

CASE REPORT

Thiamine transporter mutation: an example of monogenic diabetes mellitus

Ali S Alzahrani¹, Essa Baitei², Minging Zou² and Yufei Shi²Departments of ¹Medicine and ²Genetics, King Faisal Specialist Hospital and Research Center, MBC 46, PO Box 3354, Riyadh 11211, Saudi Arabia

(Correspondence should be addressed to A S Alzahrani; Email: aliz@kfshrc.edu.sa)

Abstract

Objective: Thiamine-responsive megaloblastic anemia (TRMA) is a rare syndrome characterized by diabetes mellitus (DM), anemia, and sensorineural deafness. We describe the clinical course and the molecular defect of a young woman who was diagnosed to have this syndrome.

Case: The patient is an 18-year-old girl who was born to non-consanguous parents. She was noted to be deaf-mute in the first year of life. She was diagnosed with DM at the age of 9 months and with severe anemia at the age of 2 years. An extensive work up could not identify the cause. She was treated with blood transfusions every 3–4 weeks for the past 16 years. A diagnosis of TRMA was suspected and the patient was treated with thiamine hydrochloride. Hemoglobin and platelets increased to normal values after a few weeks of thiamine therapy. Diabetic control significantly improved but she had no noticeable changes in the deafness.

Methods: Peripheral blood DNA was extracted from the patient, her mother, aunt, and a healthy sister. Exons and exon–intron boundaries of the thiamine transporter gene *SLC19A2* were PCR amplified and directly sequenced.

Results: A G515C homozygous mutation was identified in the *SLC19A2* gene of the patient. This mutation changes Gly to Arg at codon 172 (G172R). The mother, an aunt, and a sister had a heterozygous G172R mutation.

Conclusions: Mutations in thiamine transporter gene, *SLC19A2*, causes a rare form of monogenic diabetes, anemia, and sensorineural deafness. Thiamine induces a remarkable hematological response and improvement in the diabetic control but has no effect on deafness.

European Journal of Endocrinology 155 787–792

Introduction

Thiamine-responsive megaloblastic anemia (TRMA), also called Rogers' syndrome, is a very rare syndrome characterized by early onset diabetes mellitus, anemia, and sensorineural deafness (1). It is inherited in an autosomal recessive pattern. Unlike the familiar forms of diabetes in which polygenetic and environmental factors interact, diabetes in TRMA syndrome is a monogenic form of diabetes secondary to mutations in the *SLC19A2* gene, which encodes a plasma membrane thiamine transporter, called THTR1 (2–6). The latter is a member of the solute carrier family that includes its homolog THTR2 and the reduced folate carrier. In this report, we present the clinical course of a young woman, who was diagnosed to have this syndrome and describe the salutary outcome of treatment with thiamine. Genetic analysis of her DNA revealed a mutation in the thiamine transporter gene *THTR1*.

Case report

SD is an 18-year-old girl who was born at full term after uneventful pregnancy and delivery to non-consanguous

parents. She was noticed to be deaf-mute in the first year of her life. At the age of 9 months, she was noticed to be lethargic with poor feeding and frequent vomiting. At that time, she was found to have high blood sugar of around 240 mg/dl but without ketoacidosis. She was diagnosed to have diabetes mellitus and was treated with insulin since then. Her initial insulin dose was 8 units/day but over the next few years, her insulin requirements progressively increased to a dose of around 20 units/day at the age of 5 years and around 35 units at the age of 10 years. The major increase in her dose occurred when she became pubertal at 14 years of age. Since that time, her daily insulin requirements ranged between 65 and 110 units. Her diabetes has been always labile and difficult to control but no definite history of diabetic ketoacidosis. At 2 years of age, she was found to have severe anemia with hemoglobin of 3 g/dl and thrombocytopenia (platelets 14 000 cells/dl). An extensive work up could not identify the cause and she was labeled as a case of transfusion-dependent anemia. She was treated with blood transfusions every 3–4 weeks for the past 16 years. She was admitted several times during her childhood and adolescence with severe anemia and

