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Epigenetic Signatures for Continuous Metabolic Health Monitoring in at Risk Populations

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ABSTRACT

Among the frequently occurring metabolic disorders that persist to cause significant morbidity among the at-risk groups are obesity, diabetes, and cardiovascular diseases. The standard biomarkers of such disorders cannot typically be monitored continuously and in real time. In the given research paper, it is discussed whether the epigenetic signatures and, specifically, DNA methylation can serve as such an indicator of the present-day metabolic health. The state-of-the-art epigenomic tools, including bisulfite sequencing and DNA methylation arrays, are being used to measure the extent of epigenetic changes in individuals in high-risk groups, including obese patients, prediabetic patients, and hypertensive patients. The study discovered that some patterns of methylation are very much associated with the indicators of metabolic health, such as insulin resistance, lipid profiles, and body mass index. Besides, this study proposes a machine learning model that will utilize these epigenetic markers to predict the metabolic changes with high accuracy, sensitivity, and specificity. It has been demonstrated that the application of epigenetic signatures is not only applicable in the context of sustained metabolic health testing but could also be applicable in the sphere of personalized medicine, and is rather an inexpensive and less invasive methodology of early disease diagnosis. This paper suggests that it can be used in future applications in real-time monitoring systems and that longitudinal studies are necessary to further validate such observations. The achievement of the goal of developing the application of epigenetic biomarkers into a routine practice with the at-risk population is preconditioned by this article to reach the desired state of improved metabolic health and prevent diseases.

Keywords: *Epigenetics; DNA Methylation; Metabolic Health; Biomarkers; Predictive Modeling; At-Risk Populations; Machine Learning.*

INTRODUCTION

Obesity, type 2 diabetes, cardiovascular diseases, and hypertension are metabolic conditions that are important health problems of the world, especially among vulnerable groups, including those with a history of these diseases in the family, the elderly, and sedentary people [1]. Although lifestyle changes and pharmacological interventions have been useful, early diagnosis and constant monitoring are essential in

enhancing health outcomes [5][6]. The conventional methods of diagnostics, such as blood analyses and physical check-ups, cannot always reflect the dynamic nature of metabolic health, and therefore, there is a need to establish new types of biomarkers that can give real-time information on the changes in metabolism [7].

The research on hereditary phenomena of gene expression without a change in DNA sequence has become promising in finding biomarkers of metabolic disorders through an investigation called epigenetics. One of the most predicted epigenetic changes is DNA methylation, which has been associated with a large number of metabolic disorders. Recently, new methods of high-throughput sequencing and epigenomic profiling have enabled the identification of certain epigenetic signatures that are associated with metabolic health. In addition to being indicative of present metabolic conditions, these signatures may also be used to forecast the future risks, which can be of great value in understanding the disease onset, well before it may manifest in the clinical setting.

The proposed research will investigate how DNA methylation can be used as a continuous metabolic health technology in vulnerable groups. This study pays attention to the determination of epigenetic markers that correlate with the indicators of metabolic health, including insulin resistance, lipid profile, and body mass index. It additionally presents a machine learning tool based on these epigenetic signatures, which together predict the alterations of metabolic health, which can offer a more specific and tailor-made approach to monitoring and treatment.

By establishing the basis of integrating the epigenetic biomarkers into the regular clinical practice, the final objective of this work is to allow a more proactive and individual approach to the management of the metabolic disorders in the at-risk populations.

Literature Review

The reports examine the transcriptional, epigenetic, and metabolic signatures of cardiometabolic syndrome, especially in individuals with severe phenotypes [2]. Their results indicate the correlation of their specific epigenetic changes with metabolic dysfunctions, including obesity, insulin resistance, and cardiovascular diseases. The research postulates that such epigenetic signatures might be employed to further characterize and comprehend the heterogeneity of cardiometabolic syndrome, which has a prospective solution of early detection and individualized therapy interventions [8].

The concept of epigenetic aging is extended in the previous study, which demonstrates the fact that increased epigenetic aging is closely associated with a number of cardiometabolic, hematologic, and renal abnormalities [3]. It shows that those people whose epigenetic aging is quicker have increased risks of developing diseases such as diabetes, hypertension, and chronic kidney disease. This research highlights the importance of epigenetic age as a biomarker in terms of predicting health outcomes over the long term and the necessity of monitoring epigenetic age as a means of early disease prevention among vulnerable populations [9].

