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RESEARCH ARTICLE OPEN ACCESS

Association of IFNL2 (IL-28A) gene polymorphisms and human herpesvirus-7 detection in patients with chronic myeloid leukaemia

Mohammed SS[1]ID, Almahbobi TF[2]ID, Ibrahim MS[3], Al-Alwany SHM[4]ID

[1]*Department of Community Health Technologies, Babylon Technical Institute, Al-Furat Al-Awsat Technical University, Babylon, Iraq*
[2]*College of Medicine, Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences*
[3]*Department of Microbiology, College of Medicine, Al-Mustansiriyah University*
[4]*Department of Biology, College of Science, University of Babylon, Iraq*

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Abstract
Objective: This study aimed to investigate the potential association between genetic polymorphisms in the IFNL2 (IL-28A) gene, detection of Human Herpesvirus-7 (HHV-7) DNA, and susceptibility to Chronic Myeloid Leukaemia (CML) in an Iraqi population.
Methods: A case-control study was conducted involving 100 CML patients and 100 apparently healthy controls (AHC). Genomic DNA was extracted from peripheral blood. IFNL2 gene polymorphisms were analysed by PCR amplification of a 604 bp fragment followed by Sanger sequencing. HHV-7 DNA was detected using conventional PCR targeting a 458 bp fragment. Genotype and allele frequencies were compared using Chi-square and Fisher's exact tests. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs), adjusting for age and sex.
Results: The distribution of IFNL2 genotypes differed significantly between CML patients and controls ($p<0.05$). The CC (wild-type) genotype was less frequent in patients (44%) than in controls (72%), while the CT (heterozygous) genotype was more frequent in patients (34%) than in controls (12%). The CT genotype was associated with significantly increased odds of CML (adjusted OR: 3.86, 95% CI: 1.21-12.33, $p=0.022$). HHV-7 DNA was detected in 19% (19/100) of CML patients but in none of the controls (0/100), a statistically significant difference ($p<0.001$).
Conclusion: The findings suggest a potential association between IFNL2 gene polymorphisms, HHV-7 infection, and CML susceptibility. The CT genotype and HHV-7 detection were significantly more prevalent in CML patients. These factors may represent host-viral interaction pathways relevant to CML pathogenesis, though further validation in larger cohorts is warranted.

Keywords: IL-28; Polymorphism; HHV7; Chronic Myeloid Leukaemia; PCR

Plain English Summary

This research studied two factors that might influence the risk of developing a blood cancer called Chronic Myeloid Leukaemia (CML): 1) variations in a specific human gene (IL-28) involved in antiviral defence, and 2) infection with a common virus (Human Herpesvirus-7 or HHV-7). We compared 100 patients with CML to 100 healthy individuals. We found that a specific variation in the IL-28 gene (the CT genotype) was more common in patients with CML. We also found that the HHV-7 virus was only present in the blood of CML patients and not in healthy individuals. This suggests that both this specific

Correspondence:
Almahbobi Taghreed F
College of Medicine
Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences
+9647812179590, taghreed.f.almahbobi@jmu.edu.iq

gene variation and HHV-7 infection might be linked to a higher risk of developing CML. More research is needed to confirm these findings.

Introduction

Chronic Myeloid Leukaemia (CML) is a myeloproliferative neoplasm characterised by the genetic translocation t(9;22) (q34;q11.2), known as the Philadelphia chromosome. This translocation results in the formation of the BCR-ABL1 fusion oncogene, which encodes a constitutively active tyrosine kinase driving uncontrolled myeloid cell proliferation (1). While BCR-ABL1 is the primary driver, additional genetic and environmental factors may influence disease susceptibility and progression (2).

The interleukin-28B (IL-28B) gene, which encodes interferon-lambda-3 (IFN-λ3), plays a crucial role in innate antiviral immunity. It signals through the JAK-STAT pathway, inducing interferon-stimulated genes (ISGs) that establish an antiviral state (3). The IFNL2 gene (encoding IFN-λ2 or IL-28A) is located adjacent to IL-28B on chromosome 19 (4). Polymorphisms in this genomic region, particularly in IL-28B, are known to influence outcomes in viral infections like Hepatitis C (5, 6). IFN-λs exhibit potent antiviral and antitumor activities, with expression levels linked to specific genetic alleles (6).

Human Herpesvirus-7 (HHV-7) is a ubiquitous betaherpesvirus that establishes lifelong latency following primary infection, typically in childhood (7, 8). While often asymptomatic, HHV-7 has been implicated in various lymphoproliferative disorders and can infect CD4+ T-cells and lymphoblastoid cell lines (9). HHV-7 infection can induce cell cycle arrest and impair T-cell cytotoxicity, potentially creating an environment conducive to oncogenesis (10, 11).

