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MITOCHONDRIAL DNA METHYLATION: A POSSIBLE BREACH IN PATHOPHYSIOLOGY OF CARDIOVASCULAR DISEASES

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ABSTRACT

Cardiovascular diseases (CVD) are a cluster of diseases that hamper cardio-vascular system and may lead to death. Several injuries and risk factors like obesity correspond to the malfunctioning of heart and blood vessels that lead to the condition of stroke, myocardial infarction, deep vein thrombosis (DVT), etc. Only a few studies have depicted the role of mitochondrial genes and their epigenetic regulation in DVT. The association of certain mitochondrial genes of electron transport chain and oxidative phosphorylation has been reported in CVD. Expression of these genes is regulated by DNA methylation. Obesity not only influences the CVD but also has been found responsible for alteration in mtDNA methylation. In present study, of the 5182 research articles, only 13 and 4 papers were found dealing with the qualitative and quantitative analysis, respectively. MT-CO1, MT-CO2, MT-CO3, MT-TL1 and PPARGC1 genes were noted to be involved in disease condition. The mitochondrial DNA methylation has profound impact on mitochondrial gene expression which may lead to the development of dysfunctional mitochondria and cause the development of various cardiovascular diseases. In present paper, a systemic analysis was done with the hypothesis that mitochondrial DNA methylation has an intriguing role in the occurrence of several CVDs in relation to obesity.

Keywords: Cardiovascular diseases, patho-physiology, methylation, mitochondrial DNA

INTRODUCTION

Cardiovascular disease (CVD) is a general term used to refer to a cluster of conditions that affects the heart and blood vessels. The major risk factors that enhance the chances of CVD are hypertension, high blood cholesterol, diabetes, obesity and overweight, smoking, physical inactivity, life style, heredity, etc. (Lesk, 2017). Heart disease is most common in people of age between 30-44 (Sharma *et al*., 2019). CVDs include stroke, myocardial infarction (MI), angina, arteriosclerosis, coronary heart disease (CHD), etc. (Zhong *et al*., 2016). Many cardiac diseases like atherosclerosis, ischemia reperfusion injury, heart failure and hypertension are believed to be associated with mitochondrial dysfunction, mostly due to the inadequate cellular energy production and uncontrolled production of reactive oxygen species. Mitochondrial DNA (mtDNA) damage is one of the instrumental phenotypic characteristics of mitochondrial dysfunction, which is characterized by the presence of dysfunctional mitochondrial enzymes, impaired mitochondrial biogenesis, decrease in mitochondrial number, fibrosis stimulation, cardiac remodelling, etc. (Siasos *et al*., 2018; Diaz-vegas *et al*., 2020). All these impaired signatures are potential factors for cardiovascular anomalies like heart failure and ischemia.

Mitochondrial nuclear genome consists of 3.3 billion DNA base pairs. On the other hand, mitochondria contain its own DNA (mtDNA) within the mitochondrial matrix which is circular,

double stranded and comprises of 16,569 bp. Its heavy stand is purine rich while the complementary strand (light stand) is pyrimidines enrich. mtDNA also possesses a non-coding control region (NCR) of ~1.1 kb that has elements to regulate replication and two major transcription initiation sites required to generate polycistronic transcripts (Lesk, 2017). Mitochondrial DNA is organized into nucleoprotein complexes 'nucleoids' which is different from nuclear DNA because the nuclear DNA is protected by arrangement into nucleosome. Mitochondrial transcription factor A (TFAM), an abundant protein, is the primary constituent of mitochondrial nucleoids. It is associated with mtDNA transcription in addition to packaging mtDNA into nucleoids. However, recent super-resolution microscopy experiments have revealed that each nucleoid is approximately 100 nm in diameter and comprises one copy of mtDNA packaged by multiple TFAM molecules (Sharma *et al*., 2019).

Mitochondria play a vital role in lipid metabolism of cell by carrying out β-oxidation of free fatty acid (Sobenin *et al*., 2013). Modulation in blood lipid profile is one of the initial recognizable risk factors of atherosclerosis, as it is the low-density lipoprotein (LDL) fraction that assists as the key source of lipids accumulating in the arterial wall (Libby *et al*., 2016). Further, the consequences of mitochondrial dysfunction are not just limited to atherosclerotic plaque development in vascular wall, but could expand to other parts of cardiovascular system. Cardiomyocytes require high energy consumption for their normal functioning and become very sensitive to aberrations of mitochondrial turnover process. Deficient mitochondrial biogenesis was earlier proved to be connected with endoplasmic reticulum (ER) stress involved in the pathogenesis of abdominal aortic aneurysm (Poznyak *et al*., 2020). Hypertension is one of the widely accepted risk factors of atherosclerosis and is involved in oxidative stress and endothelial dysfunction (Ward *et al*., 2006). The hypertension is also the resultant of mitochondrial ATP synthase dysfunction in cardiomyocyte. The mitochondrial Ca^{2+} overload also contributes to the development of hypertension (Iuv, 2001).

