A novel 3600+11.5 kb C>G homozygous splicing mutation in a black African, consanguineous CF family

EDITOR—Cystic fibrosis (CF), a common autosomal recessive disease in white populations, appears to be rare in the black African population and very few cases have been reported. However, the disease is probably underdiagnosed and its frequency is still undetermined. Among the few mutations reported to date in the black African population, a first study identified the presence of the 3120+1 G>A mutation in five out of eight CF chromosomes, a mutation previously described in black American CF patients. The three other mutations identified in that study were ΔF508, G1249E, and 3196del54. More recently, a systematic screening of the 3120+1G>A transition performed in different populations of African origin led to a carrier frequency estimation of one out of 91 in southern Africa and one out of 315 in central Africa, while no carrier was detected in a population of 109 subjects originating from west Africa. The frequency of CF in African populations may therefore be higher than generally estimated, as observed in southern Africa. It is likely that many CFTR mutations remained undetected. We report here the identification of a new homozygous mutation in two children from a consanguineous Guinean family in association with an unusual phenotype.

The parents are first cousins and have had five children. The first child died after a dramatic episode of myocarditis. The second child, now aged 9, is healthy. The third child, a girl born at term, was admitted to our hospital because of failure to thrive and massive daily steatorrhoea with foul smelling stools, which had been noted by her mother for about six months. At 4 years of age, her weight was 12.2 kg (–2 SD) and her height 91 cm (–3.5 SD) with a poor nutritional status (BMI=14.7). She was then diagnosed as having CF on the basis of two independent abnormal sweat tests. In the first test, the chloride concentration measured on 119 mg of sweat was 105 mEq/l with a sodium concentration of 132 mEq/l. On the second sweat test done on 105 mg of sweat was 105 mEq/l with a sodium concentration of 95 mEq/l. At the age of 6 years, a colorectal biopsy was performed and a frameshift insertion of 214 bases was found in sputum from Pseudomonas aeruginosa 011 was found in sputum from P. aeruginosa. This insertion contained a stop codon. Alignment of the translation product clearly showed a frameshift insertion of 214 bases (fig 2, panel B). Direct sequencing of the amplification product of the 3501-4157 fragment of the cDNA which includes exons 18 to 21 (fig 2, panel B). Direct sequencing of the amplification product clearly showed a frameshift insertion of 214 bases at the junction of exons 18 and 19 (data not shown). This insertion contained a stop codon. Alignment of the gene was carried out on DNA samples from the two affected children. None of the 25 major CFTR mutations responsible for most of the CF chromosomes in white populations were detected. A complete sequencing of the 27 exons of the CFTR gene including the 5' and 3' exon-intron junctions was performed after PCR amplification. No sequence alteration of PCR products and no polymorphisms were detected. Taken together with the haplotyping of the CF chromosomes performed in the family using intragenic microsatellites and presented in fig 1, these results confirmed the homozygosity of the CFTR gene in the two affected children. We then had the opportunity to extract mRNA from a colorectal biopsy of one of the affected children (patient III.5) using an acid guanidinium thiocyanate-phenol-chloroform method. Reverse transcription and amplification of overlapping fragments of cDNA were performed using Long Expand Reverse Transcriptase (Roche, Switzerland) and Taq Plus Precision Polymerase (Stratagene, USA) according to the manufacturer’s instructions.

When the sizes of the RT-PCR products were controlled by electrophoresis on an agarose gel, an aberrant homozygous band was observed after amplification of the 3501-4157 fragment of the cDNA which includes exons 18 to 21 (fig 2, panel B). Direct sequencing of the amplification product clearly showed a frameshift insertion of 214 bp at the junction of exons 18 and 19 (data not shown). This insertion contained a stop codon. Alignment of the two diallelic markers KM19 and XV1c and the four intragenic dinucleotide markers IVS1, IVS6, IVS8, IVS17 (Tf) for the two parents and the two affected children. The presence or absence of the 3600+11.5 kb C>G mutation is indicated by +/- . No DNA could be obtained from the two unaffected children. The markers are arranged from top to bottom in their centromere to telomere localisation.

Figure 1 Haplotyping of the CF locus in the affected family. Haplotypes of the two diallelic markers KM19 and XV1c and the four intragenic dinucleotide markers IVS1, IVS6, IVS8, IVS17 (Tf) for the two parents and the two affected children. The presence or absence of the 3600+11.5 kb C>G mutation is indicated by +/- . No DNA could be obtained from the two unaffected children. The markers are arranged from top to bottom in their centromere to telomere localisation.
insertion fragment with the 3’ end of the CFTR gene (clone 133K23, GeneBank entry AC000061) showed that this fragment was part of intron 18, located at -1.3 kb from exon 19. As shown in panel A of fig 2, a putative acceptor splice site was present at the 5’ end and a potential donor splice site could be present if a C>G transition occurred at nucleotide +1 from the 3’ end of the fragment. The intronic fragment was then amplified from the CF genomic DNA sample and sequenced. A homozygous C>G mutation was clearly present in the DNA of the affected child. This mutation led to a frameshift additional exon. We named this splicing mutation 3600+11.5 kb C>G according to the notation used for cystic fibrosis mutations. The genomic intron 18 fragment was amplified from the CF genomic DNA sample and sequenced. A homozygous C>G mutation was identified. Unfortunately, no DNA sample was available from the first child who died from myocarditis, a rare clinical situation already previously reported in CF.12 This C>G transition was not found in 100 chromosomes from the general population making it unlikely to be a polymorphism. Furthermore, this mutation was not found in a panel of 24 unrelated CF chromosomes with unidentified mutations. This panel included 10 chromosomes from black southern African children and 14 CF chromosomes from European children; all these patients had a clear CF phenotype and a positive sweat test.

In the reported family the 3600+11.5 kb C>G mutation is associated with two severe forms of CF with pancreatic insufficiency but with a different mode of presentation when considering the pulmonary function. The observed variability both in the onset and in the evolution of the disease might be associated with factors involved in the tissue specific regulation of alternative or aberrant splicing which can result in variable expression of CFTR alteration.13

This new type of splicing mutation that took place in an intronic region and created an aberrant cryptic exon has been previously reported in the CFTR gene. Two mutations of this kind are quite common in the Hispanic population, the 3849+10 kb C>A mutation14 and the 1811+1.6 kb A>G mutation.15 We would like to emphasise that this type of mutation, as well as large genomic deletions, are not detected at the DNA level by the mutation screening procedures usually used by most laboratories. This could account for the large number of mutations which remain undetected even after complete sequencing of the exons (about 10% in France according to the French CF Consortium).

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