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ORIGINAL ARTICLE

Molecular Characteristics, Clinical and Immunologic Manifestations of 11 Children with Omenn Syndrome in East Slavs (Russia, Belarus, Ukraine)

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Abstract
Background Omenn syndrome [Mendelian Inheritance (OMIM 603554)] is a genetic disease of the immune system, characterized by the presence of fatal generalized severe erythroderma, lymphoadenopathy, eosinophilia and profound immunodeficiency.
Objective We studied clinical and immunologic presentation of the disease manifestation among East Slavs population with genetically confirmed Omenn syndrome.
Results We collected clinical and immunologic data of 11 patients (1 from Belarus, 5 – Ukraine, 5 – Russia): 6 females, 5 males. The age of Omenn syndrome manifestation varied from the 1st day of life to 1 year and 1 month, the age of diagnosis – 20 days to 1 year and 10 months. Nine out of 11 patients had classic immunologic phenotype T(+/−)B-NK+, 1 pt had TlowB + NK+ with CD3 + TCRgd + expansion and 1 had TlowB+/−NK+ phenotype. Eight out of 11 pts had mutation in *RAG1* gene, 4 out of 8 had c.368-369delAA (p.K86fsX118) in homozygous state or heterozygous compound. In our cohort of patients, we also described two new mutations in *RAG* genes (p.E722Q in *RAG1* and p.M459R in *RAG2*). At present, 7/11 were transplanted and 5 out of the transplanted are alive.
Conclusion This study demonstrates that the most popular genetic abnormality in East Slavs children with Omenn syndrome is c.368-369delAA (p.K86fs118) in *RAG1* gene, which may be connected with more favorable prognosis because 4/4 patients survived after hematopoietic stem cells transplantation.

Keywords Omenn syndrome · *RAG1/2* genes · mutations · East Slavs children

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Introduction

Omenn syndrome (OS) is a rare inherited primary immunodeficiency characterized by severe combined immunodeficiency in combination with autoimmune features leading to squamous erythroderma, alopecia, lymphadenopathy, hepatosplenomegaly, and intractable diarrhea [1]. The syndrome was first described in 1965 by Gilbert Omenn, a student of Harvard Medical School. The disease was characterized by reticuloendotheliosis and eosinophilia and was observed in 12 patients in consanguineous marriage [2].

Typical OS is presented during the first year of life with chronic diarrhea, pneumonitis, failure to thrive, enlarged

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lymphoid tissue, generalized, exudative erythroderma. The protein loss through the skin (in addition to that, often due to chronic diarrhea) results in hypoproteinemia and generalized edema [3, 4]. Laboratory findings show high T cell counts with restricted clonality of T cell receptor (TCR) [3], which are predominantly of T helpers type 2 and infiltrate the skin, the gut, the liver, and the spleen [5]. These abnormal T cells have a predominantly activated phenotype (CD25+, HLA-DR+, CD45RO+) and secrete a big amount of cytokines that promote autoimmune and allergic inflammation in the absence of proper regulation by other components of the immune system [1, 3, 4]. The majority of patients had low amount or no B cells, which was accompanied by hypogammaglobulinemia. However, elevated serum IgE level with eosinophilia was frequently observed [1].

Moreover, some patients present some but not all signs consistent with OS. These patients are commonly referred to as affected with atypical OS [3]. Hypomorphic recombination activating genes 1 and 2 mutations lead to the defect in the lymphoid-specific *V(D)J* recombination process that was first described in patients with OS [6]. At present, defects in *Artemis* [7, 8], *IL7-R alpha* [9], *ADA* [10], *common gamma chain IL2-R* [11], *DNA Lig IV* [12], *CHD7* [13] genes were shown in the association with OS.

Considering the polygenetic basis of OS, the mutation finding may be time-consuming. There are some ethnic distributions linked with immunodeficiencies in different countries. For example, Nijmegen syndrome with deletion in *NBS1* gene is common in Slavic countries [14, 15].

Here we report a cohort of patients from Russia, Belarus and the Ukraine as East Slavic origin with clinical and immunologic features of OS with defined genetic basis.

Patients, Material and Methods

Ethics Statement

For all patients written informed consent for the performed studies and photo were obtained from the patients' families in accordance with the guidelines of the ethics committee of each country where a patient was diagnosed and treated.

Data Collection

Data about all genetically CONFIRMED patients with OS were collected among all clinical immunologists of Russia, Ukraine and Belarus. Clinical, immunologic and genetic data were collected from paper forms which were filled by clinical immunologists in Minsk (Belarus), Moscow, St. Petersburg (Russia), Kiev and Lvov (Ukraine). The following information was extracted from the clinical records: (i) **Personal data**: family history, age of onset, age of diagnosis, nationality; (ii) **Clinical data**: respiratory tract infections, lymphoproliferation (hepatosplenomegaly and lymphadenopathy), age of erythroderma (location and spreading), age of diarrhea (pathogen), vaccination; (iii) **Immunologic data** included absolute lymphocyte counts and counts of lymphocyte subsets, percentage of naïve CD45RA+ T cells among CD4+ T cells and/or recent thymic emigrants (RTE) and percentage of γ/δ T cells among CD3+ T cells, concentration of IgE at presentation of disease; serum concentration of IgG, IgM, IgA, IgE; (iv) **Genetic data:** gene, mutation.

