Genetic associations with carotid intima-media thickness link to atherosclerosis with sex-specific effects in sub-Saharan Africans

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Supplementary Note 1

1. F-box and leucine rich repeat protein 17 (*FBXL17*)

Our study identified rs552690895 (p = 2.5E-08) in *FBXL17* in the combined set. F-box and leucine rich repeat protein 17 (*FBXL17*) is characterized by an approximately 40-amino acid F-box motif. SCF complexes are formed by *SKP1* (S-phase kinase-associated protein 1), cullin (*CUL1*), and F-box proteins, and act as protein-ubiquitin ligases. F-box proteins interact with SKP1 through the F-box, and they interact with ubiquitination targets through other protein interaction domains (1). Evidence suggest that the SCF is a key complex in the ubiquitine-proteasome system (UPS) that is involved in 70.0-90.0% of protein degradation processes (2). It has been found that protein degradation by the UPS play a central role in cardiovascular physiology and disease: from endothelial function, the cell cycle, atherosclerosis, myocardial ischaemia, cardiac hypertrophy, inherited cardiomyopathies, and heart failure (3–7). A GWAS in Lithuanian families found that variants in *FBXL17* were associated with coronary heart diseases (8). Other reports on genetic associations for *FBXL17* include studies for educational attainment and mathematical ability (9), intelligence (10), and pulse pressure (11).

2. Signal regulatory protein alpha (*SIRPA*)

Our combined analysis identified also genetic association of rs6045318 (p = 4.7E-08) with cIMT in the *SIRPA* gene. Signal regulatory protein alpha (*SIRPA*) is a regulatory membrane glycoprotein from the SIRP family, which inhibits the cytoskeleton-intensive process of phagocytosis by the macrophage. *SIRPA* is activate cancer cells (through high expression of CD47) and upregulated *SIRPA* inhibits macrophage-mediated destruction. This mediation of phagocytosis and polarization of macrophages is important in the pathophysiology of atherosclerosis (12). There is evidence that *SIRPA* is involved in discrete stages of cardiovascular cell lineage differentiation (13) and that defects in the gene (knock out) reduces atherosclerosis in mice (14). *SIRPA* expression has been found as a signature of inflamed atherosclerotic plaque (15). Previous reports on GWAS studies associate *SIRPA* with blood protein (16,17), and the percentage of basophil in granulocytes (18).

3. Sorting nexin 29 (*SNX29*)

On the chromosome 16, rs147978408 (p = 6.3E-09) was the top cIMT associated variant in *SNX29* for the male-specific analysis. The sorting nexin (*SNX*) family is a diverse group of cytoplasmic- and membrane-associated phosphoinositide-binding proteins that play pivotal roles in the regulation of protein trafficking. *SNX* gene variants are associated with CVDs, and dysfunction of the *SNX* pathway is involved in several forms of cardiovascular disease (CVD) (19). In a study of genes that regulate smooth muscle cell differentiation and disease risk, *SNX29* was involved in pathways for occlusion of blood vessels and atherosclerosis (20). Ito and collaborators identified sex-dependent differentially methylated regions close to *SNX29* in mouse liver and found that this methylation status was influenced by testosterone and contributed to sex-dimorphic chromatin decondensation (21). This might explain the sex-specific effect observed in our study. A study in children with sickle cell disease, identified *SNX29* variants as suggestive of association with systolic blood pressure (22). Also, variants in *SNX29* were found in suggestive association with subcutaneous adipose tissue in women (Sung *et al*. 2016). In patients with pulmonary arterial hypertension, *SNX29* variation was reported for differential responses to vasodilator treatment (24). Further GWAS analysis stratified by hypertensive status showed that the association was driven by the hypertensive group (effect three times higher in hypertensives compared to the non-hypertensives), therefore demonstrating that the association of *SNX29* with cIMT might be mediated by the vascular remodeling caused by hypertension. GWA studies reported *SNX29* variants for association with educational attainment, mathematical ability and cognitive function measurement (Lee *et al*. 2018), intelligence (10,25), bone mineral density (26), and smoking (Liu *et al*. 2019).

