Review article

Hereditary fructose intolerance

Manir Ali, Peter Rellos, Timothy M Cox

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 homozygotes develop hypoglycaemic and severe abdominal symptoms after taking foods containing fructose and cognate 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.
(*J Med Genet* 1998;35:353–365)

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-glyceraldehyde 3-phosphate, and D-glyceraldehyde[1] (fig 1). There are two different processes for the generation of the stable carbanion substrate enzyme complexes involved in the aldolase reaction: aldolases of the class II type, which are confined to yeasts and prokaryotes, form the carbanion using a bivalent metal cation (Zn^{2+}).[2] 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.[3]

There are three genetically distinct isozymes of class I vertebrate aldolase which can be distinguished on the basis of their antigenic and catalytic properties.[4 6] 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 X07092) is present in the brain. Aldolases A and C are constitutively expressed, but the B isoform is under dietary control.[7] 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
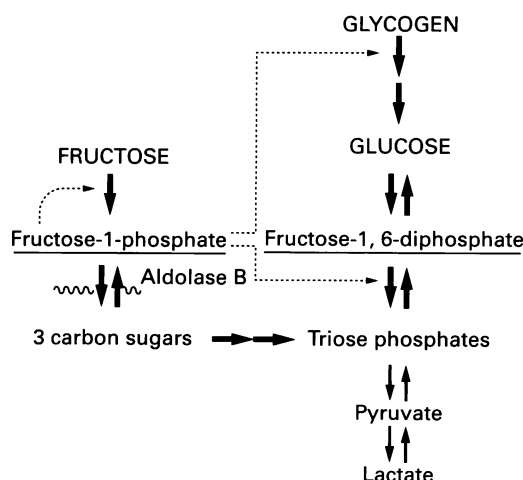

Figure 1 The metabolism of fructose in normal subjects. The enzyme aldolase B catalyses the reversible cleavage of fructose 1-phosphate in the specialised pathway of fructose metabolism, as well as fructose-1,6-bisphosphate in the glycolytic/gluconeogenic pathway. 3-carbon sugars: D-glyceraldehyde 3-phosphate, D-glyceraldehyde, dihydroxyacetone phosphate. Dotted arrows indicate point of secondary metabolic arrest in aldolase B deficiency (see text).

University of Cambridge, Department of Medicine, Level 5, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK
M Ali*
P Rellos
T M Cox

Correspondence to: Professor Cox.

*Present address: Molecular Medicine Unit, Clinical Sciences Building, University of Leeds, St James's University Hospital, Leeds LS9 7TF, UK.

enzyme is activated by dissociation of the aldolase B-cytoskeletal protein complex in the presence of accumulated intermediate metabolites[8-13] 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.[14-16] 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.[17 18]

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),[19 20] the demethylation of a cytosine residue (position -129),[21] and the simultaneous appearance of transcription factors, AIF-A (identical to liver specific factor HNF-1[22]) 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.[23 24] 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[25 26] suggesting that competition between these activators/repressors regulates aldolase B gene expression.[27] Recently, Sabourin et al[28] 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 isozyme 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.[6 29-31] The aldolases show less affinity for the fructose 1-phosphate substrate (aldolase B K_M ~1 mmol/l), compared with the bisphosphate, (aldolase B K_M 50 μmol/l).[6 32 33] The similarity between the isozymes, in terms of their structural and general catalytic properties, led Rutter[34] to conclude that the isozymes evolved by a process of gene duplication and divergence, according to the mechanism proposed by Ingram[35] to explain the differences observed in the haemoglobins. 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.[36]

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.[14 37-39] 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.[40 41] 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[42 43] and the A domain of pyruvate kinase,[44] as well as 14 other enzymes with little sequence, but significant structural homology (reviewed by Farber and Petsko[45]). Since human aldolase B has 69% amino acid identity to its paralogue 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,[46] the substrate binding residues Lys 146, Arg 148,[47] and Lys 229 (which forms the Schiff base[3]) 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,[40] experiments on the evolutionarily conserved terminal tyrosine residue, either by carboxypeptidase treatment[1 48] or site directed mutagenesis of recombinant aldolase expressed in *Escherichia coli*,[49] 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.[40] 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.[41]

The gene for human aldolase B has been mapped to chromosome 9q22.3.[50-52] The structural organisation shows 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.[53] The cloning of the aldolase B cDNA shows that the mRNA encodes a 364 amino acid polypeptide including the initiator methionine.[54] The A and C isoforms of human aldolase have also been cloned and their cognate genes map to chromosomes 16 and 17, respectively.[36 55] Kitajima et al[56] observed that there are seven common conserved and four isozyme group specific regions in the sequences.[56] The carboxyl tail shows significant sequence divergence, thus the unique functional properties of the individual isozymes may be invested in this region. Indeed, there is clear evidence to support the contention that isozyme 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.[57] The single primary transcript undergoes alternative splicing to give rise to the three isozyme specific aldolases with variable carboxy-terminal sequences and differing catalytic properties.[58 59]

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.[60]

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 "idiosyncrasy 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.[62 63] Infants with this condition are the most vulnerable to exposure of dietary fructose, especially at weaning.[64 65] 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 unbeknown 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.[64-66]

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 domain[68] 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.[69-71]

A particular hazard for people with undiagnosed HFI has been the indiscriminate use of fructose infusions as a source of parenteral feeding[72 73]; more than 20 fatal or near fatal instances resulting from this cause have been and continue to be reported in HFI[74-77] (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,[78] their use is not only fatal in patients with HFI,[79 80] but is potentially toxic in normal subjects.[81 82] 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.[83 84] 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 Medic-Alert 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 Pratt[61] is alive and well known to the authors. She is in good physical health at the age of 67 years and is a grandparent.

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
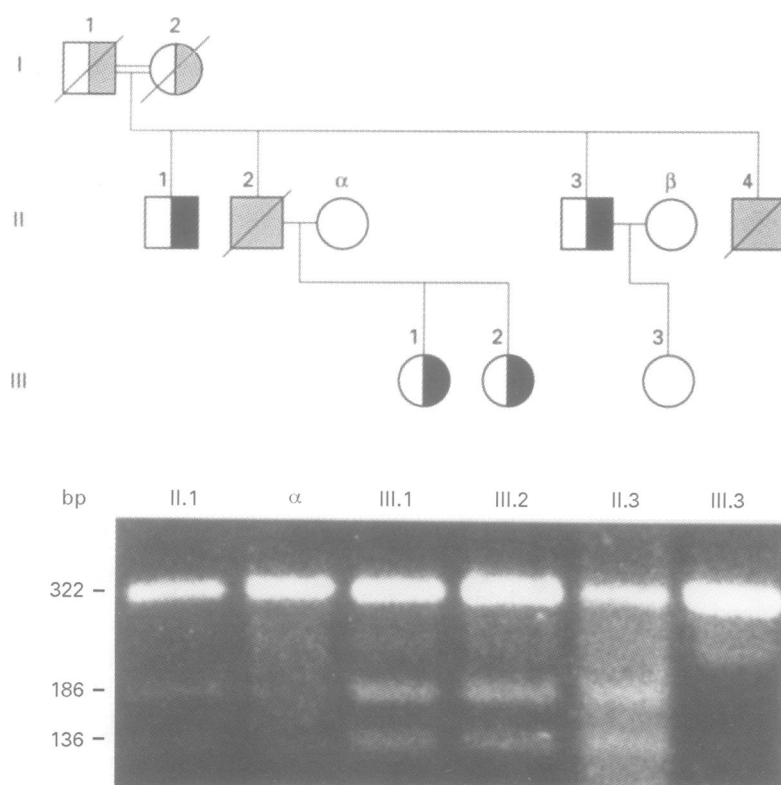
Figure 2   Retrospective diagnosis of HFI by molecular analysis of aldolase B genes. The unexpected death in a German hospital of a local 42 year old patient (II.2) with a history of peculiar eating habits (disliked sweet foods), who received intravenous infusions of fructose and its derivative, sorbitol, to correct dehydration after a minor diarrhoeal illness, as well as the death of his younger brother (II.4) under similar circumstances, prompted lawyers representing the widow and family to request analysis of aldolase B genes in surviving first degree relatives. Agarose gel of PCR amplified DNA containing exon 5 fragments of the aldolase B gene after digestion with the restriction endonuclease AhaII.[117] Partial cleavage of the 322 base pair fragment into 186 and 136 base pairs confirms the presence of a single copy of the aldolase B mutant allele A149P. Both daughters, as well as the surviving brothers, of the dead man (II.2) are indeed carriers of HFI suggesting that the dead brothers had fructose intolerance having inherited a single copy of the disease allele from each of their consanguineous parents. Dark shading, genotype determined from the gel; light shading, genotype determined by inference; I.1 and I.2, natural death; II.2 and II.4, premature death.

