

ORIGINAL ARTICLE

Different Gene Preferences of Maple Syrup Urine Disease in the Aboriginal Tribes of Taiwan



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Background: Maple syrup urine disease (MSUD) is a rare inborn error of metabolism caused by a deficiency of the branched-chain α -ketoacid dehydrogenase (BCKD) complex. Mutations in any one of the three different genes encoding for the BCKD components, namely, *BCKDHA*, *BCKDHB*, and *DBT*, may be responsible for this disease. In Taiwan, few MSUD cases were diagnosed clinically, and most of these patients are from Aboriginal tribes.

Materials and methods: To identify and detect the carrier frequency of MSUD in Taiwanese Aboriginal tribes, we performed biochemical and molecular studies from peripheral blood in MSUD patients and dried blood on filter paper in the enrolled screened populations.

Results: Homozygous A208T and I281T of *BCKDHA* were found in two patients from Hans (non-Aboriginal Taiwanese), respectively; compound heterozygous mutations of the *DBT* gene [4.7 kb deletion/c.650-651insT (L217F or L217fsX223) and c.650-651insT/c.88-89delAT] were found in two patients from Amis, respectively, after direct DNA sequencing and polymerase chain reaction-restriction fragment length polymorphism studies. There were no cases of deleted 4.7-kb heterozygote out of 302 normal people (Hans, $n = 125$; Atayal, $n = 156$; and Saisiyat, $n = 21$); by contrast, the *DBT* mutations c.650-651insT and deleted 4.7-kb heterozygote were noted in 2/121 and 1/121, respectively, from the general population of the Amis, a southeastern Taiwanese tribe.

Conclusion: Although the Taiwanese Austronesian Aboriginal tribes are considered to share a common origin, different gene preferences of MSUD were noted. The novel *DBT* mutation c.650-651insT was more prevalent than the deleted 4.7-kb heterozygote in the Amis

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population. The reported 4.7-kb deletion indicating a possible founder mutation may be preserved in the southern and eastern, but not in northern Aboriginal tribes of Taiwan.

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1. Introduction

Maple syrup urine disease (MSUD) is an autosomal recessive metabolic disorder, which is caused by mutations in any one of the three different genes (*BCKDHA*, *BCKDHB*, and *DBT*) that encode components of the branched-chain α -ketoacid dehydrogenase (BCKD) complex (E1 α , E1 β , and E2 subunits).¹ The MSUD derives its name from the sweet, burnt sugar, or maple syrup smell of the urine. If not detected and treated early in life, the accumulation of branched-chain amino acids (leucine, isoleucine, and valine) and their metabolites may result in acute and chronic brain dysfunction.^{1–3} The MSUD has been described in all ethnic groups and occurs in approximately 1/185,000 and 1/101,624 newborns in the United States and Taiwan, respectively.^{1,4,5} As an autosomal recessive disorder, MSUD is more prevalent in selected inbred populations with a high frequency of consanguinity, such as the Mennonites in Pennsylvania, USA (whose incidence may be as frequent as 1/176 newborns).^{1,6,7} Founder mutations including Y393N/Y438N of the *BCKDHA* gene in the Mennonite/non-Mennonite communities, R183P of the *BCKDHB* in Ashkenazi Jews, a 239-bp insertion after exon 10 of the *DBT* gene in Filipino population, and a c.117delC-alpha (p.R40GfsX23) deletion in the *BCKDHA* gene in a Portuguese Gypsy population have been reported.^{8–11} Since the incorporation of tandem mass spectrometry into the existing newborn screening program, more patients with MSUD were detected earlier, and thus an in-time therapy became possible.^{3,12,13} Recently, MSUD has been found to have the tendency to occur in the Austronesian Aboriginal tribes of southern Taiwan.^{5,13,14} Aboriginal Austronesian tribal populations in Taiwan are considered as highly homogeneous within each tribe due to geographic isolation over a long period.¹⁵ Therefore, screening for some specific genetic mutations underlying MSUD in certain populations may potentially facilitate the prenatal diagnosis and carrier detection of MSUD. A previous report indicated a founder effect after characterizing a *DBT* gene 4.7-kb deletion of intron 10 found in the general population from the Paiwan Austronesian Aboriginal tribe in southern Taiwan.¹⁴ Because members of the Aboriginal tribe in Taiwan may be at a higher risk of classic form of MSUD,^{5,14} we enrolled the Aboriginal patients and collected the genetic epidemiological data in the general population to identify carriers of MSUD among northern and eastern Taiwanese Aborigines, including the Atayal, Amis, and Saisiyat tribes. This study was done together with the Lohkata Medical Service Team for Aborigines of Chang Gung Medical School.

