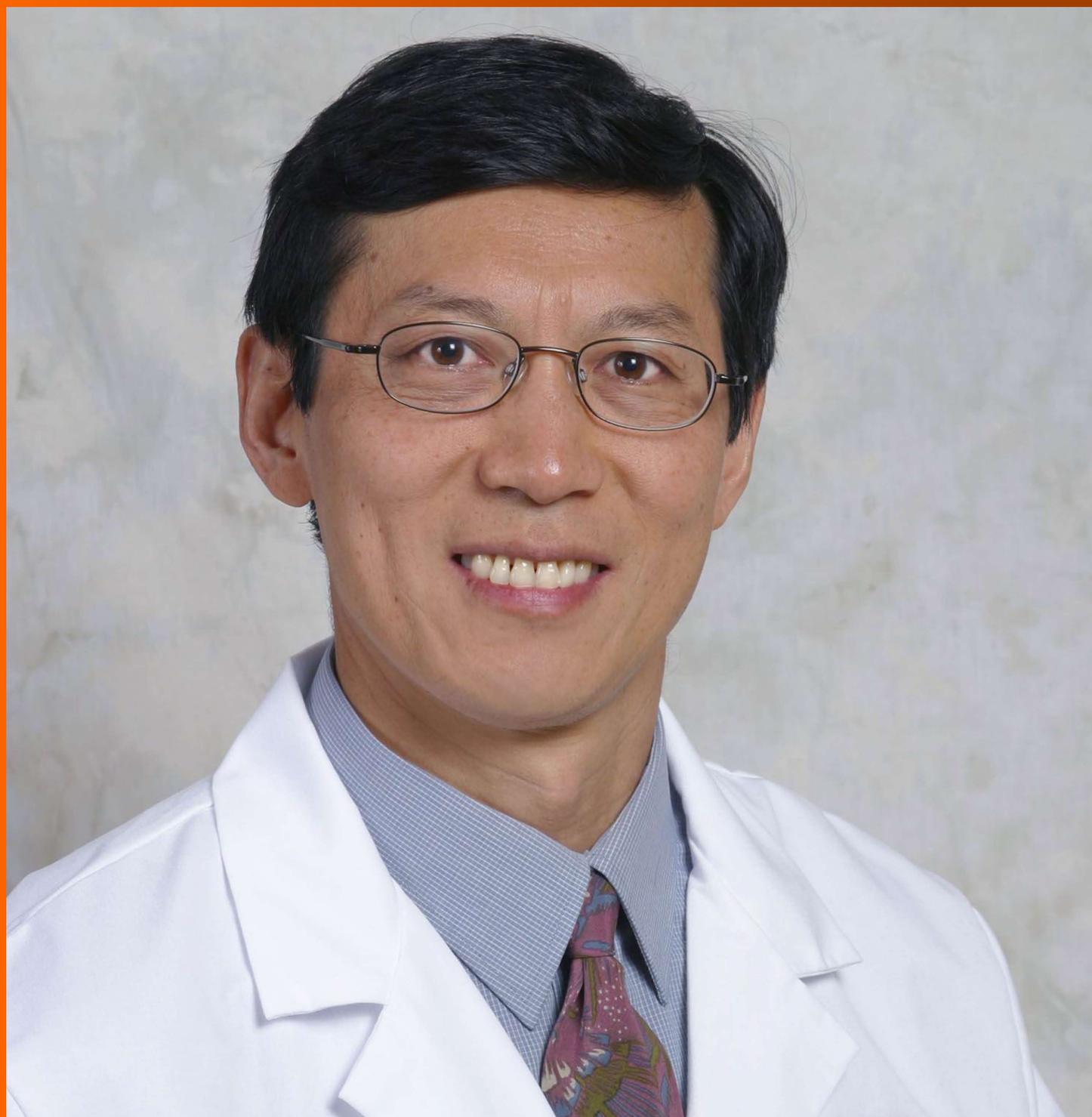


# World Journal of *Medical Genetics*

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## New era of cystic fibrosis: Full mutational analysis and personalized therapy

