Health Technology Assessment Of The Genetic Tests For Cystic Fibrosis Carrier Screening In Italy

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Abstract

Introduction: Cystic fibrosis (CF) is a genetic disorder caused by mutations in the CFTR gene. In Italy, the reported prevalence is approximately 0.70 per 10,000 inhabitants. Our aim was to summarize the available evidence, using the HTA approach, on the genetic tests for cystic fibrosis carrier screening.

Methods: Systematic literature search was used to find the best available international and national evidence on genetic tests for CF carrier screening. We addressed health problem of disease, description and technical characteristics of tests – its analytic and clinical validity, and clinical utility. Economic evaluation of different scenarios was synthesized from literature. Ethical, organizational, and social aspects of CF and genetic screening were also considered.

Results: Several screening strategies have been evaluated in the literature and screening options can be characterized by different timing, model and place of screening. The reported cost of a screening test ranged from €25 to €212. Ethical analysis emphasized that the use of these tests is an advantage in terms of the acquisition of knowledge and of responsible management of choices, but at the same time raises many ethical questions. Social considerations reported an overall positive attitude among patients and their families towards CF carrier screening.

Conclusions: Advances in the molecular genetics technology have made CF carrier testing reliable and affordable. The multidisciplinary approach of this HTA provided an evidence-based evaluation of the genetic tests and offers a firm scientific background for the decision-makers to consider the implementation of a screening for cystic fibrosis carriers into the Italian health care system.

Keywords: HTA, cystic fibrosis, genetic tests, carrier screening.

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Cystic fibrosis (CF) is an autosomal recessive genetic disorder caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene located on chromosome 7. The gene encodes for a membrane protein which has the properties of a chloride channel. CFTR however exhibits a number of function beyond controlling chloride movement through epithelial membranes and is now considered as a hub protein involved in different intracellular and membrane processes. Over 2000 mutations have been found but only for a minority there is clear relation to clinical manifestations. These are quite variable and involve various organs. Exocrine pancreas, lung, vas deference are most commonly involved leading to the classical form of CF and causing fat malabsorption, malnutrition, recurrent pulmonary infections and male infertility. Clinical manifestations vary, however, widely and non-classical, atypical forms of CF are known to occur. The incidence of CF may vary in different population but is approximately 1:2500-3500 neonates/year among Caucasians (Chapter 2.1 for further details). The frequency of healthy carriers bearing a single mutation is of the order of 1:25 in the general population [1].

The classical forms are already clinically observed soon after birth due to steatorrhea and poor growth. Early airway colonization by specific bacteria (i.e. Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa) can occur precociously. Muco P aeruginosa lower airways infection is considered almost pathognomonic of the disease. Pulmonary disease and bronchial obstruction are the result of highly dense and viscous bronchial secretions which are the main characteristic of the respiratory disease (hence mucoviscidosis) favoring bacteria adhesion and growth. This has been linked to the basic defect in bronchial epithelia which causes the production of a low water content of mucus layer on the bronchial epithelial surface.

Exogenous pancreas insufficiency can be corrected by replacement treatment with exogenous enzymes allowing normal growth in the majority, but not all, patients. A minority of patients are pancreatic sufficient at birth (15-25%), some of which can lose exocrine sufficiency later in life. Uncontrolled lung infection leads to chronic colonization and recurrent pulmonary exacerbation causing the development of bronchiectasis and chronic purulent lung disease progressing to pulmonary insufficiency, the major cause of death.

Other manifestations include salt losing syndrome due to excessive NaCl content in sweat, chronic liver disease which can progress to liver cirrhosis in about 5% of cases, paranasal sinus disease, nasal polyps, chronic allergic aspergillosis, growth retardation, intestinal obstruction, meconium ileus. Around 20-30% of patients develop a peculiar form of diabetes in the adult age know as CF-related diabetes exhibiting properties which differ both from classical type I or II. The disease causes also the formation of renal or gallbladder stones. It is still unclear whether CF is associated with an increased frequency of colonic cancer. Beyond the classical appearance many cases can evolve slowly being apparent in late childhood or even adulthood. Diagnosis is made according to an abnormal sweat test (high chloride content), presence of CFTR mutations in at least one allele, clinical manifestations. Several guidelines for diagnosis are available [2]. In a minority of cases sweat tests are in a borderline range, mutations are not found in a first approach genetic screening and clinical manifestations are mild and more elaborated tests could be necessary (i.e. nasal potential differences, whole gene screening).

CF may however be recognized at birth by neonatal screening consisting in the measurement of immunoreactive trypsinogen in the blood of the neonate followed or couple to genetic tests and confirmed by sweat test. Newborn screening
programs are implemented in the majority of European countries, Russia and North-America. In these cases more than 90% of cases are recognized in the first weeks of life [3].

Modifying-disease drugs have been made available only in recent years, the first one being Kalydeco a CFTR potentiator active in specific mutations accounting for 3-8% of CF cases. More recently a fixed combination of a potentiator and a corrector known as Orkambi has proved to have some activity on the major mutation worldwide, the F508del mutation accounting for 30->50% of cases in different countries. No data are still available on the long term effects of these drugs, but in a 2-5 years term there has been clear improvement of lung function, reduction of pulmonary exacerbation, weight gain (Kalydeco) or decreased rate of pulmonary exacerbations (Orkambi). Several other compounds are now in clinical development or undergoing clinical trials (phase I-III). Due to the very active research on novel therapeutic strategies it is foreseeable that in the few next years the clinical spectrum of CF will undergo profound changes.

Other treatments include antibiotic therapies for acute and chronic infections, chronic nebulized antibiotics in case of airway colonization, pancreatic replacement therapy, treatment of complications including ear-nose and throat surgery, GI surgery for intestinal obstruction, liver or renal stones, oral hypoglycemic agents and insulin for diabetes. Almost all patients need daily respiratory physiotherapy to remove pulmonary secretions. All this represents a high burden of care for patients and their families.

However, so far around 30% of patients develop in early adulthood a severe respiratory disease leading to chronic obstructive pulmonary disease with chronic, multidrug resistant bacteria, mainly Pseudomonas aeruginosa, but other less frequent and multiantibiotic-resistant bacteria can be found. This causes respiratory failure, need of oxygen treatment, non-invasive respiratory support and lung transplant, of which CF is the leading cause in children or young adults.

A minority of patients develop portal hypertension and may require liver transplantation or surgery to relief portal elevated pressure. Chronic ursodeoxycholate has been recommended for CF related liver disease, but apparently does not affect the evolution of severe cases. Since respiratory failure is the principal cause of death, monitoring respiratory function through spirometry is necessary. Forced expiratory volume in 1 sec (FEV1) is the major parameter related to the progression of lung disease and is related to quality of life, rate of hospitalization, survival. Therefore, FEV1 is the major surrogate endpoint in CF studies. Typically, FEV1 decreases progressively throughout life and abruptly decreases during acute pulmonary exacerbations. Once values of 40% of the normal range predicted for age is reached respiratory disease is considered severe and with lower levels patients enter in a LTtx program.

Median survival for CF has considerably increased in the last decades. Once considered a cause of childhood death today the great majority of patients reach adulthood with a median estimated survival of around 50 years in industrialized countries. Rate and age of death varies however based on the provision of cures in specialized center, access to cure, welfare, economic sustainability, gross national income, health system. In several industrialized countries adults account for over 50% of the CF population and it is foreseeable that their number will rapidly grow in the next two decades making CF a disease of adults and even elderly subjects. Late onset complications i.e. bone and joints disease, cancer, endocrine, heart involvement are always more frequently recognized [4].

1.1.2 Genetic testing for CF: methodology of evaluation

Baroukh Maurice Assael

More than 2000 mutations of the CFTR gene are known. Finding a mutation is a decisive step in the process of diagnosis. However, gene/phenotype correlation is not reliable for individual use, with the only possible exception of exocrine pancreatic status [5]. A genetic test for CF can be performed for a variety of reasons:

Diagnostic purpose

1. In the process of neonatal screening after a positive trypsin test at birth
2. In the process of diagnosis in a patient exhibiting symptoms suggesting CF
3. In special conditions where the CFTR gene is known to play a role: chronic or recurrent pancreatitis, male infertility
4. Prenatally to assess the fetal risk in the presence of fetal signs associated to CF (i.e echogenic fetal bowel) after testing parents

For screening purposes

5. In a close relative of a patient
6. In a partner of a person carrying a CF mutation
7. Preconceptionally
8. Prenatally when parents are known carriers of CF mutations
9. A genetic test for CF can also be performed in persons with no clinical indication or genetic risk (general population).

The genetic test can be performed looking for specific mutations or screening for a panel of mutations. Panels usually include mutations known to be associated with the disease and/or prevalent mutations in the population. Panels may be customized to reach a sensitivity of at least 95% in the population under study. The choice of the testing procedure determines the sensitivity of the test and its cost in a rapidly changing panorama.

Furthermore, methods are rapidly changing and new methods based on Next Generation Sequence (NGS) technologies may be customized to include up to hundreds of
mutations, increasing thus the sensitivity of the test. Approximately 250 mutations are now labelled as CF-causing by the CFTR2 database (www.cftr2.org), and in the next few years it is expected to reach a total of 500 mutations. All these mutations will probably soon be included in a single carrier test, with very high sensitivity.

In most regions in Italy, CF genetic tests should be prescribed by a geneticist or after the indication of a CF specialist in order to be partially reimbursed by the National Health (NH) system. Panels including from around 50 to around 80 mutations are most widely used, however new generation gene scanning is rapidly gaining popularity and new recommendations are currently being developed to include gene screening as a standard diagnostic procedure.

Specific recommendations are given by the Italian Medical Genetic Society (SIGU, Società Italiana di Genetica Medica) and by the Italian CF Society but do not include so far gene screening.

Genetic testing contributes to the estimation of the carrier frequency in the population. CFTR mutations frequencies are reported to the Italian CF registry and to the Italian database in the European CF Patients Registry.

References


Chapter 2

2.1. EPIDEMIOLOGY OF THE CYSTIC FIBROSIS IN ITALY

Vladimir Vukovic, Giancarlo Ripabelli

2.1.1 Prevalence and incidence of cystic fibrosis and carriers of related genes in Italy and by region

Epidemiological data on the incidence and prevalence of cystic fibrosis (CF) have been collected from the available literature. Studies published on PubMed, Scopus, ISI web of science up to February 1st, 2017, dealing with the prevalence and incidence of cystic fibrosis and carriers of related genes in Europe and in Italy, were examined to describe the frequency of the disease. The following combination of keywords was used for the search: (“Cystic Fibrosis” OR “CFTR gene carriers” OR “Cystic Fibrosis Transmembrane Conductance Regulator gene” OR “CF gene carriers” OR mucoviscidosis OR “fibrocystic disease” OR “pancreatic fibrosis”) AND (prevalence OR frequency OR incidence OR occurrence OR epidemiology) AND (Europe OR Ital*).

Also, detail search of specific CF websites – European Cystic Fibrosis Society Patient Registry, Cystic Fibrosis Database, Fondazione per la Ricerca sulla Fibrosi Cistica – Onlus, Lega Italiana Fibrosi Cistica, Società Italiana per lo studio della fibrosi cistica, was carried out for the same time period, in order to draw additional information about the Italian epidemiological context. Several studies have largely used a population-based approach, and the majority have provided data on CF incidence and prevalence for the populations of the regions of northern Italy, while accurate and recent data are still lacking for the rest of the national territory.

The discovery of the Cystic fibrosis transmembrane conductance regulator (CFTR) gene in 1989 increased the awareness of the disease, and thus a lot of scientific attention has improved CF research. Since then, there have been attempts in Italy to evaluate the prevalence and incidence of CF and its changes during time. A registry of all CF patients in Italy has been established and annually updated since 1988.

In Europe, among Caucasians, the most widely reported incidence for CF is 1 case per 2000-3000 live births [1]. On the other hand, the incidence of CF reported for Italy ranges between 1 in 4854 [2] and 1 in 2438 [3], based on data from different regional populations. Combining data of 35,806 CF patients from 27 EU countries in 2004, Farrell et al. reported a mean birth prevalence of CF to be 0.74 per 10,000 in this population, ranging from 2.98 in Ireland to 0.12 per 10 000 in Finland [4]. Mehta et al. (2010) in their study investigated the cross-sectional CF demography, by focusing on country-specific population profiles collected from CF centers across 35 European countries and demonstrated that there were approximately 4 CF children for every 3 CF adults (57% versus 43%, respectively) in their 29,095 sample, across Europe [5].

In 2016, Italian Cystic Fibrosis Register Report 2010 [2] was published and have reported the estimated prevalence of 7 per 100,000 residents in Italy, same as the one published in previous Report (2006) and almost the double with respect to the number reported in 1988 (3.62 per 100,000 inhabitants) when the Registry was activated [6]. The large increase in the reported prevalence has to be probably associated with the decreasing mortality occurring every year. The number of deaths is much lower than the number of people with a new CF diagnosis. The increased life expectancy is the likely cause of
increasing population prevalence, and not to the increase in
the number of patients diagnosed. Among the Italian regions,
the population prevalence is highly variable, from a minimum
of 4.3 per 100,000 inhabitants in the Friuli-Venezia Giulia
region, to a maximum of 10.2 per 100,000 inhabitants in the
Basilicata region (Figure 1).

Considering the number of live births in Italy in 2010 (N = 549,794, ISTAT), an incidence of 20.6 per 100,000 live
births (1 in 4854) was estimated, assuming a delay in dia-
gnosis for those born in 2010 (similar to that of 2008 and
2009), and taking into account the missing data from the
centers of Campobasso, Cagliari and Livorno [2].

The estimated median CF incidence at birth for the pe-
riod 1988-2004 was 1:4079 live births, with a minimum of
1:4762 in the 1998, and a maximum of 1:3425 in 2000. Par-
ticularly, the incidence data of CF for each Italian region
according to the year of birth ranging from 1988 to 2004 are
reported in Table 1 [6].

Table 1. Incidence of CF (per 10,000 live births) among different Italian regions (by years and place of birth) (Modified from: Società Ita-
liana per lo studio della Fibrosi Cistica. Report del Registro Italiano Fibrosi Cistica. 2006.) *CI 95%: Confidence Interval 95%

<table>
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<tr>
<th>Region of birth</th>
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(Continua)
In a study conducted in the regions of Veneto and Trentino Alto-Adige, in the north-eastern part of Italy, a total of 593 patients (283 males and 310 females) born and living in those areas, and diagnosed at the Cystic Fibrosis Center of Verona from 1958 to 2000, were enrolled. The authors reported an estimated incidence of cystic fibrosis of 1:2650 live births per year. The incidence resulted from 129 diagnosis during 1993–1999 on a total of 341,947 screened neonates. In Veneto and Trentino Alto-Adige regions, neonatal screening has been implemented since 1973, being among the first programs in Europe, and have been enhanced during time, reaching virtually the whole cohort of newborns (98.4 to 99.1%) in the late 1990’s [7].

In another cohort study in Veneto and Trentino Alto-Adige, 228 newborns with CF were found among a total of 807,191 newborns screened at birth for CF between 1990 and 2005. Hence, the CF incidence at birth in Veneto/Trentino Alto-Adige regions was estimated to be 1:3540 (95%CI 1:4,031 – 1:3,109). A significant decrease in the CF incidence at birth was observed over this 16-years period, with an average annual percent change (AAPC) of -4.7% (95% CI -7.3 – -2). The decrease was clearly noticeable after 1995, corresponding to an increased use of prenatal diagnosis in those areas at the time, and it was reported to be even higher in later years, when terminations of pregnancy after prenatal carrier testing began. By including the number of pregnancy interruptions, the overall incidence of CF would be 1:3116 in Veneto/Trentino Alto-Adige, instead of 1:3540 [8]. Later, a successive study was conducted by a CF center in the area of Verona city, Italy, on the same resident population; particularly, between 1993 and 2007, 779,631 newborns were screened for CF, and 195 newborns with CF were detected through this neonatal screening. The overall incidence of CF was estimated to be 1 in 3998, or 1 in 3821 including false-negative test results [9].

In order to evaluate the effects of CF carrier screening on birth prevalence trends and the newborn screening (NBS) efficiency, a study has been conducted where, the above mentioned Italian regions – with and without carrier screening program, were compared for a period of 20 years. From 1994 to 2013 year, 174,494 carrier tests were performed; 5966 carriers and 150 carrier couples were detected from this cohort. In the region without carrier screening, the estimated birth prevalence was calculated to be 1:3589 in 1993 and 1:3870 in 2013; while in the region where carrier screening was performed the prevalence was 1:2730 and 1:14,200 in 1993 and in 2013, respectively. Over the whole study period 1,112,620 neonates were screened, with an annual average of 52,982, and a total of 259 newborns with CF were detected. The average CF birth prevalence for the indicated period was estimated to be 1:4296 (1:4,106 including false-negative results). A time-related decrease in birth prevalence was confirmed for the period under study, with a mean annual percentage decrease of 9% (95% CI: 4–15%; P = 0.002) [10].

In a study evaluating the CF carrier frequency conducted at the Pediatrics Department at the University of Padua the results of a 10-year CF-carrier screening program (from 1996 to 2006) on 57,999 subjects with no prior family history of CF (28,026 males and 29,973 females, ranging from 20 to 50 years of age) were reported. Among the persons screened, 1879 CF carriers were identified, with an overall frequency in the general population of 1:31 (3.23%), as a priori risk for Italian northeastern populations. By including the individuals with a family history of CF in the calculation (541 CF carriers were identified out of 1783 analyzed), the frequency of carriers increased from 1:31 to 1:25 [11]. The estimated incidence of CF in the regions of Veneto and Trentino Alto-Adige is one of the highest reported so far, and is also higher than the overall incidence reported for Italy, where it is still lacking a national screening program.

### Table 1 (continua)

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<table>
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<td>1/4700</td>
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<tr>
<td>Latium</td>
<td>28,500</td>
<td>1/3150</td>
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<td>Liguria</td>
<td>11,000</td>
<td>1/4400</td>
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<tr>
<td>Lombardy</td>
<td>92,000</td>
<td>1/4600</td>
</tr>
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<td>Marche</td>
<td>13,000</td>
<td>1/5200</td>
</tr>
<tr>
<td>Piedmont</td>
<td>37,000</td>
<td>1/2650</td>
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<td>Tuscany</td>
<td>30,000</td>
<td>1/3500</td>
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<td>Veneto/Trentino-Alto Adige</td>
<td>52,000</td>
<td>1/4150</td>
</tr>
<tr>
<td>Western Sicily</td>
<td>20,000</td>
<td>1/2500</td>
</tr>
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</table>

A study performed on 200,000 newborns between the second and fifth days of life, conducted from 1992 to 2000 in the Lazio region, have reported that the incidence of affected subjects (screening + symptoms/overall newborns) was 1:2982, which corresponds to the carrier frequency in the general population of 1:27 [12].

