Novel and characteristic CFTR mutations in Saudi Arab children with severe cystic fibrosis


Abstract
More than 600 different CFTR (cystic fibrosis transmembrane conductance regulator) gene mutations have been identified so far that are considered to cause the fatal genetic disorder cystic fibrosis (CF). We have investigated 15 Arab children from 12 families, who were diagnosed as having CF, for mutations in the coding region and in the flanking intron sequences of the CFTR gene. Six different CFTR mutations were identified including two novel mutations, 1548delG in exon 10 and 406-2A→G in intron 3. Prominent mutations were the splice mutation 3120+1G→A (intron 16) followed by N1303K (exon 21) and 1548delG (exon 10). Most CF children were homozygotes who presented with a severe form of the disease including failure to thrive, recurrent chest infections, particularly with Pseudomonas aeruginosa, and frequent hospital admissions. Identification of the CFTR mutations facilitates molecular investigation of the disease and better understanding of its pathophysiology in Arab children, among whom CF is probably an underdiagnosed disease.

(Keywords: cystic fibrosis; CFTR gene; Saudi Arabia)

Cystic fibrosis (CF) is an inherited disease of the exocrine glands whose clinical symptoms include pulmonary disease, pancreatic exocrine insufficiency, male infertility, and an increase in the concentration of sweat electrolytes. The cloning of the gene responsible for the disease, the CFTR gene, in 1989 has allowed the characterisation of the mutational spectrum of the CFTR gene in many populations and has helped the elucidation of the molecular pathogenesis underlying variant forms of the disease.

The incidence of CF varies in different nations with the highest incidence of about 1:2000 in white populations. Although CF is generally considered to be rare in Arabic and African children, it is likely that many of these children remain undiagnosed owing to the lack of proper diagnostic facilities and public awareness of the disease. In Saudi Arabia, in 1 out of 12 families the parents were first degree cousins, that is, a consanguinity rate of 83%. Severity of the disease was judged by incidence of recurrent chest infections, in particular with Pseudomonas aeruginosa, failure to thrive, and repeated hospital admissions. The chest presentation was assessed by the four chest sounds: pneumonia, crepitations, rhonchi, and tachypnoea.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Nucleotide change</th>
<th>Location</th>
<th>Allele frequency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3120+1G→A</td>
<td>3120+1G→A</td>
<td>Intron 16</td>
<td>(21%)</td>
<td>14</td>
</tr>
<tr>
<td>N1303K</td>
<td>4041C→G</td>
<td>Exon 21</td>
<td>(4%)</td>
<td>12</td>
</tr>
<tr>
<td>1548delG</td>
<td>1548delG</td>
<td>Exon 10</td>
<td>(14%)</td>
<td>This study</td>
</tr>
<tr>
<td>406-2A→G</td>
<td>406-2A→G</td>
<td>Intron 3</td>
<td>(7%)</td>
<td>This study</td>
</tr>
<tr>
<td>1224V</td>
<td>3832A→G</td>
<td>Exon 12</td>
<td>(17%)</td>
<td>13</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td>Exon 19</td>
<td>(29%)</td>
<td>15</td>
</tr>
</tbody>
</table>

Mutations identified in 12 Arab CF families are listed by decreasing frequency. Only one sib of a family was counted, and only one occurrence of the mutation in consanguineous unions was considered to achieve more accurate estimates of the allele frequencies.
CFTR mutations in Saudi Arab children with severe cystic fibrosis

Following informed consent of the parents, blood samples were collected from the patients, their parents, and affected sibs. Isolation of high molecular weight DNA from leucocytes was performed according to standard procedures.

