Preventive Treatment of Hypercholesterolemia and Atherosclerosis: A Genetic Approach

Brian Vail

Florida Atlantic University

Abstract

Hypercholesteremia is characterized by high levels of LDL-C, and leads to atherosclerosis. This is a largely preventable disease given the proper treatment. Historically, statins are the most widely used form of treatment in reducing cholesterol in those at-risk or already diagnosed with atherosclerosis. Recently, genetic risk factors have been identified, such as the PCKS9, LDLR and ANKS1A genes. Researchers have begun investigating their mechanisms and potential treatment options to lower risk for individuals at high-risk of developing atherosclerosis. This review outlines some of the major findings in the preventative treatment of hypercholesteremia and atherosclerosis and suggests future lines of research.

Background

High levels of cholesterol have been associated with increased risk of atherosclerosis and heart disease for decades. Atherosclerosis is a disease where deposits of plaques and fatty acids can build up within the artery walls, thereby impeding blood and oxygen flow and cause further conditions such as heart attacks, strokes, and peripheral vascular diseases. In general, atherosclerosis is the result of long-term, increased levels of low-density lipoproteins, or cholesterol, where the cholesterol builds up in the arteries as obstructing plaques. However, these coronary artery diseases are largely preventable (Roberts, 2014). Reducing risk factors, such as cholesterol, through preventative care have shown up to a 30% to 40% reduction in morbidity and mortality (Shepherd et al., 1995; Downs et al., 1998). Others argue that with time, we could prevent up to 90% of a coronary heart diseases (McGill, McMahan & Gidding, 2008).

McGill, McMahan & Gidding (2008) argue lowering risk factors in adolescence and young adults through preventative care and treatment will minimize the risk of atherosclerosis and further coronary heart disease later in life. Several studies provide evidence that slowing the effects of atherosclerosis earlier in life delay the onset of more severe medical issues (Lloyd, et al., 2006; Stamler et al., 1999; Cohen, Boerwinkle, Mosley, & Hobbs, 2006). Statins are the most widely used form of treatment in lowering cholesterol levels and lowering the risk of hypercholesterolemia and atherosclerosis. A statin is defined as any drug that inhibits cholesterol synthesis. One study found statins administered to those at high risk of coronary heart disease significantly lowered the risk of a cardiovascular event (Thavendiranathan, Bagai, Brookhart, &

Choudhry, 2006). However, the use of statins has only put a dent in the overall cases of atherosclerosis in at-risk individuals. One such study found that only 28% of individuals using statins achieved the recommended level of cholesterol (Seidah, 2003), suggesting that statins as the only means of preventative care is just not sufficient. Recently, researchers have begun focusing on the genetic risk factors of hypercholesterolemia and atherosclerosis as a means of potential treatment. As discussed below, they have found additional and complementary preventative treatments for those at risk of developing atherosclerosis and related diseases by determining genetic risk factors and investigating the mechanisms that lead to elevated cholesterol levels.

Methods

Literature Review

For the literature review section of this paper, research articles were found using the online resources Google Scholar, The National Center for Biotechnology Information (NCBI), and PubMed. Key terms used for search results included “Genetics”, “Atherosclerosis”, “Hypercholesteremia”, “Prevention”, “Treatment”, “Risk Factors”, and others.

Wikipedia and WebMD were utilized for further understanding of biological and medical concepts relevant to this review.

GO Term Enrichment

Identified genes from the literature review were further identified on the HUGO Gene Nomenclature Committee (HGNC) website, confirming correct gene identification within their

database. GO enrichment analysis was performed using Gene Ontology (www.geneontology.org). This provided a list of related GO Terms and their statistical significance in terms of their p values. To summarize and visualize the GO term results, GO terms and corresponding p values were entered into ReviGO to generate a scatterplot, interactive graph and treemap. The related GO terms were further explored using the GO database in Web Protégé.

The gene ontology database and Wikipedia were utilized for further understanding of relevant biological concepts.