Given the roles of IFNL2 in immune surveillance and the potential oncogenic associations of HHV-7, this study aimed to investigate the association between IFNL2 gene polymorphisms, HHV-7 DNA detection, and CML susceptibility in an Iraqi population.

Materials and Methods

Study Population

A case-control study was conducted with 100 CML patients and 100 apparently healthy controls (AHC). CML patients were recruited based on WHO diagnostic criteria, with ages ranging from 19 to 72 years. The control group consisted of age- and sex-matched healthy volunteers with no history of malignancy. Ethical approval was obtained from the institutional review board (Project No. M250942, dated March 15, 2023), and informed consent was acquired from all participants.

DNA Extraction

Approximately 2 mL of venous blood was collected from each participant into EDTA tubes. Genomic DNA was extracted from 200 µL of whole blood using the G-Spin Total DNA Extraction Kit (iNtRON Biotechnology, Korea) according to the manufacturer's instructions. The extracted DNA was stored at -20°C until analysis.

Primer Selection and PCR Amplification

Specific primers were used for amplification:

HHV-7
Forward: 5'-AGTTCCAGCACTGCAATCG-3',
Reverse: 5'-CACAAAAGCGTCGCTATCAA-3'
(amplicon: 458 bp).

IFNL2 (IL-28A)
Forward: 5'-TCCTCCAATCCCACCAGGAT-3',
Reverse: 5'-CTGCTCAGAGCTCACAGACC-3'
(amplicon: 604 bp).

PCR was performed in a 25 µL reaction volume containing 12.5 µL Master Mix, 1 µL of each forward and reverse primer, 5 µL of template DNA, and 5.5 µL nuclease-free water. The thermal cycling conditions are summarised in Table 1.

Table 1: Thermal Cycling Conditions for Conventional PCR

Gene	Step	Temperature	Time	Number of Cycles
HHV-7	Initial Denaturation	95°C	5 min	35
	Denaturation	95°C	1 min	
	Annealing	58°C	45 sec	
	Extension	72°C	2 min	
	Final Extension	72°C	5 min	
IL-28	Initial Denaturation	95°C	5 min	40
	Denaturation	95°C	1 min	
	Annealing	59.5°C	45 sec	
	Extension	72°C	2 min	
	Final Extension	72°C	5 min	

DNA Genotyping and Sequencing
PCR products were electrophoresed on a 1.5% agarose gel and visualised. The IFNL2 amplicons were purified and sent for Sanger sequencing (Macrogen, South Korea). Sequence analysis was performed using BioEdit software, and variants were identified by comparison with the NCBI reference sequence (GenBank acc. no. DQ126336.2).

Statistical Analysis
Data were analysed using SPSS software. Continuous variables were compared using Student's t-test. Categorical variables were compared using the Chi-square (χ^2) or Fisher's exact test. Genotype associations with CML were assessed using logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs), adjusted for age and sex. A p-value of < 0.05 was considered statistically significant.

Results

Demographic Characteristics of the Study Groups
The mean age of CML patients was 47.6 ± 10.3 years, and that of controls was 44.8 ± 11.1 years. The difference in age distribution was not statistically significant (p=0.06). The gender distribution was also similar between patients (56% male, 44% female) and controls (58% male, 42% female), with no significant difference (p=0.06). The distribution of CML patients across different age strata is shown in Table 2.

Table 2: Distribution of CML Patients According to Age Strata and Gender

Age Stratum (Years)	Male (n, %)	Female (n, %)	Total (n, %)
18-35	20 (20%)	14 (14%)	34 (34%)
36-55	26 (26%)	18 (18%)	44 (44%)
56-72	10 (10%)	12 (12%)	22 (22%)
Total	56 (56%)	44 (44%)	100 (100%)

Detection of HHV-7 DNA
HHV-7 DNA was detected in 19% (19/100) of CML patients, while none of the controls (0/100) tested positive, a highly significant difference (p<0.001) (Table 3, Figure 1).

Table 3. PCR Results for HHV-7 DNA in Blood Specimens of Study Groups

HHV-7 Result	CML Group (n=100)	AHC* Group (n=100)	P-value
Positive	19 (19%)	0 (0%)	<0.001
Negative	81 (81%)	100 (100%)	

*AHC: Apparently Healthy Control

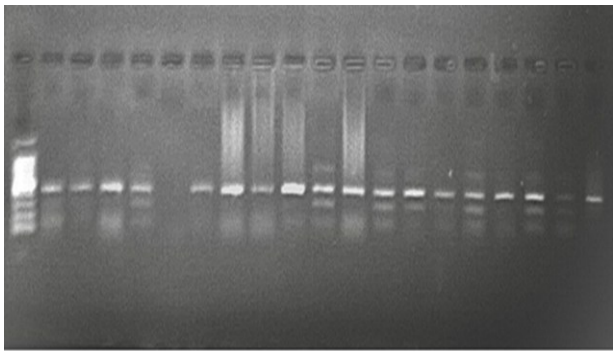

Figure 1: Agarose gel electrophoresis (1.5%) showing PCR detection of HHV-7 DNA (458 bp) in patients with CML. Lanes: M, DNA ladder; P, positive samples; N, negative control

Among HHV-7 positive patients, the highest frequency was observed in the 36-55 years age group (52.6%, p=0.04) (Table 4).