4. Mitogen-activated protein kinase kinase kinase 7 (*MAP3K7*)

In the male-specific analysis, we found rs284509 (p=5.3E-08) in *MAP3K7* region on chromosome 6 to be associated with cIMT. Mitogen-activated protein kinase kinase kinase 7 (*MAP3K7*) also called *TAK1* encodes a serine/threonine protein kinase family member, with a central role as regulator of cell death. Because of its role in kinase pathway, and regulation of transforming growth factor beta (TGF-β), *MAP3K7* plays a role in growth inhibition in vascular smooth muscle cells and can be atheroprotective or atherogenic (28). More biological evidence of the contribution of *MAP3K7* to atherosclerosis is through its regulation by micro-RNAs (29,30). In a study of women receiving hormone replacement therapy, variants in *MAP4K4,* a gene targeting *MAP3K7* (31) were associated to cIMT (32). The sex-specific association observed might be related to the fact that *MAP3K7IP3* (located on the X chromosome), which is known to form a ternary complex with *MAP3K7* in response to inflammatory stimuli, has shown sex-differential expression in ischemic stroke (33,34). In a study on expression of androgen-modulated micro-RNAs, it has been reported that *MAP3K7* was a target of mmu-miR-467h and mmu-miR-669i in the angiogenesis and transforming growth factor beta receptor signalling pathways (35). Despite biological relevance to atherosclerosis, GWA studies reported variants in gene region to be associated with cancer progression (36), anti-TNF response in rheumatoid arthritis (37), attention deficit hyperactivity disorder (38), adolescent idiopathic scoliosis (Liu *et al*. 2018), and sporadic amyotrophic lateral sclerosis (Xie *et al*. 2014). Our study is the first to report *MAP3K7* association with a CVD phenotype.

5. La-related protein 6 (*LARP6*)

LARP6 (La-related protein 6) is a ribonucleoprotein domain family member 6. Studies showed that it has a role in collagen regulation by targeting mRNA encoding Type I collagen (Cai *et al*. 2010; Zhang and Stefanovic 2016; Glenn, Wang, and Schwartz 2009; Stefanovic *et al*. 2019). Sukhanov and collaborators found that *IGF1R* deficiency downregulated collagen mRNA-binding protein *LARP6* and vascular collagen, and showed an atheroprotective effect (45,46). Collagen is a hallmark of atherosclerotic plaque stability, thus alteration of the collagen balance may lead to an instability of atherosclerotic lesions, and therefore promote plaque formation and rupture (15,47). In the Taiwanese population, the *LARP6* locus was found to be associated with coronary artery disease (48). In European ancestry populations, *LARP6* was found associated with insulin measurement (49). However, Mendelian randomization for cIMT found that despite the limited effects of proinsulin-increasing SNP scores on cIMT, proinsulin was unlikely to have causal effects on cIMT (50). Myocardial gene expression in non-ischemic human heart failure found *LARP6* to be differentially expressed between men and women (1.36 fold) (51). The female-specific effect of the loci may find its explanation in the enhancer function of rs78172571 in high LD with rs150840489 (the top SNP associated in our female-specific) on *THAP10* gene (FDR = 2.03E-17) known to be regulated by oestrogen.

6. Prokineticin 1 (*PROK1*)

Prokineticin 1 (*PROK1*), also called endocrine gland derived vascular endothelial growth factor (*EG-VEGF*), is a specific placental angiogenic factor which play a role in the control of normal (e.g endometrial decidualization) and pathological placental angiogenesis (52). It is involved in pathologies such as recurrent pregnancy loss, gestational trophoblastic diseases, foetal growth restriction, and preeclampsia (53–57). The gene is known to be predominantly expressed in the steroidogenic glands, such as ovary, testis, and adrenal cortex, and is often complementary to the expression of vascular endothelial growth factor (*VEGF*), suggesting that these molecules function in a coordinated manner. The function and particular pattern of this gene's activity might explain why we identified the locus only in our female-specific analysis. Our study is the first to report *PROK1* for any trait in a GWAS.

