignatti PF, Bombieri C, Marigo C, Benetazzo M, Luisetti M (1995) Increased incidence of cystic fibrosis gene mutations in adults with disseminated bronchiectasis. Hum Mol Genet 4(4):635-639. doi: 10.1093/hmg/4.4.635
97. Girodon E, Cazeneuve C, Lebargy F, Chinet T, Costes B, Ghanem N, Martin J, Lemay S, Scheid P, Housset B, Bignon J, Goossens M (1997) CFTR gene mutations in adults with disseminated bronchiectasis. Eur J Hum Genet 5(3):149-155
98. Castellani C, Bonizzato A, Mastella G (1997) CFTR mutations and IVS8-5T variant in newborns with hypertrypsinaemia and normal sweat test. J Med Genet 34(4):297-301. doi: 10.1136/jmg.34.4.297
99. Highsmith WE, Burch LH, Zhou Z, Olsen JC, Boat TE, Spock A, Gorvoy JD, Quittel L, Friedman KJ, Silverman LM, et al. (1994) A novel mutation in the cystic fibrosis gene in patients with pulmonary disease but normal sweat chloride concentrations. N Engl J Med 331(15):974-980. doi: 10.1056/NEJM199410133311503
100. Wilschanski M, Zielenski J, Markiewicz D, Tsui LC, Corey M, Levison H, Durie PR (1995) Correlation of sweat chloride concentration with classes of the cystic fibrosis transmembrane conductance regulator gene mutations. J Pediatr 127(5):705-10. doi: 10.1016/s0022-3476(95)70157-5
101. Sugita M, Yue Y, Foskett JK (1998) CFTR Cl- channel and CFTR-associated ATP channel: distinct pores regulated by common gates. EMBO J 17(4):898-908. doi: 10.1093/emboj/17.4.898
102. Schwiebert EM, Morales MM, Devidas S, Egan ME, Guggino WB (1998) Chloride channel and chloride conductance regulator domains of CFTR, the cystic fibrosis transmembrane conductance regulator. Proc Natl Acad Sci U S A 95(5):2674-9. doi: 10.1073/pnas.95.5.2674
103. Castellani C, Cuppens H, Macek M Jr, Cassiman JJ, Kerem E, Durie P, Tullis E, Assael BM, Bombieri C, Brown A, Casals T, Claustres M, Cutting GR, Dequeker E, Dodge J, Doull I, Farrell P, Ferec C, Girodon E, Johannesson M, Kerem B, Knowles M, Munck A, Pignatti PF, Radojkovic D, Rizzotti P, Schwarz M, Stuhrmann M, Tzetis M, Zielenski J, Elborn JS (2008) Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros 7(3):179-96. doi: 10.1016/j.jcf.2008.03.009
104. Kiesewetter S, Macek M Jr, Davis C, Curristin SM, Chu CS, Graham C, Shrimpton AE, Cashman SM, Tsui LC, Mickle J, et al. (1993) A mutation in CFTR produces different phenotypes depending on chromosomal background. Nat Genet 5(3):274-8. doi: 10.1038/ng1193-274
105. Rohlfs EM, Zhou Z, Sugarman EA, Heim RA, Pace RG, Knowles MR, Silverman LM, Allitto BA (2002) The I148T CFTR allele occurs on multiple haplotypes: a complex allele is associated with cystic fibrosis. Genet Med. 4(5):319-23. doi: 10.1097/00125817-200209000-00001
106. de Prada Merino A, Bütschi FN, Bouchardy I, Beckmann JS, Morris MA, Hafen GM, Fellmann F (2010) [R74W;R1070W;D1270N]: a new complex allele responsible for cystic fibrosis. J Cyst Fibros 9(6):447-449. doi: 10.1016/j.jcf.2010.08.014
107. Lucarelli M, Narzi L, Pierandrei S, Bruno SM, Stamato A, d'Avanzo M, Strom R, Quattrucci S (2010) A new complex allele of the CFTR gene partially explains the variable phenotype of the L997F mutation. Genet Med 12(9):548-555. doi: 10.1097/GIM.0b013e3181ead634
108. Rowntree RK, Harris A (2003) The phenotypic consequences of CFTR mutations. Ann Hum Genet 67(Pt 5):471-85. doi: 10.1046/j.1469-1809.2003.00028.x
