Different genotypes in a large Italian family with recurrent hereditary fructose intolerance.

Different genotypes in a large Italian family with recurrent hereditary fructose intolerance

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Objectives **Hereditary fructose intolerance is caused by a deficiency of the aldolase B enzyme, which is expressed in the liver, small intestine and kidneys. Patients usually show a marked aversion to fruits and sweets; if, however, it is not diagnosed, persistent or incidental ingestion of fructose might be lethal. Our paper aims at improving the clinical and molecular characterizations of these patients, to avoid dangerous misdiagnoses.**

Methods **Here we report the molecular results in an Italian cohort: on the occurrence of *aldolase B* mutations and, in particular, on the clinical and molecular characterization of a large family with recurrent hereditary fructose intolerance.**

Results **Patients included in our cohort showed the three most common mutations (p.A150P, p.A175D and p.N335K). Such molecular tests were enough to cover all the mutated alleles of hereditary fructose intolerance found in our patients. The allele frequencies of hereditary fructose intolerance mutations detected were 69.2% for p.A150P, 23.1% for p.A175D and 7.7% for p.N335K. The proband of the family with recurrence of the disease was heterozygous for the known p.A150P and p.A175D mutated alleles of the *aldolase B* gene. Molecular characterization of at-risk family members also identified the p.N335K mutation. In addition, the oldest affected patients exhibited mild clinical impairment.**

Conclusions **Our results indicate that the diagnosis of hereditary fructose intolerance can be complicated by clinical and genetic intrafamilial variability. A knowledge of the clinical and geographical history of each family member is thus essential, to reduce potentially lethal misdiagnoses and to facilitate such patients to receive appropriate genetic counselling. *Eur J Gastroenterol Hepatol* 20:118–121 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Hereditary fructose intolerance (HFI; Online Mendelian Inheritance in Man + 229600), which is caused by the absence or reduction of the aldolase (EC 4.1.2.13) enzyme, B isoform (ALDOB), has an estimated frequency of 1 in 20 000 births [1]. Despite eating habits that include increased sugar consumption in industrialized countries, patients' distinct aversion to fruit and sweets is the major reason for underdiagnoses of HFI [1,2]. Untreated patients, being unable to assimilate dietary fructose, can develop a broad range of gastrointestinal symptoms, lactic acidosis and hypoglycaemia. Persistent ingestion of fructose and related sugars can lead to severe liver and kidney damage, seizures, coma and risk of death [1].

The human *ALDOB* gene has been cloned and characterized: the gene consists of nine exons encoding a polypeptide containing 364 amino acids [3]. So far, about 35 mutations in the *ALDOB* gene have been reported [4,5]. The most common *ALDOB* mutations, p.A150P, p.A175D and p.N335K, account for about 72–76% of HFI mutated alleles worldwide [4–6]. The p.A150P mutation has a greater frequency in Northern Europe, whereas the p.A175D mutation is predicted to be more frequent in Southern Europe, and the p.N335K in Central and Eastern Europe [5,7]. These general population studies included the Italian population. In addition, Italian HFI patients have been analysed as an exclusive group [8,9]. The estimated frequencies of the p.A150P and p.A175D mutated alleles in Italy are reported to be about 50 and 30%, respectively [8,9]. Seven mutations have, however, been proposed to account for about 95% of Italian *ALDOB* mutated alleles [10].

Given the high prevalence of a small number of *ALDOB* mutations, a rational mutation-screening programme could be introduced. It seems likely that the frequency of HFI has been underestimated: hence, such a

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programme could have a dramatic impact for potential patients, as dietary treatment can prevent this potentially lethal disease [5].

To increase the number of characterized HFI patients and to ascertain the prevalence of each mutation in the Italian population better, we report the allele frequencies of HFI mutations that were detected in the *ALDOB* gene of 13 index patients. This HFI cohort includes index patients from a large Italian family with recurrent HFI and atypical molecular characterization due to the presence of the three common *ALDOB* mutations.

Methods

Patients

The clinical presentations of the HFI patients, belonging to the large Italian family here reported, are summarized in Table 1. The eight additional unrelated Italian patients, who were included in our cohort, presented classic HFI phenotypes.

Genomic DNA isolation and analysis

Consent statements for genetic analysis were obtained from all patients. Genomic DNA was obtained from patients' lymphocytes using a commercial DNA-extraction kit (Qiagen, Hilden, Germany). The oligonucleotides and the PCR-amplifying conditions have been described previously [11]. The three most common *ALDOB* mutations were screened. Three *ALDOB* fragments, encompassing exons 5 and 9 and containing the p.A150P, p.A175D and p.N335K mutations, were amplified and directly sequenced on both strands.