The prevalence of CF carriers reported by the Lega Italiana Fibrosi Cistica – Marche Region Section, was 1/25 at 31 December 2012 in the resident population. On the other hand, the prevalence of the CF patients in the Marche region was calculated to be 1:3495. The resulting number of CF patients to the Regional Reference Center for Cystic Fibrosis in Marche region was 166 [13].

The epidemiology of CF in other Italian regions has not been investigated with so much attention as in the northern areas of the country. In a large survey on newborn screening (NBS) for cystic fibrosis, from the Members of European Cystic Fibrosis Society Neonatal Screening Working Group, data of the current practice from 26 regional and national CF NBS programs in Europe were collected. They reported the epidemiological data for each Italian region (Table 2). The CF incidence at birth for the Italian regions was calculated to vary from a minimum of 1:5200 in the Marche region to the maximum of 1:2500 in Western Sicily [14].

Taking into account the very dynamic migrations that occurred in the past decades to the European continent and within it, a population-based cohort study using the 1991–2005 database of the newborns screening of Tuscany, Italy, was performed to evaluate the incidence of CF in the Albanian population born in Tuscany region during the time under study, with both parents of Albanian origin. Moreover, it was verified whether the estimated incidence was different from the population of Tuscany, and evaluated the prevalence of CF healthy carrier status among this ethnic group. The CF incidence among children born to both Albanian parents in Tuscany was significantly higher (1:555; 99%CI 1:2980-1:306) than the rest of the Tuscan population (1:4101; 99%CI 1:5564-1:3248). The distribution of AF508 mutation of CFTR gene found in the Albanian CF children was further reported to be very high (70%). The prevalence of CF carrier status among Albanians living in Tuscany was estimated to be 1:12 (99%CI 1:27-1:9), while in the rest of the Tuscan population was 1:32 (99%CI 1:37-1:28) [15].

Data from another study on the newborns cohorts of Veneto and Trentino-Alto Adige, screened for CF at birth between 1990 and 2005, did not support the finding of higher CF incidence among newborns of non-Italian origin. In this study, the birth incidence was calculated to be 1:3090 in the subgroup of newborns with both parents of Italian origin (95% CI 1:3578 – 1:2669), and resulted significantly higher compared to the subgroup of newborns with both parents from a foreign country (1:10.359; χ² = 4.82; P = 0.0273). More precisely, the birth incidence was 1:6113 in the subgroup of newborns with non-Italian Caucasian parents, and 1:18,852 in the subgroup of newborns with non-Italian or non-Caucasian parents [8].

Several variables, not always computable, may affect the differences in the reported CF prevalence observed among Italian regions; it might include a different incidence of certifications of disease at birth and in adulthood; different methods of diagnosis (some regions with high rate of screening for CF); different care; different frequency of non-European citizens; different frequency of voluntary abortions; greater or lesser awareness of the CF problems among the pediatricians and general practitioners; etc. In addition, the estimates are calculated on a small number of patients in some regions, and therefore not always reliable. Indeed, significant differences were observed in the incidence of CF depending on region of birth and, within the same region in Italy [6].

2.2. PROVISION OF GENETIC TESTS FOR CYSTIC FIBROSIS IN ITALY

Baroukh Maurice Assael

There are no systematic data on the provision of genetic tests for CF in Italy. The Istituto Superiore di Sanità runs a quality control study on the genetic test for CF, data have been irregularly collected. Data from the Italian Human Genetic Society SIGU (Società Italiana Genetica Umana) suggest that CF is the most genetically tested disease in Italy.

Most tests are performed neonatally in the process of diagnosis after neonatal screening. Test are recommended and performed in close relatives of patients, before or in early pre-
Pregnancy in case of the woman and/or the partner is a known carrier of CF mutations. Test are performed as well in case of male infertility or medically assisted pregnancy. In all these cases regional programs provide the tests free of charge or with a small co-payment. Other indications are considered inappropriate. However, as shown by the Veneto experience testing for carriers in the general population seems to gain popularity. In Veneto it has been calculated that almost 200,000 persons have been tested in the last decades, which has been paralleled by a decrease in the disease incidence [16]. The authors of the paper report that the reasons for which the incidence of CF decreased might be attributable to different choices, i.e. prenatal diagnosis and abortion, preimplantation diagnosis, or not to have children. However there are no data to know which eventually were the procreative decisions. There are no data on the provision of the genetic test to the persons with no increased risk for CF.

2.3. GENOTYPE-PHENOTYPE CORRELATION AND CLINICAL PATHWAYS IN PATIENTS WITH CYSTIC FIBROSIS

2.3.1 Cystic fibrosis: the genetic basis of the disease

Fiorella Gurrieri, Giovanna Elisa Calabrò

Cystic Fibrosis (CF) (OMIM 219700) is the most common severe genetic disease among Caucasian population, affecting approximately 1 in 2500 live births [17]. All other ethnic groups are affected to a lesser extent [18]. It is an autosomal recessive disease caused by mutations of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene, which encodes an epithelial anion channel [19].

CFTR (OMIM, 602421) was identified in 1989 as the abnormal gene for CF [20–22]. The CFTR is a transmembrane multifunctional protein expressed mainly at the apical membrane of epithelial cells. It is located on the long arm of chromosome 7 (7q31.2) and consists of a TATA-less promoter and 27 exons spanning about 215 kb of genomic sequence (Figure 2a) [23]. The CFTR gene encodes for a 1480-residue long transmembrane protein, an ion channel that belongs to the adenosine triphosphate binding cassette transporter family of proteins [24]. Consistent with the adenosine triphosphate binding cassette transporters, the CFTR structure has a symmetrical, multi-domain structure, consisting of two membrane-spanning domains (MSD1, MSD2), two nucleotide-binding domains (NBD1, NBD2) and a central, highly charged regulatory domain (R) with multiple phosphorylation consensus sites (Figure 2b, c) [25]. The principal function of CFTR is that of cAMP-regulated chloride transport at the apical membranes of epithelial cells but has also been implicated in many other processes such as regulation of other ion channels, membrane trafficking, pH regulation and apoptosis [26].

To date, 2006 different CFTR variants have been identified and collected from the international CF genetics research community [27,28]. These variants have been found mostly in European-derived populations and with much less frequent...
cy in African and East Asian populations [29]. All types of variants are represented (missense, frameshift, nonsense, splice, small and large in-frame deletions or insertions), and are distributed throughout the entire gene. Missense variants are the most prevalent (40%), compared with frame shift (16%), splicing (12%), and nonsense (8%) [29,30]. The most common variant (70% of patients) is ΔF508, a recessive 3-bp deletion in exon 10 (c.1521_1523delCTT; Refseq transcript ID, NM_000492.3) that induces a loss of phenylalanine at the aminoacid position 508 of the protein product (p.phe508del; Refseq protein ID NP_000483.3) [27,28]. All CF patients carry two CFTR variants alleles, whereas heterozygotes with one mutated copy do not display CF manifestations [17,20].

The biological consequences of CFTR mutations are numerous and have been extensively studied, particularly for the p. F508 del mutation. The defective ion transport caused by CFTR mutations results in reduced apical airway surface liquid, which leads to impaired mucociliary clearance that progressively induces obstruction of the airways with thickened mucus. This in turn creates a favorable environment for bacterial contamination and colonization. Recent observations using a piglet model of CF suggest that mucociliary transport is a primary defect in CF [31]. Patients with CF have an impaired host immune response especially against Pseudomonas Aeru-ginosa, which is characterized by an exaggerated and ineffective airway inflammatory response. There is evidence suggesting that CFTR dysfunction affects innate immunity pathways, notably those associated with toll-like receptors [32] and that the initial predisposition to infection in infants with CF may represent a primary defect in local mucosal immunity [33].

CF is a disease with a complex, multifaceted clinical phenotype characterized by multiorgan involvement. Clinical manifestations are caused by an impaired function of exocrine gland sin many organs, mainly the respiratory and the gastrointestinal tracts. This genetic disorder is mostly characterized by recurrent lower respiratory infections, exocrine pancreatic insufficiency, and increased electrolyte concentration in sweat. A subset of patients also presents with a variety of other less common symptoms, such as meconium ileus, liver disease, diabetes, and pancreatitis [19]. Clinical consequences of the CFTR defect for various organs affected in CF patients are site-specific and range from severe (lungs, pancreas, male reproductive tract) to mild (intestine) to asymptomatic (sweat glands). Due to the large number of pathogenic variants in the CFTR gene with variable effect on the protein function, a wide range of phenotypic manifestations can be observed in CF [25].

2.3.2 The genetic epidemiology of CF in Italy and by region

Vladimir Vukovic, Giancarlo Ripabelli

The frequency of mutations is variable in terms of geographical distribution of the populations, and some mutations might be more frequent in specific populations compared to others. A large cohort study by De Boeck et al. conducted in 2009, was the first to publish relative frequencies of the CFTR mutation classes in wider European population. They analyzed data from the European Cystic Fibrosis Society Patient Registry (ECFSPR) of more than 25,000 patients with CF from 23 European countries. It was concluded that patients with CF hold a wide spectrum of CFTR mutations, with class II mutations, containing the F508del, being the most frequent in a European population. Overall 22,144 patients (87.2%) carried at least one class II mutation (G85E, F508del, I507del, R560T, N1303K). Their proportion was highest in Denmark (97.1%, 95% CI 95.1 – 98.5), while the lowest proportions were recorded in Israel (46.8%, 95% CI 42.5 – 51.1) and Hungary (57.30%, 95% CI 53.1 – 61.4). All other class mutations were described with noticeably lower frequencies [34].

One of the earliest studies to deal with this issue in Italy was published in 1997, and have reported data on the relative frequencies of 60 Cystic Fibrosis (CF) mutations in Italy and the geographical distribution of the 12 most frequent CF mutations screened in 3492 CF chromosomes (1746 CF patients) [35]. Overall, the 12 most frequent mutations characterized about 73% of the Italian CF chromosomes. The most common mutation in Italy was ΔF508, a deletion of the phenylalanine amino acid in position 508, with an average frequency of 51%. The highest (>60%) frequency of ΔF508 was found in Piedmont, Umbria and Campania regions, the lowest (<40%) in Friuli and Marche. The next more frequent mutations reported were N1303K and G542X (mean frequency of 4.84% and 4.83%, respectively), with the N1303K mutation being relatively common (8-10%) in central and south-eastern regions (Abruzzo, Marche, Lazio, Molise and Puglia), and rare (<1%) in Piedmont and Trentino Alto Adige. The G542X mutation showed a frequency from 2% (Trentino Alto Adige) to up to 13% in Basilicata, Friuli and Liguria regions, with a low number of chromosomes tested. The 2183AUG mutation was the fourth most common mutation in Italy (mean frequency of 3%), reaching up to 7% in Trentino Alto Adige and Veneto regions. Mutation R1162X showed an average frequency of 2.42%, but a frequency of 14% and 9% have been reported in Trentino Alto Adige and Veneto, respectively. The 1717-1GAU mutation accounted for 4.6% of the CF chromosomes in Lombardy, Piedmont and Liguria, but was rare (<2%) or absent in the central and southern regions.

The remaining mutations were less frequent (about 1%) and more geographically isolated: W1282X in Abruzzo and Friuli (both with small sample sizes); 7115GAU was only reported in Marche, Trentino Alto Adige and Veneto, whereas T338I is an exclusively Sardinian mutation. The 48 mutations not selected for further analysis accounted for 2.6% (93/3492) of CF chromosomes [35].

Later studies have further confirmed this wide genetic heterogeneity among and within Italian regions. A pilot study for the simultaneous detection of up to 31 cystic fibrosis mutations was performed in a cohort of 4476 Italian new-
borns from Pavia, Turin and San Giovanni Rotondo cities, up to 1999 year. The observed carrier frequency was 1:31.1. Forty-two carriers were detected from 1341 samples in Pavia (1:31.9), 53 from 1574 in Turin (1:29.7), and 49 from 1561 in San Giovanni Rotondo (1:31.8). One individual in 3868 was expected to be either a homozygous mutant or a heterozygote for a CFTR mutant allele. The most common mutation was found to be ΔF508, accounting for more than half of the total sample (90 of 144, 0.625). Other mutations found with greater than normal frequency were G542X (0.11), which is particularly common in southern Italy (0.29 from San Giovanni Rotondo), N1303K (0.06), and R117H (0.06), which was only detected in the northern centers [36].

A nationwide study has assessed 1742 CF subjects (857 females, 885 males) born between January 1988 and December 2001, taken from data collected in the Italian National CF Registry. The ΔF508 was found to be the most common mutation in Italy, but its frequency was only 49% compared to 70-75% as detected in other countries. The ΔF508 frequency was of 41%, 52% and 61% in Calabria, Lombardy, and Emilia Romagna regions, respectively. The other common mutations presented in the Italian study population were the N1303K and G542X alleles (6% of chromosomes), and 2183AA→G, 1717-1G→A, R1162X, and W1282X (in about 2-3% of chromosome). Regarding the ΔF508 allele, 26.6% of the patients were homozygotes, 44.6% were ΔF508 heterozygotes, and the remaining 28.8% did not carry this allele [1].

The Italian Society for CF published in 2016, a report with the frequency of mutations found in 4094 CF patients, and marked ΔF508 mutation to be present in 45.1% of the samples. This result is in line with the results published at the European level, for the same year [37], where the mutation ΔF508 was the most commonly identified, with a frequency that varies from 23.8% (Israel) to 82.6% (Denmark). Another interesting fact is observed in relation to the frequency of N1303K mutation. The reported frequency of this mutation is very high in Italy (5.3%) and among the highest reported in Europe [37]. Other recorded mutations had a frequency of 5% and lower (G542X 5.0%, 2789+5G→A 3.0%, 2183AA→G 2.0%, W1282X 2.0%) (Table 3) [2].

A study reflecting the CF gene mutation frequencies in the southern Italian was conducted on 371 unrelated CF patients from Campania, Puglia and Basilicata regions, and revealed that several mutations, rare or absent in other ethnic groups, are frequent (1.0 to 6.0%) in CF patients from this geographic area. The data are related to about 90% of the known CF patients from Campania, Basilicata and Puglia regions, and thus may reflect the distribution of CF mutations in southern Italy. The 4016insT, R1158X, 711+1G>T and L1065P had a cumulative frequency of 6.3% in CF chromosomes from Campania region; G1244E and 852del22 a cumulative frequency of 9.6% in CF chromosomes from Basilicata; and 4382delA, 1259insA, 1502T, 852del22, 4016insT, D579G, R1158X, L1077P and G1349D a cumulative frequency of 19.6% in CF chromosomes from Puglia. The ΔF508 mutation was detected with a frequency of 51.5% (55.6% in Campania, 55.8% in Basilicata, 46.8% in Puglia), the N1303K with a frequency of 7.3% (7.3% in Campania, 3.8% in Basilicata, 7.7% Puglia), and the G542X with a frequency of 5.9% (5.0% in Campania, 3.8% in Basilicata, 7.1% in Puglia) [38].

Further, the number of patients carrying at least one mutation with allelic frequency ≥1%, are listed in Table 4. In particular, more than 68% of patients are characterized by the mutation ΔF508, whether in homozygous (21.9%) or heterozygous form (46.5%), 10% had N1303K mutation, while G542X mutation was present in 9.45 of patients [2].

### Table 4. Number of the patients carrying at least one mutation with allelic frequency ≥1%, year 2010.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF508</td>
<td>2799</td>
<td>68.4</td>
</tr>
<tr>
<td>N1303K</td>
<td>411</td>
<td>10.0</td>
</tr>
<tr>
<td>G542X</td>
<td>386</td>
<td>9.4</td>
</tr>
<tr>
<td>2789+5G→A</td>
<td>236</td>
<td>5.8</td>
</tr>
<tr>
<td>2183AA→G</td>
<td>161</td>
<td>3.9</td>
</tr>
<tr>
<td>1717-1G→A</td>
<td>155</td>
<td>3.8</td>
</tr>
<tr>
<td>W1282X</td>
<td>154</td>
<td>3.8</td>
</tr>
<tr>
<td>G85E</td>
<td>103</td>
<td>2.5</td>
</tr>
<tr>
<td>R553X</td>
<td>101</td>
<td>2.5</td>
</tr>
<tr>
<td>R1162X</td>
<td>97</td>
<td>2.4</td>
</tr>
<tr>
<td>D1152H</td>
<td>94</td>
<td>2.3</td>
</tr>
</tbody>
</table>

In 2004, the World Health Organization in association with many international organizations devoted to CF, have released a publication, after several joint meetings and workshops, in order to highlight the worldwide relevance of the CF and the up-to-date CF genetic knowledge. In this report, the CF birth incidence for Italy was 1:2438. A detailed distribution and frequency of mutations for many Italian regions are reported in Table 5 [3].

A study reflecting the CF gene mutation frequencies in the southern Italy was conducted on 371 unrelated CF patients from Campania, Puglia and Basilicata regions, and revealed that several mutations, rare or absent in other ethnic groups, are frequent (1.0 to 6.0%) in CF patients from this geographic area. The data are related to about 90% of the known CF patients from Campania, Basilicata and Puglia regions, and thus may reflect the distribution of CF mutations in southern Italy. The 4016insT, R1158X, 711+1G>T and L1065P had a cumulative frequency of 6.3% in CF chromosomes from Campania region; G1244E and 852del22 a cumulative frequency of 9.6% in CF chromosomes from Basilicata; and 4382delA, 1259insA, 1502T, 852del22, 4016insT, D579G, R1158X, L1077P and G1349D a cumulative frequency of 19.6% in CF chromosomes from Puglia. The ΔF508 mutation was detected with a frequency of 51.5% (55.6% in Campania, 55.8% in Basilicata, 46.8% in Puglia), the N1303K with a frequency of 7.3% (7.3% in Campania, 3.8% in Basilicata, 7.7% Puglia), and the G542X with a frequency of 5.9% (5.0% in Campania, 3.8% in Basilicata, 7.1% in Puglia) [38].
In the analysis conducted at the Fetal-Maternal Medical Centre in Rome, 25,393 samples collected through invasive procedures – amniocentesis (92%) and villocentesis (8%) were examined, in order to identify the most frequent mutations in the cystic fibrosis gene in population, and have identified 922 heterozygous and 9 homozygous affected fetuses.

### Table 5. Distribution and frequency of CF-causing CFTR mutations among different Italian regions (Modified from: World Health Organization. The molecular genetic epidemiology of cystic fibrosis. 2004.)

