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Molecular characterization of mt-DNA mutations associated with hypertension and cardiovascular diseases

Umer bin Zakaria[1]*, Muhammad Arshad[1*2], Aleem Ahmed[1*3], Kaleem Ahmed[1*4], Ahad Mehmood[5]

[1]Department of Biological Sciences, International Islamic University Islamabad, Pakistan

[2]Department of Microbiology, Abbottabad University of Science and Technology

Corresponding Author Email ID:umerbinzakaria@gmail.com

Abstract

Hypertension, a physical condition characterized by elevated blood pressure, stands as a significant contributor to various severe illnesses such as cardiovascular diseases, renal diseases, strokes, and numerous vascular conditions. While environmental factors play a function in the development of hypertension, a substantial portion of its origins can be attributed to genetic factors. Notably, hypertension serves as a primary catalyst for numerous cardiovascular diseases, often culminating in fatalities on a global scale. The multifaceted etiologies of cardiovascular diseases encompass lifestyle choices, environmental factors, and genetic predispositions. This research delved into a relatively unexplored facet of hypertension-related cardiovascular diseases by investigating mitochondrial mutations in patients diagnosed with hypertension. Surprisingly, despite the prevalence of hypertension, a definitive link between this condition and the development of cardiovascular diseases has yet to be conclusively established. The data from 80 patients was included in the study and samples were meticulously gathered from various hospitals, specifically targeting individuals grappling with hypertension and concomitant heart-related ailments. The methodology involved the procurement of saliva followed by the extraction and sequencing of mitochondrial DNA (mtDNA). Subsequently, advanced bioinformatics tools were employed to scrutinize the genetic data, with the prime focus to identify and characterize any mutations associated with mitochondrial physiology. The outcomes of this study hold the potential to be invaluable for both the general population and medical practitioners. By uncovering mitochondrial mutations linked to hypertension, the research provided individuals with hypertension insights into their specific risk factors for cardiovascular diseases. This knowledge, in turn, empowers the population to adopt preventative measures and make informed

lifestyle choices. Furthermore, physicians stand to benefit from a deeper understanding of the genetic underpinnings of cardiovascular risks in hypertensive patients, enabling more personalized and effective medical interventions. Conclusively, this research endeavored to bridge gaps in current knowledge, offered a foundation for enhanced care and supervision of persons at stake of cardiovascular diseases due to hypertension.

1. INTRODUCTION

Cardiovascular diseases (CVDs) stand as a formidable global health challenge, contributing significantly to morbidity and mortality rates worldwide. Within this broad spectrum of cardiovascular disorders, hypertension holds a central position, often acting as a precursor and exacerbating factor for severe cardiovascular events (Rehman, S et al., 2021). Coronary artery disease (CAD), accompanied by its primary outcome, myocardial infarction, stands as the majority widespread cardiovascular ailment and the foremost global contributor to morbidity and death ratio. The significant impact of CAD on both society and healthcare systems results in substantial socioeconomic costs (Lopez, A.D et al., 2006). In meticulous, coronary heart disease (CHD) every twelve months results in 502000 casualties in the US and >700 000 deceases in China (Zhang XH et al., 2008). Coronary heart disease (CHD) is a widespread convoluted condition that can be carried on by a single gene or a combination of causes arising from the interaction of inherited and ecological risk aspects (Khot UN et al., 2003, Sing CF et al., 2003).

According to the American Heart Association's 2019 report, around 48% of patients in the US who were over 20 years old had one or more cardiovascular disease (CVD) among 2013 and 2016. These conditions included heart failure (HF), hypertension, coronary heart disease, and stroke (Benjamin, E.J et al., 2019, Townsend, N et al., 2016, World health statistics overview 2019).

It seems that a plateau has been reached, signaling the necessity for innovative approaches. From this standpoint, there is a proposal to shift attention to circulating cells for a more comprehensive understanding of cardiovascular disease (CVD) pathophysiology and the discovery of novel biomarkers. Given that CVDs are generally systemic conditions, emerging evidence suggests that assessing the respiratory capacity of circulating platelets and peripheral blood mononuclear cells (PBMCs) could serve as an indicator to identify mitochondrial dysfunction in various organs, including the heart (Rose, S et al., 2019).

Substantial advancements have been achieved in the realm of cardiovascular disease (CVD) diagnosis and treatments, with notable emphasis on neuro-hormonal modulation, exemplified by approaches like natriuretic peptide (NP)-guided therapy (Bárány, T et al., 2017). Several major genome-wide organization studies and meta-analyses have recognized abundant regular genetic variants connected to the risk of coronary artery disease (CAD) over the last 14 years (Deloukas, P et al., 2013). Despite these results, the combined effect of these discrepancies only contributes a small amount (20%) to the disease's heritability (Lempiäinen, H et al., 2018).

Recent scientific endeavors have shifted the spotlight towards the intricate molecular landscape, especially the mitochondrial genome, as a key player in the etiology of hypertension and associated cardiovascular complications (Mensah, G. A. et al., 2019). Often neglected, genetic variants of the mitochondrial DNA (MT) reveal an unanticipated potential explanation for the "missing heritability" of several complex features, ranging from CAD (Vilne, B. et al., 2022).

Mitochondria, once viewed solely as cellular powerhouses, have emerged as integral regulators of cellular homeostasis. Comprising their possess genetic material in the appearance of mt-DNA, mitochondria play a pivotal character in power production, apoptosis, and redox signalling (Rossmann, M.P et al., 2021).

The link among mitochondrial dysfunction and cardiovascular diseases has become increasingly apparent, with a growing body of substantiation implicating mt-DNA mutations in the pathogenesis of hypertension (Bray, A.W. and Ballinger, S.W., 2017). Understanding the molecular intricacies of mt-DNA mutations requires a nuanced exploration of the functional consequences of these genetic aberrations (Taylor, R.W. and Turnbull, D.M., 2005).

