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Diverse Mutations in the Aldolase B Gene That Underlie the Prevalence of Hereditary Fructose Intolerance

To the Editor:

Hereditary fructose intolerance (HFI), first recognized in 1956 by Chambers and Pratt, is an autosomal recessive disorder with a wide ethnic distribution. It is caused by deficiency of liver aldolase (aldolase B) (Hers and Joassin 1961) and is characterized by vomiting, failure to thrive, liver disease, and metabolic disturbances—including hypoglycemia—that occur on exposure to fructose and related sugars, at weaning (Gitzelmann et al. 1989). Diverse mutations have been identified in the human aldolase B gene in association with fructose intolerance, several of which are sufficiently widespread to be of diagnostic utility (Cross et al. 1990; Cox 1994). The frequency of HFI has not been determined with precision in any population, and many pediatricians consider it to be rare (Cornblath and Schwartz 1991). Nonetheless, recognition of HFI is of critical importance, since it responds favorably to dietary exclusion of fructose, sucrose, and sorbitol; survival to adulthood is associated with the development of strong aversions to sweet-tasting foods and drinks.

There is mounting evidence that mutant alleles of aldolase B are more frequent in the population than was first recognized: (1) there are multiple reports of parent-to-offspring transmission of fructose intolerance in nonconsanguineous pedigrees (Cox. et al. 1982); (2) after surviving the stormy period of infancy, individuals adjust their dietary habits and escape formal diagnosis—they may later come forward in response to articles in the public domain (Cross and Cox 1989); and (3) the effects of administration of fructose-based solutions have resulted in at least 16 deaths of patients not known to have been suffering from HFI but in whom the diagnosis has been subsequently confirmed (Gitzelmann et al. 1989; Ali et al. 1993; Cox 1993). We have investigated key members of a large kindred from the United States who are affected by HFI that vividly illustrates all these features.

The pedigree is depicted in figure 1. The original fructose-intolerant subjects came from two families, of maternal Swiss-German descent, within a large kindred and were first cousins (Landau et al. 1971). Diagnosis was based on characteristic symptoms and the induction of hypophosphatemia and hypoglycemia after controlled fructose challenge (Froesch et al. 1963). Since then, one patient has died at age 10 years, from acute hepatorenal failure after intravenous infusion of invert sugar during a minor surgical procedure. There have been additions to the pedigree—notably the marriage of the fructose-intolerant subject III-22 to an unrelated woman, γ, with the birth of offspring IV-16 and IV-17. Their son, IV-16, and his female cousin, IV-1, the oldest member of generation IV, avoid sugary foods and fruit and have dietary preferences that, combined with their immediate family history, are highly suggestive of fructose intolerance (Chambers and Pratt 1956; Froesch et al. 1963; Gitzelmann et al. 1989). IV-1 had suffered severe nutritional disturbances, including adolescent scurvy, since early infancy. Thus, because IV-1 and IV-16 questioned, as adults, whether they suffered from HFI, first-degree and collateral relatives of the original fructose-intolerant subjects requested diagnostic confirmation and clarification of the mode of transmission of disease within their kindred. Accordingly, blood samples were obtained from key members, for DNA extraction and aldolase B genotyping based on the PCR (Cross et al. 1990). Detailed inquiry has failed to reveal consanguinity in the kindred: subjects α, β, γ, and δ are of English, German, French, and Anglo-American descent, respectively.

To screen systematically for aldolase B gene mutations that are associated with HFI and widespread in the European population, exon 5 sequences were first amplified in the PCR for study. Aliquots were digested with the restriction enzyme *Bsa*HI (GPu↓CGPyC̲), an isoschizomer of *Aha*II, that cleaves the products at the site of the common missense mutation, A149P (Ala^{149}→Pro; G→C) (Cross et al. 1988). One copy of this allele was detected in DNA samples from subjects α, III-22, and IV-17. To identify other alleles segregating in this kindred, DNA obtained from the brother of the child who had suffered lethal fructose intoxication (III-36) was examined as the index case. Direct genomic sequencing revealed two distinct mutations. In exon 4, the 4-bp deletion elsewhere described in a patient of British ancestry (Cox et al. 1983; Dazzo and Tolan 1990) was identified. To screen for the presence of this mutation, heteroduplex analysis—which facilitates detection of small deletions in PCR products that also contain wild-type sequence (Nagamine et al. 1989)—was undertaken. The hybrid amplified exon 4 sequences were identified by their anomalous mobility during electrophoresis through polyacrylamide. Heteroduplex analysis con-