<table>
<thead>
<tr>
<th>Region</th>
<th>Detection rate (total number of CFTR alleles studied)</th>
<th>Number of mutations found</th>
<th>Mutations (proportion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central: Rome</td>
<td>0.867 (586)</td>
<td>37</td>
<td>F508del (0.558) / N1303K (0.087) / G542X (0.070) / W1282X (0.026) / S549R(A&gt;C) (0.014) / 621+1G&gt;T (0.012) / 1717-1G&gt;A (0.009) / G85E, R553X (0.007 each) / H139R, R347P, L1065P, L1077P (0.005 each)</td>
</tr>
<tr>
<td>Central: Tuscany</td>
<td>0.775 (382)</td>
<td>19</td>
<td>F508del (0.455) / G542X, N1303K (0.050 each) / 2789+5G&gt;A (0.037) / R347P (0.031) / 2183AA&gt;G, L1065P (0.029 each); T338l, R553X (0.018 each) / W1282X (0.013) / G85E (0.010) / 1898+1G&gt;A, 3849+10kbC&gt;T (0.008 each) / 1717-1G&gt;A (0.005)</td>
</tr>
<tr>
<td>North-Central: Milan</td>
<td>0.816 (1160)</td>
<td>67</td>
<td>F508del (0.500) / G542X (0.051) / N1303K (0.050) / 1717-1G&gt;A (0.037) / 2183AA&gt;G (0.015) / W1282X (0.014) / R1152H (0.009) / R334W, R352Q, R553X, R1066H (0.008 each); 3849+10kbC&gt;T (0.007) / R117H (0.005) / R347P, E555X, L1077P (0.004 each); M1V, G85E, D110E, D1158X, L1065P, L1077P (0.005 each)</td>
</tr>
<tr>
<td>North-East: Friuli-Venezia Giulia</td>
<td>0.825 (126)</td>
<td>22</td>
<td>F508del (0.492) / N1303K (0.063) / G542X (0.048) / 2183AA&gt;G (0.032) / 621+1G&gt;T, 1717-1G&gt;A, R1162X (0.024 each); G85E, D110H, 677delTA, 711+3G&gt;A, T338l, S466X(TAA), I507del, G551D, 2368del, 2789+5G&gt;A (0.008 each); F1052X, R1070Q, 3659delIC, K1177R, 401insT (0.008 each)</td>
</tr>
<tr>
<td>North-East: Turin</td>
<td>0.839 (316)</td>
<td>20</td>
<td>F508del (0.611) / G542X (0.051) / N1303K (0.035) / R347P, 2183AA&gt;G, 2789+5G&gt;A, R1162X (0.016 each); G85E, 1717-1G&gt;A (0.013 each) / R117H (0.009) / 711+5G&gt;A, 2789+5G&gt;A, S1235R, W1282X (0.006 each)</td>
</tr>
<tr>
<td>North-East: Veneto</td>
<td>0.904 (312)</td>
<td>29</td>
<td>F508del (0.487) / 2183AA&gt;G, R1162X (0.074 each) / 711+5G&gt;A (0.038) / N1303K (0.035); /1717-1G&gt;A (0.026), Q552X (0.019); G85E, G542X, R553X, 2789+5G&gt;A (0.016 each) / I507del (0.013); 621+1G&gt;T, Q553X, 898+3A&gt;G, 2790-2A&gt;G, 3132delITG, W1282X (0.006 each)</td>
</tr>
<tr>
<td>North-East: Veneto and Trentino-Alto Adige</td>
<td>0.911 (225)</td>
<td>24</td>
<td>F508del (0.476) / R1162X (0.098) / 2183AA&gt;G (0.093) / N1303K (0.040) / 711+5G&gt;A, G542X (0.027 each) / 1717-1G&gt;A (0.022); G85E, Q552X, R553X, 2789+5G&gt;A (0.013 each) / 621+1G&gt;T, 2790-2A&gt;G, 3132delITG, W1282X (0.009 each)</td>
</tr>
<tr>
<td>Sardinia</td>
<td>0.941 (186)</td>
<td>22</td>
<td>F508del (0.491) / T338l (0.151) / G542X, 2183AA&gt;G (0.059 each) / N1303K (0.043) / 3849+10kbC&gt;T (0.022) / G1244E (0.016); 991del5, 170del17, 1717-1G&gt;A, S912X (0.011 each); S13F, G85E, D110E, D1158X, L1065P, L1077P, 2184insT, 2789+5G&gt;A, H1054D, D1270N+R74W, 401insT (0.005 each)</td>
</tr>
<tr>
<td>South-East: Apulia</td>
<td>0.922 (374)</td>
<td>31</td>
<td>F508del (0.449) / N1303K (0.080) / G542X (0.072) / 4382delA (0.040) / 852del22 (0.032) / 1259insA (0.032); I502T, L1077P (0.019 each) / R553X, D579G, R1066C, 3849+10kbC&gt;T, G1349Q (0.016 each); R1158X, 401insT (0.013 each) / 1717-1G&gt;A (0.011) / R347P, 2183AA&gt;G, 2789+5G&gt;A (0.008 each) / G1244E, W1282X (0.005 each)</td>
</tr>
<tr>
<td>South-East: Basilicata</td>
<td>0.923 (52)</td>
<td>12</td>
<td>F508del (0.558) / 852del22, 2183AA&gt;G (0.058 each) / G542X, G1244E, W1282X, N1303K (0.038 each) / 1717-1G&gt;A, S549R(A&gt;C), L558S, Y849X, 3849+10kbC&gt;T (0.019 each)</td>
</tr>
<tr>
<td>South-East: Campania</td>
<td>0.915 (340)</td>
<td>27</td>
<td>F508del (0.556) / N1303K (0.074) / G542X (0.050) / W1282X (0.035) / 1717-1G&gt;A, 2183AA&gt;G (0.024 each) / 401insT (0.021) / 711+1G&gt;T, R553X, R1158X (0.015 each) / L1065P (0.012) / G1244E, 2522insC (0.009 each); G85E, L1148T, G178R, E555X, 2789+5G&gt;A, L1077P (0.006 each)</td>
</tr>
</tbody>
</table>

The frequency of heterozygous was 1:27.5, whereas for the affected homozygous was 1:2821. The most frequent mutation was the ΔF508, detected in the 63.2% of cases. The second mutation in terms of relative frequency was the N1303K mutation (14.3%), followed by G542X (6.6%), W1282X (3.1%), R553X (2.6%), G85E (2.4%), R1162X.
including the ethnic groups. It is important to know which mutations distribution in Italy from a new nationwide study (70% of the CF patients). As well, some of these mutations may have originated in specific groups (southern Italy), and among them, the more frequent are the p.Thr338Ile (T338I). Other mutations may have originated in specific groups (southern Italy), and among them, the more frequent are the p.Thr338Ile (T338I) (65.0%), followed by the p.Phe508del (F508del) (22.5%). The p.Asn1303Lys, c.2051_2052delA-AinsG, c.489+1GNT, c.54-5811_164+2187del1808ins182, and p.Arg347Pro mutations had a frequency of 2.5% each. Since 38 CF carriers were identified of 1000 subjects with no family history of CF, the overall carrier frequency observed in the general Sardinian population was 1:25 (4.0%) [40].

These studies on the relative frequency of CF mutations in Italy allowed to confirm a wide genetic heterogeneity among different Italian regions. The northern areas of the country were clearly differentiated from the central-southern regions, and there was a difference even within the northern regions, with the north-western regions (Piedmont and Lombardy) being characterized by 171721GR A mutation, and the north-eastern regions (Trentino Alto Adige and Veneto) by R1162X, 2183AAR G, and 71115G R A mutations. The population from the northern Italy is genetically and anthropologically closer to central European ethnic groups, while those from the southern areas are more affiliated to other Mediterranean groups, thus reflecting the pre-Romanic colonization of the Italian regions, where the ancient Greeks colonized the south, and populations of Celtic origin the northern areas of Italy and central Europe [35]. Sardinia, on the other hand, is reported to be genetically very different from the rest of Italy and Europe, representing the unique CF mutation- the p.Thr338Ile (T338I). Other mutations may have originated in specific groups (southern Italy), and amplified through consanguineous marriages (852del22, L1065P and others that were identified in homozygosity in several CF patients). As well, some of these mutations may have been introduced by more recent immigrations [38].

It would be useful to have a better knowledge of the CF mutations distribution in Italy from a new nationwide study with large number of CF patients from all Italian regions, including the ethnic groups. It is important to know which mutations occur in the population, and their relative frequencies, in order to offer an effective diagnostic service to the citizens, and also to establish the provision of the CF carrier screening at a national level.

2.3.3 Genotype-phenotype correlation in patients with CF

Fiorella Gurrieri, Giovanna Elisa Calabrò

Genotype and phenotype represent the most distant categories in a causal succession of events leading to a genetic disease. In a monogenic, recessive disease such as CF, genotype as a primary cause constitutes the starting point and is typically represented by two disease-causing mutations residing on separate alleles. Phenotype, as the clinical outcome, which is characterized by observable and/or measurable clinical features manifested in a patient, has three main components: specific configuration of signs and symptoms, their severity, and time course [25].

There is strong correlation between the general type of CFTR mutation and disease phenotype. Various mutations can be grouped into different classes based on their known or predicted molecular mechanisms of dysfunction and functional consequences for the CFTR protein. The classification, which was first proposed by Tsui [41], has subsequently been expanded and refined to accommodate more data [42–44]. CFTR gene mutations are categorized into six classes [45,46] (Figures 3–4).

Class I mutations result in the total or partial lack of production of a functional CFTR protein. Such mutations may arise either due to (a) a nucleotide substitution introducing an in-frame premature termination codon (PTC) – UAA, UAG or UGA –, (b) frame-shifting insertions or deletions, (c) mutations at the invariant dinucleotide splice junctions, or introduction of a PTC, resulting in complete skipping of an out of frame exon, (d) a complete or partial deletion of the CFTR gene or (e) a rearrangement in the gene altering the exon sequence. The p.Gly542* mutation is the most frequent worldwide mutation of its class, affecting at least one allele of up to 4% of CF patients [18].

Class II mutations, including Phe508del, have folding or maturation defects, which can result in premature CFTR degradation [47].

Class III and IV mutations, however, are typified by aberrant channel function rather than reduced quantities of CFTR. Class III mutations result in limited channel gating that arises from ineffectual binding of nucleotide; an example is G551D which accounts for 2–3% of CFTR mutations worldwide [46]. Class IV mutations are mostly located within membrane spanning domains implicated in the constitution of the channel pore. The missense mutations located in these regions produce a protein efficiently inserted in the membrane, which retains a cAMP-dependent Cl–channel activity, but with a reduced channel conductance. Alleles in
Figure 3. **Classification of CFTR mutations based on CFTR structure–function** (edited by Emilie Vallières et al, 2014)

<table>
<thead>
<tr>
<th>Normal</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
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<td>Molecular defect</td>
<td>No synthesis</td>
<td>Block in processing</td>
<td>Block in regulation</td>
<td>Reduced conductance</td>
<td>Reduced synthesis</td>
<td>Reduced half-life</td>
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<td>Channel opening defect</td>
<td>Ion transport defect</td>
<td>Decreased protein synthesis</td>
<td>Decreased half-life of the protein</td>
</tr>
<tr>
<td>Type of mutations</td>
<td>Nonsense; frameshift; canonical splice</td>
<td>Missense; Aminoaicid deletion</td>
<td>Missense; Aminoacid change</td>
<td>Missense; Aminoacid change</td>
<td>Splicing defect; Missense</td>
<td>Missense; Aminoacid change</td>
</tr>
<tr>
<td>Main mutations</td>
<td>Gly542X Trp128X Arg553X 621+1G&gt;T</td>
<td>Phe508del Asn1303lys Ile507del Arg560Thr</td>
<td>Gly551Asp Gly178Arg Gly551Ser Ser549Asn</td>
<td>Arg117His Arg347Pro Arg117Cys Arg334Trp 3849+10kbG&gt;T 2789+5G&gt;A 3120+1G&gt;A 5T</td>
<td>4326delITC Gln1412X 4279insA</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. **Classes of CFTR mutations.** Mutation classes I, II, V and VI result in an absence or reduced quantity of CFTR protein at the cell membrane, whereas mutation classes III and IV influence the function or activity of CFTR at the cell membrane. Class I mutations are associated with the greatest disruption to CFTR-mediated chloride transport; in general, chloride transport gradually increases through the remaining five classes, with the greatest activity being observed in Class IV–VI mutations (by Nico Derichs, 2013).
Clinical manifestations of CF

| Sweat gland | Excessive secretion of sodium chloride by sweat glands is characteristic of CF. In healthy individuals, CFTR protein is responsible for reabsorption of chloride (and subsequently sodium) in the reabsorptive duct of the sweat gland. Absence or dysfunction of CFTR impedes this process, giving rise to hyperionic beads of sweat as seen in CF [51]. |
| Pancreas and GI tract | Pancreatic insufficiency is thought to occur in 90–95% of CF patients. Defective secretion of digestive enzymes and resulting fat malabsorption in the gastrointestinal system has several consequences including steatorrhoea and failure to thrive. In addition, ionic imbalance in the biliary tract may lead to increased risk of gall stone and hepatobiliary disease [50]. Pancreatic abnormalities also can cause CF-related diabetes, which has a prevalence of 225% in CF patients aged >25 yrs [46]. |
| Reproductive system | Around 99% of adult males with CF have congenital bilateral absence of the vas deferens, which is a developmental defect that blocks the transport of spermatozoa from the testes to epididymis to the vas deferens resulting in azoospermia [52]. Generally speaking, infertility in females with CF has a less serious outcome than in males and is thought to be due to thickening of cervical mucus [53]. |
| Airways | The most significant clinical manifestations of CF are in the lung and airways. In bronchial tissues, CFTR protein is expressed in the submucosal glands and the apical surface of ciliated epithelial cells [54] and has been shown to play a critical role in epithelial wound repair. Normally, effective mucociliary transport is facilitated by sufficient hydration of the Airway Surface Liquid (ASL) [55]. ASL hydration is achieved through establishment of an osmotic gradient by a predominant efflux of chloride ions through CFTR channels, coupled with a moderate influx of sodium ions through epithelial sodium channels in the apical membrane. Absence or dysfunction of CFTR leads to a lack of chloride efflux and unregulated hyperabsorption of sodium ions. This imbalance of the osmotic gradient causes ASL dehydration, increased mucoviscosity and impaired mucociliary transport [56]. This situation gradually deteriorates causing plugging of small airways, bacterial infection, chronic local inflammation and eventually bronchiectasis [57]. Furthermore, repeated pulmonary exacerbations in CF have been shown to contribute to an accelerated decline in lung function [58]. |

this class are typically associated with a milder form of the disease. The p.Arg117His mutation is the best-characterized class IV mutation [48].

While **Class V mutations** can lead to the production of normal CFTR, a limitation of transcriptional regulation results in a reduced quantity of the protein being produced [49]. Finally, the relatively novel **Class VI mutations** are characterized by high turnover of CFTR at the channel surface [46].

The clinical manifestations of CF have been noted in several organ systems (Table 6) with the respiratory system being the worse affected. With the exception of the sweat gland, regardless of organ or tissue, the absence or dysfunction of CFTR results in an ionic imbalance that results in the secretion of thick, dehydrated mucus [50]. Mucobstruction of exocrine glands is the main cause of CF-associated pathology.

Loss of function variants in CFTR have also been linked to a variety of conditions collectively termed “CFTR-opathies” [59] as, for example, male infertility and pancreatitis. The concept that dysfunction of CFTR could create disorders other than CF was first illustrated by obstructive male infertility due to congenital bilateral absence of the vas deferens (CBAVD; OMIM 277180) [60]. The anatomical features of CBAVD are identical to those seen in males with CF, and some individuals with CBAVD have subtle features of CF, such as mildly elevated sweat chloride concentration or minimal airway disease [61]. However, a fraction of males with CBAVD manifest no evidence of CF in detailed studies of the lungs, pancreas and sweat gland [62]. Furthermore, the distribution of CFTR variants differs between CBAVD and CF, and a much higher fraction of variants is associated with residual function occurring in CBAVD [63].

Thus, CBAVD is part of the CF spectrum caused by CFTR dysfunction but it is also viewed as clinically distinct, particularly in males with features limited to the vas deferens.

Furthermore, discovery of a pathological role for CFTR has proved to be particularly instructive for the study of pancreatitis. Pancreatitis is a known complication of CF, primarily occurring in individuals with preserved pancreatic exocrine function [64]. Pancreatitis in the general population is a heterogeneous disorder with heritable and idiopathic sporadic forms. A subset of heritable forms of recurrent acute and chronic pancreatitis can be attributed to CFTR dysfunction [65]. As with CBAVD, the distribution of CFTR variants differs from that of CF. Recent evidence suggests that cells expressing CFTR bearing variants associated with pancreatitis, but not with CF, manifest defective bicarbonate transport, while chloride channel function is preserved [66]. This concept aligns well with the role of CFTR as an important mediator of bicarbonate transport in the pancreatic ducts [67].

Another classification of CFTR mutations is based on the clinical consequences. The first description of CF was done in 1938 by Dorothy Hansine Andersen, describing an abnormal pancreas, which presented with cysts and fibrosis [68]. The clinical spectrum of CF has since greatly expanded, giving rise to diagnoses of **classic and non-classic CF** [69,70] (Figure 5).

In the diagnostic algorithms proposed by the European Consensus Group, patients with one or more phenotypic characteristic suggestive of CF are classified according to their pilocarpine sweat test result (chloride >60 mmol/L, 30–60 mmol/L or <30 mmol/L) [29]. To date, no test has proven to be as practical or reliable as the sweat test for clinical diagnostic purposes.
However, as time passes and knowledge of CF clinical spectrum increases, the border between classical CF and non-classical CF is not sharp enough to sustain such distinction. Therefore we may end up in lumping all CF manifestations under a unique continuum spectrum.

The majority of CF patients suffer from classic CF, yet their organs are affected to varying degrees. Patients are diagnosed with classic CF if they have one or more phenotypic characteristic and a sweat chloride concentration of >60 mmol/L (Figure 5) [18]. They may have exocrine pancreatic insufficiency (PI) or pancreatic sufficiency (PS). The disease can have a severe course with rapid progression of symptoms or a milder course with very little deterioration over time. Classic CF patient mortality is mainly due to progressive respiratory disease. From a genetic standpoint, classical CF is characterized by one established CF-causing mutation on each CFTR allele.

Non-classic CF describes individuals with at least one CF phenotypic characteristic and a normal (<30 mmol/L) or borderline (30–60 mmol/L) sweat chloride level, in whom detection of one mutation on each CFTR allele, or direct quantification of CFTR dysfunction by Nasal Potential Difference (NPD) measurement has been confirmed [71,72] (Figure 5). These patients have either multi- or single-organ involvement. Most of them have exocrine PS and milder lung disease. As described by the CF Diagnostic Working Group, “some patients with single-organ involvement resulting from CFTR dysfunction may be more appropriately given an alternative diagnostic label, as recommended in the World Health Organization diagnostic list” [73]. These alternative diagnoses for non-classic CF, or CFTR-related disorders, include isolated obstructive azoospermia, chronic pancreatitis, disseminated bronchiectasis, allergic bronchopulmonary aspergillosis, diffuse panbronchiolitis and sclerosing cholangitis, and all have at least one identified associated CFTR mutation. In 2007, the Consensus Conference, organized by the European Cystic Fibrosis Society with the partnership of the European Society of Human Genetics and the EuroGentest Network of Excellence, allowed for the establishment of a classification of CFTR mutations, into the following four groups, according to their clinical consequences (Table 7). Group A comprises mutations that cause

![Figure 5. The clinical spectrum of cystic fibrosis. This diagram depicts the phenotypic characteristics of CF and distinguishes between classic and non-classic CF clinical descriptions (by Pascale Fanen et al. 2014).](image-url)
classic CF (CF-causing), Group B includes mutations that cause non-classic CF (associated to CFTR-related disorders), Group C comprises mutations with no known clinical consequences, and Group D consists of mutations with unknown or uncertain clinical relevance (also referred to as VUs: variants of unknown significance) [29].