109. Dörk T, Wulbrand U, Richter T, Neumann T, Wolfes H, Wulf B, Maass G, Tümmler B (1991) Cystic fibrosis with three mutations in the cystic fibrosis transmembrane conductance regulator gene. Hum Genet 87(4):441-6. doi: 10.1007/BF00197165
110. Kälin N, Dörk T, Tümmler B. A cystic fibrosis allele encoding missense mutations in both nucleotide binding folds of the cystic fibrosis transmembrane conductance regulator. Hum Mutat. 1(3):204-210. doi: 10.1002/humu.1380010305
111. Fanen P, Clain J, Labarthe R, Hulin P, Girodon E, Pagesy P, Goossens M, Edelman A (1999) Structure-function analysis of a double-mutant cystic fibrosis transmembrane conductance regulator protein occurring in disorders related to cystic fibrosis. FEBS Lett 452(3):371-4. doi: 10.1016/s0014-5793(99)00647-x
112. Clain J, Lehmann-Che J, Girodon E, Lipecka J, Edelman A, Goossens M, Fanen P (2005) A neutral variant involved in a complex CFTR allele contributes to a severe cystic fibrosis phenotype. Hum Genet 116(6):454-60. doi: 10.1007/s00439-004-1246-z
113. Wei L, Vankeerberghen A, Jaspers M, Cassiman J, Nilius B, Cuppens H (2000) Suppressive interactions between mutations located in the two nucleotide binding domains of CFTR. FEBS Lett

473(2):149-53. doi: 10.1016/s0014-5793(00)01519-2
114. Romey MC, Guittard C, Chazalette JP, Frossard P, Dawson KP, Patton MA, Casals T, Bazarbachi T, Girodon E, Rault G, Bozon D, Seguret F, Demaille J, Claustres M (1999) Complex allele [-102T>A+S549R(T>G)] is associated with milder forms of cystic fibrosis than allele S549R(T>G) alone. Hum Genet 105(1-2):145-50. doi: 10.1007/s004399900066
115. Zangerl B, Wickström K, Slavik J, Lindauer SJ, Ahonen S, Schelling C, Lohi H, Guziewicz KE, Aguirre GD (2010) Assessment of canine BEST1 variations identifies new mutations and establishes an independent bestrophinopathy model (cmr3). Mol Vis 16:2791-804. PMID: 21197113
116. Duarte A, Amaral M, Barreto C, Pacheco P, Lavinha J (1996) Complex cystic fibrosis allele R334W-R1158X results in reduced levels of correctly processed mRNA in a pancreatic sufficient patient. Hum Mutat 8(2):134-9. doi: 10.1002/(SICI)1098-1004(1996)8:2<134::AID-HUMU5>3.0.CO;2-B
117. Casals T, Bassas L, Ruiz-Romero J, Chillón M, Giménez J, Ramos MD, Tapia G, Narváez H, Nunes V, Estivill X (1995) 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 95(2):205-11. doi: 10.1007/BF00209403
118. Hojo S, Fujita J, Miyawaki H, Obayashi Y, Takahara J, Bartholomew DW (1998) Severe cystic fibrosis associated with a deltaF508/R347H + D979A compound heterozygous genotype. Clin Genet 53(1):50-3. doi: 10.1034/j.1399-0004.1998.531530110.x
119. Clain J, Fritsch J, Lehmann-Che J, Bali M, Arous N, Goossens M, Edelman A, Fanen P (2001) Two mild cystic fibrosis-associated mutations result in severe cystic fibrosis when combined in cis and reveal a residue important for cystic fibrosis transmembrane conductance regulator processing and function. J Biol Chem. 276(12):9045-9. doi: 10.1074/jbc.M008979200
120. Teem JL, Berger HA, Ostedgaard LS, Rich DP, Tsui LC, Welsh MJ (1993) Identification of revertants for the cystic fibrosis delta F508 mutation using STE6-CFTR chimeras in yeast. Cell 73(2):335-46. doi: 10.1016/0092-8674(93)90233-g
121. Polizzi A, Tesse R, Santostasi T, Diana A, Manca A, Logrillo VP, Cazzato MD, Pantaleo MG, Armenio L (2011) Genotype-phenotype correlation in cystic fibrosis patients bearing [H939R;H949L] allele. Genet Mol Biol 34(3):416-20. doi: 10.1590/S1415-47572011000300008
