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In-Silico Databases based Study on the Effects of Single Nucleotide Polymorphisms in the 3' UTRs of Mycobacterium tuberculosis-Associated Host Gene IFNG in Human Populations

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ABSTRACT: This In-Silico Databases based study examines the effects of single nucleotide polymorphisms (SNPs) in the 3' untranslated regions (3' UTRs) of the interferon gamma gene (IFNG) associated with Mycobacterium tuberculosis in human populations through in-silico analysis. Tuberculosis (TB) continues to pose a major health threat globally, with significant mortality rates, particularly in low- and middle-income countries (WHO, 2021). This study utilizes comprehensive genomic databases to evaluate SNPs in the IFNG gene, which is crucial for the immune response against TB. Focused on regulatory mechanisms, the research identifies how SNPs can alter microRNA (miRNA) binding and subsequently influence gene expression. Notably, variations such as rs2069723, rs186409216, and rs147926889 exhibit differing frequencies across populations, revealing the potential for significant effects on immune responses. For example, although rs147926889 shows moderate binding affinity with several miRNAs, its rare occurrence limits its impact on the overall human population. These findings emphasize the need for population-specific studies to understand genetic variations better and their links to TB susceptibility. By integrating SNP data with predictive modeling, this study aims to cover the way for targeted interventions and personalized strategies in TB management. The research underscores the importance of understanding genetic factors influencing TB outcomes and provides a foundation for future studies aimed at confirming functional implications of identified SNPs.

Keywords: Mycobacterium tuberculosis, Single Nucleotide Polymorphism (SNP), Interferon Gamma (IFNG), 3' Untranslated Region (3' UTR), MicroRNA.

INTRODUCTION

Mycobacterium tuberculosis (M. tuberculosis) is widely recognized as the causative agent of tuberculosis (TB), which remains one of the deadliest infectious diseases globally (Bali *et al.*, 2009). According to the World Health Organization (2021), TB is responsible for approximately 1.5 million deaths each year, with millions more infected, predominantly in low- and middle-income countries. Despite significant advancements in diagnostics, treatment, and vaccination, TB continues to pose a severe public health challenge. Understanding the genetic factors that contribute to an individual's susceptibility to TB is crucial for developing more effective prevention and treatment strategies. One area of particular interest is the role of single nucleotide polymorphisms (SNPs) within immune-related genes, especially in the context of the 3' untranslated regions (3' UTRs) of mRNAs.

The 3' UTRs play a critical role in post-transcriptional regulation of gene expression, influencing mRNA stability, localization, and translation efficiency (Bartel, 2009). These regions contain important regulatory elements, including binding sites for microRNAs (miRNAs) and RNA-binding proteins, which together modulate gene expression in response to cellular signaling and environmental stressors. Variations in 3' UTRs, particularly through SNPs, can significantly affect gene function by altering these binding sites, thereby impacting the immune response to TB (Hidayah *et al.*, 2022). Among the genes implicated in the host's defense against M. tuberculosis, the interferon gamma gene (IFNG) is particularly noteworthy, as it is vital for orchestrating the immune response against intracellular pathogens. Research indicates that IFNG is crucial for activating macrophages, enhancing their ability to kill pathogens such as M. tuberculosis (Kak *et al.*, 2018). Elevated levels of IFNG during infection have been correlated with increased resistance to TB, highlighting the gene's importance in the host's control of the disease. However, genetic variations within the IFNG gene and its regulatory elements can lead to differing expression levels and functional efficiency, which may influence an individual's susceptibility to

TB. For example, certain SNPs in the IFNG gene have been reported to be linked with varying immune responses, ultimately affecting the host's ability to control TB infections (Bastos, 2017).

Recent advances in bioinformatics and computational biology have facilitated in-silico studies that evaluate the effects of genetic variations, particularly SNPs, on gene regulation. Researchers can leverage databases such as dbSNP and the UCSC Genome Browser to analyze SNP data, integrating tools that predict the functional impact of specific variants within non-coding regions (Sherry *et al.,* 2001; Kent *et al.,* 2002). Particularly, studies focusing on SNPs located in the 3' UTR of the IFNG gene can shed light on how these genetic alterations may enhance or diminish gene expression, contributing to individual susceptibility to TB. Furthermore, the distribution of these SNPs among human populations may reflect geographical, environmental, and genetic factors, emphasizing the necessity of analyzing these polymorphisms across diverse populations. Such analyses can enhance our understanding of genetic variability and its implications for TB outcomes, allowing for targeted approaches in public health strategies (McHenry, 2021). This research article zeroes in on an in-silico study examining the impacts of SNPs located in the 3' UTR of the IFNG gene. The primary objective is to assess how these variations influence gene regulation and to establish associations with susceptibility to M. tuberculosis infection in various human populations. By integrating SNP data with predictive modeling tools, this study aims to predict the functional consequences of these genetic variants, contributing to a deeper understanding of host-pathogen interactions and the genetic foundations of TB susceptibility after wet lab experiments.