Results

In a review article by Roberts (2014), GWAS identified single nucleotide polymorphism (SNP) variants of the genes LPA, APOB, SORT1, LDLR, APOE, ABCG5 – ABCG8, and PCSK9 were associated with high levels of low-density lipoprotein cholesterol (LDL-C) which lead to increased risk of atherosclerotic cardiovascular disease. Furthermore, he identified an SNP variant of the gene ANKS1A that is associated with low levels of high-density lipoprotein (HDL) which leads to increased risk of high cholesterol and atherosclerotic cardiovascular disease. Roberts (2014) points out that while statins are historically the only method of both prevention and treatment to high cholesterol and atherosclerosis, identification of these at-risk genes is leading to new forms of both prevention and treatment. For example, the PCSK9 gene creates the enzyme PCSK9 that increases degradation of LDL-C receptors, leading to excess LDL-C and hypercholesterolemia (Seidah, 2003). PCSK9 inhibitors can lead to lower levels of LDL-C, thus reducing risk of atherosclerotic cardiovascular disease (Roberts, 2014). Mutations of the LDLR

receptor gene may lead in hypercholesterolemia as the receptors are a major mechanism for the removal of LDL-C. The ANSK1A gene produces high-density lipoproteins (HDL), which remove cholesterol from the blood. Mutations of this gene lead to increased cholesterol levels and increased risk of atherosclerosis. While unclear the connection to the ANSK1A gene, cholesteryl ester transfer protein (CETP) has recently been investigated as a source of increasing levels of HDL. However, in one such study, they found that a CEPT increased blood pressure and lowered serum potassium thereby increasing mortality rate of patients and forcing the trials to end prematurely (Barter et al., 2007; Feig, 2014). Other genetic risk variants of atherosclerosis have been identified in terms of triglycerides, hypertension and risk of myocardial infarction. These genes are summarized in Table 1. Other genes in this review have been implicated with hypercholesterolemia but their mechanisms are unclear. To date, the best method of prevention and treatment are statins which inhibit cholesterol synthesis (Roberts, 2014).

Sun et al. (2018) investigated genetic variants associated with familial hypercholesterolemia (a genetic disorder) which causes lifelong levels of increased LDL-C and enhances risk of atherosclerotic cardiovascular disease. They found additional specific variants or SNPs of genes to be significantly associated with high levels of LDL-C. One variant of the gene PCSK9, two variants of the gene APOB, and 5 variants of the gene LDLR.

In a study on the prevention and treatment of ischemic heart disease (IHD) which is generally caused by atherosclerosis, Gerloni et al. (2017) examined several preventive treatments as a means of coping with atherosclerosis. β-blockers reduce blood pressure by altering heart rhythm. Calcium channel blockers reduce peripheral vascular resistance by inducing vasodilation. And

nitrates allow for coronary arteriolar and venous vasodilation (Gerloni et al., 2017). However, no clear link was given between medication and genetic interaction.

A summary of the review findings can be found in Table 1.

Gene	Variant (SNP ID)	Gene Products	Phenotypic Expression	Preventative Treatment
PCSK9	rs11206510 rs141502002	PCSK9 Enzyme	Hypercholesterolemia	PCSK9 Inhibitors Statins
APOB	rs12713559 rs5742904 rs515135	-	Hypercholesterolemia	Statins
LDLR	rs768563000 rs151207122 rs121908030 rs201573863 rs137853964	LDL Receptor	Hypercholesterolemia	Statins
LPA	rs3798220	-	Hypercholesterolemia	Statins
APOE	rs2075650	-	Hypercholesterolemia	Statins
SORT1	rs599839	-	Hypercholesterolemia	Statins
ABCG5-ABCG8	rs6544713	-	Hypercholesterolemia	Statins
ANKS1A	rs12205331	HDL	Hypercholesterolemia	CETP
TRIB1	rs10808546		Risk Variant: Triglycerides	Statins
ZPR1	rs964184		Risk Variant: Triglycerides	Statins
APOA5	rs964184		Risk Variant: Triglycerides	Statins
APOA4	rs964184		Risk Variant: Triglycerides	Statins
APOC3	rs964184		Risk Variant: Triglycerides	Statins
APOA1	rs964184		Risk Variant: Triglycerides	Statins
SH2B3	rs3184504		Risk Variant: Hypertension	Statins
CYP17A1	rs12413409		Risk Variant: Hypertension	Statins
CNNM2	rs12413409		Risk Variant: Hypertension	Statins
NT5C2	rs12413409		Risk Variant: Hypertension	Statins
GUCY1A1	rs7692387		Risk Variant: Hypertension	Statins
FURIN	rs17514846		Risk Variant: Hypertension	Statins
ABO	rs579459		Risk Variant: Myocardial Infarction	Statins

Table 1: Identified genes variations associated with risk of hypercholesterolemia and atherosclerosis.

