GENETIC VARIATION OF OVER EXPRESSED GENES IN THE CAROTID ARTERY PLAQUES AND THEIR INFLUENCE ON CAROTID ARTERY INTIMA MEDIA THICKNESS

Michael Smee
Syventävien opintojen kirjallinen työ
Tampereen yliopisto
Lääketieteen yksikkö
Toukokuu 2013

Tampereen yliopisto
Lääketieteen yksikkö
Kliininen Kemia

Genetic variation of over expressed genes in the carotid artery plaques and their influence on carotid artery intima media thickness

Kirjallinen työ, 18 s.
Ohjaaja: professori Terho Lehtimäki ja FM Nina Mononen

Toukokuu 2013

Avainsanat: Subkliininen ateroskleroosi, SNP, GWA, geneettinen variaatio

Ateroskleroosi on tulehdustauti jota rasva-aineenvaihdunnan häiriö edesauttaa. Sen kardiometaboliset riskitekijät ja molekyylitason mekanismit tulevat ilmi jo lapsuudessa.

Hyvä non-invasiivinen tutkimus ateroskleroosi muutosten monitorointiin on ultraääni. Kasvanut kaulavaltimosuonten pinta- ja keskikerroksen paksuuntuminen on aikainen taudin muutos. Geneettinen profilointi ja genomin laajuiset assosiaatiotutkimukset ovat tuoneet lisävaloa sydän-ja verisuonitauteihin.
Kyseisen tutkimuksen tarkoituksena oli etsiä geneettisen variaation (SNP) rooli geeneissä jotka ovat yliilmentyneet kolminkertaisesti kaulavaltimon ateroskleroottisissa plakeissa ja löytää uusia funktionaalisia geneettisiä muutoksia joilla olisi merkitystä ateroskleroosin ilmetymisessä varhaisessa iässä.

Näytteet ja aineisto saatiin Cardiovascular young finns study ja Tampere vascular study potilasaineistosta. Kaulavaltimosuonten sisä-ja keskikerroksen paksuus (cIMT) mitattiin ultraäänellä ja yhteensä 2442 henkilöä genotyypitettiin. TVS aineistosta ateroskleroosisuonia verrattiin histologisesti normaaleihin suoniin. YFS aineiston 546 677 genotyypitetystä SNP:stä valittiin lisätutkimuksiin 235 geeniä.

20 näistä olivat tilastollisesti merkityksellisiä cIMT kanssa. Geenit TBXAS1 ja CD36 ovat aikaisemmin assosioituneet ateroskleroosin kanssa.
Tutkimuksen tulokset ovat alustavia, mutta näyttävät että 20 itsenäisellä geneettisellä variaatiolla on viitteellistä assosiaatiota ateroskleroosin subkliiniselle kehittymiselle.

Sisällysluettelo

Genetic variation of over expressed genes in the carotid artery plaques and their influence on carotid artery intima media thickness

AtheroRemo Consortium substudy

Michael Smee[1], Nina Mononen[1], Leo-Pekka Lyytikäinen[1], Kari-Matti Mäkelä[1], Terho Lehtimäki[1]

[1]Department of Clinical Chemistry, Fimlab Laboratories and University of Tampere, School of Medicine, Tampere, Finland

Address for correspondence:

Michael Smee

Department of Clinical Chemistry

Fimlab Laboratories PO Box 66

Finn-Medi 2, 3rd floor Biokatu 8

33520 Tampere, Finland

Email: Michael.smee@uta.fi

Abstract

Background: Atherosclerosis is lipid derived immune-inflammatory disease. Cardiometabolic risk factors of atherosclerosis and the molecular mechanisms responding to these risk factors in the artery wall are in place and start their effect soon after birth even cardiometabolic disorders can have roots already in utero. A good noninvasive way to monitor subclinical effects of atherosclerosis is to measure these indices by ultrasound technique. Increased carotid intima-media thickness (cIMT) and involvement of carotid artery plaques are markers of early structural atherosclerosis. Genetic profiling with the aid of genome-wide association studies has found novel variants associating with myocardial infarction and cardiovascular diseases.

Objectives: The present study aimed to investigate the role of genetic variation (single nucleotide polymorphism, SNPs) in the genes over-expressed in carotid artery atherosclerotic lesions in the development of early atherosclerotic changes in the carotid artery wall and to find new functional genetic changes which affect the onset of cardiovascular diseases at early age.

