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Hereditary fructose intolerance: Frequency and spectrum mutations of the aldolase B gene in a large patients cohort from France—Identification of eight new mutations

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ABSTRACT

We investigated the molecular basis of hereditary fructose intolerance (HFI) in 160 patients from 92 families by means of a PCR-based mutation screening strategy, consisting of restriction enzyme digestion and direct sequencing. Sixteen different mutations of the aldolase B (*ALDOB*) gene were identified in HFI patients. As in previous studies, p.A150P (64%), p.A175D (16%) and p.N335K (5%) were the most common mutated alleles, followed by p.R60X, p.A338V, c.360_363delCAAA (p.N120KfsX30), c.324G>A (p.K108K) and c.625−1G>A. Eight novel mutations were also identified in 10 families with HFI: a one-base deletion (c.146delT (p.V49GfsX27)), a small deletion (c.953del42bp), a small insertion (c.689ins TGCTAA (p.K230MfsX136)), one splice site mutation (c.112+1G>A), one nonsense mutation (c.444G>A (p.W148X)), and three missense mutations (c.170G>C (p.R57P), c.839C>A (p.A280P) and c.932T>C (p.L311P)). Our strategy allows to diagnose 75% of HFI patients using restriction enzymatic analysis and to enlarge the diagnosis to 97% of HFI patients when associated with direct sequencing.

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Hereditary fructose intolerance (HFI, MIM# 229600) is an autosomal recessive disease caused by a deficiency in aldolase B (E.C.4.1.2.13), the isoenzyme expressed in the liver, kidney and intestine tissues that metabolizes fructose. The incidence of the disease was estimated in Caucasian population to be one in 20,000 births [1].

Clinical manifestations of HFI, which occur at the time of weaning when fructose or sucrose is added to the diet, are recurrent vomiting, abdominal pain, liver failure, tubulopathy and hypoglycaemia that may be fatal [2]. A complete exclusion of fructose results in dramatic recovery, and thus early diagnosis is essential. Later, there is a remarkable aversion for sweets and fruit (and an absence of dental caries) which can also lead to the diagnosis. In case of unknown diagnosis, the disease may be life-threatening after fructose, sucrose or sorbitol infusions leading to iatrogenic death [2].

For many years, diagnosis of HFI has been confirmed by the metabolic response to an intravenous fructose load test or by the enzymatic assay on liver or intestinal biopsy samples which were either dangerous or invasive. Both do not allow carrier detection [3] and intravenous fructose load is less accurate in adults. Therefore, molecular genetic techniques provide a useful tool for the diagnosis of HFI.

The human gene is located on chromosome 9q31.1, spans 14.5-kbp and consists of nine exons which code for 364 amino acids [4]. To date, 45 enzyme-impairing mutations have been reported in the aldolase B gene (The Human Gene Mutation Database, http://www.hgmd.org, [2,5]). They are spread throughout the entire gene and their frequencies differ between ethnic groups. Among them, three amino acid substitutions p.A150P [6], p.A175D [1] and p.N335K [7] are frequently found while others seem to be rare or confined to single families.

Here we report the spectrum of aldolase B mutations observed in France and their relative carrier frequencies. We also describe eight new aldolase B gene mutations identified in ten unrelated HFI patients.

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Table 1
Sense (sn) and antisense (asn) primers used for sequencing of ALDOB exons 2–9 with flanking intron sequences

Exon		Primer sequences (5′–3′)
2	sn	caaacctcatttgcttatcca
	asn	gagtcttctgctcccactcc
3	sn	gtggagaagggtgacaggaa
	asn	tggaaaagggtgagaagagaa
4	sn	gggtccctcgcactaataca
	asn	tttgtttttccttgcttccttc
5	sn	ccttccctttattagaagccc
	asn	ctagcctactctttttcagccc
6	sn	ctaggttctgaggcagctag
	asn	tgcccaagatcccacaag
7	sn	gattaggggtgatgaagacagg
	asn	atttgggcagacttgggatg
8	sn	tcaacatttcactgccttcct
	asn	aaacaatgcttctcccgtgtt
9	sn	ggatggtatccccagcaa
	asn	ccctttcagccctcctac

Materials and methods

Patients

Mutations analysis for aldolase B gene was realized in 160 individuals from 92 unrelated families including at least one patient with a diagnosis of HFI. DNA samples were collected from 1994 to 2007. Diagnosis was based either on (i) a positive conventional test result, *i.e.*, an enzymatic aldolase B assay on liver biopsy specimen (before 1998) or an intravenous fructose load test (before 2002) or (ii) the detection of mutations by DNA analysis. The ethnic origin of patients was French in 70 families and Belgian in 3 families. The others were immigrants mainly from Mediterranean countries. All individuals tested and/or their parents were informed about the nature of the study and gave their consent. Fifty unrelated controls were tested for sequence analysis.