Although classic CF most often presents with a severe multi-organ phenotype, and non-classic CF with milder single-organ phenotypes, this is not always the case. Whether the phenotype is “severe/mild” or “multi/single-organ” is not intrinsically linked to the classic or non-classic CF diagnosis. The great phenotypic variability of CF has been shown to implicate not only the type of CFTR mutations, but also other factors, such as the environment (lifestyle, treatment), modifier genes and the progression of the disease with age, can also affect the clinical heterogeneity of patients carrying these large spectrum mutations.

Individuals with CF show a high degree of variability in disease severity, complications and survival. It was initially postulated that a substantial fraction of phenotypic variability would be explained by allelic heterogeneity in the dysfunctional gene [74]. CFTR genotype correlates well with pancreatic exocrine disease severity and modestly with sweat chloride concentration [75,76]. However, it has been difficult to detect a relationship between lung function and CFTR genotype [77] with a few notable exceptions [78]. Genetic modifiers have considerable influence on lung function variation in CF lung disease with pancreatic sufficiency. Group D mutations have unknown or uncertain clinical relevance to CF phenotypic characteristics [18].

### Table 7. Classification of CFTR mutations, into four groups, according to their clinical consequences (by Pascale Fanen et al. 2014).

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic-CF</td>
<td>Non-classic CF</td>
<td>No clinical consequence</td>
<td>Unknown clinical relevance</td>
</tr>
<tr>
<td>CF-causing mutations</td>
<td>CFTR-related disorder associated mutations</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mutations that may belong either to Group A or to Group B.

*Mutations that may belong either to Group B or to Group C.

### Table 8: American College of Medical Genetics (ACMG) recommended panel of 23 classic CF-causing mutations (by Pascale Fanen et al. 2014).

<table>
<thead>
<tr>
<th>23 ACMG recommended panel of classic CF-causing mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>G85E</td>
</tr>
<tr>
<td>R117H</td>
</tr>
<tr>
<td>R334W</td>
</tr>
<tr>
<td>R347P</td>
</tr>
<tr>
<td>A455E</td>
</tr>
</tbody>
</table>

Additional or alternative mutations present at significant frequencies in an ethnic population served by a newborn screening program may be assessed.
For example, Beucher et al. [80] assessed, in CF patients, the association of lung disease severity with the variant AGER -429 T/C, coding for RAGE, a pro-inflammatory protein. The contribution of genetic modifiers to four other traits that are relevant to survival in CF has been estimated [81] (Figure 6): genetic modifiers and non-genetic factors both contribute to airway obstruction and infection with Pseudomonas aeruginosa, two traits that define lung disease in CF; CFTR genotype is the primary determinant of the degree of pancreatic exocrine dysfunction. The presence of CFTR variants associated with severe pancreatic exocrine dysfunction is essentially a pre-requisite for the development of diabetes and intestinal obstruction; in the setting of severe endocrine dysfunction, genetic modifiers determine when, and if, diabetes occurs and whether neonatal intestinal obstruction occurs; genetic variation plays the predominant part in nutritional status as assessed by body mass index (BMI) [82].

Clinical and molecular characterization is of great importance, especially in diseases such as CF, where the impact is multisystemic with variable expressivity, high morbidity and mortality. The ability to identify genes, or genomic regions, that influence disease processes hold enormous promise for better understanding those processes and for manipulating them for therapeutic gain. However, an important caveat of genetic screens is that they may identify genes involved in a process, but as such they are only markers; they do not provide information about the mechanisms by which the variants exert their phenotypic effects. Thus, for the results of any genetic study to have more than prognostic value, the genes must be followed up by functional studies to have clinical utility. In this context, genetic has played and will continue to play a key part in achieving a normal lifespan for individuals with CF.

### 2.3.4 Manifestations of disease in CF carriers

Fiorella Gurrieri, Giovanna Elisa Calabro

Manifestations of disease may also be present in CF carriers. Indeed, CFTR variants also act as risk alleles for multigenic disorders in the general population. Idiopathic disseminated bronchiectasis is a relatively rare pulmonary airway...
disease that manifests features similar to those observed in the lungs of individuals with CF. Several studies have shown a higher frequency of deleterious CFTR variants in individuals with bronchiectasis than in control subjects [83]. Bronchiectasis that is complicated by infection with non-tuberculous Mycobacteria or with the fungus Aspergillus fumigatus has also been associated with an increased frequency of CFTR variants [84,85]. Chronic rhinosinusitis (CRS) is an aetiologically heterogeneous condition affecting ~15% of the general population. CRS is a common complication in patients with CF. Genotyping of subjects meeting rigorous criteria for CRS revealed an excess of carriers of a single deleterious CFTR variant compared to disease-free controls [86]. In these studies, the entire coding region of CFTR was examined to exclude a second deleterious variant.

Support for the concept that presence of a single loss of function variant in CFTR predisposes to CRS was derived from the observation that the obligate heterozygous carriers of the deleterious CFTR variant, that is the parents of individuals with CF, had a threefold increase in prevalence of CRS [87]. Assessing whether pancreatitis, bronchiectasis or sinusitis can be attributed to CFTR dysfunction in a heterozygous cystic fibrosis carrier requires detailed phenotyping to exclude other conditions, including mild forms of CF [88]. Associating CFTR variants with common multigenic disorders has substantial implications, as there are an estimated 20 million heterozygous carriers of CF in the world. However, the penetrance of most CFTR variants for the traits discussed above is not known. Establishing the penetrance of variants in disease-associated genes is a considerable and important challenge [89].

2.3.5 Clinical pathways for patients with CF

Fiorella Gurrieri, Giovanna Elisa Calabrò

In 2004 the European Cystic Fibrosis Society (ECFS) convened a Consensus Conference on standards of care for CF. CF professionals from many European countries, and some from outside Europe, produced a document that was later published in the Journal of Cystic Fibrosis [90]. The aim of this consensus document was to define standards for the routine evaluation, monitoring and treatment of patients with CF in Europe. In this document were indicated the characteristics of a CF center:

1. the center should have the staff and facilities to provide comprehensive care and be capable of treating all CF associated complications;
2. the center should be an integral part of a university or teaching hospital with funding guaranteed by the provider of medical care;
3. a CF center should normally care for a minimum of 50 patients;
4. the center director should be an experienced CF physician working in close collaboration with at least one other physician knowledgeable in CF medicine;
5. the CF centers should have varied numbers of specialist CF nurses, dietitians, physiotherapists, social workers, psychologists, pharmacists and microbiologists according to patient numbers;
6. the CF center should have close links with consultants within the hospital or at hospitals nearby specializing in gastroenterology, hepatology, endocrinology, ear, nose and throat (ENT) surgery, general, hepatobiliary and pediatric surgery, radiology, obstetrics and gynecology, infectious diseases and infection control, rheumatology, ophthalmology and nephrology;
7. the center should have the following facilities available: a radiology department with CT scanning facilities; expertise in bronchial artery embolization for pulmonary hemorrhage; a pulmonary function laboratory; expertise in the placement of totally implantable venous access devices, nasogastric and gastrostomy tubes; a microbiology service expert in examining specimens from people with CF with established contacts with a CF microbiology reference laboratory; a full diagnostic capability including reliable sweat testing and CFTR gene mutation analysis;
8. patients should have 24-h access to the CF center for telephone advice or for emergencies or other consultations.

CF is a disease with a complex, multifaceted clinical phenotype characterized by multiorgan involvement therefore it is essential to provide multidisciplinary care for patients with CF. Furthermore, this consensus document has indicated “the routines of CF care”. For example, for the outpatient care it indicated the following: “Patients should be seen every 1–3 months, preferably every month. Newly diagnosed infants or patients with severe disease should be seen more often, and those with mild phenotypes or atypical CF may be seen less often, every 3–6 months”. Yet, for the inpatient care it indicated that a CF specialist center must have sufficient beds available at all times to allow immediate admission and that each center should have a clear infection control policy [90].

Ten years later the ECFS has developed a specific field of the standards of care related to these three areas: a) the required framework of the CF Centre; b) best clinical practice; and c) quality management in CF care.

The Centre Framework document [91] emphasizes the unquestionable role of dedicated CF centres for achieving optimal outcomes. The paper highlights the importance of the multidisciplinary team, describes the general structure of pediatric and adult CF centres and stresses the need for their cooperation and for a transition programme. All disciplines are strongly encouraged to be members of their national and international specialist groups, to update themselves by attending meetings, and to participate in audit and research whenever possible.
The *Best Practice* document [92] describes the services that centres should provide using a question and answer model. Castellani et al. [93] describe the contents of this document summarizing them with the following key messages:

1. Early and accurate detection through neonatal screening and expertise to undertake biochemical and genetic diagnostic testing are vital and enable access to specialist care from early in life,
2. Prompt recognition of pulmonary infection and deterioration permits intervention with timely treatments which are proven to reduce the impact of CF on the lungs;
3. Nutritional and metabolic complications require regular monitoring and timely and effective intervention;
4. CF professionals need to have experience and expertise in all common complications and to have developed referral pathways with other disciplines to support more complex situations;
5. Appropriate management and communication are particularly important at transplantation referral and end of life;
6. Despite excellent improvement in clinical outcomes and survival for people with CF, psychological complications are common for the patient and their family and require early recognition, assessment and management with the active involvement of experienced psychological health care professions.

The *Quality Management* document [94] reviews management of quality of care for patients with CF at several levels: patient, centre, regional, national and international. Improvement issues are examined with particular reference to annual assessment, patient quality management charts, CF team sessions, therapy goals, certification, peer review, public reporting, quality groups, ranking, learning from best practice, interaction with registries, benchmarking, and cooperation with national organizations.

Certainly, the professionals/experts of ECFS are aware of the barriers that some countries, in which CF services are absent or minimal, may encounter in implementing the new standards of care. They know that their accomplishment is made challenging by deficiencies in political prioritization, inappropriate funding and a lack of staff recruitment and training. However, all European nations should strive to achieve a model of CF care in accordance with the ECFS recommendations.

### 2.3.6 Spots on CF Therapy

*Fiorella Gurrieri, Giovanna Elisa Calabrò*

Until recently, treatment of CF has focused on lessening the manifestations of CF disease in the affected organs. Iva-caftor is the first prescribed drug that targets CFTR. This compound potentiates the opening probability of CFTR to aid chloride and bicarbonate ion transport in CF-epithelia [95]. Ivacaftor was shown to significantly improve lung function in individuals with at least one G551D-CFTR allele [96], and is now approved for use in CF individuals with gating mutations. More recently, ivacaftor has been combined with lumacaftor (VX-809), which is a CFTR corrector that improves the Phe508del CFTR processing to increase the amount of cell surface-localized protein, demonstrating improved outcomes in a clinical trial [97]. However, the average improvements appear less striking with respect to what observed in the ivacaftor trial. It has been propose that the variability in response to these compounds is likely mediated by a common variant in *SLC26A9*, already known as a CF modifier, which may predict the response to CFTR-directed therapeutics [98].

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3.1. SUMMARY OF THE ACCE (ANALYTIC VALIDITY, CLINICAL VALIDITY, CLINICAL UTILITY, ETHICAL, LEGAL AND SOCIAL ASPECTS) REPORT OF THE CDC

3.1.1 The ACCE project

Antonella Agodi, Martina Barchitta

The ACCE (Analytic validity, Clinical validity, Clinical utility and Ethical, legal and social implications) project has been funded by the Centers for Disease Control and Prevention (CDC) to establish a model process, on DNA and related testing for disorders with a genetic component, in order to allow policy makers to have access to up-to-date and reliable information for decision making, also taking into account gaps in knowledge that will help to define future research agendas.

The ACCE model is composed of a standard list of 44 targeted questions that address the disorder and setting (7 questions), the analytic (10 questions) and clinical validity (8 questions) and utility (16 questions) of the genetic test and its ethical, legal and social implications (3 questions) [1].

The first disorder to undergo an ACCE review is the Cystic Fibrosis (CF) in the prenatal setting. In this chapter a summary of the model system reported in the “ACCE review of the population-based prenatal screening for CF via carrier testing” [2] is described.

Disorder and Clinical Setting

CF, an autosomal recessive genetic disorder, primarily affects the lungs and the gastrointestinal tract of patients in which the exocrine glands produce abnormally dense secretions of mucus. This leads to a wide variety of progressive respiratory and gastrointestinal problems, and impaired fertility in males. The disease reduced life expectancy, however, during the past quarter of last century, the long-term prognosis has improved substantially due to more effective treatments to prevent or ameliorate the clinical complications of CF, couples who underwent prenatal diagnosis need to be fully informed about the potential outcomes. Moreover, carrier individuals will be encouraged to share this information with their family.

Given that both the CF prevalence and the frequency of mutations vary greatly, depending upon race and ethnicity [5], it is most effective to offer prenatal CF screening to couples who are Caucasian and of European or Ashkenazi Jewish descent, and who are planning a pregnancy or who are in a variety of ways. Because, to date, there are no in vitro treatments to prevent or ameliorate the clinical complications of CF, couples who underwent prenatal diagnosis need to be fully informed about the potential outcomes. Moreover, carrier individuals will be encouraged to share this information with their family.

It is possible to detect both normal and mutant CF alleles in any sample, blood and buccal swab, with the exception of mature red blood cells. Of the wide variety of testing methodologies available, most screening trials have chosen forward dot-blot, reverse dot-blot, or amplification refractory mutation system (ARMS) technologies. At the time of the ACCE process, in the United States, however, no kits have been approved by the Food and Drug Administration (FDA) for CF testing, and none is known to be under review.

In the United States, protocols promote CF carrier testing in the prenatal setting: pregnant women and their partners are the target group in order to identify couples in whom both partners are carriers of a CF mutation (carrier testing). The carrier state itself has no medical implications and such testing is done solely to assist in reproductive decision-making in a variety of ways. Because, to date, there are no in utero treatments to prevent or ameliorate the clinical complications of CF, couples who underwent prenatal diagnosis need to be fully informed about the potential outcomes. Moreover, carrier individuals will be encouraged to share this information with their family.

Table 9. The core panel of 25 mutations for prenatal screening of CF (from Grody et al., 2001 [7]).

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Mutation</th>
<th>Mutation</th>
<th>Mutation</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>delF508</td>
<td>delE507</td>
<td>G542X</td>
<td>G551D</td>
<td>W1282X</td>
</tr>
<tr>
<td>N1303K</td>
<td>R553X</td>
<td>G21+1G&gt;T</td>
<td>R117H</td>
<td>1717+1G&gt;A</td>
</tr>
<tr>
<td>A455E</td>
<td>R560T</td>
<td>R1162X</td>
<td>G85E</td>
<td>R334W</td>
</tr>
<tr>
<td>R347P</td>
<td>711+1G&gt;T</td>
<td>1898+1G&gt;A</td>
<td>2184delA</td>
<td>1078delT</td>
</tr>
<tr>
<td>3849+10kbC&gt;T</td>
<td>2789+1G&gt;A</td>
<td>3659delC</td>
<td>1148T</td>
<td>3120+1G&gt;A</td>
</tr>
</tbody>
</table>
screening question concerning race/ethnicity to determine which couples would be offered screening.

3.1.1.1 Analytic validity

Domenico Coviello, Maria Baffico

In pre-conception carrier screening for CF the purpose is to identify couples, not a-priori at increased risk of CF, in which both the mother and her partner have identifiable CF mutations. Their offspring have a 1 in 4 risk of having cystic fibrosis. This allows either standard prenatal diagnosis in the first or second trimesters, or no testing of the foetus. Subsequent pregnancies have the full range of reproductive options [8].

The DNA test results are qualitative. For DNA-based tests, analytical validity requires establishing the probability that a test will be positive when a detectable mutation is present and this is the “Analytical sensitivity” (also called the true positive rate). For DNA-based tests, analytical validity requires establishing the probability that a test will be positive when a detectable mutation is present. The analytical sensitivity is 97.9% (95 percent CI 96.9 to 98.7%), after removing challenges involving delI507 [8].

The probability that the test will be negative when the mutation is absent is the “Analytical specificity” (also called the true negative rate). The probability that the test will be negative when the mutation is absent would be the proportion of positive test results when no detectable mutations are present. The analytical specificity is 99.4% (95 percent CI 98.7 to 99.8%), after removing challenges involving delI507 [8].

False negative results could be due to technical errors in the analytic phase (e.g., sample placement, contamination, expired reagents and cross-reactivity). Analytical sensitivity and specificity are statistical measures of the test performance and must be determined before it is made available in clinical practice.

For CF mutation analysis, laboratories can currently choose between development of in-house developed testing methodologies or use of commercial specific reagents. Actually most of the commercial diagnostic kits, used for the CF molecular analysis, are CE-marked in vitro diagnostic devices (IVDD); however, assay performance should always be verified by laboratories before diagnostic use.

Analytical validation of a new genetic test includes performing replicate determinations to ensure that a single observation is not spurious (false) and needs “blind” testing of coded positive samples and negative control samples. A sufficient number of patient samples are necessary to have statistical confidence in the validation.

“Internal quality control” is a set of laboratory procedures designed to ensure that the test method is working properly. An internal quality control program includes documentation that high standards are being practiced to ensure that:

- reagents used in all aspects of genetic testing are of high quality to allow successful test completion,
- all equipment is properly calibrated and maintained,
- good laboratory practices are being applied at every level of genetic testing. To the extent possible, all steps of the testing process must be controlled.

The purpose of the quality control procedures is to rigorously control all steps of the DNA testing process to minimize the potential for test failure.

One additional consideration might be that laboratories perform differently when testing proficiency testing samples than when testing clinical samples on a routine basis. This difference might make the form of less good performance because the sample is handled outside of the laboratory routine. Alternatively, the performance might be better because extra attention might be paid to obtaining a reliable result. Future analyses should be aimed at providing reliable method- and, possibly, mutation-specific analytic performance estimates.

Appropriate sample size for determining analytic specificity can be derived by choosing an acceptable target specificity and an acceptable lower limit that should be excluded in the 95 percent confidence interval. The higher the specificity chosen and the tighter the confidence interval, the larger is the sample size that will be necessary to provide a definitive answer. For example, if a laboratory chose a target specificity of 98 percent and wanted to rule out a specificity of 90 percent, it would need to correctly identify at least 49 of 50 known negative samples (estimated using the binomial distribution).

The table 10 shows guidelines, recommendations and checklists that address internal quality control issues and requirements.

