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Next-generation sequencing of *CCR5*, *CXCR4*, and *IFNAR1* variants in relation to HIV-1 disease progression and ART response

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HIV, which causes acquired immune deficiency syndrome (AIDS), invades the host cell via the CD4 receptor and CCR5 or CXCR4 co-receptors. Interferons induced early in HIV infection induce an antiviral defense mechanism through IFNAR signaling. Our study aimed to examine the relationship between *CCR5*, *CXCR4*, and *IFNAR1* gene variations as a risk factor in HIV + patients and their response to their clinical parameters. Targeted next-generation sequencing (tNGS) was used to perform molecular genotyping analysis of the *CCR5*, *CXCR4*, and *IFNAR1* genes in genomic DNA from 22 HIV + patients and 25 healthy individuals as controls. We detected 13 rare mutations in the study, including 3 missense, 1 synonymous, 2 5′UTR, 4 3′UTR, and 1 frameshift variation. We also analyzed 6 common variants in the *IFNAR1* and *CXCR4* genes. HIV + patients carrying the homozygous TT genotype of the *IFNAR1* intronic rs2856973:T > A variant had higher CD4 + T cell counts compared with patients carrying the TA + AA genotypes of the rs2856973 variant in the naive and first month of the ART ($p = 0.001$ and $p = 0.001$, respectively). Similarly, participants receiving ART with a TT genotype of rs2856973:T > A showed a significantly higher CD4 + T cell count in the third month ($p = 0.001$). Patients carrying the homozygous wild-type genotype of the *CXCR4* intronic rs2680880:A > T SNP had lower CD4 + T cell count compared with subjects carrying the AT + TT mutant genotypes of rs2680880:A > T in the naive and first-month period ($p = 0.015$ and $p = 0.025$, respectively). Our results demonstrate that intronic variations in the *IFNAR1* rs2856973:T > A and *CXCR4* rs2680880:A > T genes can contribute to modifications in HIV progression and CD4 + T recovery under ART.

Keywords Next-generation sequencing, HIV, *CCR5*, *CXCR4*, *IFNAR1*, Polymophism, Genetic variation

By the end of 2023, an estimated 39.9 million individuals were living with HIV globally. Among adults aged 15–49 years, the estimated global prevalence was 0.6%. Despite global efforts to curb the epidemic, substantial disparities in HIV burden persist across countries and regions[1]. Although HIV prevalence in Turkey remains low (0.1–0.3%), recent years have seen a marked increase in new diagnoses, particularly among younger individuals[2]. Host genetics have been a focus of researchers since it became clear that molecular mechanisms in different HIV/AIDS-associated gene alleles affect different populations in various ways[3]. Successful HIV invasion requires the presence of the CD4 receptor and one of the C–C chemokine receptor type 5 (CCR5) or C-X-C chemokine receptor type 4 (CXCR4) co-receptors on the host cell[4,5]. Death of the host cell causes a dramatic decrease in the number of CD4 + T lymphocytes[6]. Since CD4 + T lymphocytes are regulators of the adaptive immune system, if their numbers decrease, the immune system weakens and the infection progresses to the AIDS stage[7]. Two biological phenotypes of HIV-1 exist and differ in receptor tropism: X4 HIV-1 has affinity for the CXCR4 receptor, while R5 HIV-1 has affinity for the CCR5 receptors[8]. The receptor genes *CXCR4* and *CCR5* are localized on chromosome 2q22.1 and 3p21.31, respectively[9].

Type I interferons (IFN-1) are potent cytokines that inhibit the replication of HIV-1 and other viruses. IFN-1 exhibits antiviral activity through the IFN-1 receptor (IFNAR) and functions as an immune system regulator.

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Cell surface density of the IFNAR receptor plays a crucial role in HIV-1 pathogenesis[10]. However, all IFN-α molecules compete for binding to a single receptor complex, IFNAR. IFNAR is a heterodimeric molecule consisting of IFNAR1 and IFNAR2 subunits[11]. Upon ligand binding, the IFNAR complex initiates signaling cascades primarily through the IFNAR1 subunit, leading to the activation of IFN-1[12].

Numerous studies have investigated the roles of *CCR5*, *CXCR4*, and *IFNAR1* receptor genes in various human diseases. However, there is a limited amount of research exploring how genetic variations in the *CCR5*, *CXCR4*, and *IFNAR1* genes are associated with the pathogenesis of HIV infection. Additionally, CD4 + T cell count is one of the most important clinical parameters indicating HIV progression. According to our knowledge, the present study is the first one in the literature to investigate the impact of variations in the *CCR5*, *CXCR4*, and *IFNAR1* genes on HIV-1 disease progression, measured through $CD4^+$ T cell counts, in both ART (antiretroviral therapy)-naive HIV + and under ART HIV + individuals in the Turkish population. Therefore, using targeted Next-Generation Sequencing (tNGS), we investigated genetic polymorphisms in *CCR5*, *CXCR4*, and *IFNAR1*, and evaluated their association with disease progression in HIV-1.

Methods

Characteristics of work groups

22 naive HIV-1 infected patients and 25 controls aged 18–64 years were examined in this research. The median age of the HIV + group was 40,72 years ± 14,99, and 60% were male and 40% female. In the control group, the median age was 41,52 ± 13,82 years, 55% were male and 45% female. The project was accepted by Haliç University Ethics Committee (Reference number 2021/148) and complied with the Declaration of Helsinki. Written informed consent was obtained from all participants between 10.01.2022 and 16.01.2023. Peripheral whole-blood samples from all HIV-1 participants and healthy individuals were obtained from the Department of Infectious Diseases and Clinical Microbiology of Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty, Istanbul, Turkey. Following enrollment, blood samples were collected from HIV + patients at multiple time points to monitor changes in CD4 + T cell counts and HIV viral load over time. HIV negativity in the healthy control group was confirmed by the 4th generation ELISA tests prior to inclusion in the study.

Patients were followed with unique ART. A group of patients was defined as ART-naive HIV + patients. Patients were defined as"ART-naive"if they had never received any form of ART prior to their enrollment in the study. This status was confirmed through clinical records and physician verification. The baseline median plasma HIV-1 RNA viral load was 5,36 ± 0,96 $\log_{10}$ copies/mL, and the median CD4 + T cell count was 2,55 ± 0,54 cells/mL. All patients were treated with the standard ART regimen consisting of bictegravir sodium, emtricitabine, and tenofovir alafenamide fumarate (respectively, 50 mg/200 mg/25 mg).

The past medical records of HIV + and healthy participants were screened. Exclusion criteria were defined as taking prior ART treatment, taking prior immunosuppressive treatment, pregnancy, and incomplete clinical or demographic records.

Next-generation sequencing and analyzing raw data

5 mL of peripheral whole blood was collected in EDTA tubes and stored at approximately –80 °C. Genomic DNA was extracted from whole peripheral blood samples using the QIAamp DNA Mini Kit (QIAGEN®, Silicon Valley, CA, USA, cat. no. 51304) according to the manufacturer's protocol. DNA purity and concentration were assessed using both NanoDrop 2000c (Thermo-Fisher Sci., MA, USA) and Qubit 3.0 Fluorometer (Invitrogen, CA, USA).

Exons and exon/intron boundaries of *CCR5*, *CXCR4*, and *IFNAR1* genes were amplified by a long polymerase chain reaction using a thermal cycler (C1000; Biorad, USA). Primers and conditions used for targeted sequencing fragments are presented in Supplementary Tables 1 and Supplementary Tables 2. After the primers were designed, Illumina® DNA Prep, (M) Tagmentation kit (Illumina, Inc., San Diego, CA, USA, cat. no. 20018705), was used.

