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# The Genetics of CFTR: Genotype – Phenotype Relationship, Diagnostic Challenge and Therapeutic Implications

Marco Lucarelli, Silvia Pierandrei, Sabina Maria Bruno and Roberto Strom  
*Dept. of Cellular Biotechnologies and Haematology - Sapienza University of Rome  
 Italy*

## 1. Introduction

Cystic fibrosis (CF; OMIM 602421, see OMIM link in the website section) is the most common lethal genetic disease of the Caucasian population, with a very variable prevalence, from 1/25000 to 1/900, depending on the geographical region (O'Sullivan & Freedman, 2009; Riordan, 2008). CF is caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene (Kerem et al., 1989; Rommens et al., 1989; Zielenski et al., 1991) (see Ensembl link in the website section), which encodes for a transmembrane multifunctional protein expressed mainly in epithelia (Trezise et al., 1993a; Yoshimura et al., 1991b) but also in several cell types of nonepithelial origin (Yoshimura et al., 1991a). It is an ATP- and cAMP-dependent Cl<sup>-</sup> channel with the main function performed at the apical membrane of epithelial cells. This function is the Cl<sup>-</sup> ion secretion in the colon and airways, or its reabsorption in sweat glands (Riordan, 2008; Vankeerberghen et al., 2002). In the lung, the main targeted organ of CF, an additional crucial function performed by CFTR is the regulation of the epithelial Na<sup>+</sup> channel (ENaC) activity. The exact mechanism of CFTR – ENaC interaction is not completely understood and contrasting evidences exist about the role of ENaC in CF. The most reliable vision of the basic defect is that, in the airway epithelia of CF patients, a CFTR deficiency causes an anomalous dual ion transport associated to an altered water absorption (Mall et al., 1998; Stutts et al., 1995; Berdiev et al., 2009) that, in turn, leads to sticky mucus and impaired mucociliary clearance (Donaldson et al., 2002; Matsui et al., 1998). The immune response greatly contributes to increased mucus viscosity through bacterial lysis and DNA release, as well as through immune cell death in the airways. Bacterial infections and inflammation produce bronchial obstruction, bronchiectasis, atrophy and, eventually, lung insufficiency. A probably non-exhaustive list of other CFTR functions includes: the bicarbonate secretion (Kim & Steward, 2009); the regulation of several other ionic channels and of the ion composition of intracellular compartments, as well the control of intracellular vesicle transport (Vankeerberghen et al., 2002); antibacterial activity exerted by epithelial cells (Pier et al., 1997; Schroeder et al., 2002) and macrophages (Del Porto et al., 2011; Di et al., 2006); maintenance of a correct level of hydration, essential for a physiologic development of male reproductive apparatus (Dube et al., 2008; Patrizio & Salameh, 1998; Trezise et al., 1993a), testis, pancreas, liver and intestine (O'Sullivan & Freedman, 2009; Ratjen & Doring, 2003); critical role in

spermatogenesis (Trezise et al., 1993a; Trezise et al., 1993b; Xu et al., 2011b), sperm fertilizing capacity (Xu et al., 2007) and inflammatory response (Belcher & Vij, 2010; Buchanan et al., 2009; Campodonico et al., 2008; Mattoscio et al., 2010). The phenotypic severity of CF is essentially referable to CFTR residual function (Estivill, 1996; Zhang et al., 2009) that in turn depends on a combination of variables acting on the CFTR gene, transcript and/or protein, as well as to the action of variables external to CFTR. Random variability and the effect of the environment also influence the final phenotype (Figure 1). Depending on this complex situation, clinical manifestations of CF are highly variable. Some mono- or oligo-symptomatic phenotypes, namely the CFTR-related disorders (CFTR-RD), should have to be distinguished from poly-symptomatic classic CF (Dequeker et al., 2009). Nearly all male CF patients and several CFTR-RD male subjects show obstructive azoospermia due to congenital bilateral absence of the vas deferens (CBAVD); over 80% of CF patients show pancreatic insufficiency. The clinical history of CF patients is characterized by progressive, age-dependent, multiresistant bacterial infections of the lung, where the main pathogens are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, and the *Burkholderia cepacia* complex. Lung colonization causes the clinical decline, characterized by respiratory impairment, that is the main cause of morbidity and mortality. Despite advances in the treatment of CF, there is no definitive cure, the survival median of CF patients being at present limited to approximately 40 years.

## 2. The genetics of cystic fibrosis

The CFTR gene is located on the long arm of chromosome 7 (7q31.2), spans about 250 kb and contains 27 exons (Zielenski et al., 1991). The most common transcript is 6128 bases long and it is translated to a protein of 1480 aminoacids. The CFTR is under control of an housekeeping-type promoter with a time- and tissue-specific regulated expression established by alternative transcription start sites and/or alternative splicing (Vankeerberghen et al., 2002). CF is a monogenic autosomal recessive disease. Affected subjects have both the alleles mutated. When the same mutation is present on both alleles they are called homozygotes, whereas when different mutations are present on the 2 alleles they are called compound heterozygotes. A carrier of only 1 mutation on 1 allele has no clinical symptoms but has a genetic risk. Two carriers have a high risk of 1/4 (25%) of having an affected child and a risk of 1/2 (50%) of having a healthy carrier child, with a residual probability of 1/4 (25%) of having a healthy non-carrier child. In a given population, the frequency of couples at high risk depends on the frequency of carrier individuals. The prevalence of CFTR mutations and carrier frequency, as well as the incidence of CF, are highly variable depending on geographical region and ethnic group. The disease is very common among Europeans and white Americans with an incidence of about 1/3000 (about 1/27 carriers), whereas the incidence is lower in African Americans (1/17000) and Asian Americans (1/30000). It is uncommon in Africa and Asia with, for example, an incidence as low as 1/350000 in Japan. A comprehensive analysis of worldwide CF incidence and ethnic variations is available (Bobadilla et al., 2002; O'Sullivan & Freedman, 2009).

The basic view of the CF genetics explained above is complicated by biological variability, gene network and technical limitations in the mutational search. A more complex view is reported below.

## 2.1 Maturation, protein domains and mutational classes

The CFTR gene codes for a symmetric transmembrane protein of 1480 aminoacids that belongs to the family of ATP-binding cassette transporters (ABC transporters). The CFTR protein undergoes a complex transport and maturation process within the cell (Rogan et al., 2011; Vankeerberghen et al., 2002). Through an initial co-translational transport, the polypeptide is included in the membranes of the endoplasmic reticulum (130 kDa form) and is N-glycosylated (150 kDa form). By interacting with chaperones the polypeptide assumes the correct folding with a relatively low efficiency of about 25%, the remainder being degraded by the proteasome. Then it is transported to Golgi apparatus where, after further glycosylation, it becomes the mature CFTR (170 kDa form). It is then transported to the cell membrane where it performs its multiple functions, with a half-life of about 12 to 24 h. The CFTR protein exists in a cAMP-regulated dynamic condition of endocytosis and recycling in clathrin-coated vesicles. Finally it is degraded within lysosomes. After this complex pathway to intracellular and plasma membranes and owing to its multiple functions, the CFTR protein contains a number of different domains, each functionally specialized (Rogan et al., 2011; Vankeerberghen et al., 2002). Its NH<sub>2</sub>-end interacts with the SNARE-proteins Syntaxin 1A (STX1A) and synaptosome-associated protein of 23 kDa (SNAP23) (Peters et al., 2001; Tang et al., 2011). The first (TMD1) and the second (TMD2) transmembrane domains, both consisting of six transmembrane helices, form the physical pore through the membrane. The nucleotide binding domains 1 (NBD1) and 2 (NBD2), functionally interacting, contain the sites for ATP binding and hydrolysis. The ATP binding to NBD1 site initiates channel activity, whereas the ATP binding to NBD2 site allows the formation of the intramolecular NBD1 – NBD2 tight heterodimer that turns the channel in a stable open state; the hydrolysis of the ATP bound at NBD2 starts the disruption of the heterodimer interface and finally leads to channel closure (Gadsby et al., 2006). The regulatory domain (R) contains most of the PKA, PKC and PKG phosphorylation sites and has a regulatory role in channel opening/closing. The ATP binding is allowed only after channel activation by PKA-dependent phosphorylation of the R domain. It also interacts with the SNARE protein Syntaxin 8 (STX8) (Bilan et al., 2004). The COOH-end interacts with PDZ domains of the CFTR-associated protein 70 (CAP70) (Wang et al., 2000a), of the Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor (NHERF, which in turn interacts with ezrin) (Seidler et al., 2009) and of the CFTR associated ligand (CAL) (Cheng et al., 2002). It also interacts with the  $\alpha$ 1 AMP-activated protein kinase (AMPK) (Hallows et al., 2000) and contains an internalization signal (Prince et al., 1999) and a binding site for the AP-2 adaptor complex (Weixel & Bradbury, 2000), needed for correct endocytosis.

