Title:
Linking Common Risk Factors to the Biological Mechanisms Behind Atherosclerosis

Authors:
Meric Odabas

Abstract:

Atherosclerosis is the narrowing and hardening of arteries due to the formation of plaque in the arterial walls that results in blockage of blood flow. A variety of diseases can occur due to atherosclerosis, depending on the specific artery affected, such as peripheral artery disease or coronary artery disease, which is the leading cause of death in the United States for both men and women. Many risk factors for the development of atherosclerosis are known, for example, smoking, exercise, diet, obesity, cholesterol, and aging, amongst other factors, have been found to correlate with atherosclerosis, and further, many of these factors are interrelated. The goal of this study is to understand the interrelations between the risk factors of atherosclerosis and their links to the underlying biological mechanisms that directly enhance the progression of this condition.

Background:

Initiation of the pathogenesis of atherosclerotic lesions occurs typically in regions of the vascular endothelial lining of the inner blood vessel wall that are more prone to lesion formation. Atherosclerosis resistant regions have the transcription factors Kruppel-like factors (KLF) 2 and 4 activated by MEK5/ ERK5/MEF2 signaling, which in turn expresses endothelial nitric oxide synthase (eNOS). Greater eNOS means increased nitric oxide (NO) production, which aids in maintenance of the barrier. [1]

Prone regions suffer from low endothelial shear stress, more common in arterial branch points and areas with inner curvature. Before atherosclerosis develops, these sites show changes in endothelial turnover and the local genetic expression. This results in adaptive intimal thickening, which sets the stage for plaque development. The intimal thickening along with plaque development can affect local flow patterns to further cause stress in the region. [2]

Genes related to inflammation, CXCR4 and ICAM-1, have been found to be differentially regulated as a result of this shear stress. In particular, the gene ICAM-1 is known to be involved in atherosclerosis and showed a significant downregulation, while the gene CXCR4 showed a strong upregulation as a result of shear stress. CXCR4 is a chemokine receptor involved in the migration inhibitory factor (MIF) function that induces lesion progression by inducing transmigration of macrophages and dendritic cells and causing plaque inflammation. [3]

Local shear stress, tissue necrosis factor-alpha (TNF-α), and tissue growth factor-beta (TGF-β) induce production of lectin-like oxidatively modified LDL (oxLDL) receptor-1, which accelerates local uptake of LDL in endothelial cells and macrophages. Further, angiotensin II, which is a mediator of the salt and water balance along with the blood pressure of the body, promotes atherosclerosis. Angiotensin II functions by enhancing NADH/NADPH oxidase activity, which oxidizes LDL in the portion of the blood vessel in which the atherosclerotic lesion has formed.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2716189/

After initiation, the presence and uptake of low-density lipoproteins (LDLs) causes atherosclerosis by accumulating within the arterial intima and subsequently acting as stimulators of immune response. Specifically, lipoproteins containing apolipoprotein B (apoB) have been found to be critical in initiating an inflammatory response. Multiple factors play can play a role in this uptake phase. Stress or injury of an arterial wall results in the infiltration of monocytes into the endothelial space, which results in the internalization of apoB containing lipoproteins by macrophages. Alternatively, CD36 and scavenger receptor class A (SR-A) have been found to be responsible for uptake of LDL by macrophages. [4] One member of the CD36 superfamily, SR-BI, is an HDL surface receptor that selectively uptakes HDL and increases its concentration in the blood plasma, thereby counteracting the proatherosclerotic effects of LDL. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2716189/

The presence of LDL within the macrophages within the arterial walls promotes the formation of "foam cells". This creates an enhanced local oxidative stress on the LDLs, and the oxLDL initiates bioactivities that drive lesion formation. [1] One such instance of this is seen with fibroblast growth factor-1 (FGF-1), which has been shown to be released directly in proportion to the concentration of oxLDL. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2716189/