Libraries for targeted sequencing were prepared using a custom amplicon-based approach. Specifically, primers were manually designed to amplify the regions of interest (targeted exons) from each sample's genomic DNA. The resulting PCR amplicons were indexed, pooled, and sequenced on the NovaSeq 6000 system (Illumina, Inc., San Diego, CA, USA). This strategy yielded very high coverage across all target regions: coverage above 100 × was achieved for 100% of the targeted bases, with an average sequencing depth of approximately 500 ×. As a quality control measure, we aimed to ensure that each targeted region had a minimum depth of 100 ×, and overall sequencing metrics confirmed this coverage was achieved.

Prior to alignment, raw sequencing read quality was evaluated using FastQC, and low-quality base calls and adapter sequences were removed using Trimmomatic (trimming bases with Phred quality score < 30). The trimmed reads were then aligned to the Genome Reference Consortium Human Build 38 (GRCh38) using the Burrows-Wheeler Aligner (BWA-MEM) algorithm implemented through Illumina's DRAGEN Bio-IT platform in High Coverage Mode. This alignment step produced a CRAM file as the final alignment format for each sequencing pool. During alignment, the DRAGEN pipeline was configured to improve mapping accuracy by removing soft-clipped reads and filtering out reads with low mapping quality (MAPQ < 40). The resulting CRAM file retained a Phred-scaled quality (Q) score for each nucleotide aligned to the reference, consistent with our previously described pipeline[13].

Variant calling was performed on the aligned reads following the Genome Analysis Toolkit (GATK) Best Practices pipeline[14]. In line with these recommendations, we employed a joint calling strategy: GATK's HaplotypeCaller was run in GVCF mode to generate an intermediate genomic VCF (gVCF) for each sample, and these gVCFs were then jointly genotyped (using GATK's GenotypeGVCFs) to produce a combined set of variant calls across all samples. This approach leverages information from multiple samples to reduce sequencing artifacts and improve variant detection accuracy. The initial variant calls were unannotated and stored in variant call format (VCF) files. The raw variant calls underwent rigorous filtering to obtain high-confidence variants. First, variants were stratified by type into single nucleotide polymorphisms (SNPs) and insertions/deletions

(Indels) using GATK's SelectVariants tool. Each subset was then filtered with GATK's VariantFiltration using standard hard-filter thresholds (GATK best practices). Variants failing to meet quality criteria – for example, those with low Quality-by-Depth or showing significant strand bias – were flagged for removal. Only variants passing all filters (marked with a "PASS" tag in the VCF) were retained for further analysis. We also imposed a minimum read depth threshold to ensure each call was well-supported: a coverage of at least 30 × supporting reads was required for a variant to be considered in downstream analysis. Any variant not meeting this depth (coverage) requirement was excluded as low-confidence. The final set of high-quality variants for each sample (i.e. variants with a "PASS" filter status and ≥ 30 × read depth support) was compiled into a VCF file per sample. These high-confidence VCFs were then carried forward into the annotation pipeline (DNAlyzer) for downstream analyses.

Annotated variant analysis was carried out using multiple tools and software to interpret the functional impact of each variant. GRCh38 was used as the reference for all annotation steps (http://genomereference.org). Variant annotation and effect prediction were performed using ANNOVAR, SnpEff, and the Ensembl Variant Effect Predictor (VEP). The annotated variants for each sample were compiled and filtered as part of our custom DNAlyzer pipeline, and the results were stored in the respective sample's folder for further investigation. Additionally, variant data management and comparative analysis between samples (or groups of samples) were facilitated by the Ilyome© software (Ilyome©, Turkey), which was used for triaging and reviewing the annotated variant lists.

For variant prioritization, we focused on rare and potentially impactful variants. We examined annotated VCF files for variants that were truncating (nonsense or frameshift mutations), missense (nonsynonymous single-nucleotide changes), synonymous, or located in the 5′ or 3′ untranslated regions (UTRs), provided that their total population minor allele frequency was below 5% in public databases. These filtered variants were then compared between study groups as part of our strategy to identify group-specific or disease-relevant genetic differences. To validate the identified variants and assess their supporting reads, we visualized each variant locus using the Integrative Genomics Viewer (IGV) by loading the CRAM file alongside the reference genome. This visual inspection step allowed us to confirm the presence and zygosity of variants and to check the local read depth and sequence context, thereby verifying variant accuracy. Finally, all high-confidence variants identified through this pipeline were evaluated in the context of the patients' genotypes and correlated with clinical information for interpretation.

Statistical analyzes

Statistical analyses were performed with SPSS v.20.0 (IBM SPSS, Inc., Chicago, IL, USA) and GraphPad Prism version 10.3.1 software. Genotype distributions of common polymorphisms of *IFNAR1* and *CXCR4* genes in study groups were performed using the chi-square test. Viral load and CD4 + T cell count at three defined time points (ART-naive, and at 1 and 3 months following ART initiation) were compared using two-way analysis of variance followed by Tukey's multiple comparison tests. Viral load and CD4 + T cell count of HIV + patients carrying the *IFNAR1 and CXCR4* genes common variants in defined time were compared using two-way analysis of variance followed by Tukey's multiple comparison tests. Multiple linear regression analyses were performed to evaluate the impact of age and gender on viral load and CD4 + T cell counts in the HIV + patients. Quantitative variables are presented as mean ± standard error of the mean (SEM). $p < 0.05$ values were considered statistically significant. The allele/genotype distributions, including Hardy–Weinberg equilibrium estimations, were calculated.

Results

Genotype distributions of common variants of the study groups

The results of statistical analyses between homozygous and heterozygous carriers of the mutant allele compared to the wild-type genotype in common variants in HIV + and control groups are shown in Table 1. Common variants; *IFNAR1* gene (NM_000629.2 for only rs2850015 and NM_000629.3 for the other variants) rs2834196:A > G, rs2856973:T > A, rs2243592:T > G, rs2850015:T > C, rs2257167:G > C[15] and *CXCR4* gene (NM_003467.3) rs2680880:A > T[16] with the highest 6 MAF values from *IFNAR1* and *CXCR4* genes were selected and analyzed. All variants identified in the *CCR5* gene (NM_001394783.1)[17] are rare. In the analysis of common variants in the *IFNAR1* and *CXCR4* genes, no statistically significant differences were observed between HIV + individuals and the control group in terms of genotype and allele frequencies ($p > 0.05$ for all variants). There was no significant deviation from the expected Hardy–Weinberg equilibrium in either the cases or controls (HWE) ($p > 0.05$).

Figure 1 shows diagrams of the different variations in *IFNAR1* and *CCR5* genes in the IGV program. The IGV illustrations in sections A and C show the missense mutation rs2257167:G > C p.V168L and the upstream homozygous variation rs2850015:T > C (NM_000629.2) of the common variations of *IFNAR1* gene. Sections B and D illustrate the heterozygous intronic changes rs2243592:T > G and rs2834196:A > G in the *IFNAR1* gene. Section E includes illustrations of the frameshift mutation rs333 (NM_001394783.1:c.554_585del) in the *CCR5* gene, while Section F presents the heterozygous missense variation rs1800452:G > A in the same gene.

