A novel mutation in SLC39A7 identified in a patient with autosomal recessive agammaglobulinemia

Melinda Erdős[1], Kristina Mironska[2], Lidia Kareva[2], Katarina Stavrik[2], Arijeta Hasani[2], Arpad Lanyi[3], Judit Kallai[3], and Laszlo Marodi[4]

[1]University of Debrecen, Faculty of Medicine
[2]Ss Cyril and Methodius University in Skopje
[3]University of Debrecen
[4]Semmelweis Egyetem

March 14, 2022

A novel mutation in *SLC39A7* identified in a patient with autosomal recessive agammaglobulinemia

To the Editor,

ZIP7 deficiency is the most recently described congenital agammaglobulinemia with autosomal recessive inheritance (1). ZIP7, encoded by *SLC39A7,* is an endoplasmic reticulum-to-cytoplasm Zn^{2+} transporter. Developing B cells are sensitive to altered Zn^{2+} distribution which cause developmental blockade beyond the pre-B cell stage (2). Complete loss of ZIP7 in cell lines causes a reduction in cytoplasmic Zn^{2+} and an increase in endoplasmic reticulum Zn^{2+} concentration. Since the original report in 2019, no additional cases of ZIP7 deficiency have been published. Here we describe a patient evaluated for recurrent respiratory tract infections, meningitis, agammaglobulinemia and B cell lymphopenia, ultimately found to carry a novel *SLC39A7*variant. We describe his clinical characteristics, immunological findings, and genetic investigations.

The patient and his family members were interviewed, examined, treated and monitored at the University Clinic for Children's Disease in Skopje. Medical records were obtained from the electronic registry of the University Clinic. The mother of the patient has given written informed consent to conduct the study and for publication of data. All procedures were performed in accordance with the ethical standards of the Institutional Research Committee. Genomic DNA from the patient and his family members was isolated with the Gen Elute Blood Genomic DNA kit (Sigma-Aldrich) and subjected to whole-exome sequencing (WES) in the patient and targeted gene sequencing in the patient and available family members (3). WES was performed at the New York Genome Center and the Rockefeller University using an Illumina HiSeq 2500 sequencing system (Illumina). Exome capture was carried out with SureSelect human all exome kit (Agilent) in accordance with the manufacturer's instructions. Putative disease alleles found by WES were validated by dideoxy Sanger sequencing in the patient as well as in family members.Exons and flanking intronic regions of *SLC39A7* were amplified by PCR. Amplicons were sequenced with the Big Dye Terminator cycle sequencing kit (Applied Biosystems) and targeted regions were analyzed by an ABI 3130 capillary sequencer (Applied Biosystems). Sequence variants were determined by comparing to the appropriate GenBank reference sequence to identify the position of mutations.

The patient, a 14-year-old male and the third child in a Macedonian family with Albanian origin was born at term (Fig. 1). Birth weight and length were in the normal ranges and neonatal adaptation was uneventful. Consanguinity in the family was not reported. The patient received only BCG vaccination at birth indicated by a small scar on the left shoulder. He had suffered from recurrent upper and lower respiratory

tract infections since 6 months of age. He was first hospitalized for *H. influenzae* pneumonia at the age of 4 years. Treatment with ceftriaxone, 50 mg/kg/day for 10 days, resulted in clinical and radiological recovery. He was suspected for immunodeficiency, but the family failed to meet the medical appointments for immunological evaluation. By $6\frac{1}{2}$ years of age he developed bilateral chronic mastoiditis which was treated with antibiotics and surgery with good therapeutic efficacy. At the age of 7 he was hospitalized for purulent meningitis. Pathogenic microorganisms did not yield from the cerebrospinal fluid which could be due to the previously started cephalosporin antibiotic therapy. After two weeks of treatment with 3$^{\text{rd}}$ generation intravenous cephalosporin, he was discharged from hospital but continued to develop recurrent upper respiratory tract infections treated mostly by the family pediatrician. At the age of 9 years, he was hospitalized again because of severe bilateral pneumonia. Respiratory aspirate yielded*Pseudomonas aeruginosa* . He received intravenous antibiotics, inhalative Colistin (polymyxin E), and antimycotic treatment because*C. albicans* was isolated from the tracheal aspirate. Multiple rigid bronchoscopic lavages were performed as part of the treatment. Chest computed tomography (CT) showed bronchiectasis and perfusion scan of the lung showed hypoperfusion in the right lower lobe. He was seen at the Immunology Department of the University Clinic for Children's Diseases in Skopje, at age $9\frac{1}{2}$. By physical examination lymphoid tissue hypoplasia (lack of tonsillar tissues, small adenoids and impalpable lymph nodes), hepatomegaly (liver edge was palpable 3 cm below the costal margin), vesicular breathing with bilateral crepitation above both lungs, and low body weight (18 kg, $<5^{\text{th}}$percentile) were found. The family history was negative for primary immunodeficiencies and hematological disorders. His mother and three siblings (2 boys and 1 girl) are healthy; his father died in a car accident at the age of 33 years.

