Molecular Diagnosis of Hereditary Fructose Intolerance: Founder Mutation in a Community from India

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Abstract Hereditary fructose intolerance (HFI) is a difficult-to-confirm diagnosis, requiring either invasive liver biopsy-enzyme assay or potentially hazardous fructose challenge test or expensive molecular genetic analysis. Therefore, worldwide there has been a trend towards finding “common mutations” in distinct ethnic groups to simplify the process of diagnosis. The nonspecific presentation of the disease often leads to diagnostic confusion with other metabolic liver disorders such as glycogenoses, galactosemia, and tyrosinemia. This leads to much delay in diagnosis with consequent harm to the patient.

We report mutations in the *ALDOB* gene, from eleven Indian patients, seven of whom belong to the Agarwal community. Six patients from the Agarwal community and two non-Agarwal patients harbored one novel mutation,

c.324+1G>A (five homozygous and one heterozygous), in the *ALDOB* gene. Haplotyping performed in families confirmed a founder effect. The community has been known to harbor founder mutations in other genes such as the *MLC1*, *PANK2*, and *CAPN3* genes, thus providing another evidence for a founder effect in the community in case of HFI. This may pave the path for a simpler and quicker test at least for this community in India. In addition to the founder mutation, we report four other novel mutations, c.112+1delG, c.380-1G>A, c.677G>A, and c.689delA, and a previously reported mutation, c.1013C>T, in the cohort from India.

Introduction

Hereditary fructose intolerance (HFI, OMIM #229600) is a potentially fatal inborn error of metabolism (IEM) resulting from deficiency of aldolase B enzyme (EC 4.1.2.13), encoded by the *ALDOB* gene (OMIM *612724) in the liver and kidneys. The disorder is extremely important to recognize as it is easy to treat and has excellent outcome on avoidance of fructose- and sucrose-containing foods and dietary products (Steinmann and Santer 2012). Therapy does not involve any expensive diets or medications. In view of its rarity and practical difficulties in diagnosis, many HFI patients remain undiagnosed and suffer permanent liver damage, increasing the morbidity and sometimes mortality. The most difficult part in HFI has been establishing a diagnosis. Traditionally, these children when suspected on clinical grounds were subjected to “fructose challenge test” which is potentially life-threatening in view of sudden hypoglycemia in patients (Steinmann and Gitzelmann 1981). It has therefore become obsolete now. Enzyme assay requires a liver biopsy, an invasive test, and

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estimation can be performed only in a few laboratories around the world. The liver sample requires to be shipped under dry ice which adds to the cost and is not often feasible for long distances. Further, the enzyme activity in the liver may be secondarily reduced in a damaged liver (Steinmann and Santer 2012). Thus, the most accurate method to diagnose is through gene studies. Worldwide, many founder and common mutations have been identified in many communities, which has simplified the *ALDOB* molecular analysis by using population-specific custom protocols (Coffee et al. 2010; Esposito et al. 2010). India is a unique mix of many populations but marriage within communities creates the background for the presence of founder mutations in various IEMs.

We performed sequencing of the *ALDOB* gene in eleven patients from nine families, who were strongly suspected to have HFI. We report the existence of a founder mutation in the Agarwal community from North India. The single most common mutation, c.324+1G>A, in the *ALDOB* gene was observed in homozygous form in five and heterozygous state in one of seven patients from the Agarwal community. This mutation was also noted in two non-Agarwal patients in heterozygous state. Sequencing of the *ALDOB* gene also revealed presence of four new mutations in the cohort.

Materials and Methods

Consecutive patients presenting to genetics clinics at Sir Ganga Ram Hospital and All India Institute of Medical Sciences, New Delhi, with history consistent with HFI were recruited. There were 11 patients in the cohort from 9 families from North India, including a trio of mother and two children.

Clinical data was collected from each patient and family including the demographic details, the ethnic group, age of presentation, main clinical features, and investigation details that were performed prior to diagnosis of HFI in each family. Few patients had been on clinical follow-up for few years before recruitment into the study.

Molecular analysis was performed for a diagnostic purpose. Informed consent was taken for the test as well as for haplotype analysis from parents of each case. As this was a diagnostic study of a small scale, no ethical clearance was required from the hospital ethics committee.