Marco Lucarelli

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### Abstract

Despite its apparently simple genetics, cystic fibrosis

(CF) is a rather complex genetic disease. A lot of variability in the steps of the path from the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene to the clinical manifestations originates an uncertain genotype - phenotype relationship. A major determinant of this uncertainty is the incomplete knowledge of the *CFTR* mutated genotypes, due to the high number of *CFTR* mutations and to the higher number of their combinations in *trans* and in *cis*. Also the very limited knowledge of functional effects of *CFTR* mutated alleles severely impairs our diagnostic and prognostic ability. The final phenotypic modulation exerted by *CFTR* modifier genes and interactome further complicates the framework. The next generation sequencing approach is a rapid, low-cost and high-throughput tool that allows a near complete structural characterization of *CFTR* mutated genotypes, as well as of genotypes of several other genes cooperating to the final CF clinical manifestations. This powerful method perfectly complements the new personalized therapeutic approach for CF. Drugs active on specific *CFTR* mutational classes are already available for CF patients or are in phase 3 trials. A complete genetic characterization has been becoming crucial for a correct personalized therapy. However, the need of a functional classification of each *CFTR* mutation potently arises. Future big efforts towards an ever more detailed knowledge of both structural and functional *CFTR* defects, coupled to parallel personalized therapeutic interventions decisive for CF cure can be foreseen.

**Key words:** Genotype - phenotype relationship; *CFTR*; Cystic fibrosis; Next generation sequencing; Functional meaning of mutations; Personalized therapy; Mutation search; Mutation functional classes

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**Core tip:** Cystic fibrosis (CF) is the most common severe monogenic disease of Caucasian population. Despite its apparently simple genetics, it has a complex genotype

- phenotype relationship. This is mainly due to the high number of mutations of the causing gene (the *CFTR*) and to the complexity of the *CFTR* cellular network. The next generation sequencing approach allows a full genetic characterization of the *CFTR* and *CFTR* network improving our diagnostic, prognostic and therapeutic ability. This is coupled to the availability of drugs acting on specific mutational classes. The synergy between massive sequencing and personalized therapy is expected to produce an unparalleled advantage for CF patients.

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## INTRODUCTION

Cystic fibrosis (CF; OMIM 219700) has a variable incidence, mostly from 1/25000 (but even with lower incidences in some Countries) to 1/900, depending on the geographical region considered<sup>[1-3]</sup>, and a high heterogeneity of its mutational spectrum and clinical manifestations<sup>[3-6]</sup>. It is considered the most common lethal genetic disease of the Caucasian population, with an average incidence among Europeans and white Americans of about 1/3000 (about 1/27 carriers). The estimated number of affected individuals and carriers worldwide is, respectively, at least 70000 and 20 millions. CF has an autosomal recessive inheritance pattern and is caused by mutations of the cystic fibrosis transmembrane conductance regulator (*CFTR*; 7q31.2; RefSeq NG\_016465.4) gene<sup>[7,8]</sup> (Figure 1). It encodes for a protein expressed mainly at the apical membrane of epithelial cells but also in some cells of nonepithelial origin. The *cfr* protein has a primary function of chloride ion (Cl<sup>-</sup>) channel but with several additional functions as, for example, the transport of other anions (mainly bicarbonate, HCO<sub>3</sub><sup>-</sup>), the regulation of other ionic channels (mainly the epithelial channel of sodium ion, Na<sup>+</sup>; ENaC) and a direct or indirect role in immunity and/or inflammation<sup>[7,9,10]</sup>. The direction of the Cl<sup>-</sup> movement depends on the specific cell type. For example, the *cfr* channel accomplishes Cl<sup>-</sup> extracellular secretion in colon and airways (Figure 1) but its reabsorption in sweat glands<sup>[9,10]</sup>.