### Table 10. Guidelines, Recommendations, and Checklists that Address Internal Quality Control Issues and Requirements.

| Clinical Laboratory Improvement Amendments of 1988 Federal Register 1992;57:7002-3 |
| Genetic Testing Under CLIA Federal Register 2000;65: 25928-24934 |
| New York State Laboratory Standards (9/00) www.wadsworth.org/labcert/download.htm |
| Molecular Diagnostic Methods for Genetic Diseases: National Committee for Clinical Laboratory Approved Guidelines Standards MM1-A Vol 20 #7 |
| College of American Pathologists Checklist www.cap.org |
| American College of Medical Genetics www.faseb.org/genetics/acmg/ stds |
| European Concerted Action on Cystic Fibrosis (BMH-4-CT96-0462) |
| Technical Standards and Guidelines for Cystic Fibrosis Supplement to the ACMG Standards and Guidelines for Clinical Genetics Laboratories |

Molecular genetics laboratories participating in CF carrier screening programmes should satisfy the test validation criteria described by guidelines. They should be accredited (ISO 15189 or equivalent) and participate annually in international EQAs for CF.
Due to the rapid growth and evolution of technologies, constant update of quality management practices is essential at all stages of the testing process to ensure the accuracy and utility of these tests. This requires a system that includes both internal and external procedures.

Internal quality assurance processes are handled by the laboratory and include measures to maintain analytic accuracy, at all stages of the testing process, pre-analytic, analytic and post-analytic phases. They include internal control reactions, negative and positive control samples that must be run with each assay, negative or "no DNA" control that should be included in each run, etc. Moreover, a regular activity of training for staff and update of personnel competency.

**External monitoring.** All clinical laboratories performing genetic testing must comply with general regulations under the Clinical Laboratory Improvement Amendments (CLIA) and a CLIA certification should be considered the minimum acceptable level of external monitoring.

This kind of external controls needs availability of positive controls. Positive controls for the standard most common cystic fibrosis mutations must be utilized to validate the assay and for each lot of reagents. These controls (or a subset of positive controls) are recommended to be routinely included in each assay run. However, obtaining these positive controls can be difficult, not all of the main recommended cystic fibrosis mutations are readily available.

External quality assessment measures include examination of laboratory procedures by a third party accreditation process and participation in external quality assessment (EQA) programs. Most of the current quality assurance practices commonly used in genetic testing laboratories are designed for well-established technologies.

Actually, regarding CF, in Italy, two major schemes for external quality assessment are yearly available:

- National EQA: “Controllo esterno di qualità dei test geneticici” organized by Istituto Superiore di Sanità (ISS);
- European EQA: Cystic Fibrosis External Quality Assessment organized by European Molecular Genetics Quality Network (EMQN)*

Genetics Quality Network (EMQN)*

Most of the commercial diagnostic kits, used for the CF molecular analysis, are CE-marked in vitro diagnostic devices (IVDD); however, assay performance should always be verified by laboratories before diagnostic use. Analytical validation of a new genetic test includes performing replicate determinations to ensure that a single observation is not spurious (false) and needs "blind" testing of coded positive samples and negative control samples. A sufficient number of patient samples are necessary to have statistical confidence in the validation [10].

- Having information about repeated measurements on the same specimen is important for determining the type and rate of errors in detecting cystic fibrosis mutations
- External proficiency testing programs are the only available source of data for repeated measurements on the same specimen by multiple laboratories

All clinical laboratories test control samples repeatedly, but results are not usually reported.

**Confirmatory testing**

Confirmatory testing is additional testing to confirm the finding of a mutation(s). Such testing should be considered when a carrier, carrier couple, or affected foetus is identified. It is likely to be useful in selected circumstances, because of occasional false positive test results. There is little information about how often confirmatory testing corrects an error. The type of confirmatory testing depends on the clinical circumstances, sample type and testing methodology. Supplementary testing might occasionally be necessary [11,12].

Confirmatory testing is performed to ensure that the initially positive test result is correct. For example, rerunning a specimen that was positive for a mutation in order to ensure that it was correct is considered confirmatory testing.

**Reflexive testing** is different from confirmatory testing in that other mutations or polymorphisms are being analysed to aid in the interpretation of positive results. For example testing an unexpected homozygous [delta]F508 individual for the presence of the benign polymorphism F508C would be considered reflexive testing. This is also the case in testing for the 5T/7T/9T polymorphism after identifying the R117H mutation.

In prenatal screening for cystic fibrosis, confirmatory testing of some type should be considered.

Four distinct types of confirmatory testing could be utilized, depending on the testing protocols in place and the circumstances in which the positive test result is obtained:

- Repeating the same test protocol on another aliquot of the same specimen
- Repeating the same test protocol on a different (or further processed)*** specimen
- Performing a different test protocol on another aliquot of the same specimen
- Performing a different test protocol on a different (or further processed)*** specimen

---

* At the moment the CF network works in close collaboration with the EuroGenTest Network of Excellence and the European Molecular Genetics Quality Network (EMQN) in order to improve harmonization of External Quality Assessment (EQA) schemes within Europe [9].

*** further processing would include, for example, culturing foetal cells obtained via chorion
Patient specimens

Both whole blood and buccal lysates are acceptable for screening. Blood samples are more expensive and require collection at a medical facility, but are associated with more generous amounts of high quality DNA. Buccal lysates are less expensive and can be collected at home, but are associated with smaller amounts of lower quality DNA.

Diagnostic testing of the foetus can be performed on:

- direct and cultured amniotic fluid cells,
- chorionic villi collected via chorionic villus sampling (CVS),
- cells obtained via percutaneous umbilical blood sampling (PUBS)

Pre-implantation diagnostic testing can be carried out on a single cell.

Results obtained in multiple laboratories using the same, or different technology.

Data derived from external proficiency testing can be used to judge the consistency of results from different CF screening laboratories. Stratification of results by methodology does not currently yield reliable information because of the small number of laboratories participating in proficiency testing and the large number of methodologies. Overall, the results from multiple laboratories appear to be similar, regardless of the methodology used, if the panel of mutations employed by individual laboratories is taken into account [13].

3.1.1.2 Clinical Validity

Domenico Coviello, Maria Baffico

The term clinical validity was proposed by the NIH-DOE Task Force on Genetic Testing to describe the accuracy with which a genetic test identifies a particular clinical condition [14]. It is described in terms of sensitivity, specificity, positive predictive value, and negative predictive value (Table 11).

Table 11. Test Properties Measuring Clinical Validity

<table>
<thead>
<tr>
<th>Test Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>Among people with a specific condition, the proportion who have a positive test result</td>
</tr>
<tr>
<td>Specificity</td>
<td>Among people who do not have the condition, the proportion who have a negative test result</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>Among people with a positive test result, the proportion who have the condition</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>Among people with a negative test result, the proportion who do not have the condition</td>
</tr>
</tbody>
</table>

The definition of clinical sensitivity and clinical specificity can be derived using a two-by-two contingency table for data from either case/control or cohort studies. If the data are from the general population cohort, both positive predictive and negative predictive values can be directly computed.

Table 12 shows the definitions of these four characteristics: DNA screening test for CF is considered positive when both partners of the couple are carrier of a identifiable mutation.

<table>
<thead>
<tr>
<th>Both partners are Cystic Fibrosis carriers</th>
<th>YES</th>
<th>NO</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Couple positive by DNA testing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>A</td>
<td>B</td>
<td>A+B</td>
</tr>
<tr>
<td>NO</td>
<td>C</td>
<td>D</td>
<td>C+D</td>
</tr>
<tr>
<td>Totals</td>
<td>A+C</td>
<td>B+D</td>
<td>A+B+C+D</td>
</tr>
</tbody>
</table>

- Clinical sensitivity \[ \frac{A}{A+C} \] is the proportion of couples in which both partners are cystic fibrosis carriers (A+C) and who are correctly identified as being positive (A) by the screening test.
- Clinical specificity \[ \frac{D}{B+D} \] is the proportion of non-carrier couples (B+D) who are correctly identified as being negative (D) by the screening test.
- Positive predictive value \[ \frac{A}{A+B} \] is the proportion of positive tests (A+B) that correctly identifies carrier couples (A).
- Negative predictive value \[ \frac{D}{C+D} \] is the proportion of negative tests (C+D) that correctly identifies non carrier couples (D).

The positive predictive value is dependent on the birth prevalence, the analytic sensitivity, the clinical specificity and the screening model employed. It is not strongly dependent on the proportion of detectable mutations.

The negative predictive value is dependent on the screening model used, the combination of test results in the couple, birth prevalence, and the analytic and clinical sensitivity. It is not strongly dependent on the analytic or clinical specific.

Figure 7 shows an example of applying prenatal screening for CF to a hypothetical cohort of 1,000,000 couples. In this example, the prevalence of CF is 1:2,500 (carrier rate 1/25), and the DNA test panel identifies 77% of the carrier couples. The analytic sensitivity is taken to be 97.9%, and the analytic specificity (after confirmatory testing) is assumed to be, in this example, 99.99% (false positive rate of 1 per 10,000 individuals tested). Among the population screened, there are 1,600 carrier couples (1,000,000 * (1/25)^2). 77% of the 1,600 carrier couples are detectable (1,232), and 1,181 of these are detected (1,232*99.99%). Among the 998,400 non-carrier couples, 76,800 will include one carrier partner, and, in six of these couples, a false positive result will occur in the non-carrier partner (76,800*.88*.999*0.0001). The numbers from Figure 3-1 can now be entered into a two-by-two table (Table 3-2) by substituting actual numbers into the
format shown earlier in Table 3-1. The clinical performance estimates can then be computed.

**Impact of the screening model on these estimates**

In Figure 7, there are a total of 76,800 couples with one partner a carrier, and all are considered as having positive test results. Only the expanded one-step (concurrent) screening model will identify all of these carriers. In that model, samples are obtained and tested from both partners.

The other two screening models (the two-step or sequential, and the one-step or couple) only identify half of the carrier/non-carrier couples, thereby reducing the number of clinical false positive results from six to three couples. The following sections consider additional issues relating to clinical sensitivity and specificity.

**Clinical sensitivity**

Clinical sensitivity refers to the proportion of carrier couples (or affected fetuses) that can be detected by screening couples during pregnancy. In contrast, analytic sensitivity describes how often the laboratory correctly identifies a mutation that is included in its panel. Because of the large number of mutations responsible for CF and the limited number of mutations that can currently be economically included in a prenatal screening setting, not all carrier individuals will be identified [12,15].

In order to estimate the proportion of carrier couples (or affected fetuses) that can be identified for any given panel of mutations, it is necessary to obtain the mutation frequencies in an unbiased sampling of individuals clinically affected with cystic fibrosis [16–19]. These mutation frequencies can be derived from the Cystic Fibrosis Genetic Analysis Consortium Report or from the Cystic Fibrosis Foundation Database.

**Prenatal cystic fibrosis screening models and test failure rates.**

The impact of a test failure (i.e., no useable result) on prenatal cystic fibrosis screening depends on the model used. If a program utilizes buccal samples, the test failure rate might be, for example, 1 percent. If that program uses a two-step model, a new sample must be requested from 1 of every 100 women initially tested.

**Genotype and phenotype of the foetus.**

The aim of prenatal screening for cystic fibrosis is two-tied; first, to identify carrier couples and then, to offer these couples a diagnostic procedure (usually amniocentesis) and testing to identify cystic fibrosis in the foetus. When both partners are confirmed as carriers, the risk of the foetus inheriting both mutations is 1 in 4 (odds of 1:3).

**Clinical specificity**

The analysis in this section is restricted to screened couples who, because of their genetic makeup, cannot have a child with cystic fibrosis. Rarely, these couples may be incorrectly classified as a carrier couple (i.e., a mutation is reported

1,000,000 Pregnant Couples

<table>
<thead>
<tr>
<th></th>
<th>1,600 (‘True’ Carrier Couples)</th>
<th>998,400 (Not Carrier Couples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>/ \</td>
<td>/ \</td>
<td>/ \</td>
</tr>
<tr>
<td>1,232</td>
<td>368</td>
<td>76,800</td>
</tr>
<tr>
<td>Detectable</td>
<td>Not Detectable</td>
<td>One Carrier</td>
</tr>
<tr>
<td>(false negative)</td>
<td></td>
<td>No Carriers</td>
</tr>
<tr>
<td>/ \</td>
<td>/ \</td>
<td>/ \</td>
</tr>
<tr>
<td>1,181</td>
<td>51</td>
<td>6</td>
</tr>
<tr>
<td>Detected</td>
<td>Not Detected</td>
<td>Positive</td>
</tr>
<tr>
<td>(True Positive)</td>
<td>(False Negative)</td>
<td>(False Positive)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(True Negative)</td>
</tr>
</tbody>
</table>

**Figure 7. A Schematic Showing the Results of Prenatal Cystic Fibrosis Screening for ‘Carrier Couples’**
Clinical specificity is a measure of how often this occurs.

According to the analysis of available data, the analytic specificity is 97.9% (i.e., a mutation is falsely reported to be present, or the wrong mutation is reported in about 2 to 3 per hundred tests). This rate is derived from external proficiency testing and, therefore, may not reflect the checks and balances routinely in place in the clinical laboratory that are designed to identify and correct analytic errors.

Clinical false positives occur when two mutations are found in the foetus, but the phenotype is not classic cystic fibrosis. One definition of a clinical false positive result would be a foetus with two of the mutations contained in the recommended panel that would not develop the phenotype generally associated with cystic fibrosis. Several of the less common mutations in the recommended panel are not associated with classic CF most of the time.

Cystic fibrosis prevalence

The population-based CF registries serve as the source for the analysis of prevalence. The present summary includes only reports from registries that summarize data from the 1970s or later. The three registries below use multiple sources of ascertainment over a relatively long time period (up to ten years) to capture nearly all clinically defined cases. An advantage of studies that include only births prior to 1989 is that they will not be influenced by prenatal diagnosis. Registry data shows the numbers of non-Hispanic Caucasians included in cohorts from each registry, along with the number of cases identified. In several instances, the numbers have been adjusted for various ascertainment biases or for mixed race/ethnicity. These adjustments are described in detail in the following sections. The overall prevalence estimate of 1:2499 is computed using a random-effects model [20].

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Total NHC</th>
<th>CF Cases</th>
<th>Birth Prevalence</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>United Kingdom 1978-87</td>
<td>7,360,000</td>
<td>3,046</td>
<td>1:2416</td>
<td>2333-2507</td>
</tr>
<tr>
<td>Canada 1970-79</td>
<td>3,041,510</td>
<td>1,168</td>
<td>1:2604</td>
<td>2462-2717</td>
</tr>
<tr>
<td>United States 1990-1992</td>
<td>7,675,221</td>
<td>3,086</td>
<td>1:2487</td>
<td>2396-2583</td>
</tr>
<tr>
<td>All</td>
<td>18,076,731</td>
<td>7,300</td>
<td>1:2499</td>
<td>2371-2633</td>
</tr>
</tbody>
</table>

Prevalence in racial/ethnic groups other than non-Hispanic Caucasians

Ashkenazi Jewish.

Data are available to estimate the birth prevalence of cystic fibrosis in the Ashkenazi Jewish population from five studies reporting carrier frequencies and one study using a population-based registry in Israel. The estimated birth prevalence after selected adjustments have been performed. Overall, the birth prevalence is 1:2271, (95% confidence interval 1:1793 to 1:2876), but there is a wide range in estimates from a high of 1:1639 to a low of 1:3123.

African Americans.

Data are available to estimate the birth prevalence of CF in African Americans from two studies reporting carrier frequencies and three studies uses population-based registries. The estimated birth prevalence after selected adjustments have been performed. Overall, the birth prevalence is 1:15,057, (95% confidence interval 1:14,800 to 1:15,300). The two population-based estimates are similar and indicate a carrier rate of about 1:61. The test panels used in the two carrier studies identify only about 48% of the mutations.

Asian Americans

There are two published estimates of cystic fibrosis in Asians in their native lands. One found a prevalence of 1:90,000 in Asian Hawaiians [21]. Another found a prevalence of 1:320,000 in Japan between 1969 and 1980 [22]. As of 1998, the literature contained only 40 detailed reports of cystic fibrosis in Asians, and many of the cases were of mixed heritage [23]. As of that time, all instances of delF508 occurring in ‘Asian’ cystic fibrosis patients could be traced to documented Caucasian admixture. Thus, the prevalence for cystic fibrosis among Asians could be 1:100,000, or even lower.

Environmental or other modifiers

Factors other than CFTR genotype, particularly other genes and environmental influences, are likely to play a role in the natural history of cystic fibrosis.

No genetic, environmental or other modifiers of phenotype have yet been defined. In the context of prenatal screening, future knowledge of such modifiers could provide information.

3.1.1.3 Clinical utility

Antonella Agodi, Martina Barchitta

The natural history of CF is well described with an incidence of CF related liver diseases that increases with age, from about 0.3 percent before the first 5 years of life to a peak of 8.7 percent in people aged between 16 to 20 years [24]. Although the exact pathogenesis of the disease is unknown, evidence suggests that defective CFTR chloride channel function may cause abnormal biliary secretions, resulting in mucus plugging of intrahepatic bile ducts [25]. Respiratory
complications are the most frequent causes of death among CF patients. The highly viscous mucus secretions in the respiratory tract cannot be adequately cleared, providing an ideal habitat for bacterial colonization and subsequent lung infection [26,27].

Severe gastrointestinal disease, in the form of intestinal obstruction caused by meconium ileus, is the first clinical manifestation in 10-18% of newborns with CF [28,29]. Because of the severity of this complication, mortality in the first month of life is higher in patients with meconium ileus.

Lung transplantation was introduced as a therapeutic intervention for CF in 1988 and patients with CF now account for an important portion of lung transplants done annually in the United States. One important hope for the future is gene therapy and research focuses on how this treatment might be effectively delivered to the lungs of affected individuals [30].

In the patient care, three models of CF prenatal screening have been published and used. The two-step or sequential model initially expects the collection and the analysis of the pregnant sample. If a mutation is identified in pregnant women, the next steps require woman counselling and to obtain a sample from the partner for DNA analysis. When a mutation is also identified in the partner, the couple will then be provided with more intensive counselling, given the 1 in 4 risk that the foetus will be affected by CF. Approximately 1 in 900 screened pregnancies will fall into this category.

In the one-step model, samples are collected from both the pregnant woman and her partner at the outset. Mutation analysis is then performed on the woman’s sample, and the partner’s sample is tested only if a mutation is identified in the pregnant woman. However, notification of a positive screening result is made only when both partners are found to be carriers of a mutation. In this model, more effort is required initially to obtain samples from both partners, but the need for counselling is reduced; being restricted to the 1 in 900 couples who will need to make decisions about diagnostic testing.

In the modified one-step model, recommended by the American College of Medical Genetics [7], samples are collected from both partners at the outset and DNA testing is performed on all of the samples from both partners. Notification is made when a mutation is found in either partner, and counselling is provided.