A comprehensive overview about the role played by mt-DNA in oxidative phosphorylation highlighted the repercussions of mutations on mitochondrial function. As oxidative phosphorylation represented the primary mechanism for ATP synthesis within mitochondria, any perturbations in this process can profoundly impact cellular energy homeostasis, potentially contributing to the expansion and evolution of hypertension (Chen, W et al., 2023).

The mt-DNA genome is a compact entity, encoding 13 crucial subunits of the oxidative phosphorylation complexes, 22 transfer RNAs, and 2 ribosomal RNAs. However, the genetic diversity within the mitochondrial genome is not evenly distributed, and variations in the form of haplogroups have been identified. These haplogroups represent specific clusters of mt-DNA polymorphisms that have evolved over time in response to different environmental pressures and geographical locations (Wallace, D.C., 2015).

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In the recent studies it revealed that the association between mitochondrial haplogroups and hypertension, uncovering intriguing correlations (Tranah, G.J et al., 2011). The researchers observed that individuals belonging to certain haplogroups exhibited a higher predisposition to hypertension, suggesting a genetic component in the susceptibility to this condition (Manosroi, W. and Williams, G.H., 2019).

Advancements in sequencing expertise, above all next-generation sequencing (NGS), have transfigured our capability to dissect the mitochondrial genome with unprecedented precision (Koboldt, D.C et al., 2013). By enabling the comprehensive analysis of mt-DNA, NGS has

facilitated the identification of novel mutations associated with hypertension and cardiovascular diseases (Papadopoulou, E et al., 2010).

The scientific exploration of the molecular characterization of mitochondrial DNA (mtDNA) transformations in the context of hypertension and cardiovascular syndromes represents a convergence of genetics, molecular biology, and clinical cardiology. This section introduces the fundamental connection between mtDNA and cardiovascular health, emphasizing the role of mitochondrial genetics in cellular energy production and broader cellular processes (Dabravolski, S.A et al., 2022).

Recent studies on mtDNA reveals that the association between mitochondrial haplogroups and hypertension, unveils intriguing correlations of the ailments (Tranah, G.J. et al., 2011). The researchers observed that individuals belonging to certain haplogroups exhibited a higher predisposition to hypertension, suggesting a genetic component in the susceptibility to this condition (Manosroi, W. and Williams, G.H., 2019).

This seminal work paved the way for subsequent studies exploring the intricate relationship between mt-DNA haplogroups and cardiovascular diseases, offering valuable insights into the diverse genetic landscape associated with hypertension (Veronese, N et al., 2019).

Various attempts have been made to study the functional impact of a specific mt-DNA mutation on mitochondrial bioenergetics in the context of hypertension. Different cellular models have been utilized for examine the identified mutation which led to different oxidative phosphorylation, amplified oxidative stress, and altered mitochondrial dynamics. These findings underscore the direct link between mt-DNA mutations and mitochondrial dysfunction, providing mechanistic insights into the pathogenesis of hypertension (Clemente-Suárez, V.J et al., 2023).

Mitochondrial dysfunction, arising from mt-DNA mutations, contributes significantly to the amplification of oxidative anxiety and inflammatory reaction, in the pathophysiology of hypertension. The configuration of reactive oxygen species (ROS) within dysfunctional mitochondria can lead to cellular harm and trigger inflammatory cascades (Dabravolski, S.A et al., 2021).

The development of hypertension may be attributed to an inefficient metabolism brought on by mitochondrial dysfunctions in skeletal and vascular smooth muscles, which can raise systolic blood pressure (Lopez-Campistrous, A. et al., 2008). Specifically, certain pedigrees have linked maternal transmission of hypertension, indicating that one or more mutations in mitochondrial (mt)DNA may be one of the genetic causes of this condition (Li R et al., 2009).

The burgeoning knowledge of mt-DNA mutations associated with hypertension holds immense promise for the development of precision medicine strategies (Padmanabhan, S. and Dominiczak, A.F., 2021). Tailoring therapeutic interventions based on an individual's genetic makeup represents a paradigm shift in cardiovascular medicine, offering targeted approaches that address the specific molecular determinants of hypertension (Leopold, J.A. and Loscalzo, J., 2018).

The current study was undertaken to catalyze a paradigm shift in the approach to hypertension, moving beyond its symptomatic management to a more proactive understanding of its genetic components by focusing on mtDNA. By fostering awareness and understanding the link between hypertension and cardiovascular diseases, it strived to pave the way for more targeted interventions, personalized healthcare strategies, and, ultimately, a reduction in the prevalence and impact of cardiovascular diseases worldwide.

2. MATERIAL AND METHODS

2.1. Identification and Enrolment of Families

The meticulous selection process involved identifying families with a history of hypertension and heart diseases, ensuring a robust and inclusive representation of demographic, socio-economic, and geographic factors. The data from 80 patients with careful consideration of statistical power and feasibility was considered. This cohort proved instrumental in providing a sufficiently large dataset for the application of advanced analytical techniques, allowing for the identification of subtle genetic markers and potential gene-environment interactions associated with the development of heart diseases in hypertensive individuals.

2.2. Consent Form Filling

The process of obtaining informed consent played a pivotal role in the realization of this research endeavor. At the commencement of the study, potential participants were meticulously briefed about the aims, procedures, and potential implications of the research. In adherence to ethical standards and guidelines, a comprehensive consent form was provided to each participant, delineating the study's objectives, the voluntary nature of participation, and the assurance of confidentiality. Participants were afforded ample time to review the consent form, seek clarification on any queries they might have had, and deliberate on their decision to participate. The consent form encompassed detailed information about the research, including the methods employed, the potential risks and benefits, and the measures in place to safeguard their privacy. Upon obtaining a thorough understanding of the study, participants were invited to provide their explicit consent by appending their signatures to the consent form.

