〈Review Article〉

Thiamine dependency and related gene mutations: recent aspects

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Summary Thiamine dependency is an inherited metabolic disorder from which patients exhibit severe symptoms of deficiency, although they are fed more than the normal requirement of this vitamin. Deficiency symptoms can be treated with pharmacologic doses of thiamine as high as 100 to 1,000 times the Dietary Reference Values. Thiamine dependency is nowadays classified as a disorder caused by genetic mutations affecting thiamine-dependent enzymes or thiamine transporter, which transports thiamine to cells. The former mutation on enzyme is known as a pyruvate dehydrogenase complex deficiency (i.e., congenital lactic acidemia and Leigh syndrome) and a deficiency in branched-chain α-ketoacid dehydrogenase complex (i.e., maple syrup urine disease). The latter are known as mutations in thiamine transporter gene that makes protein transporting thiamine into cells (encoded by *SLC19A2* and *SLC19A3* genes) and protein transporting thiamine diphosphate into the mitochondria (encoded by *SLC25A19* gene). Thiamine-responsive megaloblastic anemia (TRMA), an autosomal recessive disease, is caused by loss of functional mutation in the SLC19A2 (ThTr-1).

Key words: Vitamin B_1 dependency, Thiamine deficiency, Maple syrup urine disease, Megaloblastic anemia, Thiamine transporter

1. Introduction

To maintain normal carbohydrate metabolism in the body, we have to ingest thiamine from our diet. The daily required amounts are 1.4 mg (4.2 μmol) for men and 1.1 mg (3.3 μmol) for women for Japanese (18-29 y)[1, 2], and 1.2 mg (3.6 μmol) for men and 1.1 mg (3.3 μmol) for women for U.S. people and Canadians (19-30 y)[3]. However, there were several patients who had experienced symptoms from the deficiency of this vitamin, although they were supplied the normal daily requirement from diets, dietary supplements, or intravenous dosage. The deficiency symptoms were better treated by taking amounts of thiamine 100-fold greater or more than that of the daily requirement, but the symptoms would reappear if the treatment was stopped[4]. The disease termed as "thiamine dependency" or "vitamin B_1 dependency" is due to inherited causes and is classified into two types as follows: The former is caused by a gene mutation encoded for enzymes that required thiamine as a coenzyme. The latter is caused by a gene mutation

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encoded for transporter proteins of thiamine that acts in thiamine uptake into cells[5]. Because we recently experienced a case indicative of the disease, we will review on the thiamine metabolism (absorption, transport and utilization), symptoms of thiamine deficiency, in addition to the above two types of thiamine dependency. [Amounts of thiamine (mg) can be converted to International System (SI) units by multiplying them by 2.965 (inverse the molecular weight of thiamine hydrochloride: 337.3 Da)].

2. Thiamine metabolism

Thiamine (cocarboxylase, or vitamin B_1) is a water-soluble vitamin necessary for energy metabolism as a coenzyme, and has a role in nervous tissue. We have to take thiamine from diets, and the nutritional status of thiamine in the body is assessed by measuring its concentration using whole blood (i.e., red blood cells), serum (or plasma), and urine specimens. In red blood cells, all vitamers of thiamine (i.e., free thiamine, thiamine monophosphate, thiamine diphosphate [also known as thiamine pyrophosphate], and thiamine triphosphate) were observed. Serum and plasma contained only free thiamine and thiamine monophosphate, but where thiamine diphosphate and thiamine triphosphate were not present. In urine, thiamine was excreted as forms of free thiamine, 2-methyl-4-amino-5-pyrimidine carboxylic acid and 4-methyl-thiazole-5-acetic acid[6].