The most critical effects on morbidity and mortality arise in the lung, where a *cfr* functional deficiency originates an anomalous dual ion transport, depending on the *cfr* channel for Cl<sup>-</sup> secretion and on the regulation of *enac* channel for Na<sup>+</sup> reabsorption<sup>[11-15]</sup>. The consequent altered water absorption produces a sticky mucus and an abnormally viscous airway surface fluid which leads to impaired mucociliary clearance<sup>[8]</sup>. Bacterial infections, often multiresistant, and an exacerbated response of immune system produce bronchial obstruction, bronchiectasis, atrophy and, finally, respiratory failure<sup>[8,16]</sup>.

Clinical manifestations and severity of CF are highly

variable. Classic CF forms and *CFTR*-related disorders (*CFTR*-RDs, also called non-classic forms) are generally recognized<sup>[17,18]</sup>. The classic CF is usually poly-symptomatic and multi-organs, with infertility for obstructive azoospermia due to congenital bilateral absence of vas deferens (CBAVD) in nearly all CF male and pancreatic insufficiency in over 80% of patients. The *CFTR*-RDs are usually oligo- or even mono-symptomatic with more than 10 different and highly heterogeneous phenotypes<sup>[7]</sup>. The most characterized *CFTR*-RDs are probably those linked to male infertility and subfertility. By now, the mono-symptomatic CBAVD (with no other symptoms of CF) is a clinical entity well known to be based on *CFTR* mutations in a considerable proportion of cases<sup>[5,6,19]</sup>. Other male reproductive defects, such as non-obstructive azoospermia, reduced sperm quality, spermatogenesis defects<sup>[20-26]</sup> and idiopathic seminal hyperviscosity<sup>[27,28]</sup>, have been proposed to be linked to *CFTR* mutations in at least part of cases, and deserve further studies. Other well-defined *CFTR*-RDs are idiopathic pancreatitis and disseminated bronchiectasis<sup>[6]</sup>.

The CF diagnosis needs the combination of *CFTR* genetic analysis, biochemical assessment (the main diagnostic test being sweat test<sup>[29]</sup>) and clinical presentation, according to recent guidelines and recommendations<sup>[17,18]</sup>. However, several variables complicate the framework.

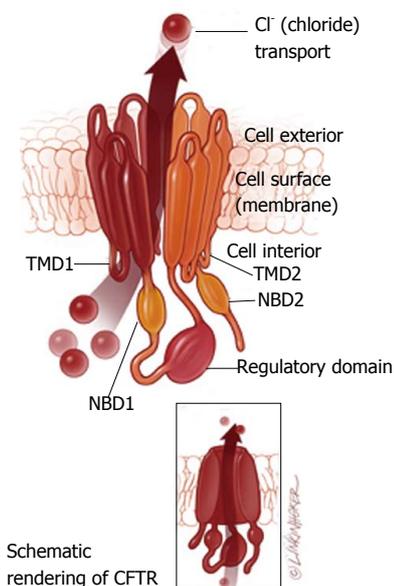
## A MONOGENIC DISEASE WITH A COMPLEX GENOTYPE - PHENOTYPE RELATIONSHIP

The genomic sequence of *CFTR* gene is about 250 kb long and contains 27 exons. The main transcript is of 6132 bases and is translated to a protein of 1480 aminoacids (Ensembl database <http://www.ensembl.org/index.html>). CF should have a simple genetics. Affected subjects have both mutated alleles, with the same (homozygotes) or different (compound heterozygotes) mutations. A carrier of one mutation on one allele has no clinical symptoms. Two carriers have a risk of 1/4 (25%) of having an affected child. However, several biochemical and genetic aspects add complexity.

At moment 2011 *CFTR* sequence variations are identified (*CFTR1* database <http://www.genet.sickkids.on.ca/Home.html>). Although a great effort directed to the recognition of *CFTR* disease-causing mutations<sup>[5,30]</sup> (*CFTR2* database <http://cfr2.net/>) is underway, pathogenic consequences are known only for a part of them. *CFTR* mutations that have been functionally characterized are grouped into 6 mutational classes<sup>[6,31]</sup> (Table 1). The class I (defective synthesis) identifies mutations with a complete lack of protein production. Most of the mutations in class I are nonsense, frameshift or severe splicing mutations, as well as deletions or insertions. In the class II (defective processing and trafficking) are grouped processing/maturation defects with increased degradation, within the endoplasmic reticulum, of the misfolded protein and a severe decrease of the protein quantity in the apical

