Nutrition 30 (2014) 485–488

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Case report

Analysis of thiamine transporter genes in sporadic beriberi

Valentina Bravatà Dr.[a], Luigi Minafra Ph.D.[a], Graziella Callari M.D.[b], Cecilia Gelfi Ph.D.[a,c], Luigi Maria Edoardo Grimaldi M.D.[b,*]

[a] Istituto di bioimmagini e fisiologia molecolare CNR—LATO, Cefalù PA, Scilly, Italy
[b] U.O. Neurologia, Fondazione Istituto "San Raffaele—G. Giglio," Cefalù PA, Scilly, Italy
[c] Department of Biomedical Sciences for Health, University of Milan, Milan, Italy

ARTICLE INFO

Article history:
Received 4 June 2013
Accepted 10 October 2013

Keywords:
Thiamine
Beriberi
Mutations
SLC19 A2
SLC19 A3
SLC25 A19

ABSTRACT

Objective: Thiamine or vitamin B_1 deficiency diminishes thiamine-dependent enzymatic activity, alters mitochondrial function, impairs oxidative metabolism, and causes selective neuronal death. We analyzed for the first time, the role of all known mutations within three specific thiamine carrier genes, *SLC19 A2*, *SLC19 A3*, and *SLC25 A19*, in a patient with atrophic beriberi, a multiorgan nutritional disease caused by thiamine deficiency.
Methods: A 44-year-old male alcoholic patient from Morocco developed massive bilateral leg edema, a subacute sensorimotor neuropathy, and incontinence. Despite normal vitamin B_1 serum levels, his clinical picture was rapidly reverted by high-dose intramuscular thiamine treatment, suggesting a possible genetic resistance. We used polymerase chain reaction followed by amplicon sequencing to study all the known thiamine-related gene mutations identified within the Human Gene Mutation Database.
Results: Thirty-seven mutations were tested: 29 in *SLC19 A2*, 6 in *SLC19 A3*, and 2 in *SLC25 A19*. Mutational analyses showed a wild-type genotype for all sequences investigated.
Conclusion: This is the first genetic study in beriberi disease. We did not detect any known mutation in any of the three genes in a sporadic dry beriberi patient. We cannot exclude a role for other known or unknown mutations, in the same genes or in other thiamine-associated genes, in the occurrence of this nutritional neuropathy.

Introduction

Thiamine or vitamin B_1 is a hydrosoluble vitamin that comprises pyrimidine and thiazole rings joined by a methylene bridge [1]. Thiamine phosphoesters are cofactors for several enzymes involved in carbohydrate metabolism, respiratory chain, synthesis of neurotransmitters, and nucleic acid precursors [2]. Thiamine deficiency diminishes the thiamine-dependent enzyme activity, alters mitochondrial function, impairs oxidative metabolism, and causes selective neuronal death [3–5]. This deficiency can be caused by an inadequate consumption of thiamine, increased need, or impaired absorption. In alcoholism, these conditions coexist and seem to be responsible for a vitamin deficiencies; in alcoholics, thiamine deficiency may result from an inadequate dietary intake, impaired thiamine absorption in the gastrointestinal tract, reduced liver storage, and decrease in the transformation of thiamine into its active form [6].

The main manifestations of thiamine deficiency affect the cardiovascular system (also known as wet beriberi), and the peripheral and central nervous systems (also defined as dry beriberi and Wernicke-Korsakoff syndrome [WKS]) [2].

Dry beriberi is a neurologic syndrome that results from intracellular deficiency of the coenzyme thiamine pyrophosphate (TPP), the active form of thiamine. The characteristic symptoms involve the lower extremities, with paresthesias and pain in the feet, decreased deep-tendon reflexes at the ankle and knee, loss of vibratory and position sense, and foot drop. Cerebral beriberi may begin with subtle affective changes and memory impairment, progressing through confusion with ataxia and ophthalmoplegia, to lethargy, coma, and death [7].

VB and LM contributed equally to this work. All authors participated in the conception, design, interpretation, and elaboration of the findings of the study. All authors read and approved the final manuscript. The authors declare that they have no competing interests.

* Corresponding author: Tel.: +39 0921 92 0362; fax: +39 0921 92 0405.
E-mail address: Luigi.grimaldi@hsr.it (L. M. Edoardo Grimaldi).

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http://dx.doi.org/10.1016/j.nut.2013.10.008

In detail, thiamine is an essential cofactor of four important enzymes in cellular metabolism: transketolase (TK) within the pentose phosphate pathway (PPP), pyruvate dehydrogenase complex (PDHC), alphaketoglutarate dehydrogenase (KGDHC) in the tricarboxylic acid (TCA) cycle and branched chain ketoacid dehydrogenase (BCKDC) of amino acid catabolism. In the central nervous system, vitamin B_1 has a key role in glucose metabolism; 80% of brain thiamine is in the form of thiamine diphosphate (cofactor for KGDHC, PDHC, and TK enzymes) [8]. Furthermore, the human body itself cannot produce this vitamin; therefore thiamine must be obtained from exogenous sources (i.e., diet), through absorption in the intestine. In the cell, imported thiamin is converted to its active forms, mainly TPP, via the action of thiamin pyrophosphokinase (TPKase), a rate-limiting enzyme that plays an important role in regulating cellular thiamin homeostasis. The cellular transport of the vitamin is mediated by specific carriers identified, characterized, and codified by genes associated to thiamine deficiency and neurologic disease.

