Hereditary fructose intolerance

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Abstract
Hereditary fructose intolerance (HFI, OMIM 22960), caused by catalytic deficiency of aldolase B (fructose-1,6-bisphosphate aldolase, EC 4.1.2.13), is a recessively inherited condition in which affected heterozygotes develop hypoglycaemic and severe abdominal symptoms after taking foods containing fructose and cocrate sugars. Continued ingestion of noxious sugars leads to hepatic and renal injury and growth retardation; parenteral administration of fructose or sorbitol may be fatal. Direct detection of a few mutations in the human aldolase B gene on chromosome 9q facilitates the genetic diagnosis of HFI in many symptomatic patients. The severity of the disease phenotype appears to be independent of the nature of the aldolase B gene mutations so far identified. It appears that hitherto there has been little, if any, selection against mutant aldolase B alleles in the population: in the UK, ~1.3% of neonates harbour one copy of the prevalent A149P disease allele. The ascendance of sugar as a major dietary nutrient, especially in western societies, may account for the increasing recognition of HFI as a nutritional disease and has shown the prevalence of mutant aldolase B genes in the general population. The severity of clinical expression correlates well with the immediate nutritional environment, age, culture, and eating habits of affected subjects. Here we review the biochemical, genetic, and molecular basis of human aldolase B deficiency in HFI, a disorder which responds to dietary therapy and in which the principal manifestations of disease are thus preventable.

Keywords: fructose intolerance; aldolase B; inborn error of metabolism

Aldolase B: a specialised enzyme of fructose metabolism
Fructaldolases (EC 4.1.2.13) are widely distributed in living organisms; they catalyse the specific and reversible cleavage of the glycolytic hexose substrates, fructose-1,6-bisphosphate and fructose-1-phosphate, into the 3-carbon sugars, dihydroxyacetone phosphate, D-glycerolaldehyde 3-phosphate, and D-glycerolaldehyde 3-phosphate (fig 1). There are two different processes for the generation of the stable carbon substrate enzyme complexes involved in the aldolase reaction: aldolases of the class II type, which are confined to yeasts and prokaryotes, form the carbonion using a bivalent metal cation (Zn²⁺). However, in class I aldolases, which are mainly of eukaryotic origin, there is transfer of a proton from a conserved Schiff base forming lysine residue located at the active site.'

There are three genetically distinct isozymes of class I vertebrate aldolase which can be distinguished on the basis of their antigenic and catalytic properties. In humans, aldolase A (Genebank Accession No M11560) exists in most tissues but predominates in the muscle; aldolase B, previously known as liver aldolase (Accession No X01098) is also found in certain cells of the kidney and small intestine; and aldolase C (Accession No X0792) is present in the brain. Aldolases A and C are constitutively expressed, but the B isoform is under dietary control. Under resting conditions, the expression of aldolase B mRNA is low and any functional enzyme present in the cytosol appears to associate with components of the cytoskeletal network rendering it catalytically inactive; after feeding a carbohydrate diet, mRNA expression is induced and the

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**Figure 1** The metabolism of fructose in normal subjects. The enzyme aldolase B catalyses the reversible cleavage of fructose-1-phosphate in the specialised pathway of fructose metabolism, as well as fructose-1,6-bisphosphate in the glycolytic/gluconeogenic pathway. 3-carbon sugars: D-glycerolaldehyde 3-phosphate, D-glycerolaldehyde, dihydroxyacetone phosphate. Dotted arrows indicate point of secondary metabolic arrest in aldolase B deficiency (see text).
enzyme is activated by dissociation of the aldolase B-cytoskeletal protein complex in the presence of accumulated intermediate metabolites. In fetal tissue, aldolase A is predominant, but as the embryo develops there is repression of aldolase A in the liver, kidney, and intestine, accompanied by expression of aldolase B. A reversion to this fetal pattern of expression is observed in transformed rat liver cells and in human hepatoma, where aldolase B has been categorised as an oncofetal antigen. The 5' flanking sequences (up to 200 base pairs) of the aldolase B gene from different species are highly conserved, as expected for common promoter regions that are important for gene expression. The developmental stage specific and tissue specific expression of aldolase B has been shown to be linked with the chromatin structure of its promoter region (which encompasses an origin of DNA replication initiation), the demethylation of a cytosine residue (position -129), and the simultaneous appearance of transcription factors, AIF-A (identical to liver specific factor HNF-1) and AIF-B (CCAAT binding protein, binds to residues at positions -129 and -128), which activate aldolase B gene transcription by binding to the promoter region. The transcription factors HNF-3 and RYB-a, which have overlapping binding sites with AIF-A and AIF-B respectively, have been identified to repress transcription from this promoter suggesting that competition between these activators/repressors regulates aldolase B gene expression. Recently, Sabourin et al. used transgenic mouse models with expression construct transgenes to identify an intronic activator region (nucleotides 650-2448) in the aldolase B gene which, in combination with the tissue specific promoter, is sufficient to mediate developmental expression of aldolase B mRNA expression but lacks the necessary site(s) for activation by dietary carbohydrate.