**Table 1 Cystic fibrosis transmembrane conductance regulator mutation classes and cystic fibrosis personalized therapy**

Mutation class	Functional effect	Structural defect	Mutation-specific intervention	Personalized therapy
I	Defective protein synthesis (complete lack of protein production)	Nonsense mutations Frameshift mutations Severe splicing mutations Deletions or insertions (a common mechanism is the onset of premature stop codons)	Restore synthesis by suppressors of premature stop codons in-frame (read-through drugs)	Suppressor in phase 3 trials: Ataluren
II	Defective protein processing and/or trafficking (severe decrease of protein in the apical membrane due to processing and/or maturation defects)	Missense mutations Small deletions or insertions	Restore processing and trafficking by correctors (chemical, molecular, pharmacological chaperones) and combined approaches (corrector + potentiator)	Combined therapy to patients: Orkambi (the corrector Lumacaftor + the potentiator Ivacaftor)
III	Defective channel regulation and/or gating (impaired channel opening)	Missense mutations Small deletions or insertions	Restore channel regulation and gating by potentiators	Potentiator to patients: Ivacaftor
IV	Defective Cl <sup>-</sup> conductance (reduced Cl <sup>-</sup> transport through the channel)	Missense mutations Small deletions or insertions	Restore the Cl <sup>-</sup> conductance by potentiators	Under evaluation
V	Reduced mRNA synthesis (reduction of the wild type mRNA)	Partial splicing mutations Promoter mutations	Restore wild-type mRNA levels by correctors, potentiators and/or antisense oligonucleotides	Under evaluation
VI	Decreased protein stability in membrane or reduced ability of other channel regulation	Missense mutations Nonsense mutations Frameshift mutations (a common mechanism is the onset of overdue stop codons because of mutations of the protein C-terminus)	Restore stability and regulation ability by potentiators, stabilizers and/or suppressors of overdue stop codons in-frame	Under evaluation



**Figure 1 Schematic representation of cystic fibrosis transmembrane conductance regulator protein.** Image from Johns Hopkins Cystic Fibrosis Center website: <http://www.hopkinscf.org/what-is-cf/basic-science/cftr/structure/>. TMD: Transmembrane domain; NBD: Nucleotide-binding domain; CFTR: Cystic fibrosis transmembrane conductance regulator.

membrane. In class III (defective regulation or gating) are included mutations leading to a defective regulation that impair channel opening. In class IV (defective Cl<sup>-</sup> conductance) are grouped the defects of reduced Cl<sup>-</sup> transport through the *cftr* channel. Most of the mutations in classes II, III or IV are missense mutations (but also small deletions or insertions can be found). Class V

(reduced synthesis) mutations are mild to moderate splicing defects, promoter mutations or, in general, mutations that by various mechanisms reduce the wild type mRNA. Class VI (decreased stability) mutations decrease the stability of *cftr* protein in membrane. Mutations producing a reduced CFTR ability in regulating other ionic channel are usually classified into this class. They can be missense mutations but also nonsense or frameshift mutations of the *cftr* C-terminus (for example generating overdue stop codons). Frequently, one mutation can raise more than one biochemical defect and, consequently, can be classified into different mutational classes. For example, the most common CFTR mutation, the F508del, belongs to at least 3 different classes. It mainly acts as a class II mutation, but also as a class III and V. In CF patients, mutations can combine in *trans* (on different alleles) to originate a great number of homozygous and compound heterozygous mutated genotypes. However, it is becoming clear that also combinations of mutations in *cis* (on the same allele) are not uncommon.

