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# Partial Aldolase B Gene Deletions in Hereditary Fructose Intolerance

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## Summary

**Hereditary fructose intolerance (HFI) is an autosomal recessive condition caused by a deficiency of aldolase B. We have recently shown that three point mutations in this gene account for ~85% of HFI alleles in Europe and the United States and are thus of diagnostic importance. In this paper we define three new lesions in the aldolase B gene: two are large deletions, one of 1.65 kb and one of 1.4 kb; the third is a small deletion of 4 bp. We have determined the breakpoints of these deletions and have demonstrated that the presence of such lesions may complicate the genotyping of individuals for diagnosis of HFI.**

## Introduction

Hereditary fructose intolerance (HFI) is an autosomal recessive disorder characterized by abdominal pain, vomiting, and hypoglycemia after the ingestion of fructose, sucrose, or sorbitol (Chambers and Pratt 1956; Gitzelmann et al. 1989). Affected individuals are most at risk as infants at weaning, when the metabolic disturbance may be fatal. Most patients, however, survive this difficult period and develop a strong aversion to foods containing the noxious sugars. Despite suffering from episodic symptoms throughout adult life, the diagnosis of HFI in these individuals is often missed. The condition may be cured by appropriate dietary treatment, and thus early recognition of the disease is critical to its effective management.

HFI is caused by a deficiency of aldolase B, the isozyme expressed exclusively in the liver, kidney, and intestine, which is responsible for the assimilation of fructose by cleavage of the sugar monophosphate to trioses. We have recently described three common mutations in the gene for aldolase B that are responsible for the enzyme defect: A149P (G→C, Ala149→Pro), A174D (C→A, Ala174→Asp), and L288ΔC (deleted C residue, Leu 288→frameshift; Cross et al. 1988, and submitted). Together these lesions account for ~85% of HFI alleles in Europe and provide the means for rapid, noninvasive genetic diagnosis and carrier detection in >95% of affected families (Cross and Cox 1989; Cross et al. 1990).

Here we report the characterization of three further defective aldolase B alleles. Each is caused by a partial gene deletion and apparently is confined to a single pedigree. We also show that the presence of such deletions may, if not detected, lead to the incorrect genotyping of individuals under investigation for HFI.

## Material and Methods

Forty-nine individuals affected by HFI, who represented 39 independent families and 78 mutant aldolase B alleles, were studied. The patients were from Britain, France, Switzerland, Italy, Yugoslavia, and Austria. The diagnosis was made by the intravenous fructose tolerance test (Steinmann and Gitzelmann 1981), and DNA was prepared from peripheral blood leukocytes (Sykes 1983).

The genotyping of these patients with respect to the common HFI lesions A149P, A174D, and L288ΔC by the polymerase chain reaction (PCR) and allele-specific oligonucleotides (ASOs) has been described elsewhere (Cross et al., submitted). Nested PCR was carried out by using 1/10,000 of the primary amplification as a substrate for the second reaction, and the extension time in each case was increased to 6 min. Oligonucleotides

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used for the amplification of aldolase B exons 2–9 have also been described elsewhere (Cross et al. 1990). New oligonucleotides used in this study were as follows: int 3+, 5′ ATCGCTTGAGCTCAGGAGTT 3′, and int 8–, 5′ TACAGAAATTAGCCAGGTAT 3′.

Fragments were subcloned into the plasmid vectors pEMBL18 or 19 and were sequenced by the chain terminator method using Sequenase (United States Biochemical). Other methods using standard laboratory techniques have been described by Maniatis et al. (1982).

## Results

DNA was extracted from 49 HFI patients, including propositi from 39 unrelated pedigrees, and was digested with *Bam*HI, transferred to nylon membranes, and probed with the aldolase B cDNA clone pHL413 (Rottmann et al. 1984). Abnormal banding patterns were detected in only two patients, designated F13 and G10 (fig. 1*A*); both showed an extra band at approximately 8 kb, in addition to that expected at 9.4 kb. F9, the asymptomatic father of F13, also showed the extra band.