Effects of ART

Figures 2A and 2B demonstrate the changes in CD4 + T cell count and HIV RNA suppression at 3rd and 6th months in patients receiving ART compared to their initial visit. The absolute CD4 + T cell count was calculated by multiplying the percentage of bead-rosetted CD4 + T lymphocytes in WBC by the total WBC count (cells/μl) and dividing the result by 100[18]. According to Fig. 2A, the change in HIV RNA suppression with viral load in patients showed no statistically significant difference between the 1 st and 3rd months ($p > 0.05$), while it showed high significance between the naive period and the 1 st and 3rd months ($p < 0.001$). In Fig. 2B, CD4 + T cell counts, there was no statistically significant difference between naive and 1 st month ($p > 0.05$), while there was

Polymorphisms	HIV + Patient (n = 22)	Control (n = 25)	*p* values
Genotype	**n (%)**	**n (%)**	
IFNAR1 rs2243592 T > G			0.343
TT	7 (31.82%)	13 (52%)	
TG + GG	15 (68.18%)	20 (48%)	
IFNAR1 rs2834196 A > G			0.343
AA	12 (54.55%)	17 (68%)	
AG + GG	10 (45.45%)	8 (32%)	
IFNAR1 rs2856973 T > A			0.427
TT	4 (18.18%)	7 (28%)	
TA + AA	18 (81.82%)	18 (72%)	
IFNAR1 rs2257167 G > C			0.097
GG	15 (68.18%)	22 (88%)	
GC + CC	7 (31.82%)	3 (12%)	
IFNAR1 rs2850015 T > C			0.521
TT	6 (27.27%)	9 (36%)	
TC + CC	16 (72.73%)	16 (64%)	
CXCR4 rs2680880 A > T			0.173
AA	18 (81.82%)	16 (64%)	
AT + TT	4 (18.18%)	9 (36%)	

Table 1. Genotype distributions of common polymorphisms of *IFNAR1* and *CXCR4* genes in study groups. For statistical analysis, a dominant genetic model was applied. The chi-square test was used to compare groups. No statistically significant differences were observed between the groups (p > 0.05 for all polymorphisms).

a significant difference between 1 st and 3rd months in terms of treatment response ($p < 0.05$). In addition, there was a high statistically significant difference between the naive period and the 3rd month ($p < 0.001$).

Sequence variants in CCR5, CXCR4 and IFNAR1 genes

The exonic regions and exon/intron boundaries of the *CCR5*, *CXCR4*, and *IFNAR1* receptor genes were sequenced by tNGS in 22 HIV+ patients and healthy 25 individuals. Rare and common genetic variations in *CCR5*, *CXCR4*, *and IFNAR1* genes have been identified in both HIV+ patients and healthy individuals. These variants were analyzed in terms of their ClinVar status, chromosomal locations, amino acid changes, protein regions, functional effects, SIFT and Polyphen2 scores, and minor allele frequencies (Table 2). We detected 19 SNPs, of which rs2243592:T > G, rs2834196:A > G, rs2856973:T > A, rs2257167:G > C and rs2850015:T > C in *IFNAR1* and rs2680880 in *CXCR4* are known common variants detected with more than 1% MAF in the populations shown in Table 2. All of the other remaining variants in Table 2 are known and rare variants that were detected with less than 1% MAF.

A total of ten variants have been identified in the *IFNAR1* gene, including intronic (rs2243592, rs2834196, rs2856973, rs180872415, rs17875880), missense (rs2257167), 5' UTR (rs112371220), upstream (rs2850015), and 3′ untranslated region variants (rs1371264018, rs74717635). Five of these (rs2243592 (intronic), rs2834196 (intronic), rs2856973 (intronic), rs2257167 (missense) and rs2850015 (upstream gene variation) are common variants. rs112371220 5' UTR variant is rare but was detected in both the patient and control groups. The rare variants rs180872415, rs1371264018, rs17875880 and rs74717635 were only observed in the patient group (Table 2).

CXCR4 gene rs2680880 intronic common variant was observed in both groups. The rare *CXCR4* gene variants rs374847203 (5' UTR), rs56400844 (missense), and rs184539659 (3' UTR) were observed only in the patient group. The rs2228014 (synonymous) variant was rare but observed in both groups.

Rare and missense variants of the *CCR5* gene rs1800452 rs1799863 were observed only in the patient group. *CCR5* gene rs333 high functional frameshift variation was only observed in the patient group. The rare *CCR5* gene rs41414147 3' UTR variant was also observed only in the control group (Table 2).

There were 18 variations of the *CCR5, CXCR4* and *IFNAR1* genes in HIV + group including 4 missense *mutations* (rs2257167:G > C,NP_000620.2:p.(Val168Leu);rs56400844:T > G,NP_003458.1:p.(Ile53Leu);rs1800452:G > A,NP_001381712.1:p.(Arg223Gln);rs1799863:T > A,NP_001381712.1:p.(Leu55Gln); 3 *3'UTR variants* (rs1371264018:T > TC, NM_000629.3:c.*436dup; rs74717635:C > T; rs184539659:C > T), 2 *5'UTR variants* (rs112371220:C > T, rs374847203:G > A), 6 *intronic variants* (rs2243592:T > G; rs2834196:A > G; rs2856973:T > A; rs180872415:T > A; rs17875880:C > T; rs2680880:A > T); 1 *upstream gene variant* (rs2850015:T > C); 1 *synonymous variant* (rs2228014:G > A) and 1 *frameshift mutation* (rs333 (NM_001394783.1:c.554_585del), (Table 2)[19].

In the control group, 9 *CCR5, CXCR4,* and *IFNAR1* gene variations were identified. We detected one *missense mutation* (rs2257167:G > C, NP_000620.2:p.(Val168Leu). 1 *3'UTR variant* (rs41414147:G>C) was only observed in the control group. 4 *intronic variants* (rs2243592:T>G, rs2834196:A>G, rs2856973:T > A, rs2680880:A > T).