Mutated genotypes of patients with varying clinical forms of CF may appear identical, despite the presence of in *cis* mutations and/or sequence variations not revealed. This usually happens for the incompleteness of diagnostic mutational search protocols that are often stopped after the finding of the first two CFTR mutations in *trans*. Although a focused experimental search for complex alleles has not yet been undertaken, about 50 of them have been so far described<sup>[7,32]</sup>. A complex allele is often originated by mutations that have a mild phenotypic effect if isolated but, if combined in *cis*, originate more severe

clinical symptoms. Also cases of one main mutation whose phenotypic effect is worsened by a second sequence variation characterized as neutral if isolated have been described. Even cases of additional mutations in *cis* that lessen the phenotypic severity exist. Furthermore, it has been demonstrated that some CFTR polymorphisms combined in specific haplotypes may have mild clinical consequences<sup>[33,34]</sup>.

CFTR transcription is controlled in a time- and tissue-specific manner<sup>[10]</sup>, with regulatory elements not limited to its own promoter but extended to the whole CFTR locus<sup>[35-39]</sup>, which encompasses about 500 kb of genomic DNA. Also miRNAs have been shown to contribute to final CFTR mRNA levels<sup>[40-44]</sup>. Within the path from the gene to the channel acting in cell membrane, the *cftr* protein undergoes complex processes of transport and maturation, channel opening and closing (as well as its other multiple functions), endocytosis and recycling, interaction with several other membrane proteins and, finally, degradation within lysosomes<sup>[10,45,46]</sup>. Several experimental evidences highlighted the modulation of the direct effects of CFTR, either wild type or mutated, by other genes. A common evidence of this effect is a high clinical variability between CF patients with a really identical CFTR mutated genotype. This property is usually attributed to the so-called modifier genes<sup>[47]</sup> or, more generally, to the CFTR interactome<sup>[48]</sup>. A number of independent studies dealt with this topic and tens of genes candidate to the modulation of original *cftr* effect have been selected<sup>[6,47,49-54]</sup>. The last investigation of the International Cystic Fibrosis Gene Modifier Consortium<sup>[55]</sup> is considered the most complete one at moment. They explored over 8 million variants in an overall number of 6365 CF patients by two genome-wide association studies. Five modifier loci of lung disease severity have been found highly significantly associated with the severity of lung disease in CF patients. The need of replication studies aimed to the consolidation of the original findings is at moment particularly felt.

Due to the above mentioned sources of variability, and also to random variability and environmental influences, the genotype - phenotype relationship in CF is very complex<sup>[54,56]</sup>. This complexity severely hampers our diagnostic, prognostic and therapeutic abilities. Although few, if any, direct experimental data exist, it is generally accepted that the clinical manifestations and severity depend on *cftr* residual function<sup>[57,58]</sup> which, in turn, depends on the sum of the events that, directly or indirectly, determine *cftr* overall functionality. Furthermore, the *cftr* levels physiologically required are tissue-specific, which means that at the same level of functional *cftr* only some organs will be affected. The general model of residual functionality cannot be easily translated into clinical practice, mainly for our poor understanding of all molecular mechanisms involved. The common sentence that "genotype - phenotype relationship in CF is unclear" appears to be supported only by our limited knowledge of molecular pathogenesis of this disease. Every effort aimed to an enhanced structural and functional characterization of *CFTR* gene and mutations, as well as of all the other

involved genes, is welcomed.

A first step towards a more functional view of CF is the moving from an allele-oriented to a genotypic-oriented view of CFTR genetics<sup>[5]</sup>. The whole CFTR mutated genotype, taking into account all the mutations both in *trans* and in *cis* present in each patient, appears to be an enhanced descriptor of the final clinical form, at least at level of phenotypic macro-categories. In agreement with this enhanced view, also the CFTR2 database has been recently improved towards the analysis of genotypes, in addition to the classic allelic view.

