Epigenetic Variation in Terms of the Extent of Methylation of the AhRR Gene as a Result of Smoking Behaviour

Shifa Siddiqui¹, Munazzah Tasleem¹,²,*

¹Department of Biochemistry, Jamia Hamdard, New Delhi, INDIA.
²BIAltesse LLC, 5109 Silverton Ln, Louisville, Kentucky, USA.

ABSTRACT
A large volume of data accumulated and numerous studies published substantiate the strong association of smoking behaviour with a variety of adverse health effects in humans. It is well established that smoking exposure increases the risk of respiratory diseases, cardiovascular diseases, and different forms of cancer, including lung, liver, and colon. A wide variety of these exposures result in epigenetic modifications of the monocytes (cells most sensitive to these exposures). DNA methylation and histone modification are crucial epigenetic modifications of the genome that are involved in regulating many cellular processes and gene activity by alteration of DNA accessibility and chromatin structure. We now recognize that genetic variation is not the only source of phenotypic variation that may be passed down through generations, since genetic variance can only account for a small portion of the diversity in complex traits. Thus, epigenetics comes into play. The pathogenesis involves the release of pro-inflammatory cytokines as a result of activation of enzymes that regulate these epigenetic modifications when exposed to cigarette smoke. Any aberration in the DNA methylation is indicative of conditions far from normal and need for immediate attention. Hypomethylation of the aryl hydrocarbon Receptor Repressor (AhRR) gene indicates long-term smoking exposure and might therefore be a monitor for smoking-induced disease risk. Here, we reviewed the concepts of epigenesis and the identification of epigenetic markers for the study of smoking behaviour and its applicability in the recognition of various risks pertaining to health of an individual. Furthermore, we also discussed about their probable translation to clinical practice and therapeutics in order to enhance risk predictions of smoking related diseases.

Keywords: AhRR, Hypomethylation, Epigenetic, Inflammatory cytokines, Therapeutics.

INTRODUCTION
Phenotypic variation results in evolution (Pigliucci et al., 2006). Apart from genotypic differences, environmental factor, behaviour etc. also tend to have a great effect on the phenotype of an individual (Angers et al., 2020). It brings about phenotypic variability in individual genotypes by means of phenotypic plasticity. Phenotypic plasticity is mediated by epigenetic modifications (Dar et al., 2022). Epigenetic modifications refer to the alterations in the chromosome that affect gene expression without any change in the nucleotide sequence (Al Aboud et al., 2023). These chemical modifications are stable as well as heritable and control gene expression by transcriptionally suppressing transposable elements, thereby regulating genome activity and promoting genomic stability (Kumari et al., 2022). They offer rates of changes that are more dynamic than mutation which serves as a source for genetic variation (Habig et al., 2021). It holds great significance in the screening, diagnosis of various diseases that occur as a result of environmental deviations, in the determination of metastatic phenotype (Chatterjee et al., 2018) and also in the induction of defence mechanisms in plants (Zhu et al., 2016). According to reports, variations in the pH of the surrounding environment are related to epigenetic polymorphism in the clonal diploid fish Chromosomus cosneogaes (Budd et al., 2022). Additionally, a severe instance of epigenetically induced phenotypic plasticity in sequential hermaphrodite fishes is documented (Roberts et al., 2021), in which an individual completely changes sex and is linked to regional variations in length at sex change.Epigenetic modifications may be the underlying cause of these regional variations. It was discovered that these alterations were caused by varying degrees of DNA methylation of sex-determining genes (Budd et al., 2022). Furthermore, protein synthesis and gene expression depend heavily on phenotypic heterogeneity. There is growing agreement that one of the missing pieces to understanding phenomena not explained by the DNA sequence alone may be epigenetics, and particularly TEI (Transgenerational Epigenetic Inheritance).
Examples of such phenomena include incomplete penetrance, which refers to individuals of a given genotype expressing different phenotypes, and variance in expressivity (Chapelle and Silvestre., 2022). The goal is to investigate how various levels of epigenetic variations specifically DNA methylation react or exhibit variability/diversity in response to changes in the environment, an individual's behaviour, and evolving ecological processes across time. For example, the degree of methylation caused by long-term smoking of the aryl hydrocarbon Receptor Repressor (AhRR) gene (Chapelle and Silvestre., 2022). It may also serve as a monitor for the risk of smoking-related diseases (Skov-Jeppesen et al., 2023).

