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Clinical and genetic analysis for a Chinese family with hereditary fructose intolerance

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Abstract Hereditary fructose intolerance (HFI) is an inheritable disorder of fructose metabolism, inherited as an autosomal recessive disorder and caused by catalytic deficiency of aldolase B, which is critical for gluconeogenesis and fructose metabolism. The affected individuals develop severe hypoglycemia after taking foods containing fructose and cognate sugars. The exons 2–9 of the aldolase B (gene symbol *ALDOB*) gene from one Chinese HFI patient were amplified by the polymerase chain reaction (PCR), and direct sequence determination was applied to the amplified fragments. The mutation of a 4-bp (AACA) deletion (479_482 del) in exon 4 of *ALDOB* gene was identified in the patient, which had been reported to cause a frameshift at codon 118 and a truncated protein of 132 amino acids in the previous study. Then, the second case with the same homozygote deletion and eight cases with heterozygotes had been found through screening for the mutation c.479_482 del AACA in the whole family. This is the first report of HFI with the mutation c.479_482 del AACA in the *ALDOB* gene in a Chinese family.

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Introduction

Hereditary fructose intolerance (HFI), first recognized in 1956 by Chambers and Pratt [1], is an autosomal recessive disorder of carbohydrate metabolism caused by catalytic deficiency of aldolase B in liver, kidney, and small intestine, which splits fructose 1-phosphate (Fru-1-P) into two trioses, enabling its entry into the glycolytic pathway. Aberrations of the aldolase B enzyme in HFI patients result in the impaired function to metabolize fructose, who will develop abdominal pain, vomiting, and such metabolic disturbances as hypoglycemia that may be fatal after taking foods containing fructose, sorbitol, or sucrose. Continued ingestion of noxious sugars causes liver and renal dysfunction, which eventually leads to liver cirrhosis and even death. Those most at risk are infants and newborn small infants [2]. The true incidence of HFI is not known, but may be estimated as high as 1 of 26,000 [3]. Confirmatory diagnosis is generally made by intravenous fructose tolerance tests, assays of aldolase B activity in liver biopsy, and molecular methods. Symptoms usually disappear once fructose is totally excluded from diet. Affected individuals can survive into adulthood by self-impose fructose restriction.

Here we report a family resident in China with HFI carrying a mutation of 4-bp deletion (c.479_482 del AACA) in exon 4 of the aldolase B gene (gene symbol *ALDOB*), and it is the first time to report a HFI family with the mutation c.479_482 del AACA in the *ALDOB* gene in Chinese.

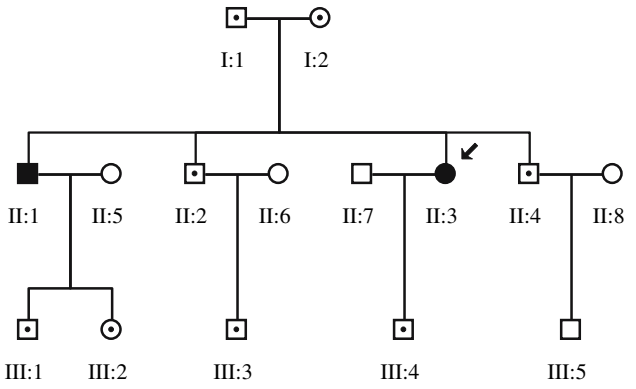

Fig. 1 Pedigree of a family with hereditary fructose intolerance. Full solid symbol represents the affected individuals. Open symbol represents the unaffected individuals. Arrow symbol represents the probands. Square symbol represents the males and circle symbol the females. Symbol with a dot in it represents asymptomatic carriers. Generations are designated by Roman numerals

