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CFTR mutations in the Algerian population

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Abstract

The nature and frequency of the major CFTR mutations in the North African population remain unclear, although a small number of CFTR mutation detection studies have been done in Algeria and Tunisia, showing largely European mutations such as F508del, G542X and N1303K, albeit at different frequencies, which presumably emerged via population admixture with Caucasians. Some unique mutations were identified in these populations. This is the first study that includes a genetic and clinical evaluation of CF patients living in Algeria. In order to offer an effective diagnostic service and to make accurate risk estimates, we decided to identify the CFTR mutations in 81 Algerian patients. We carried out D-HPLC, chemical-clamp denaturing gradient gel electrophoresis, multiplex amplification analysis of the *CFTR* gene and automated direct DNA sequencing. We identified 15 different mutations which account for 58.5% of the CF chromosomes. We used a quantitative PCR technique (quantitative multiplex PCR short fragment fluorescence analysis) to screen for deletion/duplication in the 27 exons of the gene. Taking advantage of the homogeneity of the sample, we report clinical features of homozygous CF patients. As CFTR mutations have been detected in males with infertility, 46 unrelated Algerian individuals with obstructive azoospermia were also investigated.

Keywords: Cystic fibrosis; Denaturing gradient gel electrophoresis; D-HPLC; Luminex™; ARMS™; QMPSF; Mutations; Polymorphisms; Algeria

1. Introduction

Since the cloning of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene [1,2], which is responsible for cystic fibrosis (CF), more than 1300 CF mutations have been reported by the members of the Cystic Fibrosis Genetic Analysis Consortium. There are marked variations in the frequency of some non-F508del CF mutations with respect to geographical location and ethnic group. In Algeria, no information is available about the incidence of CF and, in general, there are few data on the molecular basis of CF in the Maghrib, probably due to underdiagnosis [3–5]. F508del is found on only 20% of the CF chromosomes in Algeria [6] and Tunisia. Up to now, about 30 other mutations have been reported, most of which were previously described in other populations. Still, 3 mutations may be specific to the Algerian [7–9] population (A141D, L227R, and N1303H) and 2 to the Tunisian population (T665S and 2766del8).

In order to carry out an effective diagnostic service, we have undertaken a complete screening of the 27 exons of the *CFTR* gene in Algerian CF chromosomes by D-HPLC, denaturing gradient gel electrophoresis (DGGE) and QMPSF analysis. Taking advantage of the homogeneity of the sample, an evaluation of the most important clinical parameters is presented.

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2. Materials and methods

2.1. Sample composition

A total of 81 related and unrelated children from Algeria presenting a reminiscent clinic picture of cystic fibrosis were investigated. The patients were referred to us by different paediatric units of Algeria. Cystic fibrosis diagnosis was based on sweat tests: normal values are <40 mM; positive values are >60 mM. Borderline values are between 40 and 60 mM. The results were considered as being discordant when repeated sweat chloride analyses yielded inconsistent results. Sweat tests carried out at the ISSAD HASSANI hospital of Beni-Messous (Algiers) gave the following results: 36 patients showed a positive sweat test (with clinical pulmonary and/or gastrointestinal findings), 19 patients showed a discordant sweat test and 26 patients a normal sweat test affected by dilatation of bronchi and chronic obstructive pulmonary and digestive disease. Forty-six unrelated azoospermia men with normal FSH values and normal testicular volume were referred to us by the endocrinology unit of Mustapha Hospital (Algiers). We had no more clinical information.

2.2. Mutation analysis

Blood samples were collected from the affected children, their parents and azoospermia men. Genomic DNA was extracted from peripheral blood leucocytes by standard methods. All DNA samples were first screened for the F508del mutation as previously described [10].

The samples of 36 children, without identified CF mutations were analysed by D-HPLC, DGGE of the entire coding sequence and flanking intronic sequences as described elsewhere [11,12,8]. For D-HPLC analysis, we chose a Touchdown PCR protocol as already described [14] and by OLA assay [13]. We used a quantitative PCR technique (quantitative multiplex PCR short fragment fluorescence analysis) to screen for deletion/duplication in the 27 exons of the gene [15–19]. In addition to F508del, 30 mutations were screened in the 19 children with borderline sweat test and in the 26 children with normal sweat test, by means of the amplification refractory mutation system (ARMS™) using a commercial kit allowing the detection of 30 mutations in the *CFTR* gene [20] (ELUCIGENE™ CF 30).

Genomic DNA from the 46 obstructive azoospermia men was screened for 29 mutations by using Luminex™ technology based on fluorescent detection using a flow cytometer, microbeads dyed with multiple fluorescent colours and laser detection [21,22].We also analysed a DNA variant (the 5T allele) in a noncoding region of CFTR that causes reduced levels of the normal CFTR protein [23].

2.2.1. QMPSF analysis and molecular characterization of the genomic rearrangements

In the present study, we used the technique of quantitative multiplex PCR amplification of short fluorescent fragments (QMPSF) to resolve a significant fraction of previously uncharacterized CFTR alleles. Mutation detection and characterization were performed as previously described [15–19].

2.2.2. Automatic DNA sequencing

Each electrophoretically altered fragment detected by DGGE, D-HPLC was reamplified from genomic DNA. PCR products were purified on Microcon 100 columns (Millipore) and sequenced by Sanger dideoxy-mediated chain termination, using the ABI PRISM™ BigDye™ Terminator Cycle Sequencing Kit (PE Applied Biosystems) using walking primers. The sequences of rearranged PCR products were compared with the CFTR reference genomic sequence (GenBank Accession Number NT_007933.13 REGION: 42296307y42485805).

3. Results

Initially, the 3-bp deletion that results in deletion of a phenylalanine residue at position 508 of the CFTR protein (F508del) was detected on a non-denaturing 12% polyacrylamide gel.

3.1. Classic CF (N=36)

The allele frequency of the F508del mutation observed in the 72 CF chromosomes was 16.7% (12/72) (Table 1). Among the 60 CF chromosomes carrying an unidentified mutated allele, 14 different mutations were identified, as reported in Table 1: N1303K(6/72), 711+1G→T (6/72), V754M(1/72), 1812−1G→A(2/72), 2183AA/G(3/72), [4332delTG−621+3A/G](1/72), G542X(1/72), V562I(2/72), 1609delCA(2/72), 4271delC(1/72), W1282X(3/72), S977F(1/72), 21Kbdel(1/72).

Our screening of the entire coding sequence of the *CFTR* gene identified 9 DNA sequence variations shown in Table 2.

A female CF patient of Berber origin was a compound heterozygote V754M/1812−1G→A. She was admitted at the age of 3 years to the Department of Paediatrics (Ain Taya Hospital, East Algeria) for bronchiolitis, a high sweat chloride test (300 mEq/l) and sputum colonized by *Pseudomonas aeruginosa*. The V754M (G to A at position 2392) mutation has previously been reported to the Cystic Fibrosis Genetic Analysis Consortium by Roger Mountford and seems to confer moderate disease when it is associated either with 1812−1G→A or G542X.

Moreover, we have identified two complex mutations: R74W–D1270N in the mother of an Algerian F508del heterozygous CF patient, and none of these variations were inherited by the child. Using intragenic polymorphisms, we have confirmed that R74W and D1270N were not inherited by the F508del heterozygous CF child. This child has inherited the 2183AA/G maternal mutation which, when associated with F508del, confers a CF phenotype [24]. As this has also been reported in Tunisian CF patients [5], and