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Epigenetics as Primary Prevention of Coronary Artery Disease

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will not be able to bear witness to my maturation within the physician assistant profession and within my education. I hope that this scholarly project will have made you proud. His unexpected and tragic passing bears forth the emphasis of cardiovascular research, specifically the continued advancement of genetics in the treatment of heart disease. Professor Sieg's guidance will continually shape and define my practice as a physician assistant. Humbly, this scholarly project would not have been this polished if not due to the guidance and assistance from those mentioned above, so thank you all. Lastly, I would like to extend an expression of gratitude to the remaining faculty and staff in the Department of Physician Assistant Studies, University of North Dakota School of Medicine & Health Sciences, along with all my remaining preceptors. Thank you all for your support in my journey of becoming a physician assistant and for seeing this goal come to fruition.

Abstract

This systematic literature review intended to ascertain if epigenetic testing for the primary prevention of Coronary Artery Disease (CAD) decreases mortality compared to the current standard of care. This systemic review used PubMed and Cochrane medical databases to assess epigenetic variances and their association with CAD. The Medical Subject Headings (MeSH) terms used were as follows: CAD, genetics, epigenomics, diabetes, hypertension, obesity, dyslipidemia, primary prevention, and standard of care. Further limiting the search results to Randomized Control Trials (RCT's), meta-analysis, peer-reviewed, and published within the last 10 years. Exclusion criteria included publications before 2011, articles that did not compare genetic variances to the development of CAD, and poor study design. The final review included twelve articles. The research demonstrates multiple epigenetic variances in patients with a prior diagnosis of CAD. The research also demonstrates statistically significant evidence that epigenetic variance in modifiable co-morbid states such as hypertension, obesity, hyperlipidemia, and diabetes exacerbates a patient's evolution of CAD. Although the research demonstrates multiple epigenetic variances in patients with CAD, questions ascend as to the external validity of the research. The systemic review produced two research articles designed at primary prevention and epigenetic risk stratification of CAD. To further assess epigenetic variances in CAD, more research is needed in the form of a longitudinal, multi-country, multi-generational cohort, multi-ethnic with environmental factors included, along with the comparison of epigenetic risk stratification and gene deletion therapy.

Keywords: coronary artery disease, genetics, epigenomics, diabetes, hypertension, obesity, dyslipidemia, primary prevention, and standard of care.

Epigenetics as Primary Prevention of Coronary Artery Disease

Introduction

Coronary Artery Disease (CAD) is the narrowing of the coronary arteries surrounding the heart. These blood vessels, specifically the left anterior descending artery (LAD), right coronary artery, circumflex, diagonals, and obtuse marginals accumulate with plaque commonly called atherosclerosis. These blood vessels supply the myocardium with oxygen and metabolic blood components during the diastolic phase of the cardiac cycle. Coronary artery blood supply is crucial for effective cardiac muscle contraction, thus leading to sufficient cardiac output, maintaining homeostasis, and the body's metabolic needs. An insidious disease in progression, in which atherosclerosis typically is asymptomatic until myocardial ischemia is present. Further progression, leading to myocardial infarction, heart failure, and later death. According to the Centers for Disease Control and Prevention (CDC, 2021):

> Heart disease is the leading cause of death for men, women, and people of most racial and ethnic groups in the United States. One person dies every 36 seconds in the United States from cardiovascular disease. About 655,000 Americans die from heart disease each year; that's one in every four deaths. Heart disease costs the United States about $219 billion each year from 2014 to 2015. This includes the cost of health care services, medicines, and lost productivity due to death. (p. 1).

Although the prevalence and mortality of CAD have been trending downward within the past two decades, additional advancements in treatment modalities and risk stratification are needed in primary care. Genetics, specifically epigenetics, have been widely studied within the last two decades, specifically on the occurrence of epigenetic variances within diseases and disease processes. According to Abi Khalil C. (2014):

> The term epigenetics refers to all the heritable changes in gene expression regulation other than nucleotide sequence and chromatin organization that depend on the DNA sequences itself. Epigenetic inheritance is an essential mechanism that allows the stable propagation of gene activity states from one generation of cells to the next. Epigenetic mechanisms have been involved in the differentiation of many cell types from progenitor or primary cells and with whom they share the same DNA sequence. They also represent a stable cellular memory for the differentiation state of a cell population. The major epigenetic features of human cells include DNA methylation, post-translational histone modifications and RNA-based mechanisms including those controlled by small noncoding micro-RNAs (p.3).

Significant advancements have been made in genetic testing in the primary prevention of diseases such as breast cancer with BRCA1 and BRCA2 gene mutations, with numerous patients now electively undergoing early bilateral mastectomies. Epigenetic testing and novel genetic treatment advancements in CAD would decrease mortality, while effectively treating patients with the most evidence-based medicine available.

Statement of the Problem

The development of CAD can now be associated with multiple epigenetic variances in modifiable risk factors such as high low-density lipoprotein (LDL) cholesterol with low high-density lipoprotein (HDL) cholesterol known as dyslipidemia, hypertension (HTN), and diabetes (DM). Identification of modifiable risk factors was made statistically evident in the longitudinal multi-cohort, Framingham Heart Study (FHS). The FHS, under the direction of the National Heart, Lung, and Blood Institute (NHLBI), began in 1948 in over 13 different countries with three generations of cohorts with an *N* of 5,209 men and women. Hence, years later, in primary

care medicine the use of the Framingham risk score stratifies cardiovascular risk prevention in which patients can electively use pharmaceuticals along with diet and lifestyle modification to prevent the development of CAD. Research, such as the FHS, pioneered the development of biochemical, environmental, and behavioral characteristics that contribute to the progression of CAD. Advancements in medical pharmaceuticals such as statins, known as HMG-CoA reductase inhibitors**,** more efficacious antidiabetic and anti-hypertensives have been manufactured and studied to combat these modifiable risk factors as secondary prevention. Although a plethora of research has been conducted to identify epigenetic variances within the genome relating to the risk and development of CAD, currently, this is not an evidence-based standard of care. Eloquently stated by (Zaina & Lund, 2010):

> Epigenomics has emerged as one of the most promising areas that will address some of the gaps in our current knowledge of the interaction between nature and nurture in the development of CVD. Unveiling the individual epigenetic landscape provides an important snapshot of the epigenetic machinery that can be eventually employed to customize diagnostic and therapeutic approaches in primary and secondary prevention of cardiovascular disease. Individual epigenetic maps could represent a novel tool in the clinical practice to stratify cardiovascular risk beyond traditional or genome-based risk calculators. Epigenetic information also helps in deciphering inter- and intra-personal variation in individual drug response. Finally, adverse epigenetic patterns are amenable to pharmacological reprogramming of chromatin modifying drugs or non-coding RNAs (p.1).

Increased evidence-based medicine with epigenetic testing and targeted therapy for the prevention of CAD is desperately needed in primary care. The development of novel and

targeted CAD therapies based on epigenetic testing would further risk stratify and target significant and specific risk-factors, leading to pathophysiologic issues of the cardiovascular system. Subsequently, this would lead to a decrease in mortality, morbidity and consequently decrease the health care cost burden.

Research Question

Does the use of epigenetic testing for the primary prevention of CAD decrease mortality compared to the current standard of care?

Research Methods

The first database used to conduct the literature search for the scholarly project was the Cochrane Library. The Medical Subject Headings (MeSH) terms used in this search were as follows: coronary disease, genetics, epigenomics, DM, HTN, obesity, and dyslipidemia. The search resulted in 35 reviews, of which all but three were eliminated due to date of publication, low *N*, and not related to the topic being researched. Lastly, the author used PubMed for the final database limiting the search results to Randomized Control Trials (RCT's), meta-analysis, and reviews all within the last ten years. The MeSH terms used in this search were as follows: coronary disease, genetics, and epigenomics. This delineated 1,735 research articles. These results were used to identify the themes within current research regarding epigenetics in CAD. DM, HTN, obesity, and dyslipidemia were later added to the MeSH terms to selectively narrow the results ranging from 300 to 800 results. Lastly, primary prevention, the standard of care, and compared to FHS were added; however, resulted in no results. The research under theme six was found spending multiple hours scouring Google Scholar, CARDIA Study, FHS, and The National Institutes of Health (NIH).