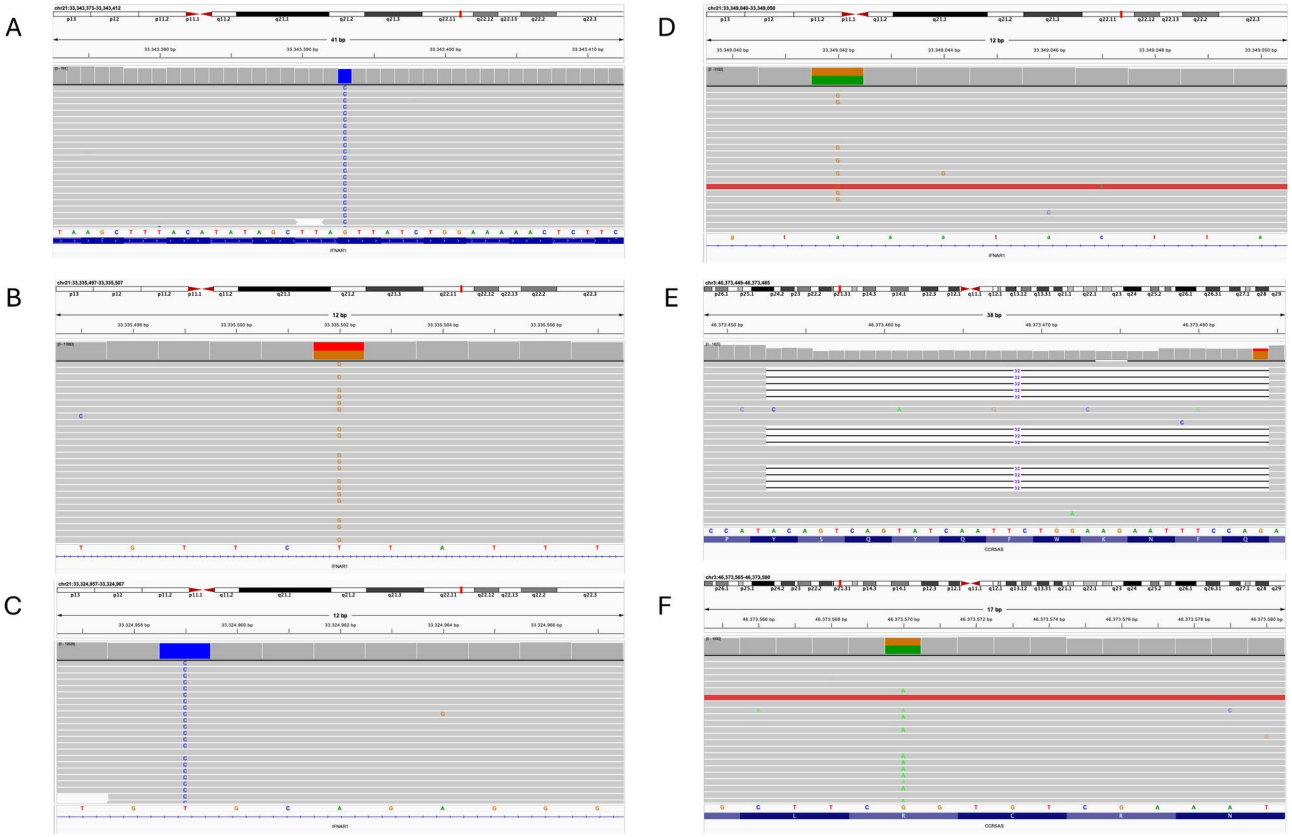


Fig. 1. The IGV illustrating the reads the *IFNAR1* gene common; missense, upstream and intronic and *CCR5* gene rare; frameshift and missense mutations. **A** and **C** show the missense mutation and the upstream homozygous variation of the common variations of *IFNAR1* gene. **B** and **D** illustrate the heterozygous intronic changes in the *IFNAR1* gene. Section **E** includes illustrations of the frameshift mutation in the *CCR5* gene, while Section F presents the heterozygous missense variation in the same gene. Integrative Genomics Viewer: IGV.

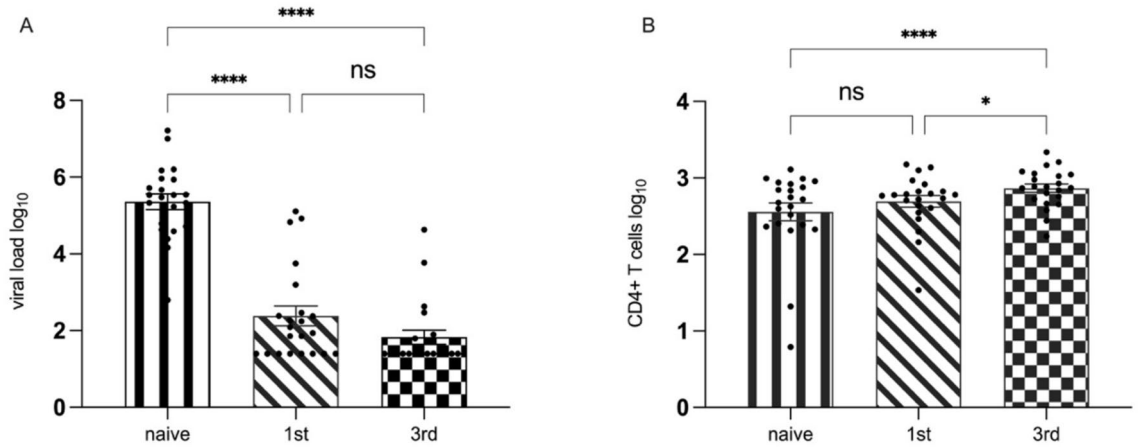


Fig. 2. Effects of ART, HIV RNA suppression, and CD4 + T cell count. **A** shows the viral load status of ART-naive HIV + and under ART HIV + patients. Data shown are the mean ± SEM (two-way analysis of variance, followed by Tukey's multiple comparisons tests, $^{****}p < 0.001$, naive vs 1 st and 3rd, non-significant 1 st vs 3rd, n = 22 for each group). HIV-1 viral load ($\log_{10}$ copies/mL). **B** shows the CD4 + T cell count of ART-naive HIV + and under ART HIV + patients. Data shown are the mean ± SEM (two-way analysis of variance, followed by Tukey's multiple comparisons tests, $^{****}p < 0.001$, 1 st vs 3rd; $^{*}p < 0.05$, non-significant naive vs 1 st, n = 22 for each group). CD4 + T cells ($\log_{10}$ cells/mL). ns: non-significant, SEM: standard error of the mean, ART: antiretroviral therapy, naive: ART-naive HIV + patient, 1st: 1 st month under ART HIV + patient, 3rd: 3rd month under ART HIV + patient.

HIV (+) Patients												
	Gene	dbSNP	Status	HGSV GRCh38	Variation type	Amino acid change	Functional effect	SIFT score	SIFT pred	Polyphen2 score	Polyphen2 pred	MAF (Ensembl)
1	*IFNAR1*	**rs2243592**[a]	Known	chr21:33335502 T > G	Intronic	-	Modifier					0.360
2	*IFNAR1*	**rs2834196**[a]	Known	chr21:33349042A > G	Intronic	-	Modifier					0.130
3	*IFNAR1*	**rs2856973**[a]	Known	chr21:33353821 T > A	Intronic	-	Modifier					0.870
4	*IFNAR1*	**rs2257167**[a]	Known	chr21:33343393G > C	Missense	V168L	Modarate	0.12	T	0.2	B	0.220
5	*IFNAR1*	**rs112371220**[c]	Known	chr21:33325011C > T	5' UTR	-	Modifier					0.004
6	*IFNAR1*	**rs2850015**[a]	Known	chr21:33324959 T > C	Upstream gene		Modifier					0.810
7	*IFNAR1*	**rs180872415**[b]	Known	chr21:33345225 T > A	Intronic	-	Modifier					0.001
8	*IFNAR1*	**rs1371264018**[b]	Known	chr21:33355983 T > TC	3' UTR/İnsertion		Modifier					0.001
9	*IFNAR1*	**rs17875880**[b]	Known	chr21:33345420C > T	Intronic		Modifier					0.011
10	*IFNAR1*	**rs74717635**[b]	Known	chr21:33356133C > T	3' UTR		Modifier					0.006
11	*CXCR4*	**rs2680880**[a]	Known	chr2:136115979A > T	Intronic	-	Modifier					0.169
12	*CXCR4*	**rs2228014**[c]	Known	chr2:136115514G > A	Synonymous		Low					0.062
13	*CXCR4*	**rs374847203**[b]	Known	chr2:136118072G > A	5' UTR	-	Modifier					0.001
14	*CXCR4*	**rs56400844**[b]	Known	chr2:136115771 T > G	Missense	I53L	Moderate	0.33	T	0.003	B	0.001
15	*CXCR4*	**rs184539659**[b]	Known	chr2:136114592C > T	3' UTR							0.001
16	*CCR5*	**rs1800452**[b]	Known	chr3:46373570G > A	Missense	R223Q	Moderate		T		B	0.012
17	*CCR5*	**rs1799863**[b]	Known	chr3:46373066 T > A	Missense	L55Q	Moderate	0	D	0.999	D	0.009
18	*CCR5*	**rs333**[b]	Known	chr3:46373452ACAGTCAGTATCAATTCTGGAAGAATTTCCAG/-	**Frameshift**	YSQYQFWKNFQ/X	**High**					0.029
Control group												
	Gene	**dbSNP**	**Status**	**Location**	**Variation type**	**a.a change**	**Functional effect**	**SIFT score**	**SIFT pred**	**Polyphen2 score**	**Polyphen2 pred**	**MAF (Ensembl)**
1	*IFNAR1*	**rs2856973**[a]	Known	chr21:33353821 T > A	Intronic	-	Modifier					0.870
2	*IFNAR1*	**rs2257167**[a]	Known	chr21:33343393G > C	Missense	V168L	Modarate	0.12	T	0.2	B	0.220
3	*IFNAR1*	**rs2850015**[a]	Known	chr21:33324959 T > C	Upstream gene		Modifier					0.810
4	*IFNAR1*	**rs2243592**[a]	Known	chr21:33335502 T > G	Intronic	-	Modifier					0.360
5	*IFNAR1*	**rs2834196**[a]	Known	chr21:33349042A > G	Intronic	-	Modifier					0.130
6	*IFNAR1*	**rs112371220**[c]	Known	chr21:33325011C > T	5' UTR	-	Modifier					0.004
7	*CXCR4*	**rs2680880**[a]	Known	chr2:136115979A > T	Intronic	-	Modifier					0.169
8	*CXCR4*	**rs2228014**[c]	Known	chr2:136115514G > A	Synonymous		Low					0.062
9	*CCR5*	**rs41414147**[d]	Known	chr3:46374251G > C	3' UTR	-	Modifier					0.008