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.[63 90 91] 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[92]: 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.[93] The biochemical changes described here immediately accompany the acute symptoms of sugar toxicity in HFI.[64 65]

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.[90 94] The reduced Pi concentration activates adenosine deaminase and the xanthine oxidase pathways, with degradation of purine nucleotides to uric acid.[95-97] Reduced intracellular concentrations of inorganic phosphate lead directly to the allosteric activation of adenosine deaminase.[98] The increased formation of uric acid leads to hyperuricaemia, a metabolic effect of fructose administration observed to a limited extent in normal subjects.[81] More recently, it has been observed that the hyperuricaemic effect is more pronounced in HFI heterozygotes than in control subjects,[99] 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 Mg^{2+} ions[100] by dissolution of the Mg-ATP complex, so causing the hypermagnesaemia observed in HFI patients following challenge with fructose.[63 101] 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.[102] As a result, transient fructosaemia (an outmoded term for hereditary fructose intolerance) and fructosuria are observed.[103 104]

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 interprandial plasma glucose release[104] without evidence of hyperinsulinism. The failure of parenteral glucagon to correct the hypoglycaemia indicates

is rapidly phosphorylated into fructose 1-phosphate[86 87] by the action of fructokinase.[88] In this specialised pathway, fructose 1-phosphate is cleaved by aldolase B, thus splitting the asymmetrical 6-carbon sugar into D-glyceraldehyde and dihydroxyacetone phosphate.[32] These intermediary triose products can be incorporated directly either into the glycolytic pathway (with the eventual formation of lactate and pyruvate, which act as substrates for the Krebs' cycle) or the gluconeogenic pathway to provide a source of glucose, or the synthesis of glycogen.

Investigations have been conducted to determine which aspects of fructose metabolism are impaired in patients with HFI. Froesch et al[89] incubated liver biopsy specimens with radiolabelled fructose and found that in patients with HFI the incorporation of fructose into glycogen was less than 6% of normal. This suggests that formation of glycogen from fructose is principally catalysed by aldolase B and that the activity of any alternative pathways for the direct conversion of fructose 1-phosphate to fructose-1,6-bisphosphate in human liver tissue is limited.

defective glycogenolysis. Although infusions of galactose raise the blood glucose concentration (indicating that the phosphoglucomutase and galactokinase pathways are intact),[91][105] a lack of response to dihydroxyacetone shows that gluconeogenesis is also impaired. The exact mode of inhibition of glycogenolysis is unknown: in vitro studies suggest that increased concentrations of fructose 1-phosphate, combined with the decrease in inorganic phosphate, block glycogen breakdown at the level of phosphorylase.[106-108] Fructose 1-phosphate also prevents the formation of the gluconeogenic intermediates, fructose-1,6-bisphosphate and glucose 6-phosphate, by competitive effects on aldolase A and glucose 6-phosphate isomerase, respectively.[109][110] 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 fasting.

Impairment of gluconeogenesis after fructose intake in patients with HFI combined with fructose 1-phosphate induced activation of pyruvate kinase[111] results in accumulation of the Krebs' cycle precursors, alanine, lactate, and pyruvate. This contributes to amino acidaemia, as well as metabolic acidosis.[82][112] Impaired function of the proximal renal tubule aggravates the acidosis and results in generalised amino aciduria, phosphaturia, and bicarbonate wasting.[113][114] As a result, adolescents present with metabolic bone disease and stunting of growth.[66] Defective acidification of the urine, as well as phosphaturia and amino aciduria, represents an acquired form of the Fanconi syndrome that has been well documented in HFI.[113] The cause of the abdominal pain[115] that is a feature in HFI remains unexplained. This pain, which follows ingestion of the offending sugars within a few minutes, may reflect autonomic afferent activity induced by release of purine nucleotides, loss of energy charge, or increased concentrations of gluconeogenic precursors such as lactate, but proof of a cause and effect relationship 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, including the synthesis of desialylated plasma transferrin isoforms.[116]

GENETIC STUDIES

Information obtained from the cloning and characterisation of the wild type human aldolase B gene[53][54] allowed Cross and colleagues 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.[117][118] 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 transversion 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 expected for a recessive disease.[68][117] 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 endonuclease *Aha*II and its isoschizomers, has been used for molecular diagnosis[68] and confirmatory testing after large scale genotyping by allele specific hybridisation.[119] 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 sequencing 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 putative 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.

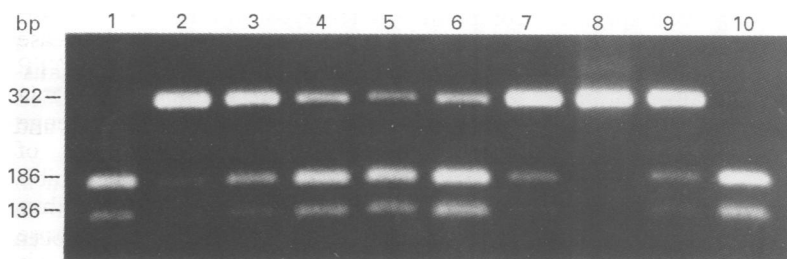As established by Cross *et al*[120] and Cox,[121] the most prominent allele causing HFI in

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 heterozygote 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 any 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).

Table 1 Human aldolase B gene mutations in hereditary fructose intolerance

Trivial name*	Systematic designation†	Mutated codon†	Ethnic distribution	Ref
C134R	c.343T→C	C135R	Amerindian (private)	135
W147R	c.441T→C	W148R	Angloamerican (private)	133
A149P	c.448G→C	A150P	European (widespread)	117
A174D	c.524C→A	A175D	S European (widespread)	120
L256P	c.770T→C	L257P	Italian (private)	149
R303W	c.910C→T	R304W	Turkish/Italian (rare)	130, 132
N334K	c.1005C→G	N335K	E European (widespread)	127
A337V	c.1013C→T	A338V	Turkish/Finnish/Swiss (rare)	130
M-1T	c.2T→C	M1T	Italian (private)	136
R3ter	c.10C→T	R4X	Turkish (private)	134
R59ter	c.178C→T	R60X	Austria (rare)	134, 135
Y203ter	c.672T→A	Y204X	Italian (private)	136
C239ter	c.720C→A	C239X	Japanese (private)	138
G→C,5' intron 5	IVS 5+1G→C	—	New Zealand (private)	130, 123
G→A,3' intron 6	IVS 6-1G→A	—	French (private)	134
Q20ΔA	c.62 del A	Q21 del	Italian (private)	132
L288ΔC	c.865 del C	L289 del	Sicilian (private)	120
G12/Δ3	IVS2-1G del GGTA	G38T39 del	Swiss (private)	137
Δ4/Δ4	c.360-363 del CAAA	N120 K121 del	European (widespread rare)	128, 133
Δ7+1 3' intron 8	IVS8-1G del GGCTAAC ins G	A334 N335 del	American (private)	164
G10/Δ 6,7	g.9912-10836 del	N181-G267 del	Swiss (private)	137
F13/Δ 4,5	g.7516-9165 del	L109-5160 del	French (private)	137

*Trivial name represents the mutated sequence of the inferred translation product after cleavage of the initiator methionine residue; similarly the codon mutation refers to the initiator methionine numbered as position (+)1.

