Novel mutation in the SLC19A2 gene in Thiamine-responsive megaloblastic anemia (Rogers' syndrome)

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Abstract

Introduction
The Thiamine Transporter gene SLC19A2 is the only gene known to be associated with TRMA. This syndrome is a trial clinical characterized by megaloblastic anemia, nonautoimmune diabetes mellitus and sensory-neural hearing loss.

Materials and Methods
Described here are three children from consanguineous Iranian families with thiamine – responsive megaloblastic anemia (TRMA) or Rogers' syndrome. Case one and two were siblings of healthy first-cousin parents and case three from a healthy second-cousin couple. These cases presented with hyperglycemia, anemia, and hearing loss. Thiamine reversed the anemia and there was a satisfactory response for the hyperglycemia as well.

Results
In all three patients, direct sequencing revealed a homozygous mutation c.38 G>A (P.E.128K) resulting in the substitution of glutamic acid to lysine at position 128 in exon 2 of the SLC19A2 gene on chromosome 1q23.3. This novel mutation was confirmed by the PCR RFLP assay of more than 100 control alleles.

Conclusion
TRMA or Rogers' syndrome should be considered for patients with diabetes (DM) and other symptoms, including hearing loss and anemia. Early diagnosis can assist families in planning future pregnancies. The administration of thiamine ameliorates the megaloblastic anemic condition and produces a better response in DM.

Key words
Rogers' syndrome, Megaloblastic Anemia, Mutation, Thiamine – Responsive Anemia

Introduction

Thiamine-responsive megaloblastic anemia (TRMA) or Rogers' syndrome is an autosomal recessive and rare disorder characterized by early onset diabetes mellitus, anemia, and sensory neural deafness.[1] The first description of this disease is attributed to Rogers' et al who reported the first patient in 1969 .[2] Other abnormalities, such as congenital heart disease, degeneration of the retina and optic nerve, and stroke-like episodes, have also been associated with this syndrome.[3] The SLC19A2 gene on chromosome 1q23.3 encodes a functional thiamine transporter. A mutation of this gene leads to abnormal thiamine transport and vitamin deficiency in cells .[4] Although thiamine treatment results in the improvement of the hematological and endocrine functions, no response is reported for the neurological symptoms.[1] This study reports three children with TRMA from consanguineous Iranian families who show a novel mutation for the SLC19A2 gene.

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Cases reports

Case One: A male neonate was delivered at term by Cesarean section. He is the second child of healthy first cousin parents. His birth weight was 3Kg and he had suffered hearing loss since he was six months-old. The patient's six-year-old sister and a cousin also had diabetes and hearing loss. The patient referred to hospital with anemia and hyperglycemia at the age of 16 months. At the time of admission, he was not febrile and had no respiratory distress. His weight was 9.350 Kg (Z score=-1.1) and length 72 cm (Z score=-3.2). An initial laboratory study revealed fasting blood glucose of 191 mg/dl, venous pH 7.36, Hco3 23.4 mmol/L, RBC 3×106, Hb 10.1gr/dl, MCV 97.2, and a platelet count of 213,000. Urea and creatinine were at normal levels. Urine specific gravity was 1010 and, ketone and glucose were positive in the urine. An evaluation of the islet cell antibody and glutamic acid decarboxylase 65 antibodies was negative. The patient displayed no hepatosplenomegaly upon physical examination, nor optic atrophy upon fundoscopy. However, audiometery revealed deep sensory neural hearing loss. As the genetic study for TRMA revealed a novel mutation in the SLC19A2 gene, thiamine therapy was administered. After treatment the patients' anemia and hyperglycemia were ameliorated. In the last visit, growth was normal and HbA1C and blood glucose were in the normal range. The patient was referred to an otolaryngologist for a cochlear implant.

Case Two: A preterm female born by normal vaginal delivery with a birth weight of 2050 gr. She was the first child of healthy first-cousin parents and the sister of Case One. At the age 3.9 years, she was hospital for failure to thrive, fever, and hyperglycemia. At the time of admission, the patient was febrile, but displayed no respiratory distress nor hepatosplenomegaly. Her weight was 9.5 Kg (Z score=-3.6) and height 78 cm (Z score=-5.1). Laboratory findings revealed a blood glucose level of 248 mg/dl, venous pH 7.39, Hco3 18.8mmol/l, a RBC of 3.6×106, Hb 11.5 g/dl, and MCV 96 with a platelet count of 205,000. Urea was18mg/dl, creatinine 0.3mg/dl, specific gravity 1036, sugar was positive with no ketones in the urine analysis. The patient had no hepatosplenomegaly upon physical examination, but showed sensory neural hearing loss upon audiometery and was a candidate for cochlear implantation by an otolaryngologist. After the diagnosis of her brother (Case One), a genetic study was performed on her and the result was compatible with TRMA syndrome; thus, thiamine treatment was started. The administration of thiamine led to the recovery from anemia, but the hyperglycemia still lingered and required control with insulin.

