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Bioinformatics Analysis of Chronic Obstructive Pulmonary Disease (COPD)-Associated *Interleukin-6* and *CHRNA3* Genetic Variations

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Abstract

Background: Chronic Obstructive Pulmonary Disease (COPD) is the fourth leading cause of death in the world. Genetics factors were found to contribute in the aetiology and progression of COPD. Recent studies demonstrated a significant association of *Interleukin-6* (*IL-6*) and *Cholinergic Receptor Nicotinic Alpha 3* (*CHRNA3*) genetic variants with increased risk of COPD in large case-control cohort. *In-silico* analysis showed that a Single Nucleotide Polymorphism (SNP) rs1818879 is located in the 3'untranslated region (3'UTR) of *IL-6* gene and a synonymous SNP rs1051730 positioned in exon 7 of *CHRNA3* gene.
Objective: This report aims to use the available bioinformatics approach to investigate the potential functional effects of these COPD-related variants on the expression of *IL-6* and *CHRNA3* gene.
Materials and Methods: Bioinformatics analysis of the *IL-6* 3'UTR SNP (rs1818879) using miRBase software was conducted to predict the micro-RNA (miRNA) binding to the target sequence. The possible impact of *CHRNA3* silent SNP (rs1051730) on RNA secondary structure was evaluated using Mfold software.
Results: Our analysis showed that the alternative allele of SNP rs1818879 in '3UTR of *IL-6* could disrupt the binding site of miRNA (mir-619-5p). With regard to *CHRNA3* exon 7 SNP (rs1051730), the single nucleotide change at this location was predicted to cause a significant variation in the secondary structure of *CHRNA3* protein-coding transcripts.
Conclusion: The current study highlighted the possible role of regulatory and silent single nucleotide polymorphisms in disease pathogenesis. An extension of our work will include experimental validation using functional analysis.

Keywords: - CHRNA3, IL-6, micro-RNA, SNP, COPD, mRNA secondary structure

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is the fourth leading cause of death in the world [1]. It is one of the conditions that affect middle-aged or older adults. COPD is a progressive and mostly irreversible disease where patients suffer shortness of breath. COPD is characterized by airflow limitation due to chronic bronchitis, emphysema, and/or small airway inflammation. In addition to airflow obstruction, the condition is related to marked extra pulmonary effect that contributes to morbidity, reduced quality of life, and possibly, mortality of this disease [1]. Cigarette smoking is the primary risk factor for COPD. Smoking accounts for only 10-15% (although this is believed to be an underestimate) of COPD cases highlighting the role of other risk factors in disease susceptibility. COPD familial aggregation suggests that genetic factors contribute to COPD pathogenesis [1]. Genetic studies have been conducted to identify causative genetic markers. To date, the only confirmed genetic factor is a single nucleotide change in Serpin Family A Member 1 (*SERPINA1*) gene. This polymorphism is associated with alpha-1 antitrypsin deficiency and accounts for (1%) of COPD cases [2].
Several genetic investigations were carried out to highlight novel genetic risk factors that could increase COPD susceptibility [3]. One of the genetic regions that could influence the development of COPD was identified to be located within chromosome 15. There are several genes in this region including Cholinergic Receptor Nicotinic Alpha 3 subunits (*CHRNA3*) gene, which encodes the alpha-subunits of nicotinic acetylcholine receptor (nAChR) [4].

nAChR is expressed in neurons as well as in different tissues such as bronchial and alveolar epithelial cells. These receptors are the target site of interaction for nicotine allowing it to exert its behavioural effect. nAChR is responsible for the body's response to smoking and can influence nicotine dependence. Thus, abnormal expression of nAChR was associated with several smoking-related disorders including COPD and lung cancer [4]. Moreover, nAChR was found to modulate synaptic signalling in the Central Nervous System (CNS) and could have a crucial role in CNS disorders such as depression, epilepsy, and Alzheimer's. Consequently, ongoing investigations are targeting to develop therapeutic agents that could regulate nAChR activity and improve patient's life. [5]

The first COPD-related Genome-Wide Association Study (GWAS) identified a significant correlation between a single-base change (rs1051730) located in *CHRNA3* gene with increased risk of COPD[4]. Uncontrolled expression of *CHRNA3* could lead to oxidative stress, a confirmed pathogenic pathway in disease development [6]. Furthermore, Wang and colleagues (2010) highlighted the association of this single nucleotide polymorphism (SNP) with increased tobacco consumption and COPD [7]. Other studies found that SNP rs1051730 was linked with COPD and lung function in many populations including Tatar and Copacetic [8, 9].