Meanwhile, thiamine concentrations in whole blood (termed as total vitamin B_1 concentrations) were the sum of free thiamine (2-18 nmol/L: 0.6-5.4 μg/L), thiamine monophosphate (4-60 nmol/L: 1.2-18.0 μg/L), thiamine diphosphate (63-229 nmol/L: 19.0-68.9 μg/L), and thiamine triphosphate (0-4 nmol/L: 0-1.2 μg/L)[7]. We reported the thiamine diphosphate concentration in red blood cells as 10^{-11} nmol/cell (3×10^{-12} mg/cell)[8], and the urinary amounts excreted in 24 hours as 37-953 μg/day (0.11-2.86 μmol/day)[9]. Butcher's meats mainly contain thiamine diphosphate and vegetables contain free thiamine. Thiamine diphosphate combined in protein is released by digestion, and then hydrolyzed to free thiamine by intestinal alkaline phosphatase (EC: 3.1.3.1). Each free thiamine from vegetables and thiamine diphosphate from meat were absorbed at jejunum of small intestine via the aforementioned transporter. [Thiamine concentrations in whole blood (nmol/L) can be converted to traditional units by multiplying them by 0.3008 (one one-thousandth of the molecular weight of free thiamine: 300.8 Da that includes pyrimidine ring, thiazole, and chloride)].

Free thiamine absorbed into enterocytes of jejunum was rephosphorylated by the enzyme, thiamine pyrophosphokinase (EC: 2.7.6.2), and yielded thiamine diphosphate, which was converted to thiamine triphosphate by thiamine-diphosphate kinase (EC: 2.7.4.15)[10]. However, once formed, thiamine diphosphate and thiamine triphosphate were dephosphorylated to thiamine monophosphate and finally to free thiamine. Free thiamine entered the blood circulation and was transported to liver, where it was again phosphorylated and stored as thiamine diphosphate[10].

Within the intestinal thiamine concentrations after intake of usual diets, free thiamine was absorbed by the active transport mechanism via thiamine transporter encoded by *SLC19A2* or *SLC19A3* gene (as described below), followed by phosphorylation in the enterocytes. While at higher concentrations after intake of high doses, i.e., intake of a dietary supplement, free thiamine was absorbed by diffusion or by a passive transport mechanism probably via the same or another transporter. However, not all free thiamine transported to the liver could be phosphorylated because of overloads to the enzyme activity of thiamine pyrophosphokinase. Consequently, free thiamine in circulating blood was greatly retarded.

In order to utilize thiamine diphosphate stored in liver, it was dephosphorylated to thiamine monophosphate, and further to free thiamine. Free thiamine circulated in the blood and then targeted organs (cells), where it was converted to thiamine diphosphate (i.e., the coenzyme form of this vitamin) and thiamine triphosphate (Fig. 1). After all, free thiamine and thiamine monophosphate have a physiological role as a transport form of this vitamin, and thiamine diphosphate and thiamine triphosphate have the following active forms: thiamine diphosphate functions

