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In-Silico Databases based Study on the Effects of SNP on the 3' UTRs of Genes which are Associated with *Mycobacterium tuberculosis* in Human

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ABSTRACT: This bioinformatics study investigates the impact of single nucleotide polymorphisms (SNPs) on the 3' untranslated regions (3' UTRs) of host genes associated with tuberculosis (TB) caused by Mycobacterium tuberculosis (Mtb) using various in-silico databases. TB remains a significant global health challenge, with millions of new cases and deaths reported annually (WHO, 2021). SNPs represent the most common form of genetic variation, influencing gene expression and the immune response, making them crucial for understanding TB susceptibility and host-pathogen interactions. The key genes analyzed in this study include SLC11A1, IFNGR1, TLR2, TNF, and IL12RB1. Utilizing resources such as GeneCards® and microSNiPer, this research identified SNPs within these genes and assessed their potential effects on microRNA interactions, which are vital for gene regulation. The results suggest that minor alleles of specific SNPs exhibit significant binding affinities with various microRNAs, potentially modulating the expression of these genes and influencing the host's immune defenses against Mtb. For instance, the SNP rs1059823 in SLC11A1 presented a minor allele frequency of 42.2% and demonstrated a strong association with microRNAs linked to immune responses. These findings underscore the complexity of genetic factors influencing TB susceptibility and highlight the potential for personalized therapeutic strategies. This in-silico approach offers valuable insights into genetic variants that may increase susceptibility to TB, contributing to improved diagnosis and treatment strategies. Further experimental validation and exploration of these genetic factors are essential for developing effective interventions against tuberculosis.

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

INTRODUCTION

Tuberculosis (TB) is a pressing global health concern, caused by the pathogen Mycobacterium tuberculosis (Mtb). According to the World Health Organization (WHO, 2021), millions of new cases and deaths are reported annually, underscoring the critical importance of addressing this infectious disease (Bali *et al.*, 2009). The complexity of TB is compounded by the pathogen's ability to evade host immune defenses, coupled with its propensity to develop drug resistance. As researchers strive to develop novel therapeutic strategies, an in-depth understanding of the genetic variations within Mtb and their ramifications for host-pathogen interactions has emerged as a focal point of inquiry. Genetic variations in Mtb, particularly single nucleotide polymorphisms (SNPs), play a significant role in influencing the bacterium's behavior, pathogenicity, and interaction with the human immune system. SNPs, which represent the most common form of genetic variation, differ at a single nucleotide position in the genome and can lead to alterations in gene expression, protein functionality, and an individual's susceptibility to disease (Bastos, 2017). A notable area of interest is the presence of SNPs within the 3' untranslated regions (3' UTRs) of genes. These regions, while not coding for proteins, have substantial regulatory roles that can affect mRNA stability, translation efficiency, and microRNA (miRNA) binding. These regulatory mechanisms ultimately modulate gene expression, influencing the pathogen's virulence and the host's immune response (Bartel, 2009).

In recent years, in-silico databases and computational tools have become instrumental in the exploration and analysis of SNPs and their consequences on gene function. These technological advancements enable researchers to predict the functional effects of specific SNPs, identify potential biomarkers for TB diagnostics, and investigate the molecular mechanisms that contribute to disease progression. By employing these in-silico approaches, scientists can efficiently analyze large datasets, significantly streamlining the process of identifying which SNPs warrant further experimental exploration. This prioritization is crucial given the extensive genetic diversity present within Mtb and the multitude of factors that can influence treatment outcomes. The integration of genetic insights into TB

research holds profound implications for developing effective therapeutic strategies. A better understanding of SNPs and their influence on host-pathogen interactions may lead to personalized treatment regimens based on an individual's genetic profile. Such an approach could enhance the efficacy of existing treatments and help in the design of new drugs that target specific SNPs associated with drug resistance. Moreover, SNP analysis can inform vaccine development by identifying genetic variations that impact vaccine efficacy, potentially leading to more robust immune responses in diverse populations.