Furthermore, genetic studies have attempted to understand the role of inflammatory gene mediators in the pathophysiology of COPD. *Interleukin-6* (*IL-6*) on chromosome 7 is a gene that encodes multifunctional pro-inflammatory cytokines secreted by many immune cells including T cells and macrophages. [10, 11]. The *IL-6* gene was found to play a role in the pathogenesis and development of inflammatory diseases such as asthma and Rheumatoid Arthritis. It is also important in the regulation of cell survival and human carcinogenesis. In addition, a study highlighted the correlation between circulating *IL-6* levels and damage caused by oxidative stress [11]. Oxidative stress plays a critical role in the pathogenesis of COPD. It is influenced by cigarette smoking and air pollutants leading to increased levels of reactive oxygen species (ROS) and nitrogen species (RNS) released from leukocytes and macrophages which in turn, are involved in the inflammatory process of COPD [12]. Grubek-Jaworska and colleagues (2012) showed a significant correlation between the disease and increased levels of *IL-6* in patients' sputum samples [13]. Several *IL-6* variants were linked to lung function and COPD pathogenesis. Yanbaeva and colleagues (2009) identified a significant association of a 3'Untranslated region (3'UTR) SNP (rs1818879) in *IL-6* gene with COPD[14]. Reports demonstrated a significant association with the rate of decline of FEV1 in turn, affecting lung function and susceptibility to acquiring COPD [14].

In order to determine the role of these genetic variants in COPD pathogenesis, it is crucial to conduct functional analysis. This could enhance our understanding on how these single nucleotides employ a far more important information than protein coding. Ultimately, this information could be then used in the development of novel medications that can be utilized in clinical settings. The current study aims to use the available bioinformatics

approach to investigate the potential functional effects of COPD-related variants in *IL-6* and *CHRNA3* genes. The analysis will target two SNPs previously found to be associated with an increased risk of COPD. *In-silico* analysis will investigate the effect of a SNP rs1818879 in 3'UTR of *IL-6* gene on target-specific miRNAs binding sites. Additionally, the bioinformatic analysis will evaluate the possible effect of a synonymous SNP (rs1051730) located in the coding region of CHRNA3 gene on RNA transcript secondary structure.

Material and Methods

IL-6 and miRNA

The miRBase software was used to determine the potential binding sites of miRNA in 3'UTR of the *interleukin-6* gene. The software provides an online search engine to publish the miRNA sequence, (available at http://www.mirbase.org).
miRBase performance depends on the degree of similarity between the miRNA sequence and the target sequence. First, *IL-6* genomic sequence was retrieved from Ensembl genome browser (https://asia.ensembl.org/index.html). The sequence of *IL-6* gene was then derived to be analysed by miRBase software. Notably, the degree of similarity between the miRNA and the target is determined by sequencing the gene in the miRBase to determine the probability of linking the miRNA and the target where the P value is less than 0.05 indicating the importance of high correlation.

CHRNA3 gene mRNA Secondary Structure

There are many structure prediction tools available [15]. In this study, 'mfold' an RNA folding algorithm was used. 'mfold' is a program for prediction and analysis of single DNA and RNA sequences (available as a web server at http://unafold.rna.albany.edu/?q=mfold) [16]. Eight transcripts existed in (*CHRNA3)* gene as ensemble displays. Non-coding transcripts were eliminated from subsequent analysis. Four coding transcripts of primary cDNA sequences were used to predict their RNA secondary structure formulations. Sequences with the wild-type and variant SNP were predicted and matched for any changes in the structure formation of RNA.

Results

IL-6 and miRNA

MiRBase software showed that SNP rs1818879 in the 3'UTR of *IL-6* gene is located in the binding site of hsa-miR-619-5p. Based on the degree of complementary, miRBase tool demonstrated a significant binding probability of hsa-miR-619-5p to its target sequence on *IL-6* 3'UTR with a p-value of 0.025. However, introducing SNP rs1818879 risk allele (A) into the binding site of hsa-miR-619-5p resulted in complete disruption of miRNA target site with a p-value of 0.13 [See figure 1].