CFTR mutations are at the present grouped into 6 classes (Table 1), according to their effects on transcription, cellular processing, final localization and quantitative level of functional protein (Amaral & Kunzelmann, 2007; O'Sullivan & Freedman, 2009; Rogan et al., 2011; Vankeerberghen et al., 2002). Class I identifies mutations with a complete lack of protein production. Usually they are nonsense mutations, severe splicing mutations (which produce only aberrant mRNA), small or large deletions or insertions. They act by generating in-frame or frameshift premature stop codons. The unstable transcripts and/or proteins formed are rapidly degraded or retain no functionality. In the class II are grouped protein trafficking defects based on ubiquitination and increased degradation, within the

endoplasmic reticulum, of the misfolded protein. These are processing/maturation defects that severely decrease the protein quantity in the apical membrane, although often in a tissue-specific manner. In class III are included mutations leading to defective regulation that impair channel opening. Although the CFTR protein is able to reach the apical membrane, it is not properly activated by ATP or cAMP. The effect is a decrease or absence of functional CFTR protein. In class IV are grouped the defects of reduced Cl<sup>-</sup> transport through CFTR. In this case the CFTR is present at the apical membrane but it is unable to properly sustain the Cl<sup>-</sup> flux. Most of mutations included in classes II, III or IV are missense ones, that produce different degrees of CFTR impairment in reaching the cell apical membrane or in functioning although correctly localized. In some cases, however, also small deletions or insertions can be found. Class V mutations are splicing defects that cause a reduction of wild-type CFTR mRNA. At variance from the splicing mutations belonging to class I, the splicing mutations grouped in this class V do not completely abolish the correctly spliced form. Mutations of class VI decrease the stability of CFTR or affect the regulation of other channels. They can be missense mutations but also nonsense mutations possibly generating overdue stop codons, that allow the production of a protein that retains a partial Cl<sup>-</sup> transport ability but is unable to correctly regulate other proteins.

## 2.2 The significance of genetics for personalized therapies

The increased knowledge about CFTR derived from over 20 years of basic and applied researches. This allowed both the development of symptom-based treatments, already in use, that greatly enhanced the life quality and lifespan of patients and the actual possibility of more effective personalized therapies. As well, a promise of primary defect correction also arose. As the most severe clinical aspect is respiratory impairment, the target tissue of these therapies is the pseudostratified epithelia of airways. A normalization of ion and water transport in respiratory epithelium can be achieved with the correction of less than 25% of the airway epithelial cells (Farmen et al., 2005; Johnson et al., 1992; Zhang et al., 2009). To classify a CFTR mutation in a functional class has recently become meaningful for a restoring strategy based on drugs acting on specific functional impairment (the so-called mutation-specific therapy) (Amaral & Kunzelmann, 2007; Becq et al., 2011; Kerem, 2005; Rogan et al., 2011) (Table 1). Particularly studied are the in-frame premature termination codons (class I). In general, many kind of tumours and more than a third of genetic diseases are originated by premature termination codons (Frischmeyer & Dietz, 1999). Also in CF, about 20% of affected subjects have at least 1 mutation that is an in-frame premature termination codon. Aminoglycoside antibiotics have shown to be useful to suppress in-frame premature termination codons by read-through and production of full-length CFTR protein (although a wrong aminoacid is inserted in each individual protein) allowing the targeting of class I mutations. The rationale of this approach is that a population of CFTR proteins each with a different wrong aminoacid will show an overall functionality greater than a population of identically truncated CFTR proteins. In this regard, recent findings, although not specifically obtained for CF, highlighted a surprisingly therapeutic potentiality for ribonucleoproteins. The authors (Karijolic & Yu, 2011) demonstrated the possibility, in vitro and in yeast, of the conversion of uridine into pseudouridine, a chemical transformation known as pseudouridylation. As all three translation termination codons contain a uridine residue at first position and the pseudouridylated nonsense codons code for serine, threonine tyrosine or phenylalanine, this may be a tool for converting nonsense into sense codons. Also in this case a wrong aminoacid will be inserted, although within a reduced



choice of 4 aminoacids. Notably, the ribonucleoprotein complex used by the authors contain a RNA guide able to target the complex to a specific nonsense mutation. Chemical, molecular or pharmacological chaperones, usually called correctors (of trafficking), have been reported to be useful, by promoting protein folding and stabilizing CFTR structure, in the targeting of class II mutations. By increasing the activation of mutated CFTR correctly localized at the apical membrane and/or by extending its half-life, some drugs act as potentiators (of function) and are suitable for the targeting of class III, IV and V mutations. Class VI mutations may be targeted by either potentiators or suppressors of in-frame termination codons. Extensive lists of promising compound are available (Amaral & Kunzelmann, 2007; Becq et al., 2011; Rogan et al., 2011). For an up-to-date description of CF clinical trials see, in the website section, the links to the U.S. National Institutes of Health Clinical trials registry and database, and to the U.S. CF Foundation drug development pipeline.

The topic of mutational classes and personalized therapy is not devoid of problems. Some mutations produce multiple effects and should be classified in multiple classes. An emblematic example is the CFTR worldwide most common mutation, the F508del, a deletion of phenylalanine at position 508 of the CFTR protein. It is a class II mutation, because most of the protein is degraded within the endoplasmic reticulum; a small proportion of it reaches however the apical membrane where it behaves as a class III mutation, with only a limited capacity to bind ATP. In addition, the F508del protein has shown a decreased stability and an enhanced degradation also in post-endoplasmic reticulum compartments (Sharma et al., 2001), a behaviour that would point to the mutational class VI. It is in general quite difficult to classify a mutation without specific experimental studies aimed to its functional characterization. Due to the complexity of such studies, they have been performed only for a very limited number of the over 1800 sequence variations found in the CFTR gene. On the other hand, only in a limited number of cases it is possible to infer, by a theoretical approach, a relationship between the functional impairment and the protein domain where the mutation is located, as well a relationship between a specific DNA sequence variation and the class it should belong to. For example, although most of class III mutations localize in the R, NBD1 or NDB2 domains and most of class IV mutations localize in TMD1 or TMD2, if a missense mutation in these domains has been found, it cannot be assumed that the effect will effectively correspond to class III or IV, since that mutation might have a prevalent effect on protein trafficking and should therefore be classified as class II. Likewise, only for nonsense and frameshift mutations it is possible to reasonably assume a direct classification in class I, while for all other kinds of mutations it is very difficult to recognize the mutational class just from DNA sequence variation. For example, for splicing mutations it can be hazardous to deduce the possible amount of anomalous splicing only by software analysis, since just a limited amount of wild type mRNA would cause the shift of that mutation from class I to class V. Taking into account these considerations, although the class-specific personalized therapeutic approach can be at the moment applied only to a limited amount of CFTR mutations, its enhancement is foreseeable when the gap between the knowledge of the structure and the effect of a mutation will be filled by increasing numbers of mutation-specific functional studies.