2.3. Collection of Saliva Samples

Liquid saliva samples were meticulously collected as a vital component of this research initiative, specifically targeting hypertensive patients afflicted with heart diseases. The sample collection process adhered to rigorous protocols and ethical guidelines to ensure the reliability and integrity of the biological specimens. Patients meeting the defined criteria were approached and provided with detailed information about the saliva sample collection procedure. Informed consent was obtained prior to sample collection, emphasizing the voluntary nature of participation and underscoring the confidentiality and anonymity of the obtained biological materials.

The collection process involved instructing participants to refrain from eating, drinking, or engaging in oral hygiene practices for a specified period before providing the saliva samples. This precautionary measure aimed to minimize potential contaminants and optimize the quality of the collected biological material. Upon obtaining consent, participants were provided with specialized collection devices, such as saliva collection tubes, designed to preserve the integrity of the samples. Detailed instructions were provided to guide participants through the proper collection technique, ensuring the acquisition of an adequate volume of liquid saliva for subsequent analyses. Careful labeling and documentation accompanied each collected sample to maintain a clear and traceable record of the participant's identity and relevant demographic and clinical information. Post-collection, the samples were appropriately stored under controlled conditions, safeguarding their stability and preventing degradation until they were ready for analysis.

2.4. DNA Isolation

The isolation of genetic DNA from the liquid saliva samples obtained by strictly following stringent protocols, the extraction process was carried out to ensure the purity and integrity of the genetic material. Upon collection of the liquid saliva samples, the DNA isolation process was initiated using established laboratory techniques. Specialized kits and reagents designed for the extraction of DNA from saliva were employed, taking into account the unique challenges associated with this biological fluid. The isolated DNA underwent a thorough quality assessment using agarose gel electrophoresis. This technique allowed for the visualization of the DNA fragments, enabling a qualitative evaluation of its integrity and purity. The agarose gel, subjected to an electric field, facilitated the migration of DNA molecules based on their size, resulting in distinct bands that provided insights into the quality of the extracted genetic material. Quantification of the isolated DNA was carried out to determine the concentration of genetic material present in each sample. Advanced spectrophotometry methods were employed to precisely measure the DNA concentration.

In the visualization step, the agarose gel was carefully examined, and the DNA bands were documented through photographic means. High-resolution photographs captured the distinctive patterns of DNA bands, forming an integral part of the comprehensive dataset generated during the course of the study.

2.5. PCR Amplification

The extracted DNA served as the foundation for a targeted exploration of the mitochondrial DNA (mtDNA). Polymerase Chain Reaction (PCR) was employed to amplify the specific regions of mtDNA relevant to the onset of hypertension and cardiovascular diseases. PCR amplification was conducted utilizing primers designed to match the specific regions of mtDNA associated with the research objectives. The carefully crafted primers were the result of a meticulous study of the genomic regions implicated in the development of hypertension and cardiovascular diseases. Following PCR amplification, the resulting products were subjected to analysis on agarose gel electrophoresis. The agarose gel separated the amplified DNA fragments based on size.

Visualization of the gel under UV light facilitated the detection of distinct bands corresponding to the amplified mtDNA fragments.

Subsequently, the gel containing the amplified gene fragments was carefully excised, and the gene products were isolated from the gel using a gene clean kit. To validate the success of the isolation process, electrophoresis was once again employed to visualize the purified gene bands. The iterative process of primer design, PCR amplification, agarose gel analysis, gene isolation, and electrophoresis constituted a sophisticated molecular workflow.

2.6. Nucleotide sequence analysis

The nucleotide sequences obtained from the purified PCR product were aligned with the reference sequences from gene banks and juxtaposed against sequences from a control group. This bioinformatic approach allowed for the identification and characterization of any variations, including point mutations or sequence alterations that might be indicative of genetic factors associated with hypertension and cardiovascular diseases.

The outcomes of this comparative analysis were meticulously reported, highlighting similarities, differences, and any novel mutations discovered. The identification of novel mutations was particularly significant, as it provided insights into potentially undiscovered genetic factors contributing to the complex interplay between hypertension and various cardiovascular diseases.

3. RESULTS

The present investigation delved into a comprehensive sequence analysis of the mitochondrial DNA (mtDNA) segment spanning positions 4,232 to 4,671, with the aim of shedding light on potential links to hypertension. The meticulous examination unveiled several noteworthy base substitution mutations situated at positions corresponding to the tRNAIle gene. Particularly, within the sample group comprising 1C, 2A, 4B, and X26, we discerned the occurrence of distinct substitution mutations: 4580G>A (rs267606829), 4439G>C (rs267606830), 4487T>G (rs267606831), and 4584C>T (rs267606832), respectively. The singularity of these mutations warrants recognition as single nucleotide variations (SNVs), which find their localization in the notably GC-rich expanse of the tRNA Ile gene. These intriguing mutations were conspicuously situated within the mitochondrial gene MTTI, responsible for encoding the tRNAIle gene. Analysis.