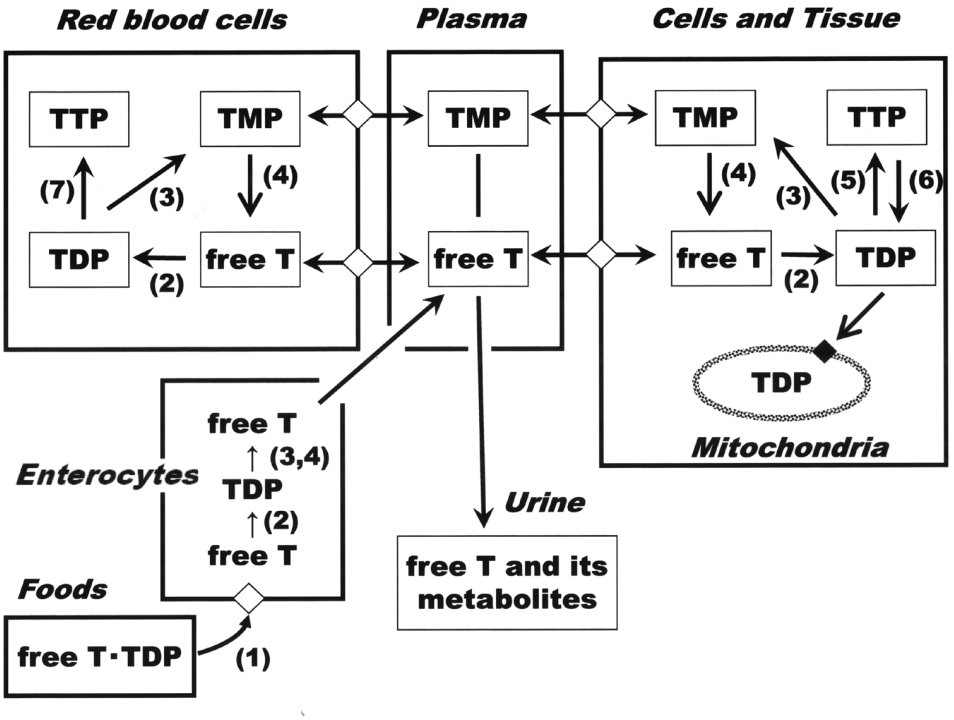

Fig 1 Distributions of thiamine vitamers in the body.
free T, free thiamine; TMP, thiamine monophosphate; TDP, thiamine diphosphate; TTP, thiamine triphosphate. (1) Intestinal alkaline phosphatase (EC 3.1.3.1), (2) Thiamine pyrophosphokinase (EC 2.7.6.2), (3) Thiamine pyrophosphatase (EC 3.6.1.15), (4) Thiamine monophosphatase, (5) Thiamine-diphosphate kinase (EC 2.7.4.15), (6) Thiamine triphosphatase (EC 3.6.1.28), (7) Erythrocyte adenylate kinase (EC 2.7.4.3), ◇, thiamine transporter; ◆, mitochondrial thiamine transporter.

as a coenzyme for pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase (as known as α-ketoglutarate dehydrogenase), branched-chain α-ketoacid dehydrogenase, 2-hydroxyphytanoyl-CoA lyase, and transketolase. Thiamine triphosphate has been considered to facilitate the release of acetylcholine from synaptic vesicle, and has a possible role in neurotransmission in nerve tissue[11-13].

3. Nutritional thiamine deficiency

Thiamine deficiency is found in subjects eating white rice greatly stripped of husks that contained thiamine, and subjects who eat unbalanced meals, and in those with insufficient intake of thiamine. Thiamine deficiency also occurs in subjects with an increased nutritional demand (i.e., pregnancy and lactation), intestinal malabsorption, diarrhea, fever, liver diseases in which the phosphorylation reaction is defective, and often in alcoholism[14-16]. Masked early symptoms of deficiency are unidentified complaints (e.g., common presenting complaints of beriberi), including fatigue, gastrointestinal upset, insomnia, epigastric pain, anorexia, abdominal pain, and diarrhea. Not only are body stores of thiamine as low as 30 mg (90 μmol), but their turnover is also quite rapid (the half-life is 9-18 days), and deficiency can therefore develop quickly[3, 17]. Thiamine diphosphate is observed in skeletal muscles, brain, heart, liver, and kidneys. Thiamine triphosphate is rich in brain and skeletal muscle.

Common types of thiamine deficiencies are beriberi (dry and wet) and Wernicke encephalopathy (Wernicke-Korsakoff syndrome), both of which lead to severe lactic acidosis (metabolic acidosis) and pyruvic acidemia[14-16]. Dry beriberi causes degeneration

of peripheral nerves, especially in the lower legs, and wet beriberi (also known as "shoshin beriberi") affects the heart and circulatory system. Symptoms of the former peripheral disease are paraesthesia, motility disturbance and muscle paralysis, and depressed Achilles tendon reflexes followed by an impaired patellar reflex. Symptoms of the latter cardiovascular disease are high-output cardiac failure, gastrointestinal disorders, and edema that is distinguished from dry beriberi.

Wernicke encephalopathy is a disease of the central nervous system characterized by triad symptoms, which are ophthalmoplegia (paralysis or weakness of the eye muscles), ataxia (difficulty standing or walking due to cerebellar ataxia), and mental confusion (loss of memory). Korsakoff syndrome is considered the aftermath of Wernicke encephalopathy, since it often develops after that disease[14-16]. Korsakoff syndrome is a memory disorder often associated with alcoholism. Beriberi is treated with oral and parenteral thiamine supplements of 10-30 mg (30-90 μmol) daily. For Wernicke encephalopathy and patients complicated with lactic acidosis, 100-400 mg (300-1200 μmol) of thiamine is administered parenterally (intravenous or intramuscular) for several days[17]. For the patients with thiamine dependency, pharmacologic doses of thiamine as high as 1,000 times (e.g., 1.8 g/day: 5.3 mmol/day) the Dietary Reference Values were given[4, 18, 19].