Obesity is the most damaging factor that contributes to the occurrence of CVDs. Obesity could be a result of unhealthy eating habits, genetics or even stress. A condition of excess nutrients or a disequilibrium may also lead to mitochondrial dysfunction, that contributes to several obesity-driven pathologies as well. For these reasons, the nutrition and epigenetics are strictly interconnected with each other. Obesity has not only been correlated to CVDs, but also to the alterations in mtDNA methylation (Bordani *et al*., 2019). Hence, mitochondrial DNA methylation acts as mediator between metabolic conditions (obesity) and mitochondrial health as well. Low grade inflammation, induced through obesity, reportedly reduces the energy generation and expenditure in adipocyte mitochondria thereby further aggravates obesity and increases fat accumulation and weight gain (Zhou *et al*., 2020). Thus revealing a nexus between obesity, mitochondrial dysfunction, mtDNA methylation and cardiovascular diseases. Interestingly, mtDNA copy number are lower in obese/overweighed people and are consistent with the reduction in metabolic flexibility reported in obesity (Smith *et al*., 2018; Galgani and Femandez-Verdejo, 2021). Apart from these factors, epigenetics plays a major role in orchestrating the biological processes underlying CVD, such as atherosclerosis, hypertension and inflammation (Gorman *et al*., 2016). Epigenetic modifications are the heritable changes in DNA that regulate the ways how genes are expressed but do not affect the nucleotide sequence itself. These includes DNA methylation, histone modification, microRNA regulation, etc. Of these phenomena, methylation significantly contributes to the development of CVDs. DNA methyl transferases or DNMTs catalyse DNA methylation by transferring a methyl group from S-adenyl methionine (SAM) to the 5^{th} carbon of a cytosine residue to form 5mc. Other DNMTs like Dnmt3a and Dnmt3b establish new patterns of methylation to unmodified DNA and hence are *de novo* DNMTs (Moore *et al*., 2013). Dnmt1 on the other hand functions during DNA replication in order to copy the DNA methylation pattern from the parental strand to the newly synthesized daughter strand (Goto *et al*., 1994). A comparison between a normal heart tissue and the tissue from patients with heart failure has revealed the presence of differential methylation of angiogenesis-related genes in diseased tissue, as well as differential expression (Feng *et al*., 2005). DNA methylation contributed to the elevated expression of inflammatory markers and preceded the formation of aortic fatty streaks (Makar and Wilson, 2004; Movassagh *et al*., 2010). The spectrum of cardiovascular epigenetics is rapidly expanding from

biological/animal studies to epidemiological studies. Methylation has been found associated with long-interspersed nucleotide repetitive elements-1(LINE-1) and ALU that contributes to CVD (Baccarelli *et al*., 2010; Kim *et al*., 2010).

The mitochondrial genome not only harbours methylated genes specific to cardiovascular diseases, but also other genetic risk factors like miRNAs and SNPs that may possibly contribute to several forms of CVD. MiR-696, miR-532, miR-345-3p and miR-690 are elevated in mitochondria of failing hearts and are also associated with oxidative stress pathway and energy metabolism (Wang *et al*., 2017). Further, miR-696 specifically targets PGC-1α in order to decrease both fatty acid oxidation in myocytes and biosynthesis of mitochondria (Aoi *et al*., 2010). Similarly, many studies have shown the connection between mitochondrial SNPs (mtSNPs) and cardiovascular diseases like myocardial infarction, diabetes and atherothrombic brain infarction (Sawabe *et al*., 2011). A study consisting of Iranian population showed m.750A > G polymorphism was 1.6 times more frequent in patients with coronary heart disease (CHD) and hence was associated with the risk of developing CHD. In addition to this, m.325C > T, m.12315G > A, m.13513G > A polymorphisms were found to have a close link with the risk of CVD (Vindis *et al*., 2005). In recent years, mitochondrial DNA methylation has gained a lot of attention as some major roles of this phenomena have been found in CVD. Mitochondrial DNA methylation changes could also throw some light on mitochondrial dysfunction which may lead to as one of the causes of CVD pathophysiology. In present study, an attempt was made to analyse all reports available so far depicting association between mitochondrial DNA methylation and cardiovascular diseases.