Table 2. List of rare and common variants of *CCR5, CXCR4 and IFNAR1* genes in study groups. MAF: minor allele frequency, SIFT: sorting intolerant from tolerant, Polyphen: polymorphism phenotyping, T: tolerable, P: pathogenic, D: damaging, B: benign,. SIFT scores: < 0.05 damaging; ≥ 0.05 tolerable; Polyphen scores: > 0.908 probably damaging; > 0.446 ≤ 0.908 possibly damaging; ≤ 0.446 benign. [a]Common variants, bit is only seen in the HIV+ group, crare but seen in both groups, dit is only seen in the control group. [a.a.] change: amino acid change, dbSNP: Database of single nucleotide polymorphisms, 5'UTR: 5' untranslated regions, 3'UTR: 3' untranslated regions.

1 *upstream gene variant* (rs2850015:T>C), 1 *5'UTR variant* (rs1123712220:C>T) and 1 *synonymous variant* (rs2228014:G > A) were also observed in the HIV+patient group.

Considering the rare variations, *IFNAR1* rs180872415:T > A, rs1371264018:T > TC, rs17875880:C > T, rs74717635:C>T; *CXCR4* rs374847203:G>A, rs56400844:T > G, rs184539659:C > T; *CCR5* rs1800452:G > A, rs1799863:T > A and rs333 (NM_001394783.1:c.554_585del) were detected in HIV + patient since they detected only patient group. On the other hand, in the only control group, *CCR5* rs41414147:G > C was detected in only one person (Table 2).

The relationship between baseline CD4 + T cell count and viral load was evaluated using multiple linear regression analysis of demographic data such as age and gender in HIV + patients. The regression model for the viral load was not statistically significant, neither age (β = 0.014, p = 0.317) nor gender (β = 0.073, p = 0.866) was a significant predictor. Similarly, the model examining CD4 levels yielded non-significant results, with neither age (β = −0.003, p = 0.721) nor gender (β = 0.007, p = 0.782) demonstrating a meaningful association.

As a result of the evaluation of the comorbidities of the patients in terms of their HIV status and mutations, it was observed that the same variations were present in different diseases. According, to 2 male patients with *IFNAR1* rs22443592:T > G and rs2856973:T > A had syphilis. In addition to these variants, the rs2257167:G > C variant was also detected in another patient with syphilis. 2 patients with *IFNAR1* rs2850015:T > C and rs2856973:T > A variants had hepatitis B. Another 2 patients with *IFNAR1* rs2850015:T > C and rs2856973:T > A variants had type 2 diabetes. 3 patient with *IFNAR1* rs2850015:T > C variant had atopic dermatitis.

Common variants from *IFNAR1* and *CXCR4* genes were selected and analyzed according to CD4 + T cell count and HIV viral load among patients. Table 3 shows that the CD4 + T cell count status of patients carrying the *IFNAR1* rs2834196:A > G, rs2856973:T > A, rs2243592:T > G, rs2850015:T > C, rs2257167:G > C and *CXCR4* rs2680880:A > T variants in the naive, 1 st and 3rd mount. Similarly, Table 4 shows that the HIV viral load of patients carrying the *IFNAR1* rs2834196:A > G, rs2856973:T > A, rs2243592:T > G, rs2850015:T > C, rs2257167:G > C, and *CXCR4* rs2680880:A > T variants in the naive, 1 st and 3rd mount.

Table 3 shows the CD4 + T cell count status of HIV + patients carrying the *IFNAR1 and CXCR4* gene's common variants in different periods. Figure 3.A showed the CD4 + T cell count status of patients carrying the rs2856973 variant in the *IFNAR1* gene, ART-naive and under ART. Patients carrying the wild type TT genotype of rs2856973:T > A variant had higher CD4 + T cell count compared to subjects carrying the TA + AA genotypes of rs2856973:T > A variant in naive and 1 st month period ($p = 0.001$ and $p = 0.001$, respectively). Similarly, patients receiving ART with TT genotype of rs2856973:T > A showed a significantly higher CD4 + T cell count in the third month ($p = 0.001$) (Table 3). Figure 3.B. shows the CD4 + T cell count status of patients carrying the rs2680880:A > T variant in the *CXCR4* gene, ART-naive and under ART. Patients carrying the AA genotype of rs2680880:A > T SNP had lower CD4 + T cell count compared with patients carrying the AT + TT genotypes of rs2680880:A > T in naive and 1 st month periods ($p = 0.015$ and $p = 0.025$, respectively). No statistically significant difference was observed in 3rd month in this variant of patients ($p = 0.149$) (Table 3).

Table 4 shows the viral load of HIV + patients carrying the *IFNAR1 and CXCR4* gene's common variants in different periods. Only the viral load change between the 1 st and 3rd months of the AA wild type genotype of the *IFNAR1* rs2834196:A > G variation was slightly statistically significant ($p = 0.048$).

Discussion

It is known that disease prognosis varies among ART-naive HIV + patients depending on genetic differences between viral and host factors[20,21]. Genetic variations in genes encoding chemokines and their receptors, such as CCR5-Δ32, play a crucial role in determining susceptibility to HIV, particularly affecting viral load and disease progression. Genetic variations in genes such as *HLA* and *CCR5*, both common and rare, are estimated to account for only about 25% of the variability in viral load among patients. From a genetic standpoint on the pathogenesis of HIV-1, host genetics are thought to explain roughly 30% of the variability in HIV-1 viral load, with approximately 5% attributable to genetic loci outside the well-characterized major histocompatibility complex and CCR5 regions[22,23]. As a result, investigating the influence of host genetics on HIV pathogenesis is essential.