Studies of enzyme function activity using purified isoforms preparations have shown that at saturating substrate concentrations, aldolases A and C display between 50- and 10-fold greater activity towards fructose-1,6-bisphosphate than to fructose 1-phosphate, whereas aldolase B has equal activity with both substrates. The aldolases show less affinity for the fructose 1-phosphate substrate (aldolase B Km ~1 mmol/l), compared with the bisphosphate, (aldolase B Km 50 mmol/l). The similarity between the isoforms, in terms of their structural and general catalytic properties, led Rutter to conclude that the isoforms evolved by a process of gene duplication and divergence, according to the mechanism proposed by Ingram to explain the differences observed in the haemoglobin. This view has been supported by the mapping of the three human aldolase genes and a pseudogene to homoeologous chromosomes that have emerged during vertebrate tetraploidisation.

Studies on the molecular architecture of class I aldolases have shown that the functional enzyme is about 160 000 Daltons and consists of four identical subunits. The most thoroughly investigated class I aldolase, rabbit muscle aldolase (aldolase A), has been crystallised and the three dimensional structure determined by x ray diffraction to a resolution of 1.9 Ångströms. The polypeptide backbone of the enzyme folds into an alternating arrangement of α helices and β sheets, which adopt a basic eight stranded β barrel type structure, similar to triose phosphate isomerase and the A domain of pyruvate kinase, as well as 14 other enzymes with little sequence, but significant structural homology (reviewed by Farber and Petsko). Since human aldolase B has 69% amino acid identity to its parologue aldolase A, it is likely to assume a similar three dimensional conformation, as does rabbit liver aldolase.

Using the structural data with the corresponding amino acid sequence, the substrate binding residues Lys 146, Arg 148, and Lys 229 (which forms the Schiff base) line the active site pocket which is located in the centre of the β barrel. Even though the spatial configuration of the carboxy-terminal tail of aldolase A could not initially be identified by Sygusch et al. experiments on the evolutionarily conserved terminal tyrosine residue, either by carboxypeptidase treatment or site-directed mutagenesis of recombinant aldolase expressed in Escherichia coli, have shown that it is involved in binding of the substrate, fructose-1,6-bisphosphate. The C terminus perhaps mediates movement of substrates in and out of the barrel structure. A more recent model based on refined data obtained from rabbit aldolase A has indicated that the C terminal residues actually project into the active site.

The gene for human aldolase B has been mapped to chromosome 9q22.3. The structural organisation showed nine exons, including the first 72 bp exon (nucleotides 924-996), which lacks the initiator methionine codon in exon 2 at nucleotides 5816-5818, to span a DNA segment of 14.5 kb. The cloning of the aldolase B cDNA shows that the mRNA encodes a 364 amino acid polypeptide including the initiator methionine. The A and C isoforms of human aldolase have also been cloned and their cognate genes map to chromosomes 16 and 17, respectively. Kita-jima et al. observed that there are seven common conserved and four isoform group specific regions. The carboxyl tail shows significant sequence divergence, thus the unique functional properties of the individual isoforms may be invested in this region. Indeed, there is clear evidence to support the contention that isoform function is dependent on changes in this single domain, since sequence analysis of the aldolase locus from Drosophila melanogaster shows a single gene with three alternative 3' terminal exons. The single primary transcript undergoes alternative splicing to give rise to the three isoform specific aldolases with variable carboxy-terminal sequences and differing catalytic properties.
Consequences of human aldolase B deficiency

Hereditary fructose intolerance (HFI, OMIM 22960) is a recessively transmitted metabolic condition caused by catalytic deficiency of liver aldolase (aldolase B) (EC 4.1.2.13) in the liver, kidney, and intestine.61

The disease, first reported in an adult in 1956 by Chambers and Pratt,61 was typified by the description of a 24-year-old woman who, after taking sugar and fructose complained of phobic symptoms, faintness, abdominal pain, and nausea; when she took glucose, these symptoms were absent, although it was noted that she did not enjoy the sweet taste. After systematic tasting with a range of sugars unknown to the patient, it was concluded that she had an "idiopathic intolerance to fructose". Chambers and Pratt suspected, but did not ever formally prove, that several of the symptoms noted in their patient were the result of fructose-induced hypoglycaemia.

Studies of HFI in infants and children soon followed and showed a more alarming disorder in those affected subjects who are unable to avoid the toxic sugars.6263 Infants with this condition are the most vulnerable to exposure of dietary fructose, especially at weaning.6465 The newborn does not develop any symptoms while taking breast milk since this contains lactose, the disaccharide of glucose and galactose. However, characteristic symptoms of vomiting, nausea, and sweating, associated with hypoglycaemia and metabolic acidosis, are induced after transfer to sweetened milk formulae and solid foods containing added sugar, as well as natural fruit and vegetables. If large quantities of sugar are consumed, the acute reaction is more severe and the infant becomes lethargic and may develop seizures or coma.

It has been observed that persistent intake of the harmful sugars in childhood leads to a syndrome of chronic toxicity.66 At this stage also irreversible damage to the liver and kidney may occur.67 This course eventually leads to cirrhosis of the liver and sometimes death. Clearly the mother, or others responsible for infant care, play a critical part in the nutrition of those affected and are usually best placed to protect the defenceless patient by the early withdrawal of foods and drinks that cause the disease, thus identifying the harmful items for the person to avoid as they become more independent. In some countries, carers other than the concerned parents may interfere with the nutrition of ailing infants with HFI: the authors are aware of several instances where grandparents have administered large quantities of honey to infants under investigation for the disease unknown to nursing staff and parents in hospital, with catastrophic results.