## NEXT GENERATION SEQUENCING AND FUNCTIONAL MEANING OF MUTATIONS

The complex genetics and genotype - phenotype relationship which underlies CF has been complicating the delineation of unequivocal mutation search strategies. It has been proved that it is not possible to set up a unique genetic test suitable for both diagnostic (including the mutational search step of neonatal screening programs) and carrier screening programs directed to the general population, as well as for all clinical forms of CF<sup>[59]</sup>. For diagnostic purpose, a multistep genetic approach is usually applied<sup>[5,59]</sup>. The first step commonly used is the search for a panel of mutations showing a suitably high detection rate (the proportion of mutated alleles detectable by the specific test; DR). Notably, there is no limitation regarding the type of mutations that can be included in panels. In fact, recently, also well characterized (particularly concerning the breakpoints) and frequent CFTR macro-deletions have been included. Depending of the geographical area and on the CF clinical form, the DR may vary greatly and, if the same panel is anyhow used, a number of alleles with no mutation detected (the so-called "unknown" alleles) remain. In these cases, a second step of sequencing, usually of all the exons, adjacent intronic zones and proximal 5'-flanking of *CFTR* gene, is often applied. The DR reached is considerably increased in respect to the first step, although some molecular lesions are not recognized yet as, for example, full intronic mutations and copy number variations (CNVs, mainly rare macro-deletions and macro-duplications of *CFTR* gene). To this purpose, a third step of search for CNVs is added for all those subjects with CF suspect but still with unknown alleles at the second step. Only in a very small proportion of cases a fourth step of RNA analysis (able to reveal anomalous splicing caused by full intronic mutations) is further added<sup>[60]</sup>. Furthermore, the common multistep approach is ending the mutational search on CFTR after the first two mutations in *trans* are found. This can often represent a severe limitation of the accuracy of CFTR genotyping, but can be seen as an unavoidable consequence of the use of mutational panels.

It is obvious that the multistep approach is inappropriate for carrier screening. The best mutational panel, optimized for ethnic origin of the tested subject and for the clinical form the program is aimed to, should be used. This seems to be appropriate even if only classic

**Table 2 Comparison of classic and next generation sequencing approaches for diagnostic mutation search in cystic fibrosis**

Feature	Classic approach	NGS approach
Analytical requirements for a full characterization of the <i>CFTR</i> gene	Multiple technical steps and different analytical platforms	Reduced number of technical steps and single analytical platform
Data elaboration	Multiple data elaboration steps handled by the laboratory itself	Reduced number of data elaboration steps often performed by internal dedicated personnel or external structures
Throughput	Low	High
Automation	Moderate	High
Timing	Time consuming	Rapid
Cost per sample	High	Low (if a reasonably high number of samples are processed in the same run)
No. of mutations analyzed	Progressively increasing from moderate (first steps) to high (last steps)	High
Detection rate	Progressively increasing from moderate (first steps) to high (last steps)	High
Possibility to analyze other genes involved in the modulation of CF clinical manifestations	Unlikely	Realistic

NGS: Next generation sequencing; CF: Cystic fibrosis; CFTR: Cystic fibrosis transmembrane conductance regulator.

CF is within the carrier screening program, as differences between mutational patterns underlying different clinical manifestations of classic CF (for example pancreas sufficiency and insufficiency) have been highlighted<sup>[59]</sup>.

The so-called next generation sequencing (NGS) is a first revolutionary hit, breaking the CFTR mutational search field<sup>[61-65]</sup> (Table 2). The possibility to sequence a huge number of extended DNA zones, rapidly and at low-cost, will allow the sequence analysis of CFTR regions usually not studied as, for example, the distal 5'-flanking, the 3'-UTR and the full introns, as well as the whole CFTR locus. It probably will also reveal an unsuspected high number of complex alleles. In addition, as at least some NGS platform also allows the simultaneous analysis of small mutations as well as CNVs, a combination of the second and third step of genetic analysis in a single step is expected. Even the actual possibility of a complete sequence analysis of genes of CFTR interactome is at hand, allowing a full analysis of both intra-CFTR and CF-related extra-CFTR structural variability. This approach raises the problem, common to every sequencing protocol but enhanced by the effect of scale of the massive parallel sequencing, of functional interpretation of the huge number of sequence variations found.

