

Prenatal screening of Cystic Fibrosis: a single centre experience

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Summary

The gene responsible for the pathogenesis of cystic fibrosis has been known for over 15 years (1, 2). Cystic fibrosis is the most common autosomal recessive disease in the European population (3). More than 1500 mutations and a large number of polymorphisms have been identified so far from 1989. In the past 10 years we examined in our centre 25393 fetuses. The exams brought to the identification of 922 heterozygous and 9 homozygous for the mutation. The frequency of heterozygous in the examined sample was 1/27,5 while that of the affected was 1/2821. We carried out the ex-

amination of the most frequent mutations which enable, according to the literature data, the identification of almost 80% of the affected alleles (4-8).

Introduction

The cystic fibrosis gene encompasses more than 250.000 bp and contains 27 exons. The cystic fibrosis transmembrane conductance regulator (CFTR) gene is located on chromosome 7 and encodes for an ion channel protein of 1.480 aminoacids which mediates chloride conductance across biological membranes. Not all of the sequence variations or mutations determine the clinical manifestation of the disease, while a certain number of nucleotidic changes are associated with atypical forms that in many cases are not defined in terms of their pathogenetic role.

The frequency of mutations is variable in terms of geographical distribution of the populations and several mutations have been found to be more frequent in some populations compared to others. The most frequently observed mutation is the $\Delta F508$, which is more common in the north of Europe (4, 5), and shows in Italy an estimated frequency of around 51% (5-11).

The first level genetic analysis allows a very quick detection of the most frequent mutations in the population through the use of techniques which are based upon commercially available kits. In Italy the commercial kits allow at present time the identification of more than thirty mutations with a detection rate estimated at almost 80% (4-8). We have examined 25.393 samples which were collected through invasive procedures such as amniocentesis (92%) and villocentesis (8%). The almost totality of patients that asked for the analysis of the most frequent mutations affecting the cystic fibrosis gene did not have any family history for cystic fibrosis, thus, with some approximation, the sample can be considered as random.

Methods

Analysis Method: DNA extraction and amplification of the CFTR gene through PCR Multiplex. Amplifications were performed of exons 2, 3, 7, 9, 10, 11, 13, 17a, 17b, 19, 20, 21 and of introns 1, 3, 4, 5, 8, 10, 11, 12, 14b, 16, 17a, 19. Reverse dot blot hybridization: Innogenetics N.V. Technologiepark, 6 Gent-Belgium. The following mutations have been examined: $\Delta F508$, $\Delta F507$, 1717 -1G-A, G542X, R553X, R560T, W1282X, 3905insT, N1303K, 711+1G-T, 1898-1G-A, 3120+1G-A, 394delTT, G85E, 621+1G-T, R117H, 1078delT, R347P, R334W, 2183AA -G, 2184delA, 2789 + 5G-A, R1162X, 3659delC, 3849 + 10kbC -T, A455E, S549N, S549R,

G551D, V520F, 3876delA, F508C, I506V, I507T. In the case a mutation was identified amongst those examined we further examined the sequences through the Italian Regional Mutation method of Innogenetics: 1259insA, 4016insT, 4382delA, 852del22, D759G, G1244E, G1349D, I502T, L1065P, R1158X, T338I, S549R(A-C), 991del5, D1152H, 1898+3A-G, R1070Q, R1066H, R347H, 621+3A-G, E217G+R334Q (Innogenetics N.V. Technologiepark, 6 Gent-Belgium).

Results

The analyses conducted in our samples to identify the most frequent mutations in the cystic fibrosis gene were performed with the following commercial kits: Innolipa CFTR 19+ and Innolipa CFTR 17 (Innogenetics). We found only 16 relevant mutations amongst the more than 30 mutations examined. As expected, the most frequent mutation found in our analysis was the $\Delta F508$ mutation, which resulted in 63,19% of the mutant alleles, followed in order of frequency by the N1303K mutation (14,26%) and G542X (6,6%), and then by the other 13 (Table I).

The identification of any of the mutations examined in the first level genetic analysis supported the extension of the analysis to the search of regional mutations, reaching in some cases up to 54 mutations. The extension up to the analysis of 54 mutations did not reveal any additional mutation on top of those found previously. Also, we performed in few cases an extended analysis that considered up to 200 mutations, however failing to reveal any additional diagnostic information. In recent years it has been possible to increment the sensitivity of the test by including in the analysis deletions of the examined region, which are frequently associated to the clinical manifestation of the disease (12, 13), or applying methods such as DHPLC (Denaturing High Performance Liquid Chromatography) (14-16).

