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INTER-POPULATION COMPARISONS AND THE IMPORTANCE IN INFECTIOUS DISEASES OF THE *IRF7, TBK1, IFNAR1, IFNAR2* AND *TLR3* GENE VARIANTS IN TURKISH INDIVIDUALS

TÜRK POPÜLASYONUNDA *IRF7, TBK1, IFNAR1, IFNAR2* VE *TLR3* GEN VARYANTLARININ POPÜLASYONLAR ARASI KARŞILAŞTIRMALARI VE ENFEKSİYON HASTALIKLARINDAKİ ÖNEMİ

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ABSTRACT

Objective: In the research conducted during the pandemic period, it has been determined that *IRF7, TBK1, IFNAR1, IFNAR2,* and *TLR3* immunity genes play an important role in the predisposition to SARS-CoV-2 infection. However, there is no information about variants of these genes in the Turkish population. The aim of this study was to determine the variants specific to the our study's population in these genes that predispose to infections and to compare them with other populations.

Materials and Methods: The variants in the exonic and flanking intronic regions of these five genes were analysed in *in-house* whole-exome sequencing data of 139 unrelated non-anonymous individuals. The allele frequencies of variants were compared with other population datasets. The DysGeNet database was used to determine human diseases associated with these genes.

Results: In our population, gene variants were detected including 28 in *IRF7*, 16 in *TBK1*, 18 in *IFNAR1*, 19 in *IFNAR2*, and 9 in *TLR3*. The allele frequencies of variants were compared with other populations. Of these variants, 9 were determined to be novel, previously unreported variants. It was shown that these genes are mainly involved in cancer and infectious diseases, especially viral infections according to the DisGeNET database.

ÖZET

Amaç: Halen devam etmekte olan pandemi sürecinde yapılan araştırmalarda, *IRF7, TBK1, IFNAR1, IFNAR2* ve *TLR3* immünite genlerinin SARS-CoV-2 enfeksiyona yatkınlıkta önemli rol oynadıkları belirlenmiştir. Ancak, Türk popülasyonunda bu genlerdeki varyantlar ile ilgili detaylı bilgi bulunmamaktadır. Bu çalışmada, enfeksiyonlara yatkınlık oluşturan bu genlerdeki toplumumuza özgü varyantların belirlenmesi ve diğer popülasyonlarla karşılaştırılması amaçlandı.

Gereç ve Yöntem: *IRF7, TBK1, IFNAR1, IFNAR2* ve *TLR3* genlerindeki ekzonik ve komşu intronik bölgelerdeki gen varyantları, 139 anonim bireye ait kurum içi tüm ekzom dizileme verilerinde analiz edildi. Varyantların allel sıklıkları, diğer popülasyonların veri setleri ile karşılaştırıldı. Ek olarak, literatürdeki bu 5 aday gen ile ilişkili hastalıkları belirlemek için DisGeNET veri tabanı kullanıldı.

Bulgular: Toplumumuzdaki immünite gen varyantları belirlenerek allel sıklıkları diğer popülasyonlar ile karşılaştırıldı. Buna göre *IRF7* geninde 28, *TBK1*'de 16, *IFNAR1*'de 18, *IFNAR2*'de 19, *TLR3*'de 9 varyant tespit edildi. Bu varyantlardan dokuzunun daha önce bildirilmemiş yeni varyant oldukları belirlendi. DisGeNET veri tabanına göre, bu genlerin çoğunlukla kanser ve enfeksiyon hastalıklarında özellikle viral enfeksiyonlarla ilgili oldukları gösterildi.

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Conclusion: The determination of immunity gene variants specific to our population and the variability of allele frequencies among populations suggest that it may cause differences in immune response, especially to SARS-CoV-2 infection. In this study, preliminary information was obtained for studies that will investigate the relationship between the clinical manifestations of infectious diseases and immunity gene variants.

Keywords: Infectious diseases, population, immunity genes, variant

Sonuç: Toplumuza özgü immünite gen varyantlarının belirlenmesi ve popülasyonlar arasında allel sıklıklarının değişkenlik göstermesi, özellikle SARS-CoV-2 enfeksiyonuna immün yanıtta farklılıklara sebep olabileceğini düşündürmektedir. Bu çalışmada, enfeksiyon hastalıklarının klinik bulguları ile immünite gen varyantları arasındaki ilişkiyi araştıracak çalışmalar için önbilgiler elde edilmiştir.

Anahtar Kelimeler: Enfeksiyon hastalıkları, popülasyon, immünite genleri, varyant

INTRODUCTION

Coronaviruses belong to enveloped, single-stranded, non-segmented, positive-sense RNA virus groups that infect a wide variety of host species including humans and several other vertebrates (1, 2). There are four types of coronavirus according to their α, β, γ, and δ genomic structures, and α and β coronaviruses only infect mammals. Human coronaviruses (HCoVs) 229E and NL63, which are of the α coronavirus strain, are responsible for the common cold and croup, which are upper respiratory tract infections. Severe Acute Respiratory Syndrome (SARS), which was reported in Guangdong province of China in 2002, and Middle East Respiratory Syndrome (MERS), which was first diagnosed in Saudi Arabia in 2012, are in beta coronavirus class that causes severe respiratory disease (3). A novel coronavirus which first appeared in Wuhan, China in late 2019 has been defined as SARS-CoV-2 due to its similarity to Acute Respiratory Syndrome Coronavirus (SARS-CoV) by the Chinese Centre for Disease Control and Prevention. The World Health Organization (WHO) has named the disease caused by this virus COVID-19 (4). This coronavirus is highly contagious and causes not only serious respiratory infections but also multi-organ involvement (5). Epidemiological studies show that especially in the elderly, individuals with existing chronic diseases such as high blood pressure, cardiovascular disease, respiratory disease, cancer, or diabetes are at greater risk. However, the symptoms and the course of the disease differ in each individual (6). Recent research suggest that the genetic background of the clinical diversity may result from immune gene variants (7).

