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A Quality Control Study of CFTR Mutation Screening in 40 Different European Laboratories

The European Concerted Action on Cystic Fibrosis

Key Words
Cystic fibrosis
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Quality control

Abstract

A quality control study was performed to determine the accuracy of cystic fibrosis (CF) transmembrane conductance regulator (CFTR) mutation screening in 40 different genetic screening laboratories throughout Europe. A total of 9 different samples were investigated blindly by the participating laboratories. Only 25/40 laboratories, i.e. 62.5%, were able to type all samples correctly for the mutations for which they routinely screened. Only 2 of the 9 samples were correctly typed in all 40 laboratories. The lowest accuracy rate was 80% for 1 sample. 12.5% of the participating laboratories interpreted the F508C polymorphism as a true CF disease mutation and 12.5% interpreted the ΔI507 mutation as a ΔF508 mutation. For the ΔF508 mutation, a false-negative result of 3.75% was obtained. It is clear that the accuracy of CFTR typing should be improved.

Introduction

Cystic fibrosis (CF) is one of the most frequent lethal autosomal recessive diseases in the Caucasian population [1]. About 1 in 2,500 newborns in Europe have CF and about 10 million European citizens are carriers of a defective CFTR gene. Mutations in the CF transmembrane conductance regulator (CFTR) gene cause this disease [2–6; The Cystic Fibrosis Genetic Analysis Consortium]. So far, more than 500 different disease mutations have been described in this gene [4–6; The Cystic Fibrosis Genetic Analysis Consortium]. The existence of such a high number of

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different mutations has hampered the genetic screening of the CFTR gene for genetic counselling purposes. A quality control study was initiated by the European Concerted Action on Cystic Fibrosis, to establish the accuracy of routine CFTR mutation screening. To a lesser extent, such a study would provide information on which mutations are routinely screened for and the type of technology that is used in different countries for screening these CFTR mutations.

Methods

The Center for Human Genetics at Leuven in Belgium was assigned as a reference laboratory. A total of 69 different laboratories throughout Europe were informed about this study and asked if they wished to collaborate. Forty (58%) agreed to do so, and, an identification number was assigned to each. These laboratories then received the same 9 samples provided by the reference laboratory, coded CM10–CM18, which they were requested to screen for CFTR mutations using their routine technology. The mutations and polymorphisms in the CFTR genes of these samples were completely known, since the coding region and exon-intron junctions had been completely sequenced. It was decided to provide only samples in which the most frequent mutations would be represented, since it was expected that most laboratories would only screen for the most common CFTR mutations. The different participating laboratories were, however, not informed about this decision. At the same time, they also received a questionnaire to obtain information about the CFTR mutations for which they routinely screened, the technology that is used and the time that is required for this screening. The results of the screening and the completed questionnaire were then sent back to the reference laboratory for analysis. The data were compiled in a table, which was made available to every laboratory. In this table, the different laboratories were only identifiable by their code number. Each code number was only known to the laboratory to which it had been assigned. They were also informed about the genotypes that they should have obtained for these samples, i.e. CM10: ΔF508/G542X; CM11: ΔI507/wild; CM12: ΔF508/F508C-S1251N; CM13: W1282X/wild; CM14: ΔF508/N1303K; CM15: ΔF508/ΔF508; CM16: ΔF508/1717-1G→A; CM17: H_2O; CM18: wild/wild. Each participant was then asked to check if the results in the compiled table were in agreement with the data they had submitted. The laboratories that found a different genotype for 1 or more samples, 15 in total, were asked to comment on the discrepancy. They were also asked if they could retype the discrepant samples. For this retyping, no new sample was provided. Ten laboratories (67%) responded to this second request.

Results

Techniques Used for Identifying CFTR Mutations

The assays used by the different laboratories are shown in table 1. Most used a combination of different techniques. For those labs that used only one technique, this was either the amplification-refractory mutation system (ARMS) or reverse dot blot.