It is evident that mutations which are different from those obtained from our analysis must show a very low or non relevant frequency if compared to that associated with the mutations found in our sample.

Several studies have been performed throughout the years on patients that showed the classical symptoms of Cystic Fibrosis and who were positive to at least one of the three validated diagnostic tests (17, 18). These tests are represented by the sweat test, the analysis of the transepithelial electric potential difference across the airway or intestinal epithelium (7, 8, 17-25). The genetic analysis is considered positive when at least two mutations that determine the disease are identified (7, 8, 17-19).

The genetic exams performed on affected patients in Italy (9-12, 14, 26-28) have revealed a quite consistent territorial heterogeneity together with a detection rate that, depending on the method and on the number of searched mutations, oscillates between 57% (9) and 90% (10-12). Our sample is constituted mostly by patients who come from the centre-south regions, whereas the number of samples from the northern regions of Italy can be considered irrelevant (less than 2%).

The genetic examinations performed on patients that manifested symptoms typical of cystic fibrosis and on samples from different regions of Italy enabled the identification of up to 90% of the mutations responsible for the disease (10-12). In our sample we identified 922 het-

erozygous and 9 homozygous affected fetuses. The frequency of heterozygous is 1/27,5 whereas that of the affected homozygous is 1/2821. The most frequent mutation, as expected, is the $\Delta F508$ found in 63,2% of cases. The $\Delta F508$ is by far the most frequent mutation (51%) but the incidence concerning the different mutations found for cystic fibrosis shows a regional variability that does not allow to easily predict their patterns and frequencies (6, 9-12). In central Italy, for example, the frequency is around 47,4%, in the north around 59,3% and in the south around 53,4% (9). The $\Delta F508$ mutation shows always to be the most frequent, nonetheless it also shows a high frequency variability in several countries where tests have been performed for the genetic characterization of the disease (5, 9-12, 29-35).

The second mutation, in terms of relative frequency, is the N1303K mutation showing a value of 14,26%, followed by G542X at 6,6%, W1282X at 3,1%, R553X at 2,6%, G85E at 2,4%, R1162X at 1,9%, G551D at 1,3%, R117H at 1,1%, the other mutations showing a much lower incidence, inferior to 1% (Table I).

The ten most frequent mutation cover 97,2% of all of the mutations detected in our sample. This result suggests that the remaining 20 examined mutations account only for 2,8% of the mutated chromosomes that we examined.

Table I - Mutations observed in the sample.

	n.	%
$\Delta F508$	594	63,2
N1303K	134	14,3
G542X	62	6,6
W1282X	29	3,1
R553X	24	2,6
G85E	23	2,4
R1162X	18	1,9
G551D	12	1,3
R117H	10	1,1
171 7 -1G->T	8	0,9
2184delA	6	0,6
621+1G->T	5	0,5
R347P	5	0,5
R560T	5	0,5
2183-AA->G	3	0,3
2789+5G->A	2	0,2
711 +1G->T	0	0,0
1078delT	0	0,0
R334W	0	0,0
A455E	0	0,0
$\Delta I507$	0	0,0
S549N	0	0,0
1898+1G->A	0	0,0
3659delC	0	0,0
3849+10kb C->T	0	0,0
3905insT	0	0,0
3120+1G->A	0	0,0
S549R	0	0,0
V520F	0	0,0
3876delA	0	0,0
F508C	0	0,0
394delTT	0	0,0
I506V	0	0,0
I507T	0	0,0
TOTAL	940	100,0

Table II - Composition of the homozygous fetuses.

ΔF508/cF508	3
ΔF508/G542X	2
ΔF508/W1282X	2
G542X/G542X	2
TOTAL	9

Discussion

On 25393 examined cases (50786 chromosomes), we have observed a total of 940 mutations which are distributed in 922 heterozygous and 9 homozygous affected. Therefore the frequency of the mutations in our samples is equal to $p=0,0185$, hence corresponding to a mutation every 54 chromosomes and, given the small proportion of mutations found in the homozygous, approximately to a carrier every 27,5 individuals.

The 95% confidence interval of the mutation frequency is comprised between 0,0173 and 0,0197. In other words we can state that, given a 95% confidence interval in the population sample we can detect a mutation every 51-58 chromosomes and a carrier (heterozygous) every 25,9-29,3 individuals.