†Mutations have been assigned according to current guidelines (Antonarakis SE (1996, 1997) website ariel.ucs.unimelb.edu.au:80/~cotton/antonara.htm; *Hum Mutat* (in press)).

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[122] 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][120] 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[121] and North America,[122] 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 heterozygous 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 prevalence of these alleles in defined populations[121] allowing speculation as to their origin. The data on haplotype linkage thus indicate that parents who appear

not to be consanguineous, but give rise to offspring with the A149P homozygous aldolase B genotype, are thus likely to be related by blood, albeit distantly.

Another mutant allele of human aldolase B associated with HFI, N334K,[127] occurs principally in central and eastern Europe, with possible origins in Balkan populations.[121] The intragenic deletion mutation in exon 4 of the aldolase B gene, Δ4, which was originally described in a British patient with HFI,[128][129] has since been identified in a German patient[130] as well as six unrelated families with HFI from Italy[131][132] and a unique extended North American pedigree of Swiss-German ancestry.[133] Hence, the Δ4 deletion appears to be widespread (though not as frequent as A149P) among European populations. The aldolase B mutation A337V has been shown to occur in three independent isolates.[130] Originally, the allele was found in homozygous form in a patient from Turkey, who was the offspring of a consanguineous marriage; after screening for this mutation, it has been detected in a compound heterozygous form in families affected by HFI from Switzerland (fig 4) and Finland. More recently, the aldolase B missense mutation R303W has been identified independently in a patient from Italy and Turkey.[130][132]

It is notable that the nonsense mutation in the aldolase B gene, R59ter, found in an Austrian patient with HFI[134] has also been reported in an Italian-American and a Native American from British Columbia, who also have the disease.[135] Although isolates of Δ4, A337V, R303W, and R59ter may each be derived from a single ancestral event and have spread by genetic drift, similar to the mutant alleles A149P and A174D, they may have arisen recurrently and more recently as a result of mutation at "hot spots" in the aldolase B locus. Indeed the mutations giving rise to A337V, R303W, and R59ter represent a C→T base transition (G→A in the antisense strand), the most frequently observed point mutation in
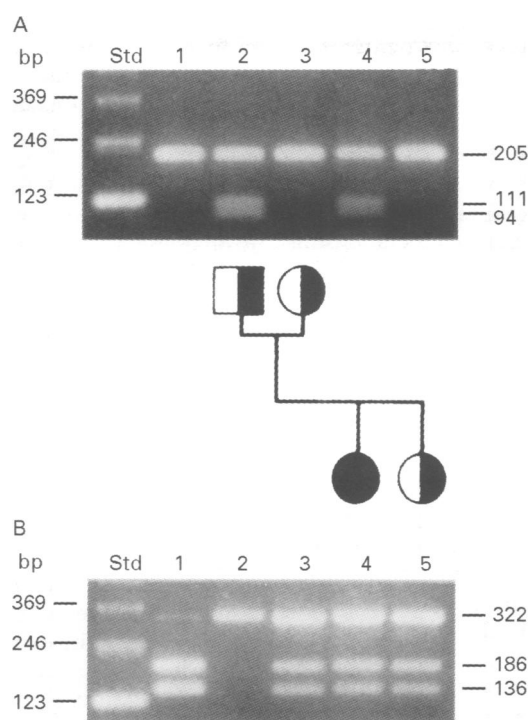
Figure 4 Compound heterozygosity for aldolase B mutations A337V and A149P in a family from Switzerland with HFI. (A) Agarose gel of MscI digests of PCR amplified exons 9 from a healthy subject (lane 1) and the Swiss family members (lanes 2-5). Cleavage of the 205 base pair fragment into two fragments of 111 and 94 base pairs indicates the presence of the A337V mutation. (B) Agarose gel of AhaII digests of exon 5 fragments of aldolase B from an A149P homozygote HFI patient (lane 1) and the Swiss family members (lanes 2-5). Digestion of the 322 base pair product into 186 and 136 base pairs signifies the presence of the A149P allele. The mendelian segregation of the A337V allele in the family and its association with the disease phenotype only in subjects with an additional aldolase B mutation on the other allele, together with the observation that no other mutation has been identified in cis, indicates that Ala 337→Val inactivates the aldolase B molecule.

eukaryotic DNA. Similarly, the event which gives rise to the Δ4 allele may represent a hot spot for recurrent frameshift mutations. The CAAA deletion occurs in a run of repeated sequence, suggesting that it may have been mediated by recurrent DNA polymerase slippage resulting in mispairing 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 muta-

tions, A149P, A174D, or N334K, or have a compound aldolase B genotype, having inherited at least one of these alleles.[121] The rare exceptions to this observation have been a family from Italy, where symptomatic subjects had the aldolase B genotype M-1T/Y203ter,[136] a pedigree of Italian-American descent where the affected patient had the R59ter/C134R genotype,[135] and a single isolate from a North American kindred of Swiss-German ancestry, where the patient had a compound genotype, Δ4/W147R.[133] Where consanguineous marriages are frequent they contribute towards homozygosity for rare mutant alleles of aldolase B in HFI, as shown in several Turkish pedigrees.[130 134] There are other illustrative examples: in a small community from Sicily the frameshift mutation L288ΔC was identified,[120] the mutation R303W was present on both alleles of an Italian patient born to consanguineous parents,[132] the aldolase B lesion, Δ3, which creates a 4 bp 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,[137] and a female Japanese child with HFI, whose parents were consanguineous, was homozygous for the mutation C239ter.[138]

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,[118 129 139 140] 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[120 134 136 138] 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 alacrity with which their parents recognised feeding difficulties presenting during infancy and childhood. Whatever 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.[141] 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,[60] 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.[118 129 143] 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.[140] An independent study by Cox et al,[118] using a radioimmunotitration 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 39 100 daltons (wild type is 38 000 daltons).[118] Subsequently, molecular analysis of aldolase B genes showed that the mutation causing HFI in this pedigree encoded the enzyme variant, Ala[149]→Pro (A149P).[117]

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*.[121 144] 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.[117] 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,[135] 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.[117 118] The expressed protein is inactive towards its specific substrate, fructose 1-phosphate, with residual activity towards the fructose-1,6-bisphosphate substrate.[121]

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 C-1 phosphate binding residues, Lys 146 and Arg 148, in the active site of the enzyme.[40 145 146] 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 carbinolamine intermediate formation,[41] a function inhibited by engineered mutations of this residue.[146]