The innate immune response forms against viral infections through various mechanisms, which are regulated by interferon immunity genes (7, 8). Interferon regulatory factor 7 *(IRF7)* (7, 9), TANK binding kinase 1 *(TBK1)*, interferon alpha and beta receptor subunit 1 *(IFNAR1)*, interferon alpha and beta receptor subunit 2 *(IFNAR2)*, and toll-like receptor 3 (*TLR3*) genes are involved in the cellular response to viral infections including SARS-CoV-2 (7-14). While *TLR3* and *TBK1* genes are associated with the interferon induction pathway, it has been stated that *IRF7*, *IFNAR1*, and *IFNAR2* genes are involved in the interferon amplification pathway (7). Recent research studies have shown that *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*, and *TLR3* genes, which are important underlying genetic factors of influenza pneumonia, also play a role in the pathogenesis of COVID-19 (7, 8). It has been suggested that the reason for the clinical table of two different dimensions, seen as asymptomatic/mild and life-threatening in SARS-CoV-2 infection, may be related to genetic variants of interferon immunity genes (7). In addition, it is reported that *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*, and *TLR3* genes variants, whose allele frequencies vary between different population groups, may lead to significant differences in the clinical course of the disease by suppressing the immune response to SARS-CoV-2 infection (7). The aim of this preliminary population study was to identify the *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*, and *TLR3* gene variants, which will provide information to future studies related to viral infections.

MATERIAL AND METHODS

Study participants

The whole-exome sequencing (WES) datasets of 139 unrelated non-anonymous individuals were analysed in this study. The informed consent was obtained from all participants and all procedures were approved by the ethics committee of the Istanbul Faculty of Medicine Clinical Research Ethics Committee, Istanbul University (Date: 28.01.2021, No: 52402).

Determination of gene variants

Whole-exome sequencing was performed on peripheral blood DNA from 139 unrelated non-anonymous individuals, in our *in-house* WES dataset called MGexome (Medical Genetics Exome). The MGexome dataset was created from whole exome data of the patient sequenced with the solo approach or the parents who have no kinship relationship sequenced with the trio approach in groups without serious paediatric disorders. The clinical data and infection histories of these individuals who randomly applied to our department are unknown, and the study group is similar to the unrelated individuals of the gnomAD population datasets in which allele frequencies were compared.

The variants of *IRF7* (NM_004031.4), *TBK1* (NM_013254.4), *IFNAR1* (NM_000629.3), *IFNAR2* (NM_207585.2) and *TLR3* (NM_003265.3) genes were screened in MGexome. The variant analyses were performed on files in variant call format (vcf) obtained from sequencing data sets using the DNA scan analysis pipeline of the Illumina HiSeq system and the IonReporter analysis pipeline of S5 Ion Torrent system. With a sequencing run yield of approximately 10 Gb, the samples generally achieved coverage of >97% of the targeted exome bases covered at >20X depth.

The information of variants in five genes of interferon immunity were determined using VarSome (UniProt, ClinVar, and PubMed), dbSNP, and their pathogenicity interpretations and conservation scores were classified according to the American College of Medical Genetics and Genomics-ACMG Standards and VarSome (https://varsome.com/) database using *in silico* tools such as MutationTaster, DANN, SIFT, PROVEAN and GERP (Genomic Evolutionary Rate Profiling) (15). The minimum allele frequencies of defined variants in MGexome were compared in African/African American, Ashkenazi Jewish, East Asian, European, Latino/American Admixed, and South Asian populations within the public Genome Aggregation Database (gnomAD) as in our previous study (16).

Investigating gene association with human diseases

Association of the *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2,* and *TLR3* genes with human diseases was investigated with a different complementary approach. This analysis was carried out via the DisGeNET database (https://www.disgenet.org), a large genes collection involved in human diseases (17).

RESULTS

A total of 80 single nucleotide variants (SNVs) and 10 small insertions/deletions (InDels) in the exonic and flanking intronic regions of the *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2,* and *TLR3* genes were detected in MGexome data of 139 Turkish individuals. The 28 variants in *IRF7*, 16 variants in *TBK1,* 18 variants in *IFNAR1*, 19 variants in *IFNAR2,* and 9 variants in *TLR3* gene were determined and their allele frequencies compared to different populations. ACMG classifications and allele frequencies comparisons of all variants detected in the study population for *IRF7, TBK1*, *IFNAR1*, *IFNAR2,* and *TLR3* genes were listed in Table 1, Table 2, Table 3, Table 4, and Table 5, respectively. For the first time, in this study, 9 novel previously unreported variants have been identified in individuals from Turkey.