Table 1 Amino-acid analysis in proband's urine

Aspartic acid	184.72 nmol/ml (31.2 ± 11.6)
Serine	450.72 nmol/ml (163.2 ± 60.8)
Glumatic acid	361.60 nmol/ml (35.1 ± 17.6)
Proline	21.70 nmol/ml (76.9 ± 32.6)
Alanine	1651.16 nmol/ml (306.8 ± 106.5)
Dicysteine	439.16 nmol/ml (94.5 ± 10.4)
Valine	143.00 nmol/ml (96.3 ± 46.1)
Methionine	29.90 nmol/ml (24.6 ± 10.5)
Isoleucine	11.46 nmol/ml (59.2 ± 21.2)
Leucine	5.20 nmol/ml (39.8 ± 28.5)
Tyrosine	109.10 nmol/ml (57.4 ± 22.4)
Phenylalanine	62.06 nmol/ml (47.6 ± 26.2)
Lysine	287.10 nmol/ml (107.4 ± 40.8)
Histidine	1223.72 nmol/ml (398.3 ± 157.2)
Arginine	53.56 nmol/ml (47.2 ± 19.4)
Tryptophane	42.28 nmol/ml (36.0 ± 14.0)

Values given in parentheses are mean ± SD of normal values

Results

Clinical features of the HFI family

The pedigree of this family is shown in Fig. 1. The physical examination (heart rate, blood pressure, respiration rate, temperature, overall health status) of all the family members seemed to be normal. The laboratory data of the proband revealed selected amino-acid uria (Table 1) and impaired glucose tolerance (IGT) with the delayed insulin response. (Table 2), her liver and renal functions were within normal limits (Table 3). There were not any significant findings in imaging examination. After a carefully controlled administration of fructose (0.25 g/kg body weight) by intravenous infusion, onset of hypoglycemia (from 4.5 mmol/L to 1.9 mmol/L) in combination with decreasing phosphorus (from 0.93 mmol/L to 0.41 mmol/L) and increasing lactic

Table 2 OGTT and IRT data of the proband

	0 min	30 min	60 min	120 min	180 min
OGTT (mmol/L)	4.2	8.0	9.3	9.4	6.5
IRT (μIU/mL)	11.7	83.5	108.0	116.0	74.6

OGTT, oral glucose tolerance test; IRT, insulin releasing test

Table 3 Routine laboratory data of the proband with HFI on admission

Complete blood counts	Within normal limits
Regular urinalysis	Within normal limits
Liver function	Within normal limits
Renal function	Within normal limits
Blood fat	
Triglyceride	2.83 mmol/L (0.56–1.70)
Cholesterol	3.44 mmol/L (2.33–5.70)
High density lipoprotein	0.82 mmol/L (0.80–1.80)
Low density lipoprotein	1.89 mmol/L (1.30–4.30)
Apolipoprotein A	1.06 g/l (1.04–2.04)
Apolipoprotein B	0.76 g/l (0.45–1.40)
Lipoprotein (a)	0.10 g/l (0–0.3)
Serum electrolytes	
Sodium	137.0 mmol/L (130–147)
Potassium	3.84 mmol/L (3.5–5.1)
Chlorine	104.0 mmol/L (95–108)
Calcium	2.12 mmol/L (2.00–2.75)
Phosphorus	1.08 mmol/L (0.80–1.60)
Urine electrolytes	
Sodium	101.5 mmol/24 h (137–257)
Potassium	33.35 mmol/24 h (36–90)
Chlorine	107.3 mmol/24 h (170–250)
Calcium	8.79 mmol/24 h (2.5–7.5)
Phosphorus	11.75 mmol/24 h (16.15–42)
Urine titratable acid	
PH	5.27 (5.1–6.5)
HCO_3^-	5.66 mEq/L (0.64–13.6)
TA	23.74 mEq/L (9.15–30.7)
$NH4^+$	52.92 mEq/L (28.8–60.2)
Arterial blood gas analysis	
PH	7.41 (7.35–7.45)
$PaCO_2$	4.95 kPa (4.67–6.00)
PaO_2	6.42 kPa (10.67–13.33)
HCO_3^-	23.3 mmol/L (22.0–27.0)
BE	–1.3 mmol/L (–3.0–3.0)
SO_2	86.3% (91.9–99.0)

Values given in parentheses are normal range. BE, base excess; SO_2, O_2 saturation