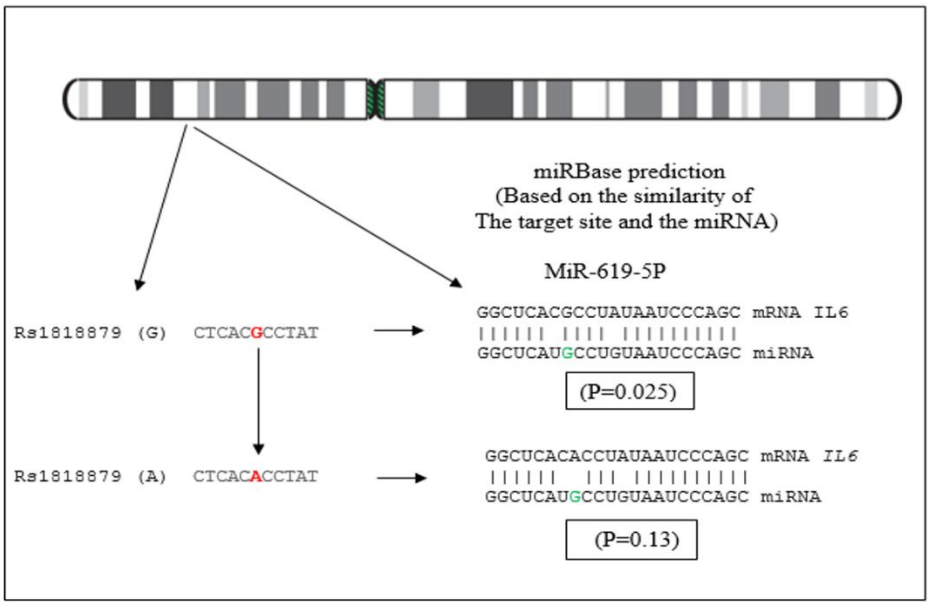

Figure 1: *IL-6* and miRNA. The figure shows the alternative allele 'A' present in mRNA reduces the binding probability of miR-619-5p.

CHRNA3 gene mRNA Secondary Structure

To investigate the possible structural effect of rs1051730 residing within exon 7 of *CHRNA3* gene, the first transcript (CHRNA3-201) consists of 3202bp including 6 exons [as shown in figure 2]. The information was retrieved from Ensembl and used to produce theoretical conformational models and the estimated minimum free-energy of both wild-type and mutated mRNA transcripts using 'Mfold' software. The energy value of wild-type (WT) and mutated models were in the range of ΔG_{WT} = -1177.10 and $\Delta G_{mutated}$ = -1175.80 respectively. Comparing the wild-type model with the mutated structure, a remarkable change was seen in the central part of RNA secondary structure which was caused by a single nucleotide substitution in rs1051730 [See figure 3].

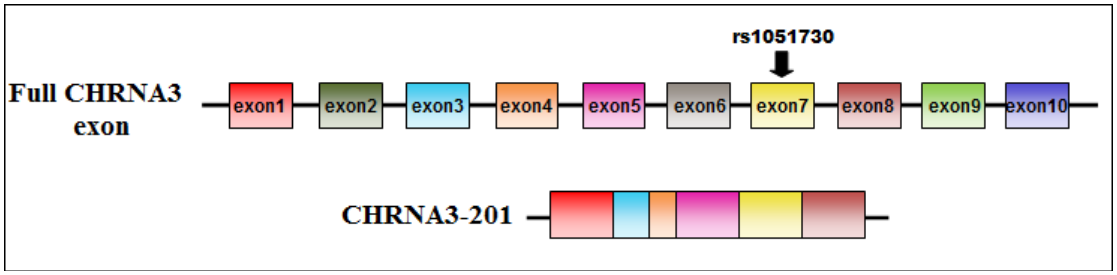

Figure 2: shows a part of the CHRNA3 gene that will encode the final mature RNA and SNP located.