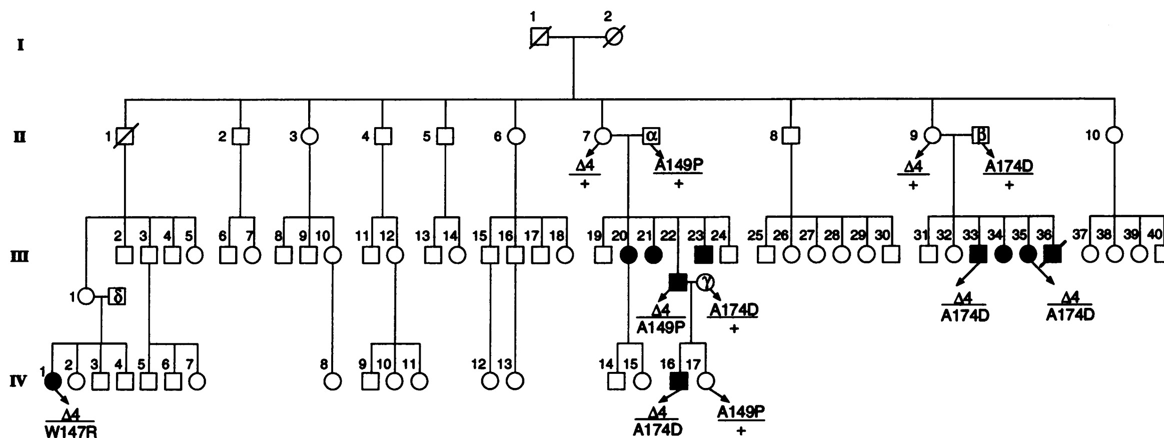

Figure 1 Kindred affected by HFI. Blackened symbols indicate patients in whom HFI had been diagnosed by Landau et al. (1971). Gray-shaded symbols show symptomatic members who requested diagnosis for suspected HFI. Blood samples for DNA extraction and molecular analysis of aldolase B genes were provided by numbered subjects whose genotype is assigned. A plus sign (+) indicates an aldolase B gene that is wild type at the A149, A174, Δ4, and W147 loci. Roman numerals indicate successive generations of the pedigree.

firmed that III-33 as well as his sister III-35 had inherited one copy of the Δ4 mutation from their mother (II-9). This mutation was also present in II-7, her son III-22, and her grandson IV-16—as well as in individual IV-1, the granddaughter of the oldest sibling in generation II.

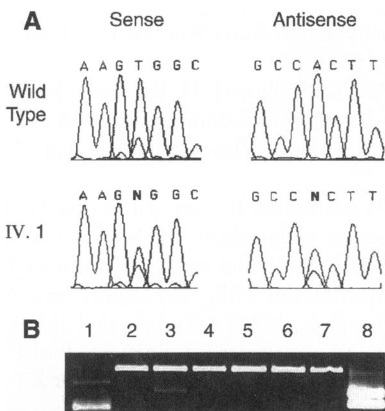

Figure 2 *A*, Identification of the W147R mutation in exon 5: automated sequencing chromatograph of exons 5 of aldolase B from subject IV-1 with suspected HFI and showing heteroallelic transition, T→C in the coding sequence (A→G antisense), compared with wild-type sequences. *B*, Confirmatory genotype analysis for the novel W147R mutation: agarose-gel electrophoresis of exons 5 of the aldolase B gene amplified by the PCR and digested with *Fnu*4HI. Lane 1, 123-bp ladder. Lane 2, Healthy subject. Lane 3, Subject IV-1. Lane 4, Member II-7, genotype Δ4/+. Lane 5, Member α, genotype A149P/+. Lane 6, Member II-9, genotype Δ4/+. Lane 7, Member β, genotype A174D/+. Lane 8, *Fnu*4HI digestion products of exon 5 sequences amplified in the PCR using DNA template containing the W174R mutation cloned in the vector pEMBL 18; the presence of the mutation permits cleavage of the 323-bp amplification product into fragments of 189 and 134 bp as is also depicted for subject IV-1 (lane 3).

Direct sequencing of amplified exons 5 from aldolase B in the index case, III-33, revealed the widespread mutation A174D (Ala^{174}→Asp; C→A) that we had also described in European patients with HFI (Cross et al. 1990). It was detected as a single copy as previously described by allele-specific hybridization to oligonucleotide probes in amplified exons 5 from subjects III-33 and III-35, who had inherited it from their father (β) and hence were compound heterozygotes with the genotype Δ4/A174D. These mutant alleles had segregated as expected for a recessively transmitted disorder.

Since the aldolase B genotypes of individuals in this kindred who were known to have suffered from intolerance of fructose had been determined, it was now possible also to study subjects IV-1 and IV-16 for the presence of diagnostic mutations, to confirm the suspicion of disease. The presence of the A174D allele in both IV-16 and his mother γ confirmed the diagnosis of fructose intolerance in IV-16, since he had inherited one copy of Δ4 from his father (III-22), a compound-heterozygote patient with the condition. This provides further evidence of parent-to-offspring transmission in a recessive disease where there is a significant frequency of mutant alleles in the general population. The sequence of exons 5 of aldolase B amplified from DNA obtained from subject IV-1 revealed a heteroallelic transition: T→C in codon 147 of the sense strand. Sequencing was carried out by the cycle sequencing method (Ruano and Kidd 1991) using fluorescent dye terminators in the Applied Biosystem 373 automated sequencer (Rosenthal and Charnock-Jones 1992); see figure 2*A*. This missense mutation, designated "W147R" (Trp^{147}→Arg), creates a novel restriction site for the endonuclease *Fnu*4H1 (G<u>C</u>↓NGC), and confirmatory digests are also depicted

(fig. 2*B*). The W147R mutation has not been previously identified in association with HFI and was detected neither in the other affected members of this kindred nor in >100 unrelated disease alleles. The tryptophan residue in position 147 is invariant in all vertebrate aldolases, and its replacement by arginine would disrupt a critical region for substrate binding in the enzyme molecule (Cross et al. 1988).

