

# A novel mutation in *SLC39A7* identified in a patient with autosomal recessive agammaglobulinemia: The impact of the J Project

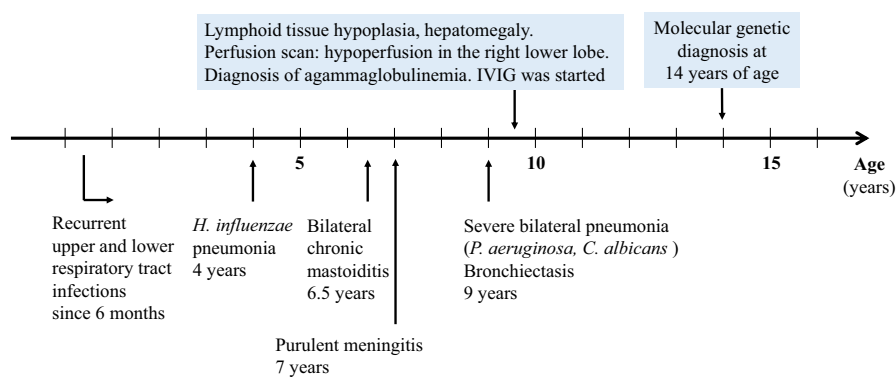
To the Editor,

ZIP7 deficiency is the most recently described congenital agammaglobulinemia with autosomal recessive inheritance.<sup>1</sup> ZIP7, encoded by *SLC39A7*, is an endoplasmic reticulum-to-cytoplasm Zn<sup>2+</sup> transporter. Developing B cells are sensitive to altered Zn<sup>2+</sup> distribution, which causes developmental blockade beyond the pre-B cell stage.<sup>2</sup> Complete loss of ZIP7 in cell lines causes a reduction in cytoplasmic Zn<sup>2+</sup> and an increase in endoplasmic reticulum Zn<sup>2+</sup> 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. We also report on the impact of the J Project (JP) in improving primary immunodeficiency (PID) patient care and research in Eastern and Central Europe (ECE).

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 the 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.<sup>3</sup> 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 AND 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 with the appropriate GenBank reference sequence to identify the position of mutations.

The patient, a 14-year-old boy, and the third child in a Macedonian family of Albanian origin was born at term. 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. Major clinical manifestations are shown in Figure 1. Treatment with ceftriaxone, 50 mg/kg/day for 10 days, resulted in the clinical and radiological recovery of pneumonia at 4 years of age. He was suspected of immunodeficiency, but the family failed to meet the medical appointments for immunological evaluation. At 7 years of age, he developed meningitis, but pathogenic microorganisms did



**FIGURE 1** Clinical manifestation, age, and diagnostic procedures

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TABLE 1 Clinical and laboratory data of patients with SLC39A7 mutation

Feature/Patient	P1	P2	P3	P4	P5	P6	Our patient
ZIP variants	P190A E363K	P190A E363K	L217P Q372X	E451X G458A	T395I T395I	P190A L217P	T351A T351A
<i>Clinical data</i>							
Age at first manifestation (m)	<1	<1	12	23	<1	26	6
Age at diagnosis of agammaglobulinemia (yr)	0.1	0	14	8	3	6	9.5
Age at description (yr)	5.5	3.5	32	16	11	18	14
Gender	M	M	F	F	F	F	M
Ethnic origin	N European	N European	N European	S Asian	Hispanic	N European	S-E European
Consanguinity	No	no	no	no	no	no	no
Bacterial infections	Yes	yes	yes	yes	yes	yes	yes
Upper and lower respiratory tract infections (age)	NA	NA	NA	NA	NA	NA	yes (since 6 m)
Bronchiectasis (age)	NA	NA	NA	NA	NA	NA	yes (9 yr)
Skin rash	blistering dermatitis	blistering dermatitis	mild eczematous rash trunk/behind ears	mild eczematous rash trunk/behind ears	seborrheic dermatitis with superinfection	transient necrotizing granulomatous rash	No
Other clinical diseases (especially liver diseases)	thrombocytopenia, profound sensorineural deafness	thrombocytopenia, profound sensorineural deafness	-	Fe-deficiency, anemia, Vitamin D deficiency, enteropathy, transaminitis	-	-	Hepatomegaly
Treatment	HSCT	HSCT	IVIG	IVIG	IVIG	SCIG	IVIG <sup>c</sup>
Height (cm) [centile] at description	101.1 [3rd]	85 [3rd]	160 [40th]	152 [5th]	140 [25th]	173 [90th]	149 [<5]
Weight (kg) [centile] at description	16.3 [10th]	13.4 [10th]	60 [50th]	38 [<0.4th]	27 [50th]	71 [73rd]	29.6 [<1]
<i>Immunoglobulins</i>							
IgG (g/L)	1	30.1 <sup>b</sup>	0.14	4.58 <sup>§</sup>	<1.4	<1.7	< 3.2
IgA (g/L)	0.25	0.16 <sup>a</sup>	<0.06	<0.06	0.17 <sup>a</sup>	<0.07	< 0.25
IgM (g/L)	< 0.22	0.19 <sup>a</sup>	<0.04	<0.04	0.23 <sup>a</sup>	0.09	> 0.32
<i>Lymphocyte subsets</i>							
CD3+ (/ul)	9036	1308	NA	1121	94.5%	6180	2586 (700 – 2200)
CD4+ T cell (/ul) (norm)	8031	1031	NA	566	61.1%	4141	789 (450 – 1400)