thrombocytopenia. Throughout her childhood, she has been noticed to be small for age with current weight of 42 kg and height of 158 cm. She had menarche at 14 years of age and her periods have been regular since then except for occasional delay of a few days. She also had normal breast development. In July 2005, she presented with fever and severe anemia. She was found to have hyperglycemia of 29 mmol/l and moderate diabetic ketoacidosis (pH 7.16, HCO₃ 14, serum and urine ketones ++). Investigations at that time showed: leucocyte count 24 000 cells/dl, hemoglobin 3 g/dl, MCV 78, MCH 27, platelets 28 000, reticulocyte count 61 (25–85), creatinine 49 µmol/l, HbA1c 12%, hemoglobin electrophoresis was normal, serum iron 20 mol/l (6–27), iron saturation 0.91 (0.08–0.41), serum ferritin 3773 g/l (13–150), vitamin B12, and red blood cell folate were normal. Bone marrow biopsy revealed erythroid hyperplasia with dysplastic changes.

Mutation analysis

After obtaining an informed consent and institutional approval, genomic DNA from peripheral blood leukocytes was isolated from the patient, her mother, an aunt, and a healthy sister following standard procedure. The *SLC19A2* coding exons and their intron–exon

boundaries were amplified by PCR from genomic DNA of the patient as detailed previously (5). Each successfully amplified fragment was directly sequenced using the BigDye Terminator V3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA), and samples were run on the ABI prism 3100 sequencer. A G515C homozygous mutation in exon 2 was detected in the patient by sequence analysis, which changes Gly to Arg at codon 172 (G172R; Fig. 1). We then directly sequenced the same region from genomic DNAs from her mother, an aunt, and a healthy sister. All had a heterozygous G172R mutation (Fig. 1). These results indicate that the patient inherited one copy of the G515C from her mother and another copy from her father in an autosomal recessive mode.

Clinical course

The patient was treated with i.v. fluids, insulin, and potassium. Diabetic ketoacidosis resolved and she was shifted to s.c. insulin. She also was transfused 3 units of packed red blood cells and 6 units of platelets. Her hemoglobin content rose to 7.6 g/dl and her platelets to 15 000 cells/dl. A diagnosis of TRMA was suspected and the patient was started on thiamine hydrochloride 100 mg i.v. BID. After 3 days, platelets and reticulocyte