MATERIALS AND METHODS

The meta-analysis for present study was carried out in accordance to the guidelines of PRISMA [Preferred Reporting Items for Systematic Reviews and Meta-analyses] statement (*http://www.prisma-statement.org/*). All the studies that showed association between mtDNA methylation and CVD were electronically searched on PubMed, Science Direct, Google Scholar and Cochrane Library till 30th June 2023. All the databases were searched using keywords methylation, cardiovascular diseases, myocardial infarction, atherosclerosis, Ischemia, coronary heart disease, hypertension, CVD, mitochondrial DNA methylation, mitochondria. A manual search was also performed to find any other potentially related articles that were previously mentioned in other meta-analysis and reviews. After that, gene-gene interaction of the obtained genes was carried out using KEGG (*http://www.genome.ad.jp/kegg/*), Genemania (*http://www.genemania.org*) and Panther (*http://www.pantherdb.org*). Lastly, protein-protein interaction of the same genes was studied using STRING (*http://string-db.org*) to understand the pathways involved in the anomalies.

Inclusion and exclusion criteria

All the potential studies were considered qualified if they showed connection between mtDNA methylation and CVD. Following parameters were used for publication selection: a) relationship between mtDNA methylation; b) case control studies; c) human studies; d) studies with detailed sample size for calculation of odds ratio and class interval; e) published in the English language; and f) full length articles. Previous meta-analysis, duplicates, review papers, dissertation thesis, publications other than in English language and studies with no sample details were excluded from the current meta-analysis.

Study selection and data extraction

After going through literature search, all the full-length articles that matched with the search terminologies were retrieved. Then the articles that met inclusion criteria were included. The data that were extracted from the selected studies were: a) first author's name, b) year of publication, c) number

of cases and controls, d) mean methylation of specific targeted genes in diseased and healthy individuals, and e) percentage of individuals belonging to different races.

RESULTS AND DISCUSSION

The detailed procedure of meta-analysis consisted of 5182 articles which were screened according to the search criteria through proper PRISMA guidelines. After removing the duplicate studies, there were 5176 articles. Records that were excluded after not matching with the search criteria were 4186. These studies were further screened for full text availability, which resulted in 998 articles. After that, 985 studies were excluded due to no relation with CVD, which resulted in only 13 articles in qualitative synthesis [Fig. 1]. Finally, after going through the detailed procedure of a systemic study, only 4 research articles were tracked down that qualified all the inclusion criteria. Baccarelli and Byun (2015) in their study targeted different genes like MT-CO1, MT-CO2, MT-CO3, MT-TL1, ATP synthase (MT-ATP6 & MT-ATP8) and NADH dehydrogenase (MT-MD5) when targeted to find the association between mtDNA methylation and CVD, and after obtaining the platelet mtDNA methylation through bisulphite-PCR pyro-sequencing, revealed significantly higher mtDNA methylation in CVD patients than healthy controls in MT-CO1 (18.53%, $p < 0.0001$), MT-CO2 (3.33%, $p < 0.0001$), MT-CO3 (0.92%, $p < 0.0001$), and MT-TL1 (1.67%, $p < 0.0001$), with these 4