The ability of NGS approaches to find sequence variations greatly overcomes our ability of their functional characterization for clinical purposes. This problem is doomed to increase in parallel with the finding of an increasing number of complex alleles and of sequence variations both within the CFTR locus and in the networking genes of CFTR interactome. Although complex alleles are at moment barely studied from functional point of view, some demonstration of their effect on the final phenotype exists<sup>[66,67]</sup> (for a review see<sup>[7,32]</sup>). On the contrary, only sporadic, if any, attempts have already been made to demonstrate long-range regulation alterations by sequence variations within CFTR locus as well as to reveal some perturbations of the CFTR interactome coordination by sequence variations in the cooperating genes.

Mainly due to these interpretation limitations, it is likely that mutational search by panels of mutations already characterized as disease-causing will persist. Also in this case, NGS approaches can greatly enhance our mutational search ability allowing the use of wider mutational panels. It is now possible to include in NGS panels hundreds of disease-causing mutations of *CFTR* gene, including complex alleles. Thanks to the increasing number of mutations of NGS panels, the gap at moment observed between the DR shown by the same mutational panel applied to different ethnicities and CF clinical forms<sup>[59]</sup> will reduce. However, due to the existence of very rare, and often individual, mutations<sup>[5]</sup> and of a high geographical heterogeneity, it is unlikely that only one large panel can be suitable for all ethnicities and CF clinical forms.

## PERSONALIZED THERAPY

The availability of a methodological approach potentially able to recognize all the molecular lesions relevant for the clinical manifestation of CF perfectly fits with the P4 medicine framework: Personalized, predictive, preventive and participatory<sup>[68,69]</sup>.

A second revolutionary hit in CF, which completely changed the therapeutic mindset, was the demonstration that the personalized therapy of CF is not anymore just a vision. At moment, in CF, the term personalized therapy mostly means the correction of the effects of specific CFTR mutations by a small-molecule therapy targeting the defective *cfr* protein (Table 1). The work of categorizing CFTR mutations in functional mutational classes according to their biochemical effects, which could appear only an academic exercise, revealed to be fundamental for the development of effective therapies<sup>[70]</sup>. Nineteen treatments aimed to the restoration of *cfr* function depending on the kind of mutations are at moment in the drug development pipeline of the United States CF Foundation (<https://www.cff.org/Trials/Pipeline>).

Two mutational class-specific treatments are now in clinical practice. The first is the Ivacaftor (also known as VX-770 or Kalydeco), a potentiator facilitating the opening of the Cl<sup>-</sup> channel in the cell membrane, released to patients in December 2014. It is effective on the so-called mutations of gating and was approved for 9 specific mutations belonging to this class. The second is a therapy that combines a corrector and a potentiator, respectively, the Lumacaftor (also known as VX-809) and the Ivacaftor (the combined therapy is also known as Orkambi), released to patients in July 2015. It is effective also on those mutations with a defective maturation as, for example, the F508del. In addition to these drugs already approved for clinical use, there are also 2 drugs in phase 3 trials: The Ataluren (Translarna), which is effective on nonsense mutations by overriding the premature stop signal, and the combined therapy of Tezacaftor (also known as VX-661) and Ivacaftor (respectively, a corrector and a potentiator). Another seven drugs are in phase 2 trials and another two in phase 1 trials. Finally, six drugs are in pre-clinical studies. The CF personalized therapy is effective for few mutations but is upcoming for a larger number of them<sup>[71-75]</sup>.

The NGS approach greatly facilitates the task of mutation finding, also taking into account the possibility of multiple molecular lesions concomitantly defining the final severity of the disease and its multifaceted clinical manifestations. It should be evident that an inaccurate mutated genotype, not recognizing all co-occurring molecular lesions, may severely hamper the therapeutic response to a mutation-specific therapy, also with economic drawback due to their high cost. However, despite the increased ability of mutation finding, the obstacle of functional characterization of sequence variations found will probably persist for some time. A central point of future CF molecular genetics is the assessment of disease liability of all the CFTR mutations, rare variants included<sup>[76]</sup>. Without a full comprehension of the biochemical mechanism of each mutation or, at least, the assignment to a functional class (a concept that should be extended to all DNA regions and genes involved) a personalized therapy would be unsuitable. In addition, the possibility of biochemical defects in multiple mutational classes for the same mutation should be taken into account. To deal with this task, it has been proposed to evaluate CFTR sequence variations, in cell-based studies, according to the effect on *ctr* protein of therapeutic drugs and, consequently, to classifying them in "theratypes". An enhanced knowledge of CFTR genetics reached thanks to NGS approaches is a relevant step that could allow the physician to apply a predictive medicine able to delineate the possible evolution of CF, as well as a preventive medicine able to deliver a personalized therapy early enough to reduce at minimum, or even to completely overcome, the clinical manifestations.

