SUPPLEMENTAL MATERIAL

Supplemental Methods

Clinical Assessment

Participants underwent a standardized medical examination and laboratory assessment of cardiovascular risk factors. Systolic and diastolic blood pressures were the average of two physician-measured readings. Body mass index was calculated as weight divided by height squared (kg/m^2). Blood was drawn for glucose, total and high-density lipoprotein (HDL) cholesterol, and triglycerides after an overnight fast. Use of lipid-lowering, antihypertensive, and hormone replacement therapies as well as cigarette smoking (regular smoker within the past year) were self-reported. Diabetes was defined as having a fasting glucose ≥126 mg/dL or taking medications to treat diabetes.

Myocardial Infarction

Original Cohort and Offspring participants were followed from the eleventh Original Cohort and first Offspring examination cycle in 1971 through 2007, a period during which data regarding incident cardiovascular events were collected. A three-investigator panel (which was unaware of CFU assay results) used standardized definitions to adjudicate all cardiovascular events occurring during this follow-up period, after a review of clinical data from the Framingham clinic, physician records, and hospital records. Adjudication of recognized MI events was based on diagnostic criteria that have been described previously,[1, 2] including a suggestive clinical

history accompanied by serial electrocardiographic changes leading to development of new pathologic Q waves and/or characteristic rise and fall of serum biomarkers of myocardial injury.

Assessment of Early Outgrowth CFUs

All blood specimens for the CFU assay were collected from fasting participants in the morning between 8 and 9 A.M. Each blood sample underwent initial centrifugation and the resulting buffy coat was further processed for CFU characterization within 4 hours of specimen collection as previous described,[3] with modifications. Specifically, buffy coat samples were diluted to 10.5 mLs with PBS (Invitrogen) and layered over 5 mLs of Ficoll (Amersham Pharmacia Biotech). Samples were centrifuged at 2200 rpm for 15 minutes at 10 degrees Celsius. Mononuclear cells were collected, washed with PBS, and then lysed with ACK lysis buffer (Fisher Scientific). Following washing with PBS, 5 million viable mononuclear cells were plated in each well of a 6 well fibronectin coated tissue culture plate (BD Biosciences) in M199/20% FBS and cultured at 37 degrees Celsius/5% CO2. After 2 days, non-adherent cells were collected and 2 million viable cells in M199/20% FBS were replated in wells of a 24-well fibronectin coated tissue culture plate (BD Biosciences). Cells were cultured for 5 additional days and then the number of colonies in each well was counted by a single, blinded technician and reported as the average number of colonies per well across up to 12 wells. In wells where the number of colonies was too numerous to count (mean of 4.7 wells from 63 individuals), the number of colonies per well was censored at 300. Initial plating of cells and counting of colonies was performed by a single investigator for all samples (RPM) except for a small subset (KSC). The re-plating step was performed by one of two investigators (RPM, RJK). Colony counts were standardized by the identity of the replater, to minimize the effects of operator variation.

Genotyping

Genotyping was performed in FHS by Affymetrix (Santa Clara, CA), using the Affymetrix 500K GeneChip array and a custom-designed gene-centric 50K molecular inversion probe. Affymetrix 500K genotypes were called using the BRLMM algorithm.[4] Individuals were excluded if they had call rates ≤97%, per subject heterozygosity ±5 SD away from the mean, or excess Mendelian errors, resulting in 8,481 individuals with genotyping (**Supplemental Figure 2**). From a total of 534,982 genotyped autosomal SNPs in FHS (Affymetrix 500K and MIPS 50K combined), we used 378,163 SNPs in imputation of Phase II HapMap CEU SNPs after filtering out 15,586 SNPs (Hardy-Weinberg p<1e-6), 64,511 SNPs (call rate < 97%), 45,361 SNPs (mishap p<1e-9), 4,857 SNPs (>100 Mendel errors), 67,269 SNPs (frequency<0.01), 2 SNPs (due to strandedness issues upon merging data with HapMap), and a further 13,394 SNPs not present on HapMap). In order to maximize coverage of the genome, imputation of ~2.5 million autosomal SNPs in HapMap was conducted using the algorithm implemented in MACH 1.0.15 (HapMap CEU release 22, build 36).[5] We used 200 biologically unrelated individuals with high quality genotype data (missingness <0.011 and low Mendelian errors) to infer model parameters first, and then subsequently applied the model to all genotyped 8,481 individuals.