The total number of white blood cells, red blood cells and platelets were normal but the patients had hypochromic anemia, neutropenia, and CD19^{+} lymphopenia (Table 1). Immunochemistry tests revealed agammaglobulinemia, and absence of pathogen-specific antibodies to tetanus toxoid, *H. influenza* type B, and *Str. pneumoniae* (Table 1). Concentrations of complement components C3 and C4 were normal, and measurement of thyroid hormones showed normal levels of fT3 and fT4. The Quantiferon-TB test was negative. Based on these findings, monthly intravenous immunoglobulin (IVIG) therapy was started. Inborn errors of immunity (IEI) related genes were analyzed based on the most recent updated classification of the International Union of Immunological Societies Expert Committee (4). Mutation screening by WES revealed that the patient harbored a private homozygous variant in *SLC39A7* . The variant was predicted to be deleterious (CADD score: 27.1). No other disease-causing variant in other IEI related genes were identified by WES. The *SLC39A7* sequence variant was validated by targeted gene sequencing, and we found that the patient was homozygous whereas his mother and three siblings were all heterozygous for the c.1051A>G, p.Thr351Ala mutation in*SLC39A7* (Fig. 1).

We report here a novel private homozygous mutation in *SLC39A7*discovered in a boy with Albanian ancestry. This mutation results in an amino acid substitution (alanine for threonine) located at position 351 of the ZIP7 protein. The biallelic variant is associated with a profound decrease of peripheral blood B cells and immunoglobulin isotypes resulting in recurrent and severe infectious diseases. As such, the present work supports earlier evidence in which homozygous or compound heterozygous mutations in *SLC39A7* were found in 6 patients with absent B cells and agammaglobulinemia and proposed as a novel PID. These vary in that they occur at different residues within the protein (1). Mutations including c.1051A>G described here resulted in changes of amino acid residues mostly at the C terminal end of the protein between residues 351 and 458 with no mutational hotspot (Fig. 1).

Transmembrane transporters of Ca^{2+}, Mg^{2+} and Zn^{2+} control the movement of these divalent cation mediators of lymphocyte signaling between the cytosol and the cytosolic organelles or the extracellular space (5-7). Monogenic germline mutations of genes may result in loss of function of the encoded transporter proteins and impaired adaptive immunity mediated by B and T cells (*ORAI1* mutation; 5), T and NK cells (*MAGT1* mutation; 6) or B cells (*SLC39A7* mutation; 7) resulting in PIDs. Structural and functional integrity of thousands of proteins are regulated by Zn^{2+} transporters (7). Biallelic hypomorphic mutations of *SLC39A7* which encodes the Zn^{2+} transporter ZIP7 has recently been discovered. Pertinent to this, in mouse B cell lines with homozygous mutation, a diminished concentration of cytoplasmic free Zn^{2+} and decreased phos-

phorylation of signaling molecules of pre-B cell and B cell receptors were found. These findings highlight the critical role for cytoplasmic Zn^{2+} in B cell receptor signaling downstream of the pre-B cell and B cell receptors.

Similarly to defects of B cell development to rearrange heavy and then light chain immunoglobulin genes that lead to AR and X-linked agammaglobulinemias, loss of function alleles of *SLC39A7* may lead to impaired B cell signaling. The patient described in this report had agammaglobulinemia, lack of B cells, and hypoplastic lymph nodes and tonsils despite recurrent upper respiratory tract infections. We thought of XLA and searched for *BTK* mutation first but found wild type sequences in the patient (not shown). Next, WES and targeted gene sequencing were used to search for known AR and AD agammaglobulinemia genes and we found in the patient's DNA a previously unknown c.1051A>G, p.Thr351Ala *SLC39A7* mutation. By using blood derived DNA samples from all available family members, we confirmed homozygosity of this *SLC39A7* mutation in the patient and heterozygosity in family members who were clinically healthy (Fig. 1). The gene encoding the zinc transporter protein ZIP7 has been implicated to cause autosomal recessive agammaglobulinemia in white European, South Asian, and Hispanic ancestries (7). To our knowledge the patient reported here is the first diagnosed with inherited ZIP7 deficiency in Central and Eastern Europe.

