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Haplotype analysis suggests that the two predominant mutations in Japanese patients with holocarboxylase synthetase deficiency are founder mutations

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Abstract Holocarboxylase synthetase (HCS) deficiency is a rare autosomal recessive disorder of biotin metabolism. Including three new Japanese patients we diagnosed in this study, ten Japanese families have, so far, been accumulated. In these families, the mutations 237Leu > Pro (seven alleles) and 1067delG (five alleles) were predominant; 508Arg > Trp and 550Val > Met mutations were identified in three families in the heterozygous form and in one patient in the homozygous form, respectively. To determine the origin of these mutations, we identified new polymorphic microsatellite markers in the *HCS* gene and analyzed the haplotypes of the patients. All the 237Leu > Pro and the 1067delG alleles were associated with haplotype 2-2. This finding is consistent with the notion that these mutations are founder mutations in the Japanese population. Three Japanese 508Arg > Trp alleles were associated with several haplotypes, including 2-3 and 1-4. The haplotype of a Taiwanese patient homozygous for the 508Arg > Trp mutation was 2-3/2-3. The haplotype of one Japanese patient homozygous for the 550Val > Met mutation was 1-4/1-4, whereas that of a Jewish patient with the same homozygous mutation was 2-3/2-3. Both mutations were associated with at least two haplotypes and were found in several ethnic groups. The changes 508Arg > Trp and 550Val > Met occurred at CpG dinucleotide. The data suggest that these two mutations represent a mutational hot-spot.

Key words Holocarboxylase synthetase · Multiple carboxylase deficiency · Biotin · Mutation · Microsatellite markers · Haplotype

Introduction

Holocarboxylase synthetase (HCS: EC 6.3.1.10) is an enzyme that catalyzes biotin incorporation into various carboxylases. Four carboxylases that require biotin as a coenzyme have been identified in mammalian cells: acetyl-CoA carboxylase (EC 6.4.1.2), a rate-limiting enzyme in fatty acid synthesis; pyruvate carboxylase (EC 6.4.1.1), a key enzyme in gluconeogenesis; propionyl-CoA carboxylase (EC 6.4.1.3), and 3-methylcrotonyl-CoA carboxylase (EC 6.4.1.4), which is involved in amino acid catabolism. Because the biotinylation of these carboxylases is essential for their enzymatic activities, a defect in HCS decreases the activity of several carboxylases, affecting various metabolic processes in cells. HCS deficiency (McKusick 253270) is therefore also referred to as biotin-responsive multiple carboxylase deficiency (MCD). Patients with HCS deficiency have acute episodes of metabolic acidosis and characteristic organic aciduria during the neonatal to early infantile period. Symptoms include tachypnea, feeding difficulties, seizures, and severe dermatitis. Patients may recover from metabolic acidosis within a few days after they start biotin therapy (Wolf 1995).

We isolated human HCS cDNA and have analyzed mutations among Japanese and other ethnic groups of patients with an HCS deficiency (Suzuki et al. 1994; Aoki et al. 1995; Aoki et al. 1999). The mutations 237Leu > Pro (nucleotide change 997T > C) and 1067delG predominated only among the Japanese patients. The 508Arg > Trp (1809C > T) mutation has been found in two unrelated Japanese patients (Sakamoto et al. 1998), as well as in non-Japanese patients (Dupuis et al. 1996). The 550Val > Met (1935G > A) mutation has also been found in many ethnic groups (Dupuis et al. 1996; Aoki et al. 1997; Zammarchi et al. 1998). To determine the origin of these mutations in

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families with an HCS deficiency, we analyzed the haplotypes of normal and mutant *HCS* alleles.

Patients and methods

Patients

The offspring of non-consanguineous Japanese parents, patient 1 developed severe ketoacidosis on the second day of life and died at the age of 5 days. Organic aciduria typical of MCD was present. HCS activity was assayed as described previously (Suzuki et al. 1996). The enzymatic activity in the patient's liver autopsy sample was 5.0% of the control value.

Patient 2 is a girl born to unrelated Japanese parents. This patient developed an upper respiratory infection at the age of 8 months that was followed by metabolic acidosis and a skin infection. Because urinary organic acid was indicative of MCD, biotin was administered. Her physical and mental development proceeded normally when she received 40mg/day of biotin. The HCS activity in lymphoblasts was 14.1% of the control value.