Molecular Analysis

DNA was isolated by salting out method from 2 mL of whole blood (Miller et al. 1988). All nine exons and exon-intron boundaries of the *ALDOB* gene were amplified using intronic primers flanking the exons (primer sequences available on request). PCR was performed using 10 pmol

of each primer, 1U of Taq DNA polymerase, 1.5 mM MgCl₂, and 0.1 mM dNTPs in the recommended buffer with the initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30s, annealing at 58°C for 30 s, and extension at 72°C for 45 s. Sequencing was carried out on ABI 3500 analyzer covering all the nine coding exons encompassing nearly 80 bp of the exon-intron boundaries (Applied biosystems, California). Sequence chromatograms obtained were analyzed with Chromas software (Technelysium, Tewantin, Australia) and blasted with UCSC *ALDOB* genome browser.

Haplotype analysis was performed to determine if mutation carriers shared a common ancestry. Members from five families were analyzed using eight single-nucleotide polymorphic markers (rs9299349, rs12686025, rs970385, rs10819926, rs2417251, rs10989471, rs10989412, rs1338739) spanning approximately 1 Mb interval at the chromosomal region 9q31.1, which contains the *ALDOB* gene.

Bioinformatics Analysis

Gene-prediction tools GENSCAN, AUGUSTUS, GeneID, and GeneMark were used to analyze the effect of intronic mutations c.112+1delG, c.324+1G>A, and c.380-1G>A (Burge and Karlin 1997; Stanke et al. 2004; Blanco et al. 2007; Lomsadze et al. 2005). The aldolase B protein structure was modeled based on 1XDL.pdb (a thermolabile A149P aldolase B mutant) using Modeller (Sali and Blundell 1993).

Results

In the cohort of 11 patients, 7 were from the Agarwal community. Consanguinity was noted in one family (family 8). Children presented in infancy with typical symptoms of failure to thrive with either vomiting and diarrhea or progressive liver disease. A detailed account of the cases is provided in Table 1. There was only one adult in the cohort, the mother of two children (family 3), who gave history of aversion to fruits and sugar since childhood. Few unusual features are also highlighted in the table such as the custom of offering honey to newborns resulting in much earlier clinical presentation than expected in a few children.

Molecular and Computational Analysis

Molecular genetic analysis of the *ALDOB* gene through sequencing revealed six new sequence variants in 22 mutated alleles, of which c.324+1G>A mutation was noted in 13 of 22 (5 in homozygous state and three heterozygous). Other mutations were: homozygous c.112+1 delG and

Table 1 Detailed clinical presentation and mutation analysis results of cases

Family number Cases (initials)	1 ST	2 AA	3 PJ	3 BJ	3 BG(mother of PJ and BJ)	4 RS	5 SR	6 SS	7 MG	8 SYK	9 B
Age at onset of Symptoms	2.5 months ^a	4 months	5 months	Birth ^a	Infancy	1 year	1.6 years	10 months	4 months	7 months	9 months
Age at diagnosis	18 months	18 months	1 year	Birth	Adult	1 year	2.5 years	2.5 years	9 months	13 months	3 years
Presenting complaints	Diarrhea, failure to thrive	Abdominal distension, failure to thrive	Vomiting, failure to thrive	Vomiting	Vomiting, aversion to sugar in milk	Vomiting, failure to thrive	Vomiting, failure to thrive	Vomiting after sugar in milk, failure to thrive	Vomiting at weaning (4 months), diarrhea at 8 months	Failure to thrive, constipation, liver disease	Vomiting, diarrhea, and jaundice
Hepatomegaly	Yes	Yes	Yes	No	Not known	Yes	Yes	Yes	Yes	Yes, 4 cm BCM	Yes, 4 cm BCM
Liver dysfunction before treatment	Yes	Yes	Yes	No	Yes (childhood and pregnancy)	Yes, mildly deranged liver enzymes	Yes	Yes	Yes	Yes	Yes, mildly deranged SGPT
Renal involvement	Yes	Yes	No	No	No	No	No	No	No	Yes	No
Liver biopsy performed, findings	Yes, granulomatous hepatitis, macrosteatosis	Yes, details not available	Yes, details not available	No	No	Yes, details not available	Yes, details not available	No	Yes, details not available	Yes, disarrayed lobular architecture, porto-portal bridging fibrosis. Panlobular macro- and microvesicular steatosis	No
Fructose challenge test	Not performed	Not performed	Performed, positive	Not performed	Not performed	Performed, positive	Performed, positive	Performed, positive	Not performed	Not performed	Not performed
Hypoglycemia	No	Yes	No	Yes ^a	Never tested	Yes	No	No	No	No	Yes
Other significant finding	Developmental delay, abnormal transferrin isoforms	Occasional epistaxis, requires vit. K. LFTs normal	No	Seizure at day 17 of life. Normal development	No	No	No	Idiopathic hypokalemia at the age of 10 months	Milk protein allergy (milk allergen specific IgE 41.8 [<0.1]), hyperreactive airway disease	No	Mild gross motor delay
Diet history	Yes (in retrospect)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Gene study: mutations in the <i>ALDOB</i> gene	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous	Compound heterozygous	Homozygous	Homozygous	Compound heterozygous	Homozygous	Compound heterozygous
	c.324+1G>A, exon 3/intron 3 boundary	c.324+1G>A, exon 3/ intron 3 boundary	c.324+1G>A, exon 3/intron 3 boundary	c.324+1G>A, exon 3/intron 3 boundary	c.324+1G>A, exon 3/intron 3 boundary	c.324+1G>A, exon 3/intron 3 boundary	c.112+1 del G, exon 2/ intron 2 boundary	c.677G>A, exon 7	c.324+1G>A, exon 3/intron 3 boundary	c.1013C> T, exon 9	c.324+1G>A, exon 3/intron 3 boundary
							c.1013C>T, exon 9		c.689delA, exon 7		c.380-1G>A intron 4/exon 5 boundary