Gene therapy would be the resolute therapeutic intervention. Although, since the discovery of CFTR gene in 1989 more than 30 clinical trials of gene therapy have been undertaken, no gene therapy has been so far approved for clinical use (Conese et al., 2011; Davies & Alton, 2011; Griesenbach & Alton, 2011). The problems arose from the repeated administration of adenovirus- and adeno-associated virus-based vectors shifted the approaches to lentiviral vectors and non-viral strategies, as well as cell therapy. The

evidence that a lot of work is still to be done in laboratory to optimize gene therapy tools arose. Two opposite approaches can be distinguished in gene therapy: the gene augmentation and the gene targeting. By the former approach, the entire wild-type CFTR gene, producing a normal gene function, is introduced into the cell without the need to know the specific CFTR mutation. On the contrary, the latter approach is a mutation-specific gene therapy strategy, as only the zone of mutation is targeted in situ, allowing the correction of the mutated zone of the gene. A recent study (Auriche et al., 2010) of gene augmentation in CF used the entire CFTR locus, including regulatory regions, cloned and delivered by a bacterial artificial chromosome (BAC), a non-viral vector. The possibility to obtain a physiologically regulated CFTR expression and activity, also of *Pseudomonas* internalization, in an in vitro cellular system has been demonstrated. The control of CFTR activity by naturally occurring regulatory elements appeared a critical aspect to obtain a physiologic CFTR expression pattern, to be taken under consideration in the planning of gene augmentation strategies. By the gene targeting, the corrected gene remains regulated by its endogenous regulatory machinery maintaining its physiologic expression pattern. Recent researches (Gruenert et al., 2003; Sangiuolo et al., 2008; Sangiuolo et al., 2002) applied to CF an intriguing gene targeting strategy, the Small Fragment Homologous Replacement (SFHR), that exchange a wild-type corrector DNA fragment with the endogenous mutated sequence, through a still undefined mechanism probably based on homologous recombination. Both approaches have to be enhanced before clinical application. The main difficulties encountered in the BAC approach are efficient manipulation and delivering to the proper cell population. The main hitches with SFHR are the low reproducibility and recombination efficiency, ranging from 0.01% to 5% (Gruenert et al., 2003). In both cases additional studies are needed to clarify the respective driving molecular mechanisms, to ameliorate our applicatory ability.

Mutation class	Functional effect	Kind of mutations	Mutation-specific therapy
I	Complete lack of protein production	Premature stop codons by: - nonsense - severe splicing - small or large deletions or insertions	Suppressors of in-frame premature termination codons
II	Processing and/or maturation protein defects	- missense - small deletions or insertions	Correctors (chemical, molecular or pharmacological chaperones)
III	Defective regulation of channel opening	- missense - small deletions or insertions	Potentiators
IV	Reduced Cl <sup>-</sup> transport	- missense - small deletions or insertions	Potentiators
V	Reduction of wild-type mRNA	- partial splicing	Potentiators
VI	Protein decreased stability or impaired ability of other channel regulation	- missense - nonsense (overdue stop codons)	Potentiators or suppressors of in-frame overdue termination codons

Table 1. Classes of CFTR mutations and possible personalized therapeutic interventions.

### 2.3 The complexity and sources of variability in the genotype – Phenotype relationship of the CF and CFTR-RD

Separation of classic CF from CFTR-RD only represents a starting attempt to organize the great clinical variability of CF (Bombieri et al., 2011; Dequeker et al., 2009; Estivill, 1996; Noone & Knowles, 2001). In fact, within classic CF are usually grouped both poly-symptomatic and oligo-symptomatic forms greatly differing in the involvement of lung, pancreas, liver, sweat gland and reproductive apparatus (to consider only the main CF targets). Not easier is the task of categorizing the even more heterogeneous oligo- and mono-symptomatic CFTR-RD. In this regard CFTR mutations have been linked to a wide series of pathologies: obstructive azoospermia for CBAVD (Claustres, 2005; Cuppens & Cassiman, 2004; Stuhmann & Dork, 2000); non-obstructive azoospermia, reduced sperm quality and spermatogenesis defects (Boucher et al., 1999; Dohle et al., 2002; Jakubiczka et al., 1999; Jarvi et al., 1998; Mak et al., 2000; Pallares-Ruiz et al., 1999; van der Ven et al., 1996); male hypofertility due to idiopathic seminal hyperviscosity (Elia et al., 2009; Rossi et al., 2004); female hypofertility due to thick cervical mucus (Gervais et al., 1996; Hayslip et al., 1997); neonatal hypertrypsinaemia with normal sweat test (Castellani et al., 2001a; Gomez Lira et al., 2000; Narzi et al., 2007; Padoan et al., 2002); idiopathic pancreatitis (Castellani et al., 2001b; Gomez Lira et al., 2000; Maire et al., 2003; Pallares-Ruiz et al., 2000); pulmonary diseases (Bombieri et al., 1998; Bombieri et al., 2000); disseminated bronchiectasis (Girodon et al., 1997; Pignatti et al., 1995); chronic rhinosinusitis (Raman et al., 2002; Southern, 2007; Wang et al., 2000b); nasal polyposis (Kerem, 2006; Pawankar, 2003); metabolic alkalosis, hypochloremia, hyponatremia, hypokalemia and dehydration (Augusto et al., 2008; Kerem, 2006; Leoni et al., 1995; Priou-Guesdon et al., 2010; Salvatore et al., 2004); primary sclerosing cholangitis, biliary cirrhosis and portal hypertension (Collardeau-Frachon et al., 2007; Gallegos-Orozco et al., 2005; Girodon et al., 2002; Kerem, 2006; Sheth et al., 2003). Several CFTR-RD are still debated, as the involvement of CFTR mutations is often inferred from small case series or even isolated case reports, as well for controversial results (as for example for non-CBAVD male reproductive defects). In addition, in several cases only one mutated allele could be found by quite non homogeneous methodological approaches of mutational search. This raises the troublesome question whether it should be assumed that 2 mutated alleles are indeed present, but the mutational search protocol applied was unable to identify both of them, or if the possibility of CFTR-RD arising in heterozygotes might also be taken into consideration. Rather than an approach for categories, a vision of a mosaic of different clinical manifestations combined in a peculiar way in each patient, overall constituting a continuous gradient of disease clinical severity, seems to better reflect the reality.

Only a rough correspondence between mutational classes and clinical outcome can be found with, for example, more severe phenotypes generated by the combination of class I and class III mutations and milder phenotypes originated by class IV and V. The variability is however so high that clinicians usually do not use genotypes for prognosis. The problem of the relationship between genotype and phenotype in CF can be partitioned in, at least, 2 steps (Figure 1). The first step concerns the production of a CFTR protein with reduced functionality starting from a mutated CFTR genotype. The second step concerns the clinical manifestations that originate owing to the protein malfunction. It is generally accepted that the clinical severity of CF and CFTR-RD is correlated with the residual function of CFTR



(Estivill, 1996; Zhang et al., 2009). It is easy to collocate high (almost physiological) levels of CFTR protein at the same end of the spectrum of the strictly mono-symptomatic patients and very low (almost absent) levels to the other end, where the poly-symptomatic patients with severe clinical manifestations are ideally collocated. Within these extreme conditions, it is however very difficult to link the values of CFTR residual activity to the severity of clinical manifestations. This not only because of the lack of systematic studies, but also for the difficulty of measuring in a real quantitative manner both the CFTR residual function and the clinical severity. Although CFTR mutated genotypes responsible for intermediate levels of residual activity often consist of a classic mutation on one allele and a mild mutation, retaining some CFTR activity, on the other allele, also the link between a specific mutated genotype and its effect on the protein functionality is elusive. Again, also in this case, the lack of systematic functional studies, addressing in vitro the effect of the mutated genotype on the protein cellular fate, have greatly hampered the knowledge at this level.