If the undiagnosed infant survives the difficult initial period of weaning, the child usually develops a self-protective aversion to foods which cause distress.61 Voluntary dietary exclusion, which is refined by trial and error over a life time, includes restriction of most sweet tasting foods. The older infant affected by HFI often proves difficult to feed and recurrent ill health and growth failure are common.6466

Adults with the condition, who have survived as a result of a self-imposed low fructose diet, can remain undiagnosed for many years until they come forward in response to articles on the subject in the public domain68 or as a result of their dentist's observations. Several dental researchers have noted that children and adults with HFI have reduced dental caries as a result of their modified dietary habits.6971

A particular hazard for people with undiagnosed HFI has been the indiscriminate use of fructose infusions as a source of parenteral feeding1213; more than 20 fatal or near fatal instances resulting from this cause have been and continue to be reported in HFI17 (see also below). These cases have lately been almost exclusively restricted to Germany where the use of parenteral fructose and sorbitol solutions has persisted. Although the use of fructose as opposed to glucose containing fluids as a source of parenteral nutrition was originally thought to be beneficial for patients with diabetes mellitus,7071 their use is not only fatal in patients with HFI,7276 but is potentially toxic in normal subjects.8082 Hence, in most countries their availability has been restricted: glucose and lipid preparations are now the preferred sources of energy. In a recent example, medical review and molecular analysis of aldolase B genes in surviving relatives of the dead patient (a German citizen) provided evidence that led to successful litigation by the wife and family of the proband; the hospital authorities settled compensation (see data and pedigree in fig 2).

Once the diagnosis of fructose intolerance has been established, a strict exclusion diet should be introduced with the assistance of an experienced dietician; provided that tissue damage has not been extensive, normal health and development returns rapidly.8384 Care should be taken to advise on the suitability of all medicines: sucrose and sorbitol are favoured excipients and coatings for tablets and are frequent components of syrups and suspensions used to deliver drugs in a palatable form to infants and children. There are numerous examples of their harmful effects in patients with HFI, who should be advised to take supplements of water soluble vitamins, including folic acid and vitamin C; “Ketovite” is a suitable preparation for this use. We recommend that patients should use warning MedicAlert bracelets advising on prohibited sugars (especially for the traveller) and on the appropriate treatment for hypoglycaemia in stricken patients (glucose or milk for parenteral or oral use as needed). At the time of writing, the original patient reported by Chambers and Pratt61 is alive and well known to the authors. She is in good physical health at the age of 67 years and is a grandmother.

CLINICAL INVESTIGATIONS AND PATHOGENESIS

The specific pathway of fructose metabolism, which was brilliantly elucidated by Hers,85 is depicted in fig 1. To summarise, once exogenous fructose is incorporated into the cell, it
Analysis of blood and urine metabolites in patients with HFI have shown that when fructose is administered, blood glucose, phosphate, and potassium concentrations decrease; this is accompanied by increased concentrations of blood magnesium, urate, alanine, and lactate. If the concentration of fructose exceeds 2 mmol/l in the blood, fructosuria, as shown by the appearance of reducing sugars in the urine, also occurs. The biochemical changes observed in HFI appear to be secondary to the fructose 1-phosphate aldolase deficiency, affecting the otherwise normal metabolic pathways of fructose metabolism in the liver and accessory tissues. Although the fructosuria of HFI patients exceeds that in normal subjects after sugar loading, only a fraction of the fructose ingested is excreted by this route. This indicates that fructose is metabolised at extrahepatic sites; the suggestion that hexokinase in leucocytes, erythrocytes, and adipose tissue may contribute to the disposal of excess plasma fructose has been supported by metabolic labelling studies using samples of rat epididymal fat. The biochemical changes described here immediately accompany the acute symptoms of sugar toxicity in HFI.

When aldolase B activity is deficient, the rapid phosphorylation of fructose creates a build up of fructose 1-phosphate with depleted intracellular inorganic phosphate and ATP in the organs where the specialised pathway of fructose metabolism is operative. The reduced Pi concentration activates adenosine deaminase and the xanthine oxidase pathways, with degradation of purine nucleotides to uric acid. Reduced intracellular concentrations of inorganic phosphate lead directly to the allosteric activation of adenosine deaminase. The increased formation of uric acid leads to hyperuricaemia, a metabolic effect of fructose administration observed to a limited extent in normal subjects. More recently, it has been observed that the hyperuricaemic effect is more pronounced in HFI heterozygotes than in control subjects, suggesting that carriers of this disorder may be predisposed to hyperuricaemia and gout. Reduced intracellular concentrations of ATP appear to account for the release of Mg2+ ions by dissolution of the Mg-ATP complex, so causing the hypermagnesaemia observed in HFI patients following challenge with fructose. High concentrations of unmetabolised fructose 1-phosphate inhibit fructokinase action by negative feedback, thus preventing further incorporation of fructose when metabolism by the aldolase B pathway is saturated. As a result, transient fructosuria (an outmoded term for hereditary fructose intolerance) and fructosuria are observed.