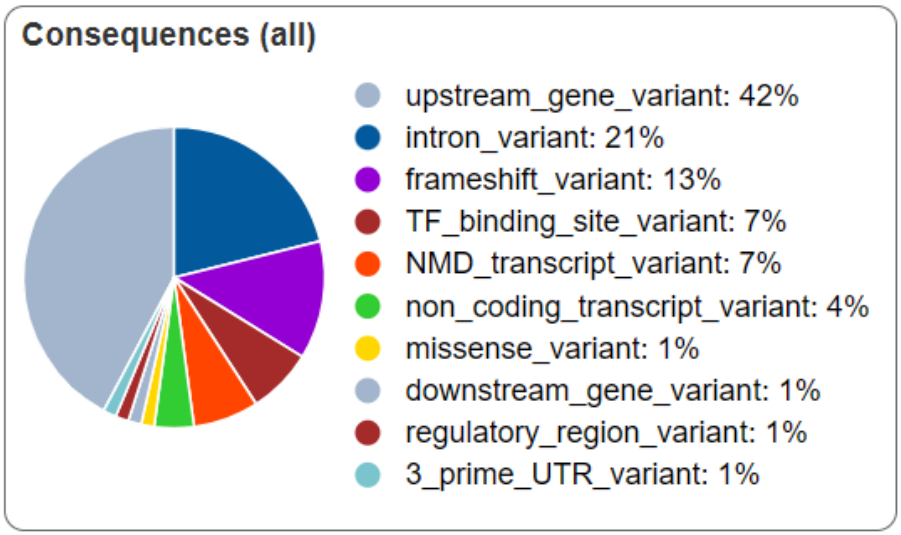

Figure 3.1: Percentages of variants from variant effect predictor analysis

Presence of such mutations within the non-coding region of MTTI were revealed in the current investigation. Intriguingly, these mutations were not confined to the region responsible for tRNA translation but were dispersed throughout the gene sequence. It is probable that mutations in the non-coding region of MTTI may impact tRNA processing, stability, or interactions with other cellular components involved in mitochondrial function. These disruptions could lead to downstream effects on mitochondrial protein synthesis, ultimately affecting energy production.

3.1. Variant Effect Prediction Analysis

Variant Effect Prediction analysis exhibited multiple effects of variants in the sequencing data. Upstream gene variants were recorded as 42 percent (Figure 3.1) which potentially affected the binding of transcription factors or other regulatory elements crucial for the initiation of transcription. This could lead to altered transcriptional activity of the MTTI gene. Frame shift mutations were found in upstream region that can possibly disrupt the reading frame of the MTTI gene, likely resulting in a non-functional or truncated tRNA molecule. This could have downstream effects on mitochondrial protein synthesis.

Transcription Factor Binding Site variations were recorded 7 percent (Figure 3.1) and predicted to alter the binding site of a known transcription factor. This could lead to differential regulation of MTTI expression, potentially affecting tRNA levels and subsequent mitochondrial protein synthesis. Overall, the variant effect prediction analysis indicated a diverse range of potential functional consequences for mutations within the non-coding region of the MTTI gene. These findings highlight the importance of considering regulatory elements and non-coding regions when studying mitochondrial genetics, as they can significantly impact mitochondrial function and contribute to mitochondrial disorders. Further experimental validation is warranted to confirm the functional relevance of these predicted effects (Figure 3.1).

3.2. Gene Ontology (GO) analysis

Gene Ontology (GO) analysis was performed to confirm the functional consequences of the MTTI gene mutations that were found. The MTTI gene sequencing data's Gene Ontology analysis offered important new information about the biological mechanisms, cellular constituents, and molecular roles connected to this gene's mutations.

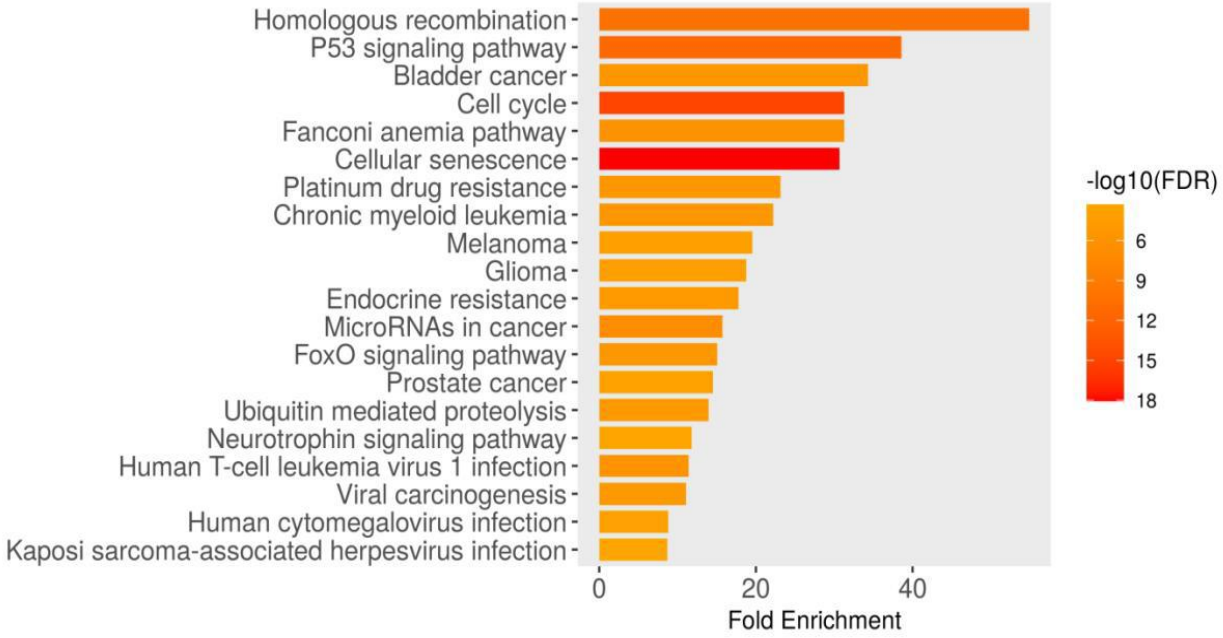

Figure 3.2: Gene Ontology analysis of sequencing data

The present study underscores the pivotal function of MTTI in mitochondrial function and underscores the plausible consequences of mutations in this gene for illnesses related to the mitochondria. Multiple phenotypes associated with these mutations were found but these can't be associated to link this gene with hypertension (Figure 3.2). These rare mutations within this gene opens avenues for further explorations into their functional and mechanistic implications, potentially unravelling new facets of mitochondrial involvement in the context of hypertension and cardiovascular disorders.