GO term enrichment analysis resulted in seven overarching categories of biological pathways associated with risk of hypercholesterolemia and atherosclerosis as related to the identified genes in Table 1: sterol metabolism, regulation of plasma lipoprotein particle levels, macromolecular complex remodeling, cholesterol transport, lipoprotein metabolism, catabolism, and organic hydroxy compound metabolism.

Sterols are a subgroup of steroids, with cholesterol being one of the more widely researched in conjunction with hypercholesterolemia and atherosclerosis. Therefore, it is no surprise gene enrichment analysis indicated that sterol metabolism and all the subgroups that belong to it were the most prominent of related biological pathways. In sterol metabolic processes, important life functions occur such as sterols forming parts of cell membranes, and sterols acting as signaling agents in cellular communication and developmental signaling. As part of these processes, catabolism is a method for breaking down complex molecules. Both metabolic and catabolic processes appear under the greater heading of sterol metabolism, affecting compounds such as alcohols, lipids, triglycerides, retinoids, glycerolipids, and isoprenoids. As discussed already, statins are used to lower cholesterol and reduce the risk of atherosclerosis. They do this by interfering with cholesterol synthesis which ultimately lowers LDL-C serum levels. Specifically, statins inhibit the activity of hydroxymethyl-glutarylCoA reductase, and enzyme used in the synthesis of cholesterol (Nissinen, Miettinen, Gylling, & Miettinen, 2010). Ratios of sterol precursors are positively related to cholesterol synthesis and negatively related to cholesterol absorption (Miettinen, Tilvis, Kesaniemi, 1989) and studies have found that the ratios of campesterol, sitosterol and cholestenol are related to the efficiency of cholesterol absorption (Miettinen, Tilvis, & Kesaniemi, 1990). In a study by Nissinen et al. (2010), they hypothesized

certain features involved in cholesterol metabolism could be used to predict statin treatment outcome, where good and poor responders would have differential serum profiles. They found that low serum level ratios of lathosterol, cholestenol and desmosterol to cholesterol predicted poor patient response to statins. Lower levels of precursor sterols and increased levels of sterol absorption predicted good patient response to statin treatment.

The results of the GO term enrichment also specified regulation of plasma lipoprotein particle levels, which share a degree of overlap with sterol metabolism. Lipoproteins are the proteins that are combined with fats and other lipids and help to transport them through the bloodstream. As discussed previously, LDL-C is a major risk factor for hypercholesterolemia and atherosclerosis, while cholesterol with higher density lipoproteins are considered healthier. In this way, it is clear to see the regulation of lipoproteins plays an important role as a risk variant for hypercholesterolemia and atherosclerosis. As well, the other identified categories in the enrichment analysis play a part in the regulation of plasma lipoprotein particle levels. In macromolecular complex remodeling, the specific GO terms include protein-lipid complex subunit organization and plasma lipoprotein particle remodeling, both of which are related regulating LDL-C levels. As well, the overarching categories of cholesterol transport, lipoprotein metabolism, catabolism, and organic hydroxyl compound metabolism all seem to have direct influence on LDL-C levels.

A summary of the identified pathways can be found in Table 2.