Table 4 Laboratory data of the proband in intravenous fructose tolerance test

	0 min	10 min	20 min	30 min	40 min	50 min	60 min
Blood glucose (3.9–6.1 mmol/L)	4.5	3.8	3.3	2.7	2.3	2.1	1.9
Blood phosphorus (0.80–1.60 mmol/L)	0.93	0.31	0.33	0.27	0.30	0.33	0.41
Blood lactic acid (0.50–2.00 mmol/L)	2.71	4.71	5.61	5.38	6.21	6.14	5.79
Blood uric acid (160–430 umol/L)	293	406	463	495	505	509	497

acid (from 2.71 mmol/L to 5.79 mmol/L) and uric acid concentrations (from 293 umol/L to 497 umol/L) were recorded (Table 4). The obvious distress shown in the process of fructose intravenous infusion, such as sweating, nausea, and abdominal pain, were clearly relieved right after glucose infusion. Thus, the fructose tolerance test signified a positive result and as one of the two methods of HFI diagnosis, this result definitely indicated HFI. As the patient's parents are cousins, and they have borne four children in all, it seems that the proband is not the only member of the family who is obviously clinically afflicted with this disease. The elder brother (II-1) has also been intolerant of fructose and had some behavioral symptoms seen in HFI patients. But he refused to be performed a fructose tolerance test. Other members of the family have had no symptoms or signs of fructose intolerance.

Mutation analysis of the affected pedigree

As shown in Fig. 2, a mutation of 4-bp (AACA) deletion (479_482 del) in exon 4 of *ALDOB* gene was first detected in the proband. This mutation would result in synthesis of a protein with a frameshift at codon 118 and a truncation of 132 amino acids [4]. By screening for the mutation 479_482 del in the whole family of proband's, his elder brother (II-1) who had several symptoms of fructose intolerance was further identified to carry the same mutation. Among other family members appearing phenotypically normal, eight heterozygotes (I-1, I-2, II-2, II-4, III-1, III-2, III-3, III-4) were also detected.

Discussion

Hereditary fructose intolerance is a serious and potentially fatal disease. The correct and effective diagnosis at the early stage decides the prognosis of affected individuals. Homozygotes are often difficult to be detected, for the intravenous fructose tolerance challenge test and an enzyme assay from a liver biopsy are both invasive. Furthermore, heterozygotes of *ALDOB* gene mutation have sufficient aldolase activity and cannot be readily identified as well. As the sequence of the human *ALDOB* gene had been known [5], the molecular diagnostic method appears to be extremely beneficial and should become routine for this hereditary disease due to its simple and noninvasive to scan for known and unknown mutations. The HFI diagnosis of the proband was based on history, clinical symptoms after transferring to harmful sugars, a fructose tolerance test, and the direct sequencing analysis of *ALDOB* gene. Since there is no abnormal manifestation of the patient's liver and renal both in imaging examination and blood analysis, we did not adopt the invasive hepatic biopsy on the patient. However, as patients from consanguineous marriages who complained of typical symptoms were investigated to find the mutant allele common to their parents [6], by screening for the 4-bp deletion in exon 4 among the other 14 family members, the suspected person (II-1) was confirmed to be another HFI patient, and eight asymptomatic persons including their parents were detected to carry the same mutation on one of their alleles, which confirmed that the mutation followed Mendelian inheritance.