The descriptions of novel variants according to *in silico* analysis

Three of 19 variants (c.433+66G>A, c.493-3C>T, and c. 886+23C>T) are described as novel SNVs, that were detected in the intronic regions of the *IRF7* gene, and were predicted as a variant of unknown clinical significance (VUS) status according to the ACMG classification. Both of c.433+66G>A and c.886+23C>T variants were identified in one individual. These SNVs had a low DANN score (0.76 and 0.94, respectively) and were also predicted as polymorphism according to the MutationTaster *in silico* tool. In the *TBK1* gene, one novel variant as c.60T>C was determined. This synonymous variant (p.Thr20Thr) was located in the second exon and was determined as likely benign according to ACMG classification. In *in silico* tools, this variant had a benign prediction (DANN pathogenicity score; 0.85) and a lower conservation score (Genomic Evolutionary Rate Profiling-GERP score; 1,09). Two novel exonic variants (c.81A>G and c.938A>G) were defined in the *IFNAR1* gene and these variants were determined as likely benign according to the ACMG classification. c.81A>G synonymous (p.Gly27Gly) variant had a low DANN score (0.83), lower GERP score (3.09), and also was predicted as polymorphic according to the MutationTaster. c.938A>G (p.Asn313Ser) missense variant was determined as damaging according to PROVEAN *in silico* tool and also was predicted as polymorphism according to the MutationTaster. This variant also had a GERP score of 3.14 and a DANN score of 0.98. The three novel intronic variants (c.709+39T>C, c.710-14T>C, and c.841-14T>C) were found in the *IFNAR2* gene. While the first two of these three variants were determined as VUS, c.841-14T>C variant was as likely benign according to ACMG classification. All of these variants were identified as polymorphism according to the Mutation Taster tool. c.709+39T>C, c.710-14T>C and c.841-14T>C had a low DANN scores (0.77, 0.92 and 0.46) and GERP scores (1.57, 4.69 and 3.95, respectively).

Inter-population comparisons of minimum allele frequencies

It is assumed that variants that cause amino acid changes may have functional effects at the protein level. The canonical splice sites (5'-GT and 3'-AG dinucleotides) and nonsense variants, which might change the polypeptide sequence, were not detected in our population. The splice-associated variants (SAVs) identified were mostly variants that disrupted non-canonical splice sites, including the third and fifth intronic bases of the donor and acceptor sites. In this population, three SAVs were detected in the *IRF7* gene (c.493-3C>T, c.223-3C>A and c.223-4T>A), two in *TBK1* (c.229-4insT and c.1341-3delT) and one in *IFNAR2* (c.841-4delT). It was observed that three (c.223-3C>A, c.223-4T>A, and c.841-4delT) of these SAVs had less minimum allele frequencies (MAF) in our population than in other populations.

Five missense variants (c.1439G>T; p.R480L, c.1274A>G; p.Q412R, c.778G>A; p.G260R, c.574A>G; p.K192E and c.431G>A; p.R131Q) were detected in the *IRF7* gene and MAF was compared with other populations (Table 1). Two

Table 1: The minimum allele frequencies of *IRF7* gene variants in Turkish and other populations and their clinical definitions according to ACMG classification

Variant Description	Allele Frequencies in GnomAD Dataset. n (allele number)									In-House Data		Position of the variant on the protein	ACMG Definition	ACMG Verdict Rules
	African, n=16250	Ashkenazi Jewish, n=10074	East Asian, n=18394	European (Finnish), n=21644	European (Non-Finnish), n=113628	Latino, n=34588	South Asian, n=30612	Other, n=6124	Total, n=251314	MGexome, n=278	dbSNP ID			
c.4863G>A	-	-	0.00752	-	-	-	-	0.000166	0.000549	0.004	rs150393275	p.A1621A	Likely Benign	BS1, BP4, BP7
g.8460G>A#	-	0.00374	0.00385	-	0.0000294	0.0000654	-	-	0.000243	0.004	rs371988773	-	Uncertain Significance	BP4
c.*60C>T	-	-	-	0.0000943	0.000118	0.000262	-	-	0.0000855	0.007	rs771059701	-	Likely Benign	BS1
c.1515C>T	-	-	-	-	0.0000353	0.0000289	0.0000653	-	0.0000279	0.004	rs755671976	p.I505I	Likely Benign	BS1, BP4, BP7
c.1439G>T	-	-	-	-	0.0000443	-	-	-	0.0000201	0.004	rs762132411	p.R480L	Uncertain Significance	PM2, BP4
c.1396-23insA	0.0529	0.162	0.339	0.359	0.275	0.139	0.237	0.238	0.242	0.029	rs34948036	-	Benign	BA1
c.1396-43A>C	0.522	0.336	0.0281	0.189	0.267	0.323	0.141	0.261	0.255	0.140	rs10902178	-	Benign	BA1, BP6
c.1395+32T>C	0.523	0.335	0.0283	0.188	0.268	0.324	0.14	0.259	0.254	0.284	rs11246213	-	Benign	BA1, BP6
c.1276+41C>G	0.519	0.336	0.028	0.187	0.263	0.319	0.139	0.257	0.25	0.273	rs1051390	-	Benign	BA1, BP6
c.1276+14T>C	0.513	0.334	0.0277	0.186	0.262	0.318	0.139	0.258	0.248	0.277	rs12422022	-	Benign	BA1, BP6
c.1274A>G	0.513	0.335	0.028	0.186	0.263	0.32	0.139	0.259	0.249	0.273	rs1131665	p.Q412R	Benign	BA1, BP4, BP6
c.1185A>C	0.515	0.333	0.0284	0.191	0.267	0.325	0.141	0.26	0.252	0.291	rs1061505	p.G382G	Benign	BA1, BP6, BP7
c.886+45G>A	-	-	-	-	-	0.000379	-	-	0.0000265	0.004	rs752603743	-	Uncertain Significance	-
c.886+43C>T	0.496	0.221	0.00643	0.0638	0.123	0.178	0.0599	0.127	0.132	0.061	rs60870990	-	Benign	BA1
c.886+28T>C	0.489	0.216	0.00704	0.0719	0.124	0.151	0.0579	0.121	0.127	0.112	rs59115876	-	Benign	BA1
c.886+23C>T	-	-	-	-	-	-	-	-	-	0.004	novel	-	Uncertain Significance	PM2, BP4
c.778G>A	0.0000718	0.0016	0.0000565	0.00117	0.000713	0.000831	0.000336	0.00269	0.000717	0.007	rs201379782	p.G260R	Uncertain Significance	PM2, BP4
c.574A>G	0.524	0.334	0.028	0.188	0.266	0.323	0.14	0.259	0.253	0.291	rs1061502	p.K192E	Benign	BA1, BP4, BP6
c.525A>G	0.0589	0.108	0.000442	0.0387	0.0441	0.0302	0.0351	0.0531	0.0411	0.065	rs11246214	p.T162T	Benign	BA1, BP6, BP7
c.493-3C>T	-	-	-	-	-	-	-	-	-	0.007	novel	-	Uncertain Significance	-
c.433+66G>A	-	-	-	-	-	-	-	-	-	0.004	novel	-	Uncertain Significance	PM2, BP4
c.431G>A	-	0.00199	-	-	0.000709	0.000859	0.000359	0.00271	0.000655	0.011	rs201036875	p.R131Q	Uncertain Significance	PM2, BP4
c.366G>A	0.839	0.907	0.738	0.858	0.869	0.763	0.817	0.855	0.834	0.809	rs1061501	p.R109R	Benign	BA1, BP6, BP7
c.223-3C>A	0.513	0.338	0.0283	0.187	0.262	0.319	0.140	0.256	0.249	0.176	rs12290989	-	Likely Benign	BA1, BP6
c.223-4T>A	0.51	0.339	0.0282	0.186	0.261	0.318	0.139	0.255	0.248	0.108	rs12272434	-	Benign	BA1, BP6
c.222+24_222+28delCCCCG	0.000223	-	-	-	0.000792	0.00014	0.00004	0.000216	0.000383	0.004	rs749444292	-	Likely Benign	BS1
c.216C>T	0.0574	0.107	0.000369	0.036	0.0436	0.0298	0.0398	0.0535	0.0409	0.032	rs113083699	p.I59I	Benign	BA1, BP6, BP7
c.123G>A	-	0.00242	0.0379	0.00275	0.000638	-	0.000985	0.00233	0.00367	0.004	rs11544075	p.E28E	Benign	BS1, BS2, BP6, BP7