Several sources of variability influence both steps and make the overall picture unclear (Figure 1).

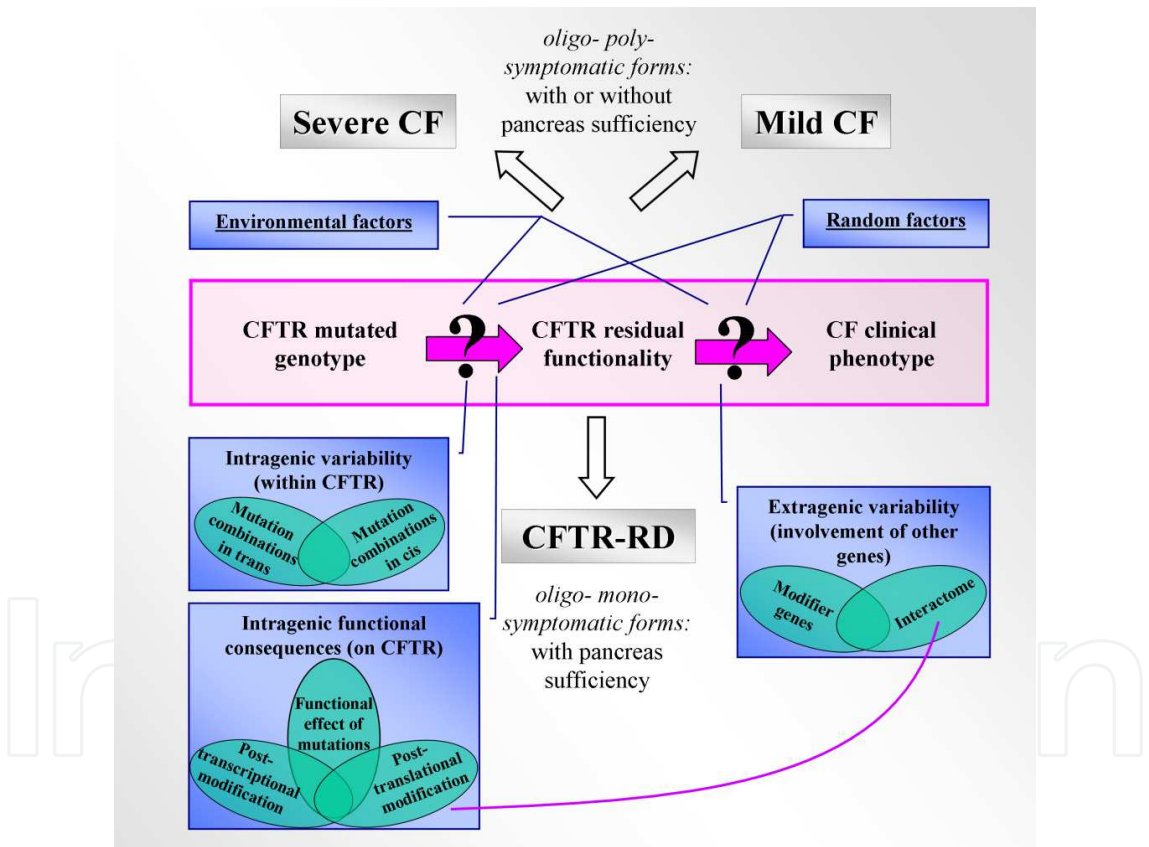


Fig. 1. The variability determinants of the genotype – phenotype relationship in CF and CFTR-RD.

The first step (from mutated genotype to residual function) is mainly influenced by structural and functional intragenic (CFTR-depending) variability. The structural intragenic variability is due to the large number of mutations and to the even larger number of their combinations both in trans, to originate homozygous and compound heterozygous genotypes, and in cis, with more than one mutation on the same allele to form the complex

alleles. Trans and cis variability may also combine leading, for instance, to genotypes with 2 complex alleles and 4 different mutations each belonging to a different mutational class. The functional intragenic variability is due on one side to the variable impairment effect of mutations and, on the other side, to the influence of both post-transcriptional and post-translational modifications, possibly with overlapping effects and interacting mechanisms. The second step (from residual function to clinical phenotype) is more likely to be influenced by extragenic variability due to genes different from CFTR. Modifier genes can indeed modulate the original effect of CFTR mutations (Collaco & Cutting, 2008; Cutting, 2010; Merlo & Boyle, 2003; Salvatore et al., 2002; Slieker et al., 2005), as evidenced by the high phenotypic variability found in some subjects with identical CFTR mutated genotypes. Reciprocal influence between modifier genes and interactome (Wang et al., 2006), as well as an effect of interactome on intragenic functional variability, might also influence this step. Furthermore, it should be taken under consideration that the CFTR levels physiologically required can be tissue-specific, with only some organs affected despite the same CFTR residual function (Estivill, 1996). For example, the male reproductive apparatus appears as the most sensitive district to CFTR impairment, as nearly all men with CF (lower levels of functional CFTR) exhibit also CBAVD while, on the contrary, men with only CBAVD (higher level of functional CFTR) do not have other organs targeted. Superimposed to these genetic sources of variability the powerful role of environmental and random factors on both steps should not be undervalued. Due to these sources of variability, the genotype - phenotype relationship in CF is still poorly understood (Salvatore et al., 2011) with, therefore, our diagnostic, prognostic and therapeutic abilities severely limited.

### 3. Complex alleles and modifier genes

#### 3.1 The relevance of complex alleles

The least addressed aspect of CFTR intragenic structural variability probably is the involvement of complex alleles, with two (or more) mutations in cis (on the same allele). Unfortunately, the most widely used protocols for a mutational search within the CFTR gene are designed only with the aim of finding the first two mutations on different alleles; additional mutations, possibly in cis with the already found mutations, may escape detection. The result is that the mutated genotypes of CF subjects with varying clinical presentations may appear identical, despite the presence of unfound complex alleles that might explain the divergent phenotypes. Undetected complex alleles may have important consequences. For example, if 2 already known disease-causing mutations have been found on both alleles (also on the allele with an in cis undetected additional mutation), the consequences will be an unclear genotype – phenotype relationship with prognostic failure. If at least 1 sequence variation with unclear functional significance in cis with an undetected additional disease-causing mutation has been found, a diagnostic error and/or misclassification of the sequence variation will arise. A systematic experimental search for complex alleles has not yet been undertaken. Probably for this reason only few complex alleles have been found so far and their prevalence is unknown. A probably non exhaustive list of CFTR complex alleles at the moment known is reported in Table 2. They have been more frequently found in patients with CF than CFTR-RD. Only in some cases an in vitro functional characterization has been performed, with the consequence that only in a limited number of these alleles it is possible to distinguish the relative functional contribution of