Literature Review

A review of the literature regarding epigenetic testing in CAD or ischemic heart failure has revealed a plethora of research that has identified epigenetic variances in the modifiable risk factors such as DM, HTN, dyslipidemia, and obesity. The research studies have demonstrated through statistical analysis that the mechanisms of epigenetic drift and imprinting involve histone modification (miRNAs) and Deoxyribonucleic Acid (DNA) hypomethylation. Limited research data in the areas of treating epigenetic variances and risk stratification was evident within the literature review. However, genetic risk stratification, diet, and lifestyle modifications have statistically been proven effective in combating epigenetic variances.

Epigenetic Drift

The 1,000 Genomes Project reported with the *N* of 185,000 CAD cases and controls, 6.7 million common Minor Allele Frequency (MAF) variances, as well as 2.7 million low-frequency MAF variances in CAD. The overarching goal of this comprehensive 1,000 genome-based project meta-analysis was to further expand on the low frequency, insertion, and deletion variances. For this meta-analysis, an *n* of 60,801 CAD cases was assembled along with a control group *n* of 123,504. Genome phase one was used for the imputation, specifically version three, which specifically had a set *n* of 38 million coronary artery disease variances. Of the 38 million variances, one-fifth were previously documented as low-frequency variances. Most of the participants in the study were of Eastern European descent, which equated to 77 % of the net *N*. Middle Eastern and Asian ancestries made up the remaining *N* with a small sample size of Hispanic and African Americans represented in the study. The inclusion criteria for the diagnosis of CAD were based upon previous medical diagnoses of MI, acute coronary syndrome, chronic stable angina, and coronary stenosis. The defined characteristic of the above-mentioned

medical diagnosis then surpassed allele frequencies with a $p = .005$ for the inclusion criteria for the study. 8.6 million Single Nucleotide Polymorphisms (SNPs) were included in this meta-analysis. Of these, 2.7 million were low-frequency variances. Manhattan plots were used to summarize the additive association variances. 2,213 variances showed a significance, with a *p*-value < .0001. The 2,213 variances had a low false discovery rate (FDR) with a *p*-value < .0021 The variances were grouped into eight new representative loci within the genome. The additive association variances found in the study also showed SNPs, which are highly statistically significant in a cardioprotective nature. Nikpay et al. (2015) also highlighted three additional low frequency SNPs that were statistically significant, which were *LPA* rs10455872 with a *p*-value of < .0001, rs3798220 with a *p*-value < .0001 and *APOE*: rs7412 with a *p*-value < .0001. According to (Nikpay et al., 2015):

> Finally, SNP rs11591147, which encodes the low frequency (MAF = 0.01) R46L variant in *PCSK9* that has been associated with low LDL cholesterol levels and cardioprotection, was imperfectly imputed (quality = 0.61). Nonetheless, these data provide the strongest evidence to date for a protective effect of this variant for CAD (*p*-value < .0001) (p.3).

Ten new CAD loci were subsequently identified: rs17087335, rs3918226, rs10840293, rs56062135, rs8042271, rs7212798, rs663129, rs180803, rs11830157 and rs12976411. All were statistically significant with *p*-values < .05. Unfortunately, due to a small $n < 50$, odds ratios, and confidence index (CI) were not conducted.

The chromosomal loci, which explains the variances in expressions, are called an expression quantitative trait (eQTLs). Majid et al. (2015) scientifically examined eQTLs to ascertain associations between prior evidence in cardiovascular risk involving genes with specific atherosclerotic processes. The goal of such examination was to demonstrate how

each loci affected CAD risk. According to (Nikpay et al., 2015):

> Chromosome 4q12 (*REST – NOA1*) locus: The lead SNP rs17087335 lies within an intron of the nitric oxide associated 1 (*NOA1*) gene; 23 SNPs in linkage disequilibrium (LD r^2>0.8) show CAD associations (*p*-value < .0001) across the *NOA1* and the repressor element-1 silencing transcription factor (*REST*) genes (p. 5).

NOA1 pathology, a protein that promotes the self-regulation of mitochondrial respiration and apoptosis, is dependent on sodium and potassium channels to maintain vascular smooth muscle cells. According to (Nikpay et al., 2015):

> Chromosome 7q36.1 (*NOS3*) locus: The lead SNP rs3918226 (MAF = 0.07) lies in the first intron of the nitric oxide synthase 3 (*NOS3*) gene. This SNP has been tentatively associated with CAD (OR = 1.14, *p* = .00014) in a candidate gene meta-analysis based on 15.6K cases and 35K controls genotyped with the HumanCVD BeadChip and firmly associated with essential hypertension (OR = 1.34, *p*-value < .0001) (p. 5).

From a physiologic standpoint, nitric oxide (NO) is a vascular smooth muscle dilator. Thus, epigenetic shifting in SNPs specific to NOS3 locus leads to primary hypertension, subsequently increasing the risk of CAD. Further discussed in the meta-analysis was the regulation of filamentous-actin networks. Chromosome 11p15.4 (*SWAP70*) locus, SNP rs10840293, materializes to be a signaling protein for filamentous-actin networks, shows a strong association with CAD with an associated *p*-value < .0001. SWAP70 is a cis-eQTL is found in subcutaneous fat skin and in the lung. Transforming growth factor-beta (TGF-β) was also indicated in this meta-analysis as being associated with the risk of CAD. Chromosome 15q22.33 (*SMAD3*), intronic to SNP rs56062135, which happens to be a TGF-β mediator within the vascular injury pathway showed a statistically significant *p* = .009. Also, within this meta-analysis, Body

Mass Index (BMI) was discussed at great length. According to Majid et al., (2015), the *MC4R* has been a well-documented variant in obesity and its relation to higher associated risk of CAD. The meta-analysis reported that chromosome 18q21.32 (*PMAIP1 – MC4R*) with the SNP identified and labeled as rs663129 demonstrated a *p*-value < .0001 (p.8). rs663129 within the same analysis showed additional risks, including lower HDL and higher triglycerides. Although statistically significant, no eQTL further implicated *MC4R* in any atherosclerotic heart disease.

With the use of the 1000 genome project, Nikpay et al. (2015) was able to ascertain on a larger scale insertion and deletion polymorphisms. Most of the data within the meta-analysis was significantly significant. However, 80 % of this research study was conducted on individuals of European descent. Questions arise as to the external validity translating to a more diverse population can not be gathered from this study. A genetic positive to this meta-analysis was the fact that the researchers used nine million SNPs to conduct this research with a 123,504 participant control group. Of notable note, 70 % of the variances were in participants with MI, leaving 30 % remaining for the other disease states listed. This would lead to more statistical evidence in patients who had an MI compared to other disease variances and CAD risk factors. Majid Nikpay, the primary author, is affiliated with the University of Ottawa and is a distinguished researcher in the field of epigenetics of CAD, with over 63 recent publications related to epigenetics. Limited to no researcher bias was assessed within the meta-analysis as there were no pharmaceutical or financial gains made from the publication.

Sharma et al. (2014) attempted to ascertain Differentially Methylated Regions (DMRs), which at the date of publication per author, no similar studies had been published with this specific genetic focus. The researchers used 1,000 human CpG Island microarray technology to identify 73 DMRs associated with CAD in correlation with a varying degree of

homocysteine levels. Inclusion criteria were defined as patients with a confirmed diagnosis of CAD. This was compared with a healthy control group that was selected by the All India Institute of Medical Sciences, New Delhi. Participants CAD had equal to or greater than 50 % stenosis of non-specified coronary arteries. The control group underwent cardiopulmonary stress examination via treadmill, which all indicated negative exams for the inclusion criteria of the control group. The geographical location of the participants was northern regions of India with ancestries of Indo-European lineages. Participant characteristics such as age, sex, diet, and smoking habits were all self-reported. The research study had an *N* of 96, with a mean average age of 52. This was further subdivided into 48 CAD participants and 48 control participants. The participants were subdivided into four groups: CAD with a normal level of homocysteine (normohomocysteinemic), hyperhomocysteinemic CAD patients, normohomocysteinemic controls, and hyperhomocysteinemic controls. All participants in this study were biological males. The author denotes this was to minimize heterogeneity and to further formulate the etiology of variances in DMRs in the CAD participants. All participants in this research study were recruited after obtaining written informed consent.