Further, this accumulation of LDLs induces the expression of adhesion molecules, cytokines growth factors, and chemoattractants from the vascular smooth muscle cells (VSMC) including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), platelet-derived growth factors (PDGF), and monocyte chemoattractant protein-1 (MCP-1). These cause the migration and differentiation of macrophages and dendritic cells, which in turn can have pro inflammatory effects and promote the next steps of atherosclerosis. [1] Further, ET-1, a powerful vasoconstrictor and mitogen of VSMC, is produced in atherosclerotic lesions by endothelial cells, VSMC, and macrophages. Due to the vasoconstrictor activity of ET-1, there is an increase in leukocyte-endothelial and platelet-endothelial interactions, which in turn increases local inflammation. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2716189/

It has also been found that because plaque development is dependent on LDLs, people with elevated levels of LDL and/or certain lipoproteins are more prone to atherosclerosis. Conversely, elevated high-density lipoprotein (HDL) and endogenous apolipoprotein E (apoE) reduce the risk of atherosclerosis as HDL functions to remove excess cholesterol from the intima and deliver it to the liver for excretion in bile, and also inhibits lipoprotein oxidation. [5]

Certain genetic disorders affecting lipoproteins and cholesterol in the blood predispose afflicted persons to be more susceptible to atherosclerosis. One such disorder affecting cholesterol is familial hypercholesterolemia (FH), which is a monogenic autosomal codominant trait in which affected persons have elevated plasma cholesterol bound to LDL. Other disorders linked to higher risk of atherosclerosis are familial hypobetalipoproteinemia (FHBL) and familial ligand-defective apoB-100 (FDB), both of which are the result of mutations in the APOB gene. FHBL is characterized by low blood plasma levels of cholesterol, LDL, and apoB, while FDB is characterized by hypercholesterolemia. [6]

Finally, the last phase of plaque formation is marked by an accumulation of a large number of these inflammatory cells, which are primarily lymphocytes and macrophages, comprising a lipid rich necrotic core. Macrophage inflammation causes chemokine and cytokine secretion, LDL oxidation, monocyte recruitment, and foam cell formation. [1]

Methods:

The background description of the processes by which atherosclerosis develops was obtained in large part through analysis of the literature on atherosclerosis research papers on Google Scholar and ncbi. These genes and gene products described in the background section have been compiled into a list, and converted into their approved symbols through genenames.org as follows:

KLF2
KLF4
eNOS
CXCR4
ICAM1
MIF
TNFA
TGFB
ACE
CD36
MSR1
SCARB1
FGF1
VCAM1
CSF1
PDAP1
CSF2
MCP1
ET1
APOE
APOB

Further, other genes found through several genome wide association studies are also implicated in the development and progression of atherosclerosis. These genes, as their approved symbols, are listed below:

vWF
FADS2
CDKN2B
FAM117B
LPL
SCARA5
ANRIL
PRDX2

GCKR
COL4A2
CLEC4M
MYBPHL
ICA1L
CDC7
CELSR2
PRKAG2
FADS1
PSRC1
PCSK9
APOC1
APOC3
KCNE2 [7]

These two compiled lists of genes from both existing literature and GWAS studies were combined and fed into the GO enrichment analysis tool at geneontology.org. The results were downloaded as a .txt file and imported to excel, converted to a form with just their GO term IDs, and this list was entered into the tool at revigo.irb.hr/. From here, the output was downloaded as a .csv file and once again cleaned in Excel by narrowing down each term to its GO ID, its process description, and its p-value. The table was sorted in ascending order by p-value, where the terms with the highest significance are at the top of the table. Further, the table was shorted from 201 terms to just the 38 terms with the lowest p-values.

The scatterplot was created using all of the 201 term output, without filtering only for the most significant p-valued terms.