Category	Biological Pathway	GO Id	GO Description	Identified in Literature Review
Sterol Metabolism	sterol metabolic process	GO:0016125	The chemical reactions and pathways involving sterols, steroids with one or more hydroxyl groups and a hydrocarbon side-chain in the molecule.	Y
	secondary alcohol metabolic process	GO:1902652	The chemical reactions and pathways involving secondary alcohol.	N
	lipid catabolic process	GO:0016042	The chemical reactions and pathways resulting in the breakdown of lipids, compounds soluble in an organic solvent but not, or sparingly, in an aqueous solvent.	Y
	steroid metabolic process	GO:0008202	The chemical reactions and pathways involving steroids, compounds with a 1,2,cyclopentanoperhydrophenanthrene nucleus.	Y
	regulation of lipid metabolic process	GO:0019216	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving lipids.	N
	neutral lipid metabolic process	GO:0006638	The chemical reactions and pathways involving neutral lipids, lipids only soluble in solvents of very low polarity.	N
	triglyceride metabolic process	GO:0006641	The chemical reactions and pathways involving triglyceride, any triester of glycerol. The three fatty acid residues may all be the same or differ in any permutation. Triglycerides are important components of plant oils, animal fats and animal plasma lipoproteins.	N
	lipid metabolic process	GO:0006629	The chemical reactions and pathways involving lipids, compounds soluble in an organic solvent but not, or sparingly, in an aqueous solvent.	Y
	organic substance catabolic process	GO:1901575	The chemical reactions and pathways resulting in the breakdown of an organic substance, any molecular entity containing carbon.	N
	retinoid metabolic process	GO:0001523	The chemical reactions and pathways involving retinoids, any member of a class of isoprenoids that contain or are derived from four prenyl groups linked head-to-tail.	N
	glycerolipid metabolic process	GO:0046486	The chemical reactions and pathways involving glycerolipids, any lipid with a glycerol backbone. Diacylglycerol and	N

			phosphatidate are key lipid intermediates of glycerolipid biosynthesis.	
	small molecule metabolic process	GO:0044281	The chemical reactions and pathways involving small molecules, any low molecular weight, monomeric, non-encoded molecule.	N
	glycerolipid catabolic process	GO:0046503	The chemical reactions and pathways resulting in the breakdown of glycerolipids, any lipid with a glycerol backbone.	N
	isoprenoid metabolic process	GO:0006720	The chemical reactions and pathways involving isoprenoid compounds, isoprene (2-methylbuta-1,3-diene) or compounds containing or derived from linked isoprene (3-methyl-2-butenylene) residues.	N
	regulation of lipid biosynthetic process	GO:0046890	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways resulting in the formation of lipids.	N
Regulation of Plasma Lipoprotein Particle Levels	regulation of plasma lipoprotein particle levels	GO:0097006	Any process involved in the maintenance of internal levels of plasma lipoprotein particles within an organism.	Y
	lipid homeostasis	GO:0055088	Any process involved in the maintenance of an internal steady state of lipid within an organism or cell.	N
	positive regulation of cellular catabolic process	GO:0031331	Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways resulting in the breakdown of substances, carried out by individual cells.	N
	regulation of lipase activity	GO:0060191	Any process that modulates the frequency, rate or extent of lipase activity, the hydrolysis of a lipid or phospholipid.	N
	regulation of lipoprotein lipase activity	GO:0051004	Any process that modulates the activity of the enzyme lipoprotein lipase.	N
	chemical homeostasis	GO:0048878	Any biological process involved in the maintenance of an internal steady state of a chemical.	N
	regulation of catabolic process	GO:0009894	Any process that modulates the frequency, rate, or extent of the chemical reactions and pathways resulting in the breakdown of substances.	N
	negative regulation of catalytic activity	GO:0043086	Any process that stops or reduces the activity of an enzyme.	N
	lipoprotein catabolic process	GO:0042159	The chemical reactions and pathways resulting in the breakdown of any conjugated, water-soluble protein in which the covalently attached nonprotein group consists of a lipid or lipids.	N

