Evaluating Evidence in Support of Multiple Genetic Links to Coronary Artery Disease

By

Mikayla Strout

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Approved by:

Dr. Lisa Rezende

Department of Molecular and Cellular Biology

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Mikayla L. Strout

University of Arizona

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Dr. Rezende

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Abstract

Coronary artery disease (CAD) is an incredibly common and severe cardiac condition that affects people all across the world. It is a condition that requires careful monitoring, and can quickly become life threatening if left untreated. Coronary artery disease has the ability to affect a patient's eligibility for cardiac clinical trials, and can make them ineligible to participate in novel life saving procedures. These procedures are oftentimes for conditions that historically have not had a potential treatment or cure, or only have treatment options that are very high risk to the patient population in question. Through the past few decades, research into CAD has expanded to explore potential genetic variants that can influence CAD pathogenesis. This review discusses the role of 9 different genes identified through GWAS studies, the molecular pathways that each gene is involved in, and evaluates the evidence that a mutation in one or more of these genes promotes CAD development. This information could allow for patients with a genetically increased susceptibility to CAD to be screened for their disease risk and monitored for premature CAD. Ideally, this would allow them to take preventative measures against CAD, or pursue necessary treatment before the disease progresses to a state of extreme severity.

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Introduction

Coronary artery disease (CAD) is a severe and very common cardiac disease, not only across the United States, but also across the world. The National Institutes of Health (NIH) states that 20.5 million adults in the United States have coronary artery disease, making it the most common type of heart disease in the U.S. Coronary artery disease is atherosclerosis of the coronary arteries within the heart, which leads to narrowing of the arteries and eventually complete blockages within the heart. If left untreated or not properly managed, it can lead to life threatening cardiac complications, specifically myocardial infarction. Many people often do not know that they have CAD until they experience a myocardial infarction, and unfortunately for some it may be too late at that point. There are many different potential causes of coronary artery disease, including build up of substances within and on the artery walls. High cholesterol is often attributed to coronary artery disease, but there are many other factors that can contribute to this pathology.

Clinical research is an incredibly important aspect of advancing the field of medicine. It requires willing and able participants to test a variety of potential medical interventions for a multitude of diseases and conditions. A common area of clinical research is trials that aim to treat cardiovascular diseases, and although it is very important that these diseases are researched, the surgical procedures in which these patients undergo do not come without risk. Like any clinical trial, it is important that we ensure the safety of these participants, and one of the best ways to do this is to create a strict list of criteria that a patient must meet in order to be eligible for a trial. This list of criteria is known as eligibility inclusion and exclusion criteria, and no two trials have the exact same requirements. For many cardiac trials, a very common exclusion

criterion is some variation on the presence of coronary artery disease. Table 1 contains a list of some currently open cardiac trials that have CAD listed as an exclusion criterion in some capacity. Often this exclusion criteria is very dependent on the severity and the ability to treat CAD before the patient participates in the trial. Although this is the case, it is not uncommon to see this exclude a patient from a trial indefinitely, or delay their possibility of receiving novel surgical treatments in a clinical trial for a certain period of time. Participants that are seeking treatment from these clinical trials are often patients who are incredibly ill, and do not have any time to waste. The time that is spent on treating and managing a patient's CAD can cause the patient to lose valuable time that they cannot gain back. It is clear that CAD is not only detrimental to a patient's health for the obvious reason of risk of myocardial infarction, but that it can also have negative implications on other aspects of a patient's life and cardiac health as well.

Table 1: Clinical Trials with CAD as an Exclusion Criteria

Clinical Trial	Clinical Indication	Exclusion Criteria
ACURATE IDE	Aortic stenosis - narrowing of the aortic valve that impedes normal blood flow	Untreated CAD that is clinically significant and requires revascularization
CHAMPION AF	Non-valvular atrial fibrillation - irregular and often rapid heart rhythm where the atria of the heart beat irregularly and can lead to blood clots within the heart	Any kind of intervention or procedure within 30 days prior to or 60 days after implant
CLASP IID/IIF	Degenerative mitral regurgitation - gaps or loose portions of the valve that develop over time and prevent the heart from being able to close properly	Clinically significant, untreated CAD
CLASP II TR	Symptomatic severe tricuspid	Clinically significant,