2. Materials and methods

2.1. Patients

This study enrolled patients with MSUD from a hospital as well as the general population of Aborigines/non-

Aborigines in Taiwan from the Lohkata Medical Service Team for Aborigines in Chang Gung Medical School over 4 years. Four affected individuals and their family members were included in the first step of the study. The patients exhibited the classical ($n = 3$, including 2 Amis boys of 4 months and 1.5 years at molecular diagnosis, and 1 Han girl of 6 months) or intermediate ($n = 1$, Han boy of 8 months) clinical phenotype of MSUD. Only one of them was diagnosed as having MSUD by newborn screening. The study was approved by the Ethics Committee of Chang Gung Memorial Hospital (IRB Number 94-779B) and informed consent was obtained from all families.

2.2. Methods

Genomic DNA for further sequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was extracted from peripheral blood in MSUD patients or dried blood on filter paper in the enrolled general population according the standard procedure. An efficient and simple method for extracting and purifying genomic DNA from dried blood spots stored on filter paper for further DNA analysis was performed.^{12,16} For each sample, six drops of approximately 100 μ L of blood were spotted on filter paper by direct application from a finger stick. They were dried at room temperature, stored in separated plastic bags, and shipped to the laboratory within 2 days of processing. Samples were immediately used for PCR analysis or stored at -20°C . Direct sequencing of the *BCKDHA* (9 exons), *BCKDHB* (11 exons), and *DBT* (9 exons) genes over the patients and their parents were performed.^{9–11,17–19} The detection of heterozygote of *DBT* 4.7-kb deletion and carrier-frequency determination in the enrolled populations were done on the genomic DNA by duplex PCR.¹⁴ The detection of c.650-651insT mutation (L217F or L217fsX223) was done with the PCR-RFLP method by the *MwoI* (New England Biolabs, Ipswich, MA, US) restriction analysis.

3. Results

The extraction and purification of genomic DNA from dried blood specimens are efficient and appropriate for genetic studies. A total of 435 blood samples were collected, including 423 from normal controls (220 males and 203 females) and 12 from MSUD patients and their parents. After PCR-RFLP and direct DNA sequencing studies, two homozygous mutations A208T and I281T of *BCKDHA* were found in two Han patients (non-Aboriginal Taiwanese), along with two compound heterozygous mutations of the *DBT* gene: 4.7-kb deletion flanking parts of intron 10 and the 3'-untranslated region of exon 11 (Figure 1)/c.650-651insT (L217F or L217fsX223) of exon 6 (Figure 2) and c.650-651insT/c.88-89delAT of exon 2, in two patients from the eastern Amis tribe, respectively. Their parents were all

heterozygous carriers. Possible maternal Paiwan lineage of the second patient was mentioned.

Large-scale screening of the mutated sites was carried out on 423 normal patients, including Hans ($n = 125$, 20–65 years of age), and non-Hans including Atayal ($n = 156$, 25–50 years of age), Saisiyat ($n = 21$, 30–45 years of age), and Amis ($n = 121$, 21–66 years of age). The first two Aboriginal tribes occupy highland areas, whereas the third one occupies lowland areas. The deleted *E2* gene (4.7 kb) or L217F heterozygote was not found in Han, Atayal, or Saisiyat patients, whereas 4.7-kb and L217F heterozygotes were found in Amis (1/121 and 2/121, respectively; Figure 3).

4. Discussion

The determination of prevalence of MSUD in a certain population plays a very important role in genetic counseling. The incidence of heterozygous carriers (i.e., carrier frequency) and the pattern are the results of consanguinity and founder effect. Certain ethnicities demonstrate higher incidence with common mutations due to endogamous practices, such as Ashkenazi Jews and Mennonites possibly attributable to a founder effect.^{8,9} Taiwan's indigenous tribes are highly homogeneous within each tribe, but diversified among the different tribes due to geographic isolation over a long period. Results of immunohematologic studies support the aforementioned theory.^{15,20} Recently, MSUD was found to occur in the Austronesian Aboriginal tribes of Taiwan. Meanwhile, a common 4.7-kb deletion on the *DBT* gene indicating a founder mutation in the Paiwan tribe in southern Taiwan was reported.¹⁴ This specific change was also noted in the Philippines,¹⁰ which is consistent with the previous description of proto-Austronesian genetic affinities with Southeast Asian populations.²¹