Transcript reference: NM_004031.4 and genomic reference #: NG_029106.1 were used for *IRF7* gene variant descriptions. Check reference 14 for the explanation of classifications and verdict rules according to ACMG.

Table 2: The minimum allele frequencies of *TBK1* gene variants in Turkish and other populations and their clinical definitions according to ACMG classification

Variant Description	Allele Frequencies in gnomAD data set. n (allele number)									In House Data	dbSNP ID	Position of the variant on the protein	ACMG Definition	ACMG Verdict Rules
	African, n=16250	Ashkenazi Jewish, n=10074	East Asian, n=18394	European (Finnish), n=21644	European (Non-Finnish), n=113628	Latino, n=34588	South Asian, n=30612	Other, n=6124	Total, n=251314	MGexome, n=278				
c.60T>C	-	-	-	-	-	-	-	-	-	0.004	novel	p.T20T	Likely Benign	BP4, BP7
c.66T>C	0.00683	0.0259	0.000163	0.0382	0.0459	0.0165	0.0315	0.0283	0.0324	0.036	rs41292019	p.N22N	Benign	BS1, BS2, BP6, BP7
c.228+81T>A	-	-	0.000641	-	-	-	-	-	0.0000319	0.007	rs940683519	-	Likely Benign	BS2
c.229-34_229-33delAT	0.0000696	0.000752	-	0.000142	0.00136	0.00139	0.00716	0.00251	0.00178	0.007	rs531707199	-	Benign	BS1, BS2,
c.229-4insT	0.718	0.81	0.79	0.853	0.811	0.828	0.719	0.792	0.797	0.831	rs57810028	-	Benign	PP3, BA1, BP6
c.978T>A	0.201	0.414	0.326	0.61	0.555	0.406	0.393	0.502	0.477	0.471	rs7486100	p.I326I	Benign	BA1, BP6, BP7
c.1062C>T	0.0000616	-	-	-	-	0.0000289	0.0000327	-	0.0000119	0.004	rs141340205	p.Y354Y	Likely Benign	BS2, BP7
c.1190-70T>C	0.184	0.0552	0.365	0.117	0.109	0.251	-	0.125	0.147	0.018	rs11175411	-	Benign	BA1, BP6
c.1341-3delT	0.000928	0.00569	-	0.0136	0.00906	0.00289	0.00562	0.00499	0.00697	0.004	rs201728462	-	Benign	BS1, BS2, BP6, PP3
c.1391T>C	0.00379	0.00901	0.0000557	0.0249	0.0199	0.00335	0.00659	0.0156	0.0135	0.004	rs35635889	p.V464A	Benign	BS1, BS2, BP4, BP6, PP2
c.1443-60C>A	0.0756	0.159	0.0884	0.336	0.296	0.222	-	0.278	0.225	0.147	rs10878177	-	Benign	BA1, BP6
c.1960-35_1960 33insGTT	0.214	0.215	0.0243	0.124	0.104	0.074	0.122	0.113	0.11	0.050	rs146676333	-	Benign	BA1, BP6
c.1960-32T>G	0.0013	0.00115	0.000227	0.000119	0.000345	0.000436	0.000761	0.000309	0.000462	0.004	rs200603336	-	Benign	BS1, BS2
c.1960-27C>T	0.0185	0.0119	0.0136	0.0789	0.0134	0.00454	0.00505	0.00796	0.0183	0.050	rs201633637	-	Benign	BA1, BP6
c.1960-24T>C	0.002	0.00166	0.000282	-	0.000525	0.000639	0.00163	0.000459	0.000681	0.004	rs756443056	-	Benign	BS1, BS2
c.-31-18A>T	0.0000882	-	-	0.0000695	0.0000748	0.0000719	-	-	0.0000584	0.007	rs1565810404	-	Likely Benign	BS2

Transcript reference: NM_013254.4 was used for *TBK1* gene variant descriptions. Check reference 14 for the explanation of classifications and verdict rules according to ACMG.