4. Thiamine dependency

Thiamine dependency is classified into two types of disorders that are caused by genetic mutations affecting thiamine-dependent enzymes, and the thiamine transporter which transports thiamine to the cell. Both dependencies manifested deficiency symptoms as described above, unless patients took large doses of this vitamin.

4.1. Dependency caused by mutation in the thiamine-dependent enzymes

This type of dependency includes enzyme mutations in pyruvate dehydrogenase complex (i.e., congenital lactic acidosis and Leigh syndrome) and branched-chain α-ketoacid dehydrogenase complex (i.e., maple syrup urine disease). With the presence of gene mutations, activities of those enzymes are functionally reduced to less than one-half of normal[18], demanding high doses of thiamine as a cofactor to accomplish healthy normal functioning.

Pyruvate dehydrogenase complex is a multi-enzyme complex composed of pyruvate dehydrogenase (E1, EC 1.2.4.1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3), that uses thiamine diphosphate as its cofactor[4, 18]. Congenital lactic acidosis occurs in most patients due to a defect of pyruvate dehydrogenase, which is caused by amino acid changes as Gly89Ser and Gly291Arg[20], encoding gene of which is the *PDHA1* gene[21]. The mutation is inherited in an X-linked recessive pattern.

Leigh syndrome, a mitochondrial disease, is a severe neurological disorder, and although most patients (75-80%) with this syndrome have a mutation in nuclear DNA, about 20-25% have a mutation in mitochondrial DNA, and a few with a mutation in nuclear DNA have a mutation in the pyruvate dehydrogenase complex[22, 23]. In the last cases, neurological symptoms were improved by treatment with thiamine[18].

Babies who have maple syrup urine disease (MSUD) seems to be normal at birth, but within three to four days after receiving lactation, unmetabolized branched-chain amino acids (BCAA) accumulate in the circulated blood, harming central nerve cells. Babies with MSUD must be treated with BCAA-restricted milk. If left untreated, the baby will have seizures, go into a coma, and die within the first few months of life[24].

MSUD is an inherited autosomal recessive disorder caused by mutations of branched-chain α-ketoacid dehydrogenase complex, which is composed of α-ketoacid dehydrogenase (E1, encoded by *BCKDHA* and *BCKDHB* genes[25, 26] located on chromosome 19q13.1-q13.2 and 6q14.1, respectively), dihydrolipoyl transacylase (E2, encoded by *DBT* gene[27] on chromosome 6q14.1), and dihydrolipoamide dehydrogenase (E3, encoded by *DLD* gene[28] on chromosome 7q31-q32).

Herein, E1 uses thiamine diphosphate as a

cofactor, and the severity of MSUD relates to the amount of residual E1 activity in the body. E1 activities in cells from a thiamine-responsive MSUD patient are reported to exhibit increased apparent K_m for α-ketoacid accompanying reduced V_{max}, and an addition of thiamine diphosphate increases the mutant E1's affinity for α-ketoacid[29]. Thus, patients with thiamine-responsive MSUD can be treated with thiamine.

4.2. Dependency caused by mutation in the thiamin transporter

Thiamine transporter belongs to a family of SLC (solute carrier) transporters which transports extracellular free thiamine into cells. The SLC transporter does not contain ATP-binding domain, and thus uptakes free thiamine without utilizing the energy released by ATP hydrolysis. The transport mechanism is classified into three distinct types: the first one uptakes single solutes (ligand or substrate) by passive diffusion, the second one uptakes multiple solutes by active transport such as symport, and the third one is an active transport by antiport process (also called counter transport)[30]. In humans, there are 386 SLC transporters[31], and free thiamine is a substrate for the SLC19A2 (ThTr-1) and SLC19A3 (ThTr-2), both of which uptake multiple solutes by counter transport of one H^+ cation[30]. Meanwhile, SLC25A19 is a mitochondrial transporter mediating uptake of thiamine diphosphate into mitochondria[32]. By the way, organic cation transporters (OCT1 and OCT3) were reported to act as high-capacity thiamine transporters[33], and OCT1 was found to regulate the prevalence of hepatic steatosis (i.e., fatty liver)[34].