Patient 3 was also a Japanese girl, the offspring of a non-consanguineous marriage. She developed severe ketoacidosis on the first postpartum day and died 5 days later. A child of the same parents (is girl) born before her had also died at the age of 4 days. An HCS deficiency was demonstrated by assaying the enzyme activity in fibroblasts from the patient (5.5% of the control value).

The other patients analyzed in this study have been described elsewhere (for references see Table 1). This study was approved by the Ethics Committee of Tohoku University School of Medicine.

Mutation in HCS-deficient patients

Genomic DNA from fibroblasts, lymphoblasts, or an autopsy sample was prepared, using a SepaGene Kit (Sanko Junyaku, Tokyo, Japan). DNA was extracted from dried blood spots obtained from the parents, the grandparents, and control individuals, as described (Ogasawara et al. 1994). The 997T > C (237Leu > Pro) and the 1067delG mutations were screened, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as described (Aoki et al. 1995). The 508Arg > Trp (1809C >

Table 1. Mutations and haplotypes of patients with holocarboxylase synthetase (HCS) deficiency

Family	Individual	HCS mutation	CAAA repeat	ATTC repeat	Haplo-type	Reference
1 (Japanese)	Father	1067delG	2/2	2/2	<u>2-2</u> ^a /2-2	Patient 1 (present study)
	Mother	237Leu > Pro	2/2	2/2	<u>2-2</u> /2-2	
	Patient	1067delG/237Leu > Pro	2/2	2/2	<u>2-2</u> / <u>2-2</u>	
2 (Japanese)	Father	1067delG	2/3	2/3	<u>2-2</u> /3-3	Patient 2 (present study)
	Mother	?	2/2	2/3	<u>2-2</u> /2-3	
	Patient	1067delG/?	2/2	2/2	<u>2-2</u> /2-2	
3 (Japanese)	Father	large deletion (LD)	2/2	3/2	<u>2-3</u> /2-2	Patient 3 (present study)
	Mother	237Leu > Pro	2/2	2/2	<u>2-2</u> /2-2	
	Patient	LD/237Leu > Pro	2/2	3/2	<u>2-3</u> / <u>2-2</u>	
4 (Japanese)	Father	237Leu > Pro	2/2	2/1	<u>2-2</u> /2-1	Patient 1 (Aoki et al. 1995)
	Mother	237Leu > Pro	2/2	2/4	<u>2-2</u> /2-4	
	Patient	237Leu > Pro/237Leu > Pro	2/2	2/2	<u>2-2</u> / <u>2-2</u>	
5 (Japanese)	Father	1067delG	2/2	2/1	<u>2-2</u> /2-1	Patient 2 (Aoki et al. 1995)
	Mother	237Leu > Pro	2/1	2/3	<u>2-2</u> /1-3	
	Patient	1067delG/237Leu > Pro	2/2	2/2	<u>2-2</u> / <u>2-2</u>	
	Sister 1	1067delG	2/1	2/3	<u>2-2</u> /1-3	
6 (Japanese)	Sister 2	normal	2/1	1/3	2-1/1-3	Patient 3 (Aoki et al. 1995)
	Father	237Leu > Pro	2/3	2/4	<u>2-2</u> /3-4	
	Mother	508Arg > Trp	2/2	3/3	<u>2-3</u> /2-3	
	Patient	237Leu > Pro/508Arg > Trp	2/2	2/3	<u>2-2</u> / <u>2-3</u>	
7 (Japanese)	Father	1067delG	<u>2/2</u>	<u>2/2</u>	<u>2-2</u> /2-2	Patient 4a (Aoki et al. 1995) Patient 4b (Aoki et al. 1995)
	Mother	237Leu > Pro	2/2	2/4	<u>2-2</u> /2-4	
	Patient 1	1067delG/237Leu > Pro	2/2	2/2	<u>2-2</u> / <u>2-2</u>	
	Patient 2	1067delG/237Leu > Pro	2/2	2/2	<u>2-2</u> / <u>2-2</u>	
8 (Japanese)	Father	1067delG	2/2	2/3	<u>2-2</u> /2-3	Patient 1 (Sakamoto et al. 1998) Patient 2 (Sakamoto et al. 1998)
	Mother	508Arg > Trp	1/2	4/3	<u>1-4</u> /2-3	
	Patient	1067delG/508Arg > Trp	2/1	2/4	<u>2-2</u> / <u>1-4</u>	
9 (Japanese)	Patient	565Arg > Ter/508Arg > Trp	2/3	3/1	<u>2-3</u> / <u>3-1</u> or <u>2-1</u> / <u>3-3</u>	
					<u>1-4</u> /2-2	
10 (Japanese)	Father	550Val > Met	1/2	4/2	<u>1-4</u> /2-2	(Aoki et al. 1997) (Hwu et al. 2000) (Livne et al. 1994)
	Mother	550Val > Met	1/2	4/3	<u>1-4</u> /2-3	
	Patient	550Val > Met/550Val > Met	1/1	4/4	<u>1-4</u> / <u>1-4</u>	
	Patient	508Arg > Trp/508Arg > Trp	2/2	3/3	<u>2-3</u> / <u>2-3</u>	
11 (Taiwanese)	Patient	508Arg > Trp/508Arg > Trp	2/2	3/3	<u>2-3</u> / <u>2-3</u>	
12 (Jewish)	Patient	550Val > Met/550Val > Met	2/2	3/3	<u>2-3</u> / <u>2-3</u>	