The comparison between the above mentioned three models shows the same clinical sensitivity. They differ for the number of individuals made conscious of their carrier status and offered counselling and the number of calls for offering counselling and foetal diagnostic testing when both partners are found to be carriers, to allow informed decision-making. Accordingly, the decision about which model to use will depend upon characteristics of the health provider and patient population, also taking into account the ethical, financial, and social concerns.

The purpose of prenatal screening for CF is to determine whether the disorder is present in the foetus. The diagnostic test is DNA testing for CF mutations in fetally derived cells, obtained via amniocentesis or chorionic villus sampling (CVS). If two disease-causing mutations are identified, the couple is counselled that the foetus will have the disorder. To date, there is no effective treatment in utero for CF, so that the couples are informed on. The couple might also choose to continue the pregnancy and plan for initiating treatment immediately after birth. According to this choice and prior to agreeing to testing, constructed and validated patient informational materials will be helpful to develop strategies for educating the public about the process and options of this testing and to alleviate the burden of CF positive results at the time of pregnancy.

Pilot trials are an important step in translating research knowledge into practice. Different pilot trials of prenatal screening for CF have been published, using successfully all three screening models above described. Many of the trials superimposed CF screening upon existing prenatal screening services. Populations were usually non-Hispanic Caucasian and/or Ashkenazi Jewish and relatively few mutations, analysed from blood and buccal samples, were included in the analysis (usually about 6 and none more than 16). Overall, about 55,000 women/couples were screened with an uptake rate that ranged between 57 and 99 percent (median 78 percent). Of the screen positive couples, 91% chose to have prenatal diagnosis. In those, diagnosis was successfully completed in 94%. Of the 18 couples with affected foetuses, 83% chose to terminate the pregnancy [31].

Either amniocentesis or CVS can be used for obtaining foetal cells for diagnostic testing. Both of these procedures are considered invasive, carrying a small, but important, risk for foetal loss. The procedure-related foetal loss rate is about 9 per 1000 procedures for amniocentesis and somewhat higher for CVS. The ratio of CF cases identified to foetal losses is about 25 to 1. If the procedures are done at the proper gestational age by experienced operators, non-fatal procedure-related risks to the foetus are minimal.

Financial costs and benefits associated with screening can be categorized in multiple ways, taking into account the purpose and perspective of the analysis. From the literature, eight key components of financial costs have been identified and reasonable estimates have been assigned, [31] as follows:

<table>
<thead>
<tr>
<th>Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Providing education/information</td>
<td>$3.00</td>
</tr>
<tr>
<td>Obtaining informed consent</td>
<td>$10.00</td>
</tr>
<tr>
<td>Obtaining a sample</td>
<td>$10.00</td>
</tr>
<tr>
<td>Performing the DNA test</td>
<td>$80 to $100</td>
</tr>
<tr>
<td>Reporting negative results</td>
<td>$2</td>
</tr>
<tr>
<td>Reporting positive results</td>
<td>$20</td>
</tr>
<tr>
<td>Performing diagnostic testing</td>
<td>$400 to $600</td>
</tr>
<tr>
<td>Accounting for procedure-related fetal loss</td>
<td>$400</td>
</tr>
</tbody>
</table>


To determine prenatal CF screening total costs, additional factors should be included. These factors comprise: screening uptake rates, partner uptake rates, proportion of carrier couples choosing diagnostic testing, the carrier rate of CF in the population being tested, the proportion of mutations detected by the DNA analysis, and the procedure-related foetal loss rate. Accordingly, the cost per case of CF identified is about $400,000 in the main target populations, thought costs are much higher in other racial/ethnic groups; this value is considerably reduced if subsequent pregnancies are taken into account. The total cost/per couple of offering screening ranges from $120 to $240, depending on the screening model chosen, and the direct annual medical costs for the average individual with CF are between $15,000 and $20,000.

Based on consensus estimates of annual medical costs, expected lifetime and recommended discount rates, the total lifetime direct medical costs are between $300,000 and $500,000.

CF mutation analysis is a complex laboratory procedure that requires laboratories with the necessary expertise, experience, and resources, in order to minimize the human error and the laboratory errors. Accordingly, a genetic testing laboratory must follow good laboratory practice guidelines and subscribe to a quality assurance program. The main constituents of generic quality assurance programs are well-established and consist among the published guidelines that are available from national and state regulatory agencies and professional organizations. These components regard quality control, inter-laboratory comparison, proficiency testing, and laboratory personnel requirements. Although multiple standards exist, only three guidelines are enforceable and require laboratory inspection for certification. These include: Clinical Laboratory Improvement Amendments (CLIA), College of American Pathologists (CAP), and New York State. The external proficiency testing is the main indicator of quality assurance and it allows laboratories to identify individual areas of weakness and potential improvements. External proficiency testing programs are in place for laboratory testing in United States and Europe.

Prenatal screening for CF needs to be viewed in the context of a combined laboratory and patient service program. To properly implement prenatal screening programs, testing facilities can be developed following the structure of the existing prenatal screening programs, including laboratory space, equipment, materials, and staff members. Moreover, a centralized administrative function should oversee all phases and aspects of the screening program, such as educational materials and consent, laboratory testing and interpretation, genetic counselling and diagnostic testing. Screening activities and personnel involved can be divided into three phases of activities: pre-analytic, analytic and post-analytic.

At the time of the ACCE report, a total of 30 or fewer laboratories are providing screening services in the United States. However, in order to implement screening activity, it requires: i) more laboratories offering prenatal screening; ii) an increase in the number of DNA clinical technologists required; iii) an increase in the number of genetic counsellors.

Prenatal screening programs need educational materials appropriate for two to types of target: providers and patients. The Secretary’s Advisory Committee on Genetic Testing (SACGT) has recently suggested a genetic test template to inform and educate health professionals [31]. Currently, 13 centres have developed educational programs based on this template, as part of pilot studies of prenatal CF screening. Anyway, laboratories could create and validate their own provider education materials. The American College of Obstetricians and Gynecologists has recently produced a Clinical and Laboratory Guideline on this subject for its members [32]. The American College of Medical Genetics has also published Laboratory Standards and Guidelines on this topic and much of that information would be useful in provider educational materials [7].

The education of pregnant women and their partners about prenatal screening for CF is necessary to provide relatively complex genetic information in order to that couples make informed choices. Patient educational materials are already available from several sources, including some that have been published as part of reports of prenatal CF pilot studies [33,34]. Other patient educational materials are available on-line. In addition, two educational leaflets have been produced by the American College of Obstetricians and Gynecologists and the American College of Medical Genetics. Overall, patient educational materials meet most content criteria and have been rated adequate with regard to objective readability standards.

Long term monitoring of the screening process can be valuable in helping to assure quality and assess public health impact. It also provides a framework for the collection of further information about clinical validity and utility, as well as ethical, legal, social and financial issues. Priorities for studying long term monitoring issues might be based on feedback from providers, laboratories, and those being offered screening. Although general methodologies for long term monitoring of program effectiveness exist, national guidelines have not been developed. In the absence of guidelines and oversight requirements, it is suspected that many screening laboratories are not collecting these types of information necessary for such monitoring and document effectiveness.

3.1.1.4 Ethical, Legal and Social Implications (ELSI)

Antonella Agodi, Martina Barchitta

Many ELSI issues are similar to those already encountered with existing prenatal screening programs for neural
tube defects and Down syndrome. Among these, the potential stigmatization of CF carriers is often mentioned as a potential ethical issue of screening. Another advisable aspect regards the quality of the informed consent decision. Education and informed consent processes could help couples to make an informed choice, prior to beginning the prenatal screening process. This requires good educational materials for patients and providers and a mechanism that encourages and allows health care providers to spend the time necessary to a full informed consent discussion about the potential implications of the screening test. One of the benefits of a CF prenatal screening program could be the cascade testing of relatives of CF carriers. However, testing involves several logistic problems, such as the possibility that the relatives of the carrier are likely not to be patients of the physician who ordered the original testing.

Additional issues regard the criteria to offer screening to populations with varying gene frequencies and prevalence. At the time of the ACCE report, the American College of Obstetricians and Gynecologists [35] recommends that testing will be made available to all couples, whatever their risk for carrying the CF gene. However, for couples in ethnic or racial groups considered at higher risk for carrying the cystic gene, physicians will specifically offer screening and will follow up with inquiries about the couple’s decision on whether to be screened.

Several potential sources of civil liability exist for the primary care physician in connection with prenatal screening for CF. First of all, a primary care physician may be liable if the standard of care dictates that a screening test should be offered to a patient and the physician fails to offer it. Second, primary care physicians can also be liable if the screening tests are conducted without securing the patient’s consent, explaining the significance of the possible results and sufficient information about the risks and benefits of the test. The third source of potential liability for negligent referral can arise if the physicians refers the test to a laboratory known or suspected to not be qualified. Finally, the primary care physician can be liable for breach of patient confidentiality in connection with the mishandling of confidential patient information or patient samples.

Although the testing laboratory usually will not have a direct with the patient, it faces a set of civil liability considerations separate from, but overlapping, the primary care physician’s. The laboratory faces risks associated with the failure to perform screening tests according to current standards and guidelines. For the CF screening, there are several sets of guidelines, recommendations and checklists, such as the Clinical Laboratory Improvement Amendments (CLIA) regulations on Genetic Testing, and, for laboratories which test samples from New York State, the New York State Department of Health Laboratory Standards.

Because few laboratories develop a direct relationship with the patients tested, there is rarely a duty on the part of the laboratory to secure the patient’s informed consent to screening. However, depending on the testing, laboratories could face liability risks associated with failing to inform patients about the meaning of test results and the risks of false negative and false positive screening results before the patient consents to proceed with the screening test. The laboratory may incur liability if it mistreats confidential patient information and patient samples. Finally, if the testing process is itself subject to patent protection or its use is otherwise restricted, the laboratory may face liability to the owner of the test without the proper license [2].

References

Chapter 4

4.1. SYSTEMATIC REVIEW OF THE ECONOMIC LITERATURE ON GENETIC TESTS FOR CYSTIC FIBROSIS CARRIER SCREENING

Carlo Favaretti, Paolo Campanella

4.1.1 CF cost-of-illness

Cystic fibrosis (CF) is the most common life-limiting genetic disorder affecting people of European ancestry with an incidence of approximately 1:2000-3500 live births [1]. With recent advances in treatment, most children with CF now can expect to survive into adulthood and life expectancy has improved considerably. CF is a progressive disease that affects many organ systems. As the disease progresses, patients require more intensive health care that includes home-based care, medications, more frequent and intensive health care that in-
prolonged hospital admissions, and, in around half of all cases, lung transplantation [2].

Through a systematic literature search in Medline, Embase, the Cochrane Library and NHS Economic Evaluation Databa-se*, we identified eleven cost-of-illness studies (Table 13).

The average annual health care cost ranged from €19,263 to €48,290**. Some studies found considerable cost variation among patients with CF depending on the patient’s lung function, whereas others found no significant effect. For example, the study by Lieu et al [8] found that the average annual health care cost for a patient with poor lung function was seven times greater than for a person with relative good lung function status. A more recent study by DeWitt et al [12] found that baseline lung function score was not a significant predictor of health care costs.

The small sample size and the data collection methods limit the ability of most of the studies to examine differences in the cost among different population groups and to provide an overall assessment of the lifetime health care costs for the entire CF population. For example, the study by Baumann et al [6] relied on children’s resource use data to estimate costs of adult patients, Horvais et al [10] included out-of-hospital costs but did not have data on inpatient treatments and Heimeshoff et al [9] focused on patients treated in one center.

However, the study of van Gool et al [13] that was based on the Australian Cystic Fibrosis Data Registry and 2255 patients estimated a mean annual health care cost for treating CF of €13,758 with differences among patients with mild moderate and severe disease estimated respectively in €8,951, €22,617, and €29,769**.

Lifetime health care costs were also approximately estimated in €255,936** (using a 3.5% discount rate) with the majority of costs accounted for by hospital inpatients (58%), followed by pharmaceuticals (29%), medical services (10%), complications (2%) and diagnostic tests (1%).

New CFTR modulator therapies designed to correct the function of the defective protein made by the CF gene with specific mutations, such as ivacaftor and lumacaftor, are not taken into account in already published studies. With their introduction, per patient expenditures are likely to multiply in the next years.

4.1.2 Review of economic evaluations related to the implementation of screening for CF carrier

Carrier screening for CF has been possible since the discovery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene [14] and can detect over 88% of carriers with a 25 mutation panel [15].

Several screening strategies have been evaluated and reported in the literature and screening options can be characterized by different timing (e.g. school-aged, while planning a pregnancy or during pregnancy), model (e.g. stepwise, couple or cascade screening) and place of screening (e.g. GP, shared care GP, obstetrician, public antenatal clinics, schools, workplace) [16].

Figures 8 and 9 report decision models for preconceptional and prenatal screenings as elaborated by Radhakrishnan et al [17].

With preconception screening, to a carrier couples who are planning a pregnancy is offered a range of reproduction options including, refraining from having (more) children, adoption, accepting the risk of giving birth to a child with CF, having prenatal diagnosis possibly followed by termination of an affected foetus and pre-implantation diagnosis.

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Table 13. Summary of cost-of-illness studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Patients</th>
<th>Mean age (age range)</th>
<th>Mean annual costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robson 1992</td>
<td>UK</td>
<td>119</td>
<td>21 (16-44)</td>
<td>£14,867</td>
</tr>
<tr>
<td>Wildhagen 1996</td>
<td>Netherlands</td>
<td>81</td>
<td>14 (0-37)</td>
<td>€20,051</td>
</tr>
<tr>
<td>Ireys 1997</td>
<td>USA</td>
<td>204</td>
<td>0-18</td>
<td>€20,147</td>
</tr>
<tr>
<td>Bauman 2003</td>
<td>Germany</td>
<td>138</td>
<td>0-18</td>
<td>€29,137</td>
</tr>
<tr>
<td>Johnson 1999</td>
<td>Canada</td>
<td>303</td>
<td>18</td>
<td>CA$10,497</td>
</tr>
<tr>
<td>Lieu 1999</td>
<td>USA</td>
<td>136</td>
<td>17 (0-56)</td>
<td>$17,546</td>
</tr>
<tr>
<td>Heimeshoff 2012</td>
<td>Germany</td>
<td>212</td>
<td>20 (0-adult)</td>
<td>€44,731</td>
</tr>
<tr>
<td>Horvais 2006</td>
<td>France</td>
<td>65</td>
<td></td>
<td>€19,230</td>
</tr>
<tr>
<td>Eidt-Koch 2010</td>
<td>Germany</td>
<td>301</td>
<td></td>
<td>€24,691</td>
</tr>
<tr>
<td>DeWitt 2012</td>
<td>USA</td>
<td>352</td>
<td>15 (5-adult)</td>
<td>$40,037</td>
</tr>
<tr>
<td>van Gool 2013</td>
<td>Australia</td>
<td>2255</td>
<td>15 (0-adult)</td>
<td>AU$21,182</td>
</tr>
</tbody>
</table>


** The cost data were converted to 2016 euro currency values using a web-based tool (CCEMG-EPPI-centre cost converter available at http://eppi.ioe.ac.uk/costconversion/default.aspx).
Figure 8. Decision model of preconceptional screening (Radhakrishnan et al Health Policy 2008).

Figure 9. Decision model of prenatal screening (Radhakrishnan et al Health Policy 2008).
With prenatal screening, pregnant women and their partners are screened as early as possible in their pregnancy. Should both parents be carriers, couples can choose to test the foetus through chorionic villus sampling (CVS) or amniocentesis. If the foetus is affected, there are couples who accept to continue the pregnancy and couples who decide to abort.

Through a systematic literature search in Medline, Embase, the Cochrane Library and NHS Economic Evaluation Database* we identified 13 studies focusing on preconception or prenatal CF screening.

The most common outcome measure used were the cost per carrier couple detected, followed by the cost per birth of an individual with CF averted. One study estimated the cost per quality adjusted life-year (QALY).

Reported participation rates for preconception screening ranged from 10% to 100%, while women’s participation in prenatal screening ranged between 50% and 100%. The reported cost of a screening test ranged from €25 to €212 and the main source of variation was the use of the less expensive single mutation test versus the multiple mutation tests. The cost of foetal diagnosis ranged from €220 to €1873. Estimated lifetime cost of care for CF patients ranged from €291,048 to €1,105,452**.

Most of the studies indicated the costing perspective but only eight (57%) justified this. Only six (43%) costed all items relevant to the stated perspective. For example, studies in Denmark claim a societal perspective but use Dutch reimbursement rates, which could vary from the actual costs. Only three (21%) showed distinction among long run and short run costs.

All studies reported how variable costs were estimated. However, only three (21%) explicitly apportioned human resources and other fixed resources like capital equipment to the cost of CF screening. Ten studies (71%) reported methods for determining the values of resources used. Although 10 (71%) reported the base year for costing, 6 studies neglected to inform readers that costs were not derived in the base year. Further, only six studies (43%) adjusted costs to the base year.

For preconceptional screening, the reviewed studies reported assumptions (based on literature) that 15–25% of carrier couples identified refrained from having children and assumed that the remaining 75–85% would make use of fetal diagnosis, following conception. Termination rates used in this case ranged from 80% to 95%.

For prenatal screening, termination of pregnancy assumed by reviewed studies ranged from as low as 30% to as high as 100%, with most assuming a rate of 75%.