Patients' age at the first reported significant infectious disease episode or the age of the first admission to hospital was noted as the age of onset.

Phenotyping of Peripheral Blood Lymphocytes

EDTA samples of venous PB obtained from the patients (№1, 9–11) were stained with the following monoclonal antibodies (MoAbs) at optimal concentrations against CD45, CD3, CD4, CD8, CD56, HLA-DR, CD19, CD45RA, CD45RO, CD31, TCRαβ, TCRγδ, CD27, IgD, IgM, CD21, CD38. Tregs were determined by CD4 + CD25++CD127-, recent thymic emigrants (CD4 + CD31 + CD45RA+), switched-memory B cells (CD19 + CD27 + IgD-), non-switched memory B-cells (CD19 + CD27 + IgD+), transitional B cells (CD19 + CD21-CD38+). B cells were analyzed on isolated mononuclear cells. The data were analyzed using CXP.analysis software, Kaluza Flow Cytometry Analysis v1.1 (Beckman Coulter).

Spectrotyping for the TCRV Beta Families

The analysis of the TCR Vβ repertoire of the lymphocytes by FACS (TCR Vβ Repertoire Kit; Beckman Coulter) was performed with cells costained for CD4–PC5 and CD8–APC-Alexa 750, CD3–APC (Beckman Coulter).

Genetic Analysis

DNA was extracted from blood samples using standard methods. *RAG1* and *RAG2* genes were amplified in several segments from coding DNA using specific primers. Primers were designed for the amplification of the *RAG* genes based on the sequences reported in databases (*RAG1*, M29474; *RAG2*, M94633). Sequencing was performed on purified polymerase chain reaction (PCR) products using the ABI Prism BigDye Terminator Cycle sequencing kit on an GeneticAnalyzer ABI 3130 automated sequencer (Hitachi, Japan), then aligning to reference sequence (NM_000448.2:c.). Ensembl Genome Browser (www.ensemble.org) was used for evaluating *RAG* variants.

Genetic analysis for pt_1, 9, 10, 11 was performed at the Belarusian Research Center for Pediatric Oncology,

Hematology and Immunology (Minsk, Belarus), pt_2, 8 at Erasmus Medical Center (Rotterdam, Netherlands), for pt_3 at INSERM U768, Hospital Necker (Paris, France), for pt_6, 7 at Pediatric Clinic (Brescia, Italy), for pt_4, 5 at the Federal Scientific and Clinical Centre of Pediatric Hematology, Oncology and Immunology named after Dmitry Rogachev (Moscow, Russia).

Statistical Analysis

Statistical analyses were performed for comparison of clinical data of patients (age of manifestation and diagnosis) and immunologic data (IgM, IgA, CD3+, CD19+, NK % and abs.) using GraphPad Prism version 5.0 (GraphPad Software Inc., SD). Differences between the groups were considered significant for $p < 0.05$, comparison data between the two groups were performed with Mann-Whitney U test. The data are presented as medians and whiskers (min - max).

Results

Eleven patients from 11 unrelated families with OS mostly with *RAG* defects are reported in this paper (Table 1). Four of them have been previously reported (patients 1, 2 in reference [16, 17]; patient 2 in 18; patient 9 in reference [17]; patient 6 in reference [18]). The family history was complicated in 4 patients (see Table 1).

On the basis of clinical presentation, age of onset (Table 1) and the immunologic data (Tables 2 and 3) of the patients were divided into 2 subgroups, 8 patients with classic OS and 3 – with atypical (late-onset) OS. In all patients, maternal fetal engraftment was excluded by HLA-typing and/or FISH (XX detection in male patients).

Eight patients manifested in neonatal period, 6/8 had marked erythroderma and 2/8 atopic dermatitis as skin manifestation, and 5 in combination with the respiratory tract lesions; 7/8 had bowel disease from the first days of life or during early neonatal period with lymphoproliferation (Pt_1 and Pt_2 in Fig. 1, Table 1).

Immunologic investigation in all classic OS patients showed a typical phenotype, absence of B cells, low to high amount of T cells, which was presented by CD45RO+ phenotype. In 5 patients, IgE was detected and in 4 out of 5 its level exceeded the upper limit of the normal range (normal range up to 15 IU/mL, Table 2).

Genetic basis of OS was mostly determined by mutations in *RAG1* gene (5/8), which were heterozygous compound, 2/8 – *RAG2* deficiency, 1 homozygous and 1 compound (p.Y434H was observed in 3 out of 4 alleles in two patients from Russia). One patient from the Ukraine had mutations in *Artemis* gene.

RAG mutations in humans associated with heterogeneous clinical phenotypes can lead to a variety of immunodeficiencies and disregulation ranging from severe combined immunodeficiency to OS and to milder immunodeficiencies [19–22].

We decided to separate the group of patients with late-onset OS with atypical clinical and immunologic manifestation (3 patients out of 11 manifested later than the age of 9 month) and to describe those patients in detail as they differ among themselves or have their own characteristics. All those patients had mutations in *RAG1* gene, the most common mutation was p.K86VfsX118, and 2 out of 3 had B cells in the peripheral blood (Tables 2 and 3).

