Thiamine responsive megaloblastic anemia syndrome (TRMAS) is a rare autosomal recessive disorder especially in countries where consanguinity is uncommon. Three main features are characteristic of the disease – megaloblastic anemia, early onset deafness, and non-type I diabetes. TRMAS is a Mendelian disorder; a gene \textit{SLC19A2} coding high affinity thiamine transporter mediating vitamin B1 uptake through cell membrane has been identified. We present the first patient with TRMAS in Lithuania – a 3-year-old boy born to a non-consanguineous family with a novel homozygous \textit{SLC19A2} gene mutation. The patient had insulin dependent diabetes (onset 11 months), respiratory illness (onset 11 months), bilateral profound hearing loss (onset at 7 months, verified at 20 months), refractory anemia (onset 2 years), and decreased vision acuity and photophobia (onset 2.5 years). The psychomotor abilities developed according to age. Phenotypic evaluation did not reveal any dysmorphic features. The clinical diagnosis of TRMAS was suspected and daily supplementation with thiamine 100 mg was started. The condition of the patient markedly improved several days after the initiation of treatment. The results of \textit{SLC19A2} gene molecular testing confirmed the clinical diagnosis – novel homozygous c.[205G>T], p.[Val69Phe]) mutation changing conserved amino acid residue or even interfering the mRNA splicing. Clinical heterogeneity, diverse dynamics, and wide spectrum of symptoms are aggravating factors in the diagnosis. The possibility of treatment demands early recognition of disorder to facilitate the improvement of the patient’s condition.

Consent: Written informed consent was obtained from the parents of patient for publication of this Clinical report and any accompanying images. A copy of the written consent is available for review by the Editor of this Journal.

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that can be attributed specifically to TRMA especially in case of consanguinity [Bergmann et al., 2009].

Although clinical characterization and treatment options of TRMA syndrome were clarified by the authors of the early papers [Rogers et al., 1969], it took almost three decades to localize the genetic locus of the disease in 1q43.2–3.3 [Neufeld et al., 1997] on the basis of linkage analysis and homozygosity mapping in big consanguineous families. The gene SLC19A2 encoding high affinity low performance thiamine transporter 1 (THTR1) was identified and functionally characterized [Fleming et al., 1999; Oishi et al., 2002]. No other gene has been implicated in pathogenesis of TRMAS so far. THTR1 is high affinity low performance thiamine transporter predominating in inner ear cells, pancreatic islets and hematopoietic stem cells. Initial manifestation of the symptoms in presence of thiamine deficiency due to the decreased activity of THTR1 occurs in most sensitive tissues. The cells lacking thiamine suffer from extensive changes in their metabolism, experience shortage of energy, impairment of DNA/RNA biosynthesis and undergo apoptosis [Boros et al., 2003; Liberman et al., 2006; Stagg et al., 1999].

We report on the first patient with TRMAS in Lithuania – a 3-year-old boy born in non-consanguineous family. The absence of consanguinity facilitates clarifying the symptoms of disease entity presenting without additional influence of co-inherited genes. Novel SLC19A2 gene mutation identified will expand the fundamental knowledge of thiamine metabolism and disease development.

**CLINICAL REPORT**

The 3-year-old propositus born to the non-consanguineous family was originally referred for genetic consultation at 20 months of age because of hearing loss. His mother experienced ketonuria during pregnancy. The patient was irritable in the early days. He suffered from insulin dependent diabetes (onset at 11 months), respiratory illness (onset at 11 months), and bilateral profound hearing loss (onset at 7 months, verified at 18 months). With time more symptoms occurred; refractory anemia (onset at 2 years), decreased vision acuity, and photophobia (onset at 2.5 years) were documented.

Phenotypic evaluation did not reveal any dysmorphic features: the patients’ height was 85 cm (~50th centile), weight 11 kg (10–25th centile). Family history did not show consanguinity or other TRMAS patients in the family.