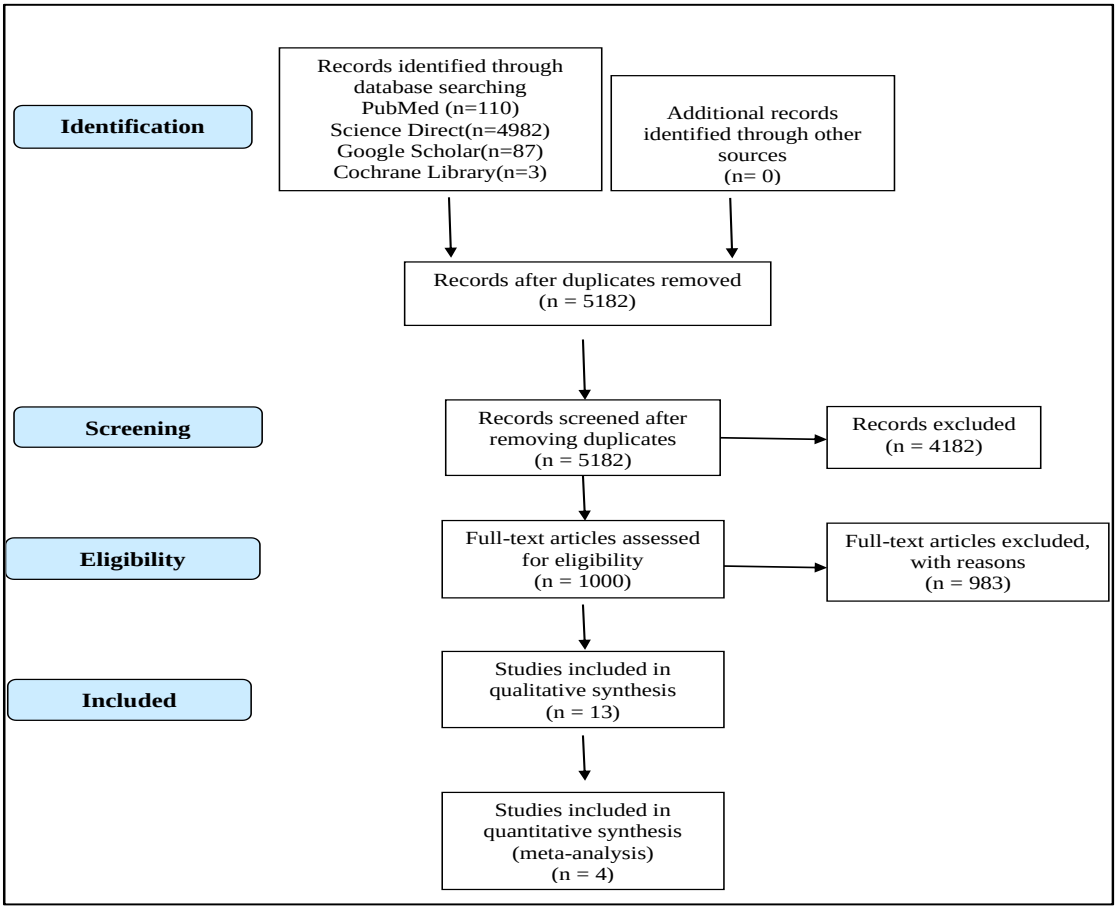

Fig. 1: Flow diagram describing the process through which articles were selected. A total of 5182 studies from different databases were considered and further screened as per the inclusion and exclusion criteria of PRISMA guidelines. Four studies were finally included for the meta-analysis which showed direct link between mitochondrial DNA methylation, cardiovascular diseases and obesity

genes playing significant roles in ATP synthesis. The predictive probability analysis of this study carried out on mixed races like Caucasian, Hispanic, African-American and Asian individuals demonstrated that platelet mtDNA methylation could be utilised as a biomarker for CVD. This study being the first to showcase the occurrence of platelet mitochondrial DNA methylation in CVD opened up a vast portal of information to dive deeper for several answers regarding the same (Baccarelli and Byun, 2015). The mean mtDNA methylation of MT-CO1 was found lesser in healthy individuals as compared to CVD patients. By using Mann-Whitney U test, the difference between mean mtDNA methylation levels between healthy individuals and CVD patients was 18.53% (p value<0.0001, 95% CI: 13.25 to 24.20). Similarly, the mean mtDNA methylation of MT-CO2 in healthy individuals was lesser in comparison to the CVD patients. The difference between mean mtDNA methylation in healthy individuals and the individuals suffering from CVD was 3.33 (p value = 0.0001, 95% CI: 2.13 to 4.71). Keeping the same process, the mean mtDNA methylation of MT-CO3 in CVD patients was higher as compared to the healthy ones. Further, mean mtDNA methylation level of MT-TL1 in healthy individuals was lower than the individuals with CVD. The other gene targets like MT-ATP6, MT-ATP8 and MT-ND5 were also selected, but due to the non-availability of any significant differences in mtDNA methylation these gene targets were excluded. Other than finding out the mean mtDNA methylation, the predictive probability of platelet mtDNA methylation to use as a CVD biomarker was performed using receiver operating characteristic (ROC) curve analysis.

Corsi *et al*. (2020) in 5-years study showed an important connection between CVD and mtDNA methylation and connecting future prediction of CVD development in obese and overweight people. Majority of the individuals in this study were Caucasian. In this study, 13CpG sites distributed within 7 mitochondrial genomic regions were analysed and the baseline mtDNA methylation was found lower in CVD-free individuals as compared to those who developed CVD during follow-up at nt6807 of MT-CO1 (CVD-free: mean = 10.8 ± 4.8%; CVD-developed: mean = 12.5 ± 4.8%; P = 0.014), nt9444 of MT-CO3 (CVD-free: mean = 0.7 ± 2%; CVD-developed: mean = 1.3 ± 1.9%; P = 0.042) and nt3254 of MT-TL1 (CVD-free: mean = 2.4 ± 1.5%; CVD-developed: mean = 3.0 ± 1.6% ; P = 0.008). For statistical analysis, χ^2 and Student's t tests were performed to compare CVD-free and CVD developed individuals for continuous variables. Besides mtDNA methylation, other factors like multivariate logistic regression, BMI (body mass index), fasting BP (blood pressure), total cholesterol/high density lipoprotein ratio (TC/HDL), SBP (systolic blood pressure) and DBP (diastolic blood pressure) were performed to observe the association between DNA methylation at each CpG site (locus) and the risk of CVD during follow-up. 95% CI were reported with increase in 5-methylcytosine (5mC) at each CpG site.