	regurgitation - a condition where the tricuspid valve does not close properly and allows blood to flow backward from the right ventricle back into the right atrium	untreated CAD requiring revascularization
HighLife	Severe symptomatic mitral regurgitation - a condition where the mitral valve does not close properly and allows blood to flow backward from the left ventricle back into the left atrium	Untreated clinically significant CAD requiring revascularization
Laminar	Non-valvular atrial fibrillation - irregular and often rapid heart rhythm where the atria of the heart beat irregularly and can lead to blood clots within the heart	Subject with a history of (CABG) surgery
MITRAL II	Severe mitral annular calcification and symptomatic mitral valve dysfunction - a build up of calcium and lipid deposits on the annulus of the mitral valve, which is located the junction of the left atrium and left ventricle	Clinically significant untreated CAD requiring revascularization
Progress	Moderate, calcific aortic stenosis - slowly progressing calcium build up on the fibrous tissue of the aortic valves that eventually prevents proper leaflet function and obstructs blood flow	Coronary anatomy that increases the risk of coronary artery obstruction post TAVR
REPAIR MR	Mitral valve regurgitation - a condition where the mitral valve does not close properly, allowing blood to flow backward from the left ventricle into the left atrium	CAD that needs to be treated if the subjects are not eligible for PCI or CABG

ShortCut	Risk of coronary obstruction following TAVR procedure - blockage in the coronary vessels following a transcatheter aortic valve replacement in which the aortic valve is replaced using a catheter inserted in through the groin or chest	Coronary disease that requires treatment or was treated less than 1 month prior to the procedure
Triluminate	Symptomatic moderate or greater tricuspid regurgitation - a condition where the tricuspid valve does not close properly and allows blood to flow backward from the right ventricle back into the right atrium	Subject has received adequate CAD treatment at least 30-day prior to index procedure.

Abbreviations:

1. CABG (Coronary artery bypass grafting): a medical procedure to increase blood flow to the heart when the coronary arteries are narrowed or blocked
2. TAVR(Transcathetar aortic valve replacement): a medical procedure that involves replacing a diseased aortic valve with a man made valve by accessing it through the femoral artery
3. PCI (Percutaneous coronary intervention) : a minimally invasive procedure that is used to treat blockages in the coronary arteries and restores blood flow to the heart

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While CAD may generally be thought of as a lifestyle condition that can be changed with lifestyle modifications, recent research conducted by Roberts et al. in 2021 states that genetic risk factors account for 40% to 60% of the predisposition for CAD. This paper identifies a vast amount of genes with various different functionalities in the body. The major biological processes in which genes were discovered, and the genes with the greatest amount of evidence supporting a role in CAD, are listed in Table 2. Many biological processes in which these genes are described seem to have an intuitive link to the presence of CAD, while some are initially less

clear. From this genetic risk stratification, the authors recognize the potential that information from these studies can assist in furthering development of therapeutic actions.

The place in which the genetics of CAD and clinical research collide is prevention. In order for these patients with CAD to be eligible for trials, it is important that the CAD does not progress to a level of high severity. As previously mentioned, the presence of CAD is not generally the problem, it is more dependent on the severity of the disease. If we are able to successfully link a variety of genes and mutations to coronary artery disease, there is high potential that we would be able to diagnose, and therefore treat CAD much earlier than we currently can. This will likely decrease the amount of patients that find themselves to be ineligible for life saving procedures that they desperately need.

Genes Implicated in Coronary Artery Disease

Table 2: Genes Implicated in Coronary Artery Disease

Gene Name	Pathway Implicated
AGT	Blood Pressure
TGFB1	Neovascularization Angiogenesis
FLT1	Vascular Remodeling
GUCY1A3	Nitric oxide-Signaling
CDKN2A	Mitosis proliferation
KLF4	Transcription Gene Regulation
APOA5	Lipid metabolism
CXCL12	Inflammation
HP	Unknown