The hypoglycaemic response to fructose experienced by patients with HFI is attributable to the impaired hepatic breakdown of fructose 1-phosphate. Experiments carried out in patients with HFI during fructose induced hypoglycaemia clearly show reduced interpan- dial plasma glucose release without evidence of hyperinsulimism. The failure of parenteral glucagon to correct the hypoglycaemia indicates
defective glycolysis. Although infusions of galactose raise the blood glucose concentration (indicating that the phospho-
fructose-1-phosphate, combined with the decrease in inorganic phosphate, block glycogen break-
down at the level of phosphorylase. Fruc-
tose-1-phosphate also prevents the formation of the
glycogen intermediate, glucose-6-phosphate, by comp-
petitive effects on aldolase A and glucose
6-phosphate isomerase, respectively. In the
absence of a fructose challenge, no such
metabolic inhibition would be expected and
this is certainly supported by the observation
that patients with HFI tolerate prolonged fast-
ing.

Impairment of glycogen synthesis after fruc-
tose intake in patients with HFI combined with
fructose-1-phosphate induced activation of
pyruvate kinase results in accumulation of the
Kreb's cycle precursors, alanine, lactate,
and pyruvate. This contributes to amino
aciduria, as well as metabolic acidosis. Impaired
function of the proximal renal tubule aggravates the acidosis and results in general-
ised amino aciduria, phosphaturia, and bicar-
bonate wasting. As a result, adolescents
present with metabolic bone disease and stunt-
ning of growth. Defective acidification of the
urine, as well as phosphaturia and amino
aciduria, represents an acquired form of the
Fanconi syndrome that has been well docu-
mented in HFI. The cause of the abdominal
pain that is a feature in HFI remains unex-
plained. This pain, which follows inges-
tion of the offending sugars within a few
minutes, may reflect autonomic afferent activ-
ity induced by release of purine nucleotides,
loss of energy charge, or increased concentra-
tions of gluconeogenic precursors such as
lactate, but proof of a cause and effect relation-

ship is lacking. The possible involvement of
fructose 1-phosphate in the regulation and
synthesis of glycoproteins has received little
attention but the high concentrations of this
ester that occur in HFI tissues after fructose
exposure might also have hitherto unsuspected
effects on hepatic glycan metabolism, includ-
ing the synthesis of desialylated plasma trans-
ferrin isoforms.

GENETIC STUDIES

Information obtained from the cloning and
characterisation of the wild type human
aldolase B gene allowed Cross and col-
leagues to clone the chromosomal aldolase B
gene from a well characterised patient suffering
from hereditary fructose intolerance, who had
an intact but functionally inactive enzyme
aldolase B polypeptide expressed in liver and
intestinal mucosa. Sequence analysis of the
entire mutated gene showed a single base
substitution in exon 5 of aldolase B from the
patient. Molecular analysis of genomic DNA
using the polymerase chain reaction followed
by restriction enzyme digestion showed that
the patient was homozygous for this mutant
allele. This mutation, a G→C transition in
the first base of codon 149, is inferred to replace
the normal alanine by a proline residue;
the variant allele was hence designated A149P.
The homozygous genotype, A149P, was found
only in association with symptomatic patients
with HFI, who had inherited one copy of the
allele from each parent, and confirmed that the
condition segregated in the families as ex-
pected for a recessive disease. It also
provided strong evidence that defects in the
aldolase B gene cause HFI. Facile detection
of the A149P mutation, which creates a novel
recognition site for the restriction endonucle-
ase AhaII and its isoschizomers, has been
used for molecular diagnosis and confirma-
tory testing after large scale genotyping by allele
specific hybridisation. Indeed we have used
this procedure to ascertain the success of
engraftment following the use of allogeneic
bone marrow transplantation from a donor
with established HFI into his HLA identical
but A149P heterozygous brother who suffered
from acquired aplastic anaemia (fig 3).

Since this original investigation, molecular
analysis of human aldolase B genes using the
polymerase chain reaction and DNA sequenc-
ing has to date identified 22 genetic lesions
in patients with HFI (table 1). The detection
of each mutation in genomic DNA using PCR
based methods has allowed screening to be
carried out in patients with uncharacterised
mutant alleles of aldolase B to confirm a puta-
tive diagnosis of HFI based on clinical grounds
alone. Molecular diagnosis by analysis of the
aldolase B gene has the obvious advantage that
it avoids invasive tests including the
intravenous fructose tolerance test and liver or
intestinal biopsy followed by enzymatic assay.
Genetic studies have also provided information
about the distribution of mutant aldolase B
alleles in different populations.