In this study, findings on the effect of variants of *CCR5*, *CXCR4,* and *IFNAR1* genes, which may be related to HIV-1 pathogenesis, are presented with the results obtained from host genetics. The analysis included HIV + patients residing in Turkey. The genetic makeup of each individual was analyzed to identify various progression patterns and to limit the influence of environmental and virogenetic factors.

Despite this, there have been relatively few genetic association studies on HIV pathogenesis so far, though some genetic variations have been identified as linked to disease progression in HIV-positive individuals. Although the genes role of *CCR5* and *CXCR4* are known to play roles in HIV entry into the host cell[4], and *IFNAR1*[24] is critical for immune system response. This study specifically examined the relationship between variants in these receptor genes and HIV pathogenesis. This study is the first preliminary investigation of the polymorphisms of the *CCR5*, *CXCR4,* and *IFNAR1* genes among HIV-infected patients in the Turkish population using tNGS.

CCR5 is a receptor that plays an important role in the immune response and acts as a co-receptor for HIV entry into CD4-positive T lymphocytes[25]. This gene has been found to lack expression of CCR5 protein due to a 32 bp deletion, (CCR5Δ32) in its coding region[26]. Homozygosity for the delta 32 allele appears to confer protection from HIV infection[27]. CCR5Δ32 in the *CCR5* gene has been found in individuals who are not infected with HIV-1, even among those with significant exposure to the virus. Although heterozygotes do not appear to be protected from HIV-1 infection, the Δ32 allele has been associated with a modest reduction in disease progression. Therefore, the Δ32 variant of CCR5 plays a crucial role as a host factor in both the transmission and pathogenesis of HIV-1[28]. The CCR5Δ32 allele has been demonstrated to confer partial protection against disease progression in HIV-infected individuals who are heterozygous for the CCR5-Δ32 allele. This is evidenced by the

Polymorphisms	Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff	*p* Value
***IFNAR1* rs2834196 A > G**				
	AG + GG, naive vs. AA, naive	−0,24	−0,6302 to 0,1502	0.460
	AG + GG, 1 st vs. AA, 1st	−0,1744	−0,5646 to 0,2158	0.769
	AG + GG, 3rd vs. AA, 3rd	−0,2046	−0,5948 to 0,1856	0.631
	AG + GG, 1 st vs. AG + GG, 3rd	−0,155	−0,5565 to 0,2465	0.860
	AA, 1 st vs. AA, 3rd	−0,1852	−0,5517 to 0,1813	0.667
***IFNAR1* rs2856973 T > A**				
	TA + AA, naive vs. TT, naive	−0,6164	−1,040 to −0,1931	**0.001**
	TA + AA, 1 st vs. TT, 1st	−0,5921	−1,015 to −0,1688	**0.001**
	TA + AA, 3rd vs. TT, 3rd	−0,6325	−1,056 to −0,2092	**0.001**
	TA + AA, 1 st vs. TA + AA, 3rd	−0,1641	−0,3960 to 0,06,773	0.301
	TT, 1 st vs. TT, 3rd	−0,2045	−0,6964 to 0,2873	0.814
***IFNAR1* rs2243592 T > G**				
	TG + GG, naive vs. TT, naive	−0,4665	−0,9765 to 0,04,347	0.090
	TG + GG, 1 st vs. TT, 1st	−0,3162	−0,8262 to 0,1938	0.449
	TG + GG, 3rd vs. TT, 3rd	−0,2009	−0,6717 to 0,2699	0.800
	TG + GG, 1 st vs. TG + GG, 3rd	−0,2021	−0,6087 to 0,2045	0.679
	TT, 1 st vs. TT, 3rd	−0,08,681	−0,6264 to 0,4527	0.996
***IFNAR1* rs2850015 T > C**				
	TC + CC naive vs. TT, naive	0,1366	−0,3890 to 0,6622	0.970
	TC + CC, 1 st vs. TT, 1st	0,004,954	−0,5207 to 0,5306	> 0.999
	TC + CC, 1 st vs. TC + CC, 3rd	−0,1884	−0,5844 to 0,2076	0.718
	TT, 1 st vs. TT, 3rd	−0,1352	−0,7149 to 0,4444	0.981
	TC + CC, 3rd vs. TT, 3rd	0,0581	−0,4675 to 0,5837	0.999
***IFNAR1* rs2257167 G > C**				
	GC + CC, naive vs. GG, naive	0,1287	−0,2941 to 0,5515	0.943
	GC + CC, 1 st vs. GG, 1st	0,122	−0,3008 to 0,5448	0.954
	GC + CC, 1 st vs. GC + CC, 3rd	−0,1931	−0,6593 to 0,2732	0.819
	GG, 1 st vs. GG, 3rd	−0,1614	−0,4799 to 0,1571	0.661
	GC + CC, 3rd vs. GG, 3rd	0,1537	−0,2691 to 0,5764	0.886
CXCR4 rs2680880 A > T				
	AT + TT, naive vs. AA, naive	0,5313	0,07,147 to 0,9911	**0.015**
	AT + TT, 1 st vs. AA, 1st	0,5008	0,04,104 to 0,9607	**0.025**
	AT + TT, 1 st vs. AT + TT, 3rd	−0,07,584	−0,6101 to 0,4584	0.998
	AA, 1 st vs. AA, 3rd	−0,1927	−0,4446 to 0,05,913	0.223
	AT + TT, 3rd vs. AA, 3rd	0,384	−0,07,584 to 0,8438	0.149

Table 3. CD4 + T cell count status of HIV + patients carrying the *IFNAR1 and CXCR4* genes common variants in different periods. Data shown are the mean ± SEM (two-way analysis of variance, followed by Tukey's multiple comparisons tests. SEM: standard error of the mean, ART: antiretroviral therapy, naive: ART-naive HIV + patient, 1st: 1 st month under ART HIV + patient, 3rd: 3rd month under ART HIV + patient.

increased frequency of the allele among HIV-infected individuals who have been infected for a long period and have not progressed to AIDS[29].

In our study, rare *CCR5* rs333 (NM_001394783.1:c.554_585del), rs1800452:G > A, rs1799863:T > A variations were observed in HIV + patients in the *CCR5* gene, whereas rs41414147:G > C variation is a rare variation seen only in the control group. The predicted effect of rs333 (NM_001394783.1:c.554_585del) on protein was determined to have a high effect on CCR5 protein by bioinformatic analysis tool Ensembl VEP (GRCh38).

Rare missense mutations which that *CCR5* rs1800452:G > A, NP_001381712.1:p.(Arg223Gln)[19] have been previously presented in ClinVar[30]. However, there are no studies in the literature investigating their phenotypic effects on HIV infection. rs333 (NM_001394783.1:c.554_585del), NP_001381712.1:p.(Ser185IlefsTer32) frameshift mutations have been previously presented in ClinVar for HIV infection[31]. *CCR5* NP_001381712.1:p.(Leu55Gln) mutation was not previously defined in the ClinVar database, and it was first identified in HIV + patients in this study. The pathogenicity of these mutations was predicted to be "moderate" for, NP_001381712.1:p.(Arg223Gln) and NP_001381712.1:p.(Leu55Gln); and "high" for NP_001381712.1:p.(Ser185IlefsTer32).

