Mutation analysis in Turkish patients with hereditary fructose intolerance

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MS received 22.01.01 Revised and accepted 22.05.01

Summary: Thirteen Turkish patients with hereditary fructose intolerance (HFI) were screened for the three common mutations, A149P, A174D and N334K, in the aldolase B gene that have been detected frequently in European population. We found that nine of the patients carry the A149P mutation in both alleles, which corresponds to a frequency of about 55%. Single-strand conformation analysis of all coding exons of the gene was also performed to detect unknown mutations in four patients not carrying the three common mutations. No aberrant migration patterns were observed in these patients.

Hereditary fructose intolerance (HFI) is an autosomal recessive disease caused by deficiency of aldolase B (McKusick 229600, EC 2.1.2.13). The patients present with vomiting, abdominal pain, hypoglycaemia, hypophosphataemia, acidosis and fructosuria after ingestion of fructose and related sugars. Affected patients usually develop an aversion to fructose-containing foods and drinks. Growth retardation may be encountered in many patients even after a restriction of dietary fructose. Therapeutic protocol in HFI consists of stringent limitation of fructose intake and avoidance of prolonged fasting (Gitzelmann et al 1995). The aldolase B gene, which consists of nine exons, maps to chromosome 9q22.3 and the cognate mRNA encodes for 364 amino acids (Rottman et al 1984; Tolan and Penhoet 1986). Since the first findings of disease-causing mutations in 1988 (Cross et al 1988), more than twenty mutations have been described and registered in the Human Gene Mutation Database–Cardiff (HGMD Cardiff). The missense mutations A149P, A174D and N334K have been found to be the most prevalent in the European population (Cross
et al 1990). In this study we present the aldolase B mutation analysis of 13 Turkish patients diagnosed with HFI on the basis of both clinical and biochemical findings.

MATERIALS AND METHODS

Thirteen Turkish patients belonging to nine apparently unrelated families were examined in this study. Five of the patients are members of a large kindred and came from the region of Haymana located in the central part of Anatolia. One patient also came from the same geographical region, but was apparently unrelated with the large kindred. The other seven patients came from different parts of Turkey. All parents were consanguineous. The patients were diagnosed upon development of clinical features following ingestion of a fructose-containing food and biochemical findings including fructosuria. The ages of the patients ranged from 1 month to 14 years. While failure to thrive and avoidance of sweet foods are the main symptoms in older patients, vomiting after intake of fructose-containing foods, hepatomegaly, increased liver function tests and fructosuria are the main presenting symptoms in infants. No fructose loading test and enzymatic studies were performed in any case.

For the mutation analysis, genomic DNA was extracted from blood leukocytes by standard procedures. All of the eight coding exons of the aldolase B gene were amplified by PCR using intronic primers labelled with fluorescein at the 5' end. The PCR products were purified from excess primers and nucleotides and subjected to SSCP analysis using a MDE non-denaturing gel (FMC Bioproducts) according to the manufacturer's recommendations. The gel was run at room temperature in 0.5× TBE buffer at 600 V for 7 h. The A149P and the N334K mutations were searched by restriction enzyme digestion of exon 5 with BsaHI and exon 9 with DdeI, and separation of the products through a long range gel (FMC Bioproducts). Electrophoretic separations were performed with an automated sequencer (ALF, Pharmacia).

RESULTS

We detected nine patients who carried the A149P mutation in the homoallelic form. Mutation A149P was not detected in the other four patients and these patients were also found not to carry two other common mutations, A174D and N334K. SSCP analysis of all coding exons of these four patients showed no aberrant migration pattern. The frequency of the A149P allele was found to be about 55% in the patients.

DISCUSSION

We report the mutation analysis of 13 Turkish patients with HFI. Although a Turkish family from the east of Turkey carrying the R3op (C→T, Arg→Ter, exon 2) mutation has been reported previously (Ali et al 1994), this is the first study established in a larger series of Turkish patients from different parts of Turkey. Nine of the patients were found to carry the A149P mutation in the homoallelic form, very