Table 4. Frequency of HHV-7 PCR Results Among CML Patients According to Age Strata

Age Stratum (Years)	Total CML Patients (n, %)	HHV-7 Positive (n, %)	HHV-7 Negative (n, %)	P-value
18-35	34 (34%)	4 (21.1%)	30 (37.0%)	0.04
36-55	44 (44%)	10 (52.6%)	34 (42.0%)	

56-72	22 (22%)	5 (26.3%)	17 (21.0%)
Total	100 (100%)	19 (100%)	81 (100%)

Furthermore, a significant gender difference was noted, with males constituting a larger proportion (63.2%) of HHV-7 positive cases (p=0.043) (Table 5).

Table 5. HHV-7 Infection Rates in CML Patients Based on Gender

Gender	HHV-7 Positive (n=19)	P-value
Male	12 (63.2%)	0.043
Female	7 (36.8%)	
Total	19 (100%)	

IFNL2 (IL-28A) Genotyping and Polymorphism Analysis

Sequencing of the 604 bp IFNL2 fragment revealed a polymorphism that was 99.5% identical to the reference sequence (DQ126336.2). The exact position of the sequenced fragment within chromosome 19 is illustrated in Figure 2.

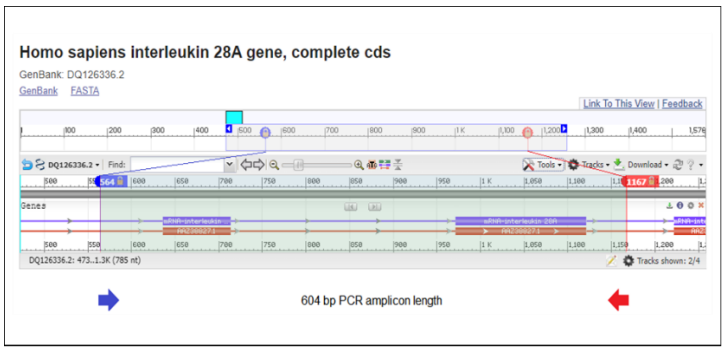

Figure 2: Schematic representation of the exact position of the sequenced 604 bp amplicon within the IFNL2 gene on chromosome 19 (GenBank acc. no. DQ126336.2). The blue arrow indicates the start point, and the red arrow indicates the endpoint

The genotype distribution of the identified SNP is shown in Table 6. A significant difference was observed between CML patients and controls ($p<0.05$). The CC genotype was less frequent in patients (44%) than in controls (72%). Conversely, the CT genotype was more frequent in patients (34%) than in controls (12%). Logistic regression analysis, adjusted for age and sex, revealed that the CT genotype was associated with a significantly increased odds of CML (adjusted OR: 3.86, 95% CI: 1.21-12.33, p=0.022). The T allele was also significantly more frequent in patients than in controls (OR: 1.44, 95% CI: 0.20-1.96, p=0.037).

Table 6: Genotyping of the IFNL2 (IL-28A) Gene Polymorphism in CML Patients and Controls

Genotype	CML Patients (n=100)	Controls (n=100)	Adjusted OR (95% CI)	P-value
CC	44 (44%)	72 (72%)	Reference	-
CT	34 (34%)	12 (12%)	3.86 (1.21 - 12.33)	0.022
TT	22 (22%)	16 (16%)	0.44 (0.12 - 1.63)	0.21
Allele	**CML (n=200)**	**Controls (n=200)**	**OR (95% CI)**	**P-value**
C	122 (61%)	156 (78%)	Reference	-
T	78 (39%)	44 (22%)	1.44 (0.20 - 1.96)	0.037

*OR: Odds Ratio; CI: Confidence Interval. Adjusted for age and sex.

After sequencing, the samples were submitted to NCBI, and the accession numbers for the IL-28 gene nucleotide sequences (new records) are: LC822750, LC822749, and LC822751

Discussion

This case-control study investigated the potential interplay between host genetics, specifically the IFNL2 (IL-28A) gene, and HHV-7 infection in Iraqi patients with CML. Our principal findings are twofold: first, we observed a significantly higher detection rate of HHV-7 DNA in CML patients (19%) compared to healthy controls (0%); and

second, we identified a marked difference in the distribution of IFNL2 genotypes between the two groups, with the CT genotype being independently associated with increased odds of CML.