4.2.1. Expression of SLC transporters in human organs (Table 1)

SLC19A2 gene encoded thiamine transporter 1 (ThTr-1) consisting of protein subunits (composed of 497 amino acid residues) with 12 transmembrane helical segments[30]. Northern blot analysis indicated that *SLC19A2* gene is highly expressed in skeletal muscle followed by placenta, heart, liver, and kidney, and poorly expressed in lung. Experiments with HeLa cells indicated that the transport of thiamine was by counter transport of H^+ in a Na^+-independent manner[30]. SLC19A2 was also expressed at the protein level in native human small intestine as found by Western blot analysis[35], and distribution of *SLC19A2* gene was reported in mice erythrocytes[36].

SLC19A3 gene encoded thiamine transporter 2 (ThTr-2) consists of protein subunits (composed of 496 amino acid residues) with 12 transmembrane helical segments[37]. At the amino acid level, ThTr-2 has a homology of 48% and 39%, respectively, to the ThTr-1 and reduced folate carrier protein 1 (RFC1). Northern blot analysis indicated that *SLC19A3* gene is highly expressed in placenta, followed by liver and kidney[37]. Semiquantitative PCR analysis revealed that *SLC19A3* gene is expressed in most tissues such as brain, heart, kidney, liver, colon, lung, small intestine, muscle, stomach, testis, placenta, salivary gland, thyroid gland, adrenal gland, ovary, prostate, and skin[37]. An experiment on the pattern of expression of ThTr-1 and ThTr-2 localized in the cell, using the

Table 1 Gene characteristics

Human gene name	Locus	Protein name	Number of amino acids	Number of transmembrane domains
SLC19A2	1q23.2-q23.3[50]	ThTr-1	497	12
SLC19A3	2q37[37]	ThTr-2	496	12
SLC25A19	17q25.3[44]	TPC	320	6
SLC22A1	6q26[45]	OCT1	544	12
SLC22A3	6q27[41]	OCT2	556	12

human colon carcinoma cell line (Caco-2), showed that ThTr-2 was expressed at the apical membrane domain of Caco-2 cells with little expression at the basolateral membrane domain of the cells. Meanwhile, expression of ThTr-1 protein was found at both the apical and the basolateral membrane domains, with slightly higher expression at basolateral compared with the apical membrane domain[38].

Mitochondrial thiamine pyrophosphate carrier (TPC) encoded by *SLC25A19* gene is composed of 320 amino acid residues, and is presumed to form proteins consisting of six transmembrane helices[39]. With Northern blot analysis the protein is expressed in large intestine and gallbladder but not the placenta[39].

SLC22A1 gene encodes OCT1 that is also related to uptakes of free thiamine. The OCT1 consists of protein subunits (composed of 554 amino acid residues) with 12 transmembrane helical segments[40]. Northern blot analysis demonstrated that the mRNA transcript of OCT1 was expressed primarily in the human liver followed by heart, skeletal muscle, and kidney, but was low in brain and placenta[40].

Furthermore, *SLC22A3* gene encodes OCT3, which consists of protein subunits (composed of 556 amino acid residues) with 12 transmembrane helical segments[41]. Northern blot analysis demonstrated that the *SLC22A3* gene was highly expressed in aorta, skeletal muscle, prostate, adrenal gland, salivary gland, liver, term placenta, and fetal lung, followed by uterus, ovary, lymph node, lung, trachea, and fetal liver[42]. In addition, the *SLC19A1* gene that encodes the RFC1 is found in human intestinal membranes. RFC1 is a transporter of 5-methyl-tetrahydrofolate and thiamine monophosphate.

The K_m for thiamine transport by ThTr-1 is 2.5 mmol/L and 27 nmol/L by ThTr-2. Moreover, RFC1 has K_m of 26 mmol/L for thiamine monophosphate and 32 mmol/L for thiamine diphosphate[43].

4.2.2. Inherited disorders caused by *SLC19A2* gene mutation (Table 2)

Mutations in this gene cause thiamine-responsive megaloblastic anemia syndrome (TRMA). TRMA is a rare condition characterized by megaloblastic anemia, diabetes, and hearing loss. Many mutations in the *SLC19A2* gene caused by a homozygous one base-