122. Terlizzi V, Centrone C, Botti M, Taccetti G (2022) G378X-I148T CFTR variant: A new complex allele in a cystic fibrosis newborn with pancreatic insufficiency. Mol Genet Genomic Med 10(9):e2033. doi: 10.1002/mgg3.2033
123. El-Seedy A, Girodon E, Norez C, Pajaud J, Pasquet MC, de Becdelièvre A, Bienvenu T, des Georges M, Cabet F, Lalau G, Bieth E, Blayau M, Becq F, Kitzis A, Fanen P, Ladeveze V (2012) CFTR mutation combinations producing frequent complex alleles with different clinical and functional outcomes. Hum Mutat 33(11):1557-65. doi: 10.1002/humu.22129
124. Krasnov KV, Tzetis M, Cheng J, Guggino WB, Cutting GR (2008) Localization studies of rare missense mutations in cystic fibrosis transmembrane conductance regulator (CFTR) facilitate interpretation of genotype-phenotype relationships. Hum Mutat 29(11):1364-72. doi: 10.1002/humu.20866
125. Viart V, Des Georges M, Claustres M, Taulan M (2012) Functional analysis of a promoter variant identified in the CFTR gene in cis of a frameshift mutation. Eur J Hum Genet 20(2):180-4. doi: 10.1038/ejhg.2011.161
126. Radivojevic D, Djurisic M, Lalic T, Guc-Scekic M, Savic J, Minic P, Antoniadi T, Tzetis M, Kanavakis E (2004) Spectrum of cystic fibrosis mutations in Serbia and Montenegro and strategy for prenatal diagnosis. Genet Test. 8(3):276-80. doi: 10.1089/gte.2004.8.276.
127. Ivanov M, Matsvay A, Glazova O, Krasovskiy S, Usacheva M, Amelina E, Chernyak A, Ivanov M, Musienko S, Prodanov T, Kovalenko S, Baranova A, Khafizov K (2018) Targeted sequencing reveals complex, phenotype-correlated genotypes in cystic fibrosis. BMC Med Genomics 11(Suppl 1):13. doi: 10.1186/s12920-018-0328-z
128. Baatallah N, Bitam S, Martin N, Servel N, Costes B, Mekki C, Chevalier B, Pranke I, Simonin J, Girodon E, Hoffmann B, Mornon JP, Callebaut I, Sermet-Gaudelus I, Fanen P, Edelman A, Hinzpeter A (2018) Cis variants identified in F508del complex alleles modulate CFTR channel rescue by small molecules. Hum Mutat. 39(4):506-514. doi: 10.1002/humu.23389
129. Petrova NV, Kashirskaya NY, Vasilyeva TA, Balinova NV, Marakhonov AV, Kondratyeva EI, Zhekaite EK, Voronkova AY, Kutsev SI, Zinchenko RA (2022) High frequency of complex CFTR alleles associated with c.1521_1523delCTT (F508del) in Russian cystic fibrosis patients. BMC Genomics 23(1):252. doi: 10.1186/s12864-022-08466-z
130. Kondratyeva E, Bulatenko N, Melyanovskaya Y, Efremova A, Zhekaite E, Sherman V, Voronkova A, Asherova I, Polyakov A, Adyan T, Kovalskaia V, Bukharova T, Goldshtein D, Kutsev S (2022) Personalized Selection of a CFTR Modulator for a Patient with a Complex Allele [L467F;F508del]. Curr Issues Mol Biol. 44(10):5126-5138. doi: 10.3390/cimb44100349
131. Farhat R, El-Seedy A, El-Moussaoui K, Pasquet MC, Adolphe C, Bieth E, Languepin J, Sermet-Gaudelus I, Kitzis A, Ladevèze V (2015) Multi-physiopathological consequences of the c.1392G>T CFTR mutation revealed by clinical and cellular investigations. Biochem Cell Biol 93(1):28-37. doi: 10.1139/bcb-2014-0042
132. Mercier B, Verlingue C, Lissens W, Silber SJ, Novelli G, Bonduelle M, Audrézet MP, Férec C (1995) 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 56(1):272-277
133. 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.; 24(2),120-129, 2004.