Results:

Revigo Output for Atherosclerotic Gene List

Description of Process	GO term ID	P-value
regulation of lipid localization	GO:1905952	3.58E-11
regulation of plasma lipoprotein particle levels	GO:0097006	3.68E-10
plasma lipoprotein particle clearance	GO:0034381	3.77E-10
positive regulation of macrophage derived foam cell differentiation	GO:0010744	2.4E-08
extracellular structure organization	GO:0043062	6.52E-08
regulation of lipid metabolic process	GO:0019216	1.04E-07
regulation of phosphate metabolic process	GO:0019220	1.26E-07
regulation of phosphorus metabolic process	GO:0051174	1.29E-07
positive regulation of molecular function	GO:0044093	1.46E-07

positive regulation of cholesterol storage	GO:0010886	1.51E-07
cholesterol transport	GO:0030301	3.92E-07
positive regulation of metabolic process	GO:0009893	3.98E-07
regulation of molecular function	GO:0065009	4.3E-07
positive regulation of phosphorylation	GO:0042327	4.37E-07
regulation of macrophage derived foam cell differentiation	GO:0010743	5.12E-07
plasma lipoprotein particle remodeling	GO:0034369	5.12E-07
protein-lipid complex remodeling	GO:0034368	5.12E-07
positive regulation of protein metabolic process	GO:0051247	5.91E-07
protein-containing complex remodeling	GO:0034367	6.13E-07
sterol transport	GO:0015918	1.08E-06
positive regulation of phosphorus metabolic process	GO:0010562	1.1E-06
positive regulation of phosphate metabolic process	GO:0045937	1.1E-06
cellular response to chemical stimulus	GO:0070887	1.47E-06
regulation of developmental process	GO:0050793	1.61E-06
regulation of phosphorylation	GO:0042325	1.81E-06
positive regulation of cellular protein metabolic process	GO:0032270	2.01E-06
positive regulation of protein phosphorylation	GO:0001934	2.58E-06
response to organic substance	GO:0010033	2.9E-06
regulation of cholesterol storage	GO:0010885	2.91E-06
positive regulation of lipid localization	GO:1905954	3.1E-06
positive regulation of cellular metabolic process	GO:0031325	3.77E-06
regulation of protein phosphorylation	GO:0001932	3.82E-06
positive regulation of catalytic activity	GO:0043085	3.82E-06
response to lipopolysaccharide	GO:0032496	4.1E-06
lipid localization	GO:0010876	4.62E-06
regulation of kinase activity	GO:0043549	4.62E-06
plasma lipoprotein particle organization	GO:0071827	5.55E-06
regulation of lipid storage	GO:0010883	5.55E-06