Complex allele	Bibliographic source
[R75Q;S549N]	Consortium for CF genetic analysis database
[(TG) <sub>m</sub> T <sub>5</sub> ;2184insA]	Consortium for CF genetic analysis database
[129G>C;R117H]	Consortium for CF genetic analysis database
[F508del;R553Q]	(Dork et al., 1991);(Teem et al., 1993)
[3732delA;K1200E]	Consortium for CF genetic analysis database
[F508C;S1251N]	(Kalin et al., 1992)
[F508del;R553M]	(Teem et al., 1993)
[R117H;(TG) <sub>m</sub> T <sub>n</sub> ]	(Kiesewetter et al., 1993; Massie et al., 2001; Peckham et al., 2006)
[125G>C;R75X]	Consortium for CF genetic analysis database
[R297Q;(TG) <sub>m</sub> T <sub>n</sub> ]	Consortium for CF genetic analysis database
[R668C;3849+10kbC>T]	Consortium for CF genetic analysis database
[deleD7S8 (CFTR 3' 500 kb); F508del]	(Wagner et al., 1994)
[1716G>A;L619S]	Consortium for CF genetic analysis database
[405+1G>A;3030G>A]	Consortium for CF genetic analysis database
[G576A;R668C]	(McGinniss et al., 2005; Pignatti et al., 1995)
[(TG) <sub>m</sub> T <sub>5</sub> ;A800G]	(Chillon et al., 1995)
[L88X;G1069R]	(Savov et al., 1995)
[S912L;G1244V]	(Clain et al., 2005; Savov et al., 1995)
[R334W;R1158X]	(Duarte et al., 1996)
[R347H;D979A]	(Clain et al., 2001; Hojo et al., 1998)
[-102T;S549R(T>G)]	(Romey et al., 1999)
[R74W;D1270N]	(Fanen et al., 1999)
[G628R;S1235R]	(Mercier et al., 1995; Wei et al., 2000)
[R117C;(TG) <sub>m</sub> T <sub>n</sub> ]	(Massie et al., 2001)
[M470V;S1235R]	(Wei et al., 2000)
[I148T;3199del6]	(Rohlf s et al., 2002)
[S1235R;(TG) <sub>m</sub> T <sub>5</sub> ]	(Feldmann et al., 2003)
[L24F;296+2T>G]	Consortium for CF genetic analysis database
[W356_A357del;V358I]	(McGinniss et al., 2005)
[V562I;A1006E]	(McGinniss et al., 2005)
[R352W;P750L]	(McGinniss et al., 2005)
[1198_1203delTGGGGCT;1204G>A]	(McGinniss et al., 2005)
[V754M;CFTRdele3_10,14b_16]	(Niel et al., 2006)
[F508del;I1027T]	(Fichou et al., 2008)
[R74W;R1070W;D1270N]	(de Prada et al., 2010)
[(TG) <sub>11</sub> T <sub>5</sub> ;A1006E]	(Tomaiuolo et al., 2010)
[R117L;L997F]	(Lucarelli et al., 2010)

Nucleotide notation: A = adenine, C = cytosine, G = guanine, T = thymine.  
Aminoacid notation: A = alanine, C = cysteine, D = aspartic acid, E = glutamic acid, I = isoleucine, K = lysine, E = glutamic acid, , F = phenylalanine, G = glycine, L = leucine, M = methionine, N = asparagine, H = histidine, P = proline, Q = glutamine, R = arginine, S = serine, T = threonine, V = valine, W = tryptophan, X = stop codon (nonsense).  
The link to the Consortium for CF genetic analysis database is reported in the website section.

Table 2. Complex alleles of CFTR (in chronological order of discovery).

each mutation. They often result in a combination of two mild mutations that, if isolated, cause CFTR-RD but if combined in cis originate CF. In some cases there is one main mutation whose phenotypic effect is worsened by a second sequence variation that may even be a neutral variant if isolated, such as F508C , R74W , S912L or M470V. Also variants that have a suppressive effect when in cis but originate a hyperactive CFTR when combined in trans, as for example the M470 and R1235, have been described. On the other hand, the finding of complex alleles also in CFTR-RD suggests the possibility that an additional mutation in cis may even lead to a lessening of the phenotypic severity (Mercier et al., 1995). This effect has been demonstrated for -102T, R553Q, R553M and R334W. The situation is further complicated by the fact that some CFTR polymorphisms, combined in specific haplotypes, may have at least CFTR-RD as phenotypic consequences (Steiner et al., 2004; Steiner et al., 2011).

### 3.2 The relevance of modifier genes

A small fraction of CF patients and a higher amount of CFTR-RD subjects remain with no CFTR mutations, also when high sensitivity methods of mutational search are used. In addition, CF and CFTR-RD patients with the same mutated CFTR genotypes often show divergent phenotypes. Also some intriguing cases have been reported: an unaffected sister who inherited the same CFTR alleles, without mutations, of her CF brother (Mekus et al., 1998) and two CF sibs, with no CFTR mutation found, who had inherited different parental CFTR allele (Groman et al., 2002). This suggested that genes different from CFTR may cause CF or CFTR-RD. The involvement of other genes in the definition of these phenotypes is relevant for the comprehension of both the molecular pathogenesis and the genotype – phenotype relationship. However, the widest action of the modifier genes probably is to modulate the CF final clinical phenotype in patients with both CFTR mutations found. Even more important, the modifier genes can represent excellent therapeutic targets, as they are able, by definition, to modify the clinical outcome of the disease but they are not mutated (on the contrary to the CFTR). A so-called bypassing approach has been proposed to correct the CF ionic imbalance by stimulating alternative ionic pathways that might compensate the impaired CFTR (Amaral & Kunzelmann, 2007). At present a comprehensive list of these genes does not exist and little is known about their effects and molecular mechanisms of action, as well as about their exact kind of interaction, if any, with the CFTR. Several putative modifier genes have been reported (Collaco & Cutting, 2008; Cutting, 2010; Merlo & Boyle, 2003; Sliker et al., 2005) to influence the second step, from CFTR residual functionality to clinical outcome. On the other hand, microRNA, known to exert a post-transcriptional regulation, have recently been shown to potentially influence the CFTR protein levels (Gillen et al., 2011; Xu et al., 2011a). Together with complex alleles, it is these genes that most probably represent the greatest source of variability in CF. Furthermore, modifier genes show tissue-specific levels of activity, that combine with equally tissue-specific CFTR levels, thus amplifying the complexity of the network. They may also influence the different CFTR functions, even in a tissue-specific manner. One of the most interesting gene complex proposed as CF modifier is the epithelial Na<sup>+</sup> channel (ENaC).

#### 3.2.1 The ENaC genes

The human functional ENaC is composed of 3 subunits coded by 3 genes with sequence similarities:  $\alpha$  (SCNN1A gene) (Voilley et al., 1994),  $\beta$  (SCNN1B gene) and  $\gamma$  (SCNN1G gene)



(Voilley et al., 1995). The ENaC protein has the functional properties of a  $\text{Na}^+$  channel with high  $\text{Na}^+$  selectivity, low conductance and amiloride sensitivity. It is expressed in human epithelial cells that line the distal renal tubule, distal colon and several exocrine glands; an ENaC-mediated amiloride-sensitive electrogenic  $\text{Na}^+$  reabsorption has been documented in the upper and lower airways (Hummler et al., 1996). Genetic diseases are caused by either loss- or gain-of-function mutations in the ENaC genes: loss-of-function mutations in one of the three subunits cause pseudohypoaldosteronism type I (PHA-I) (Chang et al., 1996) characterized by severe renal dysfunction, arterial hypotension and reduced reabsorptive capacity of both kidney and lung; gain-of-function mutations in either SCNN1B or SCNN1G are responsible for Liddle's syndrome, a severe form of hypertension (Shimkets et al., 1994). Interestingly, some PHA-I patients, without CFTR mutations, also exhibit CF-like lung symptoms, such as recurrent bacterial infection of the airways (Hanukoglu et al., 1994).