CFTR Mutations Routinely Screened

The 9 CFTR mutations most frequently screened are given in table 2. For the W1282X mutations, 3 of the 21 contributing laboratories that routinely screened for this mutation could, however, not exclude that the R1283M mutation was present in a positive sample, since their asssay could not discriminate between these two mutations [13].

The number of different mutations screened by each individual laboratory varied from 3 to more than 20. The latter always used indirect mutation detection assays such as denaturing gradient gel electrophoresis (DGGE) and single-strand conformation polymorphism (SSCP), but even then only a limited number of exons of the CFTR gene were routinely screened. However, the number of mutations that are actually routinely screened by some laboratories are underesti-

Table 1. Techniques used for screening CFTR mutations

Assay	Number of laboratories
Restriction enzyme analysis	26
Amplification-refractory mutation system [7]	25
Heteroduplex analysis [8]	11
Reverse dot blot [9]	7
Allele-specific oligonucleotides	6
Sequencing	4
Denaturing/temperature gradient gel electrophoresis [10]	3
Single-strand conformation polymorphism [11]	3
Restriction-site-generating-polymerase chain reaction (RG-PCR) [12]	1
Others (not specified)	2

For the more unfamiliar techniques, only one reference is given. It is possible, however, that a modified assay was used by some of the contributing laboratories.

mated. Indeed, some which only screened a limited number of mutations in this study, where only isolated DNA samples were provided, would actually screen for more mutations depending on clinical and pedigree information for the individual under investigation.

Given the variety of techniques used, the time needed for genetic screening therefore varied between laboratories and these data were therefore not interpreted in detail. In general, the laboratories that screened for a limited number of mutations with a direct test could perform the screening in 2–3 days. Others, such as those that used a DGGE assay, had more complex screening protocols. Here, screening was performed in different phases, such as the analysis of the most frequent mutations in a first phase followed by a second phase screening for rarer mutations for those samples in which a frequent mutation could not be identified. Here, the screening could last for up to 2 weeks.

Table 2. Nine most frequent CFTR mutations that were routinely screened

Mutation	Number of laboratories that screened for the mutation
ΔF508	40
G551D	38
G542X	36
R553X	32
621 + 1G→T	26
ΔI507	22
W1282X/R1283M	21
N1303K	20
1717-1G→A	18

For the W1282X mutation, 3 of the laboratories that routinely screened for this mutation could not exclude that the R1283M mutation was present in a positive sample, since their assay could not discriminate between the W1282X and R1283M mutations [13].

Table 3. Number of incorrectly typed CFTR alleles made by the different laboratories

Number of alleles incorrectly typed	Number of laboratories	Number of laboratories that confirmed their data
0	25	
1	6	4
2	5	3
3	1	1
4	1	0
5	1	1
6	1	1

A positive result for the H_2O control was counted as one allele that was not correctly typed. This error occurred in 1 laboratory. All laboratories that confirmed their data also performed a reanalysis of the wrongly typed samples. Two laboratories found that the mistyping of 1 allele was due to an administrative mistake. For the remaining mistyped alleles, the expected allele was found after retyping.

Quality Control

Since the different mutations that were routinely screened differed between laboratories, it was possible that a particular mutation, present in the samples to be tested, was not screened by a particular laboratory. For this reason, the information on the mutations that were routinely screened was taken into consideration in the analysis of the deduced genotypes. In cases in which a mutation was not found because the relevant mutation was not screened, the deduced genotype was interpreted to be correct.

Scoring of the Different Laboratories

All samples were correctly typed in 25/40 (62.5%) laboratories (table 3). In 15 laboratories, at least 1 sample was clearly wrongly typed. All laboratories that confirmed their incorrect data also performed a reanalysis. Two laboratories found that the mistyping of 1 allele was due to an administrative mistake (error in reporting). For the remaining mistyped alleles, the expected allele was found after retyping. In 1 of the 6 laboratories that incorrectly typed 1 allele, the error occurred when a PAGE heteroduplex assay was used. Here, unfamiliar heteroduplexes were recognized, however they were incorrectly interpreted.