As established by Cross et al and Cox, the
most prominent allele causing HFI in

Figure 3 Aldolase B genotyping after bone marrow transplantation for severe aplastic anaemia. Agarose gel of AhaII restriction digests using amplified exons 5 of aldolase B from DNA obtained from peripheral blood of the HFI donor (lane 1); the recipient before (lane 2) and after the transplant at 1, 3, 18, and 21 months (lanes 3, 4, 5, and 6), a healthy subject (lane 8), a heterozygous carrier of A149P (lane 9), and an HFI patient homozygous for A149P (lane 10). Lane 7 represents AhaII digestion of amplified DNA extracted from a saline mouthwash sample obtained from the transplant recipient 21 months after the transplant. Before transplantation, the donor was homozygous and the recipient heterozygous for the aldolase B gene mutation A149P. The proportion of the recipient's leucocyte cells with the A149P allele increased after the transplant, illustrating that the recipient had bone marrow derived cells from the donor. This observation of blood chimaerism, combined with the absence of recurrent disease symptoms four years after the transplant, confirms that the engraftment has been curative and that spontaneous
recovery of the aplastic state had not occurred as a result of cytotoxic conditioning therapy (courtesy of Drs P S Rohrlich and E Vilmer, Service Hemato-immunologie, Hôpital Robert Debré, Paris).
patients of European descent, A149P (accounting for 65% of European HFI alleles so far studied), has a wide distribution among European populations, including Ashkenazi Jews. It has been found to be prevalent in North America, where it accounts for 55% of HFI alleles studied and has recently been found in New Zealand.123 This allele is the most frequent cause of the disease in populations of northern European descent, accounting for over 85% of mutant aldolase B alleles that have been studied in the UK; it therefore has a powerful discriminatory capacity in relation to HFI diagnosis.68 124 These findings provide evidence that the mutation arose early during the evolution of modern human populations and possibly before the European expansion that characterised the late Bronze Age.124 The A174D allele, which is also widespread,120 accounts for 14% and 11% of HFI alleles so far studied in Europe and North America, respectively. It is particularly frequent in central and southern European locations.121 125

A study to investigate haplotype linkage analysis of A149P alleles, using allele specific oligonucleotides, has shown absolute association with informative linked aldolase B polymorphisms in intron 8 (c→t at nucleotide 84 and a→g at nucleotide 105) and provides convincing evidence that the mutation arose on a single ancestral chromosomes and spread throughout populations by a combination of mass migration and random genetic drift.126 These two biallelic single base pair polymorphisms were found to be in absolute linkage disequilibrium between themselves and are informative, since 47% of control subjects were found to be homozygous at these loci. Allele specific hybridisation in 15 HFI patients homozygous for the A149P aldolase B mutation showed that they were also homozygous for the intron 8 84T/105G alleles. These findings accord with the population frequency of these alleles in defined populations,127 allowing speculation as to their origin. The data on haplotype linkage thus indicate that parents who appear

Table 1 Human aldolase B gene mutations in hereditary fructose intolerance

<table>
<thead>
<tr>
<th>Trivial name*</th>
<th>Systematic designation†</th>
<th>Mutated codon‡</th>
<th>Ethnic distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>C134R</td>
<td>c.343T→C</td>
<td>C135R</td>
<td>Amerindian (private)</td>
</tr>
<tr>
<td>W147R</td>
<td>c.441T→C</td>
<td>W148R</td>
<td>Anglo-American (private)</td>
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<td>A149P</td>
<td>c.448G→C</td>
<td>A150P</td>
<td>European (widespread)</td>
</tr>
<tr>
<td>A174D</td>
<td>c.524C→A</td>
<td>A175D</td>
<td>S European (widespread)</td>
</tr>
<tr>
<td>L256F</td>
<td>c.770T→C</td>
<td>L257P</td>
<td>Italian (private)</td>
</tr>
<tr>
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<td>c.910C→T</td>
<td>R304W</td>
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</tr>
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<td>N334K</td>
<td>c.1005C→G</td>
<td>N335K</td>
<td>E European (widespread)</td>
</tr>
<tr>
<td>A337V</td>
<td>c.1013C→T</td>
<td>A338V</td>
<td>Turkish/Finnish/Swiss (rare)</td>
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<td>M-1T</td>
<td>c.27→C</td>
<td>M1T</td>
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</tr>
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<td>c.10C→T</td>
<td>R4X</td>
<td>Turkish (private)</td>
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<td>c.178C→T</td>
<td>R60X</td>
<td>Austria (rare)</td>
</tr>
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<td>c.603T→A</td>
<td>Y115X</td>
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<td>c.720C→A</td>
<td>C239X</td>
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<td>IVS 5+1G→C</td>
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<td>IVS 6→G→A</td>
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<tr>
<td>F13/A 4,5</td>
<td>g.7516-9165 del</td>
<td>L109-5160 del</td>
<td>French (private)</td>
</tr>
</tbody>
</table>

*Trivial name represents the mutated sequence of the inferred translation product after cleavage of the initiator methionine residue; similarly the codon number refers to the initiator methionine numbered as position +1.
†Mutations have been assigned according to current guidelines (Antonarakis SE (1996, 1997) website ariel.ucsf.edu.au:80/—cotton/antonara.htm; Hum Mutat (in press)).
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Consanguineous pedigrees observed the transition. Lase B mutations may result from transitions and C→T changes, which arise from mispairing in mispairs of strands during replication or occurred at a single ancestral event. However, in the absence of sufficient numbers for haplotype analysis, this question cannot be answered with certainty.

All the other genetic lesions in human aldolase B which have so far been identified and give rise to the disease (table 1) have not been convincingly detected in more than one country and so may represent unique mutations that remain confined to the local community (“private” mutations). Although CpG dinucleotides may represent mutational “hot spots” in genes, of the 15 base substitutions in the human aldolase B gene mutated in HFI, only three are C→T transitions and one is caused by a G→A transition.