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.[40] This region has long been considered critical for functional differentiation of the aldolase isozymes A, B, and C.[56 147 148] 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.[145] 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.[41] It interacts with the C-terminal region residue Glu 354 and forms a hydrogen bond with the C_2 carbonyl oxygen of bound dihydroxyacetone phosphate which is derived from the C_1 moiety of the hexose
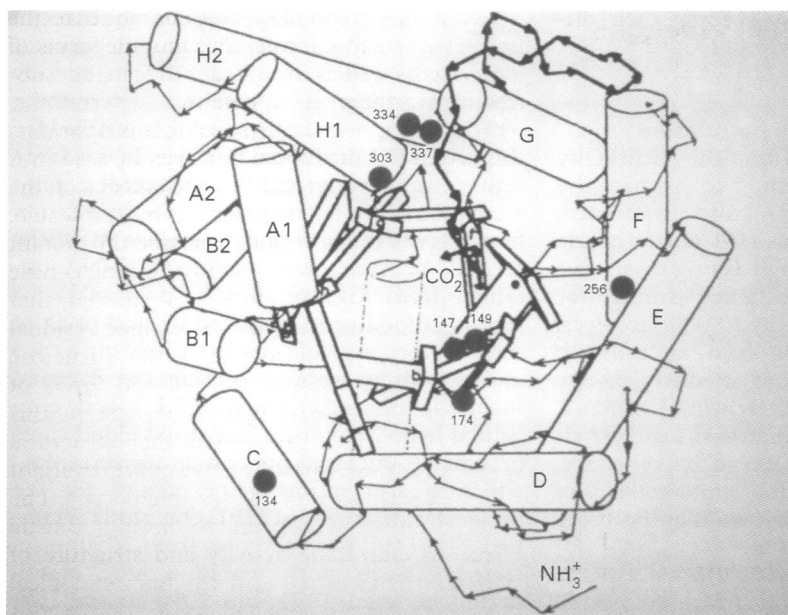
Figure 5   Schematic diagram of three dimensional structure of human aldolase A at 2.0 Angström resolution (after Gamblin et al,[145] with permission). The α helices and β sheets are represented as barrels and arrows, respectively. The carboxy-terminus, which is depicted as bold, is postulated to extend from the tail of helix H2 to the centre of the α/β 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 keto form of the incoming substrate at the active site.

Tetramer formation required for functional aldolase B involves close contacts between hydrophobic residues[40]; it may be the case that the exon 7 mutation, L256P,[149] 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.[150] [151] However, the exact consequences of all these mutations on the catalytic function of the aldolase B isozyme 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 aldolases, 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.[65] [83] [84] Confirmatory studies hitherto have relied either on direct assay of fructaldolase activity in tissue biopsy specimens[60] [142] [152] or inducing the characteristic biochemical changes after carefully controlled administration of fructose (0.2-0.25 g/kg body weight) by intravenous infusion.[89] [153] More recently, ^{31}P 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.[99] Indeed this method, and enzymatic assay of intestinal biopsy specimens,[152] 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[154] 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.[130] Initial screening for the previously identified aldolase B mutations A149P,[117] A174D (Ala 174→Asp), and N334K,[120] [127] 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 or 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 off-spring onto potentially harmful foods.[121]

Frequency of HFI in the population

An independent study from the University Children's Hospital, Zurich,[155] to estimate the prevalence of hereditary fructose intolerance, recorded five patients with HFI out of nearly 100 000 live births in a single centre over a five year period. Thus, the frequency estimate for HFI was approximately 1 in 20 000; however, the confidence limits for such an estimate based on small numbers of affected subjects would be wide (approximately 1 in 11 000 to 1 in 100 000). Even so, paediatricians and physicians consider the condition to be very rare, despite growing evidence that mutant alleles of aldolase B causing the disease may be more frequent than at first realised.

(1) As a rule, the parents of affected patients are not consanguineous. For rare autosomal recessive diseases, clearly, as noted by Garrod,[156] consanguinity would be expected.

(2) There are numerous reports of parent to offspring transmission resulting from the marriage between affected homozygotes with asymptomatic heterozygous carriers.[133 152 157–159] Because this disorder is inherited as a recessive trait, this observation signifies an appreciable frequency of mutant alleles in the population at large. Indeed, several large pedigrees affected by HFI have been reported that suggest, in the absence of consanguinity, that mutant alleles of aldolase B are prevalent.[91 133 152 160] In one family,[133] 10 affected subjects were identified because of the independent segregation of four mutant alleles (Δ4, W147R, A149P, A174D); there was an example of pseudodominant vertical transmission of disease and all affected patients were compound heterozygotes; no consanguinity was identified.

(3) After surviving the initial traumatic period of weaning and infancy, many affected subjects as adults adjust their eating habits accordingly and can live a normal life. In this way, they escape formal diagnosis. However, people continue to come forward in response to articles in the lay press about the disorder.[68 123]

(4) The effects of administration of fructose based solutions during surgery have resulted in at least 17 deaths of patients not previously known to be suffering from HFI.[75 76 133 136 161–163]

Since heterozygotes, who are asymptomatic, manifest no obvious advantage compared with normal subjects, there is no known mechanism to account for the prevalence of aldolase B mutations, apart from the observation that affected homozygotes have a reduced incidence of dental caries. This may have favourably contributed to fitness in earlier times. It has been suggested that although mutant alleles of aldolase B may have been accumulating over the years as a result of genetic drift, homozygotes would have escaped detection when sugar, honey, and fruit were scarce. Only the increasing consumption of sugars in the western diet over the last century has led to the emergence of HFI as a prevalent disorder.[121]

Given the potentially severe consequences of untreated fructose intolerance and the value of diagnosis as well as the efficacy of strict dietary treatment, there is a case for determining whether mass neonatal screening is justified for this avoidable nutritional disorder. In this laboratory we have 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.[119] 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^2) of 1 in 23 000 homozygotes to be predicted for this allele[119] (giving a disease frequency of 1 in 18 000 assuming the A149P variant accounts for ~80% of mutant alleles).[68 121] 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 UK[121] and so is diagnostically significant, diverse alleles give rise to the disease in other populations, such as the Italian population.[120 125 131 136 149] So far, 10 aldolase B mutations, M-1T, ΔA20, Δ4, 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, physical studies will be able to explore the tantalising 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 mutant aldolases B, 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.