The number of alleles wrongly typed, including a positive result for the H_2O, was 6 for 1 laboratory, where an ARMS assay was used. The discrepancies were explained by the difference in concentration of DNA compared to the concentration of DNA they normally used. On retyping, using techniques other than their ARMS assay, the expected results were obtained. This laboratory will be referred to as laboratory A.

In another laboratory, 5 alleles were wrongly typed. This was explained by the fact that they used a nonoptimized SSCP assay. Retyping of the samples with their optimized SSCP method clearly identified all genotypes correctly. This laboratory will be referred to as laboratory B.

Scoring of the Different Samples

Two samples were correctly typed in all 40 laboratories: CM15 (ΔF508/ΔF508) and CM18 (wild/wild).

Sample CM17, which only contained H_2O, was correctly typed in 39/40 laboratories. A ΔF508 heterozygote was concluded in laboratory A. Retyping of the sample there gave the expected negative result, and contamination of this sample can therefore be excluded. In a second laboratory, a ΔF508 heterozygote was initially concluded on the basis of a heteroduplex assay for the ΔF508 mutation. However,

Table 4. Results of typing the different samples

Sample	Allele 1	Allele 2	Number of laboratories	Comment from the respective laboratories
CM10	ΔF508	G542X	31	
(ΔF508/G542X)	ΔF508	–	2	
	ΔF508	R553X	2	no answer
	ΔF508	not found	1	retyping gave the expected result
	Not found	G542X	2	no answer from 1 lab, administrative mistake in the other
	Not found	not found	1	retyping gave the expected result
	H_2O		1	retyping gave the expected result
CM11	ΔI507	wild	20	
(ΔI507/wild)	–	wild	11	
	ΔI507 (possible)	wild	1	no answer
	ΔF508	wild	5	3 labs: retyping gave the expected result; 2 labs: their assay does not discriminate between ΔF508 and ΔI507
	Not found	wild	1	retyping gave the expected, weakly positive, result
	ΔI507	ΔF508	1	no answer
	ΔI507	mutation in exon 21?	1	retyping gave the expected result
CM12	ΔF508	F508C	4	
(ΔF508/F508C-S1251N)	ΔF508	–	26	
	ΔF508	exon 10 variant	1	
	ΔF508	strange heteroduplex	1	
	ΔF508	faint wild	1	
	Deletion	–	1	
	ΔF508	ΔI507	2	1 lab: ARMS test is not optimized; second lab: retyping gave the expected result
	ΔF508	ΔI507 (strange heteroduplex)	1	no answer
	ΔF508	ΔF508	1	retyping gave the expected result
	R553X	ΔI507	1	no answer
	Not found	F508C	1	administrative mistake
CM13	W1282X	wild	15	
(W1282X/wild)	–	wild	19	
	W1282X (or R1283M)	wild	3	
	W1282X	mutation in exon 21?	1	retyping gave the expected result
	W1282X	faint ΔF508 and S1251N	1	no answer
	Not found	wild	1	no answer
CM14	ΔF508	N1303K	17	
(ΔF508/N1303K)	ΔF508	–	20	
	Not found	N1303K	1	retyping gave the expected result
	ΔF508	not found	2	1 lab: no answer; second lab: retyping gave the expected result
CM16	ΔF508	1717-1G→A	17	
(ΔF508/1717-1G→A)	ΔF508	–	20	
	ΔF508	not found	2	1 lab: no answer; second lab: retyping gave the expected result
	Not found	–	1	retyping gave the expected result

For CM12, the results of the screening of S1251N are not included in this table. The samples CM15 (ΔF508/ΔF508) and CM18 (wild/wild) were correctly typed in all 40 laboratories. Sample CM17 (H_2O) was typed as a ΔF508 heterozygote in 1 laboratory. – = No screening for this mutation.

subsequent analysis of other mutations in the blind trial, and even of the ΔF508 mutation by a different assay in this CM17 sample, showed no amplification.