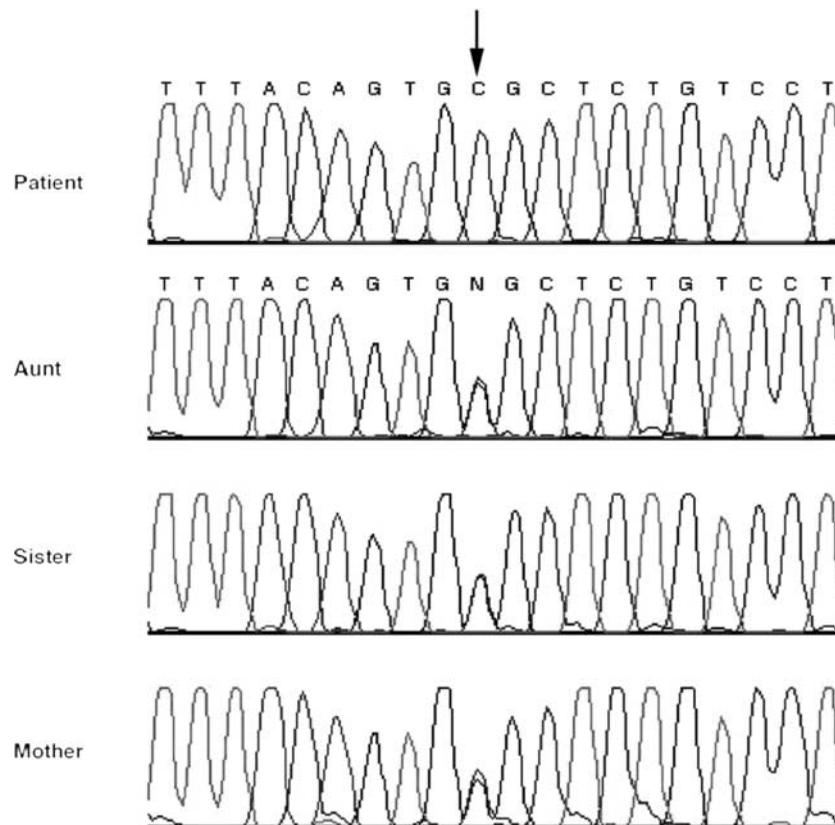


Figure 1 Direct sequencing of *THTR1* gene reveals G515C homozygous mutation (arrow) in exon 2 in the patient, which changes Glycine to Arginine at codon 172 (G172R). A heterozygous G172R mutation was found in the patient's aunt, sister, and mother.

counts started to increase reaching their maximum responses and exceeding the upper limits of normal ranges after 3 weeks of treatment with i.v thiamine hydrochloride (Fig. 2). Over time, these brisk responses of platelets and reticulocyte counts came down and remained in normal ranges. The response of hemoglobin was slower reaching a maximum level of 14.7 g/dl after about 2 months of thiamine therapy (Fig. 2). She was maintained on oral thiamine hydrochloride 100 mg daily and her hemoglobin and platelets remained in normal range for the past 10 months. Diabetic control improved with fewer and less severe fluctuations in her blood sugar on frequent

capillary glucose measurements. In addition, HbA1c level decreased from 12% before starting thiamine therapy to 8.6% 5 months later. She continued, however, to require insulin at a dose of around 60 units/day (around 1.4 units/kg per day). No noticeable changes occurred in the deafness.

Discussion

In this report, we described a new case of the rare TRMA syndrome. The case illustrates the importance of thiamine as a cofactor for cellular function in many