**Course of Disease**

**Audiologic findings.** Newborn hearing screening was not performed to the boy (universal newborn screening started in Lithuania in 2014). According to the parents the child responded to environmental sounds and speech until the 7th month of age. Hearing impairment was suspected by a therapist at 18 months of age. Profound sensorineural hearing loss in both ears was diagnosed. There was no benefit from hearing aids after a 1-month trial and right ear cochlear implantation (CI) was performed at 21 months. During the first year after surgery cochlear implant aided hearing thresholds improved to 30 dB, hearing and speech perception effects were positive, though slow.

**Non-type 1 diabetes.** At 11 months of age the patient suffered an episode of syncope. Hyperglycemia 13–18 mmol/l, and decreased C-peptide 0.27 nmol/l (n. 0.3–0.97 nmol/l) were revealed. Negative insulin, antiGAD65, anti-IA2, transglutaminase antibodies were assessed and normal thyroid function was determined. Diagnosis of insulin–dependent diabetes was made and insulin treatment 0.8–1 U/kg/day administered. Despite treatment with the insulin pump the glycemia control was not satisfactory – glucose in the blood scaled up to 14 mmol/l.

**Hematologic features.** The mild refractory anemia was noticed at 2 years of age. The patient did not receive any treatment, just observation. He was admitted to hospital because of mild refractory anemia for bone marrow aspiration. Mild normochromic, normocytic anemia (RBC 3.48 × 10^{12}/l, Hb 98 g/l; MCV 85.6 fl, MCH 28.2 pg), mild neutropenia (WBC 5.74 × 10^{9}/l), normal platelets count (PLT 245 × 10^{9}/l) was revealed. Blood biochemistry: normal folate 26.14 pmol/l (n. 7–39), slightly elevated vitamin B12–522 pmol/l (n. 127–517), and elevated ferritin – 190 mcg/ml (n. 15–150) concentrations were measured. Bone marrow aspiration showed normal granulopoiesis, count and morphology of megakaryocytes were normal. Erythropoiesis was very active, megaloblastic: erythroblasts 42% (normal E:M ratio 1:2–4), ringed sideroblasts were observed. The diagnosis of megaloblastic syndrome was made.

**Ophthalmologic features.** Decreased vision acuity and photophobia were noticed at 2.5 years of age. Bilateral maculopathy was revealed after examination of ocular fundi.

**Sudden deterioration of the patient’s health condition related with acute viral infection.** At the age of 2 years 10 months the propositus suffered an acute respiratory infection. A hemorrhagic rash appeared on the skin. Blood testing in the outpatient clinic showed anemia and severe thrombocytopenia (Hb 84 g/l, PLT 25 × 10^{9}/l). The boy was admitted to hospital with severe normochromic anemia (RBC 2.52 × 10^{12}/l; Hb 74 g/l; MCV 86 fl, MCH 29 pg) with moderate neutropenia (WBC 4.72 × 10^{9}/l) and severe thrombocytopenia (PTL 3 × 10^{9}/l). Also hyperglycemia (18–20 mmol/l) was identified. At that time, a diagnosis of TRMA syndrome was suspected and daily thiamine 100 mg was initiated.

Successful treatment with pharmacologic thiamine doses. Four days after beginning of thiamin therapy, a marked improvement in hematopoiesis was documented (Hb 94 g/l, PLT 63 × 10^{9}/l). After 1.5 months with thiamine treatment normal erythrocyte count was observed but thrombocytosis occurred (RBC 4.02 × 10^{12}/l, Hb 123 g/l, WBC 8.1 × 10^{9}/l, PLT 419 × 10^{9}/l).

The control of glycemia also improved – it varied between 4 and 9 mmol/l range, 87% of time normoglycemia was registered, HbA1c 5.1% (normal < 7.5%), insulin requirement decreased to 0.4 U/kg/day.