To assess homocysteine levels and genetic variances, venous blood was obtained from and stored in an anticoagulant tube and froze until further analysis could be conducted. DNA methylation profiling was conducted using the microarray method. The flanking region of the DNA segment, which contains the promoter, enhances other protein binding sites along with the CpG island that extends from 300 to 3,000 base pairs were used to scientifically and statistically analyze the DMRs in this study. To ascertain the epigenetic variances in DNA methylation, the researchers mapped the variances and subsequently referenced and separately mapped this sequence. The accuracy of the mapping was reported to be 99.9 % accurate and was within ten

base pairs of the variants on either side. In total, 86 DMRs were identified within the four groups. As stated by (Sharma et al.,2014):

> We observed 19 DMRs in group I that were significantly hypermethylated in CAD patients compared to controls. Group II showed 13 DMRs that had higher methylation in hyperhomocysteinemic CAD patients compared to hyperhomocysteinemic controls. In group III, we detected 24 DMRs that are hypermethylated in CAD patients with high levels of homocysteine in comparison to CAD patients with normal levels of homocysteine. We observed 30 DMRs, the largest number of differentially methylated regions, in group IV which is a comparison between controls with varying homocysteine levels. (p. 4).

The baseline characteristics such as diet, BMI, DM, and smoking, demonstrated *p*-values that were not significant when compared to the 12 prioritized DMRs. The researchers then compared ten CAD participants to ten control participants that DMRs aligned immediately upstream and downstream of the CpG island. According to (Sharma et al., 2014), these regions were STRADA gene (Homo sapiens STE20-related kinase adaptor alpha) flanking CCDC47 and LIMD2, two were in the first exon of C1QL4 gene flanking TROAP and FLJI3236 while the other was in the intronic region of HSP90B3P gene flanking CDC7 and TGFBR3 (p 7.).

There are several factors when assessing the validity of this research that arise further questions as to external validity. First, when assessing the 12 prioritized DMRs, only 48 CAD participants were compared to a control of 48 participants. This is a relatively low *N*. There was no comparison made to the female gender, leaving all data being related to males. This subsequently leads to questions regarding the external validity of this research. Another limitation to this article was no reference to the subject's ethnicity. This further makes it

difficult to assess homocysteine risk within a diverse population. However, with a relatively low *N* in this study, no ratio between the sexes, and no data representing the ethnicity of the participants, this article does highlight the significance of homocysteine levels along with the development of CAD in males, which was statistically significant. A search of the author showed that she is associated with the National Bureau of Genetic Resources with over 100 research studies published in genetics. This article referred to being the first research article assessing the statistical significance of homocysteine levels related to CAD. There is a possibility of bias within this research, although not from a monetary standpoint. Lastly, there is also the possibility of selection bias due to the fact the researchers selected the control participants, rather than having the control randomly selected within a population.

Epigenetic Variances in DM

Wu et al. (2012) performed a meta-analysis using 35 research studies involving the CAD risk factor of DM2, 36 research studies involving obesity, and 22 research studies involving CAD. With the already established evidence involving the overlap of DM2, obesity, and CAD, this meta-analysis aimed to discover communal genetic variances within the three diseases listed above and to further expand on risk stratification. The databases that were used for this meta-analysis were the GWAS (www.genome.gov/gwastudies) and PubMed. The inclusion criteria of this research study included publication dates from 1998 to 2012, and only primary GWAS were included. The meta-analysis used the most robust evidence for specific genomic regions that were later termed “bins”, which can also be referred to by chromosome. The chromosomes that were identified in the GWAS were overlaid on a grid, this divided the chromosomes equally into paired bins. Only statistically significant bins with p-values $< .005$ were included in the evidence. According to (Wu et al., 2012):

> For linkage evidence of 2DM, bin 6.2 reached genome-wide significance (p = .000143), with two adjacent bins (6.4 and 6.5) showing nominal significance Three other bins, 9.3, 10.5, 16.3, also reached suggestive level (p = .001767, p = .003829 and p = .005043, respectively). Bin 8.5 and 11.2 showed nominal significance. For obesity, bin 11.2 and 16.3 reached suggestive level (p = .001822 and p = .004428, respectively, and six other bins (19.3, 6.3, 6.4, 4.5, 9.3 and 9.4) showed nominal significance. For CAD, two bins, 10.5 and 9.3, reached the suggestive level (p = .001135 and p = .007895, respectively) and six other bins,16.3, 11.5, 15.3, 7.3, 9.4 and 7.4, showed nominal significance (p.4).

Of note, bin 9.3 and 6.5 demonstrated statistically significant data shared by all three disease states. In addition, bin 9.3 was statistically significant for DM2 and CAD with a p = .002362. Also, bin 9.3 showed moderately statistically significant evidence for CAD and obesity with p = .0405. From a genomic wide perspective, bin 9.3 attained broad genomic wide significance with a p = .000276 within all three disease states. Bin 6.5 statistically demonstrated a p = .006592 between CAD and obesity, however, was not statistically significant for DM2 and CAD. Amongst the labeled bins that were analyzed, bin 9.3 demonstrated a more statistically significant analysis of linkable diseases. According to (Wu et al., 2012):

> The increased statical power for this region suggested a mutually reinforcing interaction among the 2DM, obesity and CAD. Within this region, 9p21.3 is a loci approximately 22 million base pairs from the 9p telomere. This region was firstly identified to be associated with CAD across different ethnicities. The genetic risk variant from this region is extremely common, with 75% of the Caucasian population having one or more risk alleles. In CAD, the locus is heterozygous in 50% of Caucasians and homozygous in 25%

> with increased risk of 15–20% and 30–40% respectively. The region of 9p21 maps two well-characterized tumor suppressor genes, *CDKN2A*/ *CDKN2B*, encoding respectively proteins p16 ^{INK4a} and p15 ^{INK4b}, both of them are involved in the regulation of cell proliferation, cell aging and apoptosis It is therefore these data supports that defects in 9p21.3 might be the common genetic factors, indicating a chronic inflammation process that predispose to the sequelae of 2DM, obesity and CAD. (p.6).

Regarding epigenetics in CAD, bin 6.5 demonstrated a particularly stout statistically significant linkage between all three diseases. Bin 6.5 is linked to gene Ectonucleotide Pyrophosphate Phosphodiesterase (ENPP1). This gene encodes for a specific glycoprotein that later inhibits insulin-receptor tyrosine kinase activity, thus decreasing insulin sensitivity. Evidence has been identified that epigenetic drift in ENPP1 precludes people to early onset of DM2 and MI. Since ENPP1was statistically significant among all three diseases, this demonstrates a communal pathophysiology between the diseases.

Although the data in this research was statistically significant in aspects of genetic variances that are shared between DM2 and CAD, there was no mention in the meta-analysis of the participants' age, sex, and ethnicity. The ability to provide an evidence-based risk analysis in primary care would be dependent on such characteristics, thus it would be desirable to appropriately risk stratify patients. This could raise questions as to the external validity of this study. The meta-analysis included statistically significant previously identified genetic variances in its inclusion criteria, thus lending to further statistically sound and valid research. The meta-analysis had a robust *N,* with DM2 participants of 378,132 including 86,253 controls. Within the cohort of obesity, an *n* of 348,887 with a control of 191,126 and finally an *n* of CAD participants of 69,828 cases with 191,262 controls. Chaoneng Wu was previously educated in Fudan

University and at the time of publication was a lead researcher at the Shanghai Institute of Cardiovascular Diseases and within the Institute of Biomedical Science in the Department of Cardiology in Shanghai, China, with a particular interest in dyslipidemia and Metabolic Syndrome. The author was provided a grant from the Outstanding Youth Grant from National Natural Science Foundation of China and the National Basic Research Program of China. Further assessment on both the foundations and the research programs found no financial gains from the data provided in this meta-analysis; thus, researcher bias was assessed to be minimal.