Park *et al*. (2021) found decreased mitochondrial copy number associated with coronary artery disease (CAD) (mtDNA copy number was lower in acute coronary syndrome (ACS) than in the patients with stable CAD (0.89 ± 0.24 vs. 1.00 ± 0.28, p = 0.013). Also, higher methylation ratio of mitochondrial D-loop region (1.11 ± 0.24 vs. 1.00 ± 0.25, p = 0.027) was reported. Increased DNA methylation in mitochondrial D-loop region means reduced mtDNA content which appears to influence the clinical phenotype of CAD. When methylation status was compared, ACS had higher DNA methylation ratio in mitochondrial D-loop region (p = 0.008) but similar DNA methylation ratio in promoter region of nuclear PPARGC1A (p = 0.978). The increased methylation in mitochondrial D-loop may affect mtDNA replication so may reduce mtDNA copy number. Mitochondrial DNA methylation is quite relevant in CAD and lower mtDNA copy number in peripheral blood may be a risk factor for progression to ACS in patients with atheromatous coronary artery plaque. Hence, mtDNA methylation not only has connection in the initiation of CVD, but also affects the copy number of mtDNA in peripheral blood that could serve as an indication as a risk factor.

Bordoni *et al*. (2022) conducted a study in a cohort of 198 individuals which comprised of 101 normal weight individuals and 97 overweight/obese individuals, mtDNA methylation levels were checked along with mitochondrial copy number. Apart from testing several correlations of mtDNA methylation with body compositions which acts as a quantitative bar for obesity measurement, methylation was found in higher percentage in light strand of mitochondrial D-loop. The lack of

Table 1: Gene-gene interaction of MTCO1, MTCO2, MTCO3 and PPARGC1A individually and also amongst these four genes using Genemania

Gene-gene interaction individually through Genemania			
MTCO1	**MTCO2**	**MTCO3**	**PPARGC1A**
MTCO2	MTCO3	MTCO1	MEF2D
BCAT1	MPO	COX5A	RNF34
COX7A2L	COX8A	COX7B	MYBBP1A
OXA1L	COX6B1	CYCS	PPARG
MTRF1L	SCO1	MTCO2	PRKAA2
COX6A1	LEF1	COX6C	CAMK4
COX7C	COX7A2L	COX7C	NR1D1
CYCS	COX4I1	ELANE	NR5A2
MTCYB	MTRF1L	COX6B1	LRPPRC
COX5A	COX6A1	COX8A	ESRRA
COX7B	COX7B	COX5B	DDIT3
FH	OXA1L	ADCY5	NCOA1
SLC25A10	MTCO1	OXA1L	PARK7
COX6C	COX5A	COX7A2L	SIRT1
COX5B	COX7C	COX4IL	NR2F2
MTATP6	TIMM17B	UCP1	ATF2
COX6B1	MTCYB	COX6A1	ESRRG
COX8A	COX6C	ATP5F1B	RORA
COX4I1	COX5B	HSPB2	IRF4
MTCO3	ATP5F1B	UCP3	CYCS
Gene-gene interaction combined (MTCO1, MTCO2, MTCO3 & PPARGC1A)			
MTCO1	**MTCO2**	**MTCO3**	**PPARGC1A**
MTFF1L	MEF2D	MTCYB	COX5A
COX7B	COX6C	COX8A	COX7A2L
COX5B	COX4IL	OXA1L	SCO1

Table 2: Protein-protein interaction of MTCO1, MTCO2, MTCO3 and PPARGC1A individually using STRING