METHODS FOR IN-SILICO STUDY

1. Gene Selection. The study began by selecting tuberculosis (TB)-related genes using GeneCards®, a comprehensive database providing detailed information on human genes, including their functions, pathways, and associations with diseases. GeneCards was utilized to identify significant genes related to TB, with particular focus on the Interferon Gamma (IFNG) gene, known for its critical role in immune response against Mycobacterium tuberculosis (Safran *et al.,* 2010).

2. SNP and MicroRNA Data Retrieval. To compile information on Single Nucleotide Polymorphisms (SNPs) within the IFNG gene and associated microRNAs, the microSNiPer database was employed. This resource is specifically designed to assess SNPs and their effects on microRNA interactions, providing insights into how genetic variations can influence miRNA targeting of mRNA sequences (Barenboim *et al.,* 2010).

3. Allele Frequency Data. SNP allele frequencies were obtained from the National Center for Biotechnology Information (NCBI) SNP Database, accessible at NCBI SNP Database. This resource provides comprehensive insights into genetic variation across human populations, enabling researchers to explore common or rare alleles associated with specific traits and diseases (Sherry *et al.,* 2001).

4. Risk Assessment of SNPs and MicroRNA Interactions. Based on the compiled data from GeneCards, microSNiPer, and the NCBI SNP Database, this study assessed the risk associated with the identified SNPs and their potential effects on microRNA interactions with the IFNG gene. Bioinformatics tools were utilized to predict changes in microRNA binding efficiency resulting from these SNPs. The analysis focused on understanding how these genetic variations contribute to the immune response related to tuberculosis susceptibility.

This in-silico study integrates various genomic databases and computational biology approaches to further our understanding of the genetic factors associated with tuberculosis susceptibility. The methodologies outlined above serve to provide a comprehensive evaluation of the IFNG gene and its interactions with microRNAs, enhancing our capacity for targeted interventions and personalized treatment strategies for TB.

RESULTS AND DISCUSSION

The detailed analysis of SNP variants in the IFNG gene, as summarized in Table 1, highlights the relationship between minor alleles and their interactions with associated microRNAs relevant to tuberculosis (TB). The findings underscore the potential influence of these genetic variations on TB susceptibility and immune response.

The SNP rs2069723 has a major allele frequency of T (0.99377) and a minor allele frequency of C (0.00623), with no reported microRNA interactions according to microSNiPer. Conversely, SNP rs186409216 presents a scenario where the minor allele frequency for T is significantly lower than that for G (0.9998). The presence of multiple associated microRNAs—including hsa-miR-1911-3p (11 bp, highest binding affinity), hsa-miR-4645-5p (8 bp, high binding affinity), and others—suggests that minor alleles may enhance the ability of these microRNAs to regulate IFNG expression effectively. The high binding affinity reflects the potential for altered immune responses, which is paramount for host defense against M. tuberculosis (Kumar *et al.,* 2018) though the mutant allele frequency in population is low hence there is not much impact of the consequences of SNP.SNP rs7957366 shows a major allele frequency of C (1.00000), with an absence of the A allele in studied human population, indicating no potential for microRNA binding studies and functional consequences in pathogenesis of TB. Additionally, for SNP rs2234687, A allele is rare (0.001072). This SNP also links to various microRNAs with moderate binding affinities. The diversity of interactions emphasizes the need for population-specific studies, as different groups may exhibit varying susceptibilities to TB based on their unique genetic backgrounds (McHenry, 2021).

Table 1: Summary of SNP Variants (minor allele) in the IFNG Gene and Associated MicroRNAs Relevant to Tuberculosis when presence of mutant allele.