Subjects and methods: The study was based on the Cardiovascular Risk in Young Finns (YFS) and Tampere vascular study (TVS) samples and data. From YFS cIMT was measured by ultrasound. Altogether 2442 YFS participants (1123 males, 1319 females) were genotyped using a Illumina Human 670K BeadChip. TVS study samples (n=9 carotid atherosclerotic cases with histological AHA classification) and six histologically atherosclerotic free controls had transcriptomics data by using Illumina's Sentrix Human-8 Expression BeadChip arrays containing probes for all known human genes (23.000 genes).

Results: From the 546.677 successfully genotyped SNPs in YFS all the SNPs that were located in the 235 over expressed genes (fold change ≥ 3.0 as compared to non-atherosclerotic

control vessels with p-value <0.05) of TVS samples or 5 kb upstream and downstream of the gene were selected for further analyses. These carotid artery expressed candidate genes contained altogether 2711 true-genotyped and 7120 imputed SNPs in YFS study. From this studied SNPs 20 were statistically suggestively associated with cIMT progression in YFS study. Two of the found SNPs located in genes TBXAS1 and CD36 and have been found before to have a significant association with atherosclerosis suggesting true positive results.

Conclusion: The results of the present study are preliminary and showed that 20 individual genetic variants have a suggestive association with progression of subclinical atherosclerosis. However for confirmation of these results further studied are needed to replicate these findings with other independent cohorts.

Keywords: Subclinical atherosclerosis, SNPs, GWA, genetic variation

Introduction

Atherosclerosis, the cause of myocardial infarction, stroke, and ischemic gangrene, is an immune-inflammatory disease (1) having several phases and modified lipid accumulation to artery wall. The development of atherosclerotic lesions is a slow process that involves several immunological factors, like macrophages and T-lymphocytes. During the pathologic process in the artery wall endothelium leukocyte adhesion molecules attract monocytes, which in turn convert into macrophages and clear up the oxidized LDL-particles to form lipid containing foam cells. Monocytes differentiated into foam-cells can eventually die which leads into the accumulation of modified cytotoxic lipids and cholesterol in the intima. In response to local antigens T-lymphocytes secrete pro-inflammatory cytokines and eventually, the artery becomes inflamed. The inflammation stimulates migration, proliferation of smooth-muscle

cells, and formation of fibrous tissue and causes the thickening of the artery wall. Intense inflammation can lead to proteolysis in the plaque and cause rupture and thrombus formation, which can lead up to ischemia, myocardial infarction or even sudden cardiac death. Atherosclerosis is a multifactorial disease. Both biochemical and physiological mechanisms for atherosclerosis are in place soon after birth, and it is convincingly shown that the disease process leading to atherosclerosis is already underway in childhood (2,3).

Increased carotid intima-media thickness (cIMT), as assessed noninvasively by ultrasound, is considered a marker of structural atherosclerosis (4,5). In healthy adults (19-90 years) followed over 4 years, cIMT was shown to be an independent predictor of stroke, MI and death (6). Accelerated progression in IMT is a marker of atherosclerosis development that increases the risk of cardiovascular events (7). A recent meta-analysis revealed that an approximately 0,1 mm change in cIMT corresponds to an increase of 15% in the risk of myocardial infarction (8).

The recent surge in genetic profiling with the aid of genome-wide association studies (GWASs) has found novel variants associating with myocardial infarction and coronary artery disease (9,10,11,12,13,14,15,16). These variants could be biomarkers themselves or point to circulating markers for further exploration (17). At present, only five SNPs associated with coronary artery disease in previous GWASs (rs10757274, rs1333049, rs6922269, rs501120 and rs2943634) have been investigated for possible associations with subclinical atherosclerosis (18, 19, 20, 21, 22). Also, Hernesniemi et al. reported that the genetic profiling of 24 variants previously associated with the risk of CAD on a genome-wide -significant level (SNPs) did not improve risk stratification for subclinical atherosclerosis beyond conventional risk factors among healthy young adults. In the meta-analysis of GWAS data from nine

studies of common cIMT genome-wide significant associations between three regions and common cIMT was identified (23). The strongest association was for rs11781551, found on 8q24 approximately 385 kb from *ZHX2* gene. The second association was for rs445925, located 2.3 kb from *APOC1* on 19q13, a region that also includes *APOE, APOC2,* and *APOC4*. The third association was for rs6601530, located within the *PINX1* gene on 8q23.1. No SNP achieved the significance threshold for follow up in the discovery analysis of internal cIMT (24). There has been a persistent, but weak relationship between sCD36 and IMT after correction of confounders, and it could have a potential to be an important marker for atherosclerosis (25).