From all the data that have been published on the identification of aldolase B mutations in patients with HFI, it has generally been observed that patients from non-consanguineous pedigrees tend to be homozygous for the prevalent aldolase B mutations, A149P, A174D, or N334K, or have a compound aldolase B genotype, having inherited at least one of these alleles. The rare exceptions to this observation have been a family from Italy, where symptomatic subjects had the aldolase B genotype M+17Y203ter, a pedigree of Italian-American descent with the associated patient had the R59ter/C134R genotype, and a single isolate from a North American kindred of Swiss-German ancestry, where the patient had a compound genotype, A4/W147R. Where consanguineous marriages are frequent they contribute towards homozygosity for rare mutant alleles of aldolase B in HFI as shown in several Turkish pedigrees. There are other illustrative examples; in a small community from Sicily the frameshift mutation L288ΔC was identified, the mutation R303W was present on both alleles of an Italian patient born to consanguineous parents, the aldolase B lesion, Δ3, which creates a 4bp deletion at the junction between intron 2 and exon 3, so removing the conserved AG splice site recognition signal, was found in the homozygous state in a member of a large consanguineous family affected by HFI from Switzerland, and a female Japanese child with HFI, whose parents were consanguineous, was homozygous for the mutation C239ter.

Several null alleles of aldolase B have been identified. These include a translation initiation mutation, nonsense mutations, splice site variants, and intragenic deletions of 1 bp to 1.65 kb (table 1). Since a significant proportion of the mutant alleles of aldolase B associated with HFI represent mutations causing synthesis of missense enzyme variants, identification of rare null alleles present in homozygous form allow the human aldolase B “knockout” to be compared with the phenotype where residual hepatic fructose 1-phosphate aldolase activity persists. Patients who have inherited two null alleles of aldolase B appear to be healthy, provided they avoid fructose, and are able to withstand starvation. This indicates that liver aldolase B activity is not critical for gluconeogenesis in the interprandial state. Aldolase B is thus a component of an accessory pathway that allows efficient metabolic assimilation of specific dietary sugars as part of the adaptive response to rapid nutritional changes; gluconeogenesis and glycolysis principally depend upon the ubiquitous aldolase A.

As far as we can establish, the presence of homozygosity for null alleles or missense variants does not determine the clinical severity of HFI. The symptoms and extent of organ damage appear principally to depend on the subject’s immediate nutritional environment. This is in part reflected by their level of education as well as the dietary habits of their culture and the acrality with which their parents recognised feeding difficulties presenting during infancy and childhood. Without the reason, in nearly all instances, the aldolase B mutations cause profound deficiency of fructose 1-phosphate aldolase activity and are effectively null mutations. In only one instance has asymptomatic HFI been associated with
disease solely under conditions of extreme fructose loading.\(^\text{11}\) Unfortunately, neither detailed enzymology nor molecular analysis of the putative mutant aldolase is available from this patient.

**MOLECULAR STUDIES**

Since human aldolase B is expressed only in the relatively inaccessible fructose metabolising tissues (the liver, kidney, and intestine), it is not easy to study precisely the effects of the mutations on enzymatic activity in situ. Liver biopsy specimens, although useful for the diagnosis of HFI by assay of fructaldolase activity,\(^\text{12}\) cannot be obtained without appreciable risk to the patient and usually provide too little tissue for detailed characterisation of mutant enzymes.

Nevertheless, studies using antibodies specific to each isozyme of human class I aldolase to investigate histological sections and for analysis of tissue extracts by immunoblotting showed that some patients with HFI express polypeptides that bind to aldolase B specific antibody.\(^\text{12} 13 14\) Experiments using double immunodiffusion gels showed that antiserum against human aldolase B partly activated the altered enzyme in several liver extracts from patients with HFI.\(^\text{16}\) An independent study by Cox et al.,\(^\text{15}\) using a radioimmunoassay procedure showed that the aldolase B from patients had a reduced affinity for the antibody, suggesting that the defect created a structural variant of aldolase B in these patients, with impaired catalytic function. Furthermore, these authors succeeded in purifying mutant aldolase B from tissue extracts obtained from a patient with HFI using immunoaffinity methods. They showed that the purified enzyme was catalytically inactive and possessed an identical electrophoretic charge to the wild type enzyme, although it had an apparent subunit molecular size of 30 000 daltons (wild type is 38 000 daltons).\(^\text{16}\) Subsequently, molecular analysis of aldolase B genes showed that the mutation causing HFI in this pedigree encoded the enzyme variant, Ala\(^{149}\)→Pro (A149P).\(^\text{17}\)

With the introduction of gene cloning to isolate human aldolase B cDNA and model host/vector expression systems, catalytically active recombinant human aldolase B has been successfully expressed in E. coli.\(^\text{12} 18\) Clearly, the missense variants of human aldolase B, obtained by site directed mutagenesis, that are responsible for disease are the most interesting to study at the molecular level using these techniques because they shed light on how subtle changes in critical residues of the protein affect its function. Hitherto, eight aldolase B missense mutations have been identified in patients with HFI (table 1). All mutated residues are invariant in the class I aldolases, except the alanine at position 149 which is B isozyme specific: aldolases A and C have a cysteine at this position.\(^\text{17}\) This suggests that the regions that harbour these mutations play an important role in the catalytic function or structural integrity of the normal tetrameric enzyme. Using in vitro mutagenesis with synthetic DNA oligonucleotides, it is possible to create mutant plasmids which encode the single amino acid substitutions so that the properties of the normal and mutant forms of recombinant human aldolase B can be subjected to detailed examination.