Table 3: The minimum allele frequencies of *IFNAR1* gene variants in Turkish and other populations and their clinical definitions according to ACMG classification

Variant Description	Allele Frequencies in gnomAD data set, n (allele number)									In House Data	dbSNP ID	Position of the variant on the protein	ACMG Definition	ACMG Verdict Rules
	African, n=16250	Ashkenazi Jewish, n=10074	East Asian, n=18394	European (Finnish), n=21644	European (Non-Finnish), n=113628	Latino, n=34588	South Asian, n=30612	Other, n=6124	Total, n=251314	MGexome, n=278				
c.28A>G	-	-	-	-	0.000046	0.0000295	-	-	0.0000249	0.004	rs751675124	p.T10A	Likely Benign	BS1, BP4
c.76+65G>C	0.00123	0.0023	-	0.00782	0.00876	0.00255	0.00331	0.00335	0.00529	0.004	rs148956118	-	Likely Benign	BS1
c.77-22T>A	-	-	-	-	0.0000191	-	-	-	0.00000874	0.176	rs2243592	-	Uncertain Significance	-
c.81A>G	-	-	-	-	-	-	-	-	-	0.004	novel	p.G27G	Uncertain Significance-Benign	BP7
c.201-10delT	0.0000244	-	-	-	0.00000147	-	-	-	0.00000132	0.004	rs747996862	-	Uncertain Significance	-
c.502G>C	0.163	0.152	0.368	0.0919	0.138	0.251	0.261	0.182	0.185	0.169	rs2257167	p.V168L	Benign	BA1, BP4, BP6
c.504T>C	-	0.0000993	0.000761	-	0.000396	0.000116	0.00364	0.00179	0.000741	0.011	rs200831107	p.V168V	Likely Benign	BS1, BP6, BP7
c.624G>A	0.000554	0.000695	0.0000544	0.000693	0.00336	0.000378	-	0.00131	0.00173	0.004	rs144040431	p.T208T	Likely Benign	BS1, BP6, BP7
c.788+60C>T	0.00434	0.00231	0.000385	0.0369	0.0266	0.00779	0.0213	0.0129	0.0176	0.007	rs17875880		Likely Benign	BS1, BP4
c.789-49A>G	0.148	0.225	0.00201	0.311	0.269	0.124	0.151	0.229	0.211	0.097	rs2834196		Benign	BA1
c.916C>T	-	0.00884	-	-	0.000282	0.00029	0.000263	0.00131	0.000586	0.004	rs201281365	p.R306C	Likely Benign	BS1, BP6, PP3
c.938A>G	-	-	-	-	-	-	-	-	-	0.004	novel	p.N313S	Likely Benign	BP4, PM2
c.1076C>T	0.175	0.00457	0.000707	-	0.000986	0.00786	0.000589	0.00719	0.0133	0.004	rs17875834	p.T359M	Benign	BA1, BP4, BP6
c.1170T>C	0.000188	-	-	-	0.00103	0.00147	0.000474	0.00244	0.000789	0.007	rs17875885	p.D390D	Likely Benign	BS1, BP7
c.1295-137G>A	0.816	0.778	0.994	0.691	0.732	0.823	0.869	0.776	0.776	0.888	rs914142		Benign	BA1
c.1427C>T	-	-	-	0.0000474	0.000226	0.0000406	0.00158	-	0.000189	0.004	rs369713150	p.S476F	Likely Benign	BS1, BP4
c.1440+38T>A	0.872	0.773	0.998	0.698	0.738	0.888	0.851	0.771	0.789	0.345	rs2856973	-	Benign	BA1
c.1441-135C>T	0.179	0.175	0.384	0.124	0.176	0.261	0.299	0.207	0.193	0.029	rs2254315	-	Benign	BA1

Transcript reference: NM_000629.3 was used for *IFNAR1* gene variant descriptions. Check reference 14 for the explanation of classifications and verdict rules according to ACMG.

Table 4: The minimum allele frequencies of *IFNAR2* gene variants in Turkish and other populations and their clinical definitions according to ACMG classification