Audiologic evaluation was conducted after 3 months (15 months after CI): and the categories of auditory performance (CAP) score was 5. The boy understood common phrases without lip reading, vocalized more short words, and worked better with a speech therapist.
**Laboratory testing.** At 1 year 9 months of age plasma lactate was 2.1 mmol/l; in plasma amino acid analysis borderline elevation of branched amino acids Val, Leu, Ile was determined.

**Genetic testing.** After clinical confirmation of TRMA syndrome the coding sequence of SLC19A2 gene was sequenced by Sanger sequencing and novel homozygous mutation c.[205G>T]; p.[Val69Phe]) identified (Fig. 1A). The mutation changes G–T at the first position of the second exon of SLC19A2 gene. The alteration can result in replacement of conserved Val69 with Phe in extracellular loop 1–2 of THTR1 protein or in loss of splice site acceptor and subsequent truncation of protein or even nonsense mediated mRNA decay. The mutation has never been published in scientific literature nor found in Human Gene Mutation Database. The change was evaluated by *in silico* analysis: SIFT prediction score 0.003 (damaging), Polyphen2 prediction score 0.998 (probably damaging), and Mutation taster predicted the change to be disease-causing. Carrier status of the parents and sister of the propositus was confirmed (Fig. 1B).

**DISCUSSION**

Despite the apparent triad of symptoms (megaloblastic anemia, deafness, non-type 1 diabetes) the diagnosis of TRMA syndrome remains challenging even in the case of consanguinity. Quite often the disorder in the family is suspected only in the second decade of propositus’ life after long lasting and exhausting efforts to treat TRMA syndrome as separate unrelated diseases [Bergmann et al., 2009]. These three main features indeed seem to look like different in their development and etiology, so only multidisciplinary approach can be beneficial in early diagnostics. TRMA syndrome has a progressive nature and the onset of signs varies from newborn period till adolescence [Bergmann et al., 2009]. Our patient was diagnosed at 2 years 10 months of age though first symptoms (deafness, non-type 1 diabetes) appeared during the first year of life. Several reasons influence the delayed diagnosis: the rarity of the disorder especially in Europe where consanguinity is rather uncommon, clinical heterogeneity, diverse dynamics, wide spectrum of symptoms, and reduced penetrance. Only a few TRMA case reports have been published to date and this is the first TRMA syndrome patient in Lithuania.

The frequent occurrence of TRMA syndrome in consanguineous families with almost each case having the novel unique SLC19A2 gene mutation represents its origin from the single founder in the particular population. Since the SLC19A2 gene discovery more than 30 different mutations have been revealed, most of them are homozygous as a consequence of consanguineous marriages, ethnic isolation or homozygosity by descent [Bergmann et al., 2009; Ghaemi et al., 2013; Yilmaz Agladioglu et al., 2012]. Most mutations are predicted to produce null alleles creating premature stop codons (nonsense or frame shift mutations). Only few of them result in amino acid change of protein structure (missense changes) [Bergmann et al., 2009; Pichler et al., 2012]. In our patient we found novel homozygous mutation of the first nucleotide in the second exon of SLC19A2 gene; the alteration could change

![FIG. 1. SLC19A2 gene mutation (red arrows) c.[205G>T]; p.[Val69Phe]): (A) homozygous state in propositus, (B) heterozygous state in parents.](image-url)
followed with protein truncation or even nonsense mediated mRNA decay. In silico analysis predicted the mutation to be pathogenic. These findings are consistent with the results of recently published study with S-component ThiT from Lactococcus lactis suggesting that extracellular protein loop 1–2 functions as a lid on the thiamine binding site to allow and/or occlude the access of substrate from the extracellular side of the membrane to the binding site [Majserowska et al., 2013]. The pathogenic change identified provides a basis for the future functional analysis of thiamine metabolism. Our findings of SLC19A2 gene sequencing are compatible with the early manifestation of disease in propositus. This novel mutation has never been described in scientific literature previously. It most probably arose from a common progeny representing identity by descent. Non-consanguinity in this case raises the possibility of other TRMA cases in the future in Lithuania.