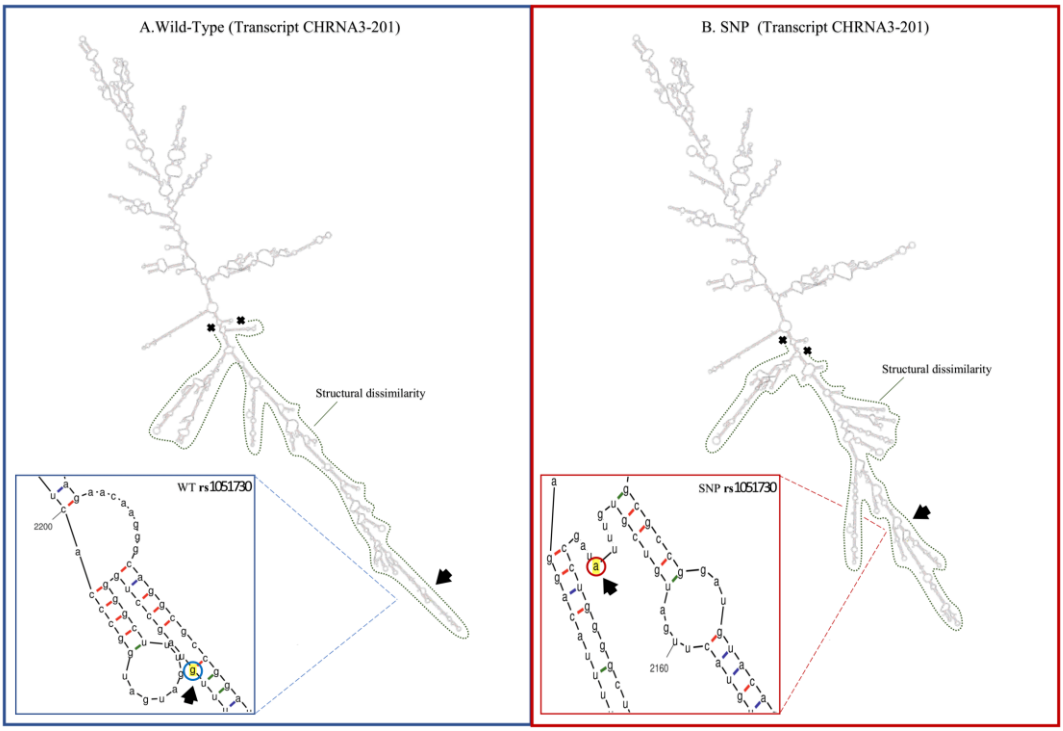

Figure 3: (CHRNA3-201) Wild-Type and SNP predicted structures. The figure shows predicted structure CHRNA3-201 WT in comparison to the structure of SNP rs1051730. The green-dotted outline surrounding the sequence demonstrates the dissimilarity between both structures and the arrows reveal the location of the single base polymorphism where the 'G' allele represents the wild type and 'A' represents the SNP or risk allele. (2173bp)

The analysis was extended to all protein-coding structures. However, other protein-coding transcripts in *CHRNA3* gene did not include the SNP. A complete coding sequence of transcript (CHRNA4-202) consisting of 3202bp was used in the analysis. This transcript included 6 exons [See figure 4]. The calculated minimal free energy for WT and mutant structure were ΔG_{WT} = -809.60 and $\Delta G_{mutated}$ = -806.80 respectively. The mutated transcript model showed a slight change on the top part of the structure around the SNP rs1051730 when compared to the wild-type model [See figure 5].

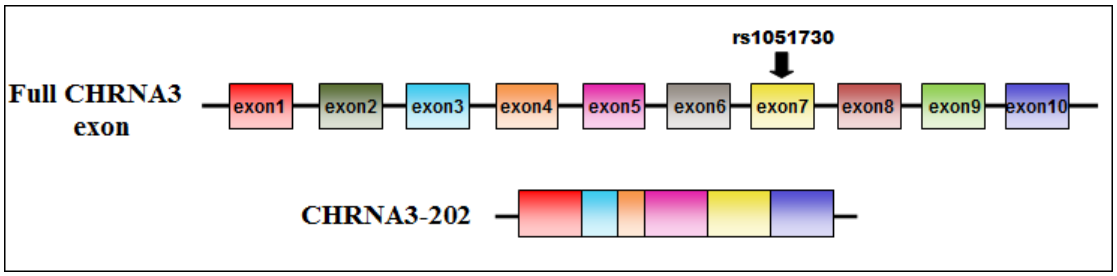

Figure 4: shows a part of the CHRNA3 gene that will encode CHRNA3-202 transcript and SNP position