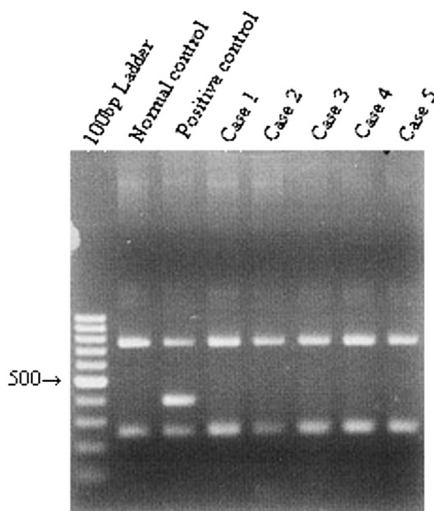


Figure 1 Identification of the heterozygous 4.7-kb deletion by duplex PCR: the 388-bp duplex PCR product representing a heterozygous deletion in the MSUD patient or carriers, whereas only two bands of 244 bp and 750 bp are present in normal controls. MSUD = maple syrup urine disease; PCR = polymerase chain reaction.

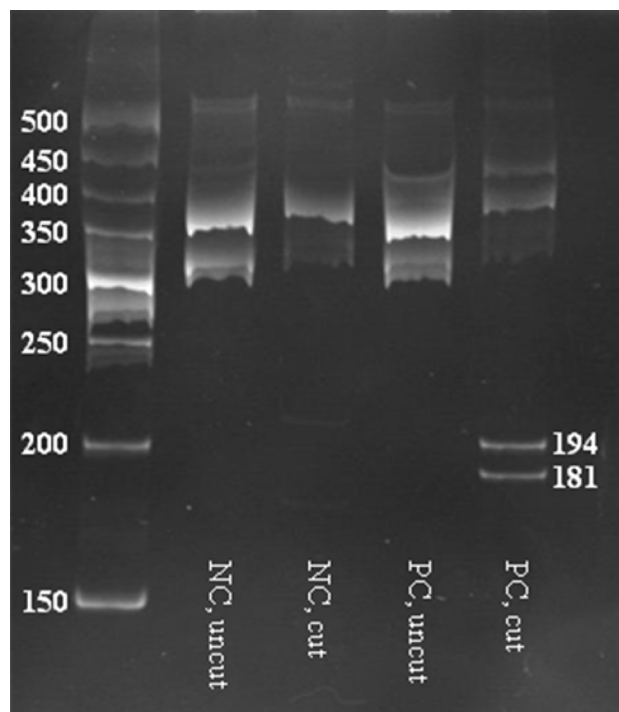


Figure 2 Polymerase chain reaction–restriction fragment length polymorphism analysis with restriction enzyme *MwoI* revealing two additional bands of 194 bp and 181 bp in the c.650-651insT (L217F) heterozygous mutation or maple syrup urine disease carrier.

This is the first comprehensive genetic study of MSUD in the Austronesian tribal groups in northern and eastern Taiwan. Mutations in Han patients occurred in the *BCKDHA* gene; and no common genetic changes were found in the Aboriginal population (Figure 3). The phylogenetic tree made from the distribution pattern of the human leukocyte antigen gene and the haplotype frequencies/immunoglobulin allotypes distinguishes Aborigines from Han populations in Taiwan or China.^{22–25} This study suggests that although the Taiwanese Aboriginal tribes are considered to share a common origin, the *DBT* gene 4.7-kb founder deletion may be preserved only in the southern¹⁴ and eastern tribes, but not in northern Aboriginal tribes of Taiwan. The novel *DBT* gene L217F mutation has more carrier frequency (2/121) than that of 4.7 deletion (1/121) in the Amis population. Moreover, we also identified a common c.88-89delAT of exon 2 found in one MSUD patient of possible maternal Paiwan lineage. Amis was delineated in a different fashion from Paiwan in terms of immunohematology and mitochondrial polymorphisms.^{20,23,26} Further dilution, distance, and selection factors, or intermixture by marriage in the past century on the founder effect may explain this condition.^{22–24}

In conclusion, our findings suggest that a limited number of mutations may exist in MSUD in certain Aboriginal Austronesian tribal populations, and this finding may potentially facilitate prenatal diagnosis and carrier detection of MSUD. A close genetic relationship between Taiwan's southern and eastern indigenous tribes and Southern Asians, such as Filipinos, exists. The identification could be performed by a simple,



F1: Frequency of *E2* 4.7-kb deletion, number of heterozygote/number of being tested

F2: Frequency of L217F, number of heterozygote/number of being tested

Figure 3 Map of Taiwan showing the studied populations and carrier frequency. *F1* = frequency of *E2* 4.7-kb deletions, number of heterozygotes/numbers being tested; *F2* = frequency of L217F, number of heterozygotes/numbers being tested.

reliable, sensitive, and cost-effective DNA extraction method from dried blood spots stored on filter paper along with a newborn screening program.

Conflict of interest

The authors state that there is no conflict of interest regarding the publication of this article.

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