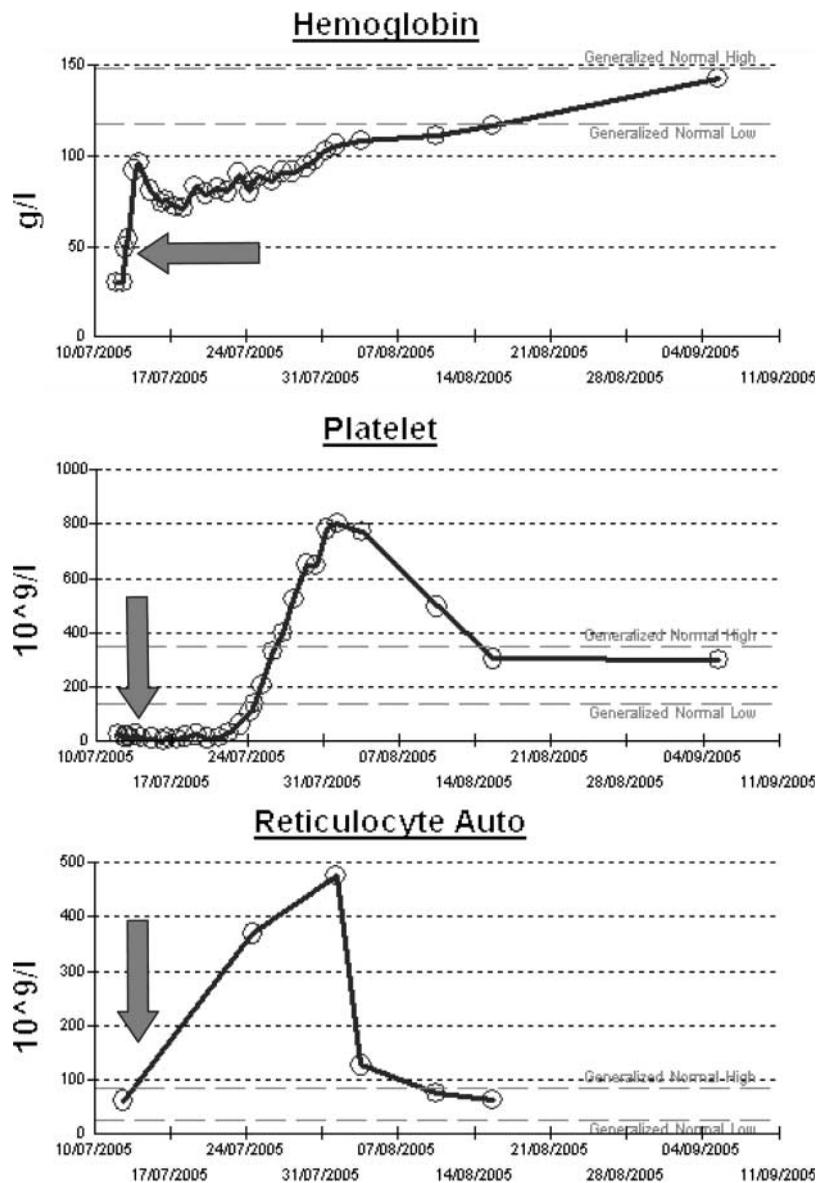


Figure 2 Response of hemoglobin, platelet, and reticulocyte counts to thiamine therapy. The arrows indicate the beginning of thiamine hydrochloride therapy. Platelet and reticulocyte count showed a brisk response exceeding the upper limits of normal ranges followed by a gradual decrease to normal levels.

tissues. The remarkable response of hematopoiesis provides clear evidence that cellular thiamine deficiency is the underlying cause of anemia and thrombocytopenia in this syndrome. It is also likely that diabetes and sensorineural deafness are due to thiamine deficiency in pancreatic islet and cochlear cells. This is further supported by the finding of a mutation in the high-affinity thiamine transporter SLC19A2. TRMA syndrome was first described by Rogers *et al.* in 1969 (1). In 1978, a second case was reported by Viana & Carvalho (7). Since then, a number of cases have been described (4, 8–13). It was postulated that this syndrome is related to defects in thiamine metabolism (1, 7, 14). However, it was not clear what the genetic defect was until 1997 when the gene causing TRMA syndrome was mapped to chromosome 1q23.2–23.3 (6). In 1999, the gene responsible was identified by three independent groups to encode a solute carrier protein, called THTR1 (2, 3, 5). This gene was cloned from a human fetal brain cDNA library and termed SLC19A2 (5). It is a member of a superfamily of solute carrier proteins which includes its homolog, THTR2 and the reduced folate transporter, SLC19A1. The protein product of SLC19A2, called THTR1, is a 497 amino acid molecule with 12 transmembrane domains. This transporter protein serves a saturable active thiamine transport process in which thiamine is transported from the extracellular to the intracellular space (11, 15–17). Point mutations and deletion/frameshift mutations of *SLC19A2* gene have been described in families from different ethnic backgrounds (11). Phenotype/genotype correlations have not clearly been established. However, these mutations cluster in a region that is important for THTR1 trafficking to cell surface. Previous work in which truncated constructs of human THTR1 were studied *in vitro* for their cellular dynamics demonstrated an essential role for the NH₃-terminal end and the backbone of THTR1 for cell trafficking and cell surface expression (3, 18). Labay *et al.* have reported a G515A mutation from an Italian family leading to an amino acid change at amino acid 172 (G172D) (3). The functional consequence of the G172D mutation has recently been characterized; this mutation leads to a misfolded protein that fails to undergo a complete glycosylation and is retained in the Golgi–ER compartment (18). Although the G172R mutation that we identified in our patient result in a different amino acid change, it likely causes similar functional defect as G172D mutation. In addition to the active transport mediated by THTR1, thiamine is also transported by another passive low-affinity non-saturable mechanism which is intact in patients with TRMA syndrome (11, 17). This may explain why patients with TRMA do not develop the usual manifestations of the clinical syndrome of severe vitamin B1 deficiency ‘beriberi’. It is likely that the passive transport mechanism protects TRMA patients from the usual manifestations of beriberi, since TRMA