Mutation analysis

Genomic DNAs were extracted from whole blood samples collected on EDTA using the QIAampDNA Mini kit (Qiagen, Courtaboeuf, France) following the manufacturer's instruction.

We first screened for the 3 most frequent aldolase B gene mutations by PCR-restriction as previously described [8]. Extensive *ALDOB* gene analyses were performed in patients with only one or no identified alleles by (i) scanning of exons 2–9 and their boundaries using PCR and sequencing (sequence of primers are given in Table 1), (ii) search for large deletion or insertion by long range PCR (LR-PCR) using forward primer exon 2 and reverse primer exon 9.

We used the recommendations for mutation nomenclature (www.hgvs.org/mutnomen/) to name aldolase B gene variations (GenBank Accession # NP_000026.2), where the A of the ATG translation start codon is +1.

Results and discussion

Hereditary fructose intolerance (HFI) is an autosomal recessive human disease that results from the deficiency of the hepatic aldolase B isoenzyme. Simple and non-invasive diagnosis is now possible by direct DNA analysis that scans for known and unknown mutations. Mutations in the human *ALDOB* gene have been characterized extensively. Using a combination of several PCR-based methods, we studied 92 HFI-patients and their family.

By screening only for the most common mutations (p.A150P, p.A175D, p.N335K), the diagnosis of HFI was confirmed in 69 of 92 index patients (75%). These widespread mutations were either homozygous (p.A150P $n=44$; p.A175D $n=8$; p.N335K $n=2$) or compound heterozygous ($n=15$).

Seventeen patients were heterozygous for only one of these frequent mutations and 6 had none of them. So we sequenced the whole aldolase B gene coding region and the intron-exon boundaries. Eleven patients with p.A150P, 2 patients with p.A175D and 1 patient with p.N335K were compound heterozygous for less common mutations (15%). Homozygosity for rare mutations was observed in 6 patients (6.5%). The second HFI allele was not identified in 3 heterozygous patients for the common mutation p.A150P.

Direct sequencing of the aldolase B gene identified 13 additional changes (Table 2): five were already known and 8 were new (Fig. 1). These new changes include 3 missense mutations, 1 nonsense mutation, 1 insertion, 2 deletions and 1 mutation affecting splicing site. None of the new HFI mutations were found in 100 control alleles or described as SNPs in the gene coding sequence of the human *ALDOB* gene. The Mendelian inheritance of the new muta-

Table 2
Mutations in the *ALDOB* gene in 92 index patients with HFI

Exon/intron	Nucleotide change	Predicted effect	Remarks	References
Intron 2	**c.112+1G>A**	***Deduced splicing***	**Compound heterozygous (p.A150P)**	**This study**
Exon 3	**c.146delT**	**p.V49GfsX27**	**Compound heterozygous (p.A150P)**	**This study**
Exon 3	**c.170G>C**	**p.R57P**	**Homozygous**	**This study**
Exon 3	c.178C>T	p.R60X	Homozygous ($n=2$) Compound heterozygous (p.A150P)	[18]
Exon 3	c.324G>A	*p.K108K*	Compound heterozygous (p.A150P)	[17]
Exon 4	c.357delAAAC	p.N120KfsX30	Compound heterozygous (p.A150P)	[15]
Exon 5	**c.444G>A**	**p.W148X**	**Compound heterozygous (p.A150P)**	**This study**
Exon 5	c.448G>C	p.A150P	Homozygous ($n=44$) Compound heterozygous ($n=29$)	[6]
Exon 5	c.524C>A	p.A175D	Homozygous ($n=8$) Compound heterozygous ($n=13$)	[1]
Intron 6	c.625−1G>A	*Deduced splicing*	Compound heterozygous (p.A175D)	[18]
Exon 7	**c.689_690insTGCTAA**	**p.K230MfsX136**	**Compound heterozygous (p.N335K), (2 twins)**	**This study**
Exon 8	**c.839C>A**	**p.A280P**	**Compound heterozygous (p.A175D)**	**This study**
Exon 8	**c.932T>C**	**p.L311P**	**Homozygous**	**This study**
Exon 8	**c.953_994del42bp**	**p.A318–A332del**	**Compound heterozygous (p.A150P), (n=6 in 3 unrelated families)**	**This study**
Exon 9	c.1005C>G	p.N335K	Homozygous ($n=2$) Compound heterozygous ($n=5$)	[7]
Exon 9	c.1013C>T	p.A338V	Homozygous ($n=2$) Compound heterozygous (p.A150P), ($n=2$)	[13]

Novel mutations described in this study are shown in bold. Reference GenBank sequences used were NM_000035.2 for c.DNA and NP_000026.2 for protein.

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