As a major factor in the ongoing struggle against TB, the exploration of genetic variations within Mycobacterium tuberculosis, particularly through the lens of SNPs, represents a promising avenue for research. Leveraging in-silico tools facilitates a deeper understanding of the complex interplay between Mtb and the human host, paving the way for innovative therapeutic and preventive measures. Addressing the challenges posed by TB, including drug resistance and immune evasion, necessitates a concerted effort to explore genetic underpinnings, ultimately contributing to a more effective fight against this global health crisis. By harnessing cutting-edge technology and genetic research, the goal of eradicating tuberculosis can become a more attainable reality. **SLC11A1**, also known as NRAMP1, encodes a protein involved in iron metabolism and host resistance to certain pathogens. This gene plays a role in the innate immune system and has been linked to susceptibility to infectious diseases like tuberculosis and leprosy. Variations in this gene can influence the body's ability to combat Mtb, making it a key focus of TB research. **IFNGR1** encodes the alpha chain of the gamma interferon receptor, crucial for antimicrobial, antiviral, and antitumor responses by activating effector immune cells and enhancing antigen presentation. Mutations and SNPs in *IFNGR1* can impair the immune response to Mtb, highlighting the importance of understanding genetic variations in this gene .**TLR2** is part of the Toll-like receptor family, which plays a key role in the innate immune system by recognizing pathogen-associated molecular patterns (PAMPs) and initiating immune responses. SNPs in *TLR2* have been associated with altered immune responses to TB, suggesting that genetic variations in this gene can influence susceptibility to the disease. **TNF** encodes a cytokine involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction (Tracey & Cerami, 1994). Variations in the *TNF* gene can affect the production of this cytokine, impacting the body's ability to control Mtb infection. **IL12RB1** encodes a subunit of the interleukin-12 receptor, which is important for the differentiation of T cells and the production of interferon-gamma, playing a critical role in the immune response against infections (Altare *et al.*, 1998). SNPs in *IL12RB1* can lead to increased susceptibility to TB by impairing the immune response to Mtb.

This study aims to utilize in-silico databases to investigate the effects of SNPs on the 3' UTRs of the genes *SLC11A1*, *IFNGR1*, *TLR2*, *TNF*, and *IL12RB1* associated with Mtb in humans. By analyzing these SNPs, we hope to identify genetic variants that contribute to TB pathogenesis, drug resistance, and host immune responses. The findings from this study could provide valuable information for the development of new diagnostic tools, therapeutic targets, and personalized treatment strategies for TB.

METHODS FOR IN-SILICO STUDY

The study commenced with the selection of tuberculosis (TB)-related genes, utilizing GeneCards®, a comprehensive database that offers detailed insights into human genes, including their functions, pathways, and disease associations. This resource was pivotal in identifying significant genes associated with TB, with particular emphasis placed on the Interferon Gamma (IFNG) gene, recognized for its essential role in the immune response against Mycobacterium tuberculosis (Safran *et al.*, 2010). Following gene selection, the next step involved the retrieval of data on Single Nucleotide Polymorphisms (SNPs) within the IFNG gene and associated microRNAs through the microSNiPer database. This specialized tool assesses the effects of SNPs on microRNA interactions, providing valuable insights into how genetic variations may influence miRNA targeting of mRNA sequences (Barenboim *et al.*, 2010).