Variant Description	Allele Frequencies in gnomAD data set, n (allele number)									In House Data	dbSNP ID	Position of the variant on the protein	ACMG Definition	ACMG Verdict Rules
	African, n=16250	Ashkenazi Jewish, n=10074	East Asian, n=18394	European (Finnish), n=21644	European (Non-Finnish), n=113628	Latino, n=34588	South Asian, n=30612	Other, n=6124	Total, n=251314	MGexome, n=278				
c.23T>C	0.0774	0.0774	0.176	0.0855	0.0808	0.154	0.125	0.0909	0.103	0.090	rs2229207	p.F8S	Benign	BA1, BS3, BP4
c.28T>G	0.19	0.322	0.582	0.391	0.331	0.474	0.475	0.354	0.383	0.417	rs1051393	p.F10V	Benign	BA1, BP4, BP6
c.98-43T>C	0.809	0.677	0.416	0.609	0.667	0.523	0.523	0.643	0.615	0.540	rs2834158	.	Benign	BA1
c.98-9_98-7delTCT	0.00351	0.0247	0.000163	0.0643	0.024	0.0129	0.0271	0.0266	0.0234	0.011	rs79402470	.	Benign	BA1, BP6
c.-238C>T	0.00612	0.0456	0.00562	0.0391	0.0309	0.0249	0.0543	0.0356	0.0243	0.032	rs17860116	.	Benign	BA1
c.-254C>A	0.217	0.327	0.582	0.403	0.324	0.398	0.492	0.315	0.322	0.144	rs17860115	.	Benign	BA1
c.-313T>C	0.548	0.538	0.693	0.538	0.562	0.577	0.642	0.544	0.564	0.263	rs9975738	.	Benign	BA1
c.-317C>G	0.00412	0.0151	0.000194	0.0409	0.0237	0.0181	0.00809	0.0223	0.0172	0.011	rs188401375	.	Benign	BS1, BS2
c.352A>G	-	-	-	-	0.00000879	-	0.0000327	0.000163	0.0000119	0.004	rs767824035	p.T118A	Uncertain Significance	BP4, PM2
c.541-50A>G	0.787	0.723	0.419	0.646	0.697	0.537	0.581	0.673	0.643	0.608	rs2236757	.	Benign	BA1
c.611C>G	0.000431	0.0179	-	0.0000924	0.00417	0.00371	0.0131	0.00751	0.00493	0.022	rs147496374	p.T204R	Benign	BS1, BS2, BP6
c.709+39T>C	-	-	-	0.0000147	-	-	-	-	0.00000657	0.004	novel	.	Uncertain Significance	-
c.710-14T>C	-	-	-	-	-	-	-	-	-	0.004	novel	.	Uncertain Significance	-
c.841-132T>C	-	-	-	-	-	-	-	-	-	0.004	novel	-	Likely Benign	BP1, BP4, PM2
c.841-44insT	0.361	0.326	0.599	0.361	0.324	0.459	0.487	0.359	0.389	0.396	rs3216172	.	Benign	BA1, BP4
c.841-33C>A	0.592	0.765	0.909	0.733	0.677	0.844	0.771	0.723	0.733	0.709	rs9984273	.	Uncertain Significance	PM2, BP4
c.841-4delT	0.409	0.398	0.479	0.424	0.433	0.455	0.446	0.429	0.438	0.076	rs34865572	.	Benign	BA1, BP4
c.1092C>T	0.000431	-	0.0178	0.000139	0.000158	0.0243	0.000294	0.00342	0.00487	0.004	rs117810077	p.S364S	Benign	BS1,BS2, BP4, BP7
c.1391A>C	-	-	-	-	-	-	-	0.000163	0.00000398	0.004	rs1226146327	p.N464T	Uncertain Significance	BP4,PM2

Transcript reference: NM_207585.2 was used for *IFNAR2* gene variant descriptions. Check reference 14 for the explanation of classifications and verdict rules according to ACMG.

Table 5: The minimum allele frequencies of *TLR3* gene variants in Turkish and other populations and their clinical definitions according to ACMG classification

Variant Description	Allele Frequencies in gnomAD data set, n (allele number)									In House Data	dbSNP ID	Position of the variant on the protein	ACMG Definition	ACMG Verdict Rules
	African, n=16250	Ashkenazi Jewish, n=10074	East Asian, n=18394	European (Finnish), n=21644	European (Non-Finnish), n=113628	Latino, n=34588	South Asian, n=30612	Other, n=6124	Total, n=251314	MGexome, n=278				
c.1-7C>A	0.155	0.156	0.235	0.188	0.189	0.169	0.164	0.183	0.183	0.147	rs3775296	-	Benign	BA1, BP6, PVS1
c.299T>C	-	-	-	-	0.0000088	-	-	0.000163	0.00000796	0.004	rs150769655	p.M100V	Uncertain Significance	BP4, PM2
c.1234C>T	0.0647	0.256	0.34	0.325	0.298	0.299	0.238	0.288	0.279	0.255	rs3775291	p.L412F	Benign	BA1, BS3, PP3
c.1377C>T	0.195	0.238	0.323	0.291	0.302	0.349	0.319	0.303	0.302	0.266	rs3775290	p.F459F	Benign	BA1, BP6, BP7
c.1660C>T	0.000308	-	-	0.000416	0.000687	0.000145	0.0000327	0.000652	0.000406	0.004	rs121434431	p.P554S	Uncertain Significance	PM2, PP3
c.1677G>A	0.0395	0.00169	-	-	0.000149	0.00165	0.000131	0.000815	0.00295	0.004	rs35617964	p. K559K	Benign	BS1, BS2, BP6, BP7
c.2278A>G	-	-	-	-	0.00001	-	-	-	0.000004	0.004	rs1226246023	p.I760V	Uncertain Significance	BP4, PM2
c.2486+50T>C	0.865	0.614	0.876	0.876	0.762	0.888	0.713	0.767	0.794	0.782	rs6830345	-	Benign	BA1
c.2553C>T	0.161	0.0356	0.000109	0.00661	0.014	0.0219	0.00212	0.0274	0.0227	0.022	rs73873710	p.F851F	Benign	BA1, BP6, BP7

Transcript reference: NM_003265.3 was used for *TLR3* gene variant descriptions. Check reference 14 for the explanation of classifications and verdict rules according to ACMG.

(c.1439G>T and c.431G>A) of these 5 variants, whose functional effects are unknown, had a higher MAF in our population than in other populations. The MAFs of the other missense variants were similar to other populations except for the East Asian population.

One missense variant (c.1391T>C; p.V464A) was determined in the *TBK1* gene. MAF of this variant was similar in the our study's population when compared with other populations. In addition, this variant was predicted as benign according to the Varsome database (Table 2).