(continued)

Table 1 (continued)

Family number Cases (initials)	1 ST	2 AA	3 PJ	3 BJ	3 BG(mother of PJ and BJ)	4 RS	5 SR	6 SS	7 MG	8 SYK	9 B
Current clinical status	5 years – mild developmental delay – attends normal school but learning difficulty +, hepatomegaly (12 cm span at 5 years), normal liver function and renal function	5 years – normal development, attending regular school. Mild hepatomegaly (span 9 cm at 5 years), Liver function tests normal, spleen 8 cm, mild nephrocalcinosis with normal renal functions	7.5 years – normal development, in grade 2 at regular school, mild hepatomegaly, normal liver and renal function	3 years of age – normal developing, attending preschool, mild hepatomegaly, normal liver and renal function	Adult – mild hepatomegaly, normal liver and renal functions	10 years – normal development. No seizures, attends regular school. Liver 5 cm below costal margin, Liver function test normal	7 years – mild developmental delay, no seizures, doing well, normal liver and renal functions	7 year 10 months – normal development. Liver palpable 3.5 cm below costal margin, Normal liver and renal function	2 year 7 months – normal development, poor weight gain, liver about 3 cm below costal margin, normal liver function and renal function	3.3 years old well child. No hepatomegaly. Normal liver and renal functions	4 years – normal development, failure to thrive, seizure, liver palpable 3 cm and spleen 2 cm below costal margins, normal liver and renal function
Response to treatment	Satisfactory	Good	Very good	Very good	Good	Very good	Satisfactory	Very good	Good (also has milk protein allergy)	Excellent	Satisfactory

^a Intake of honey

c.677G>A in one patient each, heterozygous c.689delA and c.380-1G>A in one patient each, and heterozygous and homozygous c.1013C>T mutation in one patient each.

Mutations c.324+1G>A, c.112+1delG, c.380-1G>A, c.677G>A, and c.689delA were noted to be novel, whereas c.1013C>T is a previously reported mutation. A widely used and one of the most accurate gene-prediction program, GENSCAN, predicted that all the three intronic mutations c.112+1delG, c.324+1G>A, and c.380-1G>A disrupt splicing and result in abnormal protein with 8, 4, and 6 amino acids deletion, respectively (Figs. 1 and 2). The GENSCAN predictions were in corroboration with those of three other tools (AUGUSTUS, GeneMark, and GeneID) for c.324+1G>A and c.380-1G>A mutations. However, for the c.112+1delG mutation, AUGUSTUS predicted an insertion of amino acid valine at position 36 (Fig. 1), while both GeneMark and GeneID predicted that the single aldolase B polypeptide would break into two smaller fragments (data not shown). The exonic mutation c.677G>A leads to a p.G226D mutation in the aldolase B protein (Figs. 1 and 2). Although homology modeling predicted that this alteration does not affect the protein structure (data not shown), a distinct single amino acid mutation p.A149P is known to cause losses in thermal stability, quaternary structure, and activity (Malay et al. 2005). The effects of p.A149P mutation could also not be predicted by homology modeling and underscores the difficulties in predicting perturbations due to single amino acid changes (Malay et al. 2005). On the other hand, the second exonic mutation c.689delA is a frameshift mutation that results in premature termination of the polypeptide and deletion of ~130 C-terminal amino acids of aldolase B protein (Figs. 1 and 2). All five mutations were detected to be damaging using SIFT, Polyphen II, and Mutation Taster (http://sift.jcvi.org/www/SIFT_enst_submit.html, <http://genetics.bwh.harvard.edu/pph2/>, <http://www.mutationtaster.org/>).