To complement this analysis, SNP allele frequencies were sourced from the National Center for Biotechnology Information (NCBI) SNP Database. This resource serves as a comprehensive platform for exploring genetic variations across human populations, allowing researchers to assess both common and rare alleles linked to specific traits and diseases (Sherry *et al.*, 2001). By compiling the data from GeneCards, microSNiPer, and the NCBI SNP Database, the study then conducted a risk assessment of the identified SNPs and their potential impacts on microRNA interactions with the IFNG gene. Bioinformatics tools were employed to predict alterations in microRNA binding efficiency due to these SNPs, ultimately focusing on how these genetic variations might affect the immune response and contribute to susceptibility to tuberculosis. Overall, this in-silico study integrates various genomic databases and computational biology approaches to enhance our understanding of the genetic factors associated with tuberculosis susceptibility. The research specifically aims to utilize in-silico methods to investigate the effects of SNPs on the 3' untranslated regions (3' UTRs) of the genes SLC11A1, IFNGR1, TLR2, TNF, and IL12RB1, thereby providing a comprehensive framework for elucidating the intricate relationships between genetic variations and TB susceptibility.

RESULTS AND DISCUSSION

This study extensively analyzed single nucleotide polymorphisms (SNPs) in TB-related genes, emphasizing the implications of minor alleles and their interactions with associated microRNAs, as shown in Table 1. Understanding these minor alleles is crucial for elucidating genetic factors that may influence susceptibility to tuberculosis (TB) and the host's

immune response to Mycobacterium tuberculosis. Focusing on the SLC11A1 gene, several noteworthy SNPs expressed minor alleles with significant implications. For instance, the SNP rs1059823 exhibited a minor allele frequency of 42.2% for G (major allele A: 57.8%). This variation correlates with binding interactions to multiple microRNAs, specifically hsa-miR-4793-5p and hsa-miR-4286, both of which display high binding affinities. The presence of the G allele potentially alters the expression levels of SLC11A1, thereby impacting the function of the immune response against TB. The high binding affinity indicates that the G allele might enhance the regulatory effects of these microRNAs, supporting a mechanism where minor alleles can modulate gene expression and influence susceptibility to the disease.

Additionally, the SNP rs11554539 presented a striking example, with a minor allele frequency of only 2.7% for C (major allele A: 97.3%). This rare variant was associated with the microRNA hsa-miR-4731-5p, which also exhibited a high binding affinity. Such a low frequency of the minor allele underscores its potential significance. If the C allele differentially affects SLC11A1 expression or function, it could pose unique risks or protections in certain populations, particularly concerning the immune response to TB. The SNP rs13062, with a minor allele frequency of 36.2% for A (major allele C: 63.8%), was associated with hsa-miR-373-3p, exhibiting moderate binding affinity. This suggests that even variants that are relatively more common can have discernible effects on gene regulation. The interaction of microRNAs with this minor allele may also influence the host's response to TB, highlighting the intricate balance between common and rare variants in disease susceptibility. Exploring the IFNGR1 gene, the minor allele A of SNP rs55665036 had a frequency of only 1.1% (major allele C: 98.9%). This minor variant was associated with several microRNAs, suggesting potential disruptions in the immune signaling pathways that rely on IFNγ signaling. Even with its low frequency, the minor allele's influence on microRNA binding could be critical for individuals carrying this variant, potentially resulting in altered immune responses during Mtb infection. For the TNF gene, the minor allele C of SNP rs3093665 also exhibited a low frequency of 2.6% (major allele A: 97.4%), and it was linked to various microRNAs. The minor allele's association with higher binding affinity microRNAs such as hsa-miR-6515-5p suggests that it may play a role in fine-tuning TNF expression and, consequently, the inflammatory response during TB infections.

The findings related to IL12RB1, particularly SNP rs3746190, indicated a minor allele frequency of 31.9% for A (major allele G: 68.1%), which linked to the moderately binding hsa-miR-2114-3p. This highlights that even more frequent minor alleles can significantly influence the gene's expression, potentially affecting the IL-12 signaling pathway critical for T-cell responses against TB. In summary, this analysis underscores the importance of minor alleles in the context of TB-related genes and their interactions with microRNAs. These findings suggest that minor alleles can significantly influence gene expression and modulate immune responses, which may ultimately affect susceptibility to TB. Continued research into these genetic factors and their functional implications is essential for developing personalized therapeutic strategies and improving our understanding of TB pathogenesis. Understanding the intricacies of how these minor alleles affect gene regulation is vital for identifying potential biomarkers for susceptibility and therapeutic targets in the fight against tuberculosis.

