

## A novel mutation in the *SLC19A2* gene in a Turkish male with thiamine-responsive megaloblastic anemia syndrome

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Received: 17th November 2017, Revised: 17th February 2018, Accepted: 10th March 2018

**SUMMARY:** Odaman-Al I, Gezdirci A, Yıldız M, Ersoy G, Aydoğan G, Şalcıoğlu Z, Tahtakesen TN, Önal H, Küçükemre-Aydın B. A novel mutation in the *SLC19A2* gene in a Turkish male with thiamine-responsive megaloblastic anemia syndrome. Turk J Pediatr 2019; 61: 257-260.

Thiamine-responsive megaloblastic anemia (TRMA) is a very rare syndrome characterized by the triad of early onset megaloblastic anemia, sensorineural deafness and diabetes mellitus. Here we report, a 5-year-old boy who presented with transfusion dependent anemia and diabetes mellitus and was diagnosed with TRMA. Besides reporting a novel mutation of the causative gene *SLC19A2*, we wanted to emphasize this syndrome in the aspect of coexistence of insulin dependent diabetes, transfusion dependent anemia and thrombocytopenia.

**Key words:** thiamine-responsive megaloblastic anemia, thrombocytopenia, diabetes mellitus, *SLC19A2* mutation.

Thiamine-responsive megaloblastic anemia (TRMA) is a rare autosomal recessive disorder caused by mutations in *SLC19A2* gene, encoding a thiamine transporter protein and expressed in a very wide range of tissues including the pancreas, heart, retina, brain and bone marrow.<sup>1-5</sup> Three cardinal findings of this syndrome are megaloblastic anemia with ringed sideroblasts, diabetes mellitus and sensorineural hearing loss. Additionally, cardiovascular abnormalities and optic atrophy have also been described in some patients. This syndrome was first described by Rogers et al.<sup>1</sup> in 1969. Pharmacological doses of thiamine (vitamin B1) therapy usually corrects hematological and endocrine function, but results on neurological outcome is not that robust.<sup>2,3,6-8</sup> TRMA has a variable phenotypic presentation which may cause a significant delay between the onset of symptoms and an accurate diagnosis and treatment.

Here we presented a 5-year-old boy who was diagnosed with TRMA during evaluations for his transfusion-dependent anemia, thrombocytopenia and insulin dependent diabetes mellitus.

A novel mutation of the *SLC19A2* gene was also reported.

### Case Report

The 5-year-old boy was referred to our outpatient clinic for a hematological consultation because of severe anemia. He was born as a second child of healthy third degree consanguineous parents at term with normal birth weight. His physical examination was normal other than pallor and tachycardia. Laboratory analysis revealed anemia with a hemoglobin level of 5.2 g/dl, hematocrit 16.5%, mean corpuscular volume 85 fl, white blood cell count (WBC) 10400/mm<sup>3</sup> and platelets (Plt) 203 000/mm<sup>3</sup>. The reticulocyte count was 0.5%. The peripheral blood smear revealed 55% neutrophil, 40% lymphocytes, 5% monocyte, poikilocytosis, anisocytosis, normocytic erythrocytes without atypical cells.

Ferritin level was 255 ng/ml. Folic acid and vitamin B12 levels were all in normal limits. Red blood cell transfusion was done. Hemoglobin electrophoresis, osmotic fragility, pyruvate kinase and G6PDH levels were also

**Table I.** Hematological Findings of The Patient During Follow up.

	Before thiamine treatment	10th day of treatment	1st month of treatment	3rd month of treatment	6th month of treatment
Hemoglobin (g/dl)	5.2	10.3	11	11.6	12
MCV (fl)	85	90	87	81	79
WBC (/mm <sup>3</sup> )	10400	6980	7000	5600	5900
PLT (/mm <sup>3</sup> )	47000	128000	250000	189000	240000

MCV: mean corpuscular volume; PLT: platelet; WBC: white blood cell.