3.3. Sequence Analysis

a. Sample 1C

The secondary structure prediction results provided a detailed and reliable characterization of a specific tRNA molecule, focusing on tRNA Type Ile with the anticodon GAT. The Infernal score, a quantitative metric indicating the alignment quality with the predicted model, is reported as a robust 114, implying a high level of confidence in the accuracy of the prediction.

The tRNA sequence under scrutiny, designated as 1C.trna1 and spanning positions 34-102, exhibits a total length of 69 base pairs. The classification as Type Ile is noteworthy, with the anticodon GAT

positioned precisely at nucleotide positions 30-32 (63-65). This information is critical for understanding the functional specificity of the tRNA molecule, particularly in the context of its role in protein translation.

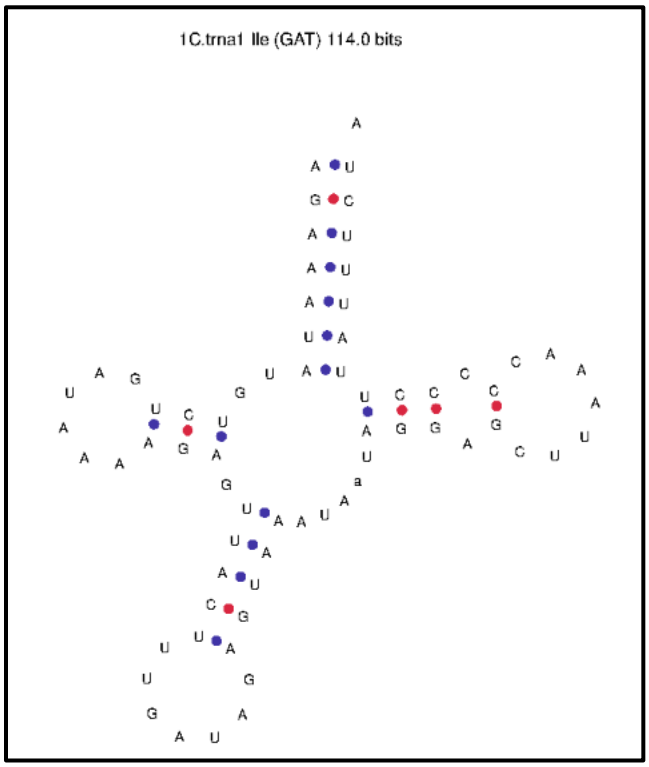

Figure 3.3: tRNAIle secondary structure predicted from sequencing data of Sample 1C

This graphical representation aids in intuitively grasping the structural features of the tRNA molecule. The actual sequence AG AAATATGTCTGATAAAAGAGTTACTTTGATAGAGTAAATAaTAGGAGCTTAAACCCCCT TATTTCTA is systematically analyzed, each nucleotide is described, revealing potential base pairing regions and the specific location of the anticodon loop denoted by a lowercase 'a.' (Figure 3.3). This sequence analysis is crucial for deciphering the intricate details of the tRNA structure and understanding how it might interact with other molecules during cellular processes. tRNA Type and Anticodon *viz.* Type Ile (Isoleucine) indicated that the tRNA molecule is associated with the amino acid Isoleucine.

b. Sample 2A

The results presented here are derived from a comprehensive analysis of a tRNA molecule, specifically identified as tRNA Type Ile with the anticodon GAT. The Infernal score of 114 suggests a high degree of confidence in the accuracy of the secondary structure prediction, emphasizing the reliability of the computational approach utilized.

The tRNA sequence, denoted as 2A.trna2 and spanning positions 443-375, is precisely 69 base pairs in length. This particular tRNA molecule belongs to the Ile type, with the anticodon GAT located at nucleotide positions 30-32 (414-412). Understanding the specific type and anticodon sequence of a tRNA molecule is crucial for unraveling its functional role in the intricate processes of protein synthesis.

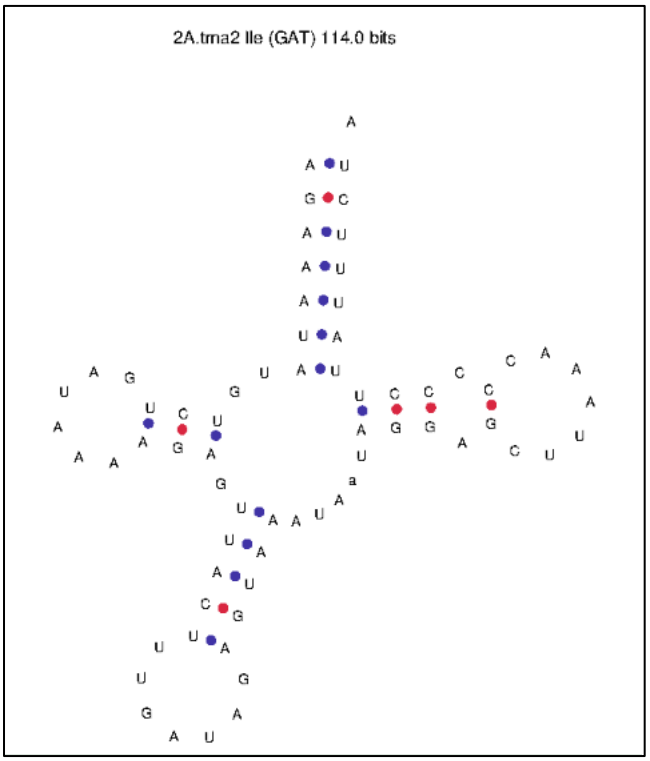

Figure 3.4: tRNAIle secondary structure predicted from sequencing data of Sample 2A

The sequence analysis revealed that the nucleotide composition of the tRNA molecule is AGAAATATGTCTGATAAAAGAGTTACTTTGATAGAGTAAATAaTAGGAGCTTAAACCCC CTTATTTCTA. Each nucleotide was meticulously detailed to provide a comprehensive understanding of the sequence structure. The lowercase 'a' in the anticodon sequence denotes the anticodon loop, a critical element in the recognition of codons during translation (Figure 3.4).