Table 1: Summary of SNP Variants in TB-Related Genes and Associated MicroRNAs in the Context of 3' UTR Variants with Seed Lengths Greater than 6 bp.

Sr. No.	Rank GeneCards for TB	Gene	Gene card score (TB)	SNP	Global (N)	Major allele	Minor allele 3 Prime UTR Variant (selection criteria >1%)	hsa microRNAs Seed length with mutant allele	Microrna Seed length (microSNiPer) cut-off criteria >6 Seed length
1	2	SLC 11A1	36.02	rs1059823	239678	A=0.578151	G=0.421849	hsa-miR-4793-5p	9 bp (Binding affinity Highest)
								hsa-miR-873-5p, hsa-miR-4286	7 bp (Binding affinity moderate)
								hsa-miR-4286	8 bp (Binding affinity High)
				rs13062	148922	C=0.638019	A=0.361981	hsa-miR-373-3p	7 bp (Binding affinity moderate)
				rs11554539	5008	A=0.9730	C=0.0270	hsa-miR-4731-5p	8 bp (Binding affinity High)
				rs2279014	197858	C=0.640298	T=0.359702	hsa-miR-5683, hsa-miR-3180-5p	8 bp (Binding affinity High)
								hsa-miR-23a-5p	7 bp (Binding affinity moderate)
2	3	IFNGR1	27.22	rs55665036	18964	C=0.98935	A=0.01065	hsa-miR-5692b , hsa-miR-5692c , hsa-miR-3145-3p	7 bp (Binding affinity moderate)
3	4	TLR 2	24.96	-	-	Minor allele frequency <1		-	-
4	5	TNF	24.2	rs3093665	252980	A=0.973694	C=0.026306	hsa-miR-6515-5p	10 bp (Binding affinity Highest)
								hsa-miR-4257	8 bp (Binding affinity High)
								hsa-miR-5197-3p, hsa-miR-3165, hsa-miR-6134, hsa-miR-575	7 bp (Binding affinity moderate)
5	6	IL12RB1	21.77	rs3746190	34272	G=0.68108	A=0.31892	hsa-miR-2114-3p	7 bp (Binding affinity moderate)

CONCLUSIONS

This study highlights the critical role of minor alleles in tuberculosis (TB) susceptibility, particularly through their influence on gene expression and interactions with microRNAs in key immune-related genes. The findings indicate that even low-frequency SNPs can significantly impact the regulation of genes such as SLC11A1, IFNGR1, TNF, and IL12RB1, potentially modulating the host's immune response to Mycobacterium tuberculosis. This underscores the complexity of genetic factors contributing to TB susceptibility, where minor alleles may either confer risk or protection depending on their interactions with microRNAs. By elucidating the relationship between these genetic variations and microRNA binding affinities, the research provides a foundation for understanding the molecular mechanisms underlying TB pathogenesis. Moreover, identifying these genetic markers can pave the way for personalized approaches to treatment and prevention strategies. Ultimately, this study emphasizes the need for further investigation into the functional implications of these minor alleles in diverse populations, which is essential for improving interventions against tuberculosis and enhancing our understanding of this global health challenge.

FUTURE SCOPE

The future scope of this study includes the potential development of targeted therapeutic strategies that leverage the identified SNPs and their interactions with microRNAs to enhance host resistance to Mycobacterium tuberculosis. Additionally, further research could explore the application of these genetic insights in personalized medicine, leading to improved diagnostic tools and treatment regimens tailored to individual genetic profiles, thereby contributing to more effective tuberculosis management and prevention.

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Conflict of Interest. None.

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