Sample CM10 (ΔF508/G542X) was correctly typed in 33/40 laboratories, i.e. 82.5% (table 4). Two laboratories found R553X instead of G542X, for which no comment was given. In 1 laboratory, this sample failed to be amplified in the first screening. After retyping, the correct result was clearly obtained when the sample was diluted 1 in 50. The failure of amplification in the blind trial was explained by the fact that they only dealt with cells from cheek scrapings and therefore had no experience with concentrated DNA samples. Nevertheless, they were able to type the other 8 (concentrated) samples correctly. Laboratory B was able to find the ΔF508 but not the G542X allele. Retyping of the sample with their optimized SSCP method clearly identified the G542X allele. Two laboratories could find the G542X but not the ΔF508 allele. One laboratory explained the error through an administrative mistake. Here the ΔF508 allele was recognized; however, the result was misrepresented on the list summarizing the data of the CM samples. No answer was obtained from the second laboratory. Finally, laboratory A found neither ΔF508 nor G542X with their ARMS assay. Analysis of this sample with other techniques gave the expected result.

Sample CM11 (ΔI507/wild) was correctly typed in 32/40 laboratories, i.e. 80% (table 4). In one, the ΔI507 allele was described as a possible ΔI507. This result was probably based on a PAGE heteroduplex assay in which unfamiliar heteroduplexes were observed. Five laboratories interpreted the ΔI507 allele as a ΔF508 allele. One of these laboratories used a heteroduplex assay in which very faint heteroduplex bands were obtained, such that they were not able to detect the characteristic ΔI507/wild heteroduplexes. The second laboratory used a dot blot assay in which the oligonucleotide probe for the ΔF508 mutation unfortunately also detected the ΔI507 mutation; however, the signal obtained with the ΔF508 oligonucleotide probe compared to that obtained with the wild-type oligonucleotide probe was much weaker. Both laboratories found the expected results after retyping. The results in two other laboratories were based on a PAGE heteroduplex assay which did not, at least in these cases, discriminate between the ΔF508 and ΔI507 mutations. The fifth laboratory was laboratory A where, after retyping, the correct genotype was obtained. In one laboratory, the ΔI507 allele was not found. After retyping, a weak positive result was obtained. Here, the ΔI507 mutation was screened with a ΔI507/ΔF508 ARMS test. Comparison with previous results of other samples led them to conclude that the ΔI507 mutation was missed due to a decreased sensitivity of the kit after prolonged (1.5 years) storage. In 1 laboratory, the ΔI507/wild sample was interpreted as a ΔI507/ΔF508 sample; no comment was received on the discrepancy. Finally, laboratory B observed a different SSCP pattern for a fragment that contained exon 21 of the CFTR gene, compared to control samples. Since exon 21 and its exon-intron junctions were completely sequenced, the presence of a polymorphism can be excluded. Moreover, it was stated that this analysis was performed by means of SSCP which at that time was still not completely optimized. Retyping of the sample with an optimized SSCP method clearly identified no aberrant SSCP pattern for this fragment.

Sample CM12 carried the ΔF508 mutation on one allele and the S1251N mutation on its second allele. The S1251N mutation also carried F508C, which is a known polymorphism. The main interest of this sample was to inves-