So far the disease causing aldolase B variants C134R and A149P have been synthesised in E. coli, to understand further the effects of the natural mutations on the enzyme molecule. Brooks and Tolan,\(^\text{19}\) who expressed the mutant aldolase B corresponding to the single base substitution C134R in E. coli, showed that although the mutant enzyme retained residual catalytic activity towards the fructose 1-phosphate substrate, the reduced levels of expression compared with wild type in this host/vector system suggested that structural instability of the partially active enzyme variant may be the cause of the disease phenotype. The A149P mutation appears to have a drastic effect on enzymatic activity and structure of aldolase B as indicated by immunological studies in tissues obtained from homozygous patients.\(^\text{20} 21\) The expressed protein is inactive towards its specific substrate, fructose 1-phosphate, with residual activity towards the fructose-1,6-bisphosphate substrate.\(^\text{22}\)

The schematic diagram of the three dimensional structure of human aldolase A (which is highly homologous to aldolase B) can be used to model the missense mutations in aldolase B associated with HFI (fig 5). The mutations C134R, W147R, A149P, and A174D on exon 5 all appear to be located in the vicinity of the substrate binding pocket and so are likely to disrupt important residues, including the C1 phosphate binding residues, Lys 146 and Arg 148, in the active site of the enzyme.\(^\text{23} 24\) In aldolase A, x ray diffraction of a resolution of 1.9 Å indicates that Lys 146 is sufficiently close to function as a proton donor or acceptor during carbamolamine intermediate formation,\(^\text{25}\) a function inhibited by engineered mutations of this residue.\(^\text{26}\)

The residues implicated in the N334K and A337V mutations (exon 9) occur in an α helix and affect the mobility of the conformationally flexible carboxy-terminal tail, a region which is thought to cover and mediate access of the substrate into the active site pocket.\(^\text{27}\) This site has long been considered critical for functional differentiation of the aldolase isoforms A, B, and C.\(^\text{27} 28\) It has been claimed that the wild type residue in R303 (exon 8) participates in binding of the phosphate at the 6-carbon position of fructose-1,6-bisphosphate, although this supposition is based on modelling of the aldolase A molecule rather than definitive x ray structural determinations and analysis.\(^\text{29}\) The change in charge and bulk of the side chain associated with this mutation is likely to affect the structural integrity of the molecule. An entirely different picture of the role of R303, however, has emerged from the solution of the aldolase A structure at the high resolution of 1.9 Å.\(^\text{30}\) It interacts with the C-terminal region residue Ghu 354 and forms a hydrogen bond with the C, carboxyl oxygen of bound dihydroxyacetone phosphate which is derived from the C, moiety of the hexose
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Figure 5. Schematic diagram of three dimensional structure of human aldolase A at 2.0 Ångström resolution (after Gamblin et al.,* with permission). The α helices and β sheets are represented as barrels and arrows, respectively. The carboxy-terminus, which is depicted as bold, is postulated to extend from the tail of helix H2 to the centre of the αβ barrel. The position of amino acid substitutions identified in the human aldolase B gene of patients with HFI are mapped onto the diagram.

substrate and stabilises the kTm form of the incoming substrate at the active site.

Tetramer formation required for functional aldolase B involves close contacts between hydrophobic residues; it may be the case that the exon 7 mutation, L256P, which appears to lie on the outer surface of the molecule disrupts this hydrophobic interaction, possibly resulting in a failure of tetrameric assembly and formation of aldolase subunits which, although catalytically active, are unstable. However, the exact consequences of all these mutations on the catalytic function of the aldolase B isoenzyme and integrity of the molecule will require systematic analysis of this particular human enzyme using protein engineering techniques. Detailed investigations of the catalytic function and definitive structure of the engineered aldolas, informed by computer modelling studies, will doubtless be critical for a full mechanistic understanding of this ancient enzyme family.

Diagnosis

Although HFI may be suspected in people who show aversions to sugary foods, confirmation of the diagnosis is important, particularly for the infant, so that a strict exclusion diet can be prescribed and the tissue injury and growth retardation can be avoided. Confirmatory studies hitherto have relied either on direct assay of fructaldolase activity in tissue biopsy specimens or on inducing the characteristic biochemical changes after carefully controlled administration of fructose (0.2-0.25 g/kg body weight) by intravenous infusion.* More recently, 31P nuclear magnetic resonance spectroscopy has been used successfully to show an increase in sugar phosphates and decrease in inorganic phosphate in the liver of HFI patients after a fructose load.* Indeed this method, and enzymatic assay of intestinal biopsy specimens, have been the only procedures known to date that allow the detection of otherwise asymptomatic carriers of HFI. Nevertheless, these techniques are either cumbersome, expensive, or have obvious risks; this, combined with the pain and inconvenience the subject may have to suffer, fully justifies efforts to develop direct methods for genetic diagnosis of HFI.