Table 2 The mutation of *SLC19A2*

Mutation	Amino acid	Exon/intron	References
152C>T	Pro51Leu	exon 1	Lagarde et al., 2004
196G>T	Glu66Ter*	exon 1	Raz et al., 2000
242insA	Ins81fs/Ter97	exon 2	Diaz et al., 1999
205G>T	Val69Phe	exon 2	Mikstiene et al., 2015
277G>C	Asp93His	exon 2	Raz et al., 2000
428C>T	Ser143Phe*	exon 2	Raz et al., 2000
429delTT	Del143fs/Ter239	exon 2	Diaz et al., 1999
484C>T	Arg162Ter	exon 2	Labay et al., 1999
724delC	Del242fs/Ter259	exon 2	Labay et al., 1999
515G>A	Gly172Asp	exon 2	Labay et al., 1999
750G>A	Trp250Ter	exon 2	Labay et al., 1999
885delT	Del295fs/Ter313	exon 2	Fleming et al., 1999
1074G>A	Trp358Ter	exon 4	Scharfe et al., 2000
1147delGT	Del383fs/Ter385	exon 4	Fleming et al., 1999
1189A>T	Arg397Ter	exon 4	Tahir et al., 2015
1223+1G>A	408+1splice	intron 4	Raz et al., 2000

*; Amino acid residues at 66 and143 were corrected.

pair substitution were reported, in which a specific amino acid (i.e., amino acid residue 496) was changed to a stop codon (terminal codon: Ter) instead; Glu66 was mutated to Ter (196G>T)[46], Arg162 to Ter (484C>T)[47], Trp250 to Ter (750G>A)[47], Trp358 to Ter (1074G>A)[48], and Arg397 to Ter (1189A>T)[49].

In addition, Ter arising from frame shift mutations which were caused by insertion or deletion of bases was reported. Insertion in amino acid residue 81 mutated the residue 97 to Ter (242insA)[50], deletion at residue 143 mutated residue 239 to Ter (429delTT)[50], deletion at residue 242 mutated residue 259 to Ter (724delC)[47], deletion at residue 295 mutated residue 313 to Ter (885delT)[51], and deletion at residue 383 mutated residue 385 to Ter (1147delT)[51].

Furthermore, missense mutations caused by one base-pair substitution that change one amino acid residue to another was reported; at a residue 51 mutation from Pro to Leu (152C>T)[52], a residue 69 mutation from Val to Phe (205G>T)[53], a residue 93 mutation from Asp to His (277G>C)[46], a residue 143 mutation from Ser to Phe (428C>T)[46], and at residue 172 mutation from Gly to Asp (515G>A)[47]. These mutations were all observed from patients with TRMA. Substitution of one amino acid for another in ThTr-1 would lead to changes in protein structure, causing ThTr-1 to lose binding with free thiamine.

4.2.3. Inherited disorders caused by *SLC19A3* gene mutation (Table 3)

Mutations in this gene cause biotin-responsive basal ganglia disease (BBGD), also known as thiamine metabolism dysfunction syndrome-2 (THMD2). Missense mutation in *SLC19A3* gene that encodes ThTr-2 was reported to be produced by a homozygous mutation (68G>T and 1264A>G). In each mutation, amino acid changes from Gly to Val were at residue 23 and from Thr to Ala at residue 422, respectively[54]. The 68G>T mutation altered the first transmembrane domain (TMD), whereas the 1264A>G mutation altered the eleventh TMD. Patients having these mutations were diagnosed with THMD2. Although homozygous nonsense mutation in *SLC19A3* gene (20C>A) was also produced at amino acid residue 7 by substituting Ser for Ter[55], this mutation was clinically classified as Leigh-like syndrome[56]. Kono et al.[57] reported cases similar to Wernicke encephalopathy in which compound heterozygous mutations (but not homozygous mutation) in *SLC19A3* gene were observed; amino acid changed from Lys to Glu at amino acid residue 44 (130A>G), and amino acid changed from Glu to Gln at amino acid residue 320 (958G>C).