1 Rutter WJ, Woodfin BM, Blostein RE. Enzymic homology. Structural and catalytic differentiation of fructose diphosphate aldolase. *Acta Chem Scand* 1963;17:5226.
2 Van der Heiden BS, Meinhart JO, Dodson RG, Krebs EG. The preparation and properties of crystalline yeast aldolase. *J Biol Chem* 1962;237:2095-8.
3 Horecker BL, Pontremoli S, Ricci C, Cheng T. On the nature of the transaldolase-dihydroxyacetone complex. *Proc Natl Acad Sci USA* 1961;47:1949-55.
4 Blostein R, Rutter WJ. Comparative studies of liver and muscle aldolase. II 44. Immunochemical and chromatographic differentiation. *J Biol Chem* 1963;238:3280-5.
5 Penhoet E, Rajkumar T, Rutter WJ. Multiple forms of fructose diphosphate aldolase in mammalian tissues. *Proc Natl Acad Sci USA* 1966;56:1275-82.
6 Penhoet EE, Kochman M, Rutter WJ. Isolation of fructose diphosphate aldolases A, B and C. Molecular and catalytic properties of aldolase C. *Biochemistry* 1969;8:4391-402.
7 Munnich A, Besmond C, Darquy S, et al. Dietary and hormonal regulation of aldolase B gene expression. *J Clin Invest* 1985;75:1045-52.
8 Adelman RC, Spolter PD, Weinhouse S. Dietary and hormonal regulation of enzymes of fructose metabolism in rat liver. *J Biol Chem* 1966;241:5467-72.
9 Rosensweig NS, Stifel FB, Herman RH, Zakim D. Time response of diet-induced changes in human jejunal glycolytic enzymes. *Fed Proc* 1969;28:323.
10 Arnold H, Pette D. Binding of aldolase and triosephosphate dehydrogenase to F-actin and modification of catalytic properties of aldolase. *Eur J Biochem* 1970;15:360-6.
11 Weiss TL, Zieske JD, Bernstein IA. Reversible microsomal binding of hepatic aldolase. *Biochim Biophys Acta* 1981;661:221-9.
12 Sato JI, Tsutsumi KI, Ishikawa M, Ishikawa K. Dietary regulation of aldolase isozyme expression in rat intestinal mucosa. *Arch Biochem Biophys* 1987;254:116-23.
13 Agius L. Substrate modulation of aldolase B binding in hepatocytes. *Biochem J* 1996;315:651-8.
14 Lebherz HG, Rutter WJ. Distribution of fructose diphosphate aldolase variants in biological systems. *Biochemistry* 1969;8:109-21.
15 Hommes FA, Draisma MI. The development of L- and M-type aldolases in rat liver. *Biochim Biophys Acta* 1970;222:251-2.
16 Numazaki M, Tsutsumi K, Tsutsumi R, Ishikawa K. Expression of aldolase isozyme mRNAs in fetal rat liver. *Eur J Biochem* 1984;142:165-70.
17 Schapira F, Dreyfus J-C, Schapira G. Anomaly of aldolase in primary liver cancer. *Nature* 1963;200:995-6.
18 Matsushima T, Kawabe S, Shibuya M, Sugimura T. Aldolase isozymes in rat tumor cells. *Biochem Biophys Res Commun* 1968;30:565-70.
19 Tsutsumi K, Tsutsumi R, Ishikawa K. Tissue-specific changes in chromatin structure of the rat aldolase B locus. *J Biochem* 1987;102:1013-21.
20 Zhao Y, Tsutsumi R, Yamaki M, Nagatsuka Y, Ejiri S, Tsutsumi K. Initiation zone of DNA replication at the aldolase B locus encompasses transcription promoter region. *Nucleic Acids Res* 1994;22:5385-90.
21 Daimon M, Tsutsumi K, Ishikawa K. DNA methylation and the regulation of aldolase B gene expression. *J Biochem* 1986;100:1279-86.
22 Courtois G, Baumhueter S, Crabtree GR. Purified hepatocyte nuclear factor 1 interacts with a family of hepatocyte-specific promoters. *Proc Natl Acad Sci USA* 1988;85:7937-41.
23 Tsutsumi K, Ito K, Ishikawa K. Developmental appearance of transcription factors that regulate liver-specific expression of the aldolase B gene. *Mol Cell Biol* 1989;9:4923-31.
24 Tsutsumi K, Ito K, Yabuki T, Ishikawa K. AIF-B, a novel CCAAT-binding transcription activator that interacts with the aldolase B promoter. *FEBS Lett* 1993;321:51-4.
25 Gregori C, Kahn A, Prichard AL. Activity of the rat liver-specific aldolase B promoter is restrained by HNF3. *Nucleic Acids Res* 1994;22:1242-6.
26 Ito K, Tsutsumi K, Kuzumaki T, Gomez PF, Otsu K, Ishikawa K. A novel growth inducible gene that encodes a protein with a conserved cold-shock domain. *Nucleic Acids Res* 1994;22:2036-41.
27 Gregori C, Kahn A, Pichard AL. Competition between transcription factors HNF1 and HNF3, and alternative cell-specific activation by DBP and C/EBP contribute to the regulation of the liver-specific aldolase B promoter. *Nucleic Acids Res* 1993;21:897-903.
28 Sabourin JC, Kern AS, Gregori C, et al. An intronic enhancer essential for tissue-specific expression of the aldolase B transgenes. *J Biol Chem* 1996;271:3469-73.
29 Peanasky RJ, Lardy HA. Bovine liver aldolase. I. Isolation, crystallisation and some general properties. II. Physical and chemical measurements on the crystalline enzyme. *J Biol Chem* 1958;233:365-73.
30 Penhoet E, Kochman M, Valentine R, Rutter WJ. The subunit structure of mammalian fructose diphosphate aldolase. *Biochemistry* 1967;6:2940-9.
31 Horecker BL, Tsolas O, Lai CY. Aldolases. In: Boyer PD, ed. *The enzymes*. Vol 7. 3rd ed. New York: Academic Press, 1972:213-58.
32 Hers HG, Kusaka T. Le metabolisme du fructose-1-phosphate dans le foie. *Biochim Biophys Acta* 1953;11:427-30.
33 Rutter WJ, Woodfin BM, Blostein RE. Enzymic homology. Structural and catalytic differentiation of fructose diphosphate aldolase. *Acta Chem Scand* 1963;17:5226.
34 Rutter WJ. Evolution of aldolase. *Fed Proc Fed Am Soc Exp Biol* 1964;23:1248-57.
35 Ingram VM. *The hemoglobins in genetics and evolution*. New York: Columbia University Press, 1963.
36 Tolan DR, Niclas J, Bruce BD, Lebo RV. Evolutionary implications of the human aldolase-A, -B, -C, and -pseudogene chromosome locations. *Am J Hum Genet* 1987;41:907-24.
37 Kawahara K, Tanford C. The number of polypeptide chains in rabbit muscle aldolase. *Biochemistry* 1966;5:1578-84.
38 Morse DE, Chan W, Horecker BL. The subunit structure and carboxy-terminal sequence of rabbit muscle aldolase. *Proc Natl Acad Sci USA* 1967;58:628-34.
39 Sia CL, Horecker BL. The molecular weight of rabbit muscle aldolase and the properties of the subunits in acid solution. *Arch Biochem Biophys* 1968;123:186-94.
40 Sygush J, Beaudry D, Allaire M. Molecular architecture of rabbit skeletal muscle aldolase at 2.7-Å resolution. *Proc Natl Acad Sci USA* 1987;84:7846-50.
41 Blom N, Sygusch J. Product binding and role of the C-terminal region in class 1 D-fructose 1,6-bisphosphate aldolase. *Nature Struct Biol* 1997;4:36-9.
42 Banner DW, Bloomer AC, Petsko GA, et al. Structure of chicken muscle triose phosphate isomerase determined crystallographically at 2.5 Å resolution. *Nature* 1975;255:609-14.
43 Alber T, Banner DW, Bloomer AC, et al. On the three-dimensional structure and catalytic mechanism of triose phosphate isomerase. *Philos Trans R Soc Lond B Biol Sci* 1981;293:159-71.
44 Stuart DI, Levine M, Muirhead H, Stammers DK. Crystal structure of cat muscle pyruvate kinase at a resolution of 2.6 Å. *J Mol Biol* 1979;134:109-42.
45 Farber GK, Petsko GA. The evolution of α/β barrel enzymes. *Trends Biol Sci* 1990;15:228-34.
46 Lai CY, Nakai N, Chang D. Amino acid sequence of rabbit muscle aldolase and the structure of the active center. *Science* 1974;183:1204-6.

47 Hartman FC, Brown JB. Affinity labelling on a previously undetected essential lysyl residue in class I fructose bisphosphate aldolase. *J Biol Chem* 1976;251:3057-62.

48 Drechsler ER, Boyer PD, Kowalsky AG. The catalytic activity of carboxy peptidase-degraded aldolase. *J Biol Chem* 1959;234:2627-34.