AGT

AGT encodes the angiotensin protein, which is an incredibly important player in regulating blood pressure across the body. More specifically, angiotensin plays this role in blood pressure regulation by causing vasoconstriction that is mediated by water and sodium uptake. Several studies have begun to investigate a connection between *AGT* and CAD in order to see if it has an effect on the development and progression of the disease. Prior research has shown that SNPs (single nucleotide polymorphisms) in *AGT* have important implications in other disease progression pathways, making it an attractive candidate for further research. A meta-analysis performed by Xu et al. analyzed 43 association studies on 2 angiotensin polymorphisms to explore the association of these polymorphisms with CAD. One of the polymorphisms that is addressed by this meta-analysis is the M235T variant, which is a change of methionine to threonine in codon 235. Overall, this study found only a modest association between the M235T variant and CAD. When the analysis of this relationship was restricted to include only the larger studies described in the meta-analysis, the association between M235T and CAD became insignificant. The authors of the meta-analysis also analyzed the relationship between the T174M polymorphism and CAD. The T174M polymorphism is a change of threonine to methionine in codon 174. The authors' analysis did not find an association between T174M and CAD. Additionally, even a haplotype analysis performed by the authors did not show any significant association between the combination of these alleles and CAD. The authors recognize that there are some limitations to their study, including potential sources of heterogeneity, and also the possibility of publication bias. Furthermore, they recognize that their study may be compromised by the quality of each study that they used, and they address the fact that the available data for their haplotype analysis for the M235T and T174M polymorphisms were not abundant. Overall,

the study was not able to demonstrate significant association between the M235T and T174M variants and CAD. Although this is the case, the authors recognize that they cannot rule out the possibility that other variants, or a combination of variants at multiple loci in the same genes, could play a role in CAD pathogenesis due to the fact that blood pressure control is a complex and multifactorial system. It is important to recognize that the study described above is an older study published in 2007, but the newer studies that investigate this relationship focus on both CAD and another disease, leading to a lack of solely CAD related data.

TGFB1

TGFB1 has many important roles within the body, including roles in enhancement of macrophage and fibroblast chemotaxis, stimulation of extracellular matrix synthesis, vascular cell proliferation abnormalities, and much more. It has significant implications across the entire body, and has been found to act within the vascular system. A meta-analysis performed by Lu et al. analyzed 27 studies and demonstrated an association of two SNPs in the *TGFB1* gene with CAD in Caucasian populations, suggesting that enhanced TGFB1 signaling may be involved in the pathogenesis of CAD. The first SNP is rs1800469, which is located in the promoter region of the *TGFB1* gene. It has been found *in vivo* and *in vitro* that recruitment of the transcription regulator AP1 to the region including rs1800469 leads to transcriptional regression of the *TGFB1* gene. The authors note that this exact functional variant in the gene needs further investigation to uncover the specifics surrounding this mechanism. The second SNP that has demonstrated an association with CAD is named rs1982073, and it is located in the signal peptide region of the *TGFB1* gene. A specific mechanism for control of this gene and its effects are not stated within the Lu et al. paper, but one can propose that this could correlate to increased levels of the TGFB1

protein that could be present in the bloodstream, the plaques, or elsewhere in the body. Data that supports this association between CAD risk and these SNPs can be found through histological studies that have demonstrated increased TGFB1 production levels in different stages of atherosclerotic plaques. Furthermore, increased *TGFB1* regulated gene expression was observed in both atherosclerotic and restenotic regions, which also lends support to the suggested role *TGFB1* plays in CAD. These SNPs were demonstrated in this paper to be CAD-associated minor risk alleles that correlate with an increase in gene expression, TGFB1 secretion levels, and plasma TGFB1 levels. Although this correlation was found in the Lu et al. paper, previous results regarding these SNPs have been inconsistent. The authors state that a possible reason for this historical inconsistency could be due to the small sample sizes that have been included in the majority of these studies, especially in combination with the fact that these SNPs seem to have a modest effect. Additionally, the authors address the fact that it has been very difficult for them to determine the exact cellular sources of TGFB1 that are relevant to the pathogenesis of CAD, warranting further research into this specific area on the role of TGFB1 in disease progression. Although the prior information on small sample sizes and lack of knowledge on the specific cellular sources is true, this did not affect the main conclusions that the authors came to regarding the association between rs1800496, rs1982073, and CAD.