The **first patient (Pt_9)** presented at the age of 11 months with some episodes of obstructive bronchitis and atopic dermatitis. At the age of 1 year and 1 month, he had enterocolitis, acute bilateral pneumonia with obstructive syndrome, relapse and progression of atopic dermatitis, oral candidiasis, neutropenia, eosinophilia in blood, no gain in body weight, psychomotor retardation.

The patient was admitted to our hospital with lymphopenia (L = 144 cells/μL), hepatomegaly (Fig. 2a), malabsorption syndrome (Fig. 2b), thymus hypoplasia revealed by ultrasound.

First immunologic investigation (Table 3) showed high percentage of B cells, but in two weeks very low level of B cells was revealed (CD19 + =2.6 %). Immunologic $T^{low}B^{low/-}NK^{+}$ was established, but in a month B cells appeared in the peripheral blood again CD19 + =14.4 %, with normal B cell memory distribution (Table 3).

Patient 9 was found to be compound heterozygote of p.R108X and p.R404Q mutations in *RAG1*. The patient inherited a nonsense mutation (p.R108X) from his mother who developed chronic myeloid leukemia at the age of 27, received the necessary treatment and is still alive.

At the age of 1 year and 9 months, the patient was transplanted with umbilical cord blood stem cells (matched 5/6, CD34–$2.0x10^5$ per kg, conditioning Flu, Treo, Campath 1). Since +30 day, autoreconstitution was established (no chimera) due to absence of engraftment. Toxic mycositis, hepatitis, pulmonary aspergillosis appeared in the posttransplantation period. Despite the full treatment, fungal complications did not resolve. The patient died in 5 months after transplantation due to the heart arrest and uncontrolled growth of fungi (postmortem photos – Fig. 2c, d).

The **second patient** from this group **(Pt_10)** was vaccinated (DTP + polio). BCG vaccination was postponed due to malnutrition. At the age of 1 month, BCG vaccination was performed. A month later, an abscess appeared at the place of injection, which transformed into an ulcer with purulent discharge and did not heal up. Izoniozid treatment was started.

Since 9 months of age, relapsing fever appeared, and at the age of 1 year and 1 month, transaminase elevation was

Table 1 Clinical and geographical manifestation of patients with Omenn syndrome

Patients	Gender	Place of birth	Age of onset	Age of diagnosis	Respiratory tract infections	Erythro-derma	Bowel disease	Hepatomegaly	Splenomegaly	Lympadenopathy	BCG vaccination/ complications	Family history
	Classical Omenn syndrome											
Pt_1	Female	Russia Moscow	1st day	2 week	Pneumonia	Yes, 1st day, total	Yes, 1 m.	yes	yes	yes	No vaccination	IV pregn., IV delivery, 3rd baby – SCID (died)
Pt_2	Male	Russia Vladivostok	1 m.	2 m.	no	Yes, 1 m. face (hair part)	no	no	no	no	No vaccination	III pregn., III delivery, 1 and 2 sisters – OS (died)
Pt_3	Male	Russia St. Petersburg	3 weeks	1,5 m.	Bronchitis	No, Atopic dermatitis	Yes, 1 month	yes	no	no	+/BCGosis	I pregnancy, I delivery
Pt_4	Male	Russia Samara	5th day	3 m.	Pneumonia	Yes, 1 m., total	Yes, 1 month	yes	yes	yes	+/no complications	I pregnancy, I delivery
Pt_5	Male	Russia Bashkotostan	1 m.	2 m.	no	No, Atopic dermatitis	Yes, 1 day	yes	yes	yes	No vaccination	IV pregn., IV delivery, 2nd baby – SCID (died)
Pt_6	Female	Ukraine Kiev	1st day	1 month	no	Yes, 2nd day, face	Yes, 11 days	yes	no	yes	No vaccination	I pregnancy, I delivery
Pt_7	Female	Ukraine Kalush	1 m.	6 m.	Broncho-pneumonia	Yes, 1 m.	Yes, 10 m.	yes	no	yes	+/BCGosis	I pregnancy, I delivery
Pt_8	Female	Ukraine Lviv	1st day	20 days	Inborn pneumonia	Yes, 1 day, total	Yes, 3d week	yes	yes	yes	No vaccination	II pregn., II delivery, 1st baby – OS (died)
	Late-onset Omenn syndrome (atypical)											
Pt_9	Male	Belarus Gomel region	11 m.	1 y. 2 m.	Bronchitis	No, Atopic dermatitis	yes	yes	no	no	+/no complications	I pregnancy, I delivery
Pt_10	Female	Ukraine Odessa	9 m.	1 y. 10 m.	yes	Yes, 1 yrs. 2 m.	Yes, 9 m	yes	yes	yes	+/BCGosis	I pregnancy, I delivery
Pt_11	Female	Ukraine Lisichansk	1 y. 1 m.	1 y. 9 m.	Pneumonia	Yes, 1 yrs. 2 m.	yes	yes	no	yes	+/no complications	I pregnancy, I delivery

Tables 2 Genetics and immunological characteristics of patients with Omenn syndrome