Hematologic stem cell transplantation (HSCT) was found beneficial in two of the 6 patients in the previous study (7); these two patients had the most severe disease phenotype including failure to thrive, blistering dermatitis and thrombocytopenia in addition to early onset infections, agammaglobulinemia and B cell depletion which were present in all patients. The other 4 patients including those two with failure to thrive, liver dysfunction and seborrheic dermatitis, responded well to intravenous immunoglobulin replacement alone. HSCT in our patient has not been performed until now, because we did not have an accurate molecular diagnosis, so we treated him with regular IVIG substitution dose of 400 mg/3-4 weeks, since his $9\frac{1}{2}$ years of age. The adherence to the treatment has often been poor and he has signs of chronic pulmonary damage. He does not attend school, does not cooperate and spirometry cannot be performed which make clinical management challenging.

In summary, the report of this patient adds to the current clinical and genetic knowledge on ZIP7 deficiency in humans. Early recognition and diagnosis of this condition is pivotal for improved outcomes and prevention of complications like lung tissue damage developed over years in this patient. Unfortunately, most patients with PID in Eastern and Central Europe still remain without molecular diagnosis (8-10). Therefore, collaborative programs with more advanced centers are critically important especially in countries with lower socioeconomic condition and limited resources to molecular diagnostics. Such a program has been established by the J Project physician education and clinical research collaboration network (www.thejpnetwork.com). The J Project program provides help for early diagnosis, treatment, and family counseling of known and even very recently described PIDs like ZIP7 deficiency (10). In conclusion, diagnosis of ZIP7 deficiency should be contemplated in patients with autosomal recessive agammaglobulinemia and recurrent, early onset infectious diseases.

KEYWORDS

zinc transporter proteins, SLC39A7 mutation, B cell deficiency, agammaglobulinemia

ACKNOWLEDGEMENTS

We thank the mother of the patient for helpful collaboration. This research was supported by the J Project and the Foundation for Children with Immunodeficiencies, and from the European Society for Immunodeficiencies to ME.

CONFLICT OF INTEREST

The authors declare that they have no competing financial interests.

AUTHOR CONTRIBUTIONS

Melinda Erdős: performing bioinformatics analysis and writing the initial draft. **Kristina Mironska:** conducting clinical research and patient care, editing the initial draft. **Lidia Kareva:** conducting clinical research and patient care, data collection.**Katarina Stavric:** conducting clinical research and patient care, data collection. **Arijeta Hasani:** conducting clinical research and patient care, data collection. **Árpád Lányi:**conducting targeted gene sequencing. **Judit Kállai:** conducting targeted gene sequencing. **László Maródi:** formulation of research goals, writing the final draft**.** All authors reviewed the manuscript.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings and the materials used in this study are available on request. The data are not publicly available due to ethical restrictions.

Figure legend

Figure 1

Homozygous *SLC39A7* mutation in a patient with autosomal recessive agammaglobulinemia.

Family Pedigree with *SLC39A7* allele segregation. The black-filled symbol indicates the proband (P) having the novel homozygous *SLC39A7* mutation. Symbols consisting of black and white colours indicate heterozygous disease carriers as determined by targeted sequencing. Diagonal bar indicates diseased individual. Generations are designated by a Roman numeral (I and II), and each individual by an Arabic numeral (from left to right). DNA was obtained for genetic analysis from all individuals to whom a number is assigned. Automated sequencing profiles show homozygous c.1051A>G, p.T351A mutation in the proband (II.3; P) and heterozygous mutations in four family members (I.2, II.1, II.2, II.4). C, Control.

B) Schematic representation of domain structure of ZIP7 and localization of mutations. Numbers above and below the scheme indicate the amino acid residue numbers. Numbers below the scheme show the borders of histidine-rich domains. Positions of the identified SLC39A7 variants including six missense (P190A, L217P, T351A, E363K, T395I, G458A) and two nonsense (Q372X, E451X) mutations. The novel T351A mutation is marked in red. Mutations in bold were observed in homozygous form. TM, transmembrane; His, histidine.