The solute carrier (SLC) group of membrane transport proteins includes more 300 members organized into 47 families [6]. Because folate and thiamine play vital roles in cellular metabolism, their specific transporters are of biological importance and, consequently their function disruption, mediated by genetic mutations, can be expected to lead to serious clinical complications [9]. Most members of the SLC group are located in the outer cell membrane, but some members are found in intracellular organelles.

The *SLC19* gene family of solute carriers is composed of three transporter proteins with significant structural similarity, transport mechanisms, and substrates with different structures and ionic charges, named *SLC19 A1, SLC19 A2*, and *SLC19 A3* [9]. The first one is a folate carrier, whereas the others are thiamine transporters.

Gene expression studies have shown that the thiamine transporters *SLC19 A2* and *SLC19 A3* are well expressed in several tissues such as the intestine, placenta, kidneys, and brain. Moreover, some data suggest that individuals with variants of these two genes may be particularly susceptible to thiamine deficiency [6].

The *SLC19 A2* gene (also known as *THTR-1*) maps on chromosome 1 at 1 q23.3, contains six exons spanning 22.5 kb, and codes for a protein containing 12 transmembrane domains and two *N*-glycosylation sites [10–15]. This transporter is very specific for thiamine and no other organic cations are recognized as substrates by themselves. Analysis of *SLC19 A2* mutations revealed significant heterogeneity, although the majority are predicted to be null, with premature translation termination due to nonsense or frame shift mutations [16].

SLC19 A3 (also known as *THTR-2*) is a second thiamine transporter expressed ubiquitously in humans and many other mammals. The *SLC19 A3* gene maps on chromosome 2 at 2 q37 and consists of five coding exons [17]. Human *SLC19 A3* exhibits particularly high expression in kidneys, liver, and placenta. Allelic variants of the *SLC19 A3* could potentially be involved in a variety of diseases including neural tube defects, diabetes, anemia, deafness, and epilepsy [18].

Cell TPP is used either in the cytoplasm or is imported into the mitochondria via a carrier-mediated process that involves the mitochondrial TPP transporter, encoded by the *SLC25 A19* gene [19–21].

The *SLC25 A19* gene maps on chromosome 17 at 17 q25, contains nine exons and codes for a protein that has been described as a mitochondria inner membrane transporter for both deoxynucleotides and TPP. *SLC25 A19* gene mutations cause a metabolic disorder characterized by severe congenital microcephaly, neuropathy, and bilateral striatal necrosis [22–24].

At present, no association has been reported between thiamine-related genes and both forms of beriberi.

To our knowledge, no studies have examined all thiamine-related mutations in the three genes simultaneously and no genetic association studies between the beriberi disease and these carriers have yet been performed.

Therefore, the purpose of our study was to analyze the role of three specific thiamine-carrier genes, *SLC19 A2, SLC19 A3*, and *SLC25 A19*, testing their known thiamine-related mutations, which may contribute to the susceptibility of thiamine deficiency in a case of sporadic dry beriberi patient.

Case presentation

A 44-year-old man with an ongoing history of alcohol abuse was admitted to our hospital because of a history of subacute progression over 2 wk of difficulty in walking, followed by dysesthesia in both hands, hypoesthesia from the transverse umbilical line downward, and bladder dysfunction. The day of admission he developed in a few hours a massive edema with pain and sensitive deficit in both lower limbs and feet. The neurologic examination showed a severe paraparesis with mild bilateral flaccid hypotonus, weak symmetrical upper limbs and absent lower limbs reflexes, hypoesthesia with a D7–D8 level, and a bilateral lower limb hypopallesthesia. Coordination was normal, standing and walking could not be assessed, and he reported difficulty initiating micturition.

Excessive alcohol use can cause neurologic or hepatic problems with signs of regional brain damage and cognitive dysfunction. Changes are more severe and other brain regions are damaged in patients who have additional vitamin B_1 deficiency [25]. Routine laboratory exam with vitamin levels (including thiamine), lactate, and pyruvate were normal. Cerebral and spinal magnetic resonance imaging, as well as cerebrospinal fluid examination, was also normal. An electromyogram showed a distal and proximal symmetrical motor axonopathy. Antiganglioside antibodies were not found.

We initially considered a subacute axonal neuropathy (acute motor-sensory axonal neuropathy type) and the patient was treated with high-dose IV steroids and subsequent immunoglobulin therapy with no benefit.

In the hypothesis of a nutritional deficiency sensory-motor axonal chronic polyneuropathy, we started a 10 d regimen of IV 100 mg/d thiamine followed by 30 d with 50 mg/d orally. Thirty minutes after the first IV dose, a significant reduction of foot edema was noted with progressive recovery of strength in the lower limbs. During the following 10 d, the patient started to walk with support for about 70 m without interruption and continued the rehabilitation therapy until complete remission. Final diagnosis was “acute thiamine deficiency neuropathy,” better known as “atrophic or dry beriberi” [26–28]. Because thiamine blood levels of this patient were in the normal range throughout his clinical course, we hypothesized a possible thiamine resistance somewhere in the metabolic cascade of the vitamin.

To assess whether mutations in thiamine metabolism-related genes were associated with beriberi, we performed a systematic mutational analysis of three specific thiamine carriers previously associated with neurologic diseases in the context of a thiamine deficiency.

For selecting the known *SLC19 A2* (NG_008255), *SLC19 A3* (NG_016359), and *SLC25 A19* (NG_008274) thiamine-related mutations the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk) was used. The *SLC19 A2* gene shows 29