Aldolase B mutations in Italian families affected by hereditary fructose intolerance

G Sebastio, R de Franchis, P Strisciuglio, G Andria, C Dionisi Vici, G Sabetta, R Gatti, N C P Cross, T M Cox

Abstract

Hereditary fructose intolerance (HFI) is an inborn error of metabolism caused by aldolase B deficiency. The aldolase B gene has been cloned and the following mutations causing HFI have been identified: A149P (a G→C transversion in exon 5), A174D (a C→A transversion in exon 5), L288ΔC (a base pair deletion in exon 8), and N334K (a G→C transversion in exon 9). We have investigated the occurrence of these mutations in 11 Italian patients affected by HFI using PCR and hybridisation to specific oligomers.

We found that four patients were homozygous for the A149P mutation, two patients were homozygous for the A174D mutation, three patients were compound heterozygotes for both the A149P and A174D mutations, one patient was homozygous for the N334K mutation, and one patient did not show any of the reported mutations (HFI diagnosis carried out by aldolase B assay). The L288ΔC mutation has not been found in this survey.

Hereditary fructose intolerance (HFI, MIM 22960) is a recessively inherited disease of fructose metabolism caused by aldolase B deficiency. After characterisation of the cDNA,1 2 the study of molecular defects in the gene for human aldolase B, in relation to HFI, has proved to be of diagnostic value in this disorder. Four mutations of aldolase B have been characterised by Cross et al.3-5 The first mutation is a G→C transversion in exon 5 resulting in an amino acid substitution Ala→Pro at position 149 of the protein (A149P). This mutation alters the substrate binding site of aldolase B, dramatically reducing its activity. A further mutation (A174D) is also a transversion C→A in exon 5, which results in a Ala→Asp substitution at position 174. A single base pair deletion (L288ΔC) has been identified in exon 8 causing a frameshift with a premature stop codon. Finally, a G→C transversion has been found in exon 9, which results in an Asn→Lys substitution at position 334 of the protein (N334K).

We have used the DNA polymerase chain reaction (PCR)6 to analyse directly the A149P, A174D, L288ΔC, and N334K mutations in 11 unrelated Italian patients affected by HFI.

Materials and methods

DNA from peripheral blood of 11 HFI patients from 11 unrelated Italian families was prepared by conventional methods. A 322 bp region of exon 5, containing the mutation site, was amplified using 30 cycles of DNA PCR as follows: 1 μg of genomic DNA was mixed with 100 μl PCR reaction buffer. Reaction temperatures were: denaturing at 93°C for two minutes, annealing at 41°C for two minutes, and extension at 71°C for two minutes using Taq DNA polymerase (Cetus, Perkin-Elmer, USA). Sequences for exon 5 were 5'CCC-TTTATTAGAAGCCCATG3' and 5'GGTCAATTGTAGTTATAGT3'. Amplified DNA was electrophoresed on a 1.2% agarose gel and Southern blotted according to standard procedures. Filters were then hybridised to two different oligomers carrying either the wild (A149) or the mutant (149P) sequence: (A149) 5'AGTGGCGTGCTGCTGA3' and (149P) 5'AGTGGCGTGCTGCTGA3' (fig 1). Filters were then rehybridised to show the presence of the A174D mutation using oligomers identifying either the wild type (A174) or the mutant (A174D) sequence: (A174) 5'TCGCTACGC-
A 279 bp region of exon 8, containing the L288ΔC mutation site, was amplified by PCR using 5’CAGGGTATATAAGGTGGGAC3’ and 5’AATGCTATCAACTTTG3’. The filter obtained after electrophoresis and Southern blotting was hybridised to two other different oligomers recognising either the L288ΔC mutation or the wild sequence: (L288wt) 5’AATGCTATCAACTTTGAGGCGG3’ and (L288ΔC) 5’AATGCTATCAACTTTGAGGCGG3’. A 207 bp region, including the N334K mutation site, was again amplified by PCR. Sequences were 5’TTCCCATGAGAGGCAGA3’ and 5’GCACCTGGAGCAGA3’.