Because of the involvement of both CFTR and ENaC in the physiologic dual ion transport, it was supposed that also ENaC deregulation and/or molecular lesions might sustain CF or CFTR-RD. There are indeed experimental evidences validating this hypothesis. The over-expressing  $\beta$ -ENaC mouse model has CF-like pulmonary symptoms, with morbidity and mortality partially reduced by preventive treatment with amiloride, an inhibitor of the ENaC channel (Zhou et al., 2008). Wild type CFTR has been shown, in a heterologous cellular system and in polarized primary human bronchial epithelial cultures, to prevent the proteolytic stimulation of ENaC, thus downregulating  $\text{Na}^+$  absorption (Gentzsch et al., 2010). Enhanced expression of all the 3 ENaC genes was shown in the nasal epithelium of CF patients (Bangel et al., 2008). In human bronchial epithelial cells, the CFTR regulates the functional surface expression of endogenous ENaC, by influencing its trafficking (Butterworth, 2010; Rubenstein et al., 2011). However, also experimental evidences against a direct involvement of ENaC and/or of CFTR - ENaC interaction in CF pathogenesis have been provided. According to one study (Joo et al., 2006), CF airway submucosal glands do not display ENaC-mediated fluid hyperabsorption, differently from the ciliated cells of the airway surface. Another study (Nagel et al., 2005) evidenced that human CFTR fails to inhibit the human ENaC channel in a heterologous experimental system of *Xenopus* oocytes. Finally, no increased sodium absorption has been found in newborn CFTR<sup>-/-</sup> pigs, an animal model with features resembling those of human CF disease (Chen et al., 2010). The differences between CF and CFTR action in humans and pigs, the fact that the study has been conducted only shortly after birth and that CF patients have a mutated CFTR and not a CFTR<sup>-/-</sup>, should however be taken into account. Following the above considerations, mutational search in the ENaC genes have been performed in CF and CFTR-RD patients. Both, loss-of-function (Huber et al., 2010; Sheridan et al., 2005) and gain-of-function (Mutesa et al., 2008; Sheridan et al., 2005) mutations have been found in the SCNN1B gene of CFTR-RD patients. Several variants of SCNN1B and SCNN1G have been also found in bronchiectasis patients, some of them with only one CFTR mutation. A significantly increased prevalence of ENaC rare polymorphisms have been found in CFTR-RD patients (Azad et al., 2009), with some of these variants producing alterations of ENaC activity (Azad et al., 2009; Huber et al., 2010). The bulk of these data allows to ascribe to ENaC some roles in CF and/or CFTR-RD. This is reinforced also by the findings of physical and functional co-regulatory interactions between SNARE proteins (in particular Syntaxin 1A) and both the CFTR and ENaC (Peters et al., 2001). It is likely that wild-type ENaC is deregulated by the mutated CFTR. Moreover, ENaC genes can also act as

additional mutated genes either when only one or no copy of CFTR is mutated (the ENaC genes behaving as concomitant pathogenetic factors with respect to CFTR) or when both copies of the CFTR gene are mutated (the ENaC genes as modifiers, modulating the CF phenotype). Little is known about the prevalence and kind of mutations, as well as about the role of other kind of ENaC alterations, such as transcriptional modifications. This last point is quite intriguing considering that a deregulation of ENaC, rather than mutations of it, seems more frequently the main pathogenetic mechanism. The topic of the regulation of ENaC activity further increases the complexity of the puzzle, as multiple biochemical and cellular pathways are involved in the lung (Bhalla & Hallows, 2008; Butterworth, 2010; Eaton et al., 2010; Edinger et al., 2006; Gaillard et al., 2010; Gentzsch et al., 2010). However, little is known about the tissue-specific expression of ENaC and the coordinated transcriptional regulation of the 3 SCNN1 genes. The structure of these genes suggested a role for DNA methylation. The SCNN1G gene has 2 CpG islands in its promoter region and exon 1 (Auerbach et al., 2000; Zhang et al., 2004), the SCNN1B gene has 1 CpG island in its promoter and exon 1 (Thomas et al., 2002) and the SCNN1A gene has a high density of CpG sites, that are however not organized in a CpG island (Ludwig et al., 1998). In effect, experimental evidences suggest that DNA methylation can control transcription of the SCNN1G gene (Zhang et al., 2004).

In general, the search for new genes involved in genetic diseases, in addition to the identification and characterization of new pathogenetic mechanisms, allows the identification of new therapeutic targets. The functional interaction between CFTR and ENaC evidenced by the vast majority of experimental data makes ENaC genes attractive therapeutic targets, since it looks easier to attempt the correction of the regulation of wild type ENaC than the correction of the mutated CFTR. The ENaC gene activity repression has been tempted by amiloride, with partially contrasting results obtained in humans (Burrows et al., 2006) and animal models (Zhou et al., 2008). Also RNA interference seems a valuable and specific tool (Caci et al., 2009; Yueksekdag et al., 2010). The experimental evidences that ENaC genes undergo a DNA methylation-dependent transcription, raised new therapeutic opportunity in epigenetics and chromatin remodelling.

#### 4. The genetics, biochemistry and clinics in the diagnosis of CF

Due to the wide range of signs and symptoms, CF and CFTR-RD diagnosis is difficult, particularly in infancy. On the other hand, CF early diagnosis, revealing pancreas insufficiency, preventing malnutrition and allowing a prompt treatment of lung infections, improves both lifespan and quality of life. In addition, it allows the early selection of high risk couples. For these reasons, neonatal screening programs have been activated worldwide (Castellani & Massie, 2010; Lai et al., 2005; Southern et al., 2007). The most used neonatal screening procedure is based on a single or double dosages (at birth and later on, between the third and fifth week of life) of immunoreactive trypsinogen (IRT), possibly combined with a I level mutational analysis (Castellani et al., 2009; Narzi et al., 2002). In addition to CF newborns, it has been demonstrated that also CFTR-RD newborns are selected by the screening programs (Boyne et al., 2000; Castellani et al., 2001a; Massie et al., 2000; Narzi et al., 2007; Padoan et al., 2002). In a part of newborns positive to the neonatal screening, only one or even no CFTR mutation, sometimes linked to borderline sweat test values, are found. This raises diagnostic uncertainty (Parad & Comeau, 2005) and provides evidence that some carriers are selected by neonatal screening (Castellani et al., 2005; Laroche & Travert, 1991;

Scotet et al., 2001). A common effect of the introduction of CF neonatal screening is the progressively increasing number of CF diagnoses performed each year by screening and the decreasing number of diagnoses performed by symptoms. By definition, neonatal screening selects a lot of false positive subjects and, consequently, is not a diagnostic procedure. On the other hand, also several other pathologies different from CF have a positive sweat test, as well as some CF and a lot of CFTR-RD subjects have a borderline or even negative sweat test. In some cases measurements of the nasal potential difference and/or intestinal Cl<sup>-</sup> flux appear to be quite useful procedures. Taking into account also the highly variable clinical manifestations of CF and CFTR-RD, some of which superimposable to those of other pathologies, it became clear that none of these measurements alone allows a full diagnosis of CF or CFTR-RD. For these reasons, as stated by recent general (Farrell et al., 2008), neonatal screening-oriented (Castellani et al., 2009; Mayell et al., 2009; Sermet-Gaudelus et al., 2010), sweat test-oriented (Green & Kirk, 2007; Legrys et al., 2007) and genetic-oriented (Castellani et al., 2008; Dequeker et al., 2009) guidelines, the diagnosis of CF and CFTR-RD may only be made by a coordinated evaluation of clinical, biochemical and genetic data (Figure 2 upper part). In the last years genetic assessment has been clearly emerging as the most crucial point. In fact if 2 CF or CFTR-RD disease-causing mutations on the different alleles are found, a reliable diagnosis can be defined. Both the finding of the CFTR mutations and their functional interpretation are however very critical points, as described below.