Protein-protein interaction individually through STRING			
MTCO1	**MTCO2**	**MTCO3**	**PPARGC1A**
MTCYB	MTCO3	MTCO2	HNF4A
COX5A	COX7C	MTND4	PPARA
MTCO3	COX5B	COX7C	PPARG
COX4I1	MTCO1	MTCO1	YY1
MTCO2	COX5A	MTCYB	NRIP1
MTND4	COX6C	COX5A	FOX
COX5B	MTCYB	MTATP6	SIRT1
UQCRC2	COX7A2	COX6B1	ESRRG
COX7C	COX6B1	COX5B	NRF1
MTND1	MTND4	COX6A1	ESRRA

metabolic flexibility in obesity could be traced back to the reduction of mtDNA copy number. The reduction in mtDNA copy number reportedly is the result of an increase in mtDNA methylation in the D-loop region. The decrease in mitochondrial copy number is related to CAD. Thus, obesity is one of the major risk factors in causing CVD. While establishing a possible connection between 5 genes obtained from systematic analysis of mitochondria's connection with CVDs with obesity as a risk factor, interactome studies were carried out between the individual genes using Genemania, KEGG pathway analysis and Panther. Later, the protein-protein analysis was performed using STRING [Fig. 2]. Genemania and Panther provided the list of genes that were connected with each gene of interest along with the pathways involved. When the analysis was performed individually among each protein and between each other using STRING, several other inter-connected proteins were found linked to each other [Table 2]. When the network study was carried out in KEGG, several interconnected networks among OXPHOS, metabolic pathways and cardiovascular diseases were found along with diseases like Parkinson's, Alzheimer's and cancers. When Panther was used to find some possible

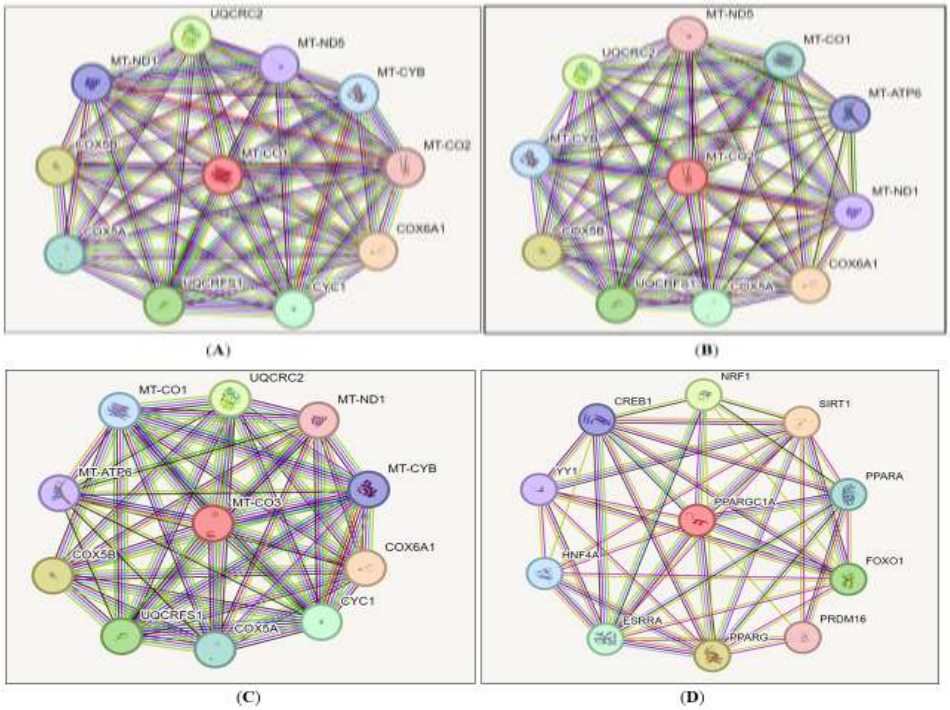

Fig. 2: Individual protein interaction using STRING. (A), (B), (C), and (D) represent the individual protein interaction of MTCO1, MTCO2, MTCO3 and PPARGC1A, respectively. Each protein (MTCO1, MTCO2, MTCO3 and PPARGC1A) is connected to 10 other proteins in immediate position