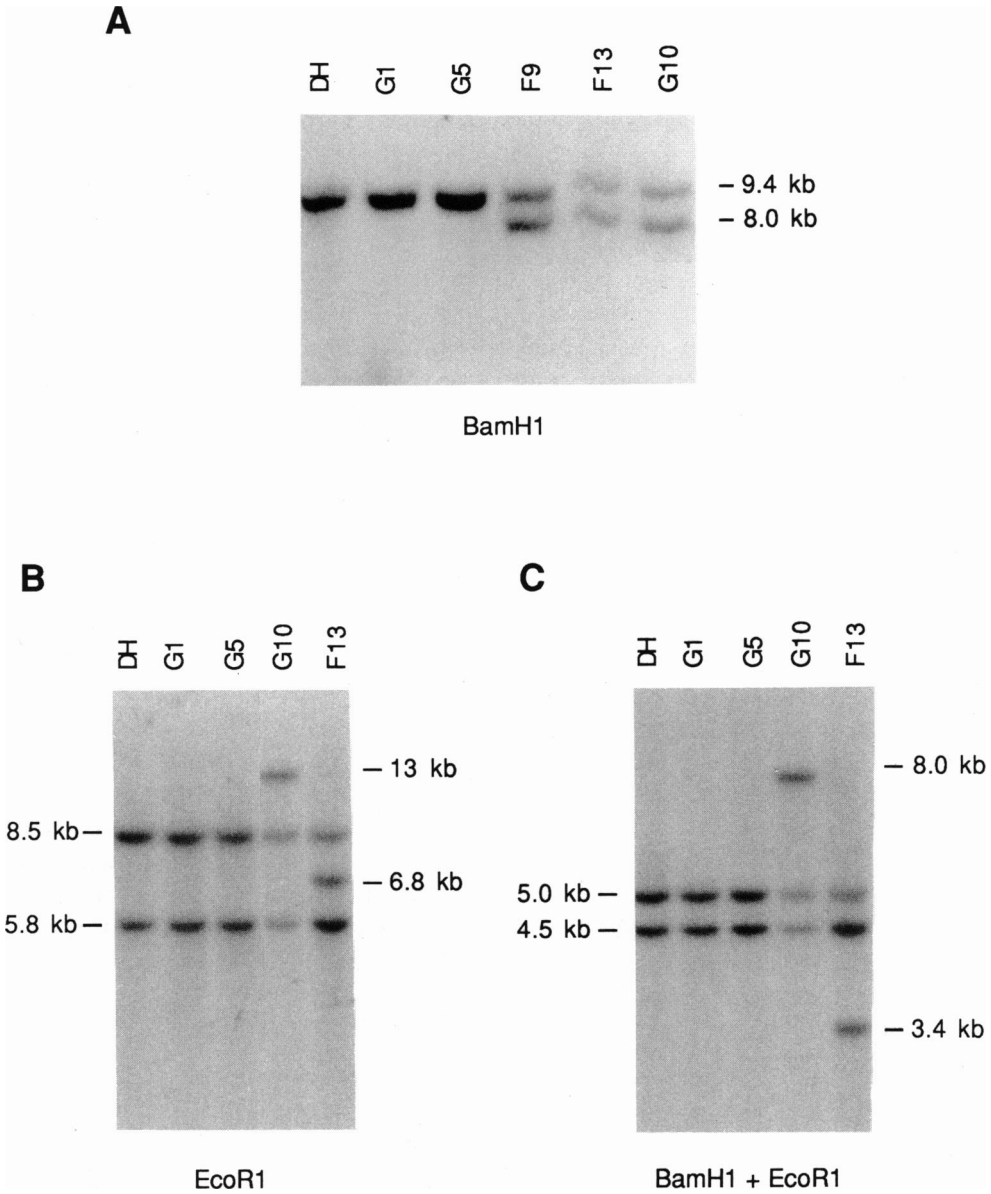


**Figure 1** Southern blot of HFI DNA probed with aldolase B cDNA. *A*, *Bam*HI. *B*, *Eco*RI. *C*, *Bam*HI+*Eco*RI. Patients DH, G1, and G5 show the normal banding patterns. Patients F13 and G10 have deletions, on one allele, of 1.65 kb and 1.4 kb, respectively.

Digestion with *Eco*RI and *Eco*RI+*Bam*HI (fig. 1*B* and 1C) demonstrated that the additional bands resulted from internal gene deletions and that the two deletions were distinct. Patient G10 showed an extra band at 13 kb on digestion with *Eco*RI and at 8.0 kb with *Eco*RI+*Bam*HI, indicating that the central *Eco*RI site in intron 6 has been deleted on one allele. Patient F13 showed extra bands at 6.8 kb with *Eco*RI and at 3.4 kb with *Eco*RI+*Bam*HI, demonstrating that the deleted region is contained within the 5.0-kb fragment from the *Bam*HI site in exon 2 to the *Eco*RI site in intron 6. This deletion is also present on one allele only.