FLT1

FLT1 is a gene that is responsible for encoding a protein within the VEGF family, which is a family of proteins that are well known for having angiogenic activity within the body. Signaling within the VEGF pathway allows for successful angiogenesis, decreased atherosclerosis, and increased blood flow within the body. Through research into FLT1 and its

role within the body, it has been seen that FLT1 may have effects on the coronary arteries and the development of coronary atherosclerosis, as it is a player in the regulation of vascular remodeling. This research was conducted by Konta et al., and consisted of a Japanese population composed of mainly male study participants. Although it may seem contradictory due to FLT1 being part of the VEGF family, this research led to the discovery that a functional SNP numbered rs74412485 and located in intron 1 of *FLT1*, leads to a soluble form of FLT1 that acts as a nonfunctional decoy receptor for VEGF. This SNP functions as a negative regulator for VEGF/FLK1 signaling in angiogenesis. Soluble FLT1 acting as a nonfunctional decoy receptor inhibits binding of the VEGF proteins that normally assist in angiogenesis within the body and traditionally combat the presence of atherosclerosis. Studies have shown that the increased levels of FLT1 cause an increased presence of atherosclerotic plaques composed of various adhesion molecules on the surface of the body's vessels, including the coronary arteries. Additionally, it is seen that FLT1 will localize on the cell surface, contributing to the atherosclerosis that can be seen in coronary vessels in patients with coronary artery disease.

GUCY1A3

GUCY1A3 is a gene that is associated with nitric oxide signaling within the cardiovascular system of the human body, as it acts as a receptor for nitric oxide (NO) and assists in the regulation of cardiovascular function. NO signaling is essential for proper vascular endothelial function and controlling vasodilation within the vessels, with coronary vessels being no exception. It is seen that with decreased levels of NO signaling, there is impaired relaxation of the vessel endothelium, leading not only to constriction of the vessels, but the potential for increased platelet aggregation, platelet adhesion, and leukocyte adhesion, which leads to

blockages within the vessels. As a gene that is known to contribute to this vascular regulation, the *GUCY1A3* gene, became a gene of interest for having potential implications on CAD. Kesseler et al. conducted a study into the *GUCY1A3* gene and looked for links that it may have to decreasing NO signaling and subsequent atherosclerosis development. It was found that a SNP numbered rs7692387, located in an intron of the *GUCY1A3* gene, has a significant association with CAD, as it is linked to decreased expression of soluble guanylyl cyclase (sGC), which directly decreases the efficacy of the NO-signaling pathway and the ability of NO to act within the vessels.

CDKN2A

CDKN2A is a gene predominantly involved in encoding proteins that regulate mitosis and cell proliferative activities. With widespread action across the body, any mutation affecting the gene's normal functionality can have large effects on the body's ability to properly regulate cell division within the various body systems. It has been found that the cardiovascular system is not immune to mutations in *CDKN2A*, proving it has implications on the vasculature within the coronary arteries, and establishing its link to coronary artery disease in humans. Studies have shown that a specific variant of *CDKN2A*, the 9p21 variant, which is a SNP numbered rs1333049, leads to higher levels of proliferation in vascular smooth muscle cells (VSMCs). This increased proliferation is known to play an important role in the pathogenesis of atherosclerosis, as the accumulation of VSMCs and the proteins they secrete largely contribute to plaque growth. The 9p21 variant leads to this increased proliferation of VSMCs through its association with decreased levels of expression of *CDKN2A* and *CDKN2B*. CDKN2A and CDKN2B are important in keeping control over excessive proliferation as they function as tumor suppressors,

mediate cell proliferation, and mediate *ANRIL* expression. Increased *ANRIL* expression is seen to be related to increased risk of atherosclerosis and plaque formation within the coronary arteries. ANRIL stands for Antisense Non-Coding RNA in the INK4 Locus, which is an RNA transcribed from the short arm of human chromosome 9 on p21.3, that overlaps a region housing three critical major tumor suppressor loci. *ANRIL* expression is a key player in important cell proliferative processes, and is said to direct cell fates that lead to cancer and cardiovascular disease. The 9p21 variant has been deemed a risk allele, which means that it is inside the gene itself, and has been associated with atherosclerotic lesion development, progression, and susceptibility to CAD. Although this has been linked to increased risk of CAD, it does not necessarily mean that there is higher risk of myocardial infarction along with it, which is one of the major concerns in patients with severe CAD. This is due the fact that the thickened plaques of VSMCs are less likely to rupture to cause thrombosis or acute ischemic events.