#### 4.1 The technical complexity of the mutational search in the CFTR gene

Over 1500 CFTR mutations and 300 polymorphisms are at moment known (in the website section see the links to the Consortium for CF genetic analysis database and to the human gene mutation database (HGMD)). The F508del is the worldwide most common mutation, accounting, on average, for about 60% of mutated alleles in northern European and North American populations. Few other single mutations account for more than about 5%. In addition the frequencies of CFTR mutations are very different depending on the geographical area (Bobadilla et al., 2002; O'Sullivan & Freedman, 2009). The simplest approach of mutational search would be to define a panel of mutations to be included in a rapid and low-cost test allowing a direct search. However, the high genetic heterogeneity has at least 2 consequences that limit such an approach. First, it is impossible to establish a general mutational panel applicable worldwide; second, the allelic detection rate, also of geographical optimized mutational panels, rarely exceeds the 80% and often is quite lower (Bobadilla et al., 2002; O'Sullivan & Freedman, 2009; Tomaiuolo et al., 2003). The detection rate is the genetic equivalent of the laboratory operative characteristics called diagnostic sensitivity. In this case it represents the proportion of mutated alleles that the specific genetic test is able to evidence. The practical consequence of a limited detection rate is that in case of a negative test, the presence of a mutation not included in the analyzed panel of mutations can not be excluded. A widely accepted approach of mutational search is the multistep one. Usually, methods of I, II and III levels are recognized (Figure 2 lower part). The I level methods are based on the search panels of the most common CFTR mutations by entry-level techniques. They are the most commonly used methods worldwide. However, due to technical and cost limitations, they show a low detection rate as at best they search for the most common CFTR mutations of the specific geographical area. At the moment commercial methods able to search near all CFTR mutations of specific geographic areas are not available. The I level genetic tests are therefore of limited prognostic and diagnostic usefulness, particularly in CFTR-RD subjects with borderline clinical

and/or biochemical values. In this case, the use of methods with higher detection rate are fundamental to resolve uncertain diagnoses. The II level methods are scanning procedures usually able to analyze all the exons, adjacent intronic zones and proximal 5'-flanking of the CFTR gene. In last years, several enhanced methods specific for CFTR scanning have been developed as for example denaturing gradient gel electrophoresis (Costes et al., 1993; Fanen et al., 1992), single-strand conformation polymorphism and heteroduplex analysis (Ravnik-Glavac et al., 1994), denaturing high pressure liquid chromatography (D'Apice et al., 2004; Le Marechal et al., 2001; Ravnik-Glavac et al., 2002), and re-sequencing (Lucarelli et al., 2002; Lucarelli et al., 2006). Due to the progressively reducing costs of the re-sequencing and to the need of further characterization by re-sequencing of positive findings of other scanning techniques, the re-sequencing has become the most used II level method. However, no mutational scan technique able to detect all the CFTR mutations exists. Also the re-sequencing, at the moment the method of mutational search with the highest detection rate, is able to select about 97% of CFTR mutations. The remaining 3% of alleles carry mutations not identified. These may be large deletions, completely intronic mutations that may reveal cryptic exons and mutations in the distal 5'-flanking as well as 3'-UTR zones. Although little is known about the geographical variability of the prevalence of this kind of mutations, due to their overall limited amount and to the extended analysis of the CFTR gene, the re-sequencing shows not only a higher, but also a more constant detection rate than mutational panel-based techniques. Automated protocol of re-sequencing, as well as software templates for automated analysis of re-sequencing data (Ferraguti et al., 2011), have also greatly reduced the time and efforts needed for both the experimental and data processing phases. It should be clear that the use of scanning techniques may raise the problem of functional interpretation of sequence variations found. In fact, whereas the mutational panels are usually planned as to include only disease-causing CFTR mutations, by using scanning procedures also sequence variations not previously characterized from functional point of view may be selected. This may complicate the genetic counselling. The III level methods should be aimed to the search for large deletions, full intronic and distal 5'-flanking, as well as 3'-UTR, mutations. In practise, commercially available products only exist either for the search of most common CFTR large deletions or for the CFTR scanning for gene dosage (gain or loss of genetic material). Although full intronic, distal 5'-flanking and 3'-UTR mutations are assessable by re-sequencing, only recently some efforts have been done to value the pathogenetic contribution of these kind of mutations to CF and CFTR-RD. Whatever technique based on PCR and/or hybridization is applied, the possibility that polymorphisms within the primer/probe recognition sequence may interfere with the pairing reactions should be taken under consideration. So, also if the detection rate is kept to a maximum by including all the 3 levels of mutational search, a full assessment of mutations is virtually impossible to reach, due to the likely, even if small, decrease in analytical sensitivity.

The practical application of this multistep approach changes depending on its use in subjects with disease suspect for diagnostic purposes or in general population subjects for genetic risk lowering. In the first case it is reasonable to progressively go through the levels up to the finding of 2 CFTR mutations on different alleles. If no mutation is found (or at least 1 mutation is not found) even at the III level, the genetic test contributes to a reasonable exclusion of the CF or CFTR-RD diagnosis. On the contrary, in the second case, since it can be difficult to apply all mutational search levels to each subject checking its carrier status, an appropriate genetic residual risk is usually chosen and the mutational search with the suitable detection rate is performed.



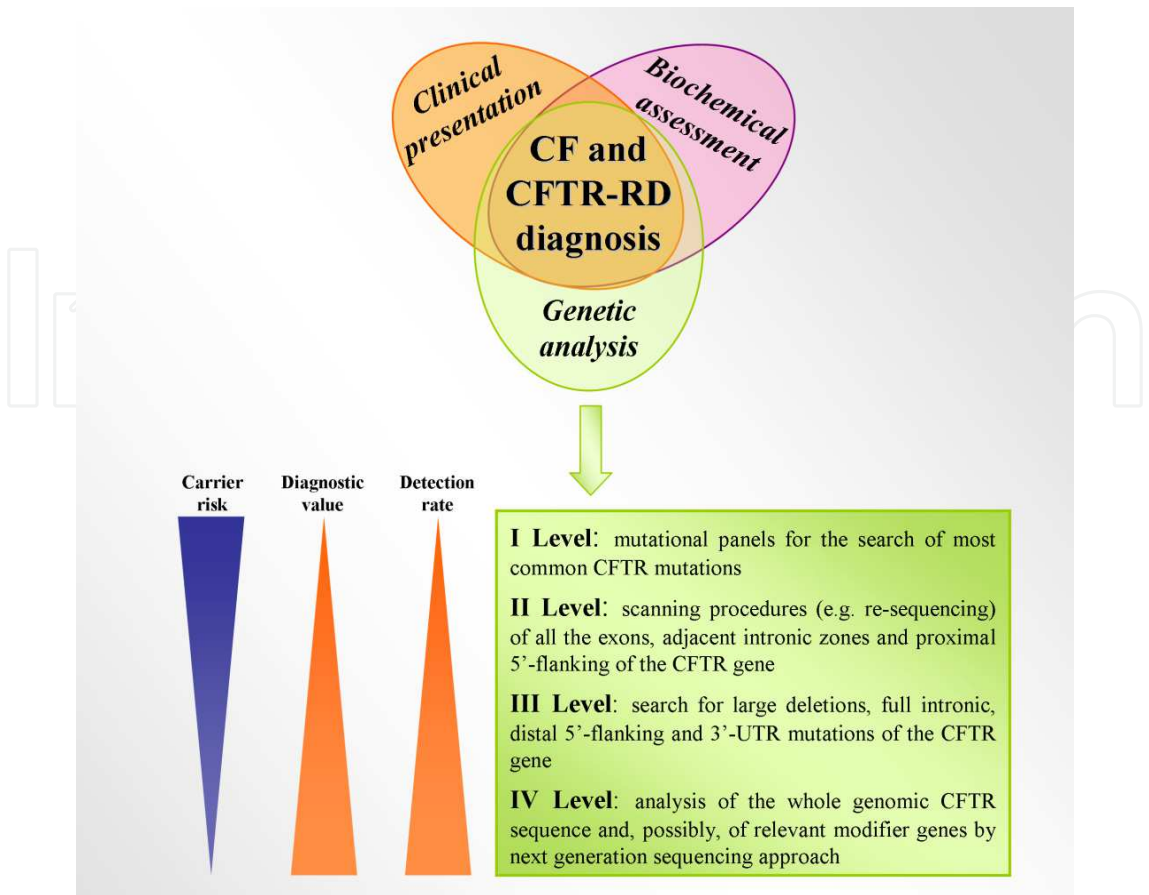


Fig. 2. The genetic analysis, biochemical assessment and clinical presentation contribute to the diagnosis of CF. The multistep genetic approach allows a progressive increase of detection rate and diagnostic value of the test in subjects with CF or CFTR-RD suspect, as well as a progressive decrease of carrier risk in general population subjects.