In this study we identified genes that were over expressed in atherosclerotic tissue obtained from carotid arteries in the Tampere Vascular study. From the genome wide association study (GWAS) made on the Young Finns Study participants we obtain the genotype data of the SNPs (single nucleotide polymorphisms) located in these over expressed genes for association studies. Our aim was to discover the genetic variation of the genes up-regulated in carotid plaque tissue and analyze their role as possible modifiers of the intima media thickness progression in the arterial wall.

Subjects

Tampere Vascular pilot Study (TVS)

The atherosclerotic vascular sample set consists of femoral arteries, carotid arteries and abdominal aortas from patients participating in the on-going Tampere Vascular Study (26).

The patients had a polyvascular disease (i.e. at least two major arterial beds affected by atherosclerotic plaques as evidenced by 1) previous transient ischemic attack and atherosclerotic plaques in the cerebral vasculature or 2) coronary atherosclerosis as evidenced by previous MI or 3) angina pectoris and atherosclerotic plaques in coronary angiography or 4) objectively verified peripheral arterial disease by ankle-brachial pressure index < 0.9 or 5) previous arterial surgery due to atherosclerosis or 6) angiographical demonstration of arterial plaques). In a histological study of atherosclerotic vessels, the atherosclerotic samples were classified as type V-VI according to the American Heart Association classification (AHA). Control samples were taken from internal thoracic arteries (ITA) from patients obligated to by-pass surgery due to coronary heart disease. The samples from ITA (internal thoracic artery) were removed from the distal end of the artery at the beginning of dissection and were verified histologically to have no atherosclerotic changes.

The Cardiovascular Risk in Young Finns Study (YFS)

The Cardiovascular Risk in Young Finns Study (YFS) is a follow-up study of atherosclerosis precursors in Finnish children and adolescents. The study has been carried out in all five Finnish university cities with medical schools (Helsinki, Kuopio, Oulu, Tampere, Turku) and in their rural surroundings. In the first cross-sectional study in 1980, altogether 4320 children and adolescents aged 3–18 years were randomly chosen from the national population register of these areas to produce a representative sample of Finnish children. Of these subjects, a total of 3596 boys and girls participated in the cross-sectional study in 1980. Since then the study cohort has participated in follow-up studies in 3-6 year intervals. The vascular ultrasound measurements of carotid intima media thickness (cIMT) were performed in 2001 and 2007 as

part of the 21-year and 27-year follow-ups. A total of 1,809 subjects had cIMT data both in 2001 and 2007, and in addition 844 had cIMT measured either in 2001 or 2007 (27).

Methods

Genome wide expression analysis (GWE) of the TVS pilot samples

The vascular samples used in GWE consist of atherosclerotic plaques from the following arterial sites: femoral artery (n=4), carotid artery (n=9) and abdominal aorta (n=7), and six control samples taken from internal thoracic arteries (ITA) during coronary artery by-pass surgery all together a total of 26 patients participating in Tampere Vascular Study (26). Of these patients, only two had polyvascular disease and all the rest had monovascular disease limited to the coronary vasculature. The analysis of the gene expression levels were made by using Illumina's Sentrix Human-8 Expression BeadChip arrays containing probes for all known human genes (23.000 genes). The method has been described in more detail in Levula et al. (26). From the GWE analysis we selected genes that showed at least 3-fold increase (n=235, p-value <0.05) in expression in carotid arteries compared to ITAs for further studies.

Genotyping and imputation of the YFS

Genotyping was done for 2,556 samples using custom build Illumina Human 670k BeadChip at Welcome Trust Sanger Institute. Genotypes were called using Illuminus clustering algorithm. After quality control there were 2,442 samples and 546,677 genotyped SNPs available for further analysis (28). Genotype imputation was performed using MACH 1.0 (29,30) and HapMap II CEU (release 22) samples as reference. Palindromic A/T and C/G

SNPs were removed before imputation. After imputation there were 2,543,887 imputed SNPs available. SNPs with squared correlation between imputed and true genotypes ≥ 0.30 were considered well imputed.