KLF4

KLF4 is an identified transcription factor that is known to play a role in inflammation, cell differentiation, cell development, cell proliferation and cell death across the body. When looking at the role of it specifically related to atherosclerosis, studies are beginning to point towards it playing at least a small role in atherosclerosis, if not a more significant one. The various studies that have looked into KLF4's role in coronary artery disease have taken different approaches, and aim to look at KLF4 in various different contexts. A study into KLF4 conducted by Liu et al. was performed in a group of Chinese male patients, and aimed to connect the presence of circulating KLF4 levels with CAD. The researchers of this study were able to conclude that increased levels of KLF4 are significantly associated with the presence and

severity of CAD. The same study by Liu et al. demonstrated that increased levels of GDF-15, which is a known biomarker of cardiovascular stress that has been associated with cardiovascular disease, are also significantly associated with the presence and severity of CAD within the body. Furthermore, the authors recognize that there have been previous studies demonstrating that KLF4 acts as a transcription factor that controls GDF-15 promoter activity, which proves the relevance GDF-15 has in their research. The authors recognize that the results of their research calls for further investigation into the interactions of KLF4 and GDF-15, as they believe that they may be in the same atherogenic pathway during the progression of atherosclerosis. Although they were able to suggest that KLF4 and GDF-15 are involved in CAD pathogenesis, their results may not be widely applicable as the study only investigated the role of KLF4 in male subjects who were specifically of Chinese descent. Another study that aimed to link KLF4 and CAD was conducted by Shyu et al., and specifically looks at the effects that high blood glucose conditions have on the regulation of KLF4 and coronary artery disease. The researchers performed a study in humans and were able to discover that in high glucose conditions, there are various steps within the pathway of KLF4 activation that happen post-transcriptionally. These steps cause KLF4 to become upregulated, leading it to have greater effects on the vasculature of the body. This increase in KLF4 will cause increased proliferation and migration of vascular smooth muscle cells, leading to coronary atherosclerosis. The authors also recognize angiotensin to be implicated in this pathway along with KLF4, which aligns with the data discussed above in the *AGT* section of this paper. This study supports the idea that KLF4 is a player in coronary artery disease, but it cannot be generalized to all patients as it is done under high glucose conditions only. With that being said, it does present promising information for developing treatment for patients with both CAD and diabetes mellitus. Contrary to the two previously discussed studies,

there has been research performed by Czepluch et. al. that finds decreased levels of KLF4 to be associated with CAD severity instead of increased levels. This study proposes that KLF4 is a transcription factor that promotes angiogenesis, and decreased levels of KLF4 lead to negative effects to the vasculature, such as endothelial dysfunction and decreased angiogenesis. The information presented in the data from the first two papers very closely agree on the role of KLF4 in atherosclerosis, even though they are done in different conditions and in mostly male subjects overall. With that said, it can be reasonably thought that the data from the first two studies may be more accurate in describing the role of KLF4 than the data from the Czepluch et al paper. Lastly, a GWAS performed by Erdmann et al. identifies a SNP named rs944172 as a significant CAD risk loci found in 29 of the studies they analyzed. Listing the identified SNP is the extent of their discussion on KLF4.

APOA5

APOA5 encodes the protein apoAV, which is integral to regulation of triglyceride and HDL metabolism. Given that lipid metabolism and regulation is known to be a major driver of atherosclerotic plaque formation, this gene has been one of clear interest for researchers looking into the development and progression of coronary artery disease. Through various studies, researchers have been able to determine that there is a causal role of this gene in the development of coronary artery disease, and there are many different variants that are suspected to play a role in the disease progression. A study published in 2018 conducted by Wang et al. explores multiple different *APOA5* variants that have been linked to CAD, with three variants being shown to have significant correlation to CAD. This study demonstrates that multiple rare variants in aggregate confer a significant risk of premature CAD. The first variant with significant association is a

