Molecular characterisation of cystic fibrosis patients in the state of São Paulo (Brazil)

To characterise the CF patient population in the state of São Paulo (Brazil), we estimated the frequency of mutations ΔF508, G542X, N1303K, G551D, and R553X, which are some of the most frequent mutations worldwide. Among the 20 most common CF chromosomes, the frequencies we obtained were as follows: ΔF508=31.7%, G542X=8.3%, N1303K=2.5%. The G551D and R553X mutations were not detected in this sample. The mutation detection rate, which was 92.5% based on the analysis of these five mutations. We conclude that further screening for more mutations will be necessary for molecular diagnosis and carrier detection because of the ethnic heterogeneity of this population. The present study represents a step towards the molecular characterisation of CF patients in the state of São Paulo.

Cystic fibrosis is the most common auto- somal recessive disease, with an incidence of 1 in 2000-3000 births in various groups. The CF gene was cloned in 1989. The major mutation causing CF is a three base pair deletion which results in the loss of phenylalanine in position 508 of the cystic fibrosis transmembrane conductance regulator (CFTR) protein. This mutation is found on about 70% of CF chromosomes worldwide and varies considerably between populations. The CF Gene Analysis Consortium has identified over 620 other sequence alterations in the CF gene. Most of these mutations are rare. Only a few mutant alleles have a worldwide frequency of 1% or greater, although they may occur at higher frequencies in selected populations.

Knowledge of the spectrum of CF gene mutations in different populations allows for the possibility of genetic testing. In Brazil, Raskin et al. and Martins et al. evaluated the frequency of ΔF508 in the state of São Paulo. Their results showed frequencies of 52% (60/116) and 31% (15/48), respectively. In view of these differing results and in order to contribute to the molecular characterisation of CF patients in the state of São Paulo, we have analysed the frequencies of CF mutations ΔF508, G542X, N1303K, G551D, and R535X.

Sixty unrelated CF patients regularly followed at the University (UNICAMP) Hospital in Campinas were studied. Blood samples were obtained from these patients, who had been diagnosed with CF on the basis of typical clinical manifestations or a positive sweat test (>60 mEq/l) or both. Genomic DNA was extracted from peripheral blood leucocytes according to standard protocols. DNA amplification was performed according to a general procedure. Primers and annealing temperatures used in each amplified region have been reported before.

Amplified samples from each subject were screened for the five previously mentioned mutations. The ΔF508 mutation was detected by heteroduplex DNA formation. The mutations G542X and N1303K were detected by digestion with BstNI and mutations G551D and R535X were detected by digestion with HincII followed by ASO hybridisation. This combination of methods was used because digestion with HincII by itself does not distinguish mutations G551D and R535X.

The frequencies of mutations ΔF508, G542X, and N1303K in the sample studied were 31.7%, 8.3%, and 2.5%, respectively. Mutations G551D and R535X were not detected.

The major objective of this study was to contribute to the molecular characterisation of CF patients in the state of São Paulo. The observed frequency of the ΔF508 mutation (31.7%) is consistent with the ethnic composition of the population, which is predominant of immigrants from Italy, Portugal, and Spain where the frequency of this mutation is lower than in the rest of the world (70%). The large result for ΔF508 differed significantly from that reported by Raskin et al. because it is in accord with that reported by Martins et al. (χ²=0.003, p<0.05). The sample studied by Raskin et al. might have been composed essentially of patients with more severe phenotypes, which are correlated with the ΔF508 mutation.

The frequencies of the next two most common mutations in the sample studied are also consistent with the above ethnic composition. The G542X mutation is fairly common throughout Europe, particularly southern Europe, and the N1303K mutation is common throughout Europe. Mutations G551D and R535X have higher frequencies in northern European countries, and for this reason they may be rare in the population of the state of São Paulo.

The most immediate application which results from determination of the spectrum of CF gene mutations in different populations is genetic testing. In the sample of CF chromosomes that we studied, 42.5% of them could be identified by the analysis of five mutations. Reaching a 90% detection rate in this population may require testing many additional low frequency mutations since São Paulo, like the whole of Brazil, is genetically very heterogeneous.

In conclusion, population molecular characterisation is an important aspect in the study of CF, but, as exemplified for the non-isolated population, it depends on the analysis of a great number of mutations. Our data should contribute further to the characterisation of CF in the state of São Paulo.

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