The need for biomarker in smoking related diseases

Smoking is thought to be the primary cause of lung cancer globally, accounting for 80% to 90% of cases of the disease (Bray et al., 2018; Schabath and Cote, 2019). It is the primary global cause of cancer-related fatalities in both men and women. In addition to cancer, the two greatest health risks linked to cigarette smoking are still cardiovascular illness and chronic obstructive pulmonary disease (Fragou et al., 2019; Mohite et al., 2017). A wealth of evidence indicates that cigarette smoke is a highly complex mixture of about 4800 different compounds, including gases like ozone, formaldehyde, ammonia, carbon monoxide, toluene, and benzene, as well as carcinogens, cocarcinogens, and/or mutagens. It has been found to shorten people’s lives by an average of 7 years (Li et al., 2022; Fragou et al., 2019). Cigarette smoke exposure lacks a cumulative measuring method, which is fundamental to an effective estimation of the future risks despite the severity of the diseases (Zhang et al., 2016). The onset of smoking could be predicted by a number of genetic, psychological (family socioeconomic position, peer and parental smoking, psychological distress), and environmental risk factors (Li et al., 2022). The other, less accurate methods for determining smoking behaviours and patterns were self-reporting, plasma cotinine, and exhaled carbon monoxide. Because of recollection bias, they only show smoking behaviours that are temporary (Skov-Jeppesen et al., 2023; Zhang et al., 2016). Additionally, self-reporting may result in underreporting, which could lead to ineffective measures, and the cotinine level is particularly insensitive to modest tobacco use. Pregnant women, for example, are unwilling to disclose their smoking status because it is socially unacceptable, (Goodwin et al., 2017). Finding more trustworthy biomarkers to determine smoking status is therefore necessary. Recent studies in the field of epigenetics and methylation profiling have created new opportunities in the search for biomarkers indicative of lifetime and current smoking exposure that may improve the ability to anticipate dangers associated with smoking (Zhang et al., 2016).

AhRR as a Tumour Suppressor Gene

AhR (Aryl hydrocarbon Receptor), a transcription factor of the bHLH/PAS family, is an intracellular receptor with a promiscuous ligand binding site that binds a variety of structurally different substances in order to get activated. It can induce gene transcription in response to xenobiotics (Baker et al., 2020). AhR remains in an inactivated state in the cytoplasm, its translocation to the nucleus occurs as a result of the conformational change when exposed to exogenous chemicals or ligands (Tsufi et al., 2014). Animals and humans experience a range of toxic reactions when the AhR is activated and it also regulates various physiological processes, tumour induction, proliferation, differentiation, inflammation and apoptosis (Esser and Rannug, 2015). AhR is highly expressed in many malignancies, including those of the breast, lung, liver, stomach, head, neck, cervical, and ovarian regions. Tetrachlorodibenzo-p-dioxin (TCDD) is a strong tumour promoter that works by activating AhR signalling for an extended period of time (Standford et al., 2016). AhRR (Aryl hydrocarbon Receptor Repressor) causes the repression of the AhR mediated activations by competing with it in its dimerization with ARNT to be able to bind to the Dioxin-Responsive Elements (DREs)(Gutierrez-Vazquez and Quintana, 2018). In fact, chromosome 5, where the human AhRR gene is found, has a short arm that is frequently deleted in different types of cancers, suggesting the presence of a tumour suppressor gene there. It was evident that overexpressed AhRR could counteract the anti-apoptotic impact of TCDD-activated AhR (Vogel and Haarmann-Stemmann, 2017). However a notable contradiction to the tumour suppression property of AhRR is supposed to be the epigenetic modification i.e. the hypomethylation of AhRR promoter in the sample of smokers (Rotroff et al., 2016). These epigenetic changes are linked to an increased risk of developing respiratory tract cancers and suggest that AhRR may have a role in the development of lung cancer. However, most studies suggest that the detoxification of toxins such as Polycyclic Aromatic Hydrocarbons (PAHs), which are the main carcinogens responsible for lung and other cancers, contained in tobacco smoke, is found to be facilitated by AhRR. Lower methylation may be a cellular response to the chemicals found in tobacco smoke, leading to greater expression of this gene (Novakovic et al., 2014). Several studies have also suggested that maternal smoking in pregnancy leads to the hypomethylation of AhRR in neonatal blood (Rotroff et al., 2016; Lee et al., 2015).