Association studies of the YFS

All the SNPs located in the over expressed (FC≥3.0, p-value <0.05) genes (plus 5 kb upstream and downstream) in plaque tissues obtained from carotid arteries were selected for further analyses. SNPs with MAF (minor allele frequency) ≥ 0.01 and p<0.01 were included in the further analysis. From each LD block we selected one SNP to represent the block by using the SPOT programme (31). As a result we had 73 independently associated SNPs. These SNPs were further analysed by using linear regression model (forward) adjusted for sex, BMI delta and principal components to control population stratification (26) in order to identify SNPs associated with changes in cIMT.

Results

In the GWE analysis of the TVS pilot samples 235 genes were ≥ 3 times up regulated in carotid plaque tissues as compared to healthy vascular tissue in Tampere Vascular study. In further analysis we studied the effect of the genetic variation of 2711 true-genotyped and 7120 imputed SNPs of these genes on changes (progression) of carotid IMT between 2001 and 2007 in the YFS population. The basic characteristics of the studied YFS population are shown in **Table 1**. In the **Table 2** we show the SNPs that had a strongest suggestive evidence

for association with the progression of carotid the intima media thickness over the period of six years from 2001 to 2007 in the Young Finns study.

Discussion

From the top 20 SNPs that we found, only two SNPs have been previously reported to have any significant association with atherosclerosis, rs41728 in TBXAS1 and rs3211883 in CD36 suggesting true positive results. TBXAS1 is a member of the cytochrome P450 superfamily of enzymes this endoplasmic reticulum membrane protein catalyzes the conversion of prostglandin H2 to thromboxane A2, a potent vasoconstrictor and inducer of platelet aggregation. The enzyme plays a role in several pathophysiological processes including hemostasis, cardiovascular disease, and stroke. The protein encoded by the CD36 gene is the fourth major glycoprotein of the platelet surface and serves as a receptor for anti-aggregator molecule, thrombospondin in platelets and various cell lines. Since thrombospondins are widely distributed proteins involved in a variety of adhesive processes, this protein may have important functions as a cell adhesion molecule. In addition variation in gene TBXAS1 has been associated with MI risk (study population, age 30-79 years, predominantly Caucasian population from western Washington State) (33), and CD36 with carotid atherosclerosis (Caucasian, age 30-60 years, from European countries) (25).

A recent meta-analysis showed that an approximately 0,1 mm change in cIMT corresponds to an increase of 15% in the risk of myocardial infarction (34). Carotid IMT is an excellent surrogate phenotype for studying the development of subclinical atherosclerosis, but its result should be interpreted with caution as not all etiological factors are identical in the

pathogenesis of coronary atherosclerosis. The results of the present study showed that individual polymorphisms do not have a substantial impact on cIMT. However, we restricted the analysis only on the genetic variation found on genes that were shown to be overexpressed in the carotid artery plaque tissue. Thus our results do not rule out the possibility that other genetic factors may affect intima media thickness and the changes in it.

Acknowledgements and funding

This study was supported with grants from the Medical Research Fund of Tampere University Hospital (TL), the Emil Aaltonen Foundation (TL), the Pirkanmaa Regional Fund of the Finnish Cultural Foundation, the Research Foundation of Orion Corporation, the Jenny and Antti Wihuri Foundation, and the Academy of Finland (grant no. 104821), the Finnish Foundation for Cardiovascular Research, the Yrjö Jahnsson Foundation and European Union 7th Framework Program, grant number 201668, Athroremo. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Table 1. Characteristics of the Young Finns Study population.

Variables	**(N)**	**Mean±SD**	**Range**
Number of participants (male/female)	1641(920/721)	-	-
Age in 2007, years		31,9	-
Body mass index delta (kg/m^2) *		0,963	
cIMT 2001 (mm)		0,579±0,093	0,967
cIMT 2007 (mm)		0,626±0,978	0,7
Change in cIMT (mm) between 2001 and 2007		0,0465±0,0839	0,76

Note* weight change between years 2001 and 2007, **Abbreviations:** cIMT = carotid intima media thickness

Table 2. Twenty top SNPs that showed the strongest suggestive association with the progression of intima media thickness in the Young Finns Study population over the period of 6 years from 2001 to 2007.