Beaney et al. (2016) conducted an observational study to further stratify chromosome 1q25, lead SNP rs10911021. Within the 1,000 genomic phase three, rs10911021 was found to be associated with cardiovascular disease in people diagnosed with DM. The researchers also tried to associate other epigenetic variances in DM with additional risk factors relating to CAD, such as low HDL levels. The researchers used 12 studies in which most of the participants were Caucasian with European ancestry. The *N* was 21,000 participants with a mean follow-up of 10 years. The 21,000 participants were then genotyped using the Metabochip. The Metabochip has approximately 200,000 SNPs that have been identified in most cardio and metabolic diseases. The inclusion criteria for characterizing heart disease were non-fatal myocardial infarction which electively underwent coronary artery bypass graft, and fatal coronary heart disease. Comparisons were made amongst participants with DM2 and participants without DM2. The non-DM2 participants were characterized into 12 different characteristics. These being: age, sex, BMI, triglyceride level, mean cholesterol, HDL, LDL, systolic and diastolic blood pressures, fasting glucose, and mean insulin level in glycated hemoglobin. (Beaney at al., 2016) stated:

> The association between rs10911021 and CHD in diabetic participants was directionally similar to that previously reported but not statistically significant, OR 0.80 (95 % CIs

> 0.60–1.06, p = .13, MAF = 0.26) for the minor allele. The results from the UCLEB studies were meta-analysis with the published data. Similar effect sizes were observed using both fixed effects and random effects models with both *p*-values strongly statistically significant, OR 0.74, 95 % CIs 0.68–0.82, *p*-value < .0001 (Fig. 1) and OR 0.75 95 % CIs 0.67–0.84, *p*-value < .0001, respectively. Heterogeneity between the studies was low (I2 = 18 %). No association between rs10911021 and CHD in those without T2D was observed, OR 1.00 (95 % CIs 0.92–1.10, MAF = 0.30) for the minor allele (p.11).

Since the association between rs1091121 was not statistically significant for participants without DM2, the researchers then tried to stratify or associate rs10911021 with the e γ-glutamyl cycle in DM2. The researchers tried to determine if the SNP was associated with elevated or decreased amino acid levels within this pathway. This was determined not to be statistically significant; thus, rs10 911021 was not observed in those people with DM2. The researchers then tried to associate rs10911021 with conventional risk factors for coronary heart disease along with DM2 and those without DM2. Statistical analysis of the characteristics included: BMI, triglycerides, cholesterol, HDL and LDL, systolic and diastolic blood pressures, fasting glucose, and glycated hemoglobin, which demonstrated that participants with DM2 with a low HDL cholesterol resulted in a *p*-value < .0005.

In summary of the research article specifically connecting DM2 and coronary heart disease found insufficient statistical analysis that is consistent with reports on rs10911021. However, lower levels of HDL in people with DM2 were found to be statistically significant. Of note, the researchers did not include the ratio between the genders within the study. This leaves questions about the external validity for risk stratification in patients who have CAD or strong

family history with DM2 and dyslipidemia. One positive of this research study was the total number of participants which was robust in nature. Also, of particular interest was the inclusion criteria of the participant group with coronary heart disease. The participants with coronary heart disease were significantly ill compared to a relatively healthy individuals. Gleaming that the data represented in this study can only be validated in ill or surgical patients. The research article mentions limitations within this research relating to kidney function which can influence coronary heart disease. Further limitations were noted within this group of significantly ill CAD participants as to whether statin therapy had been administered prior to surgical intervention or death. This leads to questions of the validity of this research specifically since the researchers linked rs10911021 as a statistically significant variant in patients with DM2 and lower levels of HDL. External validity related to races such as African American and Asian is unknown from the study. A brief search of Katherine E. Beaney revealed that she is associated with the University of Bristol, and this research was conducted within their epidemiology department. Assessment of any financial or pharmaceutical gains within this research was found to be negative. Thus, the assessment of research bias is minimal.

Jansen et al. (2015) conducted a meta-analysis to illustrate the genetic variants that are communal between DM2 and CAD. Within the CARDIoGRAM GWAS, the researchers included all genomic variances that carried a statistical p-value < .0001. This included 22,233 CAD cases with an n of 64,762 controls. The researchers included of the 44 documented DM2 SNPs ten that were found to be statistically significant in CAD with a p-value < .05.

The CARDIoGRAM GWAS is currently comprised of several different cardiovascular genomic-wide research studies. CAD inclusion criteria in the German MI Family Study one and two were defined as those having a premature myocardial infarction prior to the age of 60 and a

first-degree relative diagnosed prior to the age of 70 with CAD and or a MI. Also, within the same study, participants were characterized by having documented coronary artery bypass grafting or having an intervention via angiography with PCI placement. All other participants within the CARDIoGRAM were characterized by having CAD as a confirmed diagnosis. Within the Welcome Trust Case Control Consortium, CAD was characterized by having a documented MI, history of PCI placement by angiography, coronary bypass grafting nonspecific to how many vessels, and angina all before the age of 66. The specific inclusion criteria regarding ethnicity were predominantly Caucasian. Within the 22,233 CAD participants, the researchers included cohorts from the FHS. This data was pulled from the original cohort along with the offspring of cohort one. The number of total *n* from the FHS was 7,872. SNPs rs972283 and rs780094 were explicitly excluded due to inconsistent statistical analysis and being related to HLD. A relative weighted risk was conducted on each participant, which included the number of risk alleles for each SNP; this was then multiplied by the expected CAD risk score. Lastly, the quantitative risk for CAD was calculated on the associated effects on the participants' DM. Out of the 48 SNPs identified as inclusion criteria, 10 were found to be moderately statistically significant with a p-value < .05. The results reported by (Jansen at al., 2015):

> Considering all 44 SNPs, the average CAD risk observed per individual T2DM risk allele was 1.0076 (95% confidence interval (CI), 0.9973–1.0180). Such average risk increase was significantly lower than the increase expected based on the published effects of the SNPs on T2DM risk and ii) the effect of T2DM on CAD risk as observed in the Framingham Heart Study, which suggested a risk of 1.067 per allele (p-value < .0001 vs. the observed effect). Studying two risk scores based on risk alleles of the diabetes SNPs, one score using individual level data in 9856 subjects, and the second score on average

> effects of reported beta-coefficients from the entire CARDIoGRAM dataset, we again observed a significant - yet smaller than expected - association with CAD (p. 5).

The research did show a mild association between DM2 SNPs, and CAD. However, only the FHS data included the ratio between genders being almost 50 % males versus females. The remaining studies in the CARDIoGRAM GWAS did not mention the ratio between genders. Also, the researchers only used the Caucasian race in their inclusion criteria. Thus, this research can not be validated diversely, decreasing the external validity of the research. Specifically related to DM2, there was no mention in the research study if the participants were being treated for their DM2; thus, average glycemic values are undetermined. Also, there was no indication within the meta-analysis as to adjustments of co-founders such as diet or lifestyle modifications with participants with DM2. This potentially could have an impact on the progression of the disease. A strength to the external validity of this study was the use of the FHS. As mentioned previously, the statistical and medical significance of the FHS is one of the best epidemiological studies that has affected cardiovascular medicine and risk stratification for patients with DM2, HLD, HTN, and CAD. The statistically significant evidence that demonstrated an association between the epigenetic variances of diabetes being a risk factor of CAD was derived from the FHS cohorts within this meta-analysis. The meta-analysis was funded by the Integrated Projects Cardiogenics and the German National Genome Network. Limited or no bias was assessed within this meta-analysis as correlations to financial or pharmaceutical gains by both companies that funded this research were not found.

Epigenetic Variances in HTN

Kato et al. (2015) performed a trans-ancestry genomic wide retrospective cohort study of 320,251 East Asian, South Asian, and participants of European descent to assess new genetic

variances within blood pressures. The net *N* within the study was 99,994 participants, with East Asians having an *n* of 31,516, Europeans having an *n* of 35,352, and lastly South Asians with an *n* of 33,126. Also, the authors performed a meta-analysis to identify specific phenotypes related to the three ancestral population groups. The genomic wide association results demonstrated 4,077 different variances with a *p*-value < .0004. This correlated to blood pressure phenotypes that distributed over 630 different genetic loci. The SNPs with the lowest *p*-values were combined with the analysis from the International Consortium Blood Pressure Genetics. The analysis from the International Consortium Blood Pressure Genetics GWAS produced an *n* of 87,205. The combination of the GWAS and the trans-ancestry genomic wide study produced 19 unreported loci, which were statistically significant with a *p*-value < .0001. Additional testing of the 19 SNPs to an additional 133,052 participants of the same ancestral populations revealed that 12 of the 19 SNPs were statistically significant with a *p*-value < .0001. Of note, with the additional testing the researchers did not have a balanced ratio between the three ancestral groups. The South Asian cohort had a *n* of 16,332, the remaining *n* was equally divided among the other two ancestral groups.