Usually, no scanning techniques are applied for genetic risk lowering , also because of the possibility to select sequence variations hardly valuable from a pathogenetic point of view. The use of the I level mutational panel approach to assess the genetic risk raises 4 possibilities. If both members of the couple are positive to the mutational search, the risk for an affected child is 1/4 (25%). If both members of the couple are negative the residual risk is so low that no other action is required, although it should be kept in mind that the risk is not zero and this should be made clear to the couple by the genetic counselling. For example, with a carrier frequency of 1/27 and a detection rate of the applied mutational panel of about 85%, the couple residual risk of having an affected child, with CFTR mutations different from those analyzed, if the genetic tests are both negative is about 1/120000. An intermediate residual risk arises when one member of the couple is carrier or CF. In these cases, considering the same above carrier frequency and detection rate, the risk is, respectively, of about 1/700 and 1/350. In these cases, in addition to the genetic counselling clarifying that a concrete risk exists, a possible extension of mutational search to further lower the genetic risk may be taken under consideration for the negative partner.

Following the above considerations, the often incomplete genetic characterization of CF and CFTR-RD patients is due to technical limitations; this constitute a further obstacle to our

understanding of the genotype – phenotype relationship. An emblematic example of this are undetected complex alleles. Patients who do not undergo full mutational assessment, have discordant sweat test and/or clinical outcome, but show at a first mutational search apparently identical CFTR mutated genotypes, should undergo the search for complex alleles. The rising, within the last years, of parallel sequencing, also called next generation sequencing (NGS) (Su et al., 2011), allows to identify a possible IV level in the CFTR mutational search (Bell et al., 2011) (Figure 2 lower part). The possibility to study and analyze data of the whole genomic CFTR sequence (including introns, distal 5'-flanking and 3'-UTR zones) by massive re-sequencing, in an almost complete automated single run-based manner, will be a real possibility within next years. The NGS also has the potentiality to simultaneously study the genetics of modifier genes and, in general, of CFTR interactome to obtain a full assessment of genetic variability determining the final phenotype. If this kind of approach will be able to completely replace the multistep approach actually used is only matter of costs, investment and, finally, commercial choices. Several websites deal with CF and CFTR genetics, from diagnostic and quality assessment point of view, for example those of the European CF thematic network and of the European CF society (links reported in website section).

5. Conclusion

The comprehension of the gene network involved in CF and CFTR-RD is increasing. This is coupled with the enhancement of mutational search methodologies that allow the search for a continuously increasing number of mutations and sequence variations in the CFTR gene and in several other CF-related genes. The huge amount of structural data has to be supported by proper functional studies of single mutations, sequence variations, complex alleles and haplotypes. Only this will produce a full comprehension of genes and their molecular lesions cooperating in the definition of the final CF and CFTR-RD phenotypes, allowing full diagnosis and prognosis. As well, this will also allow the actual clinical use of mutation-specific therapies. When, in the mid-term, this objectives will be reached, the effect-oriented therapy now used will be turned into a cause-oriented therapy (Figure 3).

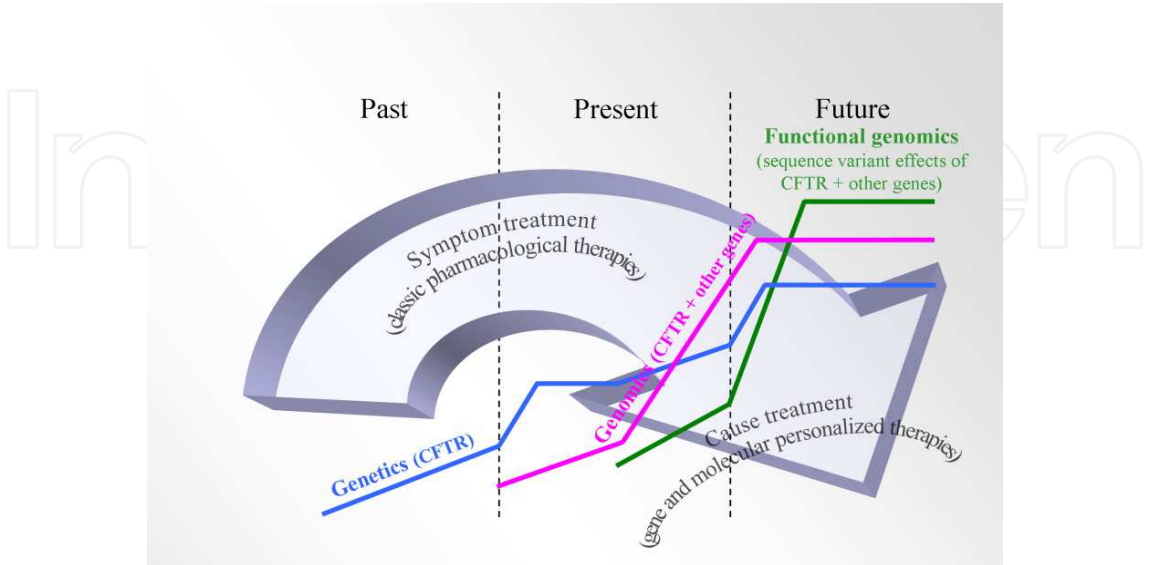


Fig. 3. A genetic-oriented view of CF and CFTR-RD therapy perspectives. The increasing knowledge about genetics, genomics and functional genomics change the therapy.

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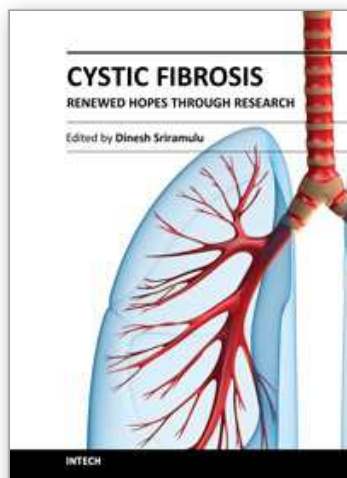
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Living healthy is all one wants, but the genetics behind creation of every human is different. As a curse or human agony, some are born with congenital defects in their menu of the genome. Just one has to live with that! The complexity of cystic fibrosis condition, which is rather a slow-killer, affects various organ systems of the human body complicating further with secondary infections. That's what makes the disease so puzzling for which scientists around the world are trying to understand better and to find a cure. Though they narrowed down to a single target gene, the tentacles of the disease reach many unknown corners of the human body. Decades of scientific research in the field of chronic illnesses like this one surely increased the level of life expectancy. This book is the compilation of interesting chapters contributed by eminent interdisciplinary scientists around the world trying to make the life of cystic fibrosis patients better.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

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### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

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