Table 1: Filtered Revigo Output Table

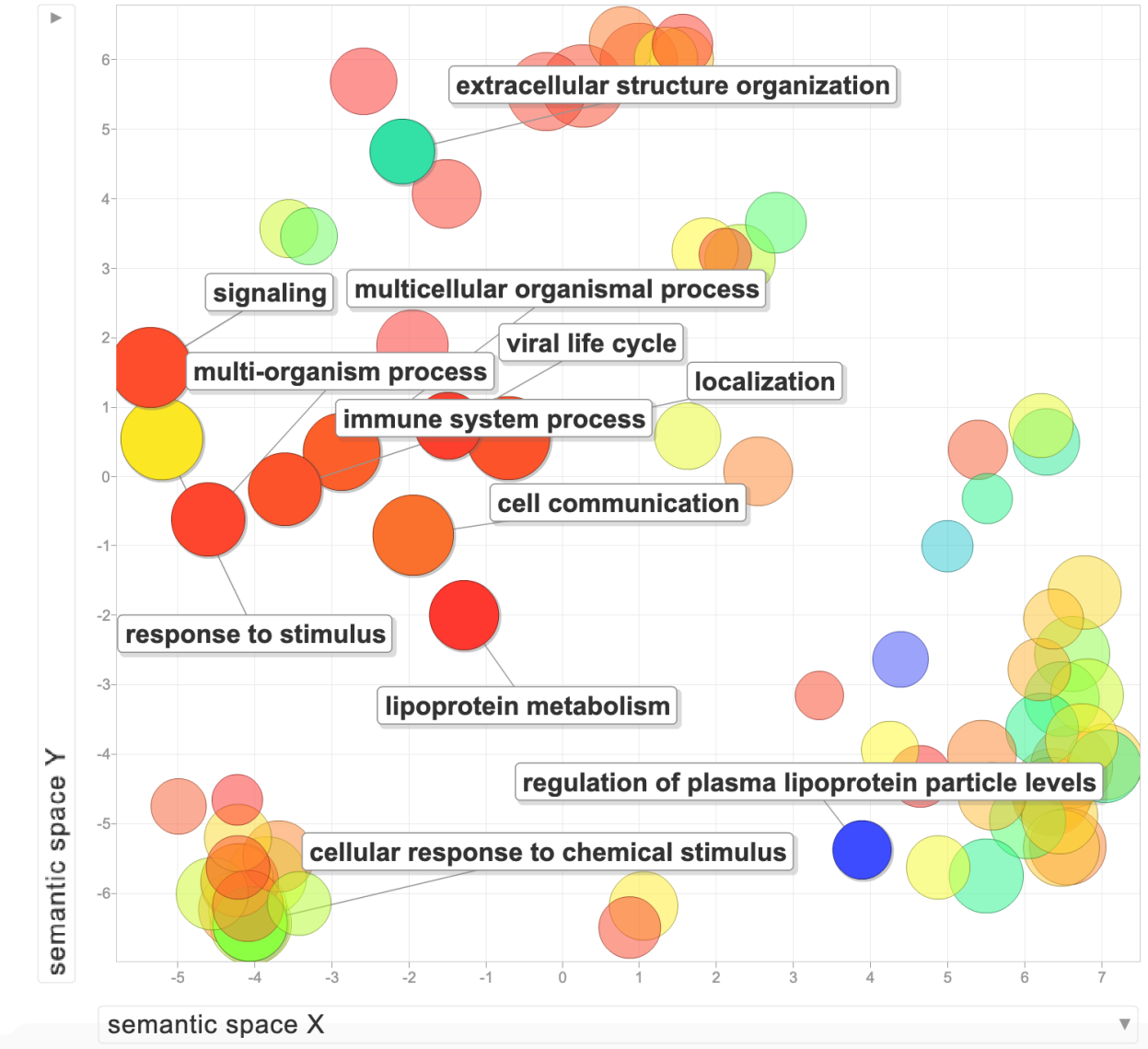

Figure 1: Scatterplot of Revigo Output

Discussion:

The output of Revigo, filtered and sorted in Table 1, allows us to verify much of our background literature of the atherosclerotic process. We see such terms as regulation of “lipid localization”, “plasma lipoprotein particle levels”, “macrophage derived foam cell differentiation”, and "Cholesterol transport”. Other significant terms that were not discussed in the literature that was reviewed in the background section include such things as phosphate/phosphorous metabolic processes, phosphorylation, kinase activity, and protein/lipid complex remodeling. These likely are processes that were found to be related to atherosclerosis from the GWAS genes list.

We can similarly apply this logic to the scatterplot in the scatterplot of Figure 1, which unlike in Table 1, used all of the data, rather than just the terms with the lowest p-values. The scatterplot shows which terms are more pertinent to our gene list. This is because the most significant p-valued terms have the largest nodes, the red-most color, and labels for their corresponding processes. Further, the scatterplot allows us to see groupings of processes by placing similar terms nearby in the semantic space, rather than just observing individual GO terms, as is done in Table 1. Here, we see certain terms that were discussed in the background such as those about lipoproteins, chemical stimuli, and localization. Other terms, such as the viral life cycle, cell communication, and signaling are also significant, but were not discussed in depth.

These undiscussed terms are likely enriched from the GWAS studies and their gene lists, and can point future explorations of atherosclerosis in a more directed manner. To better understand atherosclerosis, specific terms like those found above in the results that are not discussed in existing literature can narrow the study of atherosclerosis.

Conclusions:

Comparing the background analysis of the development of atherosclerosis with the table of associated processes generated by Revigo shows that there may be processes not previously explored or not explored fully. The functions of these processes that were shown to be significant through Revigo but not explored in the literature can be linked back to the underlying biological mechanisms causing atherosclerosis. Studies such as this one can efficiently direct the search for understanding atherosclerosis.

References:

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