The researchers continued to expand on their research by using regional association plots of the 12 newly identified loci. However, there was little evidence to support heterogeneity between the ancestral groups in the genomic wide association cohort or the replicated data cohort. Therefore, to further assess the blood pressure SNPs, the researchers hypothesized a relationship with DNA methylation. Of the total blood pressure SNPs, 35 were associated with methylated markers with a *p*-value < .0001. The researchers then took cord blood samples from 237 participants and compared the relationship to the sentinel blood pressure SNPs that were

previously reported. This produced a $p = .0004$, suggesting that the sequent variants directly affect the methylated sites since this blood was prior to any significant environmental exposures.

To further support the statistical significance of the blood pressure SNPs that were reported the variances were then tested for association with CAD, DM2, and kidney function. The SNPs were compared to the GWAS data, which produced a *p*-value ranging from .0038 to < .0001. Comparing the new variances to predetermined risk scores, these new variances showed prediction in elevated levels of NT-pro BNP along with left ventricle hypertrophy which was assessed by electrocardiogram. The comparison of the SNP's reported in this research study was associated with blood pressure and represented a cause-and-effect relationship to mortality that was statistically significant with a *p*-value of .04 to $p = .0000086$. Thus, contributing to adverse cardiovascular outcomes. As stated by (Kato et al., 2015):

> Among the genetic loci and candidate genes identified, several have been implicated in other cardiovascular and metabolic phenotypes through genome-wide association. *IGFBP3, KCNK3, PDE3A* and *PRDM6* have a role in vascular smooth muscle cell biology. PDE3A is a phosphodiesterase involved in cyclic GMP (cGMP) metabolism, vascular smooth muscle contraction and cardiovascular function. Pharmacological inhibitors of PDE3A lower blood pressure. KCNK3 is a potassium channel involved in the regulation of vascular tone; mutations in *KCNK3* are associated with pulmonary hypertension. PRDM6 acts as an epigenetic regulator of vascular smooth muscle cell phenotypic plasticity by suppressing differentiation and maintaining proliferative potential. Genetic variants near *PRDM6* are associated with intracranial aneurysm. IGFBP3 modulates the actions of insulin-like growth factors (IGFs),

> circulating hormones that influence vascular smooth muscle cell function. Serum levels of IGFBP3 are associated with cardiovascular disease (p. 12).

This combined retrospective cohort study along with meta-analysis produced multiple variances that had statistical significance. The researchers were able to correlate this specifically to poor outcomes related to CAD. However, of the 320,000 participants in the study, there was no mention of the gender ratio in the cohort groups. As mentioned earlier, there was an unequal proportion of the three ancestral groups in the associated data used to correlate their findings. Blood pressure variables were directly measured in millimeters of mercury, and participants whose blood pressure was augmented by antihypertensive values of plus 15 were added to the systolic and diastolic blood pressures. As reported by the researchers in the study, the average onset of antihypertensive treatment was less than 65 years of age at onset, and the participants who were not on antihypertensive treatment were greater than 50 years of age. This produces a narrow range on their overall data of 15 years. Therefore, external validity can only be related to late middle to elderly populations of only eastern European descent. There was no mention as to the number and pharmacological type of antihypertensive that the subjects were on. This research study was funded by multiple organizations, the National Cardiovascular Research, the Japanese Atherosclerosis Prevention fund, and supported by the US National Institute of Health (NIH). Norihiro Kato M.D, at the time of publication, was the Director of the Department of Gene Diagnostics and Therapeutics Research Institution. Doctor Kato is now the director-general of Medical Genomics Center and the National Center of Global Health and Medicine. He has authored several genomic studies within his career and his education in medicine. No bias was assessed within the research, thus promoting its validity.

Epigenetic Variances in Dyslipidemia

Braun et al. (2017) performed a retrospective cohort study using 725 participants within the Rotterdam Study. The original Rotterdam Study was conducted in the Netherlands. This was a longitudinal study that began in the 1980s. As of 2008, it had over 15,000 participants within the study. The mean age of the participants within the original Rotterdam Study was 55 years of age and older. The researcher focused on the genetic epidemiology of cardiovascular diseases specifically related explicitly to atherosclerosis and coronary heart disease risk factors. The participants' characteristics were gender, age, BMI, waist circumference, triglycerides, HDL, LDL, total cholesterol, lipid lowering medications, and coronary heart disease.

Assessing the discovery and replication groups of DNA methylation sites between the two cohorts revealed that the triglycerides and the total cholesterol were not statistically significant. However, the methylations sites compared to the discovery and replication with HDL were statistically significant with a $p = .001$. In analyzing coronary heart disease, the discovery and replication cohorts were roughly the same in percentages that varied from six to eight percent with a statistically significant $p = .003$. According to (Braun et al. 2017):

> Of the five CpG sites significantly associated with triglycerides in the discovery cohort, four replicated in the replication cohort, including *CPT1A* (cg00574958 and cg17058475), *ABCG1* (cg06500161), and *SREBF1* (cg11024682). Of the three CpG sites significantly associated with HDL-C in the discovery cohort, two replicated in the replication cohort, including *ABCG1* (cg06500161) and *DHCR24* (cg17901584) (p. 4).

Adjustments were made to account for lipid lowering medications. Performing analysis on the DNA methylation sites already identified in the replication and discovery cohorts to a lipid level, although slightly lower than previously analyzed, this remained slightly statistically significant. Using a linear regression model, the researchers then tried to account for dietary fat intake

associated with the lipid levels. Using the statistically significant related sites already identified the researchers, found no statistical evidence that dietary fat intake correlates negatively to DNA methylated sites.

Of note, within this study is the pathophysiology related to the specific DNA methylated variances were found to be statistically significant between the discovery and the replication cohorts. CPT1A is an encoding protein that is related to transporting long chain fatty acids into the mitochondria. Hypomethylation of this gene decreases the expression of the proteins; thus, leading to decreased transport in fatty acids. In previous meta-analysis, CPT1A, ABCG1, and SREBF1 have been linked to negative cardiometabolic traits such as myocardial infarction. These variances have also been statistically proven to show a correlation between increased BMI and insulin insensitivity. There were additional hypomethylated CpG sites found within the replication cohorts with combined meta-analysis. LDLR gene and CMIP have been linked to LDL receptors, explicitly relating to protein coding genes. These genes have been linked to familial hypercholesterolemia.

This research study did however have limitations. There was a wide variation between age ratios that were found to be statistically significant between the replication and discovery cohorts. The Rotterdam Study's mean age of their cohort was 55 years of age. This does not account for DNA hypomethylation sites for the early onset of CAD. Another limitation to this study, again relating to the Rotterdam Study, is the geographical location of the cohorts, which was a well-defined small region within the Netherlands. Questions to the external validity to other racial and ethnic backgrounds cannot be determined from this research. A positive aspect of this research is that the replication of DNA methylations sites from ancestral populations can be replicated in other cohorts' groups. However, this research did not indicate the geographical

location or the ancestral population of the replication cohort. The research study overcame questions asked to participants on lipid-lowering medications with adjustment to the data. They did not specify which pharmacological agents the participants were on. The study did indicate that the lipid profiles were of fasting nature. Variables in the data could be questioned if the participants followed the fasting guideline. Although the study had a relatively small *N*, the Rotterdam cohorts have been followed for generations, and hereditary and environmental variables were under tighter control than the GWAS, where environment and hereditary variables are not considered. Lastly, there was no mention of the inclusion and exclusion criteria in the replication cohort. Kim VE Braun, at the time of this research publication, was employed by Erasmus AGE, a company that researches aging. This research was funded by Nestle Nutrition. It was stated explicitly, in this research that the funders did not have any role within the research design, or data collection. Lastly, the authors signed a declaration that there was no competing interest within their research. Researcher bias was assessed to be non-present.