Table 14. Summary of economic evaluations related to the implementation of screening for CF carrier.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Evaluation type</th>
<th>Perspective</th>
<th>Focus</th>
<th>Screening strategy</th>
<th>Outcome measure</th>
<th>Reported results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weijers-Poppelaars 2005 [18]</td>
<td>Netherlands</td>
<td>CBA</td>
<td>Societal</td>
<td>Preconception</td>
<td>Step wise</td>
<td>Net savings</td>
<td>No</td>
</tr>
<tr>
<td>Verheij 1999 [19]</td>
<td>Netherlands</td>
<td>CBA</td>
<td>Societal</td>
<td>Preconception</td>
<td>Step wise</td>
<td>Net savings</td>
<td>Yes</td>
</tr>
<tr>
<td>Wildhagen 1998 [20]</td>
<td>Netherlands</td>
<td>CBA and CEA</td>
<td>Societal</td>
<td>Preconception and prenatal</td>
<td>Couple, step wise and individual</td>
<td>Net savings</td>
<td>Yes</td>
</tr>
<tr>
<td>Nielsen 2002 [21]</td>
<td>Denmark</td>
<td>CBA and CEA</td>
<td>Societal</td>
<td>Prenatal</td>
<td>Step wise</td>
<td>Net savings</td>
<td>Yes</td>
</tr>
<tr>
<td>Rowley 1998 [22]</td>
<td>USA</td>
<td>CEA and CUA</td>
<td>Societal</td>
<td>Prenatal</td>
<td>Step wise</td>
<td>Cost per CF birth averted</td>
<td>$1,608,861</td>
</tr>
<tr>
<td>Asch 1998 [23]</td>
<td>USA</td>
<td>CEA</td>
<td>Societal</td>
<td>Prenatal</td>
<td>Couple and step wise</td>
<td>Cost per CF birth averted</td>
<td>$367-594,000</td>
</tr>
<tr>
<td>Ginsberg 1994 [24]</td>
<td>Israel</td>
<td>CBA</td>
<td>Societal</td>
<td>Prenatal</td>
<td>Step wise</td>
<td>Net savings</td>
<td>No</td>
</tr>
<tr>
<td>Morris 1995 [16]</td>
<td>UK</td>
<td>CEA</td>
<td>Health sector</td>
<td>Preconception and prenatal</td>
<td>Couple, step wise and individual</td>
<td>Cost per carrier detected</td>
<td>£35-40,000</td>
</tr>
<tr>
<td>Vintzileos 1998 [26]</td>
<td>USA</td>
<td>CBA</td>
<td>Health sector</td>
<td>Prenatal</td>
<td>Step wise</td>
<td>Net savings</td>
<td>Yes</td>
</tr>
<tr>
<td>Cuckle 1995 [27]</td>
<td>UK</td>
<td>CEA</td>
<td>Health sector</td>
<td>Prenatal</td>
<td>Couple and step wise</td>
<td>Cost per affected pregnancy</td>
<td>£65-75,000</td>
</tr>
<tr>
<td>Doyle 2003 [28]</td>
<td>USA</td>
<td>CBA</td>
<td>Health sector</td>
<td>Prenatal</td>
<td>Step wise</td>
<td>Net savings</td>
<td>No</td>
</tr>
<tr>
<td>Lieu 1994 [29]</td>
<td>USA</td>
<td>CBA and CEA</td>
<td>Health sector</td>
<td>Prenatal</td>
<td>Step wise</td>
<td>Net savings</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: CEA (cost-effectiveness analysis), CUA (cost-utility analysis), CBA (cost-benefit analysis).


** The cost data were converted to 2016 euro currency values using a web-based tool (CCEMG-EPPI-centre cost converter available at http://eppi.ioe.ac.uk/costconversion/default.aspx).

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Results, as reported in Table 14, vary significantly in terms of outcome measure and incremental cost-effectiveness ratio (ICER) used in cost-effectiveness analyses (CEA) that however can be generally considered at least as cost-effective both for preconception screening and prenatal screening. The only cost-utility analysis (CUA) included in our review reported a surely cost-effective results with cost per QALY of €16,572 for prenatal screening.

Two out of three cost-benefit analyses (CBA) reviewed reported cost-savings results for preconception screening, as well as three out of six CBA for prenatal screening.

References


25. Warren E, Anderson R, Proos AL, Burnett LB, Barlow-Stewart K, etc.

*The cost data were converted to 2016 euro currency values using a web-based tool (CCEMG-EPPICentre cost converter available at http://eppi.ioc.ac.uk/costconversion/default.aspx).


Chapter 5

5.1. CYSTIC FIBROSIS CARRIER TESTING: ETHICAL EVALUATION

Maria Luisa Di Pietro, Adele Anna Teleman

5.1.1 Introduction

Cystic Fibrosis (CF) is a life-threatening, autosomal-recessive disease that results from a chloride channel defect, attributed to mutations in the CFTR gene [1]. Approximately 2000 mutations have been discovered since 1989, when the most common CFTR allele, known as ΔF508, was described [2]. More than 280 of these mutations are responsible for the majority cases of this disease [3].

The incidence of CF varies depending on region, ancestry, and CFTR allele prevalence in each population [4]. CFTR gene mutation, that codifies a modified protein responsible of chloride channel defect, can affect many organs (epithelial cells of sweat glands; pancreas; upper respiratory tract; lungs; liver; intestine; reproductive system), with symptoms varying in appearance, entity and development.

The prevention of loss of electrolytes in sweat, malabsorption, malnutrition, and research regarding therapies for pulmonary diseases are modifying the clinical history of this pathology. For 5% of the CF population, all the patients with the p.Gly551Asp and type III mutation, known as “gating mutation”, is a newly developed treatment (Ivacaftor) that improves chloride transport through an increasing chloride channel function CFTR-related [5]. Crozier optimistically, but not realistically, stated CF is “a not-so-fatal disease” anymore [6]. In fact, the survival of CF patients has been increasing during the past 4 decades. Although many factors contribute to lowering the morbidity and mortality of CF patients, it has become clear during the past decade that age of diagnosis, sex, genotype, pancreatic functional status, socioeconomic status, and respiratory flora are especially significant.

CF lung damage starts very early in life and may already be present at the time of diagnosis during the screening of 4-6 weeks newborns [7–9]. 20-30% of CF infants have residual exocrine pancreatic function at birth, lost over the following 6-12 months [10]. The early diagnosis in infancy due to a positive family history or new-born screening (i.e. pre-symptomatic identification) improves nutritional outcomes and may also be associated with less severe lung disease [4].

CF genetic testing is currently being offered to: newborns after a positive sweat testing; adults with late expression of the disease, without pancreatic failure; adults with mono-organic pathologies (i.e., chronic pancreatitis, bronchiectasis, chronic obstructive broncho-pneumopathies with mucorma), which may be CFTR-correlated, or in order to search for healthy carriers. The screening of healthy carriers is being proposed [11] with a double objective: 1. to offer a tool for a responsible procreative choice of an individual/couple; 2. to draw attention on the possible presence of a new-born with CF in the absence of a positive family history.

The proposal of a healthy carrier genetic test raises questions on its analytical validity, clinical validity and utility, including its use within a context of limited economical resources. Along with these questions, which already have an ethical value, there are other questions that relate to the management of a genetic test on a healthy carrier (i.e. information, the respect of autonomy) and to the use of the obtained results.

An answer to these questions can be found through a process of Health Technology Assessment (HTA), that takes into consideration not only the technical, clinical and economical aspects [12], but also the social and ethical ones [13,14]. It is a fact that the ethical evaluation is an integral part of HTA process: HTA has been defined as “a multidisciplinary field of policy analysis. It studies the medical, social, ethical and economic implications of development, diffusion and use of health technology” [15].

Considering that the objective of a HTA is to connect the scientific (research) and political (the decisional process) world, an ethical contemplation fills in the gap left by technology. In fact, even if technology is capable of answering questions regarding safety, efficacy, efficiency, and economical impact, it is not capable of responding ethical questions. Even the ACCE protocol – created for the evaluation of genetic tests – determines that the elements to take into consideration are not only the analytical validity (A), the clinical validity (C) and the clinical utility (C), but also the ethical, legal and social aspects (ELSI), which are an integral part of the decision-making process [16,17].

Unlike the other fields of a HTA process, which are based on standardized methods of analysis, the ethical analysis is influenced not only by the characteristics of the technology in question and the available evidences, but also by the ethical choice and the method of analysis [18]. Despite the variety of ethical methodologies, some authors doubt their
appropriateness [19,20] and point out the difficulty of integrating ethics into a HTA process. The true difficulty derives from not taking into consideration the epistemological differences of the disciplines involved. In fact, an ethical analysis does not produce scientific evidence but uses the evidence reported by the other disciplines to highlight the moral issues and make an evaluation. Therefore, the issue is not to determine the correct methodology for an ethical analysis in HTA, but to acknowledge that the use of any health technology will inevitably raise questions of meaning and value [21].

For this reason, it is important to clarify the ethical orientation and the values hierarchy. In this assessment, ethics has a “cognitive” approach, according to which a moral value can be known, and focuses on the human being (person-centred ethics) [22].

If the barrier between what is licit and illicit is the human being, we must act – according to Immanuel Kant (Groundwork of the Metaphysic of Morals, 1788) – “in such a way as to treat humanity, whether in your own person or in the person of any other, never simply as a means, but always at the same time as an end”. The morality of an act, therefore, depends on the respect for each human being, including their life, health, and freedom of expression.

Materials and Methods

Based on person-centred approach, this ethical evaluation includes three phases:

- review of the available evidence on the specific technology and the assessment of the proportionality risks/benefits;
- analysis of data in the light of the ethics of reference to understand whether or not the new technology respects the human being;
- elaboration of ethical assessment through the examination and evaluation of the use of the specific technology.

5.1.2 The epistemological moment

Presently, the sequence variations known in the CFTR gene are approximately 2000, even though many of these have no pathological significance. The genetic testing that is available today through gene sequencing (Next generation sequencing) can identify 98% of possible gene alteration, even if it does not identify all the possible mutations of the CFTR gene, especially in the cases of a mixed or non-Caucasian ethnicity and with variability even within the same ethnicity.

However, there is an increasing scientific knowledge regarding the ethnic specific mutations of this gene. According to Castellani et al., carrier screening panels ideally should include all the mutations with a frequency of 0.5-1% or more in the CF population [11]. The Italian Society for the Study of Cystic Fibrosis recommends that tests should provide “a number and type of mutations that cover at least the prevalence of approximately 85% of the mutations (editor’s note: using a Kit that provides the study of 187 mutations) identified in the population of origin of the tested subject, although it is highly desirable that with the new methods of analysis such accuracy should be closer to 95% ” [23].

The new methods based on Next-Generation Sequencing technologies (NGS) have undoubtedly a greater sensitivity, with a mutation detection rate of 99% [24], identifying also variations of unknown clinical significance. Furthermore, even in the presence of mutations, known to be associated with the disease, it is often hard to predict the severity of the individual clinical manifestations [25].

The analytical validity of a genetic test is fundamental for an exact diagnosis and the clinical use (clinical utility), but the diffusion of CF genetic testing should depend especially on the sufficient acquisition of the diagnostic aspect.

5.1.3 The anthropological moment

In the “person-centred” ethics, the reference criteria are: the defence of physical life, as a fundamental value; health promotion; respect for the subject’s responsible freedom; search for the common good. Therefore, in the ethical evaluation of CF genetic testing for identifying healthy carriers, the following aspects should be analysed: the proportionality between the possible risks and the expected benefits; the pre-test counselling for the subject’s consent; the post-test counselling; the inequalities in accessing the genetic tests.

5.1.3.1 Assessing benefits and harms

The desired benefit of a genetic test is usually evaluated in terms of prevention of disease’s clinical manifestations, or symptomatic treatments.

In the case of genetic testing for identifying healthy carriers, the purpose is to inform the individuals about the relationship between the identified mutations and the likelihood of the disease in their offspring. The benefit is, therefore, the acquisition of knowledge useful in the reproductive choices and the opportunity of affected newborn’s early treatment.

Even if knowledge is in itself good, it cannot be excluded that a genetic test for identifying CF healthy carriers can lead to psychosocial harms. The first problem could be the fear of stigmatization and discrimination because of one’s genetic state. For example, in a carrier screening program, a positive result could create difficulty in finding a partner, in health and life insurance options and in reproductive choices [11]. When such genetic knowledges are used to stigmatize people, the potential for injustice is great. In fact, the most important risk is “geneticization”, that is the human being’s identification through genes, overemphasizing their role in disease aetiology, medical practice and social attitudes towards patients [26]. The second problem is the possible anxiety of the “genetic” relatives’ involvement in an information they did not seek [11].
5.1.3.2 The respect of autonomy

Access to a genetic test on healthy carrier raises questions regarding information management and respect for individual’s autonomy [27]. The situation becomes more complex when the test is inserted in a screening program with the aim of identifying CF healthy carriers in a population. It should also be considered that the test to identify CF healthy carriers is usually proposed not only to the individual but also to the couple to avoid calling the partner in a second moment. In this way, the couple has more time to decide and each individual can be personally informed on the results and eventually choose if change own’s partner [28].

Genetic testing for CF healthy carriers might be offered in other contexts besides pre-conception counselling: for example, in schools or in the workplace [29]. Considering that in these contexts there could be a very high level of indifference towards the execution of the test as well as a high risk of social stigmatization in case of a positive result [30], the rules that should be followed in the acquisition of consent should be the same as in any medical environment.

In fact, respect for an individual’s autonomy comprises the right to make autonomous decisions regarding health care and to voluntarily seek a genetic test that might determine consequences in his or her own’s life.

Therefore, it is important not to have any pressure from the family, the professionals involved or others, since control by third parties would invalidate the given consent. The choice of whether or not to pursue genetic testing belongs to the individual.

Respect for an individual’s autonomy is ensured by obtaining an adequate informed consent, and this means more than a simple signature on a piece of paper. In fact, it is essential to offer pre-test counselling for evaluating the individual’s capacity of autonomous decision-making and to provide a realistic view of the test’s implications (risks, benefits, efficacy, alternatives, entity and potential treatments, social and ethical implications) [31]. It is necessary to explain that genetic knowledge has an individual, predictive and probabilistic nature, and, furthermore, that the results of genetic testing have implications not only for the subject, but also for their biological kin [32]. Genetic counselling should be offered before submitting the individual to the test, as well as after, when the results are disclosed.

Finally, respect for an individual’s autonomy also entails that all the information acquired should be presented in a confidential setting, and should not be disclosed without the individual’s consent. It is necessary, however, not to forget that genetic information often regards other people (e.g. genetic relatives, partners), who would be entitled to be informed of the presence within the family or couple of a healthy carrier’s state. Thus, autonomy is not appreciated in its full sense if it does not encompass the responsibility towards others who are somehow involved in the decisions.

5.1.3.3 Decision making and scarce resources: a problem of justice

In general, the economical evaluation within a HTA has to take into consideration, on one hand, the costs of the technology being used, and on the other, the direct health costs (collecting specimens, laboratory testing, counselling, follow-up testing, treatment and prevention), the direct non-health costs (sufferings, pain, loss of self-sufficiency) and all other indirect costs (for example, the loss of working hours) [33].

As regards to tests on CF healthy carriers, the economic evaluation should take into consideration the possibility to give access to the genetic testing to all the potential carriers of cystic fibrosis and the sustainability of this possibility for the public health systems. It is, however, necessary to highlight the difficulty in making an economic analysis that does not evaluate in the cost/benefit ratio also the different options that arise after a positive test in the pre-conception phase. In fact, the study by Norman et al. includes in the economical evaluation also the possible choice to abort the affected foetus, which would reduce the health costs determined by the disease, and the use of artificial fertilization techniques for the selection of embryos [34].

It is quite clear, therefore, that the economic evaluation is also strongly influenced by the ethics of reference. The person-centred ethics accepts an evaluation that analyses, on one side, the cost of the genetic testing, and on the other, the number of CF healthy carriers that could benefit from the testing results of the tests for procreative responsible choices and promotion of an early treatment of any CF newborn. It would be a very different situation putting on one hand the cost of this genetic test, and on the other the reduction in the number of CF patients, obtained through interventions after fertilization. In fact, the objective should be the reduction of CF incidence, rather than the reduction of already conceived affected individuals.

5.1.4 The ethical evaluation

Even though knowledge is in itself good, it is also true that the comprehension and the management of genetic knowledge are both very complex. For this reason, anyone who is offering (or referring for) genetic testing must provide (or refer for) appropriate genetic counselling before and after testing. Genetic counselling is the only context to help people correctly cope with such health issues [35].

5.1.4.1 The methodology of counselling

One of the principles underlying the methodology of counselling is non-directiveness [36]. It implies that professionals should not impose any decision as more correct or advantageous for an individual or society. However, is it acceptable, in
the name of non-directiveness, to place all the options on the same level, leaving the choice solely to the individual? Is this really what individuals expect? Should the counsellor engage in nondirective counselling and only present all the alternatives, without advising for or against any choice? Or does the counsellor have the responsibility of presenting his/her moral view? The answer to these questions derives from the consideration of the characteristics of medicine. Medicine regards itself ultimately as a helping and healing profession. In such a concept, value-neutrality is not an appropriate position to guide medical activities. In fact, according to this view, physicians adhere to professional norms that go beyond the neutrality of values [37]. Therefore, if there are options that do not ensure the respect for human life, dignity and health, the counsellor has the duty to express this, since these elements also constitute a part of the truth that he/she is called to represent as a professional and a person [38].

5.1.4.2 Pre-test and post-test counselling

There are two phases in genetic counselling: pre- and post-test counselling. An adequate understanding of the implications of CF healthy carrier genetic testing is, in fact, a prerequisite for the tests. It is also necessary to evaluate the individual’s capacity for autonomous decision-making.

2.1 Pre-test counselling

Pre-test counselling for CF healthy carriers should include:
1. exploration of all pros and cons of testing; 2. elucidation of an individual’s motives for testing; 3. identification of areas in which the individual’s expectations might be unrealistic; 4. understanding the phenomenon of false negatives; 5. information about psychological, familiar, social and ethical aspects and the economic consequences.

Since information regarding CF healthy carrier genetic testing is very complicated, the information offered to the subject should be correct, complete and communicated in a comprehensible manner. In signing the consent form, subjects are asked to state that they fully understand the terms and have had adequate opportunities to ask questions.

2.2 Post-test counselling

In post-test counselling for CF healthy carriers, the counsellor should communicate the test results and help in understanding their meaning. It could be useful to evaluate through a questionnaire if the subject has obtained an adequate level of comprehension and representation of the genetic risk.

It is also necessary to remember that the tested individuals have the right to decide not to know the test results [39]. The great majority of people think that genetic testing would be a good idea and, when hypothetically asked, they would want to be tested. However, when genetic testing is actually offered, the adherence rate is considerably lower.

Even among families at high risk for a genetic disorder, many individuals choose not to know. On the other hand, the right to know is valuable for persons, so that they can obtain information regarding their genetic constitution and, therefore, make responsible choices.

5.1.4.3 Other issues

There are also issues stemming from the responsibilities (e.g. parental, social) that emerge from the knowledge of the other person’s genotype.

3.1 The right to choose “not to know”

In contrast, the right not to know is sustained by various arguments: 1. knowledge can cause distress, even if it has been observed that the benefits of knowledge could outweigh the disadvantages and that uncertainty can also cause anguish; 2. since the human condition is by nature of limited knowledge, it does not make sense to say that we ought to know or that there is a duty to know.

It would thus seem more ‘human’ to assert a right to hope versus a right to certainty. Nevertheless, an apparent contradiction remains: how could a person decide not to know without knowing what is there to know? The moral problem, in conclusion, lies not so much at the level of wanting or having a duty to know or not to know, but rather it lies in how to make meaningful use of the available genetic information [40]. This points to the importance of adequate counselling, at the end of which the subject may even decide not to take the test. In fact, the information obtained by the test could be so inconclusive and probabilistic that the person involved could be unable to take any subsequent measures.

If a person has decided to know, he/she becomes the subject of information. Therefore, confidentiality and privacy are important in genetic testing, not only because of the possibility of discrimination, but also because they are crucial to preserving a person’s autonomy. Sometimes, there could be others who may be interested in the information for other reasons; in these cases, there is a conflict between autonomy and responsibility towards others. For example, blood relatives or other family members (i.e. partner) have the right to be informed [41]. Counsellors may invite tested subjects to disclose this information to biological relatives, who could benefit from it. What if the subject refuses to disclose? The choice whether or not to inform relatives at high genetic risk against a subject’s wish (or without his/her consent) is ethically difficult. In fact, the duty to preserve confidentiality is in conflict with the responsibility to warn third parties of possible harm.