The five missense variants (c.28A>G; p.T10A, c.502G>C; p.V168L, c.916C>T; p.R306C, c.1076C>T; p.T359M, c.1427C>T; p.S476F) were detected in *IFNAR1* gene (Table 3). The MAFs of c.28A>G, c.916C>T and c.1427C>T variants were higher in the our study's population than other population groups. In addition, c.28A>G and c.916C>T were predicted disease-causing according to MutationTaster although they were determined as likely benign according to the Varsome database.

Five missense variants (c.23T>C; p.F8S, c.28T>G; p.F10V, c.352A>G; p.T118A, c.611C>G; p.T204R, c.1391A>C; p.N464T) in *IFNAR2* gene were determined in Turkish individuals (Table 4). The MAFs of c.352A>G, c.611C>G, c.1391A>C variants were higher in the our study's population than other population groups. Only c.611C>G variant was predicted as damaging according to Mutation Taster, SIFT and PROVEAN tools. The MAFs of c.23T>C and c.28T>G variants were similar to other populations.

Four missense variants were detected in the *TLR3* gene, the other gene for which variant analysis was performed in our study population (Table 5). The MAFs of c.1234C>T; p.L412F variant was similar in our population compared with other populations. The MAFs of c.299T>C; p.M100V, c.1660C>T; p.P554S and c.2278A>G; p.I760V was higher than the other populations.

Human diseases associated with *IRF7, TBK1, IFNAR1, IFNAR2* and *TLR3*

The disease groups associated with the *IRF7, TBK1, IFNAR1, IFNAR2,* and *TLR3* genes identified in the screening performed used the DysGeNET database was listed in Table 6. These genes were found to strongly associate with neoplasms and infections especially viral infections. Interestingly, the digestive system, nervous system, urogenital, immune system, and respiratory diseases, which are seen in the tissues most frequently affected by viral infections such as SARS-CoV-2, were found to be associated with these genes.

DISCUSSION

For the first time, in this preliminary study, the exonic and flanking intronic variants in *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*,

Table 6: Human diseases associated with *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2* and *TLR3* genes

Disease Classification	Number of Different Types of Diseases Related to the Following Genes				
	IRF7	***TBK1***	***IFNAR1***	***IFNAR2***	***TLR3***
Infections	29	17	42	34	76
Viral	24	10	28	28	54
Parasitic and bacterial	5	7	14	6	22
Mental disorders	4	23	3	-	8
Immune system diseases	13	5	8	9	34
Behaviour and behaviour mechanisms	-	11	3	-	6
Neoplasms	41	39	36	18	90
Skin and connective tissue diseases	12	12	15	15	34
Nervous system diseases	12	15	16	8	48
Digestive system diseases	12	14	20	12	50
Cardiovascular diseases	1	4	6	4	23
Hemic and lymphatic diseases	8	3	9	5	15
Female urogenital diseases and pregnancy complications	1	5	7	5	34
Male urogenital diseases	3	7	7	5	39
Nutritional and metabolic diseases	5	17	2	3	8
Respiratory tract diseases	11	17	7	4	33
Congenital, hereditary, and neonatal diseases and abnormalities	5	7	4	5	15
Pathological conditions	12	47	18	10	15
Endocrine system diseases	5	6	6	4	-
Eye diseases	1	15	-	1	-
Animal diseases (Rift Valley Fever, Bluetongue infection, Borna Disease vs.)	6	1	2	2	-
Stomatognathic diseases	4	4	-	2	-
Otorhinolaryngologic diseases	3	-	-	-	-
Musculoskeletal diseases	3	7	1	1	-
Chemically-induced disorders	1	-	1	-	-

and *TLR3* genes were determined in-house whole-exome data set of 139 unrelated Turkish individuals. It is known that immunity genes *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*, and *TLR3* play a role in the immune response against SARS-CoV-2 infection and also other viral and bacterial infections. During the ongoing pandemic period, research was mainly on the pathogenesis of SARS-CoV-2 infection (1-3,18-20). Accordingly, SARS-CoV-2 enters the cell when the viral spike (S) protein is recognized by the angiotensin-converting enzyme (ACE2) receptors and proteolysis by transmembrane serine protease 2 (TMPRSS2) of the host cell, and then this situation causes an innate immune response. The structures of SARS-CoV-2 in the form of lipid, protein, and nucleic acid are recognized by toll-like receptors (TLR). It has been defined that the viral S protein is recognized by TLR4, the ssRNA is recognized by TLR7/8, and the dsRNA is recognized by TLR3. TLR3 and TLR4 induce IRF3 (interferon regulatory factor 3) via TRIF (toll-interleukin 1 receptor domain-containing adapter-inducing interferon-β) and TRAM (TRIF-related

adaptor molecule), and IRF3 goes to the nucleus and interferon (IFN) initiates its synthesis. The IFN mediated immune response is the innate immune system developed against SARS-CoV-2 and other viral infections. This system does not produce an immune response or create a delayed response due to both viruses and congenital disorders. The late interferon response is suggested to be associated with the cytokine storm (18). According to the latest studies summarized in a review published by Ricci et al., various immune gene variants are found to be effective in the immune response in SARS-CoV-2 infection (18). In a previous study, the whole exome and genomes sequencing techniques were performed in patients with severe life-threatening manifestations of COVID-19 and asymptomatic patients, and the genetic defects in *TLR3*, *IRF7*, *IRF9*, *TICAM1/TRIF*, *TRAF3*, *TBK1*, *IRF3*, *NEMO/IKBKG*, *IFNAR1*, *UNC93B1*, *IFNAR2*, *STAT1*, and *STAT2* genes cause differences in immune response was determined and also twenty-four variants of eight genes were identified to be causing inborn errors of immunity (7). The detected 24 variants in *TLR3*, *UNC93B1*, *TICAM1*, *TBK1*, *IRF3*, *IRF7*, *IFNAR1*, and *IFNAR2* genes were loss of function, loss of expression, and extremely hypomorphic. This study showed that in addition to factors such as gender, age, and having a chronic disease, inborn immune deficiency also plays a role in the immune response differences against SARS-CoV-2 infection (7). In our population study, a total of 90 variants were detected in *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*, and *TLR3* genes.