Patients	Immunophenotype	Gene	Mutations		CD3+		CD19+		NK		CD4 + CD45RA+	CD4 + CD45RO+	CD3 + TCRαβ+	CD3 + TCRγδ+	IgE, IU/mL
			Allele 1	Allele 2	%	cells/μL	%	cells/μL	%	cells/μL					
			Classical Omenn syndrome												
Pt_1	$T^-B^-NK^+$	*RAG1*	p.K866fsX118	p.E722Q	21	56	0.1	2	29.8	601	0 %	69.6 %	99.2 %	0.1 %	201.9
Pt_2	$T^+B^-NK^+$	*RAG1*	p.K866fsX118	p.R559S	79	4330	0.2	10	17	930	n.d.	n.d.	n.d.	n.d.	n.d.
Pt_3	$T^+B^-NK^+$	*RAG2*	p.Y434H	p.M459R	72	11,612	0	0	26	4193	n.d.	n.d.	n.d.	n.d.	n.d.
Pt_4	$T^{low}B^-NK^+$	*RAG1*	p.R561C	p.M977fsX991	88	373	0	0	7.7	33	n.d.	n.d.	n.d.	n.d.	4377.9
Pt_5	$T^+B^-NK^+$	*RAG2*	p.Y434H	p.Y434H	64	2603	0,1	4	35.1	1441	n.d.	n.d.	n.d.	n.d.	12.5
Pt_6	$T^+B^-NK^+$	*RAG1*	p.A444V	p.R624H	8.3	16,933	0.03	5	9.16	1737	0.35 %	83.2 %	n.d.	n.d.	2000
Pt_7	$T^{low}B^-NK^+$	*RAG1*	p.R829S	p.P901X p.K830X	33	546	2	33	49	645	0.3 %	21 %	93.8 %	5 %	2500
Pt_8	$T^+B^-NK^+$	*Artemis*	p.I14T	Del1-4 ex.	69	6066	0	0	11	967	0.1 %	13.8 %	81 %	7.8 %	n.d.
			Late-onset Omenn syndrome (atypical)												
Pt_9	$T^+B^+NK^+$	*RAG1*	p.R108X	p.R404Q	13.3	19	23.5	34	64.2	92	4.6 %	73.3 %	97.2 %	2.8 %	n.d.
Pt_10	$T^+B^+NK^+$	*RAG1*	p.K866fsX118	p.K866fsX118	92.4	3068	5	166	2.1	70	0.3 %	29.1 %	60.9 %	39 %	55,000
Pt_11	$T^-B^-NK^+$	*RAG1*	p.K866fsX118	p.A444V	25,9	47	0	0	66.5	122	0.2 %	16.8 %	92.1 %	7.1 %	<0.1

revealed (Fig. 3a). As a result, rash on the abdomen and legs with urticaria elements developed. Subtotal appendectomy and ileum resection was performed due to necrosis. At 1 year and 10 months, the female had a body weight of 7.2 kg (Fig. 3b). Generalized lymphadenopathy and hepatosplenomegaly, catarrhal proctosigmoiditis complicated by severe anemia (Hb = 15 g/L), active CMV infection, BCGosis, and primary hypoparathyroidism were diagnosed. The heart arrest required cardiopulmonary resuscitation (CPR) and red blood cells transfusion.

Immunologic investigation at the age of 1 yr. and 10 m. (Table 3) revealed the presence of B cells in the peripheral blood and an extremely high level of IgE. Immunosuppressive therapy with metilprednisolone (8 mg) decreased the IgE level to 2296 IU/L in a month. The second immunologic investigation performed on immunosuppression revealed normal number of B cells (Table 3) with expansion of CD3 + TCRgd + (Table 2).

Phenotype of B cells in the peripheral blood was characterized by heightened percentage of switched memory B cells, normal range of non-switched with expansion of transitional B cells = 31 %.

At the age of 2 yrs., total colitis, serous peritonitis, pneumoperitoneum, pneumomezoterium with multiple microperforation of the colon were established (Fig. 3c, d). In 3 months, suppurative left lymphadenitis with DNA of mycobacterium was removed surgically. Mycobacterium treatment (isoniazid and rifampin) was added to immunosuppressive therapy, as well as antibiotics, IVIg and anti-viral treatment.

Heightened IgE level and abnormality in TCRVβ clonality, in spite of normal number of B cells, were the reason for *RAG1* and *RAG2* sequencing. Genetic investigation revealed homozygous c.368-369delAA mutation in *RAG1* gene. The parents were the carriers of the same heterozygous mutation but were not in consanguineous marriage.

At the age of 2 years 8 months, the patient underwent allogeneic stem cell transplantation (SCT) with a mismatched graft and received mycobacterium therapy after SCT. Now she is in a good condition (Fig. 3f).

Patient 11, female. Up to 1 year she was absolutely healthy and demonstrated normal weight gain. At the age of 1 year and 1 month, she was vaccinated against measles, mumps and rubella (MMR). On the same day, redness appeared at the site of injection, three days later – diarrhea, and on the seventh day after inoculation the girl was hospitalized with a diagnosis of acute respiratory viral infection, and intestinal syndrome. In two weeks, she developed purulent conjunctivitis, and rhinitis. At the age of 1 year and 3 months, upper (acute laryngitis, purulent etmoiditis) and lower (pneumonias) respiratory infection was diagnosed. At the same time, erythema on the cheeks and rash all over the body were seen.