	cholesterol homeostasis	GO:0042632	Any process involved in the maintenance of an internal steady state of cholesterol within an organism or cell.	N
	regulation of Cdc42 protein signal transduction	GO:0032489	Any process that modulates the frequency, rate or extent of Cdc42 protein signal transduction.	N
Macromolecular Complex Remodeling	macromolecular complex remodeling	GO:0034367	The acquisition, loss, or modification of macromolecules within a complex, resulting in the alteration of an existing complex.	N
	regulation of endocytosis	GO:0030100	Any process that modulates the frequency, rate or extent of endocytosis.	N
	protein-lipid complex subunit organization	GO:0071825	Any process in which macromolecules aggregate, disaggregate, or are modified, resulting in the formation, disassembly, or alteration of a protein-lipid complex.	N
	negative regulation of multicellular organismal process	GO:0051241	Any process that stops, prevents, or reduces the frequency, rate or extent of an organismal process, the processes pertinent to the function of an organism above the cellular level; includes the integrated processes of tissues and organs.	N
	plasma lipoprotein particle remodeling	GO:0034369	The acquisition, loss or modification of a protein or lipid within a plasma lipoprotein particle, including the hydrolysis of triglyceride by hepatic lipase, with the subsequent loss of free fatty acid, and the esterification of cholesterol by phosphatidylcholine-sterol O-acyltransferase (lecithin cholesterol acyltransferase; LCAT).	N
	regulation of intestinal absorption	GO:1904478	Any process that modulates the frequency, rate or extent of intestinal absorption.	N
	extracellular structure organization	GO:0043062	A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts, or disassembly of structures in the space external to the outermost structure of a cell.	N
Cholesterol Transport	cholesterol transport	GO:0030301	The directed movement of cholesterol, cholest-5-en-3-beta-ol, into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore.	N
	organophosphate ester transport	GO:0015748	The directed movement of organophosphate esters into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore. Organophosphate esters are small organic molecules containing phosphate ester bonds.	N

	organic hydroxy compound transport	GO:0015850	The directed movement of an organic hydroxy compound (organic alcohol) into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore.	N
	lipid localization	GO:0010876	Any process in which a lipid is transported to, or maintained in, a specific location.	N
	phospholipid efflux	GO:0033700	The directed movement of a phospholipid out of a cell or organelle.	N
	organic anion transport	GO:0015711	The directed movement of organic anions into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore.	N
Lipoprotein Metabolism	lipoprotein metabolic process	GO:0042157	The chemical reactions and pathways involving any conjugated, water-soluble protein in which the covalently attached nonprotein group consists of a lipid or lipids.	Y
Catabolism	catabolic process	GO:0009056	The chemical reactions and pathways resulting in the breakdown of substances, including the breakdown of carbon compounds with the liberation of energy for use by the cell or organism.	N
Organic Hydroxy Compound Metabolism	organic hydroxy compound metabolic process	GO:1901615	The chemical reactions and pathways involving organic hydroxy compound.	N

Table 2: Biological pathways associated with risk of hypercholesterolemia and atherosclerosis.

Discussion

One of the most studied genetic links to hypercholesteremia and atherosclerosis has been the PCKS9 gene. Studies have led to innovative solutions using PCKS9 inhibitors to lower levels of LDL-C. However, preventative treatments such as this used with individuals at high risk of developing atherosclerosis are not meant to be used as a sole treatment, but a complement to other treatments. Stein et al. (2012) found that treatment of at-risk individuals with statins alone results in a 17% reduction of LDL-C, while treatment of a statin paired with the PCSK9 antibody results in a LDL-C reduction of 72%. While many of the genes and gene mutations associated with high risk of hypercholesteremia and atherosclerosis have no developed preventative

treatments, the research and development of the PCKS9 gene should be used as a model case for the development of treatments associated with these genes and their gene products.

For the LDLR gene, where the receptors are a major mechanism for the removal of LDL-C, future research should focus on restoring the functionality of these receptors. Much like in the PCKS9 gene where the PCKS9 inhibitors complement the use of statins, so to for the LDL-C receptors would treatment focused on restoring the functionality of these receptors be complemented by the use of statins. Looking to the ANKS1A gene, it is unique among the other gene variations as it produces HDLs that remove cholesterol from the blood. While CEPT is used to combat mutations of this gene by increasing the HDL levels, the side effects of increased blood pressure and lowered serum potassium are counterproductive. One solution is pair CEPTs with other medications to address the side effects. Though, adding additional medications may not lead to optimal results. Further treatment options should be researched that would restore functionality to mutated genes or replace the functionality of HDL with an alternative treatment, aside from the use of statins alone.