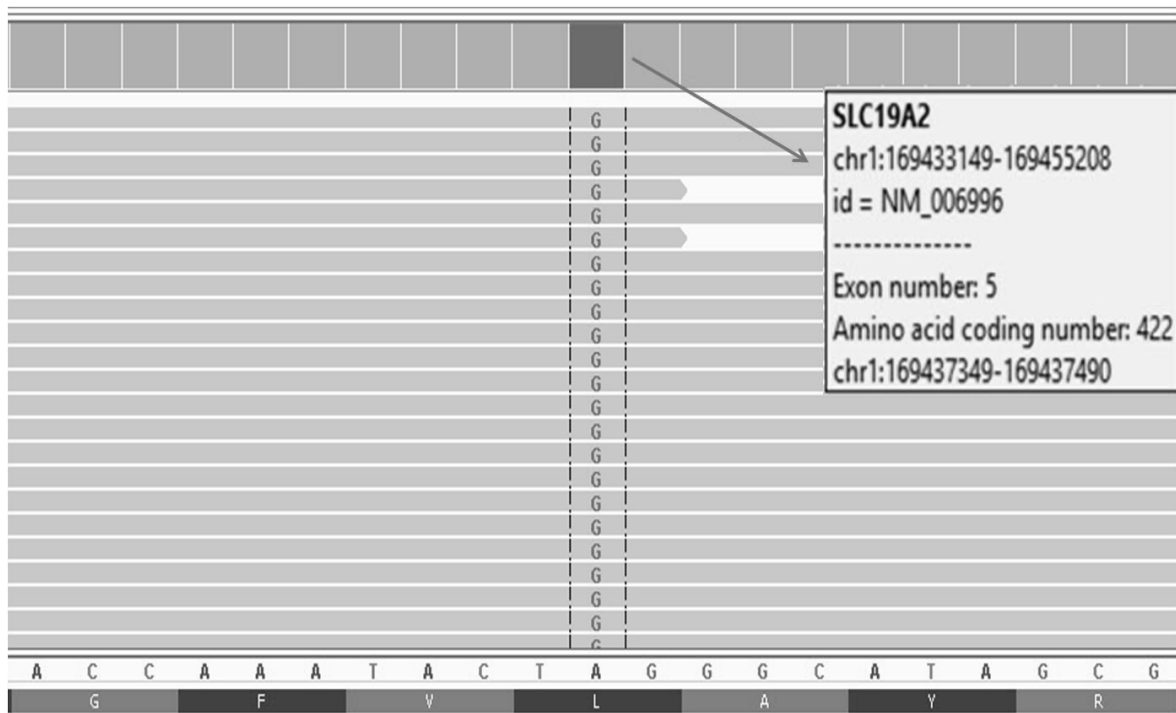
in normal range. Direct Coombs test was negative. In the follow up, three months after the first admission, a sudden decrease in hemoglobin level and thrombocytopenia (20000/mm<sup>3</sup>) was detected. Bone marrow aspiration (BMA) was performed to rule out leukemic infiltration, myelodysplastic syndromes and other pathologies that may cause maturation arrest in granulopoiesis. BMA showed 6% promyelocytes, 12% myelocyte-metamyelocyte, 39% neutrophil, normal granulopoiesis, count and morphology of megakaryocytes were normal. Megaloblastic changes and ringed sideroblasts (>15%) were observed in erythroid precursors. Monosomy 7 and trisomy 8 FISH analysis obtained for genetic evaluation of myelodysplastic syndromes were also negative. During the follow-up, five months after the first admission, a random laboratory test revealed that his blood glucose level was 365 mg/dl. After endocrinological consultation he was diagnosed with insulin dependent diabetes mellitus and multiple dose insulin therapy was started. Autoantibodies for type 1 diabetes were not detected and with the help of concomitant hematologic abnormalities he was diagnosed with TRMA. His thrombocytopenia and anemia improved very rapidly with oral thiamine nitrat therapy (75 mg/day) (Table I). He was transfusion dependent before thiamine supplementation and red blood cell transfusion was given every two weeks but he did not need any transfusion afterwards. Moreover, insulin doses were reduced after thiamine supplementation. With an informed consent from the parents, the diagnosis of TRMA was confirmed by sequence analysis of the *SLC19A2* gene using by next generation sequencing (Mi-seq Illumina) platform which showed a homozygous c.1265T>C (p. Leu422Pro) mutation (Fig.