Rank Gene Cards for TB	Gene	Gene card score (tuberculosis)	SNP	Global (N)	Major allele	Minor allele	has microRNAs Seed length on mutant allele (microSNiPer)	Microrna (microSNiPer)
1	IFNG	36.63	rs2069723	47316	T=0.99377	C=0.00623	Not reported	
			rs186409216	5008	G=0.9998	T=0.0002	11 bp ((Binding affinity Highest)	hsa-miR-1911-3p
							8 bp (Binding affinity High)	hsa-miR-4645-5p
							7 bp (Binding affinity moderate	hsa-miR-29b-3p
							7 bp (Binding affinity moderate	hsa-miR-29a-3p
							7 bp (Binding affinity moderate	hsa-miR-1256
							7 bp (Binding affinity moderate	hsa-miR-29c-3p
			rs7957366	14040	C=1.00000	A=0.00000	8 bp (Binding affinity High)	hsa-miR-4774-5p
							7 bp (Binding affinity moderate	hsa-miR-4647
							7 bp (Binding affinity moderate	hsa-miR-200a-5p
							7 bp (Binding affinity moderate	hsa-miR-200b-5p
							7 bp (Binding affinity moderate	hsa-miR-556-3p
							7 bp (Binding affinity moderate	hsa-miR-1193
							7 bp (Binding affinity moderate	hsa-miR-125b-5p
			rs2234687	102574	G=0.998928	A=0.001072	7 bp (Binding affinity moderate	hsa-miR-5580-5p
							7 bp (Binding affinity moderate	hsa-miR-4647
							7 bp (Binding affinity moderate	hsa-miR-641
							7 bp (Binding affinity moderate	hsa-miR-3166
							7 bp (Binding affinity moderate	hsa-miR-3617-5p
							7 bp (Binding affinity moderate	hsa-miR-4437
			rs55991209	5008	C=0.9942	T=0.0058	7 bp (Binding affinity moderate	hsa-miR-5580-5p
							7 bp (Binding affinity moderate	hsa-miR-4647
							7 bp (Binding affinity moderate	hsa-miR-641
							7 bp (Binding affinity moderate	hsa-miR-3166
							7 bp (Binding affinity moderate	hsa-miR-3617-5p
							7 bp (Binding affinity moderate	hsa-miR-4437
							7 bp (Binding affinity moderate	hsa-miR-3158-5p
							7 bp (Binding affinity moderate	hsa-miR-5588-5p
							7 bp (Binding affinity moderate	hsa-miR-4738-3p
							7 bp (Binding affinity moderate	hsa-miR-34a-5p
			rs147926889	14050	G=0.99986	A=0.00014	8 bp (Binding affinity High)	hsa-miR-5580-5p
							8 bp (Binding affinity High)	hsa-miR-634
							7 bp (Binding affinity	hsa-miR-

							moderate	6715a-3p
							7 bp (Binding affinity moderate	hsa-miR-6715b-3p
							7 bp (Binding affinity moderate	hsa-miR-497-3p
							7 bp (Binding affinity moderate	hsa-miR-3910
							7 bp (Binding affinity moderate	hsa-miR-4530
							7 bp (Binding affinity moderate	hsa-miR-4519
							7 bp (Binding affinity moderate	hsa-miR-4274
			rs182958286	10678	A=1.000000	G=0.000000	7 bp (Binding affinity moderate	hsa-miR-412
							7 bp (Binding affinity moderate	hsa-miR-802
							7 bp (Binding affinity moderate	hsa-miR-643
							7 bp (Binding affinity moderate	hsa-miR-6500-3p
			rs2069722	48232	G=0.99635	A=0.00365	7 bp (Binding affinity moderate	hsa-miR-5580-5p
							7 bp (Binding affinity moderate	hsa-miR-6715a-3p
							7 bp (Binding affinity moderate	hsa-miR-371a-5p

The allele distribution of SNPs such as rs55991209 further highlights the intricate relationship between genetics and TB susceptibility. The occurrence of the mutant allele almost 0.6% and its moderate-affinity microRNA interactions suggest these SNPs can contribute to altered immune responses, possibly affecting infection outcomes. It emphasizes the importance of incorporating genetic variability into public health frameworks aimed at combating TB.

In particular, the SNP rs147926889 in its mutant form exhibited associations with multiple microRNAs that demonstrated moderate binding affinities; however, it does not significantly impact the human population due to the rarity of this mutation.

CONCLUSIONS

This online available database based study provides critical insights into how minor alleles within the IFNG gene influence the regulatory interactions with microRNAs, shaping immune responses and susceptibility to tuberculosis. The evidence indicates that the presence of minor alleles enhances the likelihood of microRNA binding, which could be vital for understanding genetic factors in TB susceptibility. Future research should focus on functional assays to confirm these predictions and deepen the understanding of SNP and microRNA interactions in the context of infectious diseases.

Future scope of the study: This in-silico study lays the groundwork for experimental validation of SNPs in the IFNG gene's 3' UTR, aiming to elucidate their functional roles in TB susceptibility and immune modulation. Additionally, the integration of multi-omics approaches, including transcriptomics and proteomics, could provide comprehensive insights into gene regulation and immune response pathways relevant to tuberculosis treatment strategies tailored to diverse populations.

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Conflict of Interest. None.

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