7. Caldesmon 1 (*CALD1*)

Our gene-based analysis identified caldesmon 1 significantly associated with cIMT in our combined set led by rs7781307 (p = 2.1E-06) on 7q33. Caldesmon 1 is calmodulin binding protein encoding for a calmodulin-and actin-binding protein that play a major role in the regulation of smooth muscle contraction, cell migration and cell invasion (58). *CALD1* was identified as key gene in the "regulation of actin cytoskeleton" module from protein-protein interaction network resulting from a bioinformatics analysis of key pathways and genes in advanced coronary atherosclerosis (59). A study screening for keys genes for abdominal aortic aneurysm found that *CALD1* was leading a KEGG enrichment signal pathways (Vascular smooth muscle contraction) of differential expressed genes (DEGs) (60). Underexpression of *CALD1* was found to be a key feature of calcification of vascular smooth muscle cells from atherosclerotic plaque (15,61,62). Additionally, studies on epigenetic modifications reported *CALD1* to exhibit differential methylation in atherosclerosis (63–65). Previous GWAS reported CALD1 for phenotypes such as Lung function (FEV1/FVC) (66), Response to paliperidone in schizophrenia (PANSS score) (Li *et al*. 2017), Attention deficit hyperactivity disorder symptom score (68), Diverticular disease (69)

8. Fms-related tyrosine kinase (*FLT4*)

FLT4 or Vascular endothelial growth factor receptor 3 (*VEGFR3*) is a major signalling protein involved in angiogenesis, vasculogenesis and maintenance of the endothelium. By acting as receptor to VEGFC and VEGFD, it plays an essential role in lymphangiogenesis in adults and in the development cardiovascular system during embryonic phase. Defect and/or downregulation of *VEGR3* was found to lead to cardiovascular failure in embryonic stage and to higher mortality after myocardial infarction in mice models (70,71). Biological studies have highlighted the role of *FLT4* in atherosclerosis in major pathological processes. The gene has been reported to be involved in plaque instability by two process: the mediation of monocytes/macrophages apoptosis and consequently alteration plaque stability (72) ; and the modulation of vascular remodelling and shear stress resulting in plaques haemorrhages and calcification in carotids (73–75). Our study is the first to report association of *FLT4* locus (rs112967731, p = 5.7E-07, female-specific) with cIMT or any cardiovascular phenotype in GWAS studies. Previous studies reported the locus for association with folic acid measurement (76) and blood protein measurements (16,17).