A comparison between *ctr* protein therapy and CFTR genetic medicine is unavoidable. Gene therapy, mRNA therapy, gene repair and mRNA repair are the genetic medicine strategies usually recognized for CF<sup>[77]</sup>. At present,

the gene therapy is the most advanced genetic medicine approach for this disease. It should however be clear that the *ctr* protein therapy is a reality whereas the CFTR gene therapy is a hope. About 27 clinical trials of gene therapy involving approximately 600 CF patients have been completed. Currently, only the UK CF Gene Therapy Consortium (<http://www.cfgenetherapy.org.uk/>) is active in gene therapy trials for CF. The state-of-the-art is the most recent non-viral phase 2b multi-dose trial that, for the first time, demonstrated the possibility of stabilizing the progression of CF lung disease<sup>[78]</sup>. A characterizing aspect of the present personalized medicine acting on *ctr* protein defects is its restriction to specific mutations or, at least, mutational classes. Each drug is effective only on a limited number of mutations possibly belonging to the same class, highly characterized in their biochemical mechanism of action. The delivery of these drugs is not restricted to the lung and can complement multi-organ defects common in CF. However, continuous drug administration is required and the physiological level of *ctr* functionality is not restored. On the contrary, the gene therapy can in theory complement any CF mutation without the need of a deep knowledge of its biochemical mechanism of action, the correction could be more stable over time and could reach physiological level of *ctr* functionality. However, the delivery of therapeutic sequence cannot be as generalized as for drugs and a multi-organ complementation is currently unreal. Usually, the lung is the target organ although the efficient delivery of therapeutic sequence to lung airway epithelial cells remains a very hard task, because of a dramatically altered composition of airway surface fluid and the presence of thick mucus.

The improvement of classic symptom-based treatments, together with dedicated Centers for the cure and multidisciplinary teams, have been warranting an increasing of the survival median of CF patients from few years (if not months) in the 1930s up to about 40 years according to a projected median survival for children born and diagnosed in 2010. A further amazing elevation of life expectancy is predictable following personalized therapy. In the meantime gene therapy will be able to solve the basic defect of the disease.

## CONCLUSION

CF is more complicated than expected. NGS platforms represent a revolutionary experimental approach allowing the mutational search in CFTR gene and CFTR gene network with unprecedented power. Within next years an effort towards a full knowledge of the CFTR gene and CFTR locus structure together with related long-range interactions, as well as of CFTR locus mutations in *cis* and *trans* is expected. This will help moving to a genotypic-oriented view of CFTR genetics. Replication studies defining what are the effective modifier genes in CF, outlining their exact role and revealing the sequence variations that disrupt the CFTR interactome coordination is also needed. All these aspects will contribute to an

enhanced comprehension of the genotype - phenotype relationship in CF. Also the possibility of a real clinical application of the personalized therapy aimed to the correction of the biochemical defects of specific mutational classes has been arising in a likewise revolutionary manner. For the widest application of the personalized therapy in CF, the functional characterization at biochemical, cellular and clinical level of the huge number of *CFTR* sequence variations that will be found is mandatory. The area of functional characterization of *CFTR* gene and *CFTR* locus mutations will probably be the most intensively pursued in the next future. The synergy between the two ground-breaking novelties of massive sequencing and personalized therapy is expected to produce an unparalleled advantage for CF patients. Without forgetting that CF is a genetic disease and a definitive correction of its molecular causes only can come from genetics.

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