Patient 11 was admitted to our hospital at the age of 1 year and 9 months. Immunologic investigation showed classic OS

Table 3 Immunological investigation for patients with atypical Omenn syndrome

	Patient_9		Patient_10		Patient_11	Normal range for children 1–3 years
	Age_1 yr. 2 m.	Age_1 yr. 3 m.	Age_1 yr. 10 m.	Age_1 yr. 11 m	Age_1 yr. 9 m.	
% CD3+ (cells/μL)	13.3 (19)	15.8 (102)	92.4 (3068)	71.9 (2200)	25.9 (48)	57 to 76 %, Median 64.4
% CD4+ (cells/μL)	11.2 (16)	13.8 (84)	67.3 (2234)	21.6 (660)	16.9 (31)	30 to 50 %, Median 42.4
% CD8+ (cells/μL)	2.7 (4)	4.1 (25)	13.3 (442)	25.7 (790)	8.5 (16)	14 to 33 %, Median 23.1
% CD19+ (cells/μL)	23.5 (34)	14.4 (91)	5 (166)	17.6 (540)	0	16 to 23 %, Median 22.7
% CD3 + DR+ (cells/μL)		5.9 (36)	71.6 (2377)	47.2 (1450)	16.1 (30)	1 to 10 %, Median 3.3
% CD3-CD16 + CD56+ (cells/μL)	64.2 (92)	69.7 (424)	2.1 (70)	7.6 (230)	66.5 (122)	5 to 21 %, Median 9.1
% RTE	3	0.9	0.45	0.1	0.3	59 to 87 %, Median 70.3
% Tregs	10.8	12.8	0.8	1.1	0.9	4 to 10 %, Median 8.5
% Switched memory B cells	n.d.	17.8	25.3	27	0	2 to 11 %, Median 4.8
% Non-switched memory B cells	n.d.	4.6	8.95	6.94	0	1 to 10 %, Median 7.6
IgG, g/L	3.62	n.d.	11.8 (IVIg+)	n.d.	2.12	
IgM, g/L	0.45	n.d.	1.86	n.d.	0.46	
IgA, g/L	0.16	n.d.	0.72	n.d.	0.39	
TCR Vβ clonality	n.d.		Increased expression of Vβ9, Vβ18 among CD8+ T cells (Fig. 3g), decreased expression of Vβ5.2, Vβ11, Vβ22 in CD4+ and CD8+ and Vβ12, Vβ14 in CD8+		slight abnormalities in CD8+ T cells but without clonal populations	

immunophenotype (T + B-NK+) but with the normal level of IgE (Table 2). TCR clonality revealed slight abnormalities in CD8+ T cells but without clonal populations. Because of the increased frequency of c.368-369delAA in *RAG1* gene in our population, we have sequenced the 2nd fragment of this gene first and OS was confirmed genetically by finding p.K86Vfs118 and p.A444V. Those mutations were described in literature more than once [18, 23, 24], and we described the same compound as atypical severe combined immunodeficiency in a 14 year-old male [25].

Immunosuppressive therapy with cyclosporin A was started to correct autoimmune manifestations in addition to standard immunodeficiency treatment. Four months after admission, the patient underwent allogenic stem cell

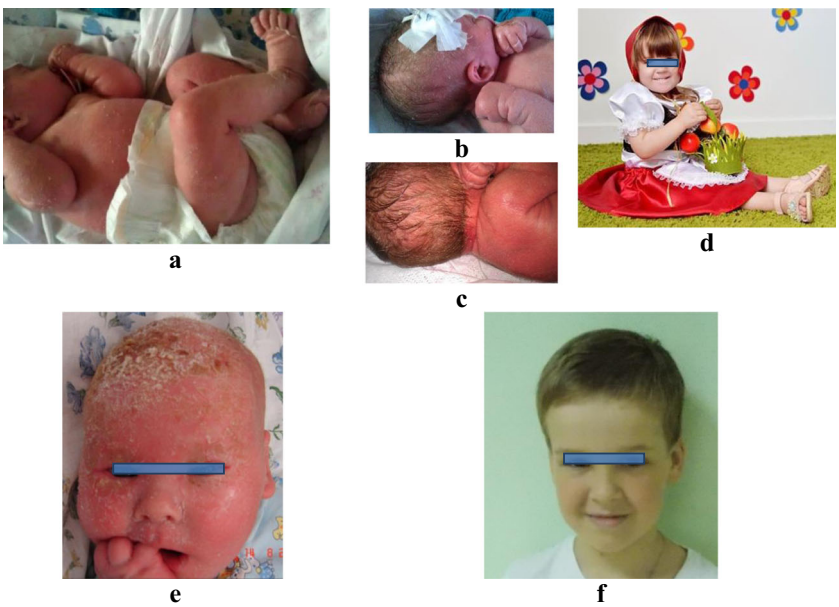

Fig 1 **a, b, c** Pt_1, female, at the 20th day of life, at the day of diagnosis. **d** Pt_1 after allo-HSCT **e** Pt_2 at the age of 4 month **f** Pt_2 at the age of 5 yrs. 8 month after HSCT