Chen et al. (2016) conducted a meta-analysis along with a compare and contrast with an aim at trying to ascertain the epigenetic variances in apolipoprotein B, EcoRI polymorphism E-verse E+ rs1042031, and CAD risk. In total, there were 24 studies associating apolipoprotein B variances and coronary artery in which 23 of the studies were used in this meta-analysis. Within the included studies, a total *N* of 2,994 CAD patients was compared to a healthy control *N* of 3,258 participants. The average age of the participants ranged from 43.2 to 66.7 years of age. However, the gender ratio was not proportional. Since not all these studies included in this meta-analysis had participant characteristics such as BMI, elevated triglycerides, total cholesterol, and elevated LDL levels, these variables were left out of the statistical analysis. The participant's ethnicities in this study were Eastern European, American, Brazilian, and Turkish. Further

inclusion criteria of these studies included specific case-control studies, available allele type summarized within the studies, and frequency of diagnosed CAD patients. According to (Chen et al. 2016):

> The overall allelic OR (95 % CI) was 1.18 (1.06–1.32). Furthermore, heterogeneity among these studies was evaluated under the dominant mode ($E^+ E^- + E^- E^-$ vs. $E^+ E^+$ genotype) for examining the association between genotypes and CHD. No statistical significance ($p = .720$) was revealed in the result of heterogeneity testing for the eligible studies; therefore, a fixed effect model was also employed to merge data. The pooled OR (95 % CI) for these studies under the dominant mode. The overall OR (95 % *CI*) was 1.18 (1.04–1.34). The pooled ORs were compared with the overall OR under different genetic models. This sensitivity analysis demonstrated that none of the studies influenced the pooled OR to any great extent. The leave one out OR estimates ranged from 1.152 (1.028–1.291) to 1.207 (1.078–1.352) for E^- vs. E^+ allele and 1.152 (1.013–1.311) to 1.210 (1.064–1.375) for $E^+ E^- + E^- E^-$ vs. $E^+ E^+$ genotype, respectively, indicating that the pooled estimates of allelic and genotypic risks obtained in this study were statistically robust (p. 8).

The data represented above was specifically related to locus SNP (rs1042031) in the ApoB gene. Results might have been different if there was an expansion of variances with the APoB gene. Apolipoprotein B plays a very precise and critical role within the metabolic pathway of cholesterol within the body. Its vital role is to transport lipids, alleviating them from the bloodstream, further decreasing total cholesterol. EcoRI has been associated with an increased risk of developing atherosclerosis and thus leading to CAD. The epigenetic drift

specifically changes glutamic acid changing the molecular structure into diaminocaproic acid, thus weakening the binding of LDL to its receptors.

The results of this meta-analysis demonstrated a moderate association in the pooled data between EcoRI and the carriers of E- and the development of CAD. However, there were several limitations to this study, the first being that the ratio of men to women was severely disproportioned, significantly favoring males over females. Secondly, other than gender, there were no other defining characteristic variables within this study. Lastly, several of the inclusion studies had a relatively small *N*, reducing the statistical power of the data. From a positive aspect, the meta-analysis included most of the meta-analysis regarding apolipoprotein B. Yelda Chen at the time of this publication, was affiliated with the Institute of Medical Systems Biology and School of Public Health Guangdong Medical University. Their research was conducted under the guidance of the University and was not funded by any grants. Furthermore, Yeda Chen signed an ethical declaration that he had no competing interests related to the data outcomes. Thus, researcher bias was assessed to be minimal due to the signing of the declaration and no financial or pharmaceutical gains were associated with this research study.

Epigenetic Variances in Obesity

Indumathi at el. (2020) conducted a retrospective cohort study specifically looking at CpG sites on premotor regions on IL-6 and TNF-alfa association with increased risk of CAD in Asian Indians who have previously been diagnosed with DM2 and obesity. Total *N* of 574 participants, was subdivided into 207 confirmed CAD patients, 100 DM2 participants, and 82 obese participants with a *n* of 187 healthy control participants. Characteristics of the DM2 patients were defined by demonstrating a fasting blood glucose level greater than 126 mg/dl, while the characteristics of the obese patients were defined as having a BMI greater than 30. The

gender amongst the two cohorts demonstrated an equal ratio. Venous blood samples were obtained with an overnight fasting of 12 hours. Previous variances that have been identified in GWAS were used as associations and to ascertain variances in IL-6 and TNF-alfa.

The statistical data reported within the variances of IL-6 in CAD and DM2 demonstrated an odds ratio of 1.96. Comparing the same data demonstrated a confidence interval of 1.32 to 2.97 with a represented $p = .001$. This data represents a significant increase in the expression of IL-6 in the participants with CAD and DM2 subjects. There was no statistical evidence found in IL-6 in the obese subjects. TNF-alfa was found to be statistically significant in both CAD and DM2 participants along with obese participants. This data showed an odds ratio of 2.04 with a narrow confidence interval of 1.36 to 3.05, representing a $p = .005$. A significant discovery of increased variance in TNF-alfa in the CAD and obese participants with a $p = .002$. Since TNF-alfa and IL-6 are acute phase reactants in the inflammation process, variances in these two proteins lead to a chronic low-grade inflammatory state that increases the pathophysiology of CAD in obese and DM2 individuals.

This study presented minor limitations. First, the total *N* within the study was relatively small compared to the other studies within this scholarly project. Second, the ethnicity of the subjects was narrow, with the inclusion criteria of only Asian Indian descent. Thus, questions arise as to the external validity of the data is diverse in nature. Third, there was no denotation as to pharmacological interventions used to control the participant's disease states. Such interventions would modify the trajectory of the disease, which might skew the statistical analysis of the research. Finally, the healthy control group had an *n* of 100 participants, again this is a low *n*. Nevertheless, this study did provide statistically significant data on the variances in chronic low-grade inflammation within CAD, DM, and obesity, as the researchers provided CI

and odds ratios to correlate the significance of the data. This research was not funded by any outside research organization or pharmacological manufacturer. The lead researcher, Bobbala Indumathi, is an expert in molecular biology and at the time of this study was a senior researcher at the Nizam's Institute of Medical Science. Therefore, limited to no researcher bias was assessed in this research.

Whal at el. (2017) performed a retrospective observational cohort study with an epigenomic wide association to demonstrate BMI and its effects on DNA methylation. As discussed by the author, DNA methylation is one of the key regulators in gene expression. The use of an epigenomic wide association demonstrated 278 genetic loci that were statistically sound, with a p = .0000001 to .000000092 that was represented in a sample size of 10,261. The ethnicities of the participants in this epigenomic acquired association were of Korean, European, and Indian Asian descent. The researchers identified these populations to be at higher risk of obesity and other related metabolic disturbances.

Of the 287 specific CpG sites identified in this study, 207 specific loci were related to BMI with a p-value < .0001. The control group within this study had an N of 4,874 subjects with European and Indian Asian descent having an equal ratio of men and women. 187 CpG sites showed a statistically significant association of DNA hypomethylation within an elevated BMI, demonstrating a p = .05 in the replicated samples. To correlate hormonal disturbances and adipocytes, the researchers further tried to correlate insulin resistance with increased adiposity. This would increase BMI, subsequently quantifying the relationship between DNA hypomethylation, BMI, and adipose tissue. The researchers found 120 CpG sites that exhibited this correlation with a p = 00013. According to data by (Whal at el., 2017):

> The strongest *cis*-signals observed are for cg09315878 with *TNFRSF4* transcription (*p*-value < .0001), cg14476101 with *PHGDH* transcription (*p*-value < .0001) and cg09152259 with *MAP3K2* transcription (*p*-value < .0001). On average a 5% absolute change in methylation was associated with a 7% change in gene expression across the 44 transcripts identified (range 1.8% for *AKAP* to 19% for *SPNS.* Amongst the 38 methylation-gene expression associations observed in blood, 3 replicated in adipose tissue *(HOXA5, BBS2, SELM)* and 3 in liver (*ANXA1, LGALS3BP, PHGDH*) at *p*= .0013 (ie *p* < .05 after Bonferroni correction for 38 tests). These criteria identified 210 unique genes, many with established roles in adipose tissue biology and insulin resistance (eg *ABGG1, LPIN1, HOXA5, LMNA, CPT1A, SOCS3, SREBF1, PHGDH.* Gene-set enrichment analyses show that the 210 candidate genes are enriched for genes involved in lipid and lipoprotein metabolism, amino acid and small molecule transport, and inflammatory pathways involving NFKB, MAPK, TAK1, IRAK2, AND TRAF6 (p. 6).