**MUTATION ANALYSIS**

DNA sequences spanning individual exons and flanking intron sequences of the CFTR gene were amplified by the polymerase chain reaction (PCR). For direct mutation analyses, PCR products were digested with the respective restriction enzyme and the fragments were separated by electrophoresis using a 3% NuSieve/1% SeaKem agarose gel. Mutagenesis primers were designed for some mutations (R117H, 1717-1G→A, G542X, 2143delT, 3272-26A→G, and N1303K) to create artifi-
an unidentified mutation on the other allele. This 1 year old girl had severe disease with failure to thrive and was already infected with *Pseudomonas aeruginosa*. The nucleotide sequence of exon 10 showing the homozygous frameshift mutation 1548delG is shown in fig 1B.

Presentation of cystic fibrosis in the majority of children in this study could be rated as severe. Many of the children presented with pancreatic insufficiency, failure to thrive, recurrent chest infections, particularly with *Pseudomonas aeruginosa*, recurrent diarrhoea, and required frequent hospital admissions, on average four to six times annually. Clinical data for all 12 children with genetically confirmed cystic fibrosis are shown in table 2.

Most children with homozygous CFTR mutations presented with a severe form of CF. One child homozygous for the 2043delG deletion died at the age of 4 months. The splicing mutation 3120+1G→A" was detected in three unrelated families from Saudi Arabia with homozygous patients in each family. Each of the four homozygous children was pancreatic insufficient and severely affected in early childhood confirming that the 3120+1G→A mutation represents a severe CF allele. The 11234V mutation was seen in the homozygous state in two sisters (aged 5 months and 5 years) who presented with failure to thrive and recurrent diarrhoea. However, the symptoms may not be solely related to CF since both girls had relatively low sweat chloride values (65 and 62 mmol/l, respectively) and the younger sister was pancreatic sufficient and free from chest infections. These two girls originated from a real Arab Bedouin tribe, whose ancestral generations have been confined to living in this region.

Discussion

Molecular studies on CFTR gene mutations in Arab populations up to the present time are scarce or undocumented. Here, we have analysed the entire coding region and flanking intron sequences of the CFTR gene in a sample of 15 Arab children with CF. Despite this extensive screening, some 30% of alleles remain unidentified. Mutations in the unscreened non-coding regions or gross deletions may account for the remaining alleles that have escaped our detection. Similar detection rates have been reported in other oriental populations, for example, from Tunisia, Israel, and Turkey (Onay et al, submitted). The major CF mutation in white populations, ΔF508, was not found in any of the Saudi families investigated. Out of 20 of the most common mutations in white populations, only the N1303K mutation was detected. This finding is in line with a high incidence and a west to east increasing frequency gradient of N1303K in the Mediterranean basin.16-18

Two new mutations were detected, the frameshift deletion 1548delG and the splice site substitution 406-2A→G. During the preparation of this manuscript we became aware of another description of the 548delG mutation in a compound heterozygous Saudi patient.16 The independent identification of this novel molecular defect in the same population favours the possibility that the mutation has arisen in Saudi Arabia. The other new mutation, 406-2A→G, was seen in a homozygous girl aged 20 months, whose pancreatic insufficiency and meconium ileus classify 406-2A→G as a severe allele. This previously unknown CF mutation might also be population specific. Many other mutations detected in this study were homozygous in the affected patient. This may be explained by the relatively high rate of consanguinity found among parents in this study (83%). In published reports, an even higher rate of consanguinity (88%) was found in Saudi families with CF children. Most children with homozygous CFTR mutations in our study presented with severe CF. The splicing mutation 3120+1G→A in intron 16, which was first reported by Mack et al19 in African American patients, is of particular interest as it was identified here in three unrelated families from Saudi Arabia with homozygous patients in each family. This mutation has recently also been detected in native African patients and it was considered to be a frequent CFTR mutation in African populations.20

Our study sheds light on the spectrum of hitherto unknown CFTR gene mutations in Saudi Arab patients and the associated presentation of cystic fibrosis in this population. The information depicted here may facilitate the design of appropriate strategies for genetic
testing of patients and carriers in this region of the world, where CF is probably an underdiagnosed disease.

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