The topic of the study was the establishment of an epigenetic signature of allostatic load, which is an indicator of the accumulated physiological burden of chronic stress [4]. Their research determines particular epigenetic features that indicate the biological effects of chronic stress, to which a variety of diseases, such as metabolic and cardiovascular ones, are associated. The demonstration of this signature in various people groups is a powerful instrument to examine the effect of stress on health and preconditions individualized interventions aimed at the diseases caused by stress [10].

All these studies indicate that epigenetic changes have the potential to be good biomarkers in predicting cardiometabolic diseases, accelerated aging, and allostatic load. They highlight the significance of epigenetic profiling in disease pathophysiology and underline the possibility of the epigenetic signature

being used in the early diagnosis, prevention, and treatment of the disease, as well as the potential of personalized treatment.

Materials and Methods

Study Design and Participant Selection

The institutional ethics review board (IRB) approved this study, and informed consent was signed by all the participants before they were enrolled. This study has enrolled 200 participants who were at-risk groups, such as the obese, pre-diabetic, and hypertensive groups, along with healthy controls. The ages of the participants ranged between 30 and 65 years, and the gender and ethnicity balance was equal.

The population at risk was included based on the following criteria: Body Mass Index (BMI) of 25kg/m^2, pre-diagnosis of prediabetes or hypertension, and the absence of metabolic disorders. The healthy controls were chosen on the basis of the lack of metabolic risk factors and normal levels of blood pressure with a BMI of 18.5-24.9 kg/m^2.

Collection of Samples and Processing

All the participants were requested to have their blood sampled following a 12-hour overnight fast. The separation of the plasma was done through centrifugation, and then it was stored at -80 °C to be analyzed. The Qiagen DNA Blood Mini Kit (Qiagen, Valencia, CA) protocol was used to extract DNA in a peripheral blood mononuclear cell (PBMC) using the Qiagen DNA Blood Mini Kit. The quality and concentration of DNA were determined with a Nanodrop spectrophotometer.

DNA Methylation Profiling

Analysis of DNA methylation was done by the Illumina Infinium Methylation EPIC Bead Chip array that encompasses more than 850,000 CpG sites in the DNA genome. They were treated with the procedure as recommended by the manufacturer, with bisulfite conversion, hybridization to the chip, followed by scanning on the Illumina iScan machine. Data of the methylation was preprocessed through the R/Bioconductor package minerfi on quality control and normalization of the data, such as background correction and elimination of subpar probes.

Bioinformatics Analysis and Discovery of Epigenetic Signature

The study determined differentially methylated regions (DMRs) in at-risk and control groups using the limma package on the R platform, and the false discovery rate (FDR) was set at less than 0.05. To investigate the functional applicability of DMRs, the study conducted pathway enrichment analysis on the clusterProfiler package and analyzed the pathways associated with metabolic diseases.

The data on methylation was also combined with gene expression data of the same participants (obtained in a separate RNA-sequencing study) to test how DNA methylation is related to gene expression in the metabolic pathways.

Predictive Modeling

The prediction of metabolic health outcomes using DNA methylation signatures through a machine learning approach was used to create a predictive model. To determine the most important epigenetic biomarkers that would be the best predictors of metabolic changes like insulin resistance and lipid abnormalities, a random forest classifier was employed.

This model was trained on a 70 % training-validation split, and the rest 30 % of the data was employed as a test. Accuracy, sensitivity, specificity, and area under the receiver operating characteristic curve (AUC-ROC) were used in the performance evaluation of the model.

Statistical Analysis

R (4.0.3) was used to conduct all statistical analyses. Because the tests used in comparing groups (Student t-test or Wilcoxon rank-sum test) are used to evaluate normally and non-normally distributed values, respectively, comparisons between groups about continuous variables were conducted.

The chi-square tests were used to analyze categorical variables. The level of significance of all the statistical tests was established at $P < 0.05$.

Results and Discussion
Participant Characteristics

The sample size consisted of 200 subjects who were separated into two groups (100 at-risk (obesity, prediabetes, hypertension) and 100 healthy controls). The mean age of the at-risk population was 45.2 years, 6.5 years old, and the mean age of the control population was 43.8 years, 5.9 years old.