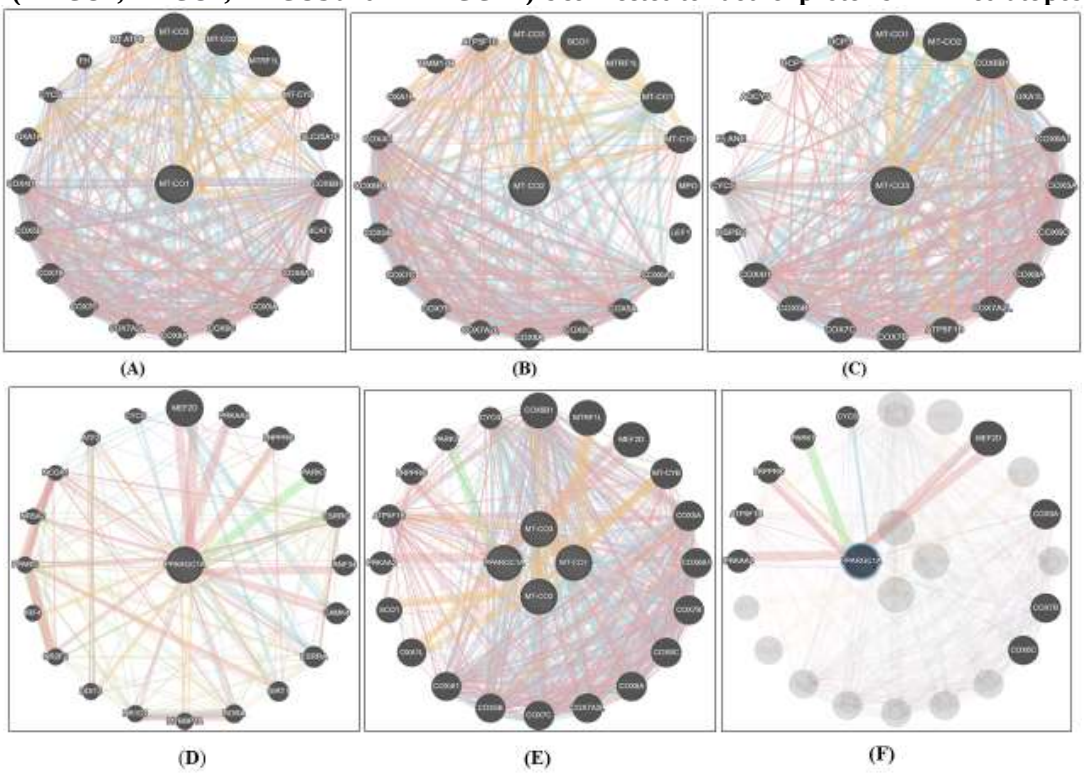

Fig. 3: The gene-gene interactome study of MTCO1, MTCO2, MTCO3 and PPARGC1A individually and also between these genes using Genemania. (A), (B), (C) and (D) shows the individual network analysis of MTCO1, MTCO2, MTCO3 and PPARGC1A respectively. (E) Combined network analysis of MTCO1, MTCO2, MTCO3 and PPARGC1A. (F) Interaction of PPARGC1A and mitochondrial COX5A, COX7B and COX6C

connections between five major genes (MTCO1, MTCO2, MTCO3, MTTL1 and PPARGC1A), ATP synthesis was highlighted majorly as the key pathway that was common and connected these 5 genes. ATP synthesis being the principle process carried out through oxidative phosphorylation and ETC, forms the energy currency of the cell. With the production of ATP, uncountable cellular and biochemical processes sustain. Similarly, Genemania was used to find out intergenic connections between five genes as well as individually too [Fig. 3]. Individual gene interactions study revealed that MTCO1 had connection with twenty other genes including MTCO2 and MTCO3. MTCO2, MTCO3 and PPARGC1A too showed connection with twenty other genes individually [Table 1]. Due to the lack of information on MTTL1, no data could be retrieved from Genemania and Panther. Lastly, when the interactome was studied between these four genes, twenty different gene networks were retrieved that were connected to these four genes. As the present study hypothesizes a link between obesity, mitochondrial methylation and CVD, the role of PPARGC1A gene became one of the major data mining sites to understand it's connection with mitochondrial dysfunction that further instigate the initiation of CVD. Individual gene-gene interaction was carried out and PPARGC1A gene's pathway connection was checked against the mitochondrially methylated genes (MTCO1, MTCO2 and MTCO3). PPARGC1A showed pathway interaction with COX5A, COX6A and COX7B. These three genes are the subunits of cytochrome C oxidase in OXPHOS chain, and dysfunction in cytochrome C oxidase was found to be associated with hypertrophic cardiomyopathy where approximately one-quarter of the individuals with cytochrome C oxidase deficiency had above mentioned cardiac anomaly. Myocardial insufficiency is even related to the reduced working efficiency of cytochrome C oxidase (Bohm *et al*., 2006; Vogt *et al*., 2018). Therefore, it is safe to say that there is definitely an important connection between PPARGC1A with the working ability of mitochondria in cardiac scenario.