patients do not have nutritional deficiency of thiamine and the passive transport mechanism is intact. Both the low-affinity non-saturable and the high-affinity saturable mechanisms mediate the intestinal absorption of thiamine. Defects in the high-affinity saturable mechanism in TRMA patients are compensated by the intact non-saturable mechanism in the intestine. This may explain why serum thiamine concentration is frequently normal in TRMA patients. It seems that a high concentration of intracellular thiamine is important for the function and integrity of certain tissues, such as islet cells, hematopoietic cells and the cochlear cells, and those defects in THTR1 result in loss of the active thiamine transport mechanism leading to inadequate intracellular thiamine concentration and apoptosis of these cells. In these and other cells, thiamine is normally converted to thiamine pyrophosphate by the enzyme thiamine pyrophosphokinase. Thiamine pyrophosphate is a cofactor to at least four important enzymes involved in the cellular metabolism. These enzymes are transketolase, pyruvate dehydrogenase, α -ketoglutarate dehydrogenase, and branched chain keto acid dehydrogenase. Reduced intracellular thiamine concentration leads to impairment of the transketolase-mediated nucleic acid ribose synthesis resulting in cell cycle arrest and apoptosis (11). In addition to the classic triad of diabetes mellitus, megaloblastic anemia, and sensorineural deafness, TRMA syndrome has been associated with a number of other organ defects. Cardiac abnormalities in the form of Ebstein’s anomaly and supraclavicular tachycardia have been described (19). A few cases of TRMA syndrome had cardiomyopathy (10, 19). Reported ocular manifestations include optic nerve atrophy and rod–cone dystrophy, which are likely to be secondary to low neuronal thiamine concentration (20). Diabetes mellitus in TRMA patients is a consistent finding and is most likely secondary to impairment of islet cell function by the intracellular thiamine deficiency. However, it resembles type 2 diabetes in that there is no insulin or islet cell antibodies and some cases of TRMA have only impaired glucose tolerance (14, 21–23). In addition, diabetic ketoacidosis is rare. Our patient presented with moderate severity diabetic ketoacidosis, which is probably secondary to the severe sepsis that she had at the time of presentation. The onset of diabetes mellitus is usually early in childhood. TRMA is one of the rare genetic disorders in which diabetes is secondary to a single gene disorder, the thiamine transporter defect. Diabetes mellitus type 2 has been linked to chromosome 1q23.2–23.3, the site of *SLC19A2* gene (24, 25). Therefore, it was logical to ask the question of whether thiamine metabolic defects could be the underlying mechanism in some patients with diabetes mellitus type 2. However, sequencing of the coding and flanking regions of *SLC19A2* gene in 20 diabetic and 20 non-diabetic Pima Indians showed no *SLC19A2* variants or mutations (26). The response of diabetes mellitus to

thiamine therapy in TRMA patients is variable (10, 23). Most cases show improvement in diabetic control and in a few cases diabetes disappeared (23). In our patient, a significant improvement in the blood sugar profile occurred with fewer fluctuations but she continued to need large doses of insulin for diabetic control. We used relatively large doses of thiamine hydrochloride (200 mg/day intravenously initially followed by 100 mg orally daily) to ensure the maximum response. These doses were chosen empirically and we did not try smaller doses which may have been equally effective. The anemia and thrombocytopenia in patients with TRMA are direct effects of intracellular deficiency of thiamine in the hematopoietic precursor cells. It is possible that the hematopoietic cells are more susceptible to the effects of thiamine deficiency than other cells since they are normally more active metabolically with rapid division and multiplication. Thiamine deficiency probably interferes with the high metabolic demand in these cells. Megaloblastic anemia with ring sideroblasts is the most common picture in TRMA (11, 27). Thrombocytopenia has been less commonly reported in TRMA patients. It is interesting that leucopenia is rare in these patients probably due to different needs of the hematopoietic progenitor cells to the intracellular thiamine. The response to thiamine has been consistently reported. In our patient, a remarkable improvement in hemoglobin and platelet counts was observed. In the first 2 weeks after the initiation of thiamine therapy, hemoglobin and hematocrit levels increased progressively and platelet and leucocyte counts vigorously responded and reached higher levels than normal (Fig. 2). This seems to reflect that the progenitor cells were in maturation arrest due to thiamine deficiency. With the availability of large amounts of thiamine; these cells rapidly progressed to mature cells. Over the next few weeks, platelet and leucocyte counts normalized indicating that the bone marrow reached a steady state. This response to thiamine was durable and constant over time. Blood counts and hemoglobin levels remained in the normal range and the patient did not need transfusion of blood products.