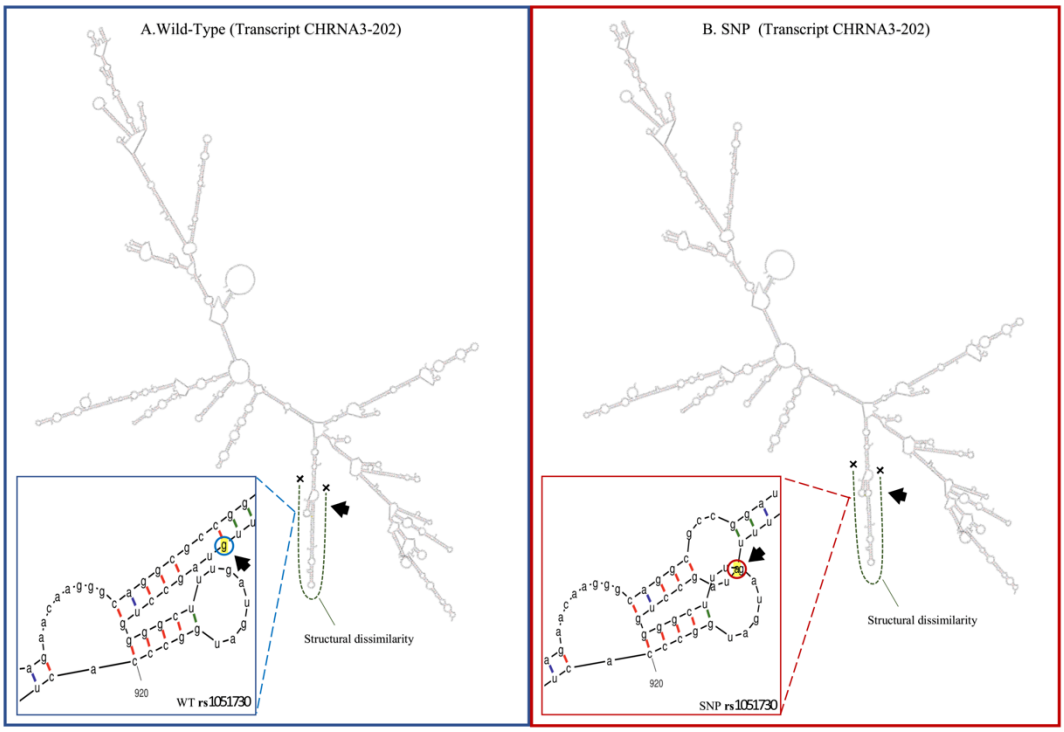

Figure 5: (CHRNA3-202) Wild-Type and SNP predicted structures. The figure shows predicted structure CHRNA3-202 WT in comparison to the structure of SNP rs1051730. The green-dotted outline surrounding the sequence demonstrates the dissimilarity between both structures and the arrows reveal the location of the single base polymorphism where the 'G' allele represents the wild type and 'A' represents the SNP or risk allele. (945bp)

A similar analysis was carried out using the sequence of (*CHRNA3-206*) transcript (including 8 exons) [See figure 6]. We proceeded to the evaluation of secondary structure by analyzing the difference between the structure of wild-type and mutant. The energy values of wild-type (WT) and mutated models were in the range of ΔG_{WT} = -930.10 and $\Delta G_{mutated}$ = -928.80 respectively. A significant difference was observed in mutated structure spanning the SNP that is located on (1190bp) in comparison to WT [See figure 7].