The PCR products were checked on a 1.5% agarose gel, and were excised and purified using Nucleospin Extract kit (Macherey-Nagel, Düren, Germany).

Sequencing reactions were performed using the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, California, USA), as recommended by the manufacturer. The nomenclature of the genetic lesions of the *ALDOB* gene is as designated previously [12–14]. The mutation frequencies of the three common HFI mutations, which refer to the index patients here analysed, are reported.

Results

Molecular analysis of HFI index patients included in our cohort showed the three most common mutated alleles (resulting in p.A150P, p.A175D and p.N335K mutations). The allele frequencies of the HFI mutations detected were 69.2% for p.A150P, 23.1% for p.A175D and 7.7% for p.N335K.

As part of our cohort, the index patients of a large Italian family with recurrent HFI were further investigated at clinical and molecular levels (Table 1, Fig. 1). The proband (patient III:6) presented at 13 months with hypoglycaemic seizures and hypotonia, after the unwilling intake of a fruit shake at kindergarten. HFI was suspected because of mild metabolic acidosis, hepatomegaly, a rapid rescue of the patient's condition after the infusion of glucosaline solutions and her aversion to sweet-tasting foods. To provide a confirmatory diagnosis, molecular analysis of the most common *ALDOB* gene mutations was opted for, and the p.A150P and p.A175D mutated alleles were detected. Two of the proband's cousins (patients III:1 and III:2) presented with a strong aversion to foods containing fructose as the only indicator of the disease. Mutation analysis again detected the p.A150P and p.A175D mutations in both patients, although patients II:6 and II:20 were reported to be nonconsanguineous.

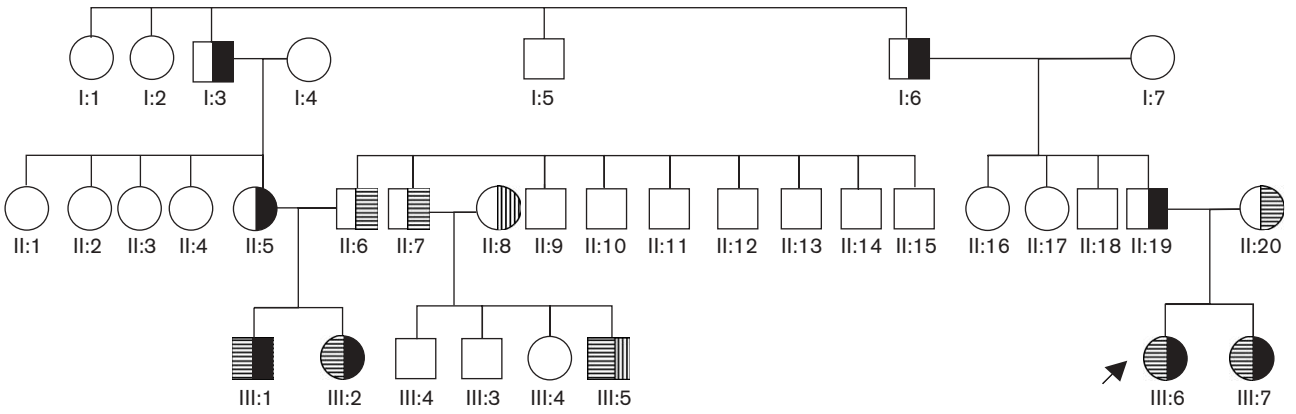
Aversion to sweet-tasting foods and abdominal pain also prompted patient III:5 to undergo *ALDOB*-gene molecular investigation. Interestingly, the p.N335K mutation was also detected along with the p.A150P mutation.