Further blots served to define the deletions more accurately: that of F13 was found to lie between the *Taq*I site in intron 3 and *Xba*I site in intron 5; the G10 deletion was delimited between the same *Xba*I site and the *Pst*I site in exon 8 (data not shown).

Initial attempts to amplify the breakpoints by the PCR for sequencing were unsuccessful, presumably because of the large size of the target sequence in each case. However, these fragments were successfully obtained by using nested PCR: F13 DNA was first amplified using E3+ and E6− oligonucleotides followed by a second amplification using int3+ and E6−. G10 DNA was amplified by first using E5+ and int8−, followed by A174wt and E8rc. In each case bands of ~1.2 kb were generated. The bands of 2.9 kb and 2.7 kb corresponding to the products of the normal length allele were not visible under the conditions used for amplification.

We experienced problems in directly subcloning the amplified fragments after treatment with polynucleotide kinase, and it appears that the 5′ ends of many of the oligonucleotides used were refractory to ligation. Accordingly, the F13 fragment was cut with *Taq*I+*Xba*I, filled in with Klenow, and ligated into the *Sma*I site of pEMBL19. The G10 band was kinased, cut with *Xba*I, and ligated to pEMBL19 cut with *Sma*I and *Xba*I. Both fragments were sequenced to reveal the nature of the deletions.

In F13, a deletion of 1,649 bp was found with breakpoints in intron 3 and exon 5 (figs. 2*A* and 2*B*) and thus completely removed exon 4 and the crucial residue implicated in substrate binding, Arg148 (Patthy et al. 1979). The regions surrounding the breakpoints show no significant direct or inverse homology. The G10 deletion is ~1,400 bp from intron 5 to intron 7 and thus removes exons 6 and 7 (figs. 2*A*, and 2*B*) and therefore the active-site residue Lys229 (Lai et al. 1974). This lesion also removes the so-called unclonable region in intron 6, an ~500-bp sequence that was not represented in a standard amplified human genomic library but that could be cloned using *Escherichia coli* strain CES200 as a host. This suggests that the region probably contains a long palindrome (Tolan and Penhoet 1986; Cross et al. 1988). The sequences of the breakpoints again show no homology with each other.

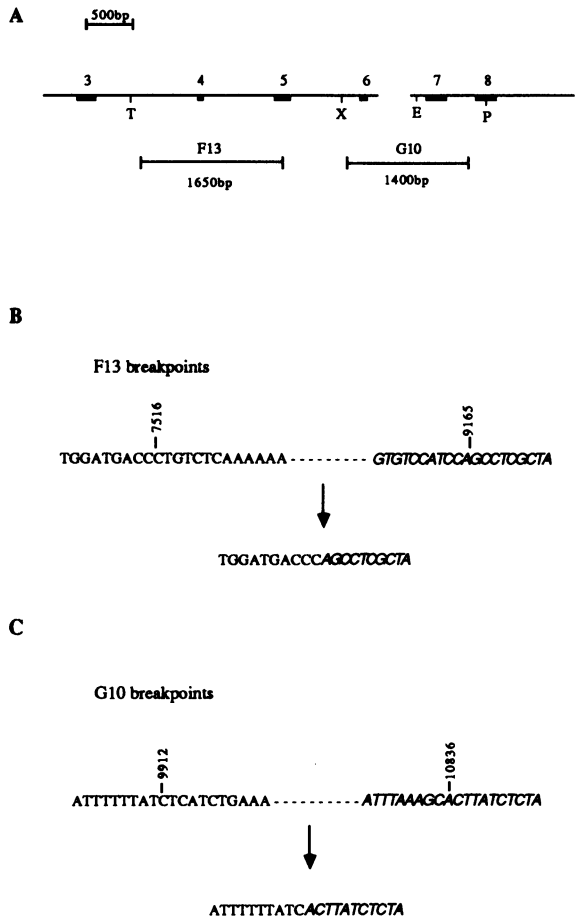


**Figure 2** *A*, Map of the human aldolase B gene, showing the extent of deletions in patients F13 and G10. Relevant restriction sites are shown: T = *Taq*I; X = *Xba*I; E = *Eco*RI; P = *Pst*I. The gap between exons 6 and 7 indicates the 'unclonable' region. *B*, Sequence of the breakpoints in F13. Numbers refer to nucleotide positions of the aldolase B gene. *C*, Sequence of the breakpoints in G10.