49 Takahashi I, Takasaki Y, Hori K. Site-directed mutagenesis of human aldolase isozymes: the role of Cys-72 and Cys-338 residues of aldolase A and of the carboxy-terminal Tyr residues of aldolases A and B. *J Biochem* 1989;105:281-6.

50 Henry I, Gallano P, Besmond C, et al. The structural gene for aldolase B (ALD B) maps to 9q13→32. *Ann Hum Genet* 1985;49:173-80.

51 Lebo RV, Tolan DR, Bruce BD, Cheung MC, Kan YW. Spot-blot analysis of sorted chromosomes assigns a fructose intolerance disease locus to chromosome 9. *Cytometry* 1985;6:478-83.

52 Lench NJ, Telford EA, Andersen SE, Moynihan TP, Robinson PA, Markham AF. An EST and STS-based YAC contig map of human chromosome 9q22.3. *Genomics* 1996;38:199-205.

53 Tolan DR, Penhoet EE. Characterisation of the human aldolase B gene. *Mol Biol Med* 1986;3:245-64.

54 Rottmann WH, Tolan DR, Penhoet EE. Complete amino acid sequence for human aldolase B derived from cDNA and genomic clones. *Proc Natl Acad Sci USA* 1984;81:738-42.

55 Santamaria R, Buono P, Paolella G, et al. Aldolase A, aldolase B and aldolalse C map to chromosome 16, chromosome 9 and chromosome 17 respectively. *Cytogenet Cell Genet* 1987;46:687.

56 Kitajima Y, Takasaki Y, Takahashi I, Hori K. Construction and properties of active chimeric enzymes between human aldolases A and B: analysis of molecular regions which determine isozyme-specific functions. *J Biol Chem* 1990;265:17493-8.

57 Shaw-Lee R, Lissemore JL, Sullivan DT, Tolan DR. Alternative splicing of fructose 1,6-biphosphate aldolase transcripts in *Drosophila melanogaster* predicts three isozymes. *J Biol Chem* 1992;267:3959-67.

58 Kai T, Sugimoto Y, Kusakabe T, Zhang R, Koga K, Hori K. Gene structure and multiple mRNA species of *Drosophila melanogaster* aldolase generating three isozymes with different enzymatic properties. *J Biochem* 1992;112:677-88.

59 Zhang R, Kai T, Sugimoto Y, et al. *Drosophila melanogaster* aldolase: characterisation of the isozymes α, β and γ generated from a single gene. *J Biochem* 1995;118:183-8.

60 Hers HG, Joassin G. Anomalie de l'aldolase hepatique dans l'intolerance au fructose. *Enzymol Biol Clin* 1961;1:4-14.

61 Chambers RA, Pratt RTC. Idiosyncrasy to fructose. *Lancet* 1956;ii:340.

62 Froesch ER, Prader A, Labhart A, Stuber HW, Wolf HP. Die herediatare Fructoseintoleranz, eine bisher nicht bekannte kongentile Stoffwechselstorung. *Schweiz Med Wochenschr* 1957;87:1168-71.

63 Levin B, Snodgrass GJAI, Oberholzer VG, Burgess EA, Dobbs RH. Fructosaemia: observations on seven cases. *Am J Med* 1968;45:826-38.

64 Baerlocher K, Gitzelmann R, Steinmann B, Gitzelmann-Cumarasamy N. Hereditary fructose intolerance in early childhood: a major diagnostic challenge. Survey of 20 symptomatic cases. *Helv Paediatr Acta* 1978;33:465-87.

65 Odievre M, Gentil C, Gautier M, Alagille D. Hereditary fructose intolerance in childhood: diagnosis, management and course in 55 patients. *Am J Dis Child* 1978;132:605-8.

66 Mock DM, Perman JA, Thaler MM, Morris RC. Chronic fructose intoxication after infancy in children with hereditary fructose intolerance: a cause of growth retardation. *N Engl J Med* 1983;309:764-70.

67 Ruecker AV, Endres W, Shin YS, Butenandt I, Steinmann B, Gitzelmann R. A case of fatal hereditary fructose intolerance: misleading information on formula composition. *Helv Paediatr Acta* 1981;36:599-600.

68 Cross NCP, Cox TM. Molecular analysis of aldolase B genes in the diagnosis of hereditary fructose intolerance in the United Kingdom. *Q J Med* 1989;73:1015-20.

69 Marthaler TM, Froesch ER. Hereditary fructose intolerance. Dental status of eight patients. *Br Dent J* 1967;123:597-9.

70 Newburn E, Hoover C, Mettraux G, Graf H. Comparison of dietary habits and dental health of subjects with hereditary fructose intolerance and control subjects. *J Am Dent Assoc* 1980;101:619-26.

71 Saxen L, Jousimies-Somer H, Kaisla A, Kanervo A, Summanen P, Sipila I. Subgingival microflora, dental and periodontal conditions in patients with hereditary fructose intolerance. *Scand J Dent Res* 1989;97:150-8.

72 Cox TM. Iatrogenic deaths in hereditary fructose intolerance. *Arch Dis Child* 1993;69:413-15.

73 Cox TM. Therapeutic use of fructose: professional freedom, pharmacovigilance and Europe. *Q J Med* 1995;88:225-7.

74 Schulte MJ, Lenz W. Fatal sorbitol infusion in patient with fructose-sorbitol intolerance. *Lancet* 1977;ii:188.

75 Muller-Wiefel DE, Steinmann B, Holm-Hadulla M, Wille L, Scharer K, Gitzelmann R. Infusionsbedingtes Nieren-und Lebervesagen bei undiagnostizierter hereditarer Fructose-Intoleranz. *Deutsche Med Wochenschr* 1983;108:985-9.

76 Steegmanns I, Rittmann M, Bayer JR, Gitzelmann R. Erwachsene mit hereditarer Fructoseintoleranz: Gefahrdung durch Fructoseinfusion. *Deutsche Med Wochenschr* 1990;115:539-41.

77 Rosien U, Cox TM, Ali M, et al. Akutes hepatorenales Versagen bei hereditarer Fructoseintoleranz. *Med Klin* 1993;88:553-4.

78 Miller M, Drucker WR, Owens JE, Craig JW, Woodward HJR. Metabolism of intravenous fructose and glucose in normal and diabetic subjects. *J Clin Invest* 1952;31:115-25.

79 Danks DM, Connellan JM, Solomon JR. Hereditary fructose intolerance: report of a case and comments on the hazards of fructose infusion. *Aust Paediatr J* 1972;8:282-6.

80 Woods HF, Alberti KGMM. Dangers of intravenous fructose. *Lancet* 1972;ii:1354-7.

81 Perheentupa J, Raivio K. Fructose-induced hyperuricaemia. *Lancet* 1967;ii:528-31.

82 Bergstrom J, Hultman E, Roch-Norlund AE. Lactic acid accumulation in connection with fructose infusion. *Acta Med Scand* 1968;184:359-64.

83 Odievre M, Gautier M, Rieu D. Intolerance hereditaire au fructose du nourisson. Evolution des lesions histologiques hepatiques sous traitment dietetique prolonge (etude de huit observations). *Arch Fr Pediatr* 1969;26:433-43.

84 Cornblath M, Schwartz R. *Disorders of carbohydrate metabolism in infancy*. 3rd ed. Boston: Blackwell Scientific Publications, 1991:325-51.

85 Hers HG. *Le metabolisme du fructose*. Brussels: Arscia, 1957.

86 Cori GT, Ochoa S, Slein MW, Cori CF. The metabolism of fructose in liver. Isolation of fructose-1-phosphate and inorganic pyrophosphate. *Biochim Biophys Acta* 1951;7:304-17.

