SHORT REPORT

Frequency of large CFTR gene rearrangements in Italian CF patients

Cristina Bombieri^{*,1}, Alberto Bonizzato², Carlo Castellani², Baroukh M Assael² and Pier Franco Pignatti¹

¹Section of Biology and Genetics, University of Verona, Italy; ²Cystic Fibrosis Center, Hospital of Verona, Italy

In most populations, an appreciable fraction of cystic fibrosis transmembrane regulator (CFTR) gene mutations in patients affected by cystic fibrosis (CF) cannot be identified, and large gene rearrangements might be missed by standard analyses. We have searched large gene rearrangements in a sample of 25 North East Italian CF patients who, after an extensive gene analysis of 188 patients, still bear one or two unidentified CF mutations. A systematic gene screening by quantitative multiplex PCR of short fluorescent fragments was performed. Overall, 5/26 (19.2%) rearranged alleles were detected, bearing mutation 3120 + 1Kbdel8.6Kb (three patients), and c.4_IVS1 + 69del119bpins299bp (two patients). These mutations were observed in compound heterozygotes with F508del or termination mutations, and a pancreatic insufficient form of CF. These findings confirm the frequency of CFTR gene rearrangements recently observed in French CF patients.

European Journal of Human Genetics (2005) 13, 687–689. doi:10.1038/sj.ejhg.5201387

Published online 2 March 2005

Keywords: CF; CFTR; QMPSPF; gene rearrangements; deletions; mutation detection

Introduction

Since the identification of the cystic fibrosis transmembrane regulator (CFTR) gene in 1989, many mutations have been identified in cystic fibrosis (CF) patients. However, a significant percentage of the CF alleles still remain unidentified in most of the studied populations, even after extensive studies of the CFTR gene. It was proposed that the presence of large genomic rearrangements in this gene could account for part of the disease-related alleles that remain unidentified. These mutations, if present at the heterozygous state, elude the common PCR-based techniques for mutation detection, including direct sequencing, and denaturing high-performance liquid chromatography (DHPLC).

In a recent article,¹ the authors reported the first systematic screening of the CFTR gene for the presence of large genomic rearrangements in CF patients. They used a recently introduced technique, the quantitative multiplex PCR of short fluorescent fragments (QMPSPF),² developing the specific conditions for the analysis of the CFTR gene. By applying this method, they resolved a significant fraction of the previously uncharacterised French CF alleles. To the best of our knowledge, no other specific study to search for rearrangements in this gene has been published, and the few previously reported large CFTR gene rearrangements were fortuitously identified.

Therefore, it would be important to determine if that¹ was just an observation limited to that population sample,¹ or if, on the contrary, it is more commonly found also in other populations.

Here, we report the results of our screening for CFTR gene rearrangements, performed on North East Italian CF patients, which confirm the data obtained on the French CF patients.

*Correspondence: Dr C Bombieri, Section of Biology and Genetics, Department of Mother and Child, and of Biology-Genetics; University of Verona; Strada Le Grazie, 8, Verona 37134, Italy.

Tel: +39 045 8027207; Fax: +39 045 8027180;

E-mail: cristina.bombieri@medgen.univr.it

Received 14 September 2004; revised 11 January 2005; accepted 19 January 2005

Subjects and methods

A sample of 188 North East Italian CF patients (for a total of 375 alleles, one allele being counted once in two cousins) was collected. The CFTR gene was extensively analysed in these patients to detect CF mutations by DGGE or DHPLC analysis (Bonizzato *et al*,³ <http://spazioinwind.libero.it/laboratoricf>), in order to determine the CF mutation frequency in the population. In this study, DGGE/DHPLC screening showed a mutation detection rate of 93% (349/375 alleles). In fact, after this study, 24 patients still bore one unidentified CF allele, and one patient had both alleles carrying unidentified CF mutations, for a total of 26 (7%) unidentified CF alleles. All patients had a classical form of CF with typical pulmonary and/or gastrointestinal findings, and positive or borderline sweat test.

The 27 exons of the CFTR gene were screened in these patients with the QMPFSF protocol as described by Audrezet *et al*.¹ QMPFSF is a semiquantitative PCR assay based on the simultaneous amplification of multiple short target sequences, followed by the rapid and reliable quantification of each amplicon. DNAs from all patients were amplified using six multiplex PCRs, designed to amplify with fluorescent primers (6-FAM) the 27 exons of the CFTR gene. Amplified DNA fragments were separated on ABI PRISM 310 DNA sequencer and analysed with Genescan software 3.1 (Applied Biosystems). Peak heights of the amplicons obtained from each patient were normalised with a reference gene (exon 2 of hemochromatosis (HFE) gene), and then compared with those generated from a normal control. A two-fold reduction in the height of a peak indicates the deletion of the corresponding exon.

Results

Overall, 5/26 (19.2%) rearranged alleles were detected, as shown in Table 1. Taking into account the total number of CF alleles of the sample, 5/375 (1.33%) alleles had a large gene rearrangement. Two different rearrangements were found in five patients. Mutation 3120+1Kbdel8.6Kb was found in three patients, and it was confirmed using a specific PCR assay, with primers from both sides of the breakpoint junction (as described by Lerer *et al*⁴).

Mutation c.4_IVS1+69del119bpins299bp was found in two patients, and it was confirmed using a specific PCR

assay with primers from both sides of the breakpoint junction (Audrezet, personal communication).

The name of these two mutations is the same adopted by the groups that first described each rearrangement. A different nomenclature and CFTR gene numbering were used by the two groups. In the first case,⁴ the current CFTR gene numbering (being the A of the ATG translation start codon numbered +133) was used. In the second case,¹ the nomenclature is in accordance with the Human Genome Variation Society (HGVS) recommendations,⁵ with the A of the ATG translation start codon numbered +1.