Homozygous mutation c.324+1G>A was noted in five of seven Agarwal patients. In addition, it also occurred in heterozygous form in one Agarwal and two non-Agarwal patients. Another child from the Agarwal community harbored a different c.112+1delG mutation in homozygous state. These findings led us to investigate the presence of a founder effect in the community, which is known to harbor other founder mutations (Gorospa et al. 2004; Bahl et al. 2005; Chabbria et al. 2007).

Haplotype Analysis

Eight single-nucleotide polymorphisms (SNPs) were analyzed flanking the mutation c.324+1G>A. These included both intragenic and flanking regions mapping up to 1 Mb. Homozygosity mapping in the families using

ALDOB_no_mutation	MAHRFPALTQEQQKELSEIAQSSIVANGKILAADES-VGTMGNRLQRIKVENTEENRRQF	59
ALDOB_splice_GENSCAN	MAHRFPALTQEQQKELSEIAQSSIVANGKILAADES-V-----RIKVENTEENRRQF	51
ALDOB_splice_AUGUSTUS	MAHRFPALTQEQQKELSEIAQSSIVANGKILAADES-VVSTMGNRLQRIKVENTEENRRQF	60
ALDOB_c.677G>A	MAHRFPALTQEQQKELSEIAQSSIVANGKILAADES-VGTMGNRLQRIKVENTEENRRQF	59
ALDOB_c.689delA	MAHRFPALTQEQQKELSEIAQSSIVANGKILAADES-VGTMGNRLQRIKVENTEENRRQF	59
***** * *****		
ALDOB_no_mutation	REILFSVDSSINQSIGGVILFHETLYQKDSQGLFRNILEKGIIVVGIKLDQGGAPLAGT	119
ALDOB_splice_GENSCAN	REILFSVDSSINQSIGGVILFHETLYQKDSQGLFRNILEKGIIV----LDQGGAPLAGT	107
ALDOB_splice_AUGUSTUS	REILFSVDSSINQSIGGVILFHETLYQKDSQGLFRNILEKGIIV----LDQGGAPLAGT	116
ALDOB_c.677G>A	REILFSVDSSINQSIGGVILFHETLYQKDSQGLFRNILEKGIIVVGIKLDQGGAPLAGT	119
ALDOB_c.689delA	REILFSVDSSINQSIGGVILFHETLYQKDSQGLFRNILEKGIIVVGIKLDQGGAPLAGT	119
***** *****		
ALDOB_no_mutation	NKETTIQGLDGLSERCAQYKKDGVDFGKWRVLRADQCPSSLAIQENANALARYASICQ	179
ALDOB_splice_GENSCAN	NKETTI-----QERCAQYKKDGVDFGKWRVLRADQCPSSLAIQENANALARYASICQ	161
ALDOB_splice_AUGUSTUS	NKETTI-----QERCAQYKKDGVDFGKWRVLRADQCPSSLAIQENANALARYASICQ	170
ALDOB_c.677G>A	NKETTIQGLDGLSERCAQYKKDGVDFGKWRVLRADQCPSSLAIQENANALARYASICQ	179
ALDOB_c.689delA	NKETTIQGLDGLSERCAQYKKDGVDFGKWRVLRADQCPSSLAIQENANALARYASICQ	179
***** . *****		
ALDOB_no_mutation	QNGLVPIIVEPEVIPDGDHDLHCQYVTEKVLAAVYKALNDHHVYLEGTLLKPNMVTAGHA	239
ALDOB_splice_GENSCAN	QNGLVPIIVEPEVIPDGDHDLHCQYVTEKVLAAVYKALNDHHVYLEGTLLKPNMVTAGHA	221
ALDOB_splice_AUGUSTUS	QNGLVPIIVEPEVIPDGDHDLHCQYVTEKVLAAVYKALNDHHVYLEGTLLKPNMVTAGHA	230
ALDOB_c.677G>A	QNGLVPIIVEPEVIPDGDHDLHCQYVTEKVLAAVYKALNDHHVYLEGTLLKPNMVTAGHA	239
ALDOB_c.689delA	QNGLVPIIVEPEVIPDGDHDLHCQYVTEKVLAAVYKALNDHHVYLEGTLLSPTW-----	233
***** * . *		
ALDOB_no_mutation	CTKKYTPEQVAMATVTALHRTVPAAVPGICFLSGMSEEDATLNLNAINLCLPKPWKLS	299
ALDOB_splice_GENSCAN	CTKKYTPEQVAMATVTALHRTVPAAVPGICFLSGMSEEDATLNLNAINLCLPKPWKLS	281
ALDOB_splice_AUGUSTUS	CTKKYTPEQVAMATVTALHRTVPAAVPGICFLSGMSEEDATLNLNAINLCLPKPWKLS	290
ALDOB_c.677G>A	CTKKYTPEQVAMATVTALHRTVPAAVPGICFLSGMSEEDATLNLNAINLCLPKPWKLS	299
ALDOB_c.689delA	-----	233