The results of studies examining the *CXCR4* gene in patients infected with HIV-1 support the conclusion that, despite the presence of the Δ32 variation, HIV-1 in patients with incomplete resistance is associated with the acquisition of *CXCR4* variants. The pathogenic potential of these viruses might vary compared to that of

Polymorphisms	Tukey's multiple comparisons test	Mean Diff	95,00% CI of diff	*p* Value
***IFNAR1* rs2243592 T > G**				
	TG + GG, 1 st vs. TT, 1st	−0,8411	−2,165 to 0,4824	0.496
	TG + GG, 1 st vs. TG + GG, 3rd	0,1587	−0,8522 to 1,170	0.999
	TT, 1 st vs. TT, 3rd	1,41	−0,07,012 to 2,889	0.072
	TG + GG, 3rd vs. TT, 3rd	0,4098	−0,9137 to 1,733	0.977
***IFNAR1* rs2834196 A > G**				
	AG + GG, 1 st vs. AA, 1st	−0,6011	−1,775 to 0,5729	0.748
	AG + GG, 1 st vs. AG + GG, 3rd	−0,1092	−1,322 to 1,103	> 0.999
	AA, 1 st vs. AA, 3rd	1,112	0,004,684 to 2,219	**0.048**
	AG + GG, 3rd vs. AA, 3rd	0,6196	−0,5545 to 1,794	0.718
***IFNAR1* rs2856973 T > A**				
	TA + AA, 1 st vs. TT, 1st	−0,00,625	−1,653 to 1,641	> 0.999
	TA + AA, 1 st vs. TA + AA, 3rd	0,6169	−0,3057 to 1,539	0.428
	TT, 1 st vs. TT, 3rd	0,286	−1,671 to 2,243	0.999
	TA + AA, 3rd vs. TT, 3rd	−0,3372	−1,984 to 1,310	0.998
***IFNAR1* rs2257167 G > C**				
	GC + CC, 1 st vs. GG, 1st	−0,4932	−1,838 to 0,8519	0.943
	GC + CC, 1 st vs. GC + CC, 3rd	0,4599	−1,044 to 1,964	0.978
	GG, 1 st vs. GG, 3rd	0,6019	−0,4254 to 1,629	0.599
	GC + CC, 3rd vs. GG, 3rd	−0,3511	−1,696 to 0,9939	0.991
***IFNAR1* rs2850015 T > C**				
	TC + CC, 1 st vs. TT, 1st	0,4091	−0,9709 to 1,789	0.982
	TC + CC, 1 st vs. TC + CC, 3rd	0,7097	−0,2561 to 1,675	0.308
	TT, 1 st vs. TT, 3rd	0,1489	−1,428 to 1,726	> 0.999
	TC + CC, 3rd vs. TT, 3rd	−0,1517	−1,532 to 1,228	> 0.999
***CXCR4* rs2680880 A > T**				
	AT + TT, 1 st vs. AA, 1st	1,545	−0,01,757 to 3,108	0.054
	AT + TT, 1 st vs. AT + TT, 3rd	1,415	−0,4426 to 3,272	0.265
	AA, 1 st vs. AA, 3rd	0,3661	−0,5094 to 1,242	0.891
	AT + TT, 3rd vs. AA, 3rd	0,497	−1,066 to 2,060	0.973

Table 4. Viral load of HIV + patients carrying the *IFNAR1 and CXCR4* genes common variants in different period. Data are presented as mean ± SEM. Statistical significance was assessed using two-way analysis of variance, followed by Tukey's multiple comparisons test.

late-stage X4 viruses or early X4 viruses acquired by individuals with different *CCR5* genotypes[32]. As these coreceptors are crucial to HIV-1 entry, they represent a promising field of research for the development of potential treatments or preventatives. Blocking cell entry effectively prevents viral replication, and when combined with other medications for sustained suppression, it can terminate disease progression. However, as the disease advances, more patients develop viral strains that can bind to the CXCR4 receptor. While most of these strains are CXCR4-targeting, some exhibit dual tropism. For these patients, developing drugs that target CXCR4 could be crucial[33]. Due to the therapeutic importance of the CXCR4 receptor, it is important to analyze the gene. Possible mutations in the gene may affect the response to treatment and progression.

According to the results of our study, in the *CXCR4* gene, 1 common and 4 rare variations have been detected. There were rs2680880:A > T intronic common, rs2228014:G > A synonymous, rs374847203: G > A 5'UTR, rs56400844:T > G, rs184539659:C > T 3'UTR. *CXCR4* rs56400844 and I53L missense mutation have been previously presented in Clinvar[34]. However, there are no studies in the literature investigating their phenotypic effects on HIV infection. In our study group, this rare variant rs56400844:T > G, (NM_003467.3, NP_003458.1:p.(Ile53Leu)) was detected in one resistant patient. The *CXCR4* gene variant NM_003467.2, p.Ile53Leu (c.157A > C) has been documented in the literature in at least three individuals: two with juvenile idiopathic arthritis and one with primary immunodeficiency[35,36]. The rs2680880:A > T intronic variant, which is common and classified as benign based on its frequency in the local population, was found in 36% of the 32 patients examined in a study of primary immunodeficiencies[37].

In our study group shows the CD4 + T cell count status of patients carrying the rs2680880:A > T variant in the *CXCR4* gene, with and without ART. Results showed that patients carrying the AA genotype of rs2680880:A > T SNP had lower CD4 + T cell count compared with patients carrying the AT + TT genotypes of rs2680880 in naive and 1 st month periods ($p = 0.015$ and $p = 0.025$, respectively). Petersen et al. previously observed a silent mutation rs2228014 in the codon 138 of the *CXCR4* gene in 1 HIV-1 seropositive patient and 1 HIV-1 seronegative control[38]. We detected this rare variant in 4 patients and 2 controls in our study. rs374847203:G > A-