missense variant named rs3135506, and it is associated with increased triglyceride (TG) and high-density lipoprotein (HDL) levels. This variant is said to have a modest, yet significantly causal and indirect effect on premature CAD. The authors come to the conclusion that there is definitely an increased risk associated with this SNP, but the variation in how causal the association is differs based on whether they're looking at triglyceride or HDL levels. The authors further state that the molecular mechanism by which this specific variant affects CAD is unknown. Another gene variant addressed by the Wang et al paper is a variant in the 5' UTR region of the *APOA5* gene named rs651821. This gene has been stated to not be conservative, but it is suggested to have an important regulatory role in CAD. Furthermore, Wang et al. cites that another study performed by Paré et al. also shows this variant to be significantly correlated with plasma lipid levels and CAD. The last variant of significant association in the Wang et al. paper is a missense variant named rs2075291 that was discovered in a meta-analysis of multi-ethnic populations from Southeast Asia. This variant is found to be significantly associated with both CAD and myocardial infarction, as well as high HDL-C levels. Additional information about this allele being present in North Indian populations was also included in the Wang et al. paper. This specific variant, rs2075291, was also discussed in another study that was published by Han et al. where it is also described as being present in multi-ethnic Southeast Asian populations. This variant is said to be independent of other variants that have previously been reported, and is also stated to have about a 60% increased risk of CAD per copy of the risk allele. This variant is found inconsistently across populations, demonstrating that it may be specific to certain populations and not others. Interestingly, while this variant has not been significantly associated with CAD risk in European populations, it has been nominally associated with myocardial infarction risk in those of European ancestry. In regards to this variant, they are unsure if the

SNP's effect on CAD is due to an atherogenic lipid profile or an undetermined function of *apoAV*. Han et al. also mentions a previous smaller scale study that states rs2075291 may be independent of traditional risk factors, and is seemingly lipid independent at this time. They also recognize that this variant will need further investigation with a larger sample size in the future in order to determine the exact degree of involvement and the mechanism by which this variant plays a role in the pathogenesis of CAD. In a separate study, published in 2018 by Chen et al., another variant named rs662799 is discussed. This SNP is located in a promoter region and influences the expression levels of *APOA5*. In the Chen et al. study they state that there have been differing results when looking into the association of this SNP with CAD, with some studies identifying it as associated, and some stating it is not. Chen et al. recognizes that the studies that do not find it to be associated with CAD may be due to a lack of statistical power, and that further studies with larger population sizes will need to be conducted in order to discover the specific relationship or lack thereof. In the Han et al. paper they do briefly mention the rs662799 SNP and state that it does confer the TG raising effect, suggesting that it plays a role in CAD development and progression.

CXCL12

CXCL12 is an important cytokine implicated in the immune response, specifically in inflammation. It is responsible for multiple functions, with those most pertinent to coronary artery disease being hematopoiesis, angiogenesis, and inflammation. CXCL12 promotes migration of hematopoietic progenitor and stem cells, endothelial cells, and most leukocytes. Multiple GWAS studies have investigated the relationship between CAD and *CXCL12*, and they have found many loci that cosegregate with the *CXCL12* gene, making it an attractive candidate

for being linked with CAD. There are a multitude of SNPs that are found within these regions that are seen to affect the functionality of the CXCL12 protein. When looking into the specifics of the association between CAD and *CXCL12*, there is some contradicting data that has been reported. A paper by Farouk et al. analyzes multiple studies that have been performed on *CXCL12* and CAD, and compiles them together in an attempt to find the mechanism behind this relationship. In the paper, before describing the study performed in 2002, they cite Abi-Younes et al., stating that *CXCL12* seems to be more highly expressed in atherosclerotic vessels as opposed to normal vessels, which is indicative that it may play a role in regulation of CAD. A study was performed in 2002 that describes CXCL12 possessing the capability to reduce inflammation in cases of unstable angina. This study was a smaller scale study that has not yet been replicated, but it helped to further suggest that CXCL12 may have anti-atherogenic effects on CAD in humans. In contrast, a study performed in 2009 demonstrated that patients with acute coronary syndromes, or ACS, have significantly higher levels of CXCL12 on the surface of platelets compared to patients with stable angina pectoris. From this study, they also discovered that CXCL12 assists in the aggregation of platelets, suggesting that CXCL12 is atherogenic, pro-thrombotic, and plaque destabilizing. Furthermore, a main finding from this study is the suggestion that CXCL12 is a potential mediator of platelet involvement in atherosclerosis and CAD. This is important as these activated platelets are crucial to rupture and early repair of unstable atherosclerotic plaques. The study performed by Farouk et al. proceeded to analyze an association between two SNPs, rs1746048 and rs501120, that were discovered in the GWAS searching for markers associated with CAD, myocardial infarction, and plasma CXCL12 levels. It was found that both of these SNPs are associated with higher plasma levels of CXCL12, suggesting that the loci in the region 80 Kb 3’ downstream of *CXCL12* may house regulatory