Furthermore, the positional information of the tRNA, beginning at position 375 and concluding at position 443, provided a clear span of the nucleotide sequence under investigation. This positional data held key significant for providing thorough insights to the context of the tRNA within the larger genomic or transcriptomic landscape.

The consistency of the Infernal score at 114 across both sequences (previously mentioned and this one) underscores the reliability and robustness of the predictive model employed. This score not only reflected the accuracy of the predicted secondary structure but also facilitated comparative analyses with other tRNA molecules or variants.

c. Sample 4B

presented results in figure (04) encompass a comprehensive analysis of a specific tRNA molecule, identified as tRNA Type Ile with the anticodon GAT. The Infernal score of 114 reflects the high confidence and reliability of the computational predictions made regarding the secondary structure of this tRNA.

The tRNA sequence, designated as 4B.trna2 and spanning positions 441-373, comprised of 69 base pairs. It belongs to the Ile type, with the anticodon GAT positioned at nucleotide positions 30-32 (412-410). This information proved to be critical for understanding the functional relevance of the tRNA in the context of protein synthesis and decoding specific codons during translation.

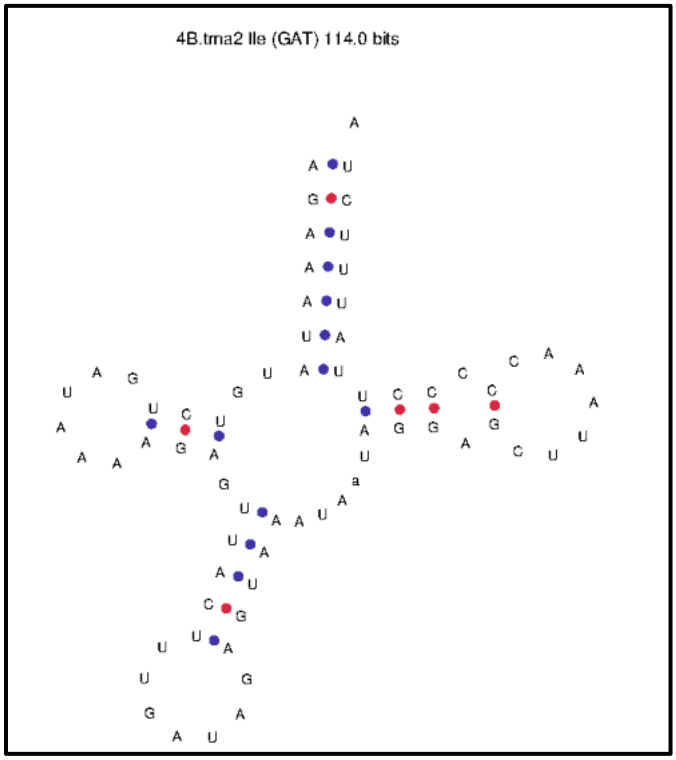

Figure 3.5: tRNAIle secondary structure predicted from sequencing data of Sample 4B

The detailed sequence analysis of AGAAATATGTCTGATAAAAGAGTTACTTTGATAGAGTAAATAaTAGGAGCTTAAACCCC CTTATTTCTA provided a nuanced view of the nucleotide composition. The lowercase 'a' in the anticodon sequence signifies the anticodon loop, (Figure 3.5) a structurally significant region for the tRNA's interaction with mRNA codons. Additionally, the positional data, indicating the tRNA's beginning at position 373 and ending at position 441, provided a contextual understanding of its placement within the larger genomic or transcriptomic framework. The consistency in the Infernal score at 114 across multiple sequences underscored the robustness and reliability of the predictive model employed in this analysis. This score not only reflected the accuracy of the predicted secondary structure but also facilitated meaningful comparisons with other tRNA molecules.

d. Sample X26

The results presented in figure (04) encompass a comprehensive analysis of a specific tRNA molecule, identified as tRNA Type Ile with the anticodon GAT. The Infernal score of 114 attests to the high confidence and reliability of the computational predictions regarding the secondary structure of this tRNA.

The tRNA sequence, labeled as x26.trna2 and spanning positions 447-379, consists of 69 base pairs. It is classified as Type Ile, with the anticodon GAT positioned at nucleotide positions 30-32 (418-416). This information is crucial for elucidating the functional significance of the tRNA in protein synthesis, especially in the accurate decoding of specific mRNA codons during translation.

A detailed sequence analysis of AGAAATATGTCTGATAAAAGAGTTACTTTGATAGAGTAAATAATAGGAGCTTAAACCC CCTTATTTCTA provides insights into the nucleotide composition, with the lowercase 'a' in the anticodon sequence signifying the anticodon loop—a region of structural importance for the interaction of tRNA with mRNA codons (Figure 3.7). The positional information indicates that the tRNA starts at position 379 and concludes at position 447, providing a contextual understanding of its placement within the larger genomic or transcriptomic framework.