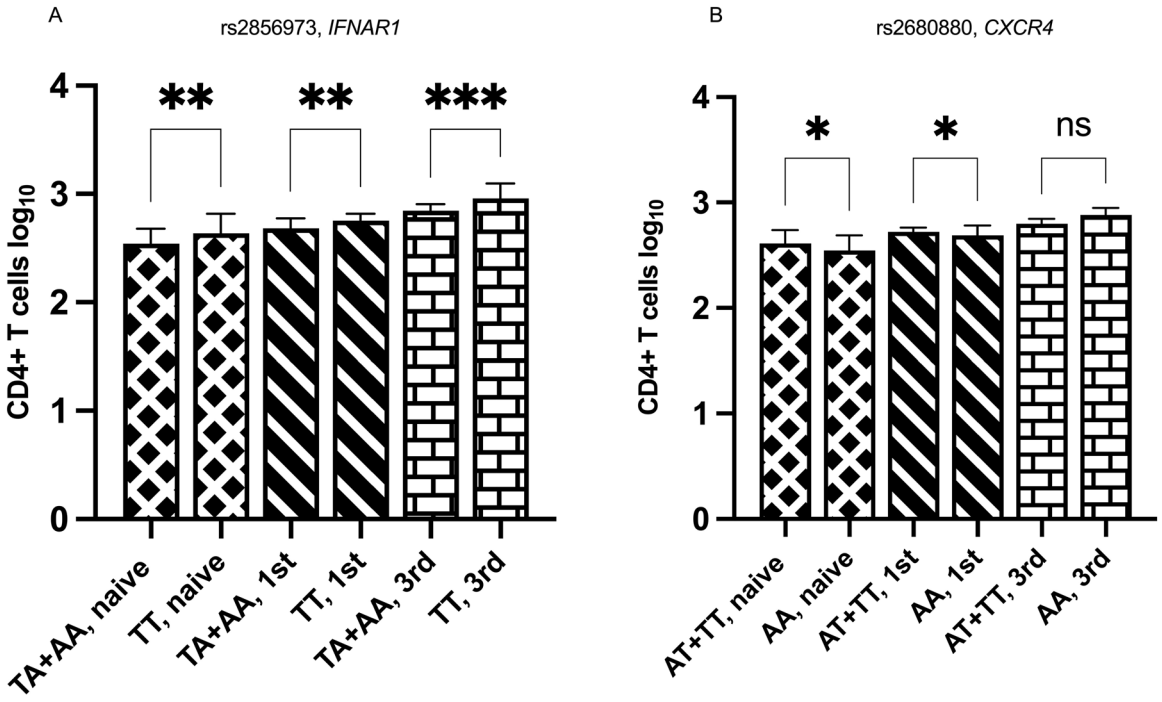


Fig. 3. Genotypic association between rs2856973 and rs2680880 SNPs and the CD4 + T cell count. Data shown are the mean ± SEM (two-way analysis of variance, followed by Tukey's multiple comparisons tests. **A**: **p < 0.01, TA + AA naive vs TT naive and TA + AA 1 st vs TT 1 st, ***$p < 0.001$, TA + AA 3rd vs TT 3rd, n = 22 for each group); **B**: *$p < 0.05$, AT + TT naive vs AA naive and AT + TT 1 st vs AA 1 st, non-significant, AT + TT 3rd vs AA 3rd, n = 22 for each group). SEM: standard error of the mean, naive: ART-naive HIV + patient, 1st: 1 st month under ART HIV + patient, 3rd: 3rd month under ART HIV + patient, SNPs: single nucleotide polymorphisms.

5'UTR and rs184539659:C > T- 3'UTR variations were not previously defined in ClinVar database, and it was first identified in two different HIV + patients in this study.

In our study, IFNAR1 subunit gene *IFNAR1* rs2243592:T > G, rs2834196:A > G, rs2856973:T > A, rs2257167:G > C and rs2850015:T > C were common variants, rs180872415:T > A, rs1371264018:T > TC, rs17875880:C > T and rs74717635:C > T variants were observed only in the HIV + group, while rs112371220 was detected in both groups, although it was rare. A female patient with the *IFNAR1* gene in addition to the rare rs180872415:T > C variant had vitiligo. A male patient with *IFNAR1* rs112371220:C > T rare variation had alopecia areata.

A study by Diop et al. analyzed the role of polymorphisms of the *IFNAR1* in the development of AIDS in a French population. In this study, rs2257167:G > C NM_000629.3:c.502G > C and intronic SNP IFNAR1_30127 variations were found to be associated with AIDS susceptibility and the promoter region of the *IFNAR1* gene was seen to be more polymorphic than the exonic regions, with eight polymorphisms[39]. These variants were also found in our study but no prognostic association was detected. Other studies have also indicated that rs2257167:G > C, NP_000620.2:p.(Val168Leu) polymorphism also plays a role in susceptibility to Multiple Sclerosis (MS) and malaria. Missense mutations which as the Val168Leu mutation have been previously presented in ClinVar[40]. However, there are no studies in the literature investigating their phenotypic effects on HIV infection. None of the other variations we detected in the *IFNAR1* gene have been previously described in the ClinVar database.

We investigated the CD4 + T cell count status of HIV + patients carrying the *IFNAR1* gene common variants in different periods. CD4 + T cell count status of patients carrying the rs2856973:T > A variant in the *IFNAR1* gene, with and without ART. Patients carrying the wild type TT genotype of rs2856973:T > A variant had higher CD4 + T cell count compared with subjects carrying the TA + AA genotypes of rs2856973:T > A variant in naive and 1 st month ($p = 0.001$ and $p = 0.001$, respectively). Similarly, patients with TT genotype of rs2856973:T > A had higher CD4 + T cell counts statistically highly significant in the 3rd month ($p = 0.001$). In a study by Diop et al. in the French population, the rs2856973:T > A variant of the IFNAR1 gene was detected, and the rs2856973:T > A variant was also detected in the SARS-CoV-2 infection study conducted in the Turkish population[39,41]. In our study is the first study investigating the polymorphisms of *IFNAR1* in HIV + individuals in the Turkish population. Our results, which indicate a possible role of the IFNAR pathway in susceptibility to HIV-1 infection or progression to AIDS, need to be confirmed by studies.

Numerous studies have investigated the roles of CCR5, CXCR4, and IFNAR1 receptors in various human diseases. However, there is a limited amount of research exploring how genetic variations in the *CCR5*, *CXCR4*, and *IFNAR1* genes are associated the pathogenesis of HIV infection. According to our knowledge, the present

study is the first one in the literature investigating the effects of variations in *CCR5, CXCR4,* and *IFNAR* genes in ART-naive HIV + and under ART HIV + Turkish patients.

In summary, the results of this study suggest that intronic variations in the *IFNAR1* rs2856973:T > A and *CXCR4* rs2680880:A > T genes can contribute to modifications in HIV progression and CD4 + T cell recovery under ART.

The study should be extended to larger patient groups to see the possible effect of rare variations. We observed that some variations in the *CCR5, CXCR4, and IFNAR1* genes have resulted in decreased and increased CD4 + T cell count and HIV copy load. Our results suggest that specific variations in the genes may be responsible for CD4 + T cell count and HIV copy load that we identified as a risk in HIV + patients.

Regarding progression and targeted therapy, it is important to investigate the effects of *CCR5* and *CXCR4*, which are the main coreceptors involved in the linkage of the virus, and the *IFNAR1* gene, which plays a crucial role in the immune response. In this context, the current study suggests that future research should focus on examining gene variations and mutations in *CCR5, CXCR4,* and *IFNAR1* that influence prognostic outcomes, using larger study populations and developing a comprehensive genetic panel.

Limitations

While the findings of this study provide valuable insights, several limitations should be considered. The relatively small sample size may limit the statistical power and generalizability of the results. The study should be extended to larger patient groups to see the possible effect of rare variations. Additionally, the study population was drawn from a single center, potentially limiting the generalizability of the results to other ethnic or geographic populations. To strengthen these findings, future studies should include larger and more diverse cohorts**.**

Data availability

Data is provided within the manuscript or supplementary information files. The datasets analysed during the current study are available in the [NCBI, ClinVAR, Variantvalidator] repository. The following list of NCBI accession identifiers contains these variants; NM_000629.3, NM_001394783.1, NM_003467.3 The following list of ClinVar accession identifiers contains these variants; VCV001249487.5, VCV000008186.2, VCV000008184.6, VCV000709395.34, VCV001170802.11 The following list of VariantValidator accession identifiers contains these variants; NP_000620.2:p.(Val168Leu); NP_003458.1:p.(Ile53Leu), NP_001381712.1:p.(Arg223Gln), NP_001381712.1:p.(Leu55Gln), NM_000629.3:c.*436dup, NM_001394783.1:c.554_585del).

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Author contributions

KCP-U: Funding acquisition, Project administration, Conceptualization, Methodology, Investigation, Writing-Original Draft, Review & Editing. ZGT-S: Methodology, Conceptualization, Investigation, Data Analysis, Manuscript preparation, Review & Editing. AB: Conceptualization, Data Curation, Review&Editing. HS: Data Curation. SM: Review & Editing. BM: Clinic Examinations. FT: Clinic Examinations, Review & Editing.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

The study was conducted by the Declaration of Helsinki on medical research consisting of a human study group. The protocol was approved by the Ethics Committee of the Haliç University (Approval Number: 2021/148). Written informed consent was obtained from all participants between 10.01.2022 and 16.01.2023.

Additional information

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