Molecular analysis of aldolase B genes in this outbred North American pedigree suggests that there is a high prevalence of aldolase B mutations associated with HFI that are widespread in European populations (Cross et al. 1990; Santamaria et al. 1993; Cox 1994). Cosegregation of A149P and A174D together with the less frequent alleles (Δ4 and the newly recognized W147R mutant described here) is in keeping with the spread of these alleles in the United States (Tolan and Brooks 1992). A previous report of five cases of HFI in maternal cousins from another family, in which there is no consanguinity, also suggests that HFI disease alleles are prevalent in the United States (Cornblath et al. 1963). Several large affected kindreds have been reported (e.g., see Rampa and Froesch 1982), but this is the first in which independent segregation of multiple mutant alleles with compound heterozygosity in numerous affected individuals has been demonstrated. Moreover, vertical transmission of disease in this kindred has resulted from marriage between an affected member and a heterozygote in a population where there is a significant frequency of defective alleles in the absence of inbreeding. Given the potentially serious consequences of untreated fructose intolerance, there is a case for determining the frequency of mutant aldolase B genes, to estimate the incidence of HFI in the population.

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Allelic Association between the HUMF13A01 (AAAG)$_n$ STR Locus and a Nearby Two-Base Insertion/Deletion Polymorphic Marker

To the Editor:

We read with interest the paper of Hammond et al. (1994) published in a previous issue of the *Journal*. The authors present an extensive analysis of the distributions and frequencies of 13 microsatellite loci in four population groups, including Caucasians, Blacks, Mexican-Americans, and Asians. We would like to emphasize the promise of the method described for DNA typing in forensic use and medical applications and to give some additional molecular information on one of the short tandem repeat (STR) polymorphisms examined, the HUMF13A01 (AAAG)$_n$.

This locus is a tetranucleotide repeat that is highly polymorphic in all population groups examined so far (Polymeropoulos et al. 1991; Wall et al. 1993; Hammond et al. 1994). In addition to the alleles due to variable numbers of four-base repeats, Hammond et al. (1994) report the finding at relatively high frequencies of a variant allele, named "3.2", that does not correspond to a change in the number of reiterations of the core sequence. However, this allele is only described as "a fragment that migrates at the position of a fragment that is two bases longer than a fragment containing three repeats of the core sequence" (p. 186). No reference is made to the exact molecular nature of the 3.2 allele in the paper.

We have been analyzing the polymorphism of the HUMF13A01 STR locus by the PCR method of Polymeropoulos et al. (1991) in various human populations belonging to three major ethnic groups, Caucasians, Africans, and Asians. We have found in all groups the same variant allele as that reported by Hammond et al. (1994). Sequencing of PCR products of this allele in three individuals, one from each ethnic group, has shown that the variation does not affect the number of reiterations, but it is due to a two-base (GT) insertion/deletion polymorphism located just one base downstream of the repeated sequence (GenBank accession number M21986). In all ethnic groups, the deletion allele was always found in association with the four-repeat allele; on the other hand, the insertion was observed in association with a minority of the four-repeat alleles and with all the other alleles (table 1). These findings are fully consistent with the data of Hammond et al. (1994) and explain the molecular nature of the 3.2 allele. The distribution of the deletion/insertion polymorphism in Caucasians, Africans, and Asians suggests that the insertion allele is ancestral and that the deletion event occurred more recently on a four-repeat background, before the radiation of *Homo sapiens sapiens*. As a corollary, the complete association between the deletion and the four-repeat allele suggests that the HUMF13A01 STR alleles are mutationally stable and have most likely arisen only once during human evolution.

Table 1

Percent Frequencies of the GT Deletion/Insertion Alleles among HUMF13A01 STR Chromosomes Carrying or Not Carrying the (AAAG)$_4$ Allele

	F13A1 STR Chromosomes				
	Four-Repeat Alleles		Other Alleles		
Population	Del (3.2)	Ins	Del	Ins	No. of Chromosomes Tested
Caucasians[a]	12.3	2.8	.0	84.9	106
Africans[b]	12.0	7.0	.0	81.0	242
Asians[c]	23.0	16.2	.0	60.8	74

[a] 53 unrelated subjects from central Italy.
[b] 121 (50 Foulbe and 71 Mossi) unrelated subjects from Burkina Faso.
[c] 37 unrelated Chinese Li.