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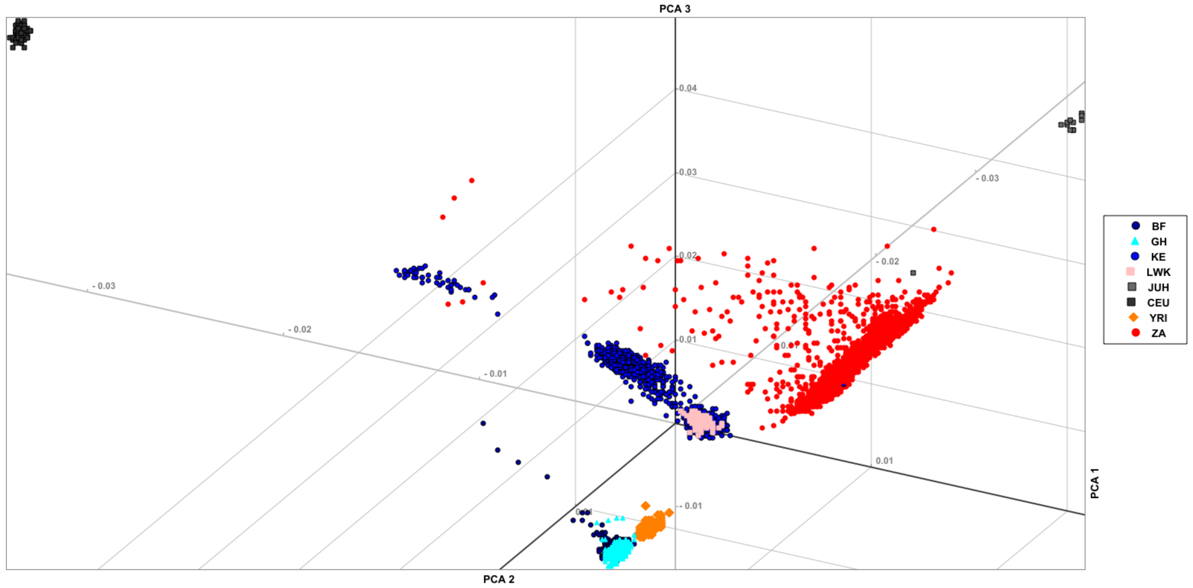
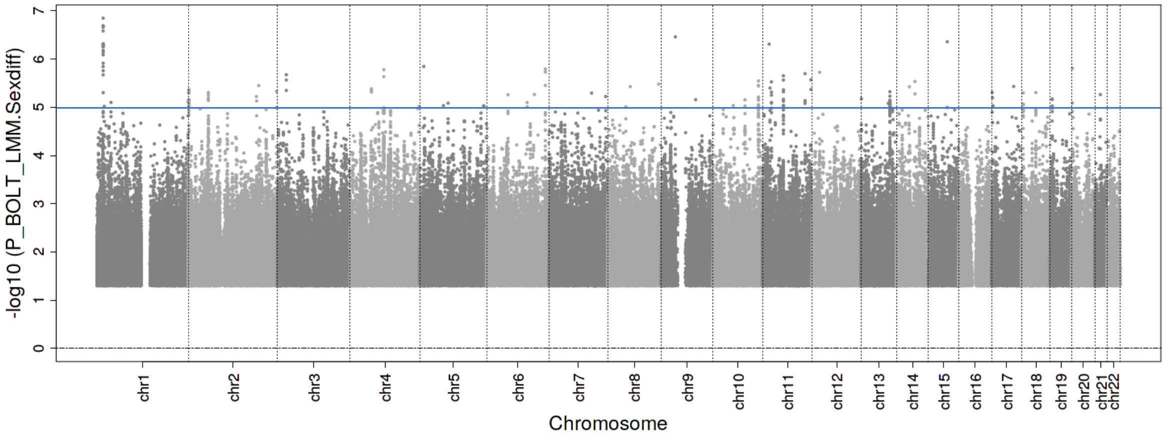