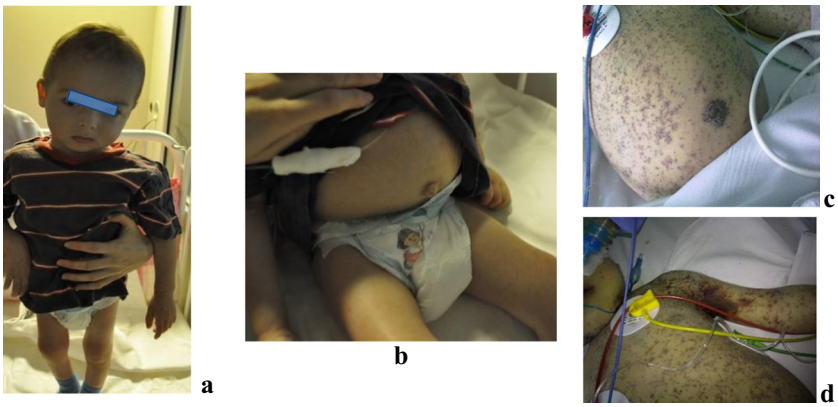

Fig 2 **a.** Patient 9 at the age of 1 year 8 months; **b** big abdomen of Pt_9 due to intestinal loops filled with liquid contents; **c** and **d** postmortem photos of Pt_9

transplantation with a full matched unrelated bone marrow. On day +8, the patient developed hyperacute GVHD, which was resolved with medrol during cardiopulmonary resuscitation. She was in a good condition on day +100 posttransplant, full chimera, no complications (Fig. 4b).

Since +90 day, overall chimerism began to fall and at the day of +160, GVHD appeared again, a skin form with liver involvement (high LDG, no viruses). Escalation of immunosuppression was accompanied by the reduction in the patient's movement.

During 3 months after the second GVHD, neurological symptoms progressively worsened (mixed hydrocephalus of brain, complete loss of movement and speech, hypersensitivity of skin).

At the day of +240, on the background of the multiple organ failure progression during the 5 days, the patient had 3 episodes of the heart arrest, which required CPR. After the last episode of the heart arrest, 10 g of IVIg were added to treatment and on the background of continuing bradycardia and decreased cardiac output anuria appeared (CVVHD was started, in 8 days stopped). Peripheral blood chimerism studies through short tandem repeat testing ultimately showed 30 % to be of donor origin, but donor chimerism in sorted CD3+ T cells was 88.5 % and in CD19+ B cells – 95 %. The patient's condition was diagnosed as systemic vasculitis (autoimmune origin: cardidtis, nephritis, polyserositis, toxic encephalopolyneuropathy, tetraparesis) due to split chimerism. Increased immunosuppression caused deterioration of the condition (destabilization of

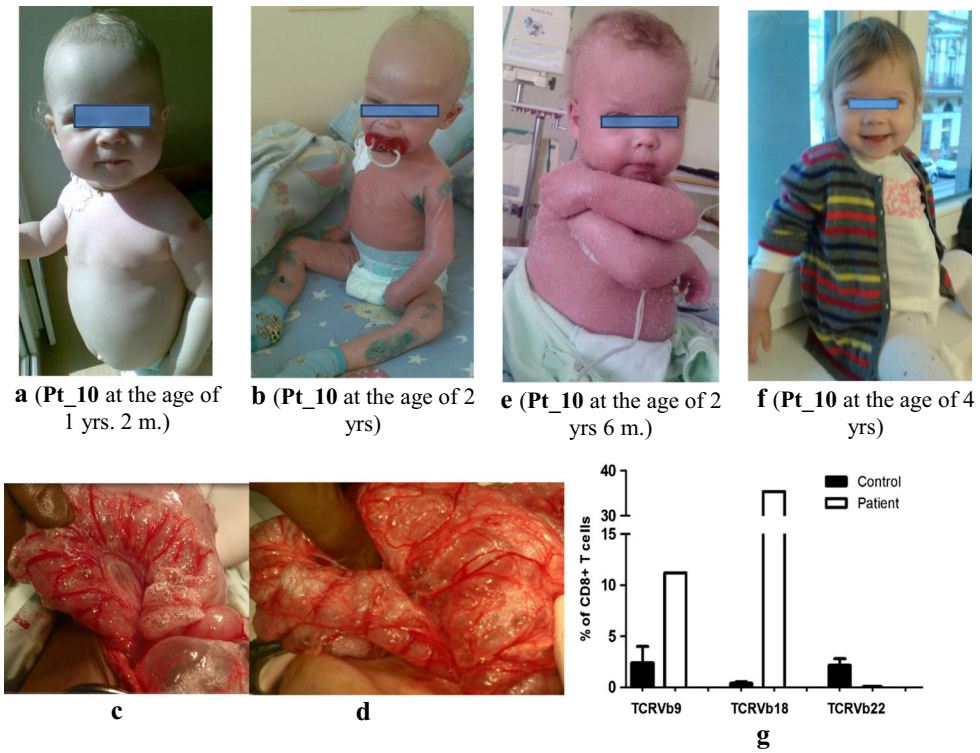

Fig 3 **a, b, e, f** Pt_10 at different age. **c** and **d** transanal bowel intubation was performed for Pt_10. It revealed diffuse imbibitions of small and medium-sized gas bubbles throughout the wall of the colon. Anaerobic total colitis, serous peritonitis, pneumoperitoneum, pneumomezoterium with multiple micro-perforation of the colon was established. *Klebsiella oxytocae* from wash water of the abdominal cavity was isolated. **g** Evaluation of T-cell receptor beta-chain usage shows two prominent clonal populations having expression of VB9 and VB18 that exceed the reference range for these isoforms