In all patients, the rearrangements are present in compound heterozygosity with a common CF mutation (F508del, G542X, or Q552X). All patients are pancreatic insufficient with a positive sweat test. FEV1 is variable in carriers of c.4_IVS1+69del119bpins299bp mutation: one patient (30 years old) had 27% predicted, while the other (8 years old) has 100%. Among carriers of 3120+1Kbdel8.6Kb mutation, FEV1 is available for only one patient (25 years old), who has 88% predicted.

Discussion

The first rearrangement (3120+1Kbdel8.6Kb) found in this study is a deletion of exons 17a–18, previously described in Palestinian Arab CF patients with a severe CF.⁴ This mutation removes about 8.6 kb of the CFTR gene, from the 3'-end of intron 16 to the 5'-side of intron 18, and was predicted to cause an in-frame deletion of 160 amino acids (from 997 to 1156), which are part of the transmembrane domains 10–12 of the CFTR.⁴ The 3120+1Kbdel8.6Kb rearrangement seems to be relatively frequent (0.8%), and therefore it is being considered for inclusion in the panel of the most frequent CF mutations utilised for diagnostic purposes in the Italian population.⁶

The second rearrangement (c.4_IVS1+69del119bpins299bp) is an ins/del mutation involving a deletion of 119 bp, which removes nearly the entire coding sequence of exon 1 (from nucleotide 4 to nucleotide 69 of intron 1), with the insertion of 229 bp, corresponding to part of the downstream sequence of intron 1, but with an inverted orientation.¹ This mutation was previously identified by QMPFSF analysis by the Audrezet *et al*¹ group in a French patient with a classical form of CF. It was predicted to

Table 1 Frequency of CFTR gene rearrangements found in Italian CF patients

Mutation ^a	Exons	Frequency of rearrangements in previously unidentified CF alleles	Frequency of rearrangements in the total of CF alleles
3120+1Kbdel8.6Kb	17a–18	3/26 (11.54%)	3/375 (0.80%)
c.4_IVS1+69del119bpins299bp	1	2/26 (7.69%)	2/375 (0.53%)
Total		5/26 (19.23%)	5/375 (1.33%)

^aSee 'Results' in the text for mutation nomenclature.

abolish the open reading frame, since the inserted sequence contains internal stop codons. This rearrangement is probably the same mutation that was reported in the CF Mutation Database⁷ with different names: 135del1120ins300 (Dork, Germany), and 136del119ins29 (Girondon, France), respectively. Therefore, this mutation could be a frequent rearrangement. It could be interesting to study this rearrangement in other populations, and to insert this mutation in the panel of common European CF mutations.

The findings of this study are in accordance with the results obtained by Audrezet *et al*¹ on the French population, where CFTR gene rearrangements accounted for 8/49 (16%) of the previously undetected CF alleles.

We agree with Audrezet *et al*¹ that QMPF is a rapid and reliable method to detect CFTR gene rearrangements, as it makes possible the analysis of the whole CFTR gene of one patient in 1 day. Taking into account the number and frequency of mutations detectable at each step, a three-step approach could be considered in CF mutation analysis. First, genotyping for common CF mutations with a commercial kit; if negative, the sample could be analysed by DHPLC. Those samples that still bear undetected alleles after this second step could be analysed by QMPF. In this way, it would be possible to maximise mutant allele detection rate in the reported populations, with evident implication for genetic counselling of CF patients and their families. Of course, the higher the mutation detection rate in the population, the higher is the possibility to detect both mutations in a CF patient, allowing a direct analysis of the family CF mutations for prenatal diagnosis and cascade screening in relatives of the CF patient.

Screening for large CFTR gene rearrangements should be extended to other populations in order to have a more general estimate of the type and frequency of these mutations, and to identify the presence of common rearrangements in a given population.

Acknowledgements

We acknowledge the financial contribution of the Italian Cystic Fibrosis Research Foundation. We are grateful to Professor C Ferec and his collaborators, MP Audrezet and O Raguene, for their help in performing this study.

References

- 1 Audrezet MP, Chen JM, Raguene O *et al*: Genomic rearrangements in the CFTR gene: extensive allelic heterogeneity and diverse mutational mechanisms. *Hum Mutat* 2004; **23**: 343–357.
- 2 Charbonnier F, Raux G, Wang Q *et al*: Detection of exon deletions and duplications of the mismatch repair genes in hereditary nonpolyposis colorectal cancer families using multiplex polymerase chain reaction of short fluorescent fragments. *Cancer Res* 2000; **60**: 2760–2763.
- 3 Bonizzato A, Bisceglia L, Marigo C *et al*: Analysis of the complete coding region of the CFTR gene in a cohort of CF patients from north-eastern Italy: identification of 90% of the mutations. *Hum Genet* 1995; **95**: 397–402.
- 4 Lerer I, Laufer-Chana A, Rivlin JR, Augarten A, Abeliovich D: A large deletion mutation in the CFTR gene (3120+1Kbdel8.6Kb): a founder mutation in the Palestinian Arabs. Mutation in brief no. 231 – Online. *Hum Mutat* 1999; **13**: 337.
- 5 Human Genome Variation Society, <http://www.hgvs.org>.
- 6 CF Mutation Database, <http://www.genet.sickkids.on.ca/cftr>.
- 7 Bombieri C, Pignatti PF: Cystic fibrosis mutation testing in Italy. *Genetic Testing* 2001; **5**: 229–233.