Case Three: A full-term male neonate born by normal vaginal delivery was a single child of healthy second-cousin parents. The patient was evaluated for anemia at 6 months of age. After one year the hematology department referred him to the pediatric department for management of hyperglycemia. The patient showed sensory neural hearing loss in the audiometery and a positive family history of TRMA syndrome in two cousins. His weight was 9 kg (Z score=-0.6) and length 73cm (Z score= -1.2). Based on his positive family history, a genetic study was performed which revealed a novel mutation in the SLC19A2 gene; accordingly, oral treatment with thiamine was given. There was a good response to thiamine and the anemia and hyperglycemia were corrected. A cochlear implantation was to be performed for the sensory neural deafness. In the last follow-up, at age four years, the patient showed normal growth and development. HbA1C was 6.8% and blood glucose in a normal range.

Material and Methods:

Subjects:

All three patients with TRMA syndrome, along with their parents, were recruited from the pediatric department of Imam Reza Hospital. An informed consent was obtained from the parents and the study was performed in compliance with the guidelines of our Ethics Committee and Research Council of Mashhad University of Medical Science (MUMS).

DNA Extraction and Amplification of SLC19A2 Exons:

Using the standard salting-out method, genomic DNA was extracted from peripheral whole blood of these three patients, the healthy members of their families, and finally, from 100 unrelated control subjects. A polymerase chain reaction (PCR) amplified the coding exons 1-6 of the SLC19A2 gene and their intron-exon boundaries with a combination of seven sets of primer pairs. PCR reactions were performed in 25 µl using 100 to 200 ng of DNA, 3-7 p mol of each primer (Table 1), 1.5 mmol/L $MgCl_2$, 200 mmol/L dNTPs, 2.0 U prime Taq DNA Polymerase and a 10X buffer (GeNet Bio Co., Chungnam, Korea). Primers and PCR conditions have previously been described by Labay et al (5). PCR products were assayed for size and purity by separation on 2% agarose gels. (Fig. 1)

DNA Sequencing:

PCR products were purified using a DNA Gel

Extraction Kit (Invitek Co., Berlin, Germany) according to the manufacturer's instructions. This was followed for patients and the healthy family members by bidirectional sequencing by Applied Biosystem's ABI 3730 XL automated DNA sequence. DNA sequences were compared to the human Gen Bank sequence for the SLC19A2 gene (OMIM * 603941) using the Sequencher sequence alignment software (version 4.10.1).

CR-Restriction Fragment Length Polymorphism (RFLP) Analysis:

The novel mutation c.382G>A (p.E128K) identified in this study caused a loss of the XbaI restriction site (5'…TCTAGA…3') in the 2a PCR product. Consequently, all sequencing results were confirmed by RFLP analysis. The 2a fragment of exon 2 was amplified for all family members.

PCR products were purified using a DNA gel extraction kit (Invitek Co., Berlin, Germany) followed by digestion with the Fast Digest XbaI restriction enzyme (Fermentase Inc., Vilnius, Lithuania). PCR product (2 µl) was digested with 1U of the Fast Digest XbaI restriction enzyme and 2 µl of the 10X Fast Digest buffer in 20 µl reaction volumes at 37°C for 20 min, and then 20 min at 65°C (optional) to inactivate the enzyme. The digested and undigested PCR products were separated according to size in a 3% agarose gel and visualized under UV light.

Results

In all three patient, DNA sequencing revealed a homozygous mutation c.382G>A (p.E128K) resulting in the substitution of glutamic acid to lysine at position 128 in exon 2 of the SLC19A2 gene on chromosome 1q23.3 (Fig. 2-B). Healthy members of the families, including parents and the third child of Case One's family, were heterozygous (Fig. 2-A). The other exons and their adjacent intronic sequences were intact and no mutations were found. All sequencing results were confirmed by the PCR-RFLP method.

XbaI digestion of the respective fragment amplified from the wild-type allele yields two bands of 214 and 284 bp. The c.384G>A mutation abolishes the restriction site for XbaI and the digestion reaction results in an uncut fragment of 498bp. Consequently, the three homozygous patients yielded an undigested fragment of 498bp. The heterozygous healthy subjects yielded an undigested fragment of 498bp and two cut fragments of 214 and 284bp. The control DNA yielded two fragments of 214 and 284bp (Fig. 3).

The absence of the mutation c.382G>A of the SLC19A2 gene was confirmed by the PCR-RFLP assay of 200 unrelated control alleles. Thus, it does not appear to be a common polymorphism.