elements that modulate plasma CXCL12 levels. Furthermore, they suggest *CXCL12* may be atherogenic, since the SNPs are associated with higher mRNA transcript and plasma levels of CXCL12. Farouk et al. recognize that it is necessary to do further studies on CXCL12 and its relationship to CAD in order to further investigate the specific mediation of and mechanisms behind this relationship. In contrast, a meta-analysis study performed by Wu et al. in 2015 analyzed 7 different studies, and describes a lack of association between CXCL12(referred to in the study as SDF-1) and CAD. In their analysis, they analyze the effects of multiple SNPs, including the previously discussed rs1746048 and rs501120, but also of a different SNP named rs1801157. The authors state that there have been multiple studies confirming the relationship between the rs1746048 and rs501120 SNPs and CAD, but the majority of their discussion focuses on the rs1801157 SNP and its potential connection to CAD. The studies that they have included in their meta-analysis are conflicting with one another in their decision on the association between *CXCL12* and CAD. Studies performed by Szalai et al., Apostolaskis et al., and Simeoni et al. suggest no correlation between rs1801157 and risk of CAD. Although those studies concluded no correlation, there were four other studies performed that demonstrated there is a correlation between rs1801157 and CAD. With that said, only one of the studies performed, which was conducted by Gu et al., demonstrated an increased susceptibility to CAD, while the rest showed a decreased susceptibility to CAD. The meta-analysis was performed to analyze the contradictions in the previous literature. However, Wu et al. do recognize there are limitations to their study that may have large implications on their results. One of their limitations is there are only a small amount of studies in their area of interest available for analysis, with each of these studies consisting of small sample sizes. Another limitation of the study is the strong heterogeneity of the study, which was not analyzed by meta-regression analysis due to the

sample size being too small. Additionally, there are multiple subgroups included in CAD, such as myocardial infarction, stable angina, and acute coronary syndrome that they state are in need of further analysis. The ability for further analysis was not available at the time due to a lack of sufficient statistical data in the literature. Ultimately Wu et al. conclude their meta-analysis by suggesting the rs1801157 polymorphism is not associated with increased susceptibility to CAD, but may be associated with decreased risk of myocardial infarction. They state larger scale and more in depth case-control studies are going to be necessary to confirm the suggested relationships that they found.

HP

Haptoglobin is an abundant plasma protein, and the main protein responsible for binding free hemoglobin and clearing it from the bloodstream. By clearing hemoglobin, it assists in maintaining normal function of the body by combating hemoglobin's inflammatory effects. When inflammation is elevated, there is a subsequent increase in haptoglobin concentration. Due to the important role it plays in healthy circulation and blood hemoglobin concentrations, *HP* has become a gene of interest for potential links to CAD. There are two known isoforms of haptoglobin, each of which plays an important part in the function of hemoglobin and its role in coronary artery disease. A study performed by Bjornsson et al. describes a novel splice mutation in the haptoglobin gene that may be a founder mutation in Iceland. The mutation is stated to be located at the first base of intron 3, and is only relevant to the presence and severity of CAD if the HP1 isoform is present. This splice mutation causes a loss of function of *HP1*, leading to higher levels of non-HDL cholesterol within the blood, and lower levels of serum haptoglobin. It is known that the presence of high cholesterol is directly linked to atherosclerosis and CAD, as it