References

1. Cupples LA, D'Agostino RB, Sr., Kiely D. Survival Following Cardiovascular Events, 30-year follow-up. In: Kannel WB, Wolf PA, Garrison RJ, eds. *The Framingham Heart Study, Section 35: an Epidemiologic Investigation of Cardiovascular Disease*. Bethesda, MD: National Heart, Lung, and Blood Institute; 1988.
2. Lloyd-Jones DM, Larson MG, Beiser A, Levy D. Lifetime risk of developing coronary heart disease. *Lancet.* 1999;353(9147):89-92.
3. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med.* 2003;348(7):593-600.
4. Rabbee N, Speed TP. A genotype calling algorithm for affymetrix SNP arrays. *Bioinformatics.* 2006;22(1):7-12.
5. Li Y, Abecasis GR. Mach 1.0: Rapid haplotype reconstruction and missing genotype inferenc. *Am J Hum Genet.* 2006;S79:2290.

Supplemental Table 1. Detailed Sample Characteristics Including Non-CFU Sample, and Participants With and Without CVD

Characteristic	Participants with CFU Measured			Non-CFU Sample N=343
	Total Sample N=1,799	CVD Sample N=187	Non-CVD Sample N=1,612	
Age, y	66 ± 9	72 ± 9	66 ± 9	66 ± 9
Female, %	54	36	56	56
Systolic blood pressure, mm Hg	129 ± 17	130 ± 20	128 ± 17	127 ± 17
Diastolic blood pressure, mm Hg	74 ± 10	69 ± 10	74 ± 10	73 ± 10
Body mass index, kg/m^2	28 ± 5	29 ± 5	28 ± 5	28 ± 5
Fasting glucose, mg/dL	106 ± 23	113 ± 28	106 ± 22	108 ± 26
Total cholesterol, mg/dL	186 ± 37	160 ± 38	189 ± 36	186 ± 38
LDL cholesterol, mg/dL	105 ± 31	85 ± 30	108 ± 31	105 ± 31
HDL cholesterol, mg/dL	57 ± 18	50 ± 14	58 ± 18	58 ± 19
Triglycerides, mg/dL	119 ± 69	130 ± 78	117 ± 68	116 ± 74
Hypertension, %	63	90	60	59
Diabetes mellitus, %	13	28	11	16
Cigarette smoking, %	7	9	7	9
Medications, %				
ACE inhibitors	24	45	22	24
Angiotensin receptor blockers	8	15	7	7
Beta-blockers	28	71	23	25
Calcium channel blockers	14	34	12	14
Diuretics	23	39	21	20
Hormone replacement	4	1	5	5
Statins	41	72	37	39
Framingham risk score	9 ± 4	12 ± 3	9 ± 4	8 ± 4
Prevalent cardiovascular disease, %	10	—	—	12

CVD, cardiovascular disease. Values shown are means ± standard deviation or percents.

Supplemental Table 2. Clinical Correlates of CFU in Analyses Adjusting for Relatedness in the Study Sample

Covariate	Age- and Sex-Adjusted		Multivariable-Adjusted	
	Regression Coefficient (standard error)	P value	Regression Coefficient (standard error)	P value
Age	-0.076 (0.027)	0.005	-0.082 (0.026)	0.001
Female Sex	-0.087 (0.051)	0.09	-0.081 (0.051)	0.11
Body mass index	-0.005 (0.025)	0.83	—	
Height	-0.043 (0.040)	0.28	—	
Weight	-0.020 (0.027)	0.47	—	
Systolic blood pressure	-0.009 (0.025)	0.73	—	
Diastolic blood pressure	0.008 (0.028)	0.79	—	
Fasting glucose	-0.024 (0.021)	0.25	—	
Total cholesterol	-0.008 (0.024)	0.74	—	
LDL cholesterol	0.001 (0.024)	0.98	—	
HDL cholesterol	0.020 (0.027)	0.47	—	
Log triglycerides	-0.051 (0.026)	0.05	-0.055 (0.026)	0.03
Hypertension	-0.008 (0.053)	0.89	—	
Diabetes mellitus	-0.090 (0.071)	0.21	—	
Cigarette smoking	-0.171 (0.093)	0.07	—	
Medications				
ACE inhibitors	-0.010 (0.062)	0.87	—	
ARBs	-0.078 (0.098)	0.43	—	
Beta-blockers	-0.026 (0.063)	0.68	—	
Calcium channel blockers	0.008 (0.079)	0.92	—	
Diuretics	0.010 (0.064)	0.88	—	
Hormone replacement	0.274 (0.142)	0.05	0.280 (0.141)	0.05
Statins	0.098 (0.054)	0.07	0.108 (0.054)	0.04