To further stratify the research, the researchers conducted a cross-sectional relationship between fasting glucose, blood insulin levels, HDL levels, total cholesterol, triglycerides, and HbA1C. The related characteristics showed statistical significance with a *p*-value ranging from .001 to < .0001. Weighted association of these genetic variances was conducted on new-onset DM2, this demonstrated a confidence interval of 2.29 with a narrow range of 2.06 to 2.5. This equates to a *p*-value < .001. This data represents that DNA hypomethylation in the above-listed gene variances is associated with a higher risk of further development of DM2 in the population of patients with increased BMI. Thus, leading to an increased risk of cardiovascular events. The epigenetic variances in ABCG1 previously discussed in this scholarly project demonstrated the statistical significance of epigenetic drifts in the pathology of phospholipid transport and

regulation of insulin secretion. These pathways have been statistically proven to increase the risk of coronary artery disease; thus, this research in the DNA methylation and epigenetic variances in people with elevated BMI who are positive for the ABCG1 variant are at an increased risk of developing obesity, type 2 diabetes, and associated cardiovascular, metabolic disturbances such as CAD.

Limited ancestry and geographical locations raise questions about the research's external validity and the supported data. However, the robust *N* within the sample and the large *N* within the control group add a degree of increased validity to the data reported. The study did not include pharmacological agents or diet and exercise characteristics of the participants with elevated BMI. As stated before, this could have a bearing on epigenetic variances and disease progression. However, the research did include a wide range of ethnicities predisposed to an increased BMI, this leading to better risk stratification with substantial external validity. The research study was funded and supported by the NIH, and there were no pharmacological or financial gains assessed from the publication of this research. The author and co-authors all signed a declaration of no competing interests. At the time of publication, Simone Wahl was the senior researcher at Helmholtz Center Munich German Research Center for Environmental Health, Neuherberg, Germany. Her areas of interest and research expertise lie in epigenetics, coronary artery disease, and DNA methylation. Due to the above mentioned, there was no research bias assessed in this research.

Epigenetic as Primary Prevention

Gallardo-Escribanoc at el. (2020) conducted an observational study of post-intervention of diet and lifestyle modifications and its correlation to methylation levels. The total *N* was 131, with the specific characteristic of the cohort group being prepubertal. The age of the participants

ranged between four and nine years of age, with a ratio of males to females roughly equating to 50 %. Consent was gathered from the children's parents. A history and physical were performed by a licensed physician and nurse who documented their specific age-related values. Baseline metabolic levels were gathered via a venous blood draw to determine levels of triglycerides, HDL, LDL, and total cholesterol. This lab draw was assessed after 12-hour fasting. Diet and lifestyle modifications included 120 minutes of physical activity in five sessions during the week. The week was classified as Monday through Friday, with off days being Saturday and Sunday. A sports medicine trainer supervised the physical exercise with a pre-determined cardiopulmonary routine. The participants also initiated the Mediterranean diet with a total caloric intake of 1,500 calories a day. The participants and their parents met with a nutritionist to receive the proper education on the diet. Data was gathered at two distinct intervals, four months, and 12 months.

There was no statistically significant data that was gathered on the anthropometric parameters at the four-month interval. However, BMI, systolic and diastolic blood pressure, glucose, HbA1C, and insulin demonstrated positive correlations to the diet and lifestyle modification changes with p-values < .001. Of note, IL-6 and TNF-alfla levels significantly increased in both male and female participants. This demonstrated a statistically significant p = .001 at the 12-month interval. Both male and female participants at the 12-month interval showed a 91.8 % increase in weight-related to their standard growth charts, while there were no significant changes in BMI between males and females.

Methylation status was correlated at specific intervals of four and 12 months. Lipoprotein Lipase (LPL) was significantly increased in the female group at four and 12 months, with a p-value ranging from .02 to .04. Methylation of leptin (LEP) increased in the female group at 12

months with a $p = .02$. Also, Liver X Receptor (LXR) decreased at the four-month interval but returned to baseline at the 12-month interval; this was represented with a *p*-value in both populations of .03. The study shows that diet, specifically the Mediterranean diet and physical activity, can alter levels of methylation related explicitly to lipid metabolism and underlying inflammation. Both epigenetic variances in lipid metabolism and methylation of inflammatory pathways have been statistically proven to increase cardiovascular events, specifically CAD. Alterations of lipid metabolism and underlying inflammation with changes in hypomethylation can be seen in the young population within this research. Education, promotion of diet, and lifestyle modifications at a younger age can be seen in this research study to decrease hypomethylation and inflammatory pathways, thus decreasing CAD risk factors later in life.

One of the limitations of this study was the low *N* that was represented in this data. The study did have a balanced ratio of males and females. Uncertain as to why there was no statistical significance in loss of weight and changes in BMI in the participants. The researchers could have strengthened their data with another assessment point at 16 months. The participants could have plateaued with their diet and exercise regimens, leading to the lack of weight loss and BMI changes. A positive aspect of this study can be attributed to it being a longitudinal study with the participants being followed over one year. Lastly, the ancestry population was of only Spanish descent; thus, questions to external validity can be made when assessing this data. The research was funded by grants from the Instituto de Salud Carlos III and co-financed by the Fondo Europeo de Desarrollo Regional-FEDER. The author and co-authors all signed a declaration of no competing interest within this research, and there were no financial or pharmaceutical gains made on this research, leading to a more substantial validity. Christina Gallardo-Escribano was a senior researcher with the institution of Salud Carlos III when this research was conducted. Her

specialty is in epigenetics in relation to obesity, diet, and exercise, which strengthens the validity of the research. There was no researcher bias assessed within the study.

Dogan et al. (2018) conducted a retrospective cohort study to further integrate epigenetics for risk stratification in patients with CAD. The statistical approach was the use of epigenetic and phenotype data from the FHS, plotted in Random Forest classification models. The researchers used Genome-wide SNPs of 2,560 participants. After quality control, 2,406 with a ratio of 1,100 males to 1,306 females. 696 participants were removed due to relatedness, leaving 1,599 participants with a ratio of 722 males to 877 females. The FHS cohort included the offspring cohort who attended the eighth examination cycle and consented to genetics research. Variable characteristics from the FHS dataset included: age, gender, systolic blood pressure, HDL, total cholesterol level, HbA1c level, smoking status, statins use, and CAD status. All phenotype data obtained during the eight-examination cycle were used for statistical analyses. Participants were categorized into groups with or without symptomatic CAD, receiving a diagnosis within the Framingham Endpoint Review Committee (FERC), at or before the eighth examination cycle. The categorization criterion decreased the participants from 1,599 to 1,545. This equated to 173 with symptomatic CAD and 1,372 without symptomatic CAD representing the control groups.

The SNPs that were specifically assessed within this research were as follows: cg26910465, cg11355601, cg16410464, cg12091641, rs6418712 and rs10275666. cg26910465, cg11355601, and cg16410464, map to *ADAL*, *JOSD1*, and *CCT6P1*. Gene *JOSD1* has been identified through research as being related to the variance in the upregulated in peripheral mononuclear cells, which have been associated with asymptomatic myocardial dysfunction.

CCT Subunit 6 gene is a pseudogene of *CCT6P1*. *CCT6P1* has not been statistically proven to affect CAD. However, the CCT complex has been proven through multiple research studies to interact with LOX-1, a receptor involved with atherogenesis. SNP rs10275666 has a tight linkage to disequilibrium (LD) with *HUS1*. HUS1 is a gene that is involved in CAD related oxidative DNA repair.

