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Low-frequency variants in a cohort of Russian primary immunodeficiency patients

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Motivation and Aim: Primary immunodeficiencies (PIDs) represent a heterogeneous group of inborn diseases varying by severity, manifestation age, clinical features, and genetic background [1]. In Russia, the mutational landscape of PID patients, especially of patients with sporadic immunodeficiencies, is understudied [2]. In this work, we conducted whole-genome sequencing (WGS) of a Russian PID cohort, classified genetic variants by clinical significance, and evaluated the percentage of novel and known pathogenic mutations. As a result, known genes enriched with rare mutations as well as several candidate genes were revealed.

Methods and Algorithms: DNA was extracted from blood samples provided by Saint-Petersburg Pasteur Institute Medical Centre and sequenced on an DNBSEQ-T7 MGI instrument. Sequence short reads were mapped to the human reference genome (hg38) using BWA. Variant calling was performed using DeepVariant [3] and SNP annotation with VEP (using CADD, Polyphen, SpliceAI etc plugins) [4]. The American College of Medical Genetics and Genomics (ACMG) guidelines were used to categorize variants [5]. The study group included 140 patients falling into several groups of diagnoses: Common variable immunodeficiency (CVID), combined immunodeficiency (CID), Bruton agammaglobulinemia (XLA), Selective IgA deficiency (sIgAD), Transient Hypogammaglobulinemia of Infancy (THI), Specific antibody deficiency, Lymphoproliferative syndrome, C1-INH-HAE, Unspecified immunodeficiency, etc. Informed consents were obtained for all the patients in accordance with the ethical standards.

Results: Germinal genetic variants in a panel of 620 genes associated with PID and other hereditary pathologies were selected in 140 patients. To reveal primarily monogenic cases of PID, we concentrated on truncating genetic changes (nonsense, frameshifts), missense mutations, and annotated splice sites in these genes. Variants with different levels of confidence were proposed based on clinical data. We concentrated on rare variants with population frequency in gnomAD and Russian population cohort of the "Biotech campus" LLC database of < 1 %. Most of detected rare variants were classified as variants of uncertain significance (VUS). 11 previously known pathogenic variants and 21 new likely pathogenic variants were identified (Table 1).

Table 1. Preliminary diagnoses and genes in which likely causal variants have been found

Preliminary diagnoses	Novel likely pathogenic variants	Known pathogenic/likely pathogenic variants
CVID	*NFKB1*, *SOCS1*, *PLCG2*	
Unspecified immunodeficiency	*SBDS*, *CD8A*	*SBDS*
CID	*TLR3*	*AIRE*, *CARD11*
C1-INH-HAE, type 1	*SERPING1*	*SERPING1*
XLA	*BTK*	*BTK*
Lymphoproliferative syndrome	*CTLA4*, *FAS*, *CARD11*	*GATA2*
Immune dysregulation	*RIPK1*	
Ataxia	*ATM*	*ATM*

Possible genetic causes of the disease were found in 34 patients. Most of them represented familial cases. In one patient with "unspecified immunodeficiency" (novel and known, presumably, in compound) mutations responsible for the Shwachman-Diamond syndrome were identified [6]. Another interesting case was a mutation in CD8A that was previously considered a VUS. In this patient, marked decrease in CD8+ T cells was observed, thereby indicating that this mutation could be pathogenic [7]. Analysis of genomic coding regions in 30 sporadic cases of CVID revealed disease-causing mutations in known genes in 4 (13 %) patients. The relatively low proportion of monogenic findings can suggest oligogenic model of inheritance in most cases [8]. We therefore considered the enrichment of rare variants in our genes panel. Several novel variants in *MAPK8* were detected, consistent with its role as a PID candidate gene [9].

Conclusion: This study highlights the potential of whole-genome sequencing to expand identification of novel variants and genes involved in PID. We evaluated frequencies of rare variants in a heterogenic cohort of Russian patients with immune system disorders, categorized them by genes, functionally predicted impacts, and clinical significance. Identified variants can be good candidates for future functional studies to establish their role in pathogenesis.

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