Rs number	A1	Beta	P-value	Imputed*	MAF	Gene abbreviation
rs3211883	T	-0,018913	1,750E-04	1	0,10	CD36
rs7201518	T	-0,0114	2,020E-04	1	0,42	n/a
rs7290147	C	0,010822	3,180E-04	1	0,43	CECR1
rs11670330	A	-0,011271	5,000E-04	1	0,32	C5AR1
rs3827784	A	-0,011195	6,090E-04	1	0,30	LY86
rs17536527	C	-0,010255	9,090E-04	1	0,47	n/a
rs10924510	C	0,020311	1,764E-03	1	0,06	GPR137B
rs6950163	C	-0,014523	1,765E-03	1	0,12	SNX10
rs1372319	G	-0,010461	1,917E-03	0	0,26	LHFPL2
rs7842	G	0,010009	2,133E-03	0	0,30	C3AR1
rs11134606	C	-0,013747	2,286E-03	1	0,16	DOCK2
rs1127203	C	-0,015393	2,860E-03	1	0,09	CA12
rs11880785	A	-0,021439	2,983E-03	1	0,04	n/a
rs1769259	G	0,018001	3,262E-03	0	0,06	FBP1
rs41728	G	0,010595	3,732E-03	0	0,21	TBXAS1
rs9982242	G	0,027777	3,914E-03	1	0,03	ABCG1
rs11785442	A	-0,012789	4,111E-03	1	0,13	n/a
rs12030938	G	-0,014979	4,586E-03	1	0,11	CD53
rs483159	A	0,010579	5,887E-03	1	0,17	n/a
rs34004019	G	-0,008071	7,215E-03	0	0,34	n/a

Gender	0,014725	4,630E-04

* 1 = SNP in question has been imputed. MAF=minor allele frequency.

References

(1) Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N.Engl.J.Med. 2005 Apr 21;352(16):1685-1695.

(2) Enos WF, Holmes RH, Beyer J. Coronary disease among United States soldiers killed in action in Korea: preliminary report. JAMA 1953:152:1090-3.

(3) Pesonen E, Norio R, Sarna S. Thickenings in the coronary arteries in infancy as an indication of genetic factors in coronary heart disease. Circulation 1975:51:218-25.

(4) O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK,Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N.Engl.J.Med. 1999 Jan 7;340(1):14-22.

(5) Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation 2007 Jan 30;115(4):459-467.

(6) Lorenz MW, von Kegler S, Steinmetz H, Markus HS, Sitzer M. Carotid intima-media thickening indicates a higher vascular risk across a wide age range: prospective data from the Carotid Atherosclerosis Progression Study (CAPS). Stroke 2006 Jan;37(1):87-92.

(7) Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, et al. The role of carotid arterial intima-media thickness in predicting clinical coronary events. Ann.Intern.Med. 1998 Feb 15;128(4):262-269.

(8) Simon A, Megnien JL, Chironi G. The value of carotid intima-media thickness for predicting cardiovascular risk. Arterioscler Thromb Vasc Biol 2010 30: 182-185.

(9) Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, et al. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007 447: 661-678.

(10) Erdmann J, Grosshennig A, Braund PS, Konig IR, Hengstenberg C, et al. New susceptibility locus for coronary artery disease on chromosome 3q22.3. Nat Genet 2009 41: 280-282.

(11) Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science 2007 316: 1491-1493.

(12) Kathiresan S, Voigt BF, Purcell S, Musunuru K, Ardissino D, et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet 2009 41: 334-341.

(13) Larson MG, Atwood LD, Benjamin EJ, Cupples LA, D'Agostino RB, Sr, et al. Framingham Heart Study 100 K project: genome-wide associations for cardiovascular disease outcomes. BMC Med Genet 2007 8 Suppl 1: S5

(14) McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, et al. A common allele on chromosome 9 associated with coronary hert disease. Science 2007 316: 1488-1491.

(15) Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, et al. Genomewide association analysis of coronary artery disease. N Eng J Med 2007 357: 443-453.

(16) Tregouet DA, Konig IR, Erdmann J, Munteanu A, Braund PS, et al. Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery dissease. Nat Genet 2009 41: 283-285.