The diverse dynamics of disease have been also considered to be responsible for the delayed diagnostics; the features are mostly not congenital and often appear one after another over the long period of time. Also, additional features have been documented in some of the TRMAS patients making it difficult to distinguish form other disorders associated with consanguinity. Furthermore, TRMA syndrome patients without one of the classical triad feature have been published [Liu et al., 2014]. Clinical heterogeneity and reduced penetrance are important disease characteristics making the process of diagnostics more complicated. Any two of the classical triad features presented in the same patient should draw the attention to the TRMA syndrome. Our patient developed the symptoms progressively, and severe anemia with thrombocytopenia and ringed sideroblasts in bone marrow despite normal folic acid and vitamin B12 in the serum was the cornerstone for correct diagnosis. The disease manifestation in our patient allows delineation of particular symptoms of TRMAS characteristic to this hereditary condition: megaloblastic anemia with ringed sideroblasts and thrombocytopenia, deafness, insulin dependent non-type 1 diabetes, and maculopathy.

The diagnostic workup of TRMA syndrome is associated with thorough clinical and laboratory investigation. The features of disease overlap with Wolfram syndrome (DIDMOAD) and mitochondrial disorders (Kearns–Sayre syndrome, Pearson syndrome). Some patients diagnosed with Wolfram syndrome responded well to the thiamine therapy [Borgna-Pignatti et al., 1989]. For this reason the re-evaluation of the Wolfram syndrome patients is strongly advisable.

Laboratory testing of TRMA syndrome also presents a challenge. Unfortunately, there are no pathognomonic findings of routine laboratory investigation and even serum thiamine concentration usually appears to be in the normal range. Refractory megaloblastic anemia despite normal serum folate and vitamin B12 concentrations could serve as a notice to consider TRMA diagnosis. Non-type 1 insulin dependent diabetes without specific antibodies against insulin, antiGAD65, antiILA2, and transglutaminase together with deafness may also raise the suspicion of this disorder. As thiamine is a ubiquitously occurring cofactor of several important enzymes of carbohydrate and amino acids metabolism, even minor changes in biochemical findings such as borderline increase of lactate, fasting branched chain amino acids, and urinary organic acids reflecting deficiency of branched chain alpha ketoacids dehydrogenase, alpha-ketoglutarate dehydrogenase, and pyruvate dehydrogenase complex should be noticed and carefully evaluated.

Basically the diagnosis of TRMA syndrome depends on thorough clinical analysis. Differential diagnostics is mainly carried out by introducing empirical treatment with pharmacologic doses of thiamine. Cellular uptake of thiamine from blood is mediated by thiamine transporters: high affinity low performance thiamine transporter THTR1 (encoded by SLC19A2 gene) [Dutta et al., 1999] predominating in inner ear cells, pancreatic islets, and hematopoietic stem cells; low affinity high performance ubiquitously expressed protein THTR2 (encoded by SLC19A3 gene) [Eudy et al., 2000], and other less important pathways via pyrophosphokinase (specific members of the human extra neuronal monoamine transporter proteins acting in neurons [Calhau et al., 2003] and alkaline phosphatase transport system having affinity to organic ions [Oshima, 1997]).

Daily thiamine supplementation has been successfully applied to most of the published TRMAS patients with significant improve in hematopoiesis and control of glycemia. In some cases early treatment even preserved hearing function [Onal et al., 2009]. The improvement of patient’s health most probably depends on intensified thiamine transport through alternative above-mentioned pathways in the presence of high thiamine level in plasma. Unfortunately, most patients in adolescence become blood transfusion and insulin dependent [Ricketts et al., 2006]. However, with proper control of anemia and glycemia normal life expectancy of the patients should be feasible.

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