Protein-protein interactome study was performed using STRING to further understand the protein-protein interaction dynamics between these 5 genes and the interaction amongst them. When individual protein interactions were carried out, each protein baring MTTL1 showed ten protein associations, whereas when protein interactome study was carried out in between MTCO1, MTCO2, MTCO3 and PPARGC1A, each protein displayed 10 individual interactions. However, the group interaction study didn't show any significant association between the three mitochondrially encoded proteins and PPARGC1A.

Mitochondria is the powerhouse of a cell and the cell's entire energy consumption is taken care by this organelle through several enzymes and biochemical pathways. Mitochondria in that way may play an important role in maintaining the normal functions of heart, and dysfunction of mitochondria in any form could be related to the pathogenesis and development of various types of heart disease (Elinor, 2012). All the 3 MT-CO subunits (MT-CO1, MT-CO2, MT-CO3) showed high mtDNA methylation in individuals with CVD. Again, sepsis mortality is directly related to lower platelet COX activity which commonly occurs with cardiac dysfunction (Zhou and Tian, 2018). The role of COX being an essential component of ETC and forming the functional core of complex IV makes it a valuable target to explore regarding diseases which involves mtDNA methylation.

Although these two studies have paramount importance because they showcase the impact of mtDNA methylation on CVD pathogenesis. But there was certain limitation of these two studies also. In case of overweight and obese individuals who have high risk of developing CVD, three healthy individuals for MT-CO1 and only one individual for MT-CO2 showed higher mtDNA methylation levels. But as the follow-up couldn't be done, it was not possible to obtain the data. Apart from important research specific points that requires thorough investigation on mtDNA methylation, certain limitations halted these studies up to a certain point. Firstly, the plasma samples size from donors was relatively small in comparison to other clinical studies. Secondly, the possibility of contamination with cell-free mitochondria in platelet pellets could be higher which were isolated for the experiment. Thirdly, the lack of statistical analysis as well as the study is limited to the prediction of more 'severe' cases (Corsi *et al.,* 2020). The hospital discharge records were used for the collection of data regarding clinical diagnosis but could under or overestimate the total number of cases being another limitation

in the second report. Lastly, a good validation of these results is required in different other ethnicities as most of the participants were Caucasian.

Apart from the clinical factors that play an active role in CVD, the role of mitochondria has also carved a niche as an important contributor in the causation of CVD. The mtDNA is sensitive enough to get affected due to oxidative stress, as it lies in close proximity with ROS production in mitochondrial matrix. The rise in ROS concentration may lead to mitochondrial dysfunction in the form of reduced electron transport chain efficiency and reduction in energy production. Alteration in mitochondrial functions have been reported to be associated with CVDs (Lorente *et al.,* 2014). In a growing array of evidences, oxidative stress has been involved as the underlying mechanism in initiating vascular events that includes thrombosis and atherosclerosis. The starting point of thrombosis is the activation of platelets which is followed by secretion and aggregation (Lorente *et al*., 2014). These energy consuming processes derive their requirements from both glycolysis and oxidative phosphorylation. Mitochondria being the organelle-in-charge of carrying out OXPHOS, handles the increase in demand of energy consumption during platelet activation and thrombus formation. Mitochondria doesn't only assist in platelet aggregation by providing ATP, but intracellular ROS generation also acts as a messenger in collagen-induced activation of platelets. Therefore, the initiation of thrombosis is being assisted by certain events in mitochondria, making it a valuable target to be studied in higher forms of thrombosis like DVT (Barquera *et al*., 2015).

Conclusion: The prevalence of obesity in India is 40.3%. When the data was divided zone wise, southern India showed highest percentage of 46.51% and eastern India being the lowest with 32.96% with obesity reaching on a pandemic level, the pathologies related to obesity are increasing exponentially including the stressful lifestyle these days. The damaging consequences that CVD burden has on the individuals, families and population requires immediate action, as there are chances of global mortality rise in annual CVD deaths up to 24.2 million by 2030 according to report published by WHO. This accounts for 32.5% of all global death. Mitochondria is the hub of energy production for the entire cell, so metabolism defects in mitochondrial machinery could jeopardise the normal functioning of those specific cells that consumes most of the energy. Epigenetic modifications were always an area of interest in CVD as no much studies have been carried out in this prospect. Mitochondrial DNA methylation being a less ventured area in CVD made it an interesting zone to explore, which tailed down three MT-COX subunits and MT-TL1 gene that were significantly methylated in the CVD patients. There is a fair possibility of involvement of more mitochondrial genes and their epigenetic regulation in disease development which is yet to be explored. Therefore, more progressive and in-depth studies are required to decipher the role of mtDNA methylation as any kind of dysfunction at gene regulation level in power house could lead to CVD pathogenesis.

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