The balance of gender was the same in the two groups (45% male in the at-risk group and 47% male in the control group). The at-risk group had much greater BMI (32.1 ± 4.4 kg/m^2 vs. 22.9 ± 2.7 kg/m^2), fasting glucose (125.5 ± 15.3 mg/dL vs. 95.3 ± 8.6 mg/dL), and blood pressure (130/85 mmHg vs. 120/75 mmHg) than controls.

Differential DNA Methylation in At-Risk vs. Control Groups

The methylation profiling of the DNA showed that there are 1,312 different methylated CpG sites (DMS) between the at-risk and control groups, 800 hypermethylated and 512 hypomethylated in the at-risk group. These DMS were overexpressing genes related to metabolic processes like insulin signaling, lipid metabolism, and inflammatory reaction.

For example, a gene related to adiponectin production (another significant insulin-sensitizing hormone, ADIPOQ) was hypermethylated in its promoter region in the at-risk group, which was associated with reduced blood adiponectin levels.

Epigenetic Signature and Metabolic Health Prediction

It utilized the methylation data to form a random forest classifier to estimate metabolic health phenotypes, such as insulin resistance, lipid profiles, and BMI. The model had an accuracy, sensitivity, and specificity of 87.2%, 84.5%and 89.3% respectively. The predictive ability of the insulin resistance was excellent, with the area under the receiver operating characteristic curve (AUC-ROC) of 0.91.

The major biomarkers that were identified during the model were PPARG, a fat cell differentiation and glucose metabolism-related gene, and FTO, an established obesity-related gene.

Epigenetic Markers validation

The ten most differentially methylated were validated using another cohort of 50 participants in a longitudinal study. The beta-hair modifications of these genes were in line with the initial results, and thus, this continues to affirm their applicability in monitoring metabolic health using the biomarkers. Also, these epigenetic signatures were strongly correlated with metabolic indicators of HbA1c and triglycerides, which once again confirms their applicability.

The present paper shows that DNA methylation markers, including those of ADIPOQ, PPARG, and FTO, can be used as biomarkers of continual metabolic health. These epigenetic changes will give information about the molecular pathways of metabolic diseases, such as insulin resistance and inflammation. Epigenetics is useful in the early detection of metabolic health problems, potentially leading to serious

health complications, as the random forest classifier designed in the current research was effective in predicting metabolic health outcomes.

The results, although they cannot be used to draw a conclusion, offer a promising technique of non-invasive real-time monitoring, despite the fact that the cross-sectional design is not to be used in a causal inference. Future studies should examine additional modifications that occur in the epigenome and the impact of lifestyle modification.

Conclusion

This paper has proposed DNA methylation signatures as an excellent biomarker for ongoing monitoring of metabolic health, especially in vulnerable groups. The findings of extensive changes in DNA methylation of the major metabolic genes ADIPOQ, PPARG, and FTO demonstrated that the DNA methylation change could be used to mirror the molecular pathophysiology of metabolic disorders, including obesity, diabetes, and cardiovascular diseases. The creation of a predictive model based on these epigenetic signatures creates an opportunity for real-time and non-invasive monitoring of metabolic health, which is essential in high-risk people to detect and intervene early. The results are capable of influencing the field of personalized medicine to go beyond the conventional forms of diagnosis, which tend to provide periodic health pictures. Nevertheless, the cross-sectional research design does not allow for a causal conclusion, and the predictive model was effective, but additional research with more extensive and varied data is essential to ensure its relevance. Moreover, other epigenetic alterations, including histone adjustments or non-coding RNAs, can be integrated to give an even greater insight into the metabolic control. Longitudinal studies are also necessary to establish the predictive utility of these epigenetic signatures, both on a long-term basis and the reversibility of the signatures through lifestyle interventions. Further efforts in the future should be made to optimize the machine learning model through the addition of more biomarkers, predictive accuracy improvement, and the implementation of this strategy into wearable devices to perform real-time monitoring. The effect of lifestyle changes, including diet and exercise, on epigenetic changes will be major in the development of individualized intervention strategies to prevent metabolic diseases. Finally, this strategy may transform the process of managing metabolic health and enhance the outcomes of the population in terms of timely diagnostics and individual treatment.

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