tigate if the F508C polymorphism could be misinterpreted as a true CF disease mutation. For this reason, the data will focus on the ΔF508 and F508C mutations (table 4). Nevertheless, this sample was screened for the S1251N mutation by 4 contributing groups, which were all able to detect it. With regard to the ΔF508 and F508C mutations, the samples were correctly scored in 34/40 contributing laboratories, i.e. 85%. Four were able to detect the F508C mutation itself. One recognized a strange heteroduplex in a PAGE heteroduplex assay. One defined the presence of a variant, although they could not define it, and this was most probably based on the presence of unfamiliar heteroduplexes in a PAGE heteroduplex assay. One recognized that the wild-type allele gave a weaker signal than expected which would also indicate the presence of a variant, but it was not interpreted as such. Finally, one scored the sample as heterozygous for a deletion. Unfamiliar heteroduplexes in a PAGE heteroduplex assay most probably prevented this laboratory from defining the actual deletion mutation. Five of the 40 contributing laboratories interpreted the F508C mutation as a true CF disease mutation. Three interpreted the samples to be derived from compound heterozygotes for the ΔF508 and ΔI507 mutations. In the first, the test was based on a PAGE heteroduplex assay, where strange heteroduplexes were nevertheless recognized. No comment was given by them. In the second laboratory, a ΔI507/ΔF508 ARMS test was used. A possible explanation for this false-positive result, proposed by them, was the relative lack of specificity of the primer which might detect not only ΔI507 and ΔF508, but also F508C. The third laboratory which concluded a ΔF508/ΔI507 genotype was laboratory B. Again, retyping of the sample with their optimized SSCP method clearly identified the expected genotype. One laboratory interpreted the sample to be derived from a ΔF508 homozygote, on the basis of an allele-specific oligonucleotide (ASO) test. In this test, a strong signal was obtained with the oligonucleotide probe that detected the ΔF508 mutation, like a ΔF508 homozygote, but a weak but definite signal was also obtained with the oligonucleotide probe that detected the wild-type allele. At that time, the CM12 sample was typed as ΔF508/ΔF508. Retyping with temperature gradient gel electrophoresis (TGGE) gave the expected genotype. Although this type of error occurred only once in this study, one of the laboratories, which typed the CM12 sample correctly, did comment that they had erroneously typed a ΔF508/F508C-S1251N patient as a ΔF508 homozygote in the past with an ASO assay. The final laboratory typed the sample as R553X/ΔI507, for which no comment was provided. Finally, a remarkable result was obtained in one laboratory that did not recognize the ΔF508 allele, but did detect the F508C allele. They commented that the ΔF508 was in fact detected during their first analysis, but was not reported due to an administrative mistake.

Sample CM13 (W1282X/wild) was correctly typed in 37/40 laboratories, i.e. 92.5% (table 4). However, 3 could not exclude that the mutant allele carried R1283M, since their assays could not discriminate between these mutations. One laboratory did not find the W1282X allele, for which no comment was given. A second laboratory obtained a very faint ΔF508 and S1251N signal, besides W1282X, for which no comment was given. Finally, laboratory B again observed a different SSCP pattern for a fragment that contained exon 21 of CFTR, compared to control samples.

Sample CM14 (ΔF508/N1303K) was correctly typed in 37/40 contributing laboratories, i.e. 92.5 % (table 4). Laboratory A failed to detect the ΔF508 mutation. Retyping of

this sample with other techniques gave the expected result. Two laboratories failed to detect the N1303K mutation. One of them was laboratory B, which obtained the expected genotype after retyping. The second laboratory did not comment on their discrepancy.

Sample CM16 (ΔF508/1717-1G→A) was correctly typed in 37/40 laboratories i.e. 92.5% (table 4). In two laboratories, the 1717-1G→A mutation was not detected. One of these used SSCP. No comment was received for the failure to detect the 1717-1G→A mutation. The second laboratory used an ARMS assay. Retyping of the sample resulted in the identification of the 1717-1G→A mutation. The failure to detect this mutation in the first screening was explained by a nonoptimal extension temperature in the ARMS reaction, which, however, did not prevent a signal being obtained for the positive control. Finally, in laboratory A, the ΔF508 mutation was not detected.

Discussion

The aim of this study was to investigate the mode of screening of CFTR mutations among different laboratories throughout Europe. The main interests were: (1) which CFTR mutations are routinely screened; (2) what techniques are used for this screening and (3) more important, how accurate is this screening?

Much care had to be taken in preparating and distributing the samples, so that every laboratory received the correct samples, free of contamination. As a control, all laboratories who found a different genotype for one or more samples were asked to retype the discrepant samples. From all the laboratories that retyped the original samples, 10 out of 15 (67%) found the expected genotype. This indicated that they indeed received the correct samples and that these were free of contamination.

Most laboratories only screened for the most common CFTR mutations. Even the limited number that made use of more laborious techniques, such as DGGE/TGGE and SSCP, only analyzed a limited number of exons and their exon-intron junctions.