TABLE 1 (Continued)

Feature/Patient	P1	P2	P3	P4	P5	P6	Our patient
CD8+ T cell (/ul) (norm)	893	268	NA	468	25.4%	2044	1668 (250 – 850)
naïve (% of CD4+)	14	48	NA	81	80	NA	NA
naïve (% of CD8+)	51	183	NA	52	71	NA	NA
CD4:CD8 ratio	8.99	3.85	NA	1.21	2.4	2	0.47
TCRab (% of CD3+)	98	NA	NA	82	93	NA	NA
HLA-DR+ of CD3+	34	1	NA	NA	NA	NA	NA
proliferation to PHA	normal	normal	NA	normal	NA	NA	NA
NK cell (/ul)	2097	78	NA	215	4.7%	163	231
CD19+ B cell (%)	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	0.1

Abbreviations: HSCT, hematopoietic stem cell transplantation; IVIG, intravenous immunoglobulin; m, month; NA, not available; PHA, phytohemagglutinin; SCIG, subcutaneous immunoglobulin.

<sup>a</sup>Declining to undetectable.

<sup>b</sup>Obtained after Ig supplementation.

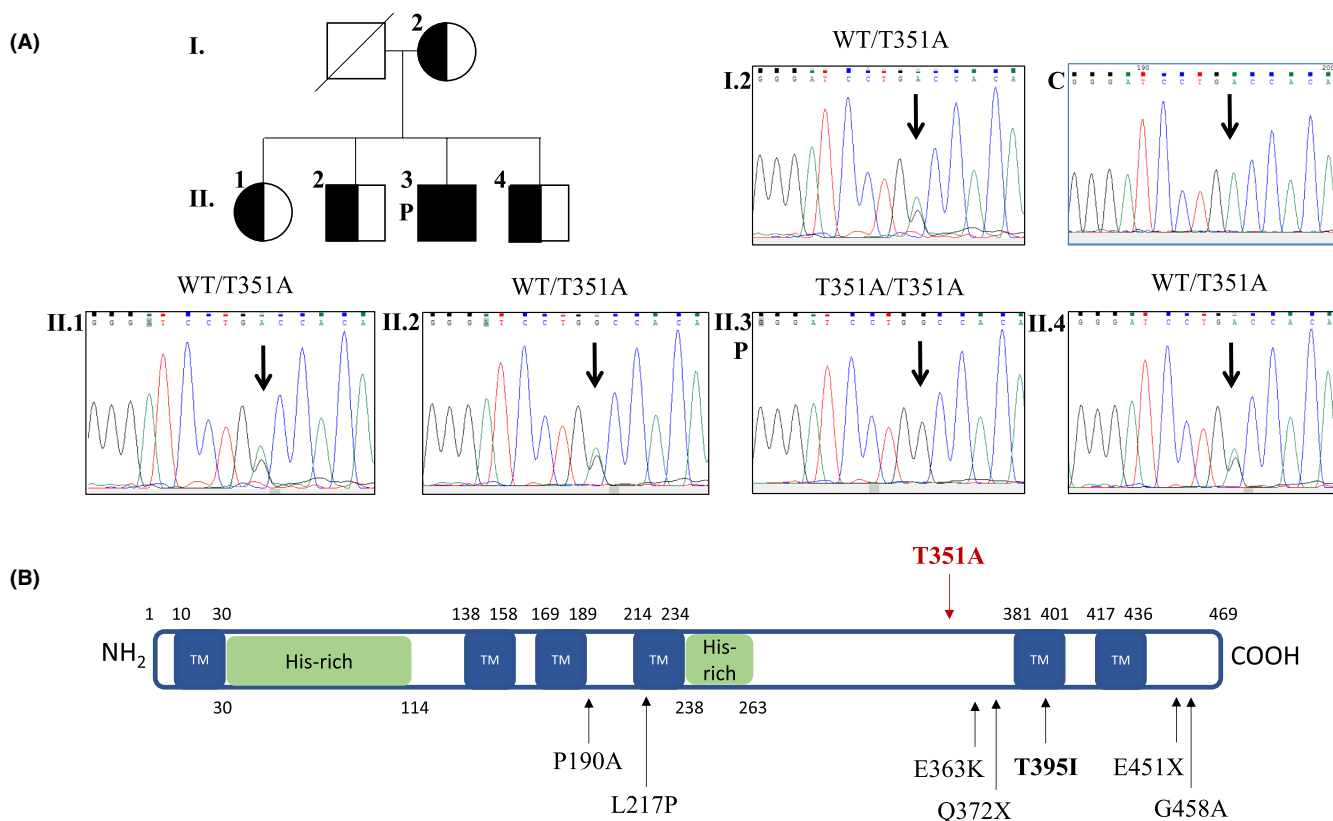
<sup>c</sup>Not regular due to the lack of collaboration.