References

- Porter FS, Rogers LE & Sidbury JB, Jr. Thiamine-responsive megaloblastic anemia. *Journal of Pediatrics* 1969 **74** 494–504.
- Fleming JC, Tartaglioni E, Steinkamp MP, Schorderet DE, Cohen N & Neufeld EJ. The gene mutated in thiamine-responsive anaemia with diabetes and deafness (TRMA) encodes a functional thiamine transporter. *Nature Genetics* 1999 **22** 305–308.
- Labay V, Raz T, Baron D, Mandel H, Williams H, Barrett T, Szargel R, McDonald L, Shalata A, Nosaka K, Gregory S & Cohen N. Mutations in SLC19A2 cause thiamine-responsive megaloblastic anaemia associated with diabetes mellitus and deafness. *Nature Genetics* 1999 **22** 300–304.
- Raz T, Labay V, Baron D, Szargel R, Anbinder Y, Barrett T, Rabl W, Viana MB, Mandel H, Baruchel A, Cayuela JM & Cohen N. The spectrum of mutations, including four novel ones, in the thiamine-responsive megaloblastic anemia gene SLC19A2 of eight families. *Human Mutation* 2000 **16** 37–42.
- Diaz GA, Banikazemi M, Oishi K, Desnick RJ & Gelb BD. Mutations in a new gene encoding a thiamine transporter cause thiamine-responsive megaloblastic anaemia syndrome. *Nature Genetics* 1999 **22** 309–312.
- Neufeld EJ, Mandel H, Raz T, Szargel R, Yandava CN, Stagg A, Faure S, Barrett T, Buist N & Cohen N. Localization of the gene for thiamine-responsive megaloblastic anaemia syndrome, on the long arm of chromosome 1, by homozygosity mapping. *American Journal of Human Genetics* 1997 **61** 1335–1341.
- Viana MB & Carvalho RI. Thiamine-responsive megaloblastic anemia, sensorineural deafness, and diabetes mellitus: a new syndrome? *Journal of Pediatrics* 1978 **93** 235–238.
- Ricketts CJ, Minton JA, Samuel J, Ariyawansa I, Wales JK, Lo IF & Barrett TG. Thiamine-responsive megaloblastic anaemia syndrome: long-term follow-up and mutation analysis of seven families. *Acta Paediatrica* 2006 **95** 99–104.
- Lagarde WH, Underwood LE, Moats-Staats BM & Calikoglu AS. Novel mutation in the SLC19A2 gene in an African-American female with thiamine-responsive megaloblastic anaemia syndrome. *American Journal of Medical Genetics* 2004 **125** 299–305.
- Ozdemir MA, Akcakus M, Kurtoglu S, Gunes T & Torun YA. TRMA syndrome (thiamine-responsive megaloblastic anemia): a case report and review of the literature. *Pediatric Diabetes* 2002 **3** 205–209.
- Neufeld EJ, Fleming JC, Tartaglioni E & Steinkamp MP. Thiamine-responsive megaloblastic anemia syndrome: a disorder of high-affinity thiamine transport. *Blood Cells, Molecules & Diseases* 2001 **27** 135–138.
- Gritli S, Omar S, Tartaglioni E, Guannouni S, Fleming JC, Steinkamp MP, Berul CI, Hafsia R, Jilani SB, Belhani A, Hamdi M & Neufeld EJ. A novel mutation in the SLC19A2 gene in a Tunisian family with thiamine-responsive megaloblastic anaemia, diabetes and deafness syndrome. *British Journal of Haematology* 2001 **113** 508–513.
- Scharfe C, Hauschild M, Klopstock T, Janssen AJ, Heidemann PH, Meitinger T & Jaksch M. A novel mutation in the thiamine responsive megaloblastic anaemia gene SLC19A2 in a patient with deficiency of respiratory chain complex I. *Journal of Medical Genetics* 2000 **37** 669–673.
- Rindi G, Patrini C, Laforenza U, Mandel H, Berant M, Viana MB, Poggi V & Zarra AN. Further studies on erythrocyte thiamine transport and phosphorylation in seven patients with thiamine-responsive megaloblastic anaemia. *Journal of Inherited Metabolic Disease* 1994 **17** 667–677.
- Oishi K, Hofmann S, Diaz GA, Brown T, Manwani D, Ng L, Young R, Vlassara H, Ioannou YA, Forrest D & Gelb BD. Targeted disruption of Slc19a2, the gene encoding the high-affinity thiamine transporter Thtr-1, causes diabetes mellitus, sensorineural deafness and megaloblastosis in mice. *Human Molecular Genetics* 2002 **11** 2951–2960.
- Balamurugan K & Said HM. Functional role of specific amino acid residues in human thiamine transporter SLC19A2: mutational analysis. *American Journal of Physiology. Gastrointestinal and Liver Physiology* 2002 **283** G37–G43.
- Rindi G & Laforenza U. Thiamine intestinal transport and related issues: recent aspects. *Experimental Biology and Medicine* 2000 **224** 246–255.
- Baron D, Assaraf YG, Cohen N & Aronheim A. Lack of plasma membrane targeting of a G172D mutant thiamine transporter derived from Rogers syndrome family. *Molecular Medicine* 2002 **8** 462–474.
- Lorber A, Gazit AZ, Khoury A, Schwartz Y & Mandel H. Cardiac manifestations in thiamine-responsive megaloblastic anemia syndrome. *Pediatric Cardiology* 2003 **24** 476–481.
- Meire FM, Van Genderen MM, Lemmens K & Ens-Dokkum MH. Thiamine-responsive megaloblastic anemia syndrome (TRMA) with cone-rod dystrophy. *Ophthalmic Genetics* 2000 **21** 243–250.