greatly contributes to plaque formation within the vessels. Although the loss of *HP1* function is linked to increased non-HDL cholesterol and CAD risk, the presence of Hp1 does not seem to be associated with lower CAD risk. With that said, the presence of Hp1 does seem to be associated with lower non-HDL levels. The data from this study suggests a causal role of the *HP1* splice mutation on cholesterol levels and CAD. Another study that was conducted regarding haptoglobin levels looks into plasma haptoglobin levels and their effect on coronary artery disease. The study conducted by Lee et al. describes three different genotypes that can be present for the *Hp* gene, which are denoted as *Hp1* homozygosity (Hp 1-1), *Hp 2* homozygosity (Hp 2-2), and *Hp* heterozygosity (Hp 2-1). The Hp 1-1 phenotype is stated to be the most successful at binding hemoglobin and demonstrates the lowest levels of plasma Hp. The Hp 2-2 phenotype is stated to have the lowest binding affinity and demonstrates the highest levels of plasma Hp. The researchers delved further into the relationship between plasma haptoglobin levels and CAD, discovering that patients with higher levels of plasma Hp have an increased risk of CAD. It would seem that this would indicate the *Hp 2-2* genotype as being directly linked to CAD, but they found no significant relationship between the two. This finding is consistent with some prior data that the author's discuss within their conclusion. From this information, it is possible that the *Hp* genotype does not directly mediate CAD, but that it likely interacts with other factors later on in order to contribute to CAD risk. Additionally, the authors recognize that Hp is involved in many pathways other than CAD, so a lack of significant and direct correlation is not overly surprising. These two studies seem to pose two opposing mechanisms for the effect of Hp on CAD risk, as one supports the claim for increased levels, and one supports the claim for decreased levels. A possible explanation for this may be due to the difference in where the Hp levels are being measured, as one study is measuring in serum, and the other is measuring in

plasma. Regardless of the difference on whether CAD risk is related to increased or decreased levels of Hp, both studies agree that Hp is in some way linked to CAD risk.

Discussion

The analysis of all of these genes in aggregate present a very compelling argument for the role of genetics in CAD. Some of the genes that are included in this analysis are more well understood than others, and have been identified as therapeutic targets for early identification, treatment, and prevention of CAD. These genes are included in Table 3, and have been directly identified by the authors of their respective studies to have direct links to CAD. Furthermore, they are identified as having the ability to be monitored and addressed in order to modulate CAD development and progression in patients with these polymorphisms.

Table 3: Therapeutic Target Genes

Gene	Function	Reference
APOA5	Lipid metabolism	Wang et al.
HP	Unknown	Asleh et al.
FLT	Vascular remodeling	Konta et al.
CXCL12	Inflammation	Farouk et al.

Additionally, some of the studies were able to establish a confident link between CAD and the respective genes, but they do not identify them to be therapeutic targets at this time. Further investigation into these genes and their mechanism of action could help to progress our current knowledge so that they too are able to act as early targets for treatment and prevention of CAD. These genes are identified below in Table 4.

Table 4: Genes with Clear Genetic Links to CAD

Gene	Function	Reference
CDKNA	Mitosis proliferation	Motterle et al.
GUCY1A3	NO-signaling	Kessler et al.

Lastly, some of the genes of interest presented conflicting results. In these studies, there is a combination of factors that have caused the authors to recognize the need for further research into these genes. This research is needed to either establish a connection between the gene and CAD or to establish the mechanism in which the gene affects CAD. One of these genes is *TGFB1*, where the authors discuss a minor association between the SNPs that they have found and CAD. They also recognize that there has been a large amount of inconsistency in data pertaining to this relationship in the past. In addition to the historical inconsistencies, they state that the exact cellular sources of TGFB1 that affect CAD are unknown, which leads to the requirement for more in depth research into *TGFB1*. Future research should be focused on determining the mechanism behind TGFB1's role in CAD. It could be possible to do this through analyzing the places in which TGFB1 levels are higher, which could include cells in the vascular walls and plaques in the coronary arteries. Larger scale studies on the gene itself and the mechanism behind its effects is important to fully understand the role that TGFB1 plays. In addition to the conflicting results seen with *TGFB1*, *KLF4* is also a gene that is in need of further research. There have been conflicting results reported on the mechanism by which *KLF4* affects CAD. The studies included in this analysis are performed in different conditions, and in mostly male studies. These studies are not reflective of, and cannot be generalized to, the entire

population. Larger scale and more diverse studies are needed in order to better understand the mechanism of KFL4 involvement in CAD pathogenesis, and to better represent the different populations around the world.

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