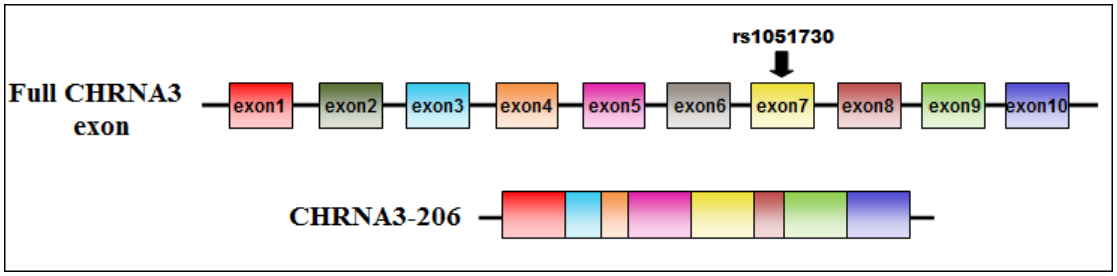

Figure 6: shows a part of the CHRNA3 gene that will encode of the final mature RNA and SNP located.

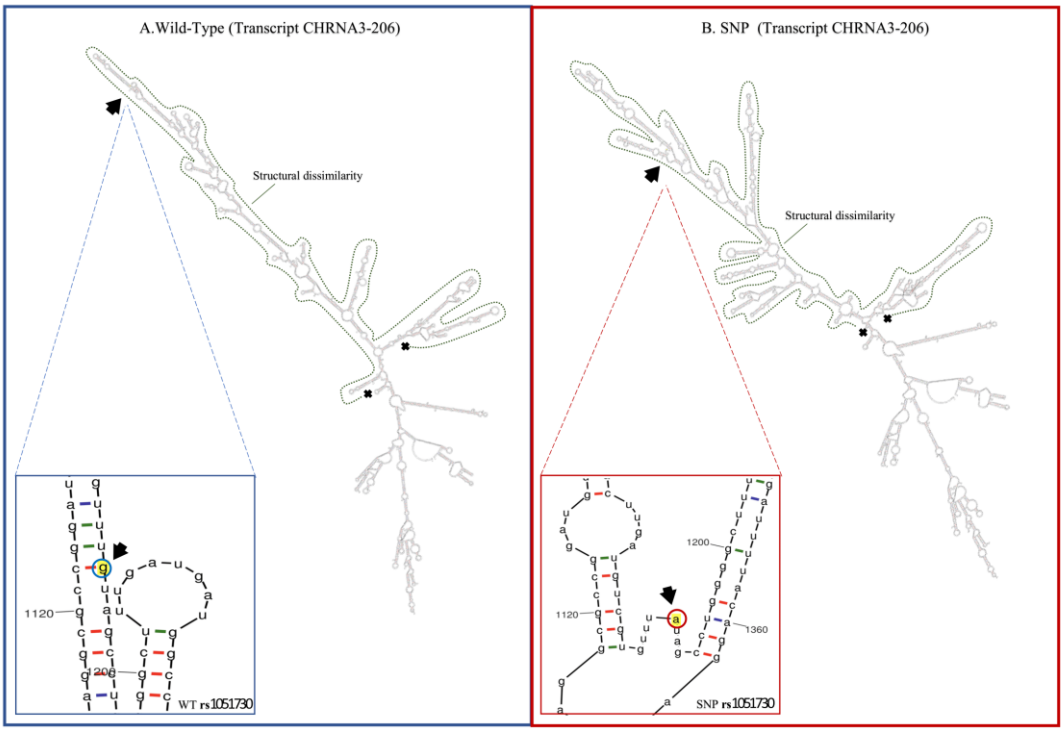

Figure 7: (CHRNA3-206) Wild-Type and SNP predicted structures. The figure shows predicted structure CHRNA3-206 WT in comparison to the structure of SNP rs1051730. The green-dotted outline surrounding the sequence demonstrates the dissimilarity between both structures and the arrows reveal the location of the single base polymorphism where the 'G' allele represents the wild type and 'A' represents the SNP or risk allele. (1190bp)

Discussion

The investigations in this report aimed to evaluate the potential functional consequences of IL-6 variant (rs1818879) and CHRNA3 variant (rs1051730) on gene expression based on bioinformatics analysis. Functional studies are crucial to identifying the mechanisms by which regulatory region variants and silent polymorphisms exert their effects. Bioinformatics analysis demonstrated the possible impact of 3'UTR IL-6 SNP (rs1818879) on the binding ability of mir-619-5p. Additionally, computational analysis of exon 7 synonymous polymorphism (rs1051730) showed a remarkable variation in the secondary structure of the mutated CHRNA3 mRNA in comparison to the wild-type structure.