3.2 The clinical management of the results

The second aim in post-test counselling is clinical management. In this phase, the information offered is essential, be-
cause of the individual differences in the perception of the risk and the consequences of made choices.

If the genetic test is conducted during the preconception phase, it should be remembered that the options presented to the individual/couple (renouncing to having a child; adoption; IVF + PGD + selection of embryos; heterologous artificial fertilization; natural conception + prenatal genetic diagnosis + abortion; natural conception with 25% chance of having a CF affected child) are not equivalent from an ethical point of view. The advice offered by a geneticist does not seem sufficient for an adequate reflection on all the issues related to each option.

In the presence of a CF affected foetus, there is a very high incidence of cases in which it is decided to undertake an abortion, ranging from 76.9% in the study conducted by HadjFredj et al. [42] to 100% in the study conducted by Massie et al [43].

Ethical advice would, therefore, also be useful to clarify the subject’s understanding of prenatal life. Interventions that are subsequent to conception (IVF + PGD + embryo selection; natural conception + Prenatal Genetic Diagnosis + abortion in case of CF) are not a form of prevention, but of selection of affected embryos/foetuses.

The right of the CF affected subject (including the newly conceived) is to be welcomed, assisted, cared for and to be able to take advantage of the options that scientific research will obtain in terms of diagnosis and treatment. To exclusively think with a “selection” mentality, should be considered, as the Italian National Committee for Bioethics states, “a defeat not only for Humanity but also for scientific progress” [44]. For example, we can remember the experience in the prevention of thalassemia in Sardinia: “In 86 monitored pregnancies occurring in couples with no previous affected offspring, 23 homozygous foetuses were identified; in all cases but two, the parents requested elective abortion” [45]. In 1994, Cao wrote: “The main reason for residual cases of thalassemia major in the Sardinian population is a complete lack of parental knowledge about thalassemia and the procedure for its prevention, followed by refusal of prenatal diagnosis, refusal of abortion when an affected foetus are identified, and false paternity” [46].

A “selection mentality”. The fact that the decision can be determined by the couple’s free choice does not change the situation: a free choice is not in itself necessarily good; a free choice could be not really “free” if it is influenced by a culture in which parents are “morally obliged to genetically modify their children” to offer “the best opportunities for a better life” [47].

5.1.5 Conclusive considerations

Using a genetic test to study the mutations in the CFTR gene, that is sensitive, safe, accurate and reliable undoubtedly represents an advantage in diagnosis and personalized therapy, as seen, for example, in nowadays treatment for patients with F508 homozygote mutation with a combination of Kalydeco and Lumacaftor [5,48].

The use of genetic tests for the detection of CF healthy carriers is undoubtedly an advantage in terms of the acquisition of knowledge and the responsible management of choices, but raises – as pointed out – many questions from an ethical point of view. The issue becomes even more complex when the genetic test is included in a screening program for CF healthy carriers. Besides the need to ensure equal opportunities in the access to screening, it is essential to also consider the management of the information, the acquisition of free consent and the confidentiality of the obtained results. In the case of a positive result for the CF genetic tests on healthy carriers, it is worth underlining that not all the proposed procreative options have the same ethical value and they should not be presented and applied in a mechanical way, without considering a question of meaning and limits. In fact, a free choice must always be confronted with its limits, including those that, being insurmountable, should simply be accepted as the limits “of any” or “of that” human life.

5.2. GENETIC TESTS FOR CYSTIC FIBROSIS CARRIER SCREENING: THE PATIENTS’ AND THE CITIZENS’ PERSPECTIVE

Carlo Castellani

Whenever a health system is considering the implementation of a screening campaign, there is a necessity to consider all its possible consequences at the social level. This entails consultations on stakeholders’ views, including patients’ and general population’s opinions.

Among patients and their families an overall positive attitude toward population CF carrier screening has been reported [49–52]. The most recent study [53], performed on a Belgian sample of 64 parents and 47 patients aged 16 or older, found that more than 80% were in favor of preconception carrier screening for CF. Some concerns were reported about potential negative consequences, i.e. the reproductive decision challenges in carrier couples detected by the system; a reduction in therapeutic research investments due to the expected decrease of CF births and of the treatable population; and health insurance issues in carriers.

Similar attitudes and concerns have been registered in the general population. In Italy, a survey conducted by DOXA examined a sample of 1006 individuals (52% females) representing the Italian population aged >15 years [54]. The survey was developed by a panel of experts in the treatment of CF patients. “About two-thirds of the sample were not aware that the carrier status may be discovered using a blood test. Among those aware about genetic analysis, 45% suggested that the test should be performed before pregnancy on all parents, while, surprisingly, 19% would have made the test available for all couples “during” pregnancy. Fre-
quency of carrier status was correctly identified by 8% of the sample.” These results highlight how in Italy public awareness of CF is still poor, and jeopardize any assessment of the CF carrier screening issue made by an uninformed public.

Reliable alternatives to effectively involve potential recipients of the test offer in the decision process are needed. Under these circumstances, deliberative democracy schemes may be used. These are based on decision-making by a group of lay people, who have no vested interests and who apply their common sense and experience, having been presented with the best possible evidence by expert witnesses. Juries of citizens constitute an example of deliberative democracy which directly involves citizens in health decisions [55–58]. People with no relevant diagnosed pre-existing medical condition have access to the best available evidence, and reflect on the advantages and disadvantages of using health care services or technologies to preserve good health.

The juries of citizens method has been applied in a project supported by the Italian CF Research Foundation. A group of 14 lay citizens with no direct or indirect experience of CF was selected, adequately informed and, after an open debate with experts, asked to answer the question: “Should the Health Service organize a screening of the population with the aim of identifying healthy people that may have children suffering from CF?” All except one member of the jury felt positively about the Health Service actively providing population carrier screening for CF [59].

The experience was later replicated in a study that involved two other juries (30 individuals altogether), providing similar results (manuscript under review). Overall the three juries included 19 males and 25 females whose age ranged from 31 to 81 years. The project included also an internet public consultation targeted to citizens ad health professionals on opinions and attitudes about screening healthy carrier. Almost one thousand responders completed the survey, with the majority in favor of a national CF carrier screening program.

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An increasing trend in the performance of CF carrier tests in women or couples with no family history of CF has been registered in the United States [1], in Australia [2,3], in Israel [4] and, to a minor extent, in Europe [5]. With the exception of Israel, which has implemented a population program supported by the national health system [4], the test offer is not part of systematic screening but instead based on the personal initiative of individual health professionals if not on direct to consumer internet offers.

Such heterogeneity of strategies and the risks connected with it has prompted various learned societies to produce statements about CF carrier screening.

Since 1997 the US National Institutes of Health [6] has recommended that carrier testing for CF should be offered not only to adults with a raised a priori reproductive risk, i.e. those with a positive family history of CF, but also to couples from the general population planning a pregnancy.

The NIH were followed by the American College of Obstetricians and Gynecologists (ACOG) [7], and the American College of Medical Genetics (ACMG) [8] in their recommendation for widespread genetic testing for CF. The 2011 ACOG update [9] specifically states that “it is important that CF screening continues to be offered to women of reproductive age.” Technical details are also considered: “complete analysis of the CFTR gene by DNA sequencing is not appropriate for routine carrier screening”.

The European CF Society produced a consensus document on standards for efficacious, safe and ethical practice of CF carrier screening, and suggested that the decision whether to implement it or not should be left to individual countries or regions in accordance with local legislation [10]. Several general recommendations are offered: “CF carrier screening should be voluntary, accessible and preceded by accurate pre-test information; CF carrier screening should be offered preferentially to prospective parents, ideally before conception but acceptably during early pregnancy as dictated by practicability; minors and other persons unable to consent should be excluded from screening; and the screening programme should be regularly monitored over the long-term”. With regard to the test itself, the document specifies that “the prevalence of CF disease mutations in the screened population must be known; carrier screening panels ideally should include all mutations with a frequency of 0.5–1% or more in the CF population; only CF-causing mutations should be screened; whenever possible, the negative partner of intermediate risk couples should be tested with a more sensitive CFTR mutation panel; people from ethnic groups that have a lower sensitivity for detection than the majority for who the screening panel was selected should be informed about the limitations of the screening programme and when possible offered a customised approach; and CFTR gene sequencing or mutation scanning should not be used in the carrier screening setting at this stage of knowledge”. Regarding communication policies, the recommendations are: “only health care professio-
The practice and recommendations for the CF carrier screening are very heterogeneous in Europe. The purpose of genetic testing on healthy carriers is to inform the individuals about the relationship between identified mutations and the likelihood of disease in their offspring. Advances in the molecular genetics technology have made CF carrier testing reliable and affordable, but still the proposal of a carrier genetic test in general population raises many questions. The aim of our work was to summarize the available evidence, using the HTA approach, on the genetic tests for CF carrier screening.

Cystic fibrosis is an autosomal recessive genetic disorder caused by mutations in the CFTR gene located on the chromosome 7. CF is a disease with a complex, multifaceted clinical phenotype characterized by multiorgan involvement and it ranges from severe (lungs, pancreas, male reproductive tract) to mild (intestine) to asymptomatic (sweat glands) cases. There is a strong correlation between the general type of CFTR mutation and disease phenotype. The defective ion transport caused by CFTR mutations results in reduced apical airway surface liquid, which leads to impaired mucociliary clearance that progressively induces ob-

### References


### Chapter 7

#### 7.1. KEY ISSUES FOR THE DECISION MAKERS

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struction of the airways with thickened mucus. Manifestations of disease may be also present in CF carriers, since CFTR variants act as risk alleles for multigenic disorders in the general population (idiopathic disseminated bronchiectasis, bronchiectasis that is complicated by infection with *non-tuberculous Mycobacteria* or with the fungus *Aspergillus fumigatus*; chronic rhinosinusitis).

Epidemiological data on the incidence and prevalence of CF have been collected from the available literature. In Europe, among Caucasians, the most widely reported incidence is 1 case per 2000-3000 live births. On the other hand, the incidence reported for Italy ranges between 1 in 4854 and 1 in 2,438, based on data from different regional populations. In 2016, Italian Cystic Fibrosis Register Report 2010 was published and has reported the estimated prevalence of 7 per 100,000 residents in Italy. Among the Italian regions, prevalence is highly variable, from a minimum of 4.3 per 100,000 inhabitants in the Friuli-Venezia Giulia region, to a maximum of 10.2 per 100,000 inhabitants in the Basilicata region. Results of a 10-year CF-carrier screening program (1996-2006) on 57,999 subjects with no prior family history of CF, indicated an overall frequency in the general population of 1:31 (3.23%), as *a priori* risk for Italian northeastern populations. Studies on the relative frequency of CF mutations in Italy confirmed a wide genetic heterogeneity among the regions in Italy. In 2016, the Italian Society for CF further published the frequency of mutations found in 4094 CF patients, and marked AF508mutation to be present in 45.1% of the samples. The reported frequency of N1303K mutation is very high in Italy (5.3%) and among the highest reported in Europe. Other recorded mutations had a frequency of 5% and lower (G542x 5.0%, 2789+5G->A 3.0%, 2183 AA->G 2.0%, W1282X 2.0%).

Genetic test for CF can be performed for diagnostic and for screening purposes. Carrier screening can detect over 88% of carriers with a 25 mutation panel. Several screening strategies have been evaluated in the literature and screening options can be characterized by different timing, model and place of screening. With *preconception screening*, to a carrier couples who are planning a pregnancy is offered a range of reproduction options including, refraining from having (more) children, adoption, accepting the possibility of giving birth to a child with CF, having prenatal diagnosis possibly followed by abortion of an affected foetus and pre-implantation diagnosis. With *prenatal screening*, pregnant women and their partners are screened as early as possible in their pregnancy. Should both parents be carriers, couples can choose to test the foetus through chorionic villus sampling (CVS) or amniocentesis. If the foetus is affected, to parents are proposed the continuing of pregnancy or abortion.

There are no systematic data on the provision of genetic tests for CF in Italy. The Italian National Health Institute (Istituto Superiore di Sanità) runs a quality control study on the genetic test for CF, but data have been incomplete. On the other hand, data from the Italian Human Genetic Society SIGU (Società Italiana Genetica Umana) suggest that CF is the most genetically tested disease in Italy. In most regions in Italy, CF genetic tests should be prescribed by a geneticist or after the indication of a CF specialist in order to be partially reimbursed by the National Health System. Panels including from around 50 to around 80 mutations are most widely used. Most tests are performed neonatally in the process of diagnosis after neonatal screening. However, as shown by the Veneto experience testing for carriers in the general population seems to gain popularity. There are no data however, on the provision of the genetic test to the persons with no increased risk for CF.

Our experience about the genetic tests for CF was carried out from a summary of the ACCE (Analytic validity, Clinical validity, Clinical utility and Ethical, legal and social implications) model system reported in the “ACCE review of the population-based prenatal screening for cystic fibrosis via carrier testing”, in the USA. The ACCE project has been funded by the Center for Disease Control and Prevention (CDC) with the main aim to establish a model process, on DNA and related testing for disorders with a genetic component. It aims at providing the policy makers access to up-to-date and reliable information for decision making, also taking into account gaps in knowledge that will help to define future research agenda. Most screening trials have chosen forward dot-blot, reverse dot-blot, or amplification refractory mutation system (ARMSTM) technologies as a testing methodologies. In the United States, protocols promote CF carrier testing in the prenatal setting (carrier testing).

The report indicated a high Analytic validity, where after removing challenges involving dell507, the analytic sensitivity was 97.9% (95 percent CI 96.9 to 98.7%), and the analytic specificity was 99.4% (95 percent CI 98.7 to 99.8%). Regarding CF, in Italy, two major schemes for external quality assessment are yearly available: National EQA: “Controllo esterno di qualità dei test genetici” organized by the Italian National Health Institute (Istituto Superiore di Sanità, ISS) and the European EQA: Cystic Fibrosis External Quality Assessment organized by European Molecular Genetics Quality Network (EMQN).

The term clinical validity was proposed to describe the accuracy with which a genetic test identifies a particular clinical condition. Among the hypothetical cohort of 1,000,000 couples screened, there are 1600 carrier couples (1,000,000 * (1/25)^2). 77% of the 1600 carrier couples are detectable (1232), and 1181 of these are detected (1,232*9792). Among the 998,400 non-carrier couples, 76,800 will include one carrier partner, and, in six of these couples, a false positive result will occur in the non-carrier partner (76,800*88*979*0.0001).

In order to estimate the proportion of carrier couples (or affected fetuses) that can be identified for any given panel of mutations (clinical sensitivity), it is necessary to obtain the...
mutation frequencies in an unbiased sampling of individuals clinically affected with cystic fibrosis (i.e. from the Cystic Fibrosis Genetic Analysis Consortium Report or from the Cystic Fibrosis Foundation Database). Clinical specificity on the other hand, is a measure of how often couples who, because of their genetic makeup, cannot have a child with cystic fibrosis and may be incorrectly classified as a carrier couple (i.e., a mutation is reported in both partners).

Regarding the clinical utility of genetic tests for CF, three models of CF prenatal screening have been published and used, in patient care. The two-step or sequential model initially expects the collection and analysis of the pregnant sample. If a mutation is identified, the next steps require woman counselling and obtaining a sample from the partner. When mutation is also identified in the partner, the couple will then be provided with more intensive counselling. In the one-step model, samples are collected from both the pregnant woman and her partner at the outset. Analysis is then performed on the woman’s sample, and the partner’s sample is tested only if a mutation is identified in the woman. However, notification of a positive result is made only when both partners are found to be carrier of a mutation. In the modified one-step model, recommended by the American College of Medical Genetics, samples are collected from both partners at the outset and DNA testing is performed on both samples and the notification with counselling is made when a mutation is found in either partner.

We synthesized the economic evaluation of different scenarios from the literature, but still there is a lack of analyses in the context of the Italian National Health Service. Results from 13 studies obtained, vary significantly in terms of outcome measure and incremental cost-effectiveness ratio (ICER) used in cost-effectiveness analyses (CEA). However, results can be generally considered at least as cost-effective both for preconception screening and prenatal screening. The only cost-utility analysis (CUA) included in our review reported a surely cost-effective results with cost per QALY of €16,572 for prenatal screening. Two out of three cost-benefit analyses (CBA) reviewed reported cost-savings results for preconception screening, as well as three out of six CBA for prenatal screening. The reported cost of a screening test ranged from €25 to €212. The cost of fetal diagnosis ranged from €220 to €1,873. Estimated lifetime cost of care for CF patients ranged from €291,048 to €1,105,452.

Based on person-centred approach, our ethical evaluation concluded that the use of genetic tests for the detection of CF healthy carriers is an advantage in terms of the acquisition of knowledge and of responsible management of choices, but at the same time raises many ethical questions. Even if knowledge is in itself good, it cannot be excluded that a genetic test on CF healthy carriers can lead to psychosocial harms (fear of stigmatization and discrimination because of one’s genetic state). The issue becomes even more complex when the genetic test is included in a screening program for CF healthy carriers. Besides the need to ensure equal opportunities in the access to screening, it is essential to also consider the management of the information in relation to the ethical difference between the different options offered to the carrier couples who are planning a pregnancy, the acquisition of free consent and the confidentiality of the obtained results.

Social considerations reported an overall positive attitude toward population CF carrier screening among patients and their families in the literature. In Italy, on the other hand results from a survey of 1,006 individuals (52% females) representing the Italian population aged ≥15 years, highlighted that the public awareness of CF is still poor, and jeopardize any assessment of the CF carrier screening issue made by an uninformed public.

The European CF Society produced a consensus document on standards for efficacious, safe and ethical practice of CF carrier screening, and suggested beside several general recommendations that the decision whether to implement it or not should be left to individual countries or regions in accordance with local legislation. The Italian CF Society (Società Italiana per lo studio della Fibrosi cistica, SIFC) has also produced the statement on CF carrier screening, endorsed by the Italian Human Genetics Society (Società Italiana Genetica Umana, SIGU), the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica, SIBIOC) and the Italian Society of Respiratory Medicine (Società Italiana di Medicina Respiratoria, SIMER) highlighting the necessity to eliminate barriers to the access to the CF carrier test and encourages the implementation of pilot CF carrier screening programs.

In conclusion, although the quality of the evidence in this area is still scarce especially from the Italian context and does not allows firm recommendations, the multidisciplinary approach of our HTA report provided an evidence-based evaluation of the genetic tests for CF carrier screening and offers a scientific basis for the decision-makers to consider the implementation of screening for CF carriers into the Italian health care system. Our investigation of the real-world practice specially underlines the need for additional economic studies taking into consideration the costs of screening by using new next-generation sequencing techniques, the new orphan medicinal products modulating CFTR synthesis and function, and the continuous improvement of life-expectancy that will add to the costs. Also, morbidity in CF adult disease is more prominent in terms of pulmonary exacerbations and systemic complications and demands for a greater economic burden.

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