a (**Pt_10** at the age of 1 yrs. 2 m.) **b** (**Pt_10** at the age of 2 yrs) **e** (**Pt_10** at the age of 2 yrs 6 m.) **f** (**Pt_10** at the age of 4 yrs)

c **d** **g**

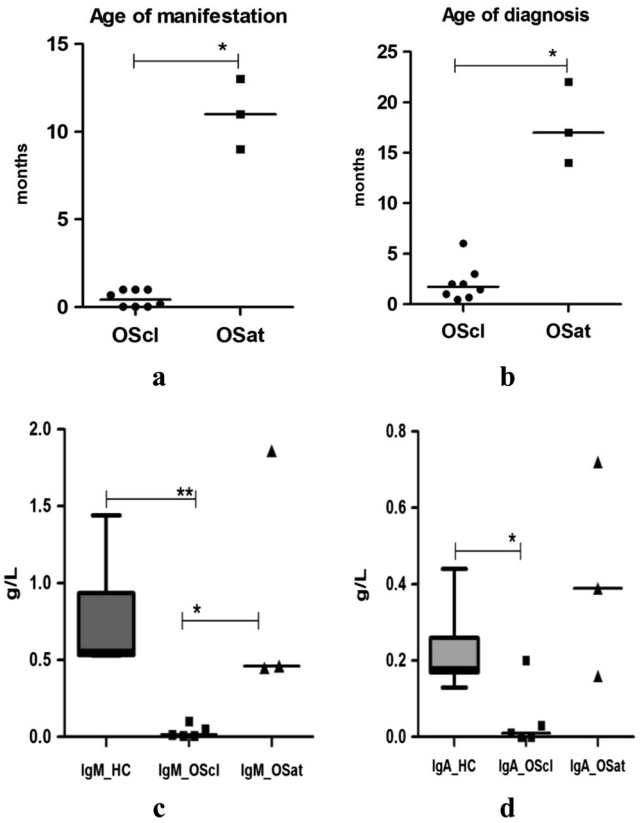

Fig 4 **a, b** Comparison of age of manifestation and age of diagnosis in patients with classic and atypical Omenn syndrome with Mann-Whitney U test**. c, d** concentration of IgM and IgA in patients with classic (n=5) and atypical Omenn syndrome (n=3) and healthy control group at the age from 2 weeks to 2 years (n=9)

hemodynamics, development of lactic acidosis, hyperglycemia). Reduction in the dose of immunosuppressive drugs and continuation of the therapy with high dose of IVIg was the reason of slow positive dynamics.

At the day of +330, the patient's condition was stable. Neurological symptoms slowly resolved, speech and movement in the arms and legs returned, but tachycardia continued.

Clinical and Immunologic Differences Between Classic and Atypical Omenn Syndrome

Classical and atypical clinical presentation of OS was observed in 8:3, respectively. Despite the age of OS manifestation, the full group of patients was presented with infections syndrome in 73 % (8/11 patients had pneumonias and bronchitis as manifestation). Autoimmune and inflammatory component was confirmed by erythroderma (8/11) and atopic dermatitis (3/11) (Table 1).

Patients with atypical form of OS were characterized by a significantly later age of manifestation and diagnosis (p = 0.017, p = 0.019; Fig. 4a, b).

The comparison of immunologic parameters between the two groups of patients showed significant differences in the level of IgA and IgM in patients with atypical OS at the time of diagnosis, while children with classic OS were characterized by a total absence of IgM (p = 0.003 as compared with healthy controls and p = 0.04 with atypical form of OS) and IgA (p = 0.02 as compared with healthy controls).

Outcomes General mortality was 6 out of 11 (54 %), in our classic group 62.5 % (5 of the 8). Five out of 8 patients were transplanted. However, the survival rate after transplantation was 60 % (3/5 all patients from Russia). Among 8 patients, 5 died due to GVHD gastrointestinal form after allo-HSCT (Pt_3); multiorgan failure, CMV-viremia, secondary hemophagocytic syndrome (Pt_4); hepatic failure (Pt_6); progressive failure of CNS, BCGosis (Pt_7); sepsis, respiratory failure after allo-HSCT (Pt_8). Among 5 patients who died, 3 were vaccinated with BCG (Pt_3, Pt_4, and Pt_7) probably due to later age of manifestation (Table 1). Five patients were not vaccinated due to erythroderma that appeared from the first days of life or positive family history, with the mortality being 2 out of 5.

Discussion

We are the first to describe a cohort of patients of the same ethnic origin (East Slavs: Belarusians, Ukrainians and Russians) and report clinical, immunologic and genetic characteristics of 11 OS patients.