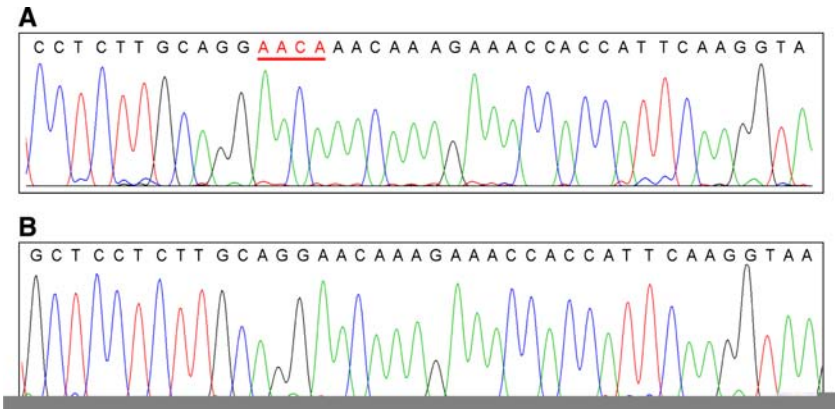

Fig. 2 Identification of mutation by direct sequencing of the *ALDOB*. The exon 4 of *ALDOB* gene, amplified by PCR, was directly sequenced in this family. Comparing with the wide type sequences (**a**), the homozygotes of ≥ 4E4 mutation (c.479_482 del AACA) presented with singlet signals on chromatogram (**b**)

Human *ALDOB* gene, located on chromosome 9q22.3, is 14.5 kb long and has 9 exons, the first of which is untranslated [6–8]. The cognate mRNA encodes 364 amino acids [9]. Until now, 43 different mutations causing HFI have been identified in *ALDOB* gene of HFI patients [10], including missense and nonsense mutations, large and small deletions (from 1 bp to 1.65 kb) and mutations in the splicing region [6, 11–13]. Three missense mutations, A149P and A174D in exon 5, and N334K in exon 9, are most frequent in the HFI population and together account for 84% of alleles in the European HFI population [14] and 68% in the North American HFI population [15]. The mutation responsible for HFI in this family is a deletion of 4-bp in exon 4, which was first reported in a A149P/Δ4E4 compound heterozygote, causing a truncated protein of 132 amino acids [4]. A protein of this size would be lacking several conserved regions important for activity of the enzyme.

It has been observed that the clinical severity of the disease phenotype appears to be independent of the nature of *ALDOB* gene mutations and found to correlate well with the immediate nutritional environment, age, culture, and eating habits of affected subjects [6]. This finding can partly reflect the alacrity of their parents when they recognized feeding difficulties during the homozygote's infancy and childhood. As a crucial feature of HFI is the ability of the fructose-metabolizing tissues to recover once the offending sugars are excluded. At the same time, it also can explain why two HFI patients in this family could grow up and develop as normal children without retardation.

Besides HFI, it is noteworthy that the proband presented with IGT (Table 2) and hyperlipidemia (Table 3) as well. It has been well documented in vivo that fructose stimulates lipid and glycogen synthesis and it is possible that a fructose-free diet or a diet poor in fructose decreases in vitro some activities involved in glucose and fructose metabolism [16]. So it is difficult to explain whether IGT and hyperlipidemia existed here is correlated with HFI or independent of that. We still need a long time to follow up the patient's health conditions and observe how the state changes.

In summary, the present study describes clinical and genetic features in a three-generation family with HFI caused by a 4-bp deletion in exon 4 of *ALDOB* gene, and it is also the first study to report the identification of *ALDOB* gene mutation in Chinese subjects. Although previous studies had got some achievements in HFI, our knowledge about this disease is still limited. The structure of the common mutant A149P protein was not published until 2005 [17] and the frequency of HFI has not been determined with precision in any population [18]. These findings unveil the case report of *ALDOB* gene mutation for HFI in China and provide us an approach to investigate the incidence of HFI in Chinese population as our future work. With artificial sweeteners become ubiquitous in many processed foods and are distributed to many countries, increased symptoms of HFI are constantly emerging. Therefore, more researches should focus on understanding the molecular basis of the disease and a more complete picture with respect to the global distribution of *ALDOB* gene mutations is required.

Materials and methods

Subjects

The study protocols were approved by the Hospital Ethics Committee for Human Research, and informed consent was obtained from every subject participating in the study.