- 21 Borgna-Pignatti C, Marradi P, Pinelli L, Monetti N & Patrini C. Thiamine-responsive anemia in DIDMOAD syndrome. *Journal of Pediatrics* 1989 **114** 405–410.
 - 22 Poggi V, Rindi G, Patrini C, De Vizia B, Longo G & Andria G. Studies on thiamine metabolism in thiamine-responsive megaloblastic anaemia. *European Journal of Pediatrics* 1989 **148** 307–311.
 - 23 Valerio G, Franzese A, Poggi V & Tenore A. Long-term follow-up of diabetes in two patients with thiamine-responsive megaloblastic anemia syndrome. *Diabetes Care* 1998 **21** 38–41.
 - 24 Elbein SC, Hoffman MD, Teng K, Leppert MF & Hasstedt SJ. A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. *Diabetes* 1999 **48** 1175–1182.
 - 25 Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns DK, Almasy L, Blangero J, Garvey WT, Bennett PH & Knowler WC. An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *American Journal of Human Genetics* 1998 **63** 1130–1138.
 - 26 Thameem F, Wolford JK, Bogardus C & Prochazka M. Analysis of slc19a2, on 1q23.3 encoding a thiamine transporter as a candidate gene for type 2 diabetes mellitus in pima indians. *Molecular Genetics and Metabolism* 2001 **72** 360–363.
 - 27 Bazarbachi A, Muakkit S, Ayas M, Taher A, Salem Z, Solh H & Haidar JH. Thiamine-responsive myelodysplasia. *British Journal of Haematology* 1998 **102** 1098–1100.
-

Received 14 August 2006

Accepted 28 September 2006