The partial gene deletion in F13 initially caused an error in the analysis of this patient's genotype: after amplification of exon 5 by the PCR and hybridization to A149P and A149wt ASOs, a positive signal was only obtained with the mutant probe; thus we classified F13 as an A149P homozygote (fig. 3*B*). The patient's father would therefore be expected to be heterozygous for A149P; however, only hybridization to the wild-type probe was found. The deletion in this family removes most of the exon 5 sequence, and thus an amplification

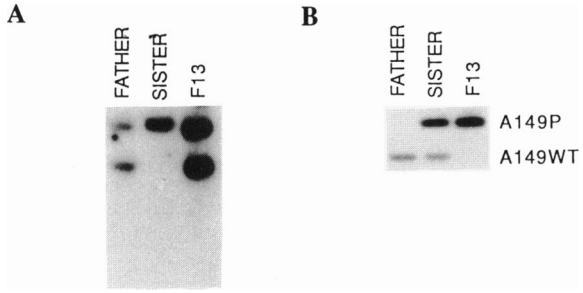


**Figure 3** *A*, Segregation of the 1.65-kb deletion. Shown is a Southern blot of a *Bam*HI digest probed with aldolase B cDNA. *B*, Hybridization of A149P and A149wt oligonucleotides to PCR-amplified exon 5. Genotypes are as follows: father, deletion/normal; sister, A149P/normal; F13 (propositus), A149P/deletion.

product is only obtained from the other, full-length, allele. This allele is A149P in the case of F13 and normal in the father. An asymptomatic sister of the patient was found to be a carrier of A149P but not of the deletion (fig. 3*A*).

The lesion of one allele of patient G10 removes exon 6 and 7; exon 5 is intact and therefore amplifies normally. In this individual we found that the full-length allele carried the A174D mutation on exon 5 (not shown).

Recently we identified two HFI patients who did not carry the common point mutations A149P, A174D, or L288ΔC on either defective allele (Cross et al. 1990). One of these individuals, designated G12, was selected for further study. Aldolase B exons from this patient were amplified, cloned, and sequenced according to methods described elsewhere (Cross et al. 1990). A 4-bp deletion was found that removes both the G residue of the AG3′ splice site of intron 2 and the first three nucleotides of exon 3 (fig. 4*A*). The lesion takes out a *Kpn*I site; no other mutations were found. The effect of this mutation on splicing is not easy to predict, though it is likely either that exon 3 would be skipped, resulting in a frameshift, or that a cryptic 3′ splice site would be activated (Green 1986). The latter is also unlikely to result in a functional protein product.

DNAs from patient G12 and from other HFI patients carrying uncharacterized aldolase B mutations were digested with *Kpn*I, were blotted, and were probed with an 840-bp *Bam*HI+*Kpn*I fragment containing intron 2. All patients except G12 revealed the band of expected size at 5.3 kb. G12 showed a larger band at 8.6 kb, confirming the deletion of the exon 3 *Kpn*I site. Furthermore, since no band is present at 5.3 kb, the patient is homozygous for this lesion (fig. 4*B*).