Analyzing the results of the GO term enrichment analysis, it is clear to see that the primary focus of these genes and identified biological pathways is the levels of LDL-C in those at risk of developing hypercholesteremia and atherosclerosis. The mechanisms identified (i.e. sterol metabolism, regulation of plasma lipoprotein particle levels, etc.) are almost entirely aimed at processing cholesterol in both metabolic and catabolic pathways, and regulating cholesterol levels. This analysis allows for a deeper understanding of the links between the identified at-risk genes and the expression of hypercholesteremia and atherosclerosis, and allows for further

hypotheses of preventative treatment other than those already identified (i.e. statins, PCKS9 inhibitors, CETP). For example, the negative regulation of catalytic activity, which is a process that inhibits enzymes, has been identified as a related biological pathway. Future research could aim as inhibiting the negative regulation of catalytic activity of molecules that reduce LDL-C levels. Or, on the flip side, increase the catalytic activity of molecules that ultimately increase LDL-C levels. However, it is important to understand that inhibiting or not inhibiting an enzyme could have far reaching consequences outside the scope of LDL-C regulation and requires study and research from both biological and medical experts. Furthermore, these results suggest the problem of elevated LDL-C levels can be approached from many fronts. Sterol metabolic processes can be targeted to increase both metabolic and catabolic processes in regards to LDL-C. Lipids, triglycerides, retinoids, glycerolipids, isoprenoids, lipase activity, Cdc42 protein, macromolecular complexes, endocytosis, and phospholipids can all allow new ways for scientists and medical professionals to target different modes of treatment and preventative care to reduce LDL-C levels and prevent hypercholesteremia and atherosclerosis.

In a review by Calpe-Berdiel, Escola-Gil, and Blanco-Vaca (2009), they examined plant sterols (phytosterols) as a means of reducing LDL-C serum levels. Research had previously shown that phytosterols lower LDL-C plasma levels by reducing intestinal cholesterol absorption, while not affecting HDL-C or triglyceride levels (Miettinen et al, 1995; Moghadasian & Frohlich, 1999). ATP-binding cassette transporter A1 (ABCA1) has been proposed as a mechanism of phytosterol induced cholesterol absorption. And others have implicated phytosterols in cholesterol esterfication, lipoprotein assembly, cholesterol synthesis, and apolipoprotein B100 in lipoprotein removal (Calpe-Berdiel, Escola-Gil, and Blanco-Vaca 2009). Preventative care could, in part, be

as simple as eating foods that contain plant sterols, such as wheat, peanuts, almonds, and Brussel sprouts.

Conclusions

The identification of genes and related biological pathways that lead to high risk of hypercholesteremia and atherosclerosis is an open door for researchers to find new methods of treatment against this disease. While statins alone have improved overall patient care in those at-risk or already diagnosed with hypercholesteremia and atherosclerosis, future advances in preventative care and treatment, such as targeting specific metabolic and catabolic processes, could lead to up to 90% prevention of coronary heart disease as McGill, McMahan & Gidding (2008) suggest. Future work should continue to include identifying the gene products and mechanisms at the cellular and molecular level of these identified genes and their associations with hypercholesteremia and atherosclerosis.

References

Barter, P.J., Caulfield, M., Eriksson, M., Grundy, S.M., Kastelein, J.J.P., . . . Revkin, J.H. (2007). Effects of Torcetrapib in Patients at High Risk for Coronary Events. *New England Journal of Medicine, 357, 2109-2122*.

Calpe-Berdiel, L., Escola-Gil, J.C., Blanco-Vaca, F. (2009). New Insights into the Molecular Actions of Plant Sterols and Stanols in Cholesterol Metabolism. *Atherosclerosis, 203, 18-31*.

Cohen J.C., Boerwinkle E., Mosley T.H. Jr., & Hobbs H.H. (2006). Sequence Variations in PCSK9, Low LDL, and Protection Against Coronary Heart Disease. *New England Journal of Medicine, 354(12), 1264–1272*.

Downs, J.R., Clearfield, M., Weis, S., Whitney, E., Shapiro, D.R., . . . Gotto, A.M. Jr. (1998). Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels. *JAMA, 279(20), 1615-1622*.

Feig, J.E. (2014). Regression of Atherosclerosis: Insights from Animal and Clinical Studies. *Annals of Global Health, 50, 13-23*.

Gerloni, R., Mucci, L., Ciarambino, T., Ventura, M., Baglio, V., . . . Gnerre, P. (2017). Management of Stable Coronary Artery Disease: From Evidence to Clinical Practice. *Italian Journal of Medicine, 11, 114-133*.