Table 1 **Synopses of the main characteristics of the members of the large family reported here**

Patient	III:6 proband	III:7	III:1	III:2	III:5
Age	3 years	2 years	23 years	21 years	18 months
Origin	Northern Italy	Northern Italy	Northern Italy	Northern Italy	Northern Italy
Growth	Normal	At the 10th centile	Normal	Normal	Normal
Metabolic presentation[a]	Hypoglycaemia, mild metabolic acidosis and vomiting	Absent	Absent	Vomiting	Absent
Gastrointestinal abnormalities	Mild hepatomegaly and hepatic steatosis	Mild hepatomegaly and hepatic steatosis	Normal	Normal	Hepatomegaly and abdominal cramps in the preceding months
Laboratory findings	Transient hyper transaminasaemia	Transient hyper transaminasaemia	Normal	Normal	Transient hyper transaminasaemia at 11 months
Crisis[a]	Hypoglycaemic seizures at 13 months	No, diagnosis at birth	Lipothymia at 19 years	No	No
Aversion to any source of fructose	Yes	No fructose ingestion	Yes	Yes	Yes
Therapy	Vitamin C, no fructose ingestion	Vitamin C, no fructose ingestion	Vitamin C, no fructose ingestion	Vitamin C, no fructose ingestion	No fructose ingestion

[a]When presenting with a crisis, symptoms have followed the ingestion of sweets and/or fruit.

Fig. 1



Family pedigree. The proband is indicated by an arrow. Horizontal lines indicate the p.A150P mutation; vertical lines indicate the p.N335K mutation; the black filling represents the p.A175D mutated allele.

Discussion

Deficient activity of ALDOB results in the accumulation of fructose 1-phosphate, which can be particularly toxic in early life [1], cause severe liver disease and, if undiagnosed, can lead to death [15].

It has been suggested that establishing the prevalence of *ALDOB*-gene mutations is a premise for rational molecular analyses in patients with suspected HFI, and for the introduction of a screening programme for newborn babies [5]. Molecular analyses also replace invasive diagnostic procedures such as the intravenous fructose load or enzyme assays that are performed on tissue biopsy samples [1]. A method that uses denaturing high-pressure liquid chromatography technology has been proposed for use in screening programmes for newborn infants [5]; however, projects in this field might be complicated by the occurrence of private or rare HFI mutations.

Our molecular results showed a distribution of the p.A150P mutation that was relatively closer to that of the pan-European population [16], than to that of Italian patients [6,9]. The p.A150P mutation has been described as being more frequent in North Europe than in Italy; however, a frequent mutation reported in the Italian population (c.865delC) has been predicted to be limited to Sicily [6]. The inclusion of Sicilian patients in a HFI survey can thus create an imbalance in the estimates of the prevalence of *ALDOB* mutations in our country.

As predicted [6,9], the frequency of the p.A175D mutation was particularly high, compared with recently reported frequencies in Central Europe (15%) or Spain (15.8%) [5,16]. The p.N335K mutation has probably spread from Eastern and/or Central Europe [4,7]. This mutated allele, however, also proved to be relatively common in Italy, with a frequency close to that in Central Europe [5,16].

A relationship between clinical symptoms and genotype has not been demonstrated [17]. As the severity of the disease might vary and as affected patients often impose fructose restrictions on themselves, adult patients might be symptom free. Patient III:7, however, despite having the same genotype as patients III:1 and III:2, and despite having avoided fructose from birth, exhibited hepatomegaly and mild steatosis; so did her sister (patient III:6), suggesting that follow-up through adulthood might be essential to assess the outcome of the disease. It is thus likely that the young patients reported herein will show an improvement in their clinical presentations as they age, like their older relatives (patients III:1 and III:2). In addition, we stress the consequences of a spread, and correct genetic counselling for the diagnoses of patients III:1 and III:2, who were essentially free of symptoms.

Interestingly, our report showed three independently segregating alleles within a family unit. Therefore, clinicians should ideally perform molecular assays of at least the three common *ALDOB* mutations. This might avoid dangerous misdiagnoses, and allow for the early introduction of a diet eliminating fructose-containing foods and for the provision of oral vitamin C supplements to offset the imbalances that a diet without fruit might entail [18]. Large population screening has previously demonstrated that HFI patients are heterozygous for at least one of those three mutations (mutated allele frequencies 94%) [5]; despite this, seven mutations are necessary to achieve the 95% prevalence of HFI alleles in Italian patients [10]. Therefore, the negative results of the molecular tests performed for the three most

common mutations do not exclude a positive diagnosis. In addition, heterozygous patients should be further investigated at the molecular level.

In conclusion, our data underline the relevance of a critical approach to the molecular analysis of common, and possible new, *ALDOB* mutations. This information could be useful to screen the *ALDOB* gene more carefully in suspected patients (i.e. those at risk), starting from the numerous parents of the large family here reported. A careful clinical and molecular diagnosis can be particularly useful in genetic counselling concerning newborn babies in affected families, as a very simple dietary therapy can prevent potentially serious damage caused by the disease.

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Conflict of interest: none declared.

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