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 failure to thrive and massive daily steatorrhoea with foul smelling stools. When the sizes of the RT-PCR products were controlled for the two diallelic markers KM19 and XV2c, these results confirmed the homozygosity of the CFTR gene in the 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 XV2c and the four intragenic dinucleotide markers IVS1, IVS6, IVS8, IVS17 (TE) 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.

The second child, now aged 9, is healthy. The third child, a girl born at term, was admitted to our hospital because of persistent abdominal pain and distension. There was no evidence of Hirschsprung 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.

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After a healthy fourth child, a fifth child was born at term. The baby girl was admitted at 26 days of age with respiratory symptoms mimicking severe bronchiolitis. Pseudomonas aeruginosa 011 was found in sputum from different samples over two months. On two different occasions, a sweat test was performed. Chloride and sodium concentrations were 106 mEq/l and 75 mEq/l respectively for 101 mg of sweat during the first test, and 115 mEq/l and 78 mEq/l for 103 mg of sweat during the second test. She was therefore diagnosed with CF like her affected sister. There was some degree of hepatic involvement with an alanine aminotransferase (ALT) activity of 64 mIU/l (normal <30), an aspartate aminotransferase (AST) activity of 87 mIU/l (normal <30), and a \( \gamma \)-glutamyl transferase activity of 220 mIU/l (normal <45). Some echoes were found in the gallbladder. She had significant steatorrhoea: 15.3, 15.5, and 13.4 g of lipids per 100 g of stool on three consecutive days. Blood concentrations of vitamin A, vitamin E, and selenium were 0.96 \( \mu \)mol/l (normal=1.4–5), 3.7 \( \mu \)mol/l (normal=12–48) and 0.31 \( \mu \)mol/l (normal=0.8–1.25), respectively. Lately, she has had many episodes of severe prolapse. There is no evidence of the disease in other members of the family but no sweat test could be obtained from the sibs owing to a lack of cooperation from the family.

Search for mutations in the CFTR 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 two affected children (patient III.5) using an acid guanidine 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

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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 addition exon. We named this splicing mutation 3600+11.5 kb C>G according to the recommendations from CFGAC. As shown in panel C of fig 2, this mutation, which abolishes a DdeI restriction site, can be easily detected after amplification. The presence of the mutation was confirmed both on the paternal and maternal CF chromosomes and the two affected girls were homozygous for this mutation. Unfortunately, no DNA sample was available from the first child who died from myocarditis, a rare clinical situation already previously reported in CF. 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.

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 mutation and the 1811+1.6 kb A>G mutation. 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).

Figure 2. Identification and screening of the 3600+11.5 kb C>T mutation in the affected family. (A) Schematic representation of the intron 18 region containing the additional exon that has been abnormally spliced in the cDNA of the affected daughter (II.5). (B) Agarose gel analysis of the PCR products resulting from amplification of the cDNA fragment spanning nucleotides 3501-5147. cDNAs were obtained as indicated in the methods section using total RNAs isolated from a control (lane 2) and the affected child's colorectal biopsies (lane 3). Lane 1 corresponds to molecular size markers. Arrows on the right indicate the size of the normal (656 bp) and abnormal (870 bp) RTPCR products. (C) Screening of the 3600+11.5 kb C>T mutation in the family. The genomic intron 18 fragment was amplified using the following forward (5'-cccagaccttattatagactagcagc) and reverse (5'-tttagtgtaaagcagaggagt) primers. After DdeI digestion, the restricted DNA was separated on a 2% agarose gel. The 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 mutation and the 1811+1.6 kb A>G mutation. 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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