The discovery of the polymerase chain reaction* has allowed direct DNA analysis of aldolase B gene sequences, obtained from somatic cells of patients, to be developed for a non-invasive diagnosis of HFI. Although three mutations in the human aldolase B gene, A149P, A174D, and N334K, and more recently Δ4, A337V, R303W, and R59Ter have been found to be widely distributed, a more complete picture with respect to the global distribution of aldolase B mutations is required, otherwise genetic screening for HFI will be limited to specific populations in which the representative mutant alleles can be predicted with confidence. The pattern of mutation frequencies appears to conform to a prototype that has been found with several other genes: a few are prevalent but most are rare. The prevalent mutations, for example, A149P, A174D, and N334K, differ in frequency between different populations as with phenylketonuria. The high prevalence of A149P in the UK in particular is very remarkable and probably explained by a founder effect early in the colonisation of these islands. For diagnostic purposes in other populations, rare mutant alleles of aldolase B can be overlooked leading to misdiagnosis.

To detect whether a suspected person has a molecular lesion in the aldolase B gene, a simple protocol has been adopted in our laboratory.* Initial screening for the previously identified aldolase B mutations A149P, A174D (Ala 174→Asp), and N334K, which are a prevalent cause of HFI, is conducted on genomic DNA, using the PCR combined with restriction enzyme digestion or oligonucleotide hybridisation. A single mutant allele in the symptomatic patient confirms the diagnosis. Subjects who have wild type alleles at these loci and if their parents were unrelated by blood were studied further only if a positive intravenous fructose intolerance or enzymatic test had been obtained previously. However, patients from consanguineous marriages who complained of typical symptoms were investigated to find the mutant allele common to their parents. Direct sequencing of aldolase B exons, including the splicing signals, was used for scanning the gene to detect any unidentified mutations because it is systematic; clearly, as automated sequencing becomes generally available, it will offer an increasingly attractive means of effecting an accurate diagnosis of this disease. Once both mutant alleles of aldolase B were identified in a proband, this information could be used in the neonatal screening of sibs for HFI using either cord blood, Guthrie card material from the child as a source of genomic DNA template and before exposure to fructose. By these means we have been able
to counsel parents before weaning their offspring onto potentially harmful foods.121

Frequency of HFI in the population

An independent study from the University Children's Hospital, Zurich,155 has recently conducted a pilot study, to obtain an estimate of the population frequency of the mutant aldolase B allele, A149P, by systematic analysis of DNA obtained from Guthrie blood spots taken at birth.108 Blood spots obtained by heel prick of the newborn are collected routinely on a card and tested for various preventable diseases, including phenylketonuria (PKU) and congenital hypothyroidism. The dried blood spots provide an unbiased population for screening, which is also relatively inexpensive for the molecular diagnosis of HFI. Our study identified 27 A149P heterozygotes from 2050 unselected subjects born within a nine month period between 1994 and 1995 in the East Anglian region of the UK (frequency (2pq) ~1.3%). These data allow for a frequency (q') of 1 in 23 000 homozygotes to be predicted for this allele119 (giving a disease frequency of 1 in 18 000 assuming the A149P variant accounts for ~80% of mutant alleles).115,121 Clearly this has important implications for establishing interventional programmes for HFI.

It is noteworthy that whereas the mutant aldolase B allele A149P, which is widespread in white populations, has been shown to account for over 85% of HFI alleles which have so far been studied in the UK121 and so is diagnostically significant, diverse alleles give rise to the disease in other populations, such as the Italian population.120,122,123,126 So far, 10 aldolase B mutations, M-1T, ΔA20, ΔA, A149P, A174D, Y203ter, L256P, L288ΔC, R303W, and N334K, have been identified to be associated with HFI in Italy, which would make for a complex diagnostic strategy based on mutation analysis. Forthcoming advanced automated methods for detecting multiple gene defects will be ideally suited for mass screening in populations such as the United States, Italy, and Switzerland with diverse mutations in aldolase B.120,122

The aim of neonatal diagnosis is to introduce therapy before disease symptoms manifest themselves, thus maintaining the health and life quality of the affected subject. Although carrier detection has no direct health implications for the child, counselling and DNA testing might be justified when decisions about reproduction are made in later life, especially in populations where consanguineous marriages occur with appreciable frequency.

Conclusions

The nature of the molecular defect in the aldolase B genes of patients with HFI does not appear to affect the phenotype appreciably; however, the individual dietary experience appears to be correlated with disease severity. The widespread mutant alleles of aldolase B which have so far been identified in certain regions of the world prompt further studies to determine the frequency of aldolase B mutant
alleles in other populations and hence determine the extent to which neonatal screening for homozygotes by genetic testing is justified. To implement a general population screening programme for HFI, the benefits of such a programme must outweigh the cost of setting up the whole procedure and there should be provision for a considerable educational and counselling effort. Ethical considerations must also be taken into account including confidentiality issues, since future access could erroneously be considered to affect the insurance status of the homozygous or compound heterozygous person who, after all, would have an avoidable clinical disorder.

The use of expression vectors in E. coli has enabled recombinant aldolases to be generated in vitro, thus opening the way for experimental manipulation. Important studies are in progress to answer questions about the precise mechanism by which the human aldolase B folds, from its linear primary translation product to the active tertiary conformation and stable homotetramer. More importantly, physiological studies will be able to explore the tautalisation relationship between regional structure and the specialised biological function of each isozyme. The naturally occurring missense mutations associated with HFI are useful in that they focus attention on critical residues in regions of the molecule which are required for aldolase B catalytic function. Detailed biochemical analysis of purified recombinant aldolases, including their crystal structure in the presence of the five known substrates, is likely to reveal much about this ancient but highly differentiated model enzyme system.

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