ALDOB_no_mutation	FSYGRALQASALAAGGKAANKEATQEA FMKRAMANCQAAGQYVHTGSSGAASTQSLFT	359
ALDOB_splice_GENSCAN	FSYGRALQASALAAGGKAANKEATQEA FMKRAMANCQAAGQYVHTGSSGAASTQSLFT	341
ALDOB_splice_AUGUSTUS	FSYGRALQASALAAGGKAANKEATQEA FMKRAMANCQAAGQYVHTGSSGAASTQSLFT	350
ALDOB_c.677G>A	FSYGRALQASALAAGGKAANKEATQEA FMKRAMANCQAAGQYVHTGSSGAASTQSLFT	359
ALDOB_c.689delA	-----	233

ALDOB_no_mutation	ACYTY 364	
ALDOB_splice_GENSCAN	ACYTY 346	
ALDOB_splice_AUGUSTUS	ACYTY 355	
ALDOB_c.677G>A	ACYTY 364	
ALDOB_c.689delA	----- 233	

Fig. 1 Alignment of aldolase B protein sequences. The intronic mutations c.112+1delG, c.324+1G>A, and c.380+1G>A result in 8, 4, and 6 amino acid deletions, respectively (shaded in yellow; GENSCAN program prediction). The residue 226 altered due to exonic mutation c.677G>A is shaded in gray. Alignment was performed using Clustal Omega at <http://www.ebi.ac.uk/Tools/msa/>

trios of child and parents revealed identical haplotypes with the c.324+1G>A mutation as shown in the Fig. 3. All patients harboring homozygous mutation had identical haplotype on both chromosomes. The Agarwal patient with heterozygous mutation showed a different haplotype on one chromosome. All the markers tested seemed to be in linkage disequilibrium with the mutation c.324+1G>A in the *ALDOB* gene. This haplotype was different from haplotype in another Agarwal patient who had a different mutation, c.112+1delG. These findings confirm the presence of a founder effect in the subpopulation.

clustalo/. ALDOB_no_mutation, wild-type protein sequence; ALDOB_splice_GENSCAN and ALDOB_splice_AUGUSTUS, predicted proteins of gene with intronic mutations by GENSCAN and AUGUSTUS, respectively; ALDOB_c.677G>A and ALDOB_c.689delA, protein sequence of gene with exonic mutations c.677G>A and c.689delA, respectively

Discussion

HFI is a unique inborn error of metabolism where the major hurdle lies in making an accurate diagnosis rather than treatment which is as simple as removal of fructose and sucrose from the diet. The outcome is related to severity of initial presentation, comorbidities, and the time taken for diagnosis after onset of symptoms and commencement of therapy. In our cases, majority of children are doing well, with no developmental delay and only mild hepatomegaly. Two children have developmental delay related to the