1). For investigating other accompanying features of the syndrome; he underwent ophthalmologic, audiologic and cardiologic examination. Unilateral sensorineural hearing loss was detected and the patient was referred to an otolaryngologist for further evaluation. His echocardiography revealed mild pulmonary insufficiency. His ophthalmologic examination was normal other than myopia and astigmatism. At the last follow-up visit he was 5 years and 8 months old and he was on multiple doses of daily insulin therapy (0.5 U/kg/day) and his whole blood count values were completely normal. Written consent was received from the family.

## Discussion

Thiamine is an essential nutrient for all tissues. Its uptake and transport through the body are relatively complex. At physiological concentrations thiamine is not transported into the cells and requires a high-affinity transporter.<sup>9</sup> TRMA is caused by mutations in this high affinity thiamine transporter gene (*SLC19A2*) and the active thiamine uptake is disturbed. Despite the apparent triad of symptoms the diagnosis of TRMA may remain challenging. Up to date, nearly 50 different mutations have been identified in *SLC19A2* gene.<sup>10</sup> We found a homozygous novel mutation, c.1265T>C (p. Leu422Pro) in our patient. In-silico prediction tools (Mutation taster, PolyPhen-2, Provean and SIFT) classify this mutation as a highly probable pathogenic mutation, as it is located within a highly conserved region.

Thiamine regulates hematopoiesis at the stem-cell level as well as plays a role in DNA metabolism and heme synthesis<sup>3</sup>. The classical hematologic profile in TRMA is megaloblastic anemia which develops early in life, but a



**Fig. 1.** Sequence analysis of the *SLC19A2* gene using by next generation sequencing (Mi-seq Illumina) platform which showed a homozygous c.1265T>C (p. Leu422Pro) mutation.

number of different types of anemia have been reported, such as sideroblastic and aplastic anemia. The bone marrow is characterized by megaloblastic changes and ringed sideroblasts, but also may reveal more diffuse myelodysplasia affecting granulocytic and megakaryocytic differentiation. As a result, thrombocytopenia and pancytopenia have been reported in association with TRMA<sup>3,6,8,11</sup>. Hematological features improve with pharmacological doses of thiamine (25-75 mg/day). However, erythrocytes may remain macrocytic, suggesting a persistent erythropoietic abnormality.<sup>12</sup> Because the bone marrow is less sensitive to thiamine deprivation, erythroid lineage can repopulate with thiamine treatment. The anemia can recur when thiamine is withdrawn. Furthermore, unresponsiveness to thiamine may develop during treatment.<sup>13</sup> In our patient, anemia and thrombocytopenia rapidly recovered with thiamine therapy, without side effects.

Pancreas cells require high level of thiamine for its normal endocrine and exocrine function. Insulin secretion is present but defective in TRMA. Non-type I diabetes mellitus may appear anytime from infancy to adolescence in many (though not all) individuals with this disorder.

In some cases, insulin requirement may drop tremendously, even disappear with thiamine therapy.<sup>2,3,6,7,14</sup> However, puberty may deteriorate diabetes control and the reinstatement of insulin therapy would become necessary. In our patient, with the initiation of thiamine treatment insulin requirement diminished, but did not disappear.

Progressive sensorineural hearing loss is generally seen early and may not be prevented by thiamine treatment.<sup>8</sup> However, there are other studies claiming early treatment may preserve hearing function.<sup>15</sup> The basis of the sensorineural deafness is not known precisely in TRMA patients; however, animal studies have suggested that selective inner hair cell loss in the cochlea might be the cause of hearing loss.<sup>3</sup> In our patient hearing loss was also detected and his treatment was pursued by an otolaryngologist.