Lloyd-Jones, D.M., Leip, E.P., Larson, M.G., D'Agostino R.B., Beiser, A., . . . Levy, D. (2006). Prediction of Lifetime Risk for Cardiovascular Disease by Risk Factor Burden at 50 Years of Age. *Circulation, 113(6), 791-798*.

McGill, H.C. Jr., McMahan, C.A., & Gidding, S.S. (2008). Preventing Heart Disease in the 21st Century: implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. *Circulation, 117(9), 1216-1227*.

Miettinen, T.A., Puska, P., Gylling, H., Vanhanen, H., Vartiainen, E. (1995). Reduction of Serum Cholesterol with Sitostanol-ester Margarine in a Mildly Hypercholesterolemic Population. *New England Journal of Medicine, 333, 1308-1312*.

Miettinen, T.A., Tilvis, R.S., Kesaniemi, Y.A. (1989) Serum Cholestanol and Plant Sterol Levels in Relation to Cholesterol Metabolism in Middle-aged Men. *Metabolism, 38, 136-140*.

Miettinen, T.A., Tilvis, R.S., Kesaniemi, Y.A. (1990). Serum Plant Sterols and Cholesterol Precursors Reflect Cholesterol Absorption and Synthesis in Volunteers of a Randomly Selected Male Population. *American Journal of Epidemiology, 131, 20-31*.

Moghadasian, M.H., Frohlich, J.J. (1999) Effects of Dietary Phytosterols on Cholesterol Metabolism and Atherosclerosis: Clinical and Experimental Evidence. *American Journal of Medicine, 107, 588-594*.

Nissinen, M.J., Miettinen, T.E., Gylling, H., Miettinen, T.A. (2010). Applicability of Non-cholesterol Sterols in Predicting Response in Cholesterol Metabolism to Simvastatin and Fluvastatin Treatment Among Hypercholesterolemic Men. *Nutrition, Metabolism & Cardiovascular Diseases, 20, 308-316*.

Roberts, R. (2014). Genetic of Coronary Artery Disease: An Update. *Methodist Debakey Cardiovascular Journal, 10(1), 7-12*.

Seidah, N.G., Benjannet, S., Wickham, L., Marcinkiewicz, J., Jasmin, S. B., . . . Chretien, M. (2003). The Secretory Proprotein Convertase Neural Apoptosis-Regulated Convertase 1

(NARC-1): Liver Regeneration and Neuronal Differentiation. *Proceeding of the National Academy of Sciences of the United States of America, 100(3), 928-933*.

Shepherd, J., Cobbe, S.M., Ford, I., Isles, C.G., Lorimer, A.R., . . . Packard, C.J. (1995). Prevention of Coronary Heart Disease with Pravastatin in Men with Hypercholesterolemia*. New England Journal of Medicine, 333(20), 1301-1308*.

Stamler J., Stamler R., Neaton J.D., Wentworth D., Daviglus M.L., . . . Greenland P. (1999) Low Risk-Factor Profile and Long-Term Cardiovascular and Noncardiovascular Mortality and Life Expectancy: Findings for 5 Large Cohorts of Young Adult and Middle-Aged Men and Women. *JAMA. 282(21), 2012–2018*.

Stein E.A., Mellis S., Yancopoulos G.D., Stahl N., Logan D., . . . Kranz, T. (2012). Effect of a Monoclonal Antibody to PCSK9 on LDL Cholesterol. *New England Journal of Medicine, 366(12), 1108-1118.*

Sun, Y.V., Damrauer, S.M., Hui, Q., Assimes, T.L., Ho, Y., Natarajan, P., . . . Wilson, P.W.F. (2018). Effects of Genetic Variants Associated with Familial Hypercholesterolemia on Low-Density Lipoprotein-Cholesterol Levels and Cardiovascular Outcomes in the Million Veteran Program. *Circulation: Genomic and Precision Medicine, 11(12)*.

Thavendiranathan P., Bagai A., Brookhart M.A., & Choudhry N.K. (2006). Primary Prevention of Cardiovascular Diseases with Statin Therapy: A Meta-analysis of Randomized Controlled Trials. *Archive of Internal Medicine*, 166(21), 2307–2313.