87 Leuthardt F, Testa E. Die Phosphorylierung der Fructose in der Leber. II. Mitteilung. *Helv Chim Acta* 1951;34:931-8.

88 Hers HG. La fructokinase du foie. *Biochim Biophys Acta* 1952;8:416-23.

89 Froesch ER, Wolf HP, Baitsch H, Prader A, Labhart A. Hereditary fructose intolerance: an inborn defect of hepatic fructose-1-phosphate splitting aldolase. *Am J Med* 1963;34:151-67.

90 Pitkanen E, Perheentupa J. Eine biochemische Untersuchung uber zwei Falle von Fructoseintoleranz. *Ann Paediatr Fenn* 1962;8:236-44.

91 Cornblath M, Rosenthal IM, Reisner SH, Wybregt SH, Crane RK. Hereditary fructose intolerance. *N Engl J Med* 1963;269:1271-8.

92 Landau BR, Marshall JS, Craig JW, Hostetler KY, Genuth SM. Quantitation of the pathways of fructose metabolism in normal and fructose-intolerant subjects. *J Lab Clin Med* 1971;78:608-18.

93 Froesch ER, Ginsberg JL. Fructose metabolism of adipose tissue. I. Comparison of fructose and glucose metabolism in epididymal adipose tissue of normal rats. *J Biol Chem* 1962;237:3317-24.

94 Woods HF, Eggleston LV, Krebs H. The cause of hepatic accumulation of fructose-1-phosphate on fructose loading. *Biochem J* 1970;119:501-10.

95 Watts RWE, Watts JEM, Seegmiller JE. Xanthine oxidase activity in human tissues and its inhibition by allopurinol (4-hydroxypyrazolo (3,4-d) pyrimidine). *J Lab Clin Med* 1965;66:688-97.

96 Maenpaa PH, Raivio KO, Kekomaki MP. Liver adenine nucleotides: fructose-induced depletion and its effects on protein synthesis. *Science* 1968;161:1253-4.

97 Bode JC, Zelder O, Rumpelt HJ, Wittkamp U. Depletion of liver adenosine phosphates and metabolic effects of intravenous infusion of fructose or sorbitol in man and in the rat. *Eur J Clin Invest* 1973;3:436-41.

98 Van den Berghe G, Bronfman M, Vanneste R, Hers HG. The mechanism of adenosine triphosphate depletion in the liver after a load of fructose: a kinetic study of liver adenylate deaminase. *Biochem J* 1977;162:601-9.

99 Oberhaensli RD, Rajagopalan B, Taylor DJ, et al. Study of hereditary fructose intolerance by use of ^{31}P magnetic resonance spectroscopy. *Lancet* 1987;ii:931-4.

100 Maenpaa PH. Fructose-induced alterations in liver polysome profiles and Mg^{2+} levels. *FEBS Lett* 1972;24:37-40.

101 Levin B, Oberholzer VG, Snodgrass GJAI, Stimmer L, Wilmers MJ. Fructosaemia: an inborn error of fructose metabolism. *Arch Dis Child* 1963;38:220-30.

102 Parks TE, Ben-Gershom E, Lardy HA. Liver fructokinase. *J Biol Chem* 1957;227:231-42.

103 Smith LH, Ettinger RH, Seligson D. A comparison of the metabolism of fructose and glucose in hepatic disease and diabetes mellitus. *J Clin Invest* 1953;32:273-82.

104 Dubois R, Loeb H, Malaisse-Lagae F, Toppet M. Etude clinique et anatomo-pathologique de deux cas d'intolerance congenitale au fructose. *Pediatrie* 1965;20:5-14.

105 Rossier A, Milhaud G, Colin J, et al. Intolerance congenitale au fructose. Deux cas familiaux avec etude biochimique *in vitro. Arch Fr Pediatr* 1966;23:533-52.

106 Kaufmann U, Froesch ER. Inhibition of phosphorylase-a by fructose-1-phosphate, α-glycerophosphate and fructose-1,6-diphosphate: explanation for fructose-induced hypoglycaemia in hereditary fructose intolerance and fructose-1,6-diphosphatase deficiency. *Eur J Clin Invest* 1973;3:407-13.

107 Van den Berghe G, Hue L, Hers HG. Effect of the administration of fructose on the glycogenolytic action of glucagon: an investigation of the pathogeny of hereditary fructose intolerance. *Biochem J* 1973;134:637-45.

108 Thurston JH, Jones EM, Hauhart RE. Decrease and inhibition of liver glycogen phosphorylase after fructose: an experimental model for the study of hereditary fructose intolerance (HFI). *Diabetes* 1974;23:597-604.

109 Froesch ER, Prader A, Wolf HP, Labhart A. Die hereditatare Fructoseintoleranz. *Helv Paediatr Acta* 1959; 14:99-112.

110 Zalitis J, Oliver IT. Inhibition of glucose phosphate isomerase by metabolic intermediates of fructose. *Biochem J* 1967;102:753-9.

111 Eggleston LV, Woods HF. Activation of liver pyruvate kinase by fructose-1-phosphate. *FEBS Lett* 1970;6:43-5.

112 Andersson G, Brohult J, Sterner G. Increasing metabolic acidosis following fructose infusion in two children. *Acta Paediatr Scand* 1969;58:301-4.

113 Morris RC Jr. An experimental renal acidification defect in patients with hereditary fructose intolerance. II. Its distinction from classic renal tubular acidosis; its resemblance to the renal acidification defect associated with the Fanconi syndrome of children with cystinosis. *J Clin Invest* 1968;47: 1648-63.

114 Richardson RMA, Little JA, Patten RL, Goldstein MB, Halperin ML. Pathogenesis of acidosis in hereditary fructose intolerance. *Metabolism: Clin Exp* 1979;28:1133-8.

115 Saxon L, Papper S. Abdominal pain occurring during the rapid administration of fructose solutions. *N Engl J Med* 1957;256:132-3.

116 Spaapen LJM. Secondary carbohydrate deficient syndromes. *Amino Acids* 1997;12:390.

117 Cross NCP, Tolan DR, Cox TM. Catalytic deficiency of human aldolase B in hereditary fructose intolerance caused by a common missense mutation. *Cell* 1988;53:881-5.

118 Cox TM, O'Donnell MW, Camilleri M, Burghes AH. Isolation and characterization of a mutant liver aldolase in adult hereditary fructose intolerance. Identification of the enzyme variant by radioassay in tissue biopsy specimens. *J Clin Invest* 1983;72:201-13.

119 James CL, Rellos P, Ali M, Heeley AF, Cox TM. Neonatal screening for hereditary fructose intolerance: frequency of the most common mutant aldolase B allele (A149P) in the British population. *J Med Genet* 1996;33:837-41.

120 Cross NCP, de Franchis R, Sebastio G, et al. Molecular analysis of aldolase B genes in hereditary fructose intolerance. *Lancet* 1990;335:306-9.

121 Cox TM. Aldolase B and fructose intolerance. *FASEB J* 1994;8:62-71.

122 Tolan DR, Brooks CC. Molecular analysis of common aldolase B alleles for hereditary fructose intolerance in North Americans. *Biochem Med Metab Biol* 1992;48:19-25.

123 Ali M, James CL, Cox TM. A newly identified aldolase B splicing mutation (G→C, 5' intron 5) in hereditary fructose intolerance from New Zealand. *Hum Mutat* 1996;7:155-7.

124 Cavalli-Sforza LL, Menozzi P, Piazza A. *The history and geography of human genes.* Princeton, New Jersey: Princeton University Press, 1993.