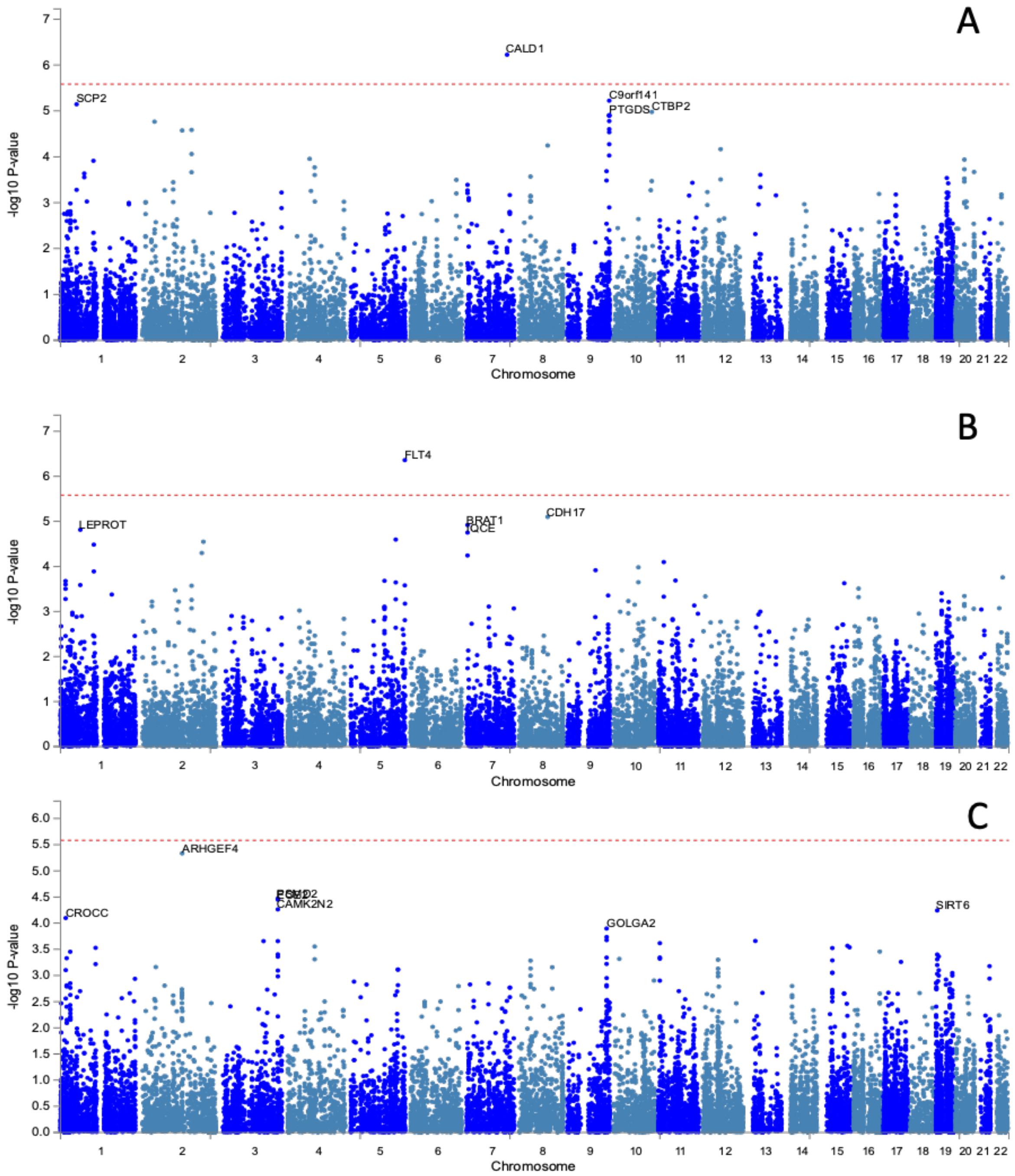
Supplementary Figure 1: Principal Component Analysis (PCA) plots showing population sub-structure in the AWI-Gen data and compared to data from selected populations from the 1000GP.

Each dot represents a participant and 1,729,661 SNPs were used in the analysis. AWI-Gen data - BF: Burkina Faso; GH: Ghana; KE: Kenya; ZA: South Africa.