^a Underlined haplotypes represent mutant alleles

T), 565Arg > Ter (1980C > T) and 550Val > Met (1935G > A) mutations were determined by direct sequencing, as described (Aoki et al. 1997; Sakamoto et al. 1998).

Analysis of polymorphic markers

Di-, tri-, and tetra-nucleotide repeats were searched for in the genomic region encompassing the *HCS* gene (accession numbers AP001726 and AP001727), and ten regions were listed as candidate regions for polymorphic markers. Only two fragments were successfully amplified and showed polymorphism. One was located in intron 8 and the other in intron 9 of the *HCS* gene. These were named the CAAA repeat and the ATTC repeat, respectively. The primer pairs used to amplify each region were: 5'-TCAGCTGTGTGAC AGTGAGACTCTATCT and 5'-GCTGGCATCGCTTC CAACCCAAACACCCAG for the CAAA repeat; 5'-TG ATCCTGCCGACTCCAGTGAAGTATAACC-3' and 5'-CTTGGTTCACTGATGGCATTGAGAAAGTGTG for the ATTC repeat. The PCR reaction mixture of 25 µl, including 0.4 µM each of sense and antisense primers and 1 unit of Taq polymerase (Takara, Tokyo, Japan) proceeded through 30 cycles of 94°C for 15s, 70°C for 30s, and 72°C for 1 min. The size of the PCR products was estimated using ALF Express II (Amersham Pharmacia Biotech, Uppsala, Sweden), with internal and external DNA standards as recommended by the manufacturer.

Results and discussion

Mutation analysis in HCS-deficient patients

Three Japanese patients with deficient HCS activity were screened for the known mutations, 237Leu > Pro, 1067delG, 508Arg > Trp, 565Arg > Ter, and 550Val > Met. The onset of symptoms in patient 1 were typically neonatal, and the 237Leu > Pro and the 1067delG mutations were identified. This genotype has already been identified in two other families (Table 1). Patient 2 had the 1067delG mutation in the heterozygous form. Patient 3 had a 237Leu > Pro mutation that was derived from her mother,

who is heterozygous for this mutation. Analysis by PCR-RFLP showed that this patient had only mutant bands. Her father, however, had only a wild-type band. All coding exons of patient 3 were directly sequenced, but no other mutations were found. These observations suggest that the paternal allele has a large deletion involving at least the exon that contains 237Leu (997T), and that patient 3 was hemizygous for 237Leu > Pro. Among 20 mutant Japanese alleles, the 237Leu > Pro and the 1067delG account for 7 and 5, respectively. The five mutations screened in this study accounted for 90% of the mutant alleles in the Japanese population.

Polymorphic markers in the HCS gene

The presence of a polymorphism at the loci of ten candidate regions containing di-, tri-, or tetra-nucleotide repeats was examined in genomic DNA from four individuals. The tetranucleotide CAAA and ATTC repeats were amplified by PCR and showed polymorphic bands. We then examined 50 normal Japanese individuals, using these two markers. All of the PCR products of these two markers were subcloned and sequenced to determine the exact numbers of the repeat. The sequence variations of the fragments obtained from 100 amplified control alleles are shown in Table 2. The numbers of CAAA repeats ranged from 6 to 8, and those of ATTC repeats ranged from 10 to 15. The frequency of each type of the two markers is shown in Table 3. The type 2 allele was the most frequent and accounted for 62% of 100 alleles in the CAAA repeat. Forty-six percent of individuals were heterozygous at this marker. In the ATTC repeat, the type 3 allele was the most frequent (47%), and four other types (1, 2, 3, and 5) accounted for about 10% each of the alleles. Type 6 was found only in one allele examined. Sixty-eight percent of individuals were heterozygous at this marker.