125 Sebastio G, de Franchis R, Strisciuglio P, et al. Aldolase B mutations in Italian families affected by hereditary fructose intolerance. *J Med Genet* 1991;28:241-3.

126 Brooks CC, Tolan DR. Association of the widespread A149P hereditary fructose intolerance mutation with newly identified sequence polymorphisms in the aldolase B gene. *Am J Hum Genet* 1993;52:835-40.

127 Cross NCP, Stojanov LM, Cox TM. A new aldolase B variant, N334K, is a common cause of hereditary fructose intolerance in Yugoslavia. *Nucleic Acids Res* 1990;18:1925.

128 Dazzo C, Tolan DR. Molecular evidence for compound heterozygosity in hereditary fructose intolerance. *Am J Hum Genet* 1990;46:1194-9.

129 Cox TM, O'Donnell MW, Camilleri M. Allelic heterogeneity in adult hereditary fructose intolerance: detection of structural mutations in the aldolase B molecule. *Mol Biol Med* 1983;1:393-400.

130 Ali M. The molecular biology of fructose intolerance. PhD Thesis, University of Cambridge, UK, 1995.

131 Santamaria R, Scarano MI, Esposito G, Chiandetti L, Izzo P, Salvatore F. The molecular basis of hereditary fructose intolerance in Italian children. *Eur J Clin Biochem* 1993;31: 675-8.

132 Santamaria R, Tamasi S, Del Piano G, et al. Molecular basis of hereditary fructose intolerance in Italy: identification of two novel mutations in the aldolase B gene. *J Med Genet* 1996;33:786-8.

133 Ali M, Cox TM. Diverse mutations in the aldolase B gene that underlie the prevalence of hereditary fructose intolerance. *Am J Hum Genet* 1995;56:1002-5.

134 Ali M, Tunçman G, Cross NCP, et al. Null alleles of the aldolase B gene in patients with hereditary fructose intolerance. *J Med Genet* 1994;31:499-503.

135 Brooks CC, Tolan DR. A partially active mutant aldolase B from a patient with hereditary fructose intolerance. *FASEB J* 1994;8:107-13.

136 Ali M, Rosien U, Cox TM. DNA diagnosis of fatal fructose intolerance from archival tissue. *Q J Med* 1993;86:25-30.

137 Cross NCP, Cox TM. Partial aldolase B gene deletions in hereditary fructose intolerance. *Am J Hum Genet* 1990;47: 101-6.

138 Kajihara S, Mukai T, Arai Y, Owada M, Kitagawa T, Hori K. Hereditary fructose intolerance caused by a nonsense mutation of the aldolase B gene. *Am J Hum Genet* 1990;47: 562-7.

139 Nordmann Y, Schapira F, Dreyfus JC. A structurally-modified liver aldolase in fructose intolerance: immunological and kinetic evidence. *Biochem Biophys Res Commun* 1968;31:884-9.

140 Gitzelmann R, Steinmann B, Bally C, Lebherz HG. Antibody activation of mutant fructose diphosphate aldolase B in liver extracts of patients with hereditary fructose intolerance. *Biochem Biophys Res Commun* 1974;59:1270-7.

141 Lamiere N., Mussche M, Baele G, Kint JA, Ringoir S. Hereditary fructose intolerance: a difficult diagnosis in the adult. *Am J Med* 1978;65:416-23.

142 Schapira F, Hatzfeld A, Gregori C. Studies on liver aldolases in hereditary fructose intolerance. *Enzyme* 1974; 18:73-83.

143 Gregori C, Shapira F, Kahn A, Delpech, Dreyfus JC. Molecular studies of liver aldolase B in hereditary fructose intolerance using blotting and immunochemical techniques. *Ann Hum Genet* 1982;46:281-92.

144 Sakakibara M, Takahashi T, Takahashi Y, Mukai T, Hori K. Construction and expression of human aldolase A and B expression plasmids in *Escherichia coli* host. *Biochem Biophys Acta* 1989;1007:334-42.

145 Gamblin SJ, Davies GJ, Grimes JM, Jackson RM, Littlechild JA, Watson HC. Activity and specificity of human aldolases. *J Mol Biol* 1991;219:573-6.

146 Morris AJ, Tolan DR. Lysine-146 of rabbit muscle aldolase is essential for cleavage and condensation of the C3-C4 bond of fructose 1,6-bis(phosphate). *Biochemistry* 1994;33: 12291-7.

147 Berthiaume L, Tolan DR, Sygusch J. Differential usage of the carboxyl-terminal region among aldolase isozymes. *J Biol Chem* 1993;268:10826-35.

148 Motoki K, Kitajima Y, Katsuji H. Isozyme-specific modules on human aldolase A molecule. *J Biol Chem* 1993; 268:1677-83.

149 Ali M, Sebastio G, Cox TM. Identification of a novel mutation (Leu^{256}→Pro) in the human aldolase B gene associated with hereditary fructose intolerance. *Hum Mol Genet* 1994;3:203-4.

150 Beernink PT, Tolan DR. Subunit interface mutants of rabbit muscle aldolase form active dimers. *Protein Sci* 1994;3: 1383-91.

151 Beernink PT, Tolan DR. Disruption of the aldolase A tetramer into catalytically active monomers. *Proc Natl Acad Sci USA* 1996;93:5374-9.

152 Cox TM, Camilleri M, O'Donnell MW, Chadwick VS. Pseudodominant transmission of fructose intolerance in an adult and three offspring. *N Engl J Med* 1982;307:537-40.

153 Steinmann B, Gitzelmann R. The diagnosis of hereditary fructose intolerance. *Helv Paediatr Acta* 1981;36:297-316.

154 Saiki RK, Scharf S, Faloona F, et al. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 1985;230:1350-4.

155 Gitzelmann R, Baerlocher K. Vorteile und Nachteile der Fructose in der Nahrung. *Padiatr Fortbild Prax* 1973;37:40-55.

156 Garrod AE. The incidence of alkaptonuria: a study in chemical individuality. *Lancet* 1902;ii:1616-20.

157 Wolf H, Zschocke D, Wedemeyer FW, Hubner W. Angeborene hereditare fructose-intoleranz. *Klin Wochenschr* 1959;37:693-6.

158 Linden L, Nisell J. Hereditar fructosintolerans. *Svenska Lakartidn* 1964;61:3185-95.

159 Barry RGG, St Colum SR, Magner JW. Hereditary fructose intolerance in parent and child. *J Irish Med Assoc* 1968;61:308-10.

160 Rampa M, Froesch ER. Eleven cases of hereditary fructose intolerance in one Swiss family with a pair of monozygotic and of dizygotic twins. *Helv Paediatr Acta* 1981;36:317-24.

161 Heine W, Schill H, Tessmann D, Kupatz H. Letale Leberdystrophie bei drei Geschwistern mit hereditarer Fruktoseintoleranz nach Dauertropfinfusionen mit sorbitolhaltigen Infusionslosungen. *Deutsche Gesundheitswesen* 1969;24: 2325-9.

162 Hackl JM, Balogh D, Kunz F, et al. Postoperative Fruktoseinfusion bei wahrscheinlich hereditarer Fruktoseintoleranz. *Wien Klin Wochenschr* 1978;90:237-40.

163 Rey M, Behrens R, Zeilinger G. Fatale Folgen einer Fructose-Infusion bei undiagnostizierter Fructose-Intoleranz. *Deutsche Med Wochenschr* 1988;113:945-7.

164 Brooks CC, Buist N, Tuerck J, Tolan DR. Identification of a splice-site mutation in the aldolase B gene from an individual with hereditary fructose intolerance. *Am J Hum Genet* 1991;49:1075-81.