Similar compound heterozygous mutations have been discovered[58], i.e., a frame shift mutation that substituted Leu at amino acid residue 26 for Ser

Table 3 The mutation of *SLC19A3*

Mutation	Amino acid	Exon/intron	References
20C>A	Ser7Ter	exon 1	Gerards et al., 2013
74dupT	Ser26Leufs/Ter44*	exon 2	Debs et al., 2010
68G>T	Gly23Val	exon 2	Zeng et al., 2005
130A>G	Lys44Glu*	exon 2	Kono et al., 2009
895_925del	VAL299fs	exon 3	Kevelam et al., 2013
958G>C	Glu320Gln*	exon 3	Kono et al., 2009
980-38dupA	*	intron 3	Debs et al., 2010
980-14A>G	*	skipping of exon 4	Debs et al., 2010
982delG	Ala328Leufs/Ter337	exon 4	Hacck et al., 2014
1264A>G	Thr422Ala	exon 5	Zeng et al., 2005
1332C>G	Ser444Arg	exon 6	Kevelam et al., 2013

*; heterozygous

(74dupT) with a resulting mutation to Ter at amino acid residue 44, and concurrently, a mutation that exchanges A for G at 14 upstream from nucleotide (nt) 980 that results in exon 4-skipping. The mutation was clinically diagnosed with THMD2. The disease is characterized by subacute encephalopathy manifesting confusion, seizures, and dysphagia, which are treated with biotin and/or high-dose thiamine supplements. Therefore, conformational change in proteins that are induced by *SLC19A3* gene mutation would manifest clinically different diseases.

Recently, mutation analysis by exome sequencing revealed mutated *SLC19A3* gene in patients with infantile Leigh-like syndrome. Deletion of G at nt 982 (982delG) produced downstream frameshift mutation at amino acid residue 328, and then amino acid residue 337 mutated to Ter[59]. Besides this, deletion between nt 895 and nt 925 (895_925del) produced downstream frameshift mutation at amino acid residue 299[60]. In addition, substitution of Ser for Arg at amino acid residue 444, caused by mutation from C to G at nt 1332 (1332C>G), was reported in *SLC19A3* gene[60].

4.2.4. Inherited disorders caused by *SLC25A19* gene mutation (Table 4)

SLC25A19 gene encodes the mitochondrial thiamine pyrophosphate carrier (TPC), which is considered to be a functional homodimer[61]. The mutation was a missense mutation in which there was one base-pair substitution; here the 373G>A mutation alters the Gly125 to Ser in the first residue of the TMD, whereas the 530G>C mutation alters the Gly127 to Ala in the first residue of the fourth TMD[62].

Homozygous mutation (373G>A) in *SLC25A19* gene is the cause of thiamine metabolism dysfunction syndrome 4 (THMD4), which is characterized by childhood onset of episodic encephalopathy, often associated with chronic progressive polyneuropathy and bilateral striatal necrosis, but most patients recover fully. Meanwhile, homozygous mutation (530G>C) causes Amish-type lethal microcephaly which is associated with severe congenital microcephaly and 2-ketogutaric aciduria, and results in early death[62]. A base-substitution mutation that exchanges one amino acid for another (i.e., under a single nucleotide in DNA such as switching G to A) in the TCP leads to conformational change in membrane protein structures, after which various clinical manifestations appear in patients in accordance with the type of nucleotide base that is substituted for another base.

5. Perspective

Total vitamin B_1 concentration in whole blood is first measured for patients manifesting symptoms of thiamine deficiency. However, if patients' symptoms are eliminated with the administration of thiamine, then no matter whether or not the total vitamin B_1 concentrations decrease, any disorder related to thiamine metabolism would be prospective. Physicians must consider which type of disorder affects the patients, i.e., thiamine deficiency or thiamine dependency, including genetic mutations of thiamine-dependent enzymes and thiamine transporters. Unfortunately, because of the lower prevalence of the cases, whole blood levels of patients' total vitamin B_1 concentration were not definitively identified in these patients with thiamine dependency, and furthermore, the distribution pattern of its vitamers, i.e., free thiamine, thiamine monophosphate, thiamine diphosphate, and thiamine triphosphate in red blood cells were not analyzed in most of the clinical laboratories. The presence of thiamine diphosphate would indicate

Table 4 The mutation of *SLC25A19*

Mutation	Amino acid	Exon/intron	References
373G>A	Gly125Ser	exon 6	Spiegel et al., 2009
530G>C	Gly177Ala	exon 7	Rosenberg et al., 2002

that free thiamine was successfully transported into cells from plasma via the thiamine transporter that was encoded by the above *SLC19A2* and *SLC19A3* genes. Thus, future studies are needed to address the composition of these vitamers in red blood cells and plasma fluctuated for the patients with suspected thiamine dependency with the different genotypes.

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