In conclusion, TRMA is a rare disorder and can be underdiagnosed by pediatric hematologists. The presence of megaloblastic anemia, sensorineural hearing loss and diabetes mellitus, must attract attention to TRMA. Genetic analysis confirms the definitive diagnosis. Early diagnosis and thiamine treatment may

prevent the complications of hyperglycemia, blood transfusions and sensorineural hearing loss. The aim of this report is to increase the awareness of this syndrome and report a novel mutation.

#### REFERENCES

1. Rogers E, Porter SF, Sidbury JR. Thiamine responsive megaloblastic anemia. *J Pediatr* 1969; 74: 499-504.
2. Yesilkaya E, Bideci A, Temizkan M, et al. A novel mutation in the SLC19A2 gene in a Turkish female with thiamine-responsive megaloblastic anemia syndrome. *J Trop Pediatr* 2009; 55: 265-267.
3. Ozdemir MA, Akcakus M, Kurtoglu S, Gunes T, Torun YA. TRMA syndrome (thiamine-responsive megaloblastic anemia): A case report and review of the literature. *Pediatr Diabetes* 2002; 3: 205-209.
4. Fleming JC, Tartaglino E, Steinkamp MP, Schorderet DF, Cohen N, Neufeld EJ. The gene mutated in thiamine-responsive anaemia with diabetes and deafness (TRMA) encodes a functional thiamine transporter. *Nat Genet* 1999; 22: 305-308.
5. Labay V, Raz T, Baron D, et al. Mutations in SLC19A2 cause thiamine-responsive megaloblastic anaemia associated with diabetes mellitus and deafness. *Nat Genet* 1999; 22: 300-304.
6. Tahir S, Leijssen LGJ, Sherif M, Pereira C, Morais A, Hussain K. A novel homozygous SLC19A2 mutation in a Portuguese patient with diabetes mellitus and thiamine-responsive megaloblastic anaemia. *Int J Pediatr Endocrinol* 2015; 2015: 6.
7. Mozzillo E, Melis D, Falco M, et al. Thiamine responsive megaloblastic anemia: a novel SLC19A2 compound heterozygous mutation in two siblings. *Pediatr Diabetes* 2013; 14: 384-387.
8. Akın L, Kurtoğlu S, Kendirci M, Akın MA, Karakük M. Does early treatment prevent deafness in thiamine-responsive megaloblastic anaemia syndrome? *J Clin Res Ped Endocrinol* 2011; 3: 36-39.
9. Brown G. Defects of thiamine transport and metabolism. *J Inherit Metab Dis* 2014; 37: 577-585.
10. Stenson PD, Ball EV, Mort M, et al. Human Gene Mutation Database (HGMD): 2003 update. *Hum Mutat* 2003; 21: 577-581.
11. Bazarbachi A, Muakkit S, Ayas M, et al. Thiamine-responsive myelodysplasia. *Br J Haematol* 1998; 102: 1098-1100.
12. Neufeld EJ, Mandel H, Raz T, et al. Localization of the gene for thiamine-responsive megaloblastic anemia syndrome, on the long arm of chromosome 1, by homozygosity mapping. *Am J Hum Genet* 1997; 61: 1335-1341.
13. Ricketts CJ, Minton JA, Samuel J, et al. Thiamine-responsive megaloblastic anemia syndrome: long-term follow-up and mutation analysis of seven families. *Acta Paediatr* 2006; 95: 99-104.
14. Yılmaz Y, Aydın BK, Baş F, et al. A thiamine responsive megaloblastic anemia presented with hypertriglyceridemia and auto-immune diabetes. *Exp Clin Endocrinol Diabetes Rep* 2015; 2: e8-e10.
15. Onal H, Baris S, Ozdil M, et al. Thiamine-responsive megaloblastic anemia: Early diagnosis may be effective in preventing deafness. *Turk J Pediatr* 2009; 51: 301-304.