The techniques used were quite variable. Errors in typing were observed when the ARMS, SSCP, heteroduplex, or ASO assay was used (assays given in decreasing order of error rate). However, it should be noted that 6 of the alleles wrongly typed with an ARMS assay occurred in a single laboratory and 5 of the alleles wrongly typed with a SSCP assay occurred in another single laboratory. Some assays were used more frequently than others (table 1), and thus the chance that a particular error will be observed with them will be higher. Finally, it was not always possible to identify the assay in which a particular error occurred. For these reasons, it is impossible to draw significant conclusions about the accuracy rate of the different assays.

Only 25/40 laboratories, i.e. 62.5%, typed all samples correctly, and only 2 of the 9 samples, i.e. 22%, were correctly typed in all 40 laboratories. Five laboratories (12.5%) actually typed the F508C polymorphism as a CF disease mutation and 5 other (12.5%) interpreted the ΔI507 mutation as a ΔF508 mutation.

This incorrect result was not always due to technical reasons. In 2 cases, there was an administrative mistake. Such an error is nevertheless important. If the most common CFTR mutation ΔF508, present on 6/16 alleles analyzed, was investigated individually, it was not found on 9/240 tested ΔF508 CFTR alleles. This would result in a false-negative result for the ΔF508 mutation of 3.75%. However, 3/9 mistypings occurred in the same laboratory, and this false-negative rate could,

therefore, be an overestimate. Nevertheless, the percentages of correctly typed samples were much lower than expected, especially since only the most common CFTR mutations were included in this study, which are well known to the CF community. It is clear that the accuracy of CFTR typing should be improved.

Since only isolated samples were provided, it is possible that some of these incorrect typings would have been recognized by the laboratories themselves when relatives of these individuals, from whom the DNA samples were obtained, had been investigated. Indeed, in most cases when CF patients, or their relatives, are analyzed, DNA of relatives, such as the parents, are available. However, for carrier screening programs [14], most people who want know their carrier status will present individually, and for these purposes our study will be quite representative.

While the results of this quality control investigation are somewhat disappointing, it is clear, however, that those who participated have been able to identify technical or procedural problems in their laboratories and that their testing services will have been improved by this experience. That such a quality control raises the standards of molecular genetic analysis is proven by the British experience. In the United Kingdom, such quality control testing has been operational for more than 3 years. For this reason, these laboratories seem to be more accustomed to the idea of quality control, because 21/40 laboratories (52.5%), that courageously submitted to the test, were of British origin. Of the 34 wrongly typed CFTR alleles in this study, only 7 occurred in laboratories from the United Kingdom: 1 laboratory wrongly typed sample CM10 as ΔF508/R553X and the CM16 sample as ΔF508/wild, for which no comment was given; a second wrongly typed sample CM10 as G542X/wild, for which no comment was given; a third typed sample CM10 as water because the DNA in this sample was too concentrated and they therefore did not obtain any amplification; a fourth typed sample CM11 as ΔF508/wild because their heteroduplex test could not discriminate between the ΔF508 and ΔI507 mutations, and finally, the fifth typed sample CM12 as ΔF508/ΔI507, although strange heteroduplexes were obtained in their heteroduplex test. A significantly lower error rate of 1.96% (95% confidence interval (CI): 0.49–3.43%) was observed in the United Kingdom laboratories compared to 8.36% (95% CI: 5.28–11.44%) in laboratories on the European continent, which is believed to be a direct consequence of their participation in the quality control scheme of the United Kingdom. It should be noted, however, that a significantly lower average number of CFTR mutations were routinely screened in these laboratories: 6.90 (95% CI: 5.27–8.53) compared to more than 12.32 (95% CI: 9.75–14.89) on the European continent.

A second round of quality control will have to be set up in the future to confirm an improvement in quality. Moreover, this type of quality control should eventually be implemented on a routine basis in diagnostic laboratories. In the meantime, we would like to acknowledge the genuine interest of all participants in this trial and the constructive atmosphere in which all steps of the procedure could be taken.

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Appendix

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