not yield from the cerebrospinal fluid, which could be due to the previously started cephalosporin antibiotic therapy. After two weeks of treatment with 3rd generation intravenous cephalosporin, he was discharged from the hospital but continued to develop recurrent upper respiratory tract infections treated mostly by the family pediatrician. At the age of 9 years, he developed pneumonia, and received intravenous antibiotics, inhalative Colistin (polymyxin E), developed pneumonia and antimycotic treatment because *C. albicans* was also isolated from the tracheal aspirate. Multiple rigid bronchoscopic lavages were also performed as part of the treatment. 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 was normal, but the patient had hypochromic anemia, neutropenia, and CD19<sup>+</sup> B cell lymphopenia (Table 1). The CD8<sup>+</sup> T cell count was high resulting in an inverted CD4<sup>+</sup>/CD8<sup>+</sup> ratio, but the CD4<sup>+</sup> cell number was normal and the HIV PCR test was negative. Immunochemistry tests revealed agammaglobulinemia, and the 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 the 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 (Figure 1). 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.<sup>4</sup> 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 variants 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* (Figure 2). We have also checked the WES files for other homozygous regions and found that the consanguinity % was below 1% (0.18105%) suggesting that this patient was not from a consanguineous marriage.<sup>5</sup>

Similar 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.<sup>6</sup> 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



**FIGURE 2** Homozygous *SLC39A7* mutation in a patient with autosomal recessive agammaglobulinemia. (A) 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 colors indicate heterozygous disease carriers as determined by targeted sequencing. Diagonal bar indicates a 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, and G458A) and two nonsense (Q372X and E451X) mutations. The novel T351A mutation is marked in red. Mutations in bold were observed in homozygous form. TM—transmembrane; His—histidine

**TABLE 2** Molecular genetic testing of disease-causing genes in 316 samples from patients in 15 J Project countries

Gene symbols → Countries ↓	ARP		CXC														
	AID	AIRE	C1B	BTK	C2	CD40L	R4	CYBB	DKC1	DOCK8	ELANE	FASG	FOXP3	G6PC3	IKBKG	IL2RG	JAGN1
1. Azerbaijan																	
2. Croatia				2		1					1						
3. Czechia																	
4. Hungary	6	12	1	18	2	17	1	7	1	2	6	3	1	1		5	4
5. Latvia						2		2								1	
6. Moldova				1													
7. Macedonia				1			1										
8. Poland				7			4										
9. Romania	1	1		9		2	3	4	2			1			1	1	
10. Russia		1		11		2		4									
11. Serbia				1													
12. Slovakia	1																
13. Slovenia				2													
14. Turkey																	
15. Ukraine		2		28		2		3			1	1				1	
SUM	8	16	1	80	2	26	9	20	3	2	8	5	1	1	1	8	4