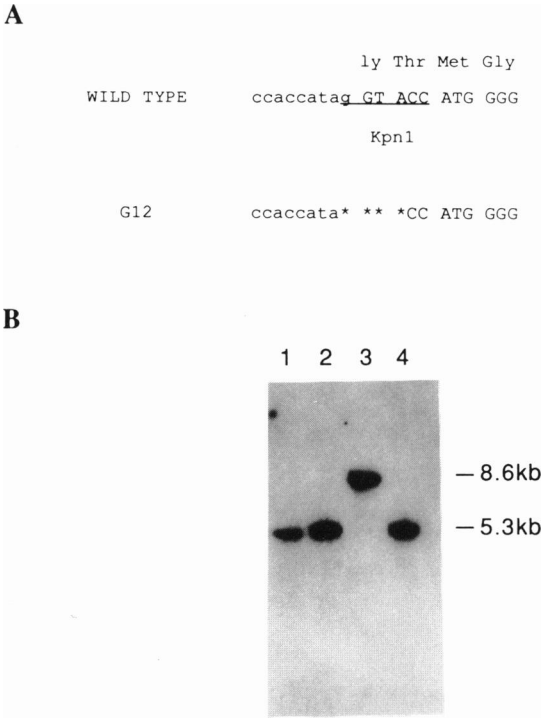


**Figure 4** *A*, Sequence of the 4-bp deletion found in patient G12 at the boundary of intron 2 and exon 3. *B*, Southern blot of HFI DNA digested with *Kpn*I and probed with an intron 2 fragment. Lanes 1, 2, and 4, Patients carrying uncharacterized aldolase B mutations. Lane 3, Patient G12, showing a homozygous deletion of the exon 3 *Kpn*I site.

## Discussion

To improve methods for the direct genetic diagnosis of HFI, we have examined further aldolase B mutations to ascertain whether there are additional widespread lesions and also to define the spectrum of mutational events at the aldolase B locus. We have identified three partial gene deletions in HFI. Two of these—i.e., one of 1.65 kb and one of 1.4 kb—were initially identified by Southern blotting and subsequently were defined by PCR amplification and sequencing. The third was a 4-bp deletion that was identified by amplifying and sequencing exons from a patient who did not carry any of the common HFI lesions. Each mutation was confined to a single family: the two patients carrying the large deletions were compound heterozygotes with either A149P or A174D on the other allele. Patient G12, carrying a 4-bp deletion, was found to be homozygous. It is noteworthy that this individual is a member of a large consanguineous Swiss family affected by HFI in which several individuals have died in infancy as a result of diagnostic errors (Froesch et al. 1963).

Patient F13 is of French origin and has previously been studied (Gregori et al. 1984). The extra band produced on digestion with *Bam*HI was noted, but, since the probe used in that study was a small 3′ cDNA clone, the authors were unable to detect any further band abnormalities by using other restriction enzymes. Thus it was not possible to decide whether the new band was due to the acquisition of a *Bam*HI site or to a heterozygous deletion. We have shown that exon 4 and part of exon 5 are deleted on one allele from this patient and that the other allele is A149P. However, genotyping by amplification of exon 5 and probing with ASOs erroneously indicated that F13 was homozygous for A149P, since no PCR product is generated from the defective allele. Such mistakes are likely to occur only rarely; we have found one instance in 49 affected individuals. However, whenever possible, we prefer to genotype the parents as well as the propositus when we attempt a genetic diagnosis for clinical purposes. Anomalous segregation of mutant alleles may indicate the presence of deletions and, moreover, the occurrence of simple Mendelian segregation serves to control for contaminated PCR reagents and sample errors.

The mechanism by which the three deletions described here have occurred is unclear. Many deletions which have been reported in other genes reveal homologous sequences flanking the breakpoints, e.g., repetitive sequence elements in the LDL receptor gene (Langlois et al. 1988) and short direct repeats in retinoblastoma (Canning and Dryja 1989). Furthermore, homologies of only 2 bp have been shown to occur more frequently at breakpoints than would be expected on the basis of random strand breakage followed by ligation (Roth et al. 1985). The deletion in patient F13 shows such a 2-bp homology: the breaks both occur after the dinucleotide CpC. Only a single base-pair homology is found at the G10 deletion, in which both breaks occur after a C residue. This lesion therefore appears to have been a random event. The 4-bp deletion in patient G12 may have been mediated by slipped-strand mispairing during DNA replication at the dinucleotide TpA.

The frequency of partial aldolase B deletions in HFI is low. In a previous study we found that 11 (13.4%) of 82 independent alleles were not accounted for by common point mutations (Cross et al. 1990). We have shown here that only two of these alleles (2.4% of the total) are large deletions, a proportion similar to that of the major structural rearrangements found in the low-density-lipoprotein receptor gene (Langlois et al. 1988). A third allele was found to harbor a much smaller deletion. The remaining eight uncharacterized mutant aldolase B alleles have a wide geographical distribution. It is therefore likely that these represent private mutations confined to single pedigrees and that the frequency of such lesions reflects the background rate of de novo mutation at the aldolase B locus. Local inbreeding will tend to increase the probability of homozygosity for rare alleles, so that individuals originating from consanguineous communities may not be readily amenable to direct genetic diagnosis.

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