(17) Gerszten RE, Wang TJ. The search for new cardiovascular biomarkers. Nature 2008 451: 949-952.

(18) Bjorck HM, Lanne T, Alehagen U, Persson K, Rundkvist L, et al. Association of genetic variation on chromosome 9p21.3 and aterial stiffness. J Intern Med 2009 265: 373-381.

(19) Cunnington MS, Mayosi BM, Hall DH, Avery PJ, Farrall M, et al. Novel genetic variants linked to coronary artery disease by genome-wide association are not associated with carotid artery intima-media thickness or intermediate risk phenotypes. Atherosclerosis 2009 203: 41-44.

(20) Kiechl S, Laxton RC, Xiao Q, Hernesniemi JA, Raitakari OT, et al. Coronary artery disease-related genetic variant on chromosome 10q11 is associated with carotid intima-media thickness and atherosclerosis. Arterioscler Thromb Vasc Biol 2010 30: 2678-2683.

(21) Samani NJ, Raitakari OT, Sipilä K, Tobin MD, Schunkert H, et al. Coronary artery disease-associated locus on chromoseome 9p21 and early markers of atherosclerosis. Arterioscler Thromb Vasc Biol 2008 28: 1679-1683.

(22) Ye S, Willeit J, Kronenberg F, Xu Q, Kiechl S. Association of genetic variation on chromosome 9p21 with suscebility and progression of atherosclerosis: a population-based, prospective study. J Am Coll Cardiol 2008 52: 378-384.

(23) Hernesniemi JA, Seppälä I, Lyytikainen L-P, Mononen N, et al. Genetic profiling using genome-wide significant coronary artery disease risk variants does not improve the prediction of subclinical atherosclerosis: The cardiovascular risk in young finns study, the bogalusa heart study and the health 2000 survey – a meta-analysis of three independent studies. PLoS ONE 2012 Jan: 7(1): e28931.

(24) Bis JC, Kavousi M, Franceschini N, Isaacs A, Abecasis GR, Schminke U, et al. Meta-analysis of genome-wide association studies from the CHARGE consortium identifie common variants associated with carotid intima media thickness and plaque. Nat Genet 2012 Apr 43: (10): 940-947.

(25) Handberg A, Hojlund K, Gastaldelli A, Flyvberg A, Dekker JM, Petrie J, et al. Plasma sCD36 is associated with markers of atherosclerosis, insulin resistance and fatty liver in a nondiabetic healthy population. J Intern Med 2011 271:294-304.

(26) Levula M, Oksala N, Airla N, Zeitlin R, Salenius JP, Järvinen O, et al. Systemic and arterial bed dependent atherosclerosis – Tampere vascular study. PLos One 2012:7(4):e33787.

(27) Juonala M, Jarvisalo MJ, Maki-Torkko N, Kahonen M, Viikari JS, Raitakari OT. Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: the Cardiovascular Risk in Young Finns Study. Circulation 2005 Sep 6;112(10):1486-1493.

(28) Smith EN, Chen W, Kähönen M, Kettunen J, Lehtimäki T, et al. (2010) Longitudinal genome-wide association of cardiovascular disease risk factors in the bogalusa heart study PLoS Genet 6(9): e1001094.

(29) Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. (2010) MaCH: Using sequence and genotype data to estimate haplotypes and unobserved genotypes Genet Epidemiol 34(8): 816-834.

(30) Li Y, Willer C, Sanna S, Abecasis G. (2009) Genotype imputation Annu Rev Genomics Hum Genet 10: 387-406.

(31) Saccone SF, Bolze R, Thomas P, Quan J, Mehta G, Deelman E, Tichfield JA, Rice JP. SPOT: a web-based tool for using biological databases to prioritize SNPs after a genome-wide association study. Nucleic Acids Res. 2010 Jul:38 (web server issue): w201-9.

(32) Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet.* 2006; 38: 904-909.

(33) Lemaitre RN, Rice K, Marciante K, Bis JC, Lumley TS, Wiggins KL, et al. Variation in eicosanoid genes, non-fatal myocardial infarction and ischemic stroke. Atherosclerosis 2009 204: e58-e63

(34) Simon A, Megnien JL, Chironi G. The value of carotid intima-media thickness for predicting cardiovascular risk. Arterioscler Thromb Vasc Biol 2010 30:182-185