DNA methylation and its use as a smoking indicator

In literal terms, the term “epigenetic” means "in addition to changes in genetic sequence. "It implies that gene activity or function can be altered without affecting the DNA sequence, resulting in changes that can be passed on to progeny cells. Gene silencing and expression are controlled by epigenetic mechanisms, which create a layer of control inside a cell. This regulation, which differs throughout tissues, is crucial to cell differentiation
status, it was found to correlate with increased risk of myocardial infarction (Langsted et al., 2020). Furthermore, it was reported that Hypomethylation of cg05575921 in the aryl hydrocarbon receptor repressor (AhRR) gene is also associated with in utero tobacco smoke exposure and therefore tobacco smoking during pregnancy is associated with metabolic dysfunction in children (Vidal et al., 2023).

Molecular mechanism associated with epigenetic changes

A significant portion of the pathophysiology of diseases caused by cigarette smoke involves inflammation and immunological changes, genetic modifications, oxidative damage, endothelial dysfunction, cell senescence, and other factors (Zong and others, 2019). In addition to genetic changes in protooncogenes and tumour suppressor genes, smoking-induced lung tumours also exhibit epigenetic modifications such as dysregulated DNA, changed histone acetylation, and abnormal microRNA expression. Unusual DNA methylation patterns in cancerous cells are linked to the activation of protooncogenes, the silencing of tumour suppressor genes, and a decrease in chromosomal stability. As a result, they are the most important epigenetic alteration in the identification of numerous diseases and also shed light on their function in normal cellular homeostasis.

Two mechanisms underlie epigenetics: first, alterations in chromosomal proteins, such as post-translational modifications in DNA and histone proteins, which modify the genome’s three-dimensional conformation; second, chemical modifications of the DNA strand itself, such as the methylation of cytosine to 5-methylcytosine DNA at the CpG sites (Locke et al., 2019). In CpG (5′cytosine-phosphate-guanine-3′) dinucleotide sites, which frequently span gene promoters and first exons, a methyl group from S-adenosyl-L-methionine is transferred to the fifth position of the pyrimidine ring of cytosines, thereby covalently binding to DNA. The silencing of tumour suppressors or regulatory areas due to DNA hypermethylation can result in transcriptional suppression, reduced gene expression, and dysregulation of cell growth. Chromosome stability is influenced by DNA hypomethylation, which primarily occurs at repeated areas. This can result in incorrect chromosome segregation during cell division and unintentional activation of transposable elements within the genome, which can cause additional genetic damage (Locke et al., 2019; Pan et al., 2018). DNA Methyltransferases (DNMTs) are primarily responsible for catalysing DNA methylation. According to Kwon et al. (2007), researchers discovered that the expression of DNMT1 was notably higher in the lung tissues of smokers than in nonsmokers. Additionally, by demethylating the genes that a CS condensate had inhibited, the inhibition of DNMT1 can restore their expression (Yang et al., 2012). Therefore, by upregulating DNMT1, which in turn causes the downregulation of target genes, CS can promote gene hypermethylation. Additionally, it