The proband (Fig. 1; the index case number is subject II-3, representing generation II, subject number 3) was a 34-year-old Chinese woman who complained of nausea, vomiting, gastrointestinal discomfort, palpitation, debilitation, and cold sweating shortly after taking sweet foods or fruit since she was born. When she suffered with these symptoms, glucose supplementation could relieve her pain. During her difficult initial period of weaning, her mother played a critical part in the nutrition and was subconsciously best placed to protect her by the early withdrawal of foods and drinks that cause distress, thus identifying the harmful items for her to avoid as she became more independent. As she grew up, she developed an aversion to sweet-tasting foods or drinks, and spontaneously to avoid of fruit and vegetables in the food intake and her voluntary dietary exclusion would be refined by trail and error over a lifetime. The maternal behavior and self-protective habits made her become symptom-free and exhibited normal growth and development. The patient had never been diagnosed of HFI or any other disease before being hospitalized. Based on her clinical history and dietary intolerance leading to hypoglycemia, and occurrence of the similar symptoms disease in one of her brother, the subject was suspected to suffer from HFI. Physical examinations and ultrasonography measurements showed no abnormalities in her liver and renal. Blood analysis showed that liver and renal functions were within normal range (Table 3). Urine analysis revealed selected amino-acid uria (Table 2). The proband did response to the intravenous fructose challenge test (0.25 g/kg body weight) with hypoglycemia, rapidly diminishing phosphorus and concentrations of lactic acid and uric acid (Table 4). Therefore these findings were useful to clinically diagnose her of HFI.

The patient is the third child, and has three brothers. The elder brother (II-1), 8 years older than the proband, was also intolerant of sweet foods and had a history of episodes

Table 5 Primers for *ALDOB* amplification

Exon	Primers	Primers Sequences(5′–3′)	Product size (bp)
2	2F	TGGAAATGGGTCAGAGGT	421
	2R	CTGGAATGTGCCTACTACTTAC	
3	3F	GAGATGATGTGGAGAAGGGTG	424
	3R	TGTGGGAAGATGACGATGG	
4	4F	GGTGCTCAATAAATGTTAGGGTAG	378
	4R	TGTGGCTCTAAGACCAGTGTAAT	
5	5F	AGGCCCGTTTAGATGACC	577
	5R	AGGGCTAAGACCTTGAGTT	
6	6F	TGATATGCTTGGCTGTT	394
	6R	GGAGGTCCATTTGTAGTT	
7	7F	TTTGGAGGATGGTGAC	693
	7R	AAGGTGAAGGGCTGAT	
8	8F	CCCACTTCAGAATACCAAGC	547
	8R	AATGCTTCTCCGTGTTGG	
9	9F	AGGAGACAGGGTCAAGGTGG	418
	9R	GTGGGTATTCTGGAGCATG	

suggestive of hypoglycemia. But he did not have the fructose intolerance test. Other two brothers (II-2, II-4) never developed symptoms of hyperglycemia. Their parents are consanguineous and have no signs or symptoms. All the third generation (III-1, III-2, III-3, III-4, III-5) of the family are symptom-free and have no significant differences in height, weight, and intelligence, compared with age-matched healthy children.

PCR and sequencing of *ALDOB* gene

Genomic DNA from peripheral blood leukocytes of the proband and her family members were extracted using a commercially available kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The 2–9 exons of *ALDOB* were amplified by polymerase chain reaction (PCR) using eight pairs of primers (Table 5), which were designed with Primer Premier 5.0 software (PREMIER Biosoft International, Palo Alto, CA). The PCR was performed in a volume of 25 μl containing standard PCR buffer2 μl, 25 mM dNTP 0.5 μl, genomic DNA200 ng, 0.5 μl of each specific primer, and 2.5 u/μl Taq DNA polymerase 0.3 μl (Sangon, Shanghai, China). Amplification was performed with preheating at 95°C for 5 min, followed by denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s for 40 cycles. A final extension step of 72°C for 10 min was used. The PCR products were purified using QIAGEN PCR purification kits (Qiagen, GmbH, Hilden, Germany) and sequenced in both sense and antisense direction on a ABI 3700 sequencer (Applied Biosystems PerkinElmer, Foster City, CA) with both sense and antisense primers.

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