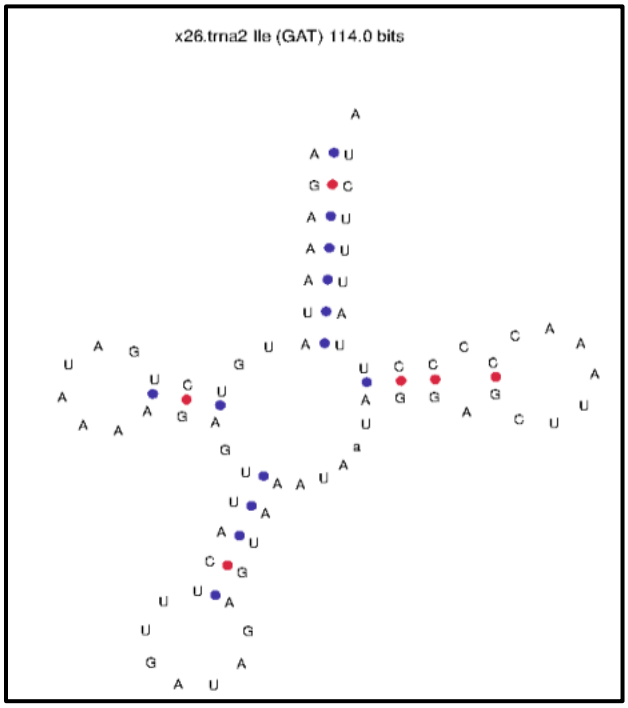

Figure 3.7: tRNAIle secondary structure predicted from sequencing data of Sample X26

The consistency in the Infernal score at 114 across multiple sequences underscores the robustness and reliability of the predictive model employed in this analysis. This score reflects not only the accuracy of the predicted secondary structure but also facilitates meaningful comparisons with other tRNA molecules.

4. DISCUSSION

Mitochondrial DNA (mtDNA) has been a subject of intense investigation due to its implications in various health conditions, including cardiovascular diseases such as hypertension. The study conducted a thorough sequence analysis of the mtDNA segment from positions 4,232 to 4,671, specifically targeting the tRNAIle gene. The identification of distinct base substitution mutations within this region, particularly the mutations 4580G>A, 4439G>C, 4487T>G, and 4584C>T, raises questions about their potential significance and their association with hypertension (Suzuki, T., & Nagao, A. (2011).

The mutations pinpointed in this study fall within the tRNAIle gene, a gene that plays a crucial role in the mitochondrial translation process. Previous research highlighted the importance of tRNA genes in maintaining mitochondrial function, and mutations in these genes have been linked to various mitochondrial disorders (Suzuki, T., & Nagao, A. (2011).

The singularity of these mutations as single nucleotide variations (SNVs) was a notable aspect of the study. Single nucleotide variations had functional consequences and could potentially disrupt normal cellular processes. Understanding the functional impact of SNVs within the tRNAIle gene was crucial for unraveling their role in hypertension. Research on SNVs in mitochondrial genes has been associated with a range of diseases, including cardiovascular disorders, highlighting the relevance of investigating the functional consequences of these mutations (Gorman, G. S et al., 2016).

The identification of intriguing mutations within the mitochondrial gene MTTI, responsible for encoding the tRNAIle gene, added a layer of complexity to the study. The focus on a specific mitochondrial gene responsible for tRNA synthesis suggested a targeted investigation into the potential functional consequences of these mutations. The study's scrutiny extended to a cohort of 80 patients, encompassing individuals afflicted by hypertension and cardiovascular conditions. This comprehensive approach involved a sizable patient cohort essential for understanding the prevalence and potential clinical implications of the identified mutations. The findings of Khera, A. V et al., 2018 emphasized the importance of large-scale cohorts in unraveling the genetic basis of cardiovascular diseases, providing a robust foundation for drawing meaningful associations.

Within the cohort, the study pinpointed the presence of these rare mutations within four individuals. The rarity of these mutations raised questions about their potential role as genetic markers or contributors to the observed cardiovascular conditions. Research on rare mutations in mitochondrial genes highlighted their significance in certain diseases, emphasizing the need for a nuanced understanding of their functional impact and potential associations with specific clinical phenotypes (Taylor, R. W., & Turnbull, D. M. 2005).

Understanding the genetic landscape of individuals afflicted by hypertension and cardiovascular conditions is crucial for advancing personalized medicine approaches. The identification of rare mutations within the MTTI gene provides a starting point for further research into the potential

diagnostic and therapeutic implications of these genetic variants in cardiovascular health (Manolio, T. A et al., 2009).

The identification of mutations within the non-coding region of the MTTI gene introduces a novel dimension to the study, as it suggests potential regulatory roles beyond the conventional focus on coding regions. Research on non-coding regions of mitochondrial genes has gained traction, recognizing their importance in orchestrating various cellular processes (Mercer, T. R et al., 2011).

Intriguingly, the mutations identified in this study were not confined to the region responsible for tRNA translation but were dispersed throughout the entire gene sequence. This dispersion raised questions about the functional significance of mutations in different regions of the MTTI gene. Understanding the distribution of mutations within the gene sequence is essential for deciphering their potential impact on various aspects of mitochondrial function (Barshad, G et al., 2018).

These disruptions in tRNA processing, stability, or interactions with other cellular components could lead to downstream effects on mitochondrial protein synthesis (Smits, P et al., 2007). Ultimately, these disruptions in mitochondrial protein synthesis have the potential to affect energy production. Mitochondrial energy production is tightly regulated, and alterations in the processes leading to energy production can have profound effects on cellular bioenergetics and overall cell function (Wallace, D. C. 2005).

The Variant Effect Prediction (VEP) analysis conducted in this study sheds light on the potential functional consequences of mutations within the non-coding region of the MTTI gene. The results were parallel to the study conducted by Zhou, H., & Rigoutsos, I. 2014 which involved the identification of various effects which were considered crucial for understanding the intricate regulatory mechanisms governing mitochondrial gene expression.