Discussion

In this report, three patients with TRMA syndrome from consanguineous families are described as having a novel mutation in the SLC19A2 gene. Case two was referred to the hospital due to diabetes at the age of 3.9 years. At first, insulin therapy was started but the response was poor. After the diagnosis of megaloblastic anemia and sensory neural deafness, thiamine therapy led to a better response than insulin therapy. The patient's brother and cousin were also similarly diagnosed after two and three years respectively. Usually anemia in TRMA syndrome is an early finding but unfortunately, these patients had not been properly evaluated when it first presented. It is, therefore, likely that the second case had had mildly chronic to moderate anemia for some period prior to diagnosis. The classical hematologic profile in this syndrome is a macrocytic anemia, sometimes associated with thrombocytopenia or neutropenia early on in life. However, in other studies, sideroblastic anemia has been described.[6] Diabetes mellitus in TRMA syndrome differs from the typical Type One diabetes. It is likely that diabetes is due to a thiamine deficiency in pancreatic islet cells and the mutation in the high-affinity thiamine transporter SLC19A2 supports this hypothesis.[7] This syndrome was first described by Rogers et al in 1969 [2] and the second, nine years later by Viana and Carvalho.[8] Since then, few cases have been reported by others .[9,10,11] In 1999, the responsible gene was identified by three independent groups to encode a solute carrier protein called THTR1.[5,12] This gene was cloned from a human fetal brain CDNA library and termed SLC19A2 .[10] The protein is a 497 amino acid molecule with 12 transmembrane domains that serve as a saturable active thiamine transport process by which thiamine is transported from extracellular to intracellular space.[13,14,15]

In addition to the active transport mediated by THTR1, thiamine is also transported by another passive low affinity and non-saturable mechanism which is intact in patients with TRMA syndrome .[13,15] This explains the absence of vitamin B1 deficiency (beri beri) symptoms in these patients. It seems that a high concentration of intra-cellular thiamine is important for the function and integrity of certain tissues, such as islet, hematopoietic, and cochlear cells. Defects in THTR1 cause a loss of the active thiamine transport mechanism, which leads to an inadequate intracellular thiamine concentration and the apoptosis of these cells (13). The DNA sequencing of the SLC19A2 gene in TRMA patients revealed a novel homozygous mutation in C.382G>A that results in the substitution of glutamic acid to lysine at position 128 in exon 2 of the SLC19A2 gene on chromosome 1q23.3. The sequencing results were confirmed by the PCR-RFLP method. The missense mutation c.382G>A of the SLC19A2 gene was confirmed by ruling out polymorphism via a PCR-RFLP assay of 200 unrelated control alleles. Alzahrani A. et al presented an AG51SC homozygous mutation in exon 2 that brought on a change of Gly to Arg at codon112x in patients and their family.[16] Lagarde HW discovered a missense mutation at amino acid 51 of the THTR and a substitution of leucine for proline.[17] Other studies by Alzahrani et al[16] , Lagarde HW et al[17] , Yesilkaya E et al[18], and Bergman et al[19] also uncovered some novel mutations in patients. A previous study of this syndrome in Iran by Zama T. et al presented consanguineous marriage in patients' families [20], as found in this report and some other studies.[21] Consanguineous marriage poses a higher risk for rare autosomal recessive diseases like TRMA. After the diagnosis of this syndrome for our patients, treatment began with a high doses of thiamine (200mg/kg), after which a significant improvement in the blood sugar profile occurred. Low dose insulin however, was still needed for diabetes control. The anemia disappeared after long-term treatment, but the hearing loss persisted. Therefore, for preventing deafness Onal has recommended starting thiamine therapy before two months of age.[22] We recommend the evaluation for TRMA syndrome in any diabetic patient with anemia or deafness. For pregnancies at increased risk, prenatal diagnosis must be performed at approximately 11-12 weeks of gestation by a DNA analysis of fetal cells using chorionic villous sampling .[23]

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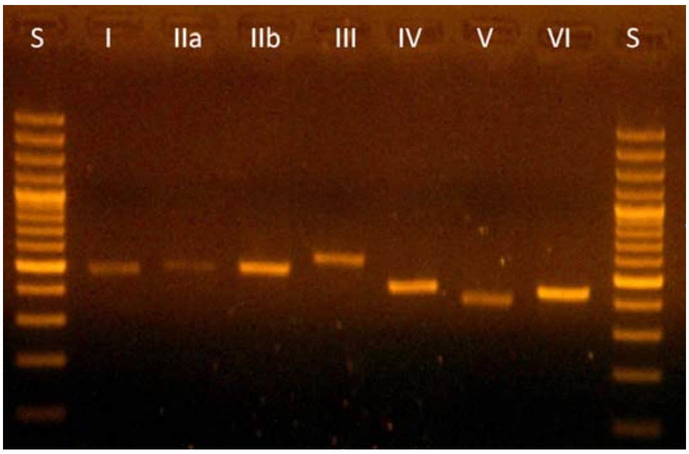
Table1. Primers used to amplify the exons 1-6 of the SLC19A2 gene.