The results agree fully with the results from previously cited studies, which have ascertained the frequency of carriers in the Italian population, estimated by neonatal screening programs to have an incidence between 1/2730 and 1/3170 newborns (7, 8, 17). From these studies we can calculate a frequency of carriers between 1/26 and 1/30. Such data suggests that the used diagnostic methodology probably has a diagnostic sensitivity which is superior to the estimated value of 80%. In more simple terms, if we hypothesize that the tests used in the analysis miss out about 20% of mutations, we would have found approximately a global frequency of mutations equal to 0,0231, which corresponds to a mutation every 43 chromosomes and approximately to a carrier every 22 individuals. The latter frequency appears by far much too high compared to the results obtained so far on the Italian population. There also would have been some non-identified pathological cases, a fact that at the moment we do not consider feasible. On a whole, 1153 fetuses should be carriers for a mutation, amongst the 1500 identified, in the CFTR gene and 13-14 should have been the affected homozygous fetuses.

The sample examined by us is in perfect agreement with the Hardy-Weimberg equilibrium, that needs not to be reached since affected patients often show reduced fertility (36, 37). This can be partly explained by the possible presence of de novo mutations which tend to maintain unaltered the allelic frequency.

Our work has shown the the rate of heterozygosity (1/27,5) agrees perfectly with that estimated for the Italian population. According to the literature data, which refer to the results obtained by the genetic examination of patients affected by cystic fibrosis, the rate of heterozygosity obtained by us appears substantially higher than what predicted. The detection rates of tests used in our work is generally accepted to be around 80%, that is to say that, based on the sensitivity of the diagnostic tests that were used, approximately 20% of the mutated chromosomes should have gone undetected. In practical terms, we should have found 1153 heterozygous individuals instead of 922, thus provoking an increment in the number of affected individuals. Such a scenario would have determined an heterozygosity rate of 1/22 individuals together with a rate of affected individuals of 1/1868, which is actually higher than the rate estimated by neonatal screening programs. Further studies on much larger random samples will certainly be useful to calculate the actual heterozygosity rate.

Our work can be considered one of the few studies performed on a substantially random and numerous sample, and, at the moment, the only one in Italy. Our results perhaps encourages us to hypothesize a correction of the detection rates of 1st level screening tests used in our centre and most commonly used for rapid and easily reproduceable answers. It is evident that a heterozygosity rate of 1/22 does not compare to any of the literature data. The rate of 1/27,5, found in our sample, appears to be very close to that estimated for the Italian population. Given the results of our study, it appears feasible that the actual sensitivity of the tests which are used can be much higher, and moreover a detection rate around 80% underestimates the true reliability of the 1st level test. One possibility is that rare or unfrequent mutation have an excessive consideration compared to the more frequent ones. Amongst the fifty or more mutations which have been examined only 16 of them have been detected at least once, while the remaining ones were never found in the analysis.

The results need to be confirmed throughout the years since the affected individuals can manifest a symptoma-

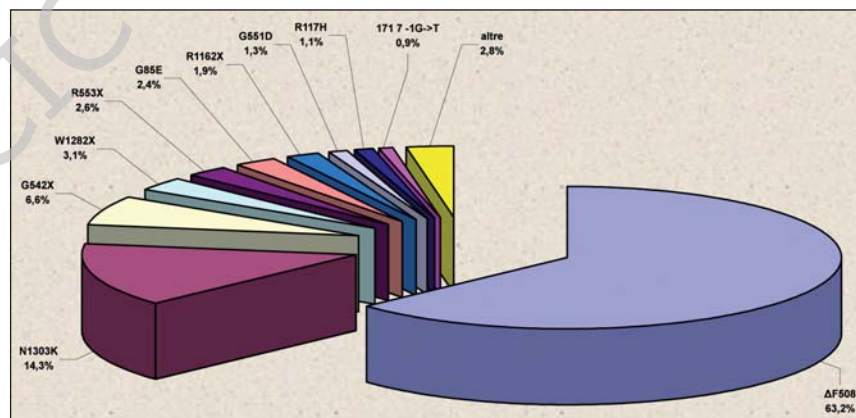


Figure 1 - Distribution of the cystic fibrosis mutations.

tology which does not enable to trace back to the disease some of the clinical manifestations typical of cystic fibrosis (38-43).

These results should also encourage new thoughts regarding the diagnostic validity of the most frequent panel of mutations amongst the Italian population, leading to the exclusion of never encountered mutations and the insertion of other more significant mutations. The actual sensitivity of these tests should be much higher; nonetheless, in order to define with certainty their diagnostic efficacy, we need to proceed in the collection of data throughout the next few years to confirm or not our results in this present work provided from the analysis of more than 25.000 fetuses.

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