The filter was then hybridised to wild and mutant oligomers whose sequences are (N334) 5’GCTAACTGCCAGGGCGG3’ and (334K) 5’GCTAACTGCCAGGGCGG3’ (fig 3). Finally, genomic DNA was digested with the restriction enzyme BamHI, electrophoresed on a 0.8% agarose gel, and blotted. Filters were hybridised to an aldolase B cDNA clone and labelled by nick translation with 32P dCTP. This probe hybridises to the entire protein coding region, but does not hybridise to exon 1 and the upstream region.

Results
The results of DNA molecular analysis, aldolase B enzymatic assay on liver biopsy, and intravenous fructose tolerance test are summarised in the table. No detectable deletions or rearrangements of the aldolase B gene were found in any of our patients on Southern blotting analysis of genomic DNA when digested with BamHI. DNA PCR amplification and hybridisation to oligomers, specific for either the wild

Genotypes of probands.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mutations</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A149P/A174D</td>
<td>AA</td>
</tr>
<tr>
<td>2</td>
<td>A149P/A149P</td>
<td>AA</td>
</tr>
<tr>
<td>3</td>
<td>A149P/A149P</td>
<td>AA</td>
</tr>
<tr>
<td>4</td>
<td>A149P/A149P</td>
<td>AA</td>
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<tr>
<td>5</td>
<td>A149P/A174D</td>
<td>AA</td>
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<tr>
<td>6</td>
<td>A174D/A174D</td>
<td>FTT</td>
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<td>7</td>
<td>A174D/A174D</td>
<td>FTT &amp; AA</td>
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<td>8</td>
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<tr>
<td>9</td>
<td>A149P/A149P</td>
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</tr>
<tr>
<td>10</td>
<td>A149P/A174D</td>
<td>AA</td>
</tr>
<tr>
<td>11</td>
<td>N334K/N334K</td>
<td>AA</td>
</tr>
</tbody>
</table>

AA=diagnosis by aldolase B enzymatic assay on liver biopsy. FTT=diagnosis by intravenous fructose tolerance test. *Diagnosis suspected on clinical grounds only.
sequence or the mutant sequence, gave the following results.

(1) Patients 2, 3, 4, and 8 are homozygous for the A149P mutation. All four were diagnosed on the basis of fructaldolase assays using homogenates of liver biopsy samples. (2) Patients 1, 5, and 9 are compound heterozygotes for the A149P and the A174D mutations and were diagnosed by enzymatic assay. (3) Patients 6 and 7 are homozygous for the A174D mutation. The former was diagnosed on the basis of the intravenous fructose tolerance test and the latter by enzymatic assay. (4) Patient 10, whose diagnosis was carried out by aldolase B enzymatic assay, does not show either the A149P or the A174D mutation. (5) Patient 11, whose diagnosis was made only on the basis of clinical signs, is homozygous for the mutation N334K. None of the patients was found to carry the L288ΔC mutation (data not shown).

**Discussion**

In this survey of Italian patients, A149P was found to be the most common mutation causing HFI (50% of independent alleles), while the A174D mutation was found in 35% of the HFI alleles. Three patients were found to be compound heterozygotes for A149P and A174D and their respective parents thus carry one or other of these two prevalent mutant alleles.

The prevalence of the A149P mutation in Italy confirms in part results of a European collaborative study by Cross et al, in which nine independent Italian patients had been already investigated. The frequency of the A149P mutation was found to be greatest in northern European countries and lowest in Italy. On the other hand, A174D is relatively more frequent in HFI patients from southern Europe and appears to be more frequent in Italy. The L288ΔC mutation, found in two Sicilian patients, was not observed in the present study, thus suggesting that it may represent a private mutation or may have been carried by migration from elsewhere in the Mediterranean region. N334K, found in patient number 11 (table), has been previously detected in four Yugoslav patients, in one Austrian, and in one British HFI patient.

The results presented here, together with those of Cross et al, allow a more accurate estimation of allelic frequencies of A149P, A174D, and ‘other’ (for example, L288ΔC and N334K) mutations in HFI Italian families: 0.52, 0.29, and 0.18 respectively. In the present study, we have been unable to establish any relationship between clinical presentation and genotype. In fact, both homozygotes for A149P and A174D mutations presented with either severe clinical manifestations in early life or mild symptoms later.

The direct analysis of aldolase B mutations in genomic DNA represents an important advance in the diagnosis of HFI since it is safe, rapid, and definitive. At the time of writing about 90% of HFI alleles can be identified by this method which, in addition, allows genetic counselling to be offered to heterozygotes.

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