The analysis revealed that 42 percent of the variants were classified as upstream gene variants, suggesting a potential impact on the binding of transcription factors or other regulatory elements crucial for the initiation of transcription. Disruptions in these upstream regions can influence the transcriptional activity of the MTTI gene, potentially leading to altered levels of tRNA production which were according to Zhou, H., & Rigoutsos, I. 2014.

A noteworthy outcome of VEP analysis identified the frame shift mutations in the upstream region of the MTTI gene. Frame shift mutations have the potential to disrupt the reading frame of the gene, likely resulting in a non-functional or truncated tRNA molecule. Ultimately leading to disrupted accurate and efficient translation by tRNA (Chang, H. H et al., 2019).

Additionally, the analysis uncovered Transcription Factor Binding Site (TFBS) variations, constituting 7 percent of the total variants resulting in the binding site of known transcription factors, suggested potential differential regulation of MTTI expression. The modulation of transcription factor binding sites in non-coding regions is linked with various diseases (Mathelier, A et al., 2016).

Gene Ontology (GO) analysis in this study offered a systematic approach to understanding the functional implications of mutations identified in the MTTI gene. GO analysis categorized genes based on their associated biological processes, cellular components, and molecular functions, and also provided a comprehensive view of the gene's role in cellular and molecular contexts (Ashburner, M. et al., 2000). Furthermore, the current investigation found multiple phenotypes linked with the identified mutations, without a clear link to hypertension. This observation highlights the complexity of gene interactions and the need for further investigation into the specific functional consequences of these mutations in the context of cardiovascular disorders (Ehret, G. B et al., 2016).

Sample 1C, exhibited characteristics consistent with a transfer RNA (tRNA) molecule specific to Isoleucine (Ile). The secondary structure prediction reveals an anticodon sequence "GAT" at positions 30-32 (63-65) within the sequence, confirming its assignment as a tRNA-Ile. The Infernal score of 114 indicated a high confidence in the predicted secondary structure. The identified tRNA-Ile molecule in Sample 1C spans positions 34-102, with a length of 69 base pairs. The anticodon sequence "GAT" is crucial for the correct amino acid incorporation during protein synthesis. Mutations or variations in this region can impact the accuracy of translation and potentially lead to functional consequences (Machnicka, M. A et al.,2013).

The analysis of Sample 2A revealed a sequence that corresponded to a transfer RNA (tRNA) molecule, specifically identified as tRNA-Ile. The secondary structure prediction, supported by an Infernal score of 114, provided confidence in the accuracy of the identification. The tRNA-Ile molecule spans positions 375 to 443, with a length of 69 base pairs, and the anticodon sequence "GAT" is located at positions 30-32 (414-412) within the sequence (Suzuki, T., & Nagao, A. 2011). The analysis of Sample 2A revealed the presence of a tRNA-Ile molecule with the anticodon sequence "GAT." This finding contributed significantly to study the mitochondrial protein synthesis and underscored the importance of accurate tRNA identification for studying the molecular processes underlying translation in mitochondria (Sissler, M. et al., 2017).

The analysis of Sample 4B revealed the presence of a tRNA molecule identified as tRNA-Ile, with the anticodon sequence "GAT." The secondary structure prediction, supported by an Infernal score of 114, provides confidence in the accuracy of the tRNA identification. The tRNA-Ile molecule spans positions 373 to 441, covering a length of 69 base pairs (Machnicka, M. A et al., 2013).

The identification of tRNA-Ile in Sample 4B is consistent with the well-established role of tRNA molecules in facilitating protein synthesis by correctly matching amino acids with their corresponding codons on messenger RNA. The Infernal score of 114 indicates a high degree of confidence in the secondary structure prediction. Infernal is a widely used tool for RNA sequence and structure analysis, providing reliable predictions for the identification of tRNA types and their respective anticodon regions (Nawrocki, E. P., & Eddy, S. R. 2013).

The analysis of Sample X26 indicates the presence of a tRNA molecule identified as tRNA-Ile, with the anticodon sequence "GAT." The secondary structure prediction, supported by an Infernal

score of 114, enhances our confidence in the accuracy of the tRNA identification. The tRNA-Ile molecule spans positions 379 to 447, with a length of 69 base pairs (Nawrocki, E. P., & Eddy, S. R. (2013).

Conclusion

It was coincided that, the multifaceted nature of hypertension and its pivotal role in contributing to severe illnesses, particularly cardiovascular diseases, underscores the urgency and importance of comprehensive research in this domain. While environmental factors are acknowledged as significant contributors to hypertension, the intricate interplay with genetic predispositions added a layer of complexity that demanded thorough exploration. Hypertension, acting as a primary catalyst for various cardiovascular diseases, emerged as a global health threat with profound implications, often leading to fatalities on a significant scale. This research, delved into the relatively unexplored realm of mitochondrial mutations in hypertensive individuals, addressed a critical gap in our understanding of the link between hypertension and cardiovascular diseases. The methodology employed involved the meticulous collection of samples from diverse hospital settings, with a specific focus on individuals grappling with hypertension and concomitant heart-related ailments.

Extraction and sequencing of mitochondrial DNA (mtDNA) from these carefully selected samples represented a methodical approach to uncovering potential genetic factors associated with mitochondrial function. The subsequent utilization of advanced bioinformatics tools to scrutinize and interpret the genetic data was utilized to identify and characterize any mutations that may serve as key indicators or contributors between hypertension and cardiovascular diseases. Furthermore, the medical community, particularly physicians, stands to benefit significantly from a deeper insight of the genetic underpinnings of cardiovascular risks in hypertensive patients. This deeper understanding enables the development of more personalized and effective medical interventions, moving beyond a one-size-fits-all approach to healthcare.

As we strive for a holistic understanding of the intricate interplay between genetic and environmental factors in the context of hypertension-related cardiovascular diseases, this research stands as a beacon guiding future endeavors to address the global impact of hypertension on cardiovascular health.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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