The previous study applied to evaluate the expression of miR-619-5p in the clinical diagnosis and prognosis of colorectal carcinoma (CRC), showed that the tumor tissues have a significantly decreased level of miR-619-5p in normal tissues. In addition, miR-619-5p shows clinicopathological features in CRC patients that show a link with lymphovascular invasion and perineural invasion [17]. Moreover, Tatyana and colleagues (2017) showed that miR-619-5p is one of three miRNAs that had a high expression in autism patients compared to control subjects. These studies highlight the potential pathological effect of miR-619-5p [18].

Several reports have highlighted the role of miRNAs in COPD. Schembri and colleagues (2008) conducted miRNAs whole genome expression on epithelial cells and found 28 miRNAs to be different between smokers and non-smokers. Additionally, Izzotti and colleagues (2009) analyzed the expression of miRNAs in mice exposed to environmental smoke. The study reported the downregulation of 24 miRNAs when compared to non-smoker controls [19]. Furthermore, a previous report demonstrated that in vitro cytokines-based stimulation of fibroblasts from COPD patients led to a significant reduction in mir-146a expression. This observation was associated with the prolonged mRNA half-life of cyclooxygenase-2, which will eventually result in increased prostaglandin (PGE2) expression [19].

Previous genetic studies highlighted the potential role of cholinergic nicotinic receptor subunit (CHRNA5-CHRNA3-CHRNB4) genes located on chromosome 15q25 region in the development of COPD [20]. These reports considered the cholinergic nicotinic cluster as a predisposing factor for COPD. Nicotine and nicotine-derived molecules in cigarette smoke excite the expression of nAChRs in lung tissue cells. Increased release

of reactive oxygen species (ROS) and reactive nitrogen species (RNS) with insufficient antioxidants to prevent oxidative damage from cigarette smoke contributes to the development of COPD [12].

An intriguing concern that has been addressed in this report is the mechanism by which a silent single nucleotide change can contribute to disease pathogenesis. The hypothesis of SNP-induced structural variations in mRNA has been supported by earlier studies. Nackley and colleagues (2006) showed that a synonymous SNP in Catechol-O- methyltransferase (COMT) gene results in altered mRNA structure and increased RNA stability. This post-transcriptional modification was associated with reduced protein expression, decreased enzymatic activity, and a rise in patients' sensitivity to pain [21].

Previous studies have reported the association of synonymous variants with conformational variation in RNA secondary structure. Duan and his colleagues (2002) evidenced the effect of Dopamine Receptor D2 (DRD2) silent or synonymous polymorphisms on the stability of mRNA by testing six naturally occurring synonymous variations within the gene. The study reported that not every synonymous or silent change in sequences is non-functional. To verify this statement, the authors reported that the 957T synonymous polymorphisms in DRD2 gene had the effect of altering the mRNA folding and in turn, altering its stability and significantly changing the protein expression [22]. Moreover, a previous study tested the impact of the minor allele of rs5888, a SNP in Scavenger Receptor class B type I (SCARB1) gene, on protein expression. The report demonstrated a significant decrease in the protein function whenever the synonymous polymorphism was present [22]. These studies supported our assumed hypothesis that silent SNPs affect protein stability and could result in phenotypic consequences and to overcome the limitation of testing the probability of our hypothesis in vivo, experimental validation using functional analysis is needed.

An extension of this work will aim to experimentally validate the predicted impact of rs1818879 and rs1051730 on miRNAs and RNA secondary structure, respectively. In addition, bioinformatics analysis together with experimental investigations will be carried out for other genome-widely COPD-associated variants located in IL-6 and cholinergic nicotinic cluster genes. In conclusion, functional studies are essential to improve our understanding of the contribution of these genomic polymorphisms in the pathogenesis of complex diseases. This will hopefully fulfil the ultimate goal of identifying genetic markers that can be used for a predictive blood test or that can be utilized in developing reliable personalized medications that provide maximum efficiency with minimum side effects.

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