and heterozygosity in family members who were clinically healthy (Figure 2). The gene encoding the zinc transporter protein ZIP7 has been implicated to cause autosomal recessive agammaglobulinemia in white European, South Asian, and Hispanic ancestries.<sup>1</sup> 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 six patients in the previous study<sup>1</sup>; 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 (Table 1). The other four patients, including those two with failure to thrive, liver dysfunction, and seborrheic dermatitis, responded well to intravenous immunoglobulin replacement alone. Our patient had hepatomegaly but no liver or skin disease. HSCT in our patient has not been performed until now because we did not have an accurate molecular diagnosis, so we treated him with a regular IVIG substitution dose of 400 mg/3–4 weeks, since his 9½ 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.

The report of this patient adds to the current clinical and genetic knowledge on ZIP7 deficiency in humans (Table 1). Early recognition and diagnosis of this condition are pivotal for improved outcomes and prevention of complications like lung tissue damage developed over years in this patient. Unfortunately, many patients with PID in ECE still remain without molecular diagnosis.<sup>7–10</sup> Therefore, collaborative programs with more advanced centers are critically important especially in countries with lower socioeconomic condition and limited resources for molecular diagnostics. Such a program has been established by

the JP physician education and clinical research collaboration network in 2004 ([www.thejpnetwork.com](http://www.thejpnetwork.com)). The JP provides help for early genetic diagnosis, prenatal genetic testing, treatment, and family counseling of known and even very recently described IELs like ZIP7 deficiency (Table 2). Supported primarily by educational grants from the European Society for Immunodeficiency (ESID), the Jeffrey Modell Foundation, and a few pharmaceutical companies, the JP coordinated by the Foundation for Children with Immunodeficiencies is now extended to 32 countries in ECE, Asia, and Egypt.<sup>9</sup> The total number of awareness and educational meetings was 344 between 2004 and 2021 and the number of JP meetings per year increased from 5–6 in the first years to 63 in 2021.<sup>11</sup> In 2002, the number of PID patients registered by the ESID registry was less than 10 in most ECE countries (Lennart Hammarström, personal communication). In contrast, a recent survey suggested that the number of PID patients in 30 JP member countries exceeded 24 thousand by the end of 2021 (submitted). The JP meeting also promotes the organization of local PID professional working groups and patient groups, national PID registry, and establishing contact with institutional and governmental health care leadership.

The JP center at the Department of Infectious Diseases and Pediatric Immunology at the University of Debrecen provided molecular genetic service for patients from ECE countries, between 2004 and 2015, free of charge. From 2016, the JP genetic diagnostics program was continued at the Semmelweis University and the Rockefeller University. Altogether, genetic analysis in these three institutions was performed on 316 samples submitted from patients from 15 JP countries (Table 2). Part of the results of these studies has been published before.<sup>3,12–20</sup> Taken together, these data suggest that the contribution of the JP to better care of patients and the advancement of clinical research in the field of PID is outstanding and unique in Eurasia over the past two decades.

IRA			SH2				SRP		SLC		STAT3		TIN		TOP		22q11		SUM
MEFV	K4	NBN	NFκB	RAG1	RAG2	RMRP	SBDS	D1A	SPI1	54	39A7	STAT1	GOF	LOF	F2	2B	WAS	.2DS	
																	1		1
																			4
											5								5
	1	3	2		2	1	2	12	1	1		5	1	11	1	1	9	1	141
		3															1		9
																	2		3
											1		1						4
													1				1		13
		4															5		34
											2	8					8		36
																			1
																			1
																			2
																	3		3
1				2			1			1		3	4				9		59
1	1	10	2	2	2	1	3	12	1	2	1	15	15	11	1	1	39	1	316

## AUTHOR CONTRIBUTIONS

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

## KEYWORDS

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

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## CONFLICT OF INTEREST

The authors declare that they have no competing financial interests.

## DATA AVAILABILITY STATEMENT

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.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/pai.13805>.

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