134. Steiner B, Rosendahl J, Witt H, Teich N, Keim V, Schulz HU, Pfützer R, Löhr M, Gress TM, Nickel R, Landt O, Koudova M, Macek M Jr, Farre A, Casals T, Desax MC, Gallati S, Gomez-Lira M, Audrezet MP, Férec C, des Georges M, Claustres M, Truninger K (2012) Common CFTR haplotypes and susceptibility to chronic pancreatitis and congenital bilateral absence of the vas deferens. Hum Mutat 32(8):912-20. doi: 10.1002/humu.21511. Epub 2011 Jun 7. Erratum in: Hum Mutat 2012 33(2):456
135. Romey MC, Pallares-Ruiz N, Mange A, Mettling C, Peytavi R, Demaille J, Claustres M (2000) A naturally occurring sequence variation that creates a YY1 element is associated with increased cystic fibrosis transmembrane conductance regulator gene expression. J Biol Chem 275(5):3561-3567. doi: 10.1074/jbc.275.5.3561
136. Pagani F, Stuani C, Tzetis M, Kanavakis E, Efthymiadou A, Doudounakis S, Casals T, Baralle FE (2003). New type of disease causing mutations: the example of the composite exonic regulatory elements of splicing in CFTR exon 12. Hum Mol Genet 12(10):1111-20. doi: 10.1093/hmg/ddg131
137. Chillón M, Casals T, Mercier B, Bassas L, Lissens W, Silber S, Romey MC, Ruiz-Romero J, Verlingue C, Claustres M, et al. (1995) Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. N Engl J Med 332(22):1475-80. doi: 10.1056/NEJM199506013322204
138. Costes B, Girodon E, Ghanem N, Flori E, Jardin A, Soufir JC, Goossens M (1995) Frequent occurrence of the CFTR intron 8 (TG)n 5T allele in men with congenital bilateral absence of the vas

deferens. Eur J Hum Genet3(5):285-293. doi: 10.1159/000472312
139. Bienvenu T, Adjiman M, Thiounn N, Jeanpierre M, Hubert D, Lepercoq J, Francoual C, Wolf J, Izard V, Jouannet P, Kaplan JC, Beldjord C (1997) Molecular diagnosis of congenital bilateral absence of the vas deferens: analyses of the CFTR gene in 64 French patients. Ann Genet 40(1):5-9
140. Fanen P, Labarthe R, Garnier F, Benharouga M, Goossens M, Edelman A (1997) Cystic fibrosis phenotype associated with pancreatic insufficiency does not always reflect the cAMP-dependent chloride conductive pathway defect. Analysis of C225R-CFTR and R1066C-CFTR. J Biol Chem 272(48):30563-6. doi: 10.1074/jbc.272.48.30563
141. Sarginson, J (1997) The Cystic Fibrosis Genetic Analysis Consortium. Newsletter, 69.
142. Claustres M, Altiéri JP, Guittard C, Templin C, Chevalier-Porst F, Des Georges M (2004) Are p.I148T, p.R74W and p.D1270N cystic fibrosis causing mutations? BMC Med Genet 5:19. doi: 10.1186/1471-2350-5-19
143. Brugnon F, Bilan F, Heraud MC, Grizard G, Janny L, Creveaux I (2008) Outcome of intracytoplasmic sperm injection for a couple in which the man is carrier of CFTR p.[R74W;V201M;D1270N] and p.P841R mutations and his spouse a heterozygous carrier of p.F508del mutation of the cystic fibrosis transmembrane conductance regulator gene. Fertil Steril 90(5):2004.e23-6. doi: 10.1016/j.fertnstert.2008.05.057
144. Schucht S, Minso R, Lex C, Reiss J, Stanke F, Tamm S, van Barneveld A, Tümmler B (2019) Functional analysis of the p.[Arg74Trp;Val201Met;Asp1270Asn]/p.Phe508del CFTR mutation genotype in human native colon. Mol Genet Genomic Med 7(2):e00526. doi: 10.1002/mgg3.526
145. Strom CM, Huang D, Buller A, Redman J, Crossley B, Anderson B, Entwistle T, Sun W (2002) Cystic fibrosis screening using the College panel: platform comparison and lessons learned from the first 20,000 samples. Genet Med 4(4):289-96. doi: 10.1097/00125817-200207000-00007.