In the study of Zhang et al., c.1660C>T (p.P554S, rs121434431) missense variant in the *TLR3* gene was found as heterozygous in a male patient of Italian origin with life-threatening COVID-19 (7). Also, in this study, it has been shown that *IFNL1* (Interferon lambda 1) mRNA levels were statistically decreased in p.P554S-mutant cells compared to wild-type cells (7). In our study, this variant was detected as heterozygous in one individual. Interestingly, the allele frequency of the p.P554S missense variant in *TLR3* was found higher in our population than in African, European, Latino, and South Asian populations. Moreover, this variant was predicted as VUS according to the ACMG classification. In previous studies, this variant has also been reported in patients with influenza pneumonia and herpes simplex encephalitis (13, 14). To support our result, it would be valuable to investigate this variant in a our study's population with wider participation and to conduct case-control studies related to susceptibility to viral diseases.

In a previous study, it has been observed that plasmacytoid dendritic cells and fibroblasts obtained from patients infected with SARS-CoV-2 and IRF-7 and IFNAR1 deficiency were not able to produce type I IFN (7). These findings were suggested to be evidence that strengthens the genotype-phenotype relationship (7). In our study, the allele frequency of p.R480L and p.R131Q variants in *IRF7*, p.T10A, p.R306C and p.S476F variants in *IFNAR1*, p.T118A, p.T204R and p.N464T variants in *IFNAR2*, p.P554S and p.I760V variants in the *TLR3* gene in the our study's population was higher compared to other populations. These missense variants were defined as benign, likely benign, and uncertain significance according to ACMG classification. On the other hand, p.T10A and p.R306C variants in the *IFNAR1* gene were predicted as disease-causing by *in silico* tools. In our study, no functional information was obtained about the effects of individuals carrying these missense variants on the immune response to viral infection. In addition, according to the GenOMICC study, a host-induced inflammatory lung injury transcriptome study with genes including IFNAR2 suggested that *IFNAR2*, which was involved in the antiviral defence mechanism, was associated with the response against SARS-CoV-2 (20). In our study, p.T204R missense variant in *IFNAR2* was identified to have higher MAF in the our study's population than other populations and it was determined as damaging according to *in silico* tools. p.T204R variant has been shown to exhibit a much higher minimum allele frequency in our population when compared to the other populations. When all these findings are evaluated, it is important to demonstrate the functional effects of the variants identified in this study experimentally and to determine their relationship in viral diseases.

In our population study, 9 of 90 variants were detected as novel variants in the immunity genes *IRF7*, *TBK1*, *IFNAR1*, and *IFNAR2*. Six of these novel 9 variants were predicted as uncertain significance (VUS) according to the ACMG classification. In addition, 13 more rare VUS has been identified in intronic and also exonic regions of these genes associated with conditions related to the immune system. However, the individuals evaluated within this study are anonymous and these variants have not been associated with the clinical conditions of individuals. In WES or GWS (genome-wide sequencing) when performed as diagnostic genetic testing, it has been recommended that the secondary findings of certain genes should be considered because they may reveal conditions for possible early diagnosis and effective treatment (21). Five genes evaluated in this study are candidate genes for the immune system and infection susceptibility, and no pathogenic-likely pathogenic variant was found in the study population, only variants of uncertain significance (VUS) were identified. The limitation of this study is that comparisons of individuals with clinical findings and family segregation studies could not be performed in order to reveal the clinical association of VUSs. In the future, the case-control association studies might be done in infectious diseases to show the clinical importance of these VUSs.

In conclusion, allelic frequencies of genes and variants that play a critical role in the immune system were determined using *in-house* WES datasets in a group of the Turkish population. In this study, preliminary information was obtained that may contribute to the determination of the relationship between life-threatening viral infections and immune system gene variants and the functional effects of the variants. The variability of variant frequencies among populations suggests that it affects the sensitivity of the immune response to infectious diseases between populations. At the same time, when compared with other populations in these candidate genes, the immune response effect of missense variants with higher allele frequency in the our study's population against viral infection such as SARS-CoV-2 might be investigated in clinically different disease groups and their contribution to the severity of infection might be determined.

Finally, in outbreaks caused by severe acute respiratory syndromes (SARS) based on coronavirus, which we still struggle with and expect to see in the future, determining the genetic background of the individuals that affects the severity of the disease will allow implementation the prevention and effective treatment approaches.

Ethics Committee Approval: All procedures performed involving participants were in accordance with the ethics committee of the Istanbul Faculty of Medicine Clinical Research Ethics Committee, Istanbul University (Date: 28.01.2021, No: 52402) and followed the Declaration of Helsinki.

Informed Consent: All participants confirmed written informed consent

Author Contributions: Drafting Manuscript- A.K.; Design of Study- A.K., E.K.B.; Data Acquisition- G.T; Data Analysis/Interpretation- A.K., E.K.B., G.T.; Critical Revision of Manuscript- A.K.B., O.U., B.K., S.B.; Final Approval and Accountability- A.K., E.K.B., G.T., O.U., B.K., S.B.

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