The sensitivity of the Randomized Forest models demonstrated 70–82 %, with a specificity range of 70–79 %. The Area Under the Curve AUC and the Receiver Operating Characteristics (ROC) demonstrated on the eight models 0.77–0.87. A comparison was made between the Forest models and conventional CAD risk factors in predicting CAD. Using the variable characteristics such as age, gender, SBP, HbA1c, total cholesterol, self-reported smoking, and HDL. Accuracies of these Forest models ranged from 70–76 %, while the sensitivity and specificity of 67–74 % and 72–79 %. The ROC AUC was 0.72–0.79. The specificity was comparable with the use of the integrated genetic-epigenetic models. While the conventional risk factors models underperformed regarding the sensitivity and ROC AUC. As stated by (Dogan et al., 2018):

> The test data comprised of individuals removed based on relatedness to those in the training dataset. This integrated classifier was capable of classifying symptomatic CHD status of those in the test set with an accuracy, sensitivity and specificity of 78%, .75 and 0.80, respectively. In contrast, a model using only conventional CHD risk factors as predictors had an accuracy and sensitivity of only 65% and .42, respectively, but with a specificity of .89 in the test set (p.9).

Regression analysis of the methylation variances listed above demonstrates the ability to further risk stratify known risk factors in CAD epigenetic pathogenesis. These results demonstrate an

integrated epigenetic approach to model symptomatic CAD.

There were several limitations to this research study. One being the lack of generalizability to CAD. Although this research provided relatively applicable sensitivities and specificities, this was only correlated to symptomatic CAD. This also could be attributed to genetic variation. Also, the only ethnicity tested in this research was of European ancestry and only on white men and women. This leads to limitations as to the external validity of the research to risk stratify a diverse population. Third, this study had a relatively low *N,* with the Forest Models only testing 142 participants. Lastly, the researchers used a small number of SNPs to conduct their Forest models. A more significant number of SNPs could equate to a more highly predictive value. This research study did not adjust or consider pharmacologic interventions, such as statins. This would lower cholesterol, specifically LDL, changing the dynamic and progression of symptomatic CAD. This could potentially be more relevant of a limitation as the cholesterol level was lower in the training set for those diagnosed with CAD than those without CAD. The assessment of researcher bias revealed that Dr. Dogan and the University of Iowa did file for intellectual property claims related to the content of the published research. Further, there are royalty rights inferred because Dr. Dogan is an officer and a stakeholder in the Cardio Diagnostics lab, which develops epigenetic biomarkers for CAD in the research markets. However, this research was funded by grants from the NIH but was also funded in the form of salaries from Cardio Diagnostics.

Discussion

The research has identified a plethora of epigenetic variances in patients with a prior diagnosis of CAD. The research has also demonstrated through statistically significant evidence that epigenetic variance in modifiable co-morbid states such as hypertension, obesity,

hyperlipidemia, and diabetes exacerbates patients to the evolution of CAD. Through research such as the FHS, pharmacological treatment modalities have been manufactured and researched to combat these modifiable risk factors. However, these pharmacologic interventions do not address underlying epigenetic variances. The systematic literature review yielded two research articles designed at primary prevention and epigenetic risk stratification of CAD. Thus, one of the conclusions of this systemic review is the need for further research in epigenetics and epigenetics as primary prevention of CAD. However, the need for further research cannot just be simply stated. The research in this systemic review produced questions about the external validity of current epigenetic research in crucial areas. Specifically, the exclusion of environmental factors within the geographical locations within all the research within the systemic review. The inclusion of environmental factors would manufacturer risk stratification pathways and protocols that could be used to assess patients in primary care medicine. Secondly, the research in this systemic review identified that most research within epigenetics in CAD and the modifiable risk factors listed above were retrospective in nature. These designs produce only a snapshot in time; thus, the research truly does not assess the epigenetic variances across the lifespan, or the impact on morbidity the epigenetic variances have on CAD. Third, many of the control groups within this systemic review were prior GWAS. It was assessed that the use of GWAS excluded participant characteristics such as ethnicity, age, BMI, environmental factors, geographical location, comorbid states such as HTN, HLD, DM, and obesity. Thus, constricted parameters on the control groups within this research were not used. The two articles produced within this literature review that could be contributory to primary prevention of CAD had significant external validity issues such as the very narrow age range of the participants, this

being preteen and significant vulnerability to bias related to financial gains. Eloquently stated by Ordovás & Smith (2010).

> However, the difficulties of the task ahead must not be underestimated. Whereas the genomic information is the same in all our cells and during our entire lifespan, the epigenomic information varies from cell to cell and during the lifetime of the individual. Moreover, not all epigenetic marks and their combinations have been identified, and how they work in concert to regulate epigenetic mechanisms and gene expression is not yet known. A complete epigenomic map will require major advances in knowledge and computing power that greatly exceed those currently available for the study of classical DNA genetic variation (p.8).

To fully assess the insidious nature of epigenetic variances within CAD and the modifiable risk factors listed above hasten the evolution of CAD, further research is needed in the form of restructuring the designs of epigenetic research. Like the FHS, longitudinal studies that are multi-country, multi-geographical, multi-generational cohort, multi-comorbid states, multi-ethnicities with the inclusion of augmentation of pharmacological agents, new risk stratification and gene deletion therapy would not only delineate the impact of epigenetic variances on CAD and its comorbid risk factors but would also thrust epigenetic research from retrospective to proactive thus decreasing overall morbidity. In addition, the benefits of such research would shepherd further research and manufacturing of gene deletion therapy, earlier treatment of the modifiable risk factors, and progressive CAD epigenetic risk stratification tools that could be used in primary care medicine.

Further expanding the diet and lifestyle modification research within this systemic review to a broader range of participant ages would further stratify methylations levels and the

importance of diet and lifestyle modifications not only in coronary artery disease but the modifiable comorbid states listed in this literature review. Further expansion is needed on the Randomized Forest Model risk stratification research within this systemic review as well. The inclusion of an increased number of identified SNPs to further assesses if Forest Models can be used as risk stratification. Broadening the inclusion criteria would make this research more applicable to primary care medicine.

An area of research that needs to be further researched and assessed is the area of gene deletion therapy. Jennifer A. Doudna, Nobel Laureate in Chemistry, Professor of Biochemistry, Biophysics and Structural Biology, and her co-inventor Dr. Emmanuelle Charpentier have pioneered a new gene deletion therapy through further research could be a way of treating epigenetic variances. Doudna & Charpentier (2014), describes the therapy which is called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9). The CRISPR-Cas9 genetic modifying system has two key molecules introducing a genetic change into the host's DNA. Cas9 is an enzyme that behaves like a pair of molecular scissors in which it cuts the two strands of DNA at a predetermined location within the host genome. Guide RNA (gRNA), which consists of a pre-designed RNA sequence typically about 20 bases long located within a longer RNA called scaffolding, binds to DNA at the pre-designed sequence. This guides the Cas9 to the correct location of the genome. This ensures that the Cas9 enzyme cuts at the right point in the genome. During this specific stage, host cells recognize that the DNA is damaged and repairs itself with no checkpoint inhibition through G_1, S, G_2, and M phases. Such treatment modalities would aid in eliminating epigenetic variances in CAD; thus, this treatment modality would decrease mortality and morbidity. Furthermore, with risk stratification in primary care, this treatment modality could be started earlier before co-morbid disease complications affect the

patient. The preliminary reports on this research are intriguing with the six participants demonstrating decrease levels of genetic variances post therapy. Currently, the research is in clinical trials. Sadly, this research had to be excluded from this scholarly project due to a very low *N* of six participants within the initial trial.

Applicability to Clinical Practice

Genetics is quickly becoming the wave of modern medicine. It has been stated that 25% of medicine will change within one year. The applicability to clinical practice for epigenetics in the primary prevention of CAD will save lives. Thus, we are already treating patients with major depression, refractory to treatment by genetically screening the patient for the most efficacious antidepressants. Such testing could be available to family medicine, although, epigenetic testing and treatment modalities will be under the specialty of cardiology. Nonetheless, referrals can be made within the clinic, ensuring the patient is receiving applicable evidence-based medicine. Furthermore, epigenetics for the primary prevention of CAD could further risk stratify and treat patients earlier to limit the progression of the disease, thus leading to a decrease in mortality long term.

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