146. Estivill X, Casals T, Nunes V (1991) Genetic analysis of cystic fibrosis. Adv Exp Med Biol 290:31-8. doi: 10.1007/978-1-4684-5934-0_4
147. Disset A, Michot C, Harris A, Buratti E, Claustres M, Tuffery-Giraud S (2005) 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 25(1):72-81. doi: 10.1002/humu.20115
148. Picci L, Cameran M, Scarpa M, Pradal U, Melotti P, Assael BM, Castellani C (2007) TG15 T5 allele in clinically discordant monozygotic twins with cystic fibrosis. Am J Med Genet A. 143A(16):1936-7. doi: 10.1002/ajmg.a.31849
149. Chu CS, Trapnell BC, Curristin S, Cutting GR, Crystal RG (1993) Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. Nat Genet 3(2):151-6. doi: 10.1038/ng0293-151
150. Niksic M, Romano M, Buratti E, Pagani F, Baralle FE (1999) Functional analysis of cis-acting elements regulating the alternative splicing of human CFTR exon 9. Hum Mol Genet 8(13):2339-2349. doi: 10.1093/hmg/8.13.2339
151. Delaney SJ, Rich DP, Thomson SA, Hargrave MR, Lovelock PK, Welsh MJ, Wainwright BJ (1993) Cystic fibrosis transmembrane conductance regulator splice variants are not conserved and fail to produce chloride channels. Nat Genet 4(4):426-31. doi: 10.1038/ng0893-426
152. Strong TV, Wilkinson DJ, Mansoura MK, Devor DC, Henze K, Yang Y, Wilson JM, Cohn JA, Dawson DC, Frizzell RA, et al. (1993) 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 2(3):225-30. doi: 10.1093/hmg/2.3.225
153. Cuppens H, Teng H, Raeymaekers P, De Boeck C, Cassiman JJ. CFTR haplotype backgrounds on normal and mutant CFTR genes. Hum Mol Genet. 1994 Apr;3(4):607-14. doi: 10.1093/hmg/3.4.607.
154. Noone PG, Pue CA, Zhou Z, Friedman KJ, Wakeling EL, Ganeshananthan M, Simon RH, Silverman LM, Knowles MR (2000) Lung disease associated with the IVS8 5T allele of the CFTR gene. Am J Respir Crit Care Med 162(5):1919-1924. doi: 10.1164/ajrccm.162.5.2003160
155. Groman JD, Hefferon TW, Casals T, Bassas L, Estivill X, Des Georges M, Guittard C, Koudova M, Fallin MD, Nemeth K, Fekete G, Kadasi L, Friedman K, Schwarz M, Bombieri C, Pignatti PF, Kanavakis E, Tzetis M, Schwartz M, Novelli G, D'Apice MR, Sobczynska-Tomaszewska A, Bal J, Stuhrmann M, Macek M Jr, Claustres M, Cutting GR (2004) 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 74(1):176-179. doi: 10.1086/381001
156. Ratbi I, Legendre M, Niel F, Martin J, Soufir JC, Izard V, Costes B, Costa C, Goossens M, Girodon E (2007) 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 22(5):1285-1291. doi: 10.1093/humrep/dem024
157. Bienvenu T, Lopez M, Girodon E (2020) Molecular Diagnosis and Genetic Counseling of Cystic Fibrosis and Related Disorders: New Challenges. Genes (Basel) 11(6):619. doi: 10.3390/genes11060619
158. de Nooijer RA, Nobel JM, Arets HG, Bot AG, van Berkhout FT, de Rijke YB, de Jonge HR, Bronsveld I (2011) Assessment of CFTR function in homozygous R117H-7T subjects. J Cyst Fibros 10(5):326-332. doi: 10.1016/j.jcf.2011.03.009
159. Osborne L, Santis G, Schwarz M, Klinger K, Dörk T, McIntosh I, Schwartz M, Nunes V, Macek M Jr, Reiss J, et al. (1992) Incidence and expression of the N1303K mutation of the cystic fibrosis (CFTR) gene. Hum Genet 89(6):653-8. doi: 10.1007/BF00221957
160. Sereth H, Shoshani T, Bashan N, Kerem BS (1993) Extended haplotype analysis of cystic fibrosis mutations and its implications for the selective advantage hypothesis. Hum Genet 92(3):289-295. doi: 10.1007/BF00244474
161. Gasparini P, Dognini M, Bonizzato A, Pignatti PF, Morral N, Estivill X (1991) A tetranucleotide repeat polymorphism in the cystic fibrosis gene. Hum Genet 86(6):625. doi: 10.1007/BF00201556