Fig. 2 Modeled structure of wild-type aldolase B. The residues that get deleted (predicted by GENSCAN program) due to intronic mutations c.112+1delG, c.324+1G>A, and c.380-1G>A have been shown in *orange*, *pink*, and *blue*, respectively. The residue 226 altered

due to exonic mutation c.677G>A is highlighted in *red*. The structure marked in *brown* gets deleted in gene with c.689delA mutation. The structure in panel B is derived by rotating the panel A structure by 260° along y-axis

severity of the symptoms at onset and delay in diagnosis. Traditionally, the disorder has either remained unconfirmed or diagnosed only after invasive liver biopsy enzymology or a fructose challenge test which can prove hazardous in view of rapid hypoglycemia after fructose intake (Steinmann and Gitzelmann 1981). Worldwide, common mutations such as p.A149P mutation, which showed a founder effect in studies, have simplified the diagnostic strategy in certain populations (Brooks and Tolan 1993). There is a paucity of literature on HFI and *ALDOB* gene studies from India, as only few case reports, without mutations, have been reported (Bharadia and Shivpuri 2012; Ananth et al. 2003). This is the first report of mutations in the *ALDOB* gene from India.

In our cohort of patients, all patients have shown remarkable improvement in clinical condition and catch-up growth once treatment is commenced after diagnosis. This is consistent with the established literature (Steinmann and Santer 2012; Steinmann and Gitzelmann 1981) and emphasizes the need for an early and accurate diagnosis.

The Indian population is a heterogeneous mix of various communities, and the common Caucasian mutations are not expected to be present. The gene pool is expected to be heterogeneous but with pockets of homogeneity because of the general trend of marriages within closed communities. The Agarwal community is a large enterprising community consisting of affluent business-class North Indian population originating from a small town of Agroha, near

Delhi and spreading all over the world (Singhal 2005). Genetic studies have suggested founder effect in Agarwals in some other genetic disorders like megalencephalic leukoencephalopathy with subcortical cysts (MLC, OMIM #604004), spinocerebellar ataxia type 12 (OMIM #183090), and pantothenate kinase-associated neurodegeneration (PKAN, OMIM#234200) (Gorospe et al. 2004; Bahl et al. 2005; Chabbria et al. 2007). Recently, two mutations have been shown to have founder effect in Agarwals in calpain-3 gene (CAPN3, OMIM *114240) in patients of LGMD type 2A (OMIM #253600) (Ankala et al. 2013).

Our molecular analysis in the Agarwal families revealed homozygous segment of DNA spanning 1 mb flanking the *ALDOB* gene, on haplotype analysis. This haplotype was in linkage disequilibrium with the mutation c.324+1G>A, as this did not match with another Agarwal patient harboring a different mutation, thus confirming a founder effect.

The information about the common mutation is likely to benefit our Indian patients who have been visiting the clinics of pediatricians, gastroenterologists, and geneticists for years without a diagnosis. It will simplify the diagnosis of this condition, at least in the Agarwal community, thus leading to an excellent outcome after early diagnosis.

We also report four other novel mutations from our cohort, including splice site mutations c.112+1 delG and c.380-1G>A as well as exonic mutations c.677G>A and c.689delA. The presence of more novel mutations than previously reported in literature is suggestive of a different

a Agarwal with homozygous mutation, c.324+1 G>A

b Agarwal with heterozygous c.324+1G>A mutation

Fig. 3 Haplotype analysis in Agarwal families

gene pool among the Indian population. Only one of six mutations, c.1013C>T, is previously reported. Interestingly this mutation was noted in a child belonging to a consanguineous family from Kashmir. The Kashmir population has been noted by our group to share the gene pool with the Caucasian population based on genetic studies in other disorders, one notable example being cystic fibrosis. We propose a stepwise molecular diagnostic approach in children suspected to have HFI, based on their ethnicity within the Indian population.

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Take-Home Message

First report of molecular mutations in HFI from India, founder mutation in the Agarwal community. Simplifying diagnosis for easily treatable yet life-threatening metabolic condition.

Conflict of Interest

All the authors (Sunita Bijarnia-Mahay, Sireesha Movva, Neerja Gupta, Deepak Sharma, Ratna D. Puri, Udhaya Kotecha, Renu Saxena, Madhulika Kabra, Neelam Mohan and Ishwar C Verma) declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

Details of the Contributions of Individual Authors

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1. Conception and design
2. Clinical management of children with HFI
3. Analysis and interpretation of data
4. Drafting the article

Sireesha Movva

1. Analysis and interpretation of data, haplotype analysis
2. Drafting the article (molecular methodology)

Neerja Gupta

1. Conception and design
2. Clinical management of children with HFI
3. Drafting the article (provided patient information)

Deepak Sharma

1. Analysis and interpretation of data (computational analysis and bioinformatics)
2. Drafting the article (methodology and results of bioinformatics)

Ratna Dua Puri

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