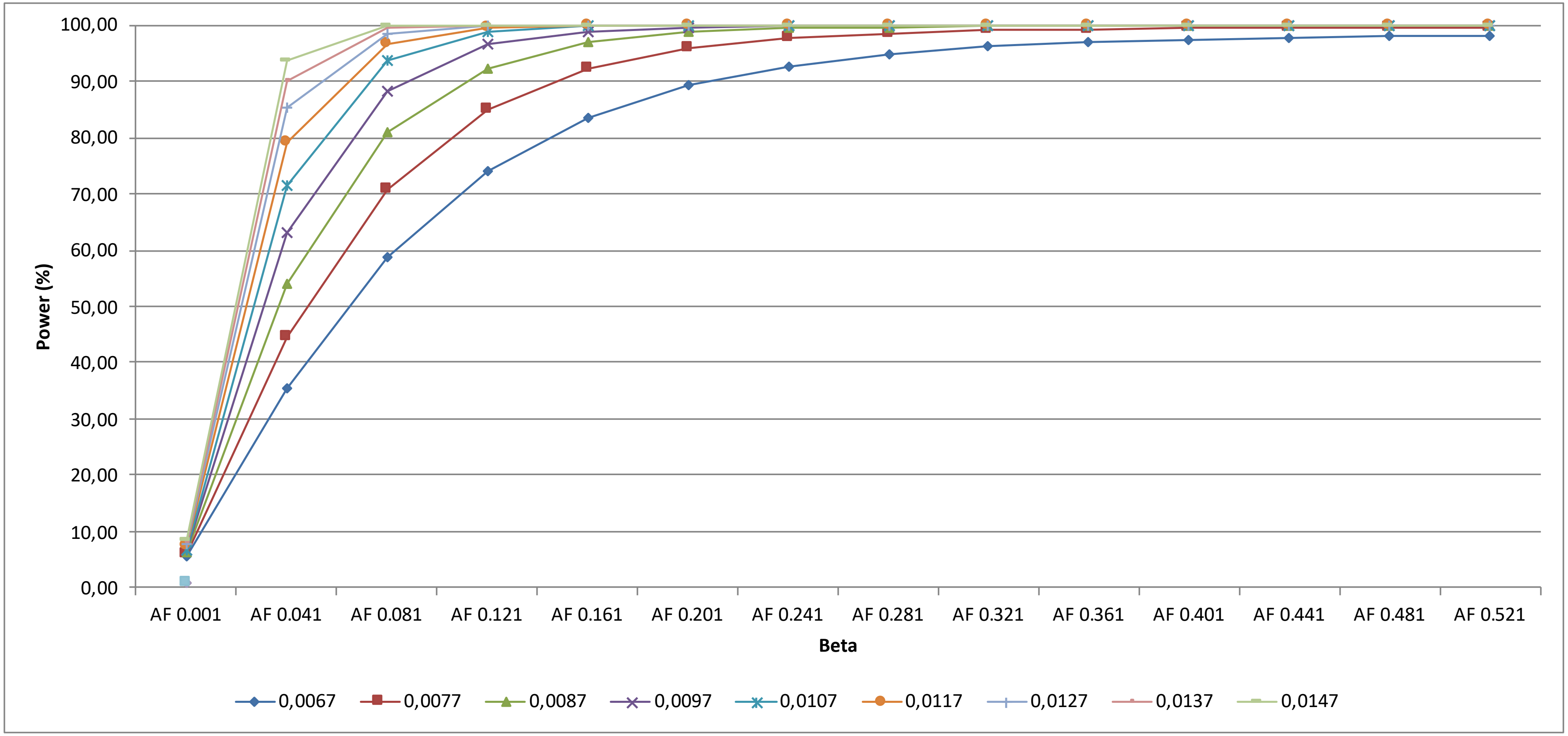
Supplementary Figure 2: Manhattan plot showing the –log10-transformed two-tailed P-value of each SNP for test of difference between female and male associations for each SNP.

The blue line indicates the threshold for suggestive association ($p < 1\times10^{-5}$). Each SNP from the GWAS for Mean Max cIMT on the Y axis and base-pair positions along the chromosomes on the X axis. The blue line indicates Bonferroni-corrected genome-wide significance (p < 1E-08); the blue line indicates the threshold for suggestive association (p < 1E-05).



Supplementary Figure 3: Manhattan plots for the gene-based test as computed by MAGMA based on our summary statistics.

Input SNPs were mapped to 19152 protein coding genes. Genome wide significance (red dashed line in the plot) was defined at P = 0.05/19152 = 2.611e-6. (A) combined dataset. (B) Female-specific. (C) Male-specific



Supplementary Figure 4: Estimation of power for the GWAS of cIMT (as a quantitative trait) considering genetic effect β ranging from 0.0067 mm to 0.156 mm.