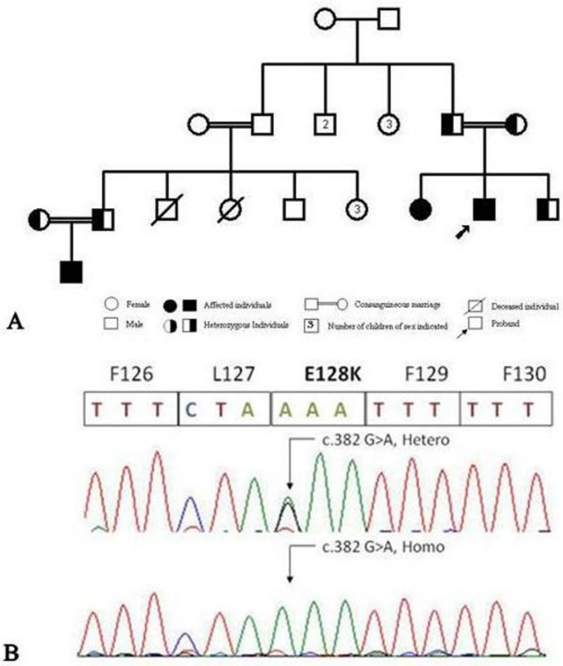
Exon	Primer	Nucleotide sequence (5′→3′)
1	1	5′-GCGTCCGCTGTGATTGGTT-3′
	2	5′-CTCCCTCTCGGTCAGGTT-3′
2a	1	5′-AGATCTTTGAGGTATTTGTAGG-3′
	2	5′-ACACAGGTAAGAGAGATGACA-3′
2b	1	5′-ACAGCCACTGAAATTGCCTA-3′
	2	5′-AGATCTACCAAGAGGGAGTTT-3′
3	1	5′-TTCGCCAGAGGGGATAAATG-3′
	2	5′-CCTGCTCCACTTGAGTACTT-3′
4	1	5′-CCCTCCATAATCTTGAGCTATT-3′
	2	5′-TTCCTCCCATTTGCCTCATTT-3′
5	1	5′-GTTGGAAAGGCAATTGACAGT-3′
	2	5′-ACTTTACATCTGTTCCCTATTG-3′
6	1	5′-CTCAGGCAGTCAGGCTTTATT-3′
	2	5′-GCTGCTGTGAAGTCAAGAAAT-3′

Fig.1: The purified PCR products of the SLC19A2 exons 1-6.



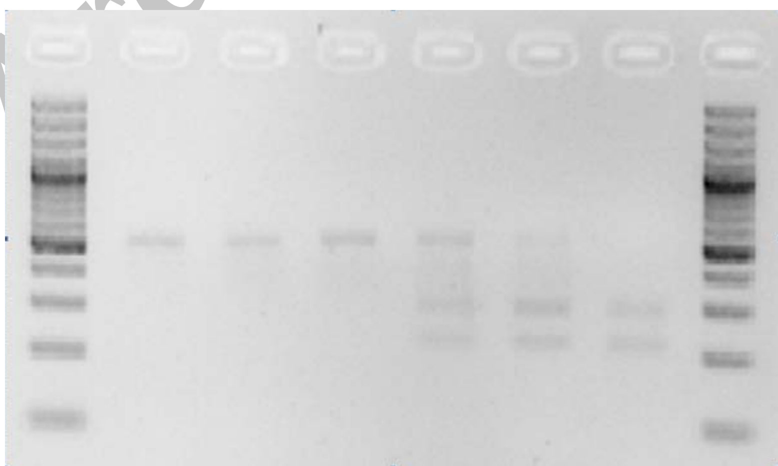
S: Ladder 100bp
I: 477bp
IIa: 498bp
IIb: 493bp
III: 560bp
IV: 440bp
V: 387bp
VI: 434bp

Fig.2: Family pedigree and mutation analysis



A, The Pedigree of two consanguineous families with TRMA.
B,TheDNAsequencingphoregramsshowingaGtoAtransitionatcodonsequence382inexon2of theSLC19A2gene.Patientsarehomozygousforthemutationandthehealthymembersoffamilies are heterozygous.

Fig.3: PCR-RFLP analysis of the SLC19A2 gene mutation.



Left to Right: S: Ladder 100bp; Patients 1-3: Homozygous mutant;
Parents1, 2: Healthy heterozygous; Control: Normal subject; S: Ladder 100bp