Melinda Erdős[1,2]

Kristina Mironska[3]

Lidia Kareva[3]

Katarina Stavric[3]

Arijeta Hasani[3]

Árpád Lányi[4]

Judit Kállai[4]

László Maródi[1,2]

[1]Primary Immunodeficiency Clinical Unit and Laboratory, Department of Dermatology, Venereology and Dermatooncology, Semmelweis University, Budapest, Hungary

[2]St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch,

The Rockefeller University, New York, NY

[3]Department of Immunology, University Clinic for Children's Disease, Skopje, Republic of North Macedonia

[4]Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Correspondence

Melinda Erdős or László Maródi, Primary Immunodeficiency Clinical Unit and Laboratory, Department of Dermatology, Venereology and Dermatooncology, Semmelweis University, Budapest, Hungary

Email: Erdos.Melinda@med.semmelweis-univ.hu

or

Marodi.Laszlo@ med.semmelweis-univ.hu

ORCID

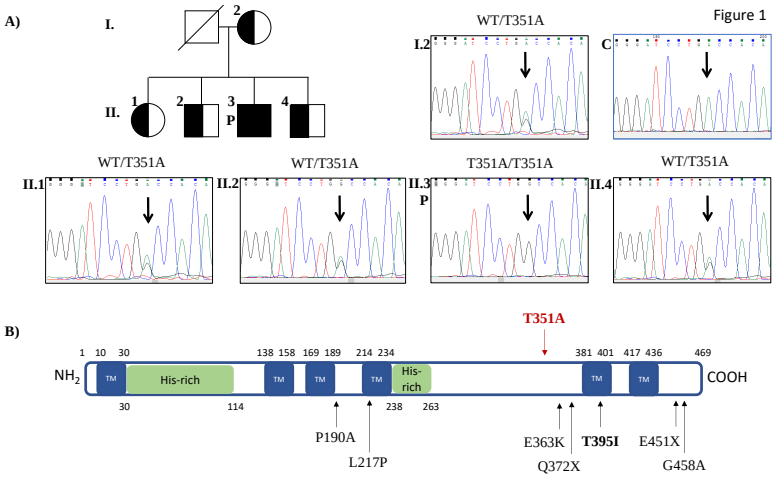
Erdős Melinda: https://orcid.org/0000-0001-6770-8490

Maródi László: https://orcid.org/0000-0002-9692-3402

REFERENCES

1. Anzilotti C, Swan DJ, Boisson B, et al. An essential role for the Zn $^{2+}$ transporter ZIP7 in B cell development.*Nat Immunol* . 2019;20:350-361.
2. Kambe T, Hashimoto A, Fujimoto S. Current understanding of ZIP and ZnT zinc transporters in human health and diseases. *Cell Mol Life Sci* . 2014;71:3281-3295.
3. Erdős M, Lányi Á, Balázs G, Casanova JL, Boisson B, Maródi L. Inherited TOP2B mutation: Possible confirmation of mutational hotspots in the TOPRIM domain. J Clin *Immunol* . 2021;41:817-819.
4. Tangye SG, Al-Herz W, Bousfiha A, et al. Human inborn errors of immunity: 2019 update on the classification from the international union of immunological societies expert committee. *J Clin Immunol* . 2020;40:24-64.
5. Feske S, Gwack Y, Prakriya M, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* . 2006;441:179-185.
6. Li FY, Chaigne-Delalande B, Kanellopoulou C, et al. Second messenger role for Mg2+ revealed by human T-cell immunodeficiency.*Nature* . 2011;475:471-476.
7. Woodruff, G. et al. The zinc transporter SLC39A7 (ZIP7) is essential for regulation of cytosolic zinc levels. *Mol Pharmacol* . 2018;94:1092-1100.
8. Maródi L and the J Project Study Group. Fifteen years of the J Project. *J Clin Immunol* . 2019; 39:636-639.
9. Tuzankina I, Bolkov M, Nabieva U, Lázár I, Maródi L. The J Daughter Siberia Project. *J Clin Immunol* . 2021;41:262-265.
10. Maródi L and the J Project Study Group. The 10thanniversary of World Primary Immunodeficiency Week: A J Project celebration. *Eur J Immunol* . 2021; 51: 2364–2366.

Figure 1



Hosted file

`Table 1.docx` available at https://authorea.com/users/400521/articles/559842-a-novel-mutation-in-slc39a7-identified-in-a-patient-with-autosomal-recessive-agammaglobulinemia