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**Table 1: Sites linked to varying degrees of DNA methylation following tobacco use.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Methylation site</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>AhRR</td>
<td>cg05575921</td>
<td>Grieshober et al., 2020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baglietto et al., 2017</td>
</tr>
<tr>
<td>PRSS23</td>
<td>cg23771366</td>
<td>Gutiérrez et al., 2021</td>
</tr>
<tr>
<td>BCOR</td>
<td>cg07764473</td>
<td>Klebaner et al., 2016</td>
</tr>
<tr>
<td>TSC2D3</td>
<td>cg21380860</td>
<td>Klebaner et al., 2016</td>
</tr>
<tr>
<td>GPR15</td>
<td>cg19859270</td>
<td>Tsaprouni et al., 2014</td>
</tr>
<tr>
<td>LRP5</td>
<td>cg21611682</td>
<td>Tsaprouni et al., 2014</td>
</tr>
<tr>
<td>F2RL3</td>
<td>cg03636183</td>
<td>Fasanelli et al., 2015</td>
</tr>
<tr>
<td>IER3</td>
<td>cg06126421</td>
<td>Tsaprouni et al., 2014</td>
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has been noted that MTHFR hypermethylation is connected to smoking-induced hypomethylation of AhRR. Since the MTHFR gene is involved in the system that determines the availability of methyl groups, its hypermethylation results in even less AhRR hypomethylation. According to Beach et al. (2017), methylation levels on the AhRR and MTHFR should be ascertained in order to accurately quantify smoke exposure.

**CONCLUSION**

Our discussion starts with the understanding of the concept of epigenesis, how it translates in the variation in phenotypes that are not resulting from mere DNA sequence changes. Epigenetic variation provides an effective explanation for the phenomenon that defies explanation based on DNA sequence. Certain examples that substantiated the above studies were also discussed followed by a discussion of the deleterious effect on health caused by smoking tobacco. Factors pertaining to smoking initiation, smoking persistence and nicotine dependence were brought to light in order to study the need for an efficient method of diagnosis and prevention and to establish a link between the tobacco smoke inhalation and the associated epigenetic modifications. We reviewed this with respect to AhR signaling pathway to understand the DNA methylation effects on AhRR gene in the context of smoking. Furthermore, light was shed on the links that could be established to develop efficient biomarkers to enhance risk predictions related to smoking and also to identify smoking cessation.

**FUTURE PROSPECTS**

Since abnormalities in methylation and these alterations are known to be associated with a number of chronic diseases, they can be a desirable feature for the creation of diagnostic tools. Therapeutic approaches that include targeted gene re-activation or re-silencing have a focus because of the potentially reversible nature of these alterations. It may be used as objective biomarkers of lifetime and current smoking exposure as well as for estimating the risk of smoking-related illnesses. In order to better understand the pathophysiology of diseases linked to cigarette smoking, it is anticipated that the molecular processes behind the epigenetic changes in aberrant inflammation will be recognized. As a result, new epigenetic therapeutics may soon be discovered. However, a number of obstacles, including technological ones, still stand in the way of a successful translation of these indicators to clinical practice.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**ABBREVIATIONS**

AhRR: Aryl hydrocarbon Receptor Repressor; TEI: Transgenerational Epigenetic Inheritance; BHLH/PAS: Basic helix-loop-helix-per-Arnt-sim; AhR: Aryl hydrocarbon Receptor; TCDD: Tetrachlorodibenzo-p-dioxin; ARNT: Aryl hydrocarbon receptor nuclear translocator; DREs: Dioxin-Responsive Elements; PAHs: Polycyclic Aromatic Hydrocarbons; COPD: Chronic Obstructive Pulmonary Disease; DNMT1: DNA Methyltransferase 1; MTHFR: Methylenetetrahydrofolate reductase; PRSS23: Serine protease 23; CHRD: Cholinergic nicotinic receptor; RPS6KA2: Ribosomal protein S6 kinase; GPR15: G-protein-coupled receptor 15.

**REFERENCES**