Haplotype of HCS gene with CAAA and ATCC repeats

Haplotypes determined by a combination of the CAAA and the ATTC are shown in Table 4. Within 100 alleles, 66 were determined with certainty (phase known). Type 2 of

Table 2. Sequences of the CAAA and ATTC repeats

Allele type	Length of PCR product	Repeat number	Sequence
CAAA			
1	98bp	6	5'-CAAAA(CAAA) ₆ CTCTCTCAA
2	102bp	7	5'-CAAAA(CAAA) ₇ CTCTCTCAA
3	106bp	8	5'-CAAAA(CAAA) ₈ CTCTCTCAA
ATTC			
1	102bp	10	5'-ATTT(ATTC) ₁₀ CA
2	106bp	11	5'-ATTT(ATTC) ₁₁ CA
3	110bp	12	5'-ATTT(ATTC) ₁₂ CA
4	114bp	13	5'-ATTT(ATTC) ₁₃ CA
5	118bp	14	5'-ATTT(ATTC) ₁₄ CA
6	122bp	15	5'-ATTT(ATTC) ₁₅ CA

PCR, Polymerase chain reaction

Table 3. Frequency and genotype of the CAAA and ATTC alleles in 50 control individuals

CAAA		ATTC	
Allele frequency			
Allele type	Frequency (%)	Allele type	Frequency (%)
1	19	1	13
2	62	2	16
3	19	3	47
		4	13
		5	10
		6	1
Genotype			
Type	Frequency (%)	Type	Frequency (%)
1/1	4	1/1	4
2/2	42	2/2	4
3/3	8	3/3	22
1/2	24	4/4	0
1/3	6	5/5	2
2/3	16	6/6	0
		1/2	4
		1/3	10
		1/4	2
		1/5	2
		1/6	0
		2/3	14
		2/4	2
		2/5	2
		2/6	2
		3/4	18
		3/5	8
		3/6	0
		4/5	4
		4/6	0
		5/6	0

the CAAA repeat and type 3 of the ATTC repeat (named haplotype 2-3) were the most frequent. Haplotype 2-2 was the second most frequent. Twelve haplotypes were found in our control samples.

We determined the haplotypes of Japanese patients with an HCS deficiency and their parents in ten unrelated families. In addition, we analyzed a Taiwanese patient who is homozygous for 508Arg > Trp, and a Jewish patient who is homozygous for 550Val > Met (Table 1). All seven 237Leu > Pro mutant alleles were associated with the 2-2 haplotype. All five 1067delG mutant alleles were also associated with this haplotype. Both the 237Leu > Pro and the 1067delG seemed to be in linkage disequilibrium with haplotype 2-2, suggesting that they are founder mutations in the Japanese population.

The 508Arg > Trp mutation was found in three unrelated Japanese families. The 508Arg > Trp mutant allele of family 6 was associated with haplotype 2-3, and that of family 8 with haplotype 1-4 (Table 1). The haplotype of the patient in family 9 could not be determined unequivocally, but should be different from haplotype 1-4. The homozygous 508Arg > Trp mutation in the Taiwanese patient was associated with haplotype 2-3. The 550Val > Met mutation was also found in a Japanese patient and in a Jewish patient in the homozygous form (Table 1). The haplotype of the Japanese patient was 1-4/1-4, whereas that of the Jewish patient was 2-3/2-3. The nucleotide change in the 508Arg > Trp and the 550Val > Met mutations is a C-to-T

Table 4. Haplotype of the *HCS* gene in 66 control alleles

Haplotype CAAA-ATTC	Frequency (%)
2-3	38
2-2	17
1-3	11
2-5	9
2-4	9
3-1	6
3-4	3
1-4	1.5
1-2	1.5
3-3	1.5
1-5	1.5
3-5	1.5

transition at the CpG dinucleotide, and is found in several ethnic groups (Dupuis et al. 1996; Aoki et al. 1997; Zammarchi et al. 1998). These data are in accord with the notion that 508Arg > Trp and 550Val > Trp represent hot-spot mutations. The spectrum of mutations in Japanese patients is different from that in other ethnic groups (Aoki et al. 1995; Dupuis et al. 1996; Aoki et al. 1999). This is probably because two founder mutations, 237Leu > Pro and 1067delG, have become predominant in the Japanese population.

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