The complete absence of HHV-7 DNA in our control group is striking and warrants careful consideration. While HHV-7 is a ubiquitous virus with high seroprevalence in the general population, the detection of its DNA in peripheral blood is typically associated with active replication or reactivation (8, 12). Our finding of a 19% prevalence in CML patients suggests that active HHV-7 infection may be more common in this patient group. This contrasts with a study by Handous et al. (13), which reported a lower prevalence (4.2%) of HHV-7 DNA in leukaemia patients. This discrepancy could be attributed to differences in patient populations, leukaemia subtypes, sample types, or the sensitivity of the molecular methods employed. The higher frequency of HHV-7 positivity in male CML patients and in the 36-55 age group may reflect sex-related immunological differences or age-associated changes in immune competence that permit viral reactivation (14). It is plausible that the immunocompromised state associated with CML or its treatment creates a permissive environment for HHV-7 reactivation (10). Alternatively, persistent HHV-7 infection could contribute to immune dysregulation or chronic antigenic stimulation, potentially creating a pro-leukemogenic microenvironment (10, 15).

The genetic component of our study revealed a significant association between IFNL2 polymorphisms and CML susceptibility. The protective CC genotype was considerably more frequent in controls (72%) than in patients (44%), while the CT genotype was significantly more prevalent in patients (34% vs. 12% in controls). This shift in genotype distribution remained significant after adjustment for age and sex, with the CT genotype conferring a nearly 4-fold increase in the odds of CML. The IFNL2 gene encodes interferon-lambda-2, a cytokine crucial for antiviral defence (3). Polymorphisms in this gene can alter cytokine expression and function, potentially impairing the host's ability to control viral infections (5, 6). Our findings align with the conceptual framework that genetic variants compromising innate immunity can increase susceptibility to virally-associated malignancies. The observed association mirrors the well-established role of IL28B polymorphisms in Hepatitis C virus infection outcomes (16), suggesting a broader role for interferon-lambda genetics in modulating host-virus interactions across different disease contexts.

A compelling implication of our study is the potential synergistic effect between IFNL2 genetic variation and HHV-7 infection. Individuals carrying the CT genotype may have a suboptimal interferon-lambda response, potentially rendering them less capable of suppressing HHV-7 replication (4, 17). The resulting persistent or recurrent viral activity could, in turn, lead to chronic immune activation and inflammation, processes known to influence hematopoietic stem cell dynamics and potentially contribute to leukemogenesis (11, 16). This model of genetic susceptibility enabling viral persistence, which then drives oncogenesis, provides a plausible pathway linking our two main findings.

Study limitations

Several limitations of this study must be acknowledged. The sample size, while adequate for detecting the strong associations presented, is moderate and from a single centre, which may limit the generalizability of the findings. The use of conventional PCR for HHV-7 detection, while specific, does not provide quantitative data on viral load; confirmation with quantitative PCR in future studies would be valuable. Furthermore, the cross-sectional, case-control design cannot establish causality or determine the temporal relationship between HHV-7 infection and the development of CML. It remains unclear whether HHV-7 reactivation is a cause or a consequence of the leukemic process. Finally, the functional consequences of the identified IFNL2 polymorphism were not characterised and represent an important avenue for future research.

Despite these limitations, our study provides novel insights into the potential roles of HHV-7 and IFNL2 genetics in CML. The significant associations observed suggest that host-virus interactions may be a previously underappreciated component of CML susceptibility. Future studies with larger, multi-centre cohorts and functional assays are needed to confirm these associations and elucidate the underlying mechanisms.

Conclusion

This study provides evidence of a significant association between a specific IFNL2 (IL-28A) gene polymorphism (CT genotype) and the detection of HHV-7 DNA in CML. The findings suggest that host genetic factors related to antiviral immunity and persistent viral infection may play a role in CML susceptibility.

List of Abbreviations

AHC: Apparently Healthy Control
CML: Chronic Myeloid Leukaemia
HHV-7: Human Herpesvirus-7
IFN: Interferon

IL-28: Interleukin-28
OR: Odds Ratio
CI: Confidence Interval
PCR: Polymerase Chain Reaction
SNP: Single Nucleotide Polymorphism

Declarations

Ethics Approval and Consent to Participate

Ethical approval was granted by the Institutional Review Board (Project No. M250942, Date: March 15, 2023). Written informed consent was obtained from all participants.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request. The sequencing data have been deposited in GenBank (Accession numbers: DQ126336.2).

Competing Interests

The authors declare that they have no competing interests.

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Author contributions

MSS: Conceptualisation, Methodology, Investigation, Writing - Original Draft.
ATF: Formal analysis, Data Curation, Writing - Review & Editing.
IMS: Methodology, Resources.
ASHM: Supervision, Validation, Project administration.
All authors read and approved the final manuscript.

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