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The Molecular Basis of Hereditary Fructose Intolerance in Italian Children[1)]

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Summary: We investigated the molecular defects of the aldolase B gene in five unrelated patients affected by hereditary fructose intolerance. The techniques used were DNA amplification, direct sequencing and allele-specific oligonucleotide (ASO) hybridization. The most frequent substitutions found in the hereditary fructose intolerance alleles analysed were the A174D and the A149P mutations, which account for 50% and 30% of the alleles, respectively. In two unrelated families, we found a rare mutation, the MDΔ4 previously described only in one British family, which may be an important cause of the disease in Italy.

Introduction

Hereditary fructose intolerance, inherited as an autosomal recessive character, is an inborn error of carbohydrate metabolism caused by a deficiency of hepatic aldolase B[2)] (1, 2). The disease is heterogeneous at the molecular level and analysis of the relative DNA mutations may allow an early diagnosis in infants and identification of the carrier status. This would avoid the routinely performed intravenous fructose tolerance test or the direct assay of aldolase activity in liver biopsy samples.

The aldolase B gene, located on chromosome 9 (3), is $14.5 \cdot 10^3$ bases long and consists of nine exons coding for a protein of 365 amino acid residues (4). Ten mutations have been described to-date; of these the most common are two single-base substitutions (A149P and A174D) in exon 5 (5). The A149P mutation leads to a protein with greatly diminished activity towards the fructose-1-phosphate and fructose-1,6-bisphosphate substrates, while the A174D mutation leads to a highly unstable protein. Other recently described mutations are rare and, in some cases, confined to single affected pedigrees (6–10). In this study we have analysed the aldolase B gene in five unrelated hereditary fructose intolerance patients from Northern Italy, using the DNA polymerase chain reaction followed by sequencing and hybridization to specific oligonucleotides. Furthermore, carrier status was established in three individuals, who are siblings of two probands.

Materials and Methods

Southern blot and hybridization

DNA, extracted from peripheral blood according to conventional methods (11), was digested with *Eco* RI and *Bgl* II, electrophoresed on 1% agarose gel and blotted according to the *Southern* method (12). The nylon membranes (Nytran, Schleicher & Schuell, Germany) were then hybridized to the human aldolase B cDNA (13) probe previously labelled by nick translation with [α-^{32}P]dATP.

Oligonucleotide synthesis

The oligonucleotides for amplification and allele-specific oligonucleotide hybridization were made on an Applied Biosystems 391 DNA synthesizer (Warrington, UK).

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[2)] Enzyme: Fructose-bisphosphate aldolase, EC 4.1.2.13