Nine out 11 patients from East Slavs cohort had classic clinical and immunologic manifestations, and we decided to analyze this group as a separate one (OScl). In the present study, we have collected a small group of patients with mutations in *RAG1* gene accompanying late and atypical presentation of OS (OSat, n = 3), but allowed for statistical analysis between the two groups. We were able to show that patients with atypical OS had significantly later age of onset and the age of diagnosis. Clinical and immunologic data of the most significant cohort of patients with atypical *RAG* mutations were published in 2011, but 18 patients with *RAG* mutations were analyzed in one group together with *LIGIV* and *Artemis* defects ($T^{low}B^{low}$ atypical severe combined immunodeficiency (SCID)) [26]. However, limitation of all cohort studies is the absence of individual clinical histories, and we decided to describe our three cases in detail.

Classic OS is characterized by the absence of B cell [1, 3]. All our patients from OScl had classic immunologic phenotype $T^{low/+/-}B^{-}$ and low level of IgG, IgM and IgA and it was comparable to that described in previous studies [3, 23]. As was shown previously [26], significant B cell numbers were documented in "B minus" atypical SCID variants. In our work, B cells were presented in the peripheral blood of 2 patients with atypical OS with normal memory distribution. Moreover, to establish the immunologic phenotype for patients with atypical OS may be not very easy. Repeated

immunologic investigation for Pt_9 and Pt_10 in a short period showed different percentage and absolute numbers of B cells in blood (Table 3). Antibody deficiency is an important feature of OS, but not a regular sign of "atypical" OS. IgM and IgA level did not differ from healthy children in our cohort, that is also consistent with previous studies [26].

One in every 2 BCG-vaccinated patients with SCID had BCG-associated manifestations, two thirds in the form of disseminated complications (≈33,000-fold increase as compared with the general population) and another third in the form of localized complications (≈400-fold increase) [27]. In our cohort of OS patients, 6 patients were BCG vaccinated (3 with typical OS and atypical - 3), 3 of them developed complications. Moreover, in the group of classic OS, 2/3 vaccinated patients had complications of vaccination and in the group of atypical Omenn only 1/3. All vaccinated patients had no family history of immunodeficiency (Table 1). There is an interesting clinical manifestation of the immunodeficiency after the vaccination against measles, mumps and rubella (MMR) in Pt_11 who was completely healthy till the age of 1 year and 1 month. Recent published data showed that vaccine rubella strain may persist in patients with PID for several years and may develop cutaneous granuloma [28]. This phenomenon of systemic vasculitis with severe neurological involvement in Pt_11 after HSCT is probably due to vaccination against rubella or complication due to split chimerism, but to prove the first version does not seem possible.

Among the Slavs, close marriages are rare, but autosomal recessive diseases of the immune system are found and are often caused by heterozygous compounds, OS is among such diseases. Nine out of 11 OS patients had heterozygous compound, two patients presented homozygous mutations (Table 2), but the parents denied consanguineous marriage.

The mutation p.K86Vfs118 in *RAG1* gene and p.Y434H in *RAG2* gene were the most common mutations in the East Slavs cohort of patients with OS. The mutation p.Y434H was present at a high prevalence in *RAG2* gene (3/4 of the mutated alleles in this group of patients, in Pt_3 and Pt_5). Moreover, p.M459R in *RAG2* in Pt_3 and p.E722Q in *RAG1* gene in Pt_1 have never been described before.

Among 11 East Slavs patients, 8 children had mutation in *RAG1* gene and the dominant p.K86VfsX118 mutation was observed (4/8) both in patients with classic OS and in atypical forms. Unfortunately, in this study we have no opportunity to study residual RAG protein activity in our two groups of patients. However, previous attempts to identify the reason of the different clinical phenotype due to the same mutation were not successful in *RAG* gene. Moreover, 18/22 patients had p.K86Vfs118 in hetero or homozygous state [18].

This mutation (p.K86Vfs118) was described earlier many times in patients with OS, atypical SCID [23, 24] of the European and American origin. Prevalence of p.K86VfsX118 mutation was described in patients from Serbia and Montenegro, Poland, Czech Republic, The Netherlands [18, 29, 30], but had never been met in Iranian [31], Israeli [32], Chinese and Southeast Asian [33, 34] and Japanese [35] cohort of patients with OS/SCID due to *RAG1* mutations.

As a result of analysis of places of birth of patients' parents with p.K86VfsX118 mutation, we determined that almost all carriers were born in Belarus (fathers of Pt_1, Pt_2 and Pt_11 were born in different regions of Belarus). Further studies are needed to investigate a possible link in the local genetic background in each Slavic country for establishing ethnically fixed mutations.

Usually carriers have no signs of a disease, but in our cohort of carriers, mother of Pt_9 (p.R108X) had developed chronic myeloid leukemia at the age of 27. It was described in literature that an adult-onset idiopathic T-cell lymphopenia due to p.K86VfsX118 in heterogeneous state in an HIV-negative male patient with no recurrent or opportunistic infections presented at the age of 38 years with chronic dermatitis, pruritus, and hyperkeratosis [36].

Survival rate of patients with p.K86Vfs118 was much higher than in patients with other types of mutations in *RAG1* gene and other genes in East Slavs OS population (4/4 patients are alive after HSCT).

The clinical outcome of patients with RAG deficiency with similar mutations is extremely difficult to predict. Determining the genetic basis of a diagnosis is necessary not only to confirm a clinical diagnosis and possibility of prenatal diagnosis in a family, but also to reveal the ethnically fixed mutations that reduce the time for diagnosis establishing.

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Compliance with Ethical Standards

Conflict of Interest None for any author

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