162. Farhat R, Puissesseau G, El-Seedy A, Pasquet MC, Adolphe C, Corbani S, Megarbané A, Kitzis A, Ladeveze V (2015) N1303K (c.3909C>G) mutation and splicing: implication of its c.[744-33GATT(6); 869+11C>T] complex allele in CFTR exon 7 aberrant splicing. Biomed Res Int 2015:138103. doi: 10.1155/2015/138103
163. Terlizzi V, Castaldo G, Salvatore D, Lucarelli M, Raia V, Angioni A, Carnovale V, Cirilli N, Casciaro R, Colombo C, Di Lullo AM, Elce A, Iacotucci P, Comegna M, Scorza M, Lucidi V, Perfetti A, Cimino R, Quattrucci S, Seia M, Sofia VM, Zarrilli F, Amato F (2017) Genotype-phenotype correlation and functional studies in patients with cystic fibrosis bearing CFTR complex alleles. J Med Genet 54(4):224-235. doi: 10.1136/jmedgenet-2016-103985
164. Farhat R, El-Seedy A, Pasquet MC, Corbani S, Megarbané A, Kitzis A, Ladeveze V (2022) Three Complex alleles associated with N1303K mutation and their molecular consequences. Cell Mol Biol (Noisy-le-grand) 68(4):52-59. doi: 10.14715/cmb/2022.68.4.7
165. Farhat R, El-Seedy A, Norez C, Talbot H, Pasquet MC, Adol-

phe C, Kitzis A, Ladevèze V (2017) Complexity of phenotypes induced by p.Asn1303Lys-CFTR correlates with difficulty to rescue and activate this protein. Cell Mol Biol (Noisy-le-grand) 63(11):106-110. doi: 10.14715/cmb/2017.63.11.18

166. Middleton PG, Mall MA, Dřevínek P, Lands LC, McKone EF, Polineni D, Ramsey BW, Taylor-Cousar JL, Tullis E, Vermeulen F, Marigowda G, McKee CM, Moskowitz SM, Nair N, Savage J, Simard C, Tian S, Waltz D, Xuan F, Rowe SM, Jain R; VX17-445-102 (2019) Study Group. Elexacaftor-Tezacaftor-Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele. N Engl J Med 381(19):1809-1819. doi: 10.1056/NEJMoa1908639
167. Heijerman HGM, McKone EF, Downey DG, Van Braeckel E, Rowe SM, Tullis E, Mall MA, Welter JJ, Ramsey BW, McKee CM, Marigowda G, Moskowitz SM, Waltz D, Sosnay PR, Simard C, Ahluwalia N, Xuan F, Zhang Y, Taylor-Cousar JL, McCoy KS; VX17-445-103 Trial Group (2019) Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. Lancet 394(10212):1940-1948. doi: 10.1016/S0140-6736(19)32597-8. Erratum in: Lancet. 2020 395(10238):1694. doi: 10.1016/S0140-6736(20)31021-7
168. Aspinall SA, Mackintosh KA, Hill DM, Cope B, McNarry MA (2022) Evaluating the Effect of Kaftrio on Perspectives of Health and Wellbeing in Individuals with Cystic Fibrosis. Int J Environ Res Public Health 19(10):6114. doi: 10.3390/ijerph19106114
169. Sondo E, Cresta F, Pastorino C, Tomati V, Capurro V, Pesce E, Lena M, Iacomino M, Baffico AM, Coviello D, Bandiera T, Zara F, Galietta LJV, Bocciardi R, Castellani C, Pedemonte N (2022) The L467F-F508del Complex Allele Hampers Pharmacological Rescue of Mutant CFTR by Elexacaftor/Tezacaftor/Ivacaftor in Cystic Fibrosis Patients: The Value of the Ex Vivo Nasal Epithelial Model to Address Non-Responders to CFTR-Modulating Drugs. Int J Mol Sci 23(6):3175. doi: 10.3390/ijms23063175
170. Chevalier B, Hinzpeter A (2020) The influence of CFTR complex alleles on precision therapy of cystic fibrosis. J Cyst Fibros 19 Suppl 1:S15-S18. doi: 10.1016/j.jcf.2019.12.008
171. Kim YJ, Krainer AR (2023) Antisense Oligonucleotide Therapeutics for Cystic Fibrosis: Recent Developments and Perspectives. Mol Cells 46(1):10-20. doi: 10.14348/molcells.2023.2172
172. Baldassarri M, Zguro K, Tomati V, Pastorino C, Fava F, Croci S, Bruttini M, Picchiotti N, Furini S, Pedemonte N, Gabbi C, Renieri A, Fallerini C; Gen-Covid Multicenter Study (2022) Gain- and Loss-of-Function *CFTR* Alleles Are Associated with COVID-19 Clinical Outcomes. Cells 11(24):4096. doi: 10.3390/cells11244096