Data are from the subset of individuals without known cardiovascular disease but with available pedigree data for analyses accounting for inter-individual relatedness (N=1,507). The colony forming unit variable is square-root transformed. Coefficients (standard error) represent change in the dependent variable for an increase in the value of the covariates shown (by 1 standard deviation for continuous variables). In age- and sex- adjusted models, the association with age is adjusted for sex and the association with sex is adjusted for age.

Supplemental Table 3. Additional SNPs with Moderate Associations with CFUs After Adjustment for Age and Sex

Chr	SNP	Position (bp)	Location relative to gene	MAF	A1-A2	Allele for higher CFU	Observed to expected variance ratio	Beta (SE)	P value	Gene(s) at or near locus
1q41	rs17008835	219046052	Intron	0.16	C-T	T	1.000	0.22 (0.05)	1.86×10^{-6}	*MOSC1*
	rs7530493	219056323	Downstream	0.16	G-T	T	0.990	0.21 (0.05)	4.47×10^{-6}	
2p16.1	rs6712720	60135112	Intergenic	0.49	G-C	C	0.968	0.16 (0.04)	4.44×10^{-6}	*BCL11A*
6q26-q27	rs1510229	160725659	Intron	0.30	C-T	T	0.979	0.18 (0.04)	2.27×10^{-6}	*SLC22A, LPAL2, LPA*
	rs420038	160728138	Intron	0.30	C-T	T	0.997	0.18 (0.04)	2.41×10^{-6}	
	rs402219	160709286	Intron	0.30	A-G	G	0.994	0.18 (0.04)	2.42×10^{-6}	
	rs443043	160709531	Intron	0.30	G-C	C	0.994	0.18 (0.04)	2.43×10^{-6}	
	rs1018234	160716048	Intron	0.30	C-T	T	0.995	0.18 (0.04)	2.44×10^{-6}	
	rs316244	160716555	Intron	0.30	G-A	A	0.996	0.18 (0.04)	2.45×10^{-6}	
	rs440962	160731144	Intron	0.30	G-A	A	0.994	0.17 (0.04)	3.18×10^{-6}	
	rs446926	160733746	Intron	0.30	A-G	G	0.994	0.17 (0.04)	3.67×10^{-6}	
	rs1510225	160738253	Intron	0.30	C-T	T	0.994	0.17 (0.04)	3.74×10^{-6}	

SNP, single-nucleotide polymorphism; MAF, minor allele frequency; A1, major allele; A2, minor allele; SE, standard error.

Supplemental Table 4. Measures of linkage disequilibrium (r^2 values) for SNPs that are most highly associated with CFU and MI

	Top CFU SNPs									
Top MI SNPs	**rs394352**	**rs1510229**	**rs420038**	**rs402219**	**rs443043**	**rs1018234**	**rs316244**	**rs440962**	**rs446926**	**rs1510225**
rs1567438	0.849	0.924	0.961	0.961	0.961	0.961	0.961	0.961	0.961	0.961
rs2063345	0.849	0.924	0.961	0.961	0.961	0.961	0.961	0.961	0.961	0.961
rs2174914	0.849	0.924	0.961	0.961	0.961	0.961	0.961	0.961	0.961	0.961
rs2457575	0.849	0.924	0.961	0.961	0.961	0.961	0.961	0.961	0.961	0.961
rs2457576	0.849	0.924	0.961	0.961	0.961	0.961	0.961	0.961	0.961	0.961
rs2661834	0.849	0.924	0.961	0.961	0.961	0.961	0.961	0.961	0.961	0.961
rs377551	0.709	0.777	0.810	0.810	0.810	0.810	0.810	0.810	0.810	0.810
rs394468	0.709	0.777	0.810	0.810	0.810	0.810	0.810	0.810	0.810	0.810
rs394487	0.709	0.777	0.810	0.810	0.810	0.810	0.810	0.810	0.810	0.810
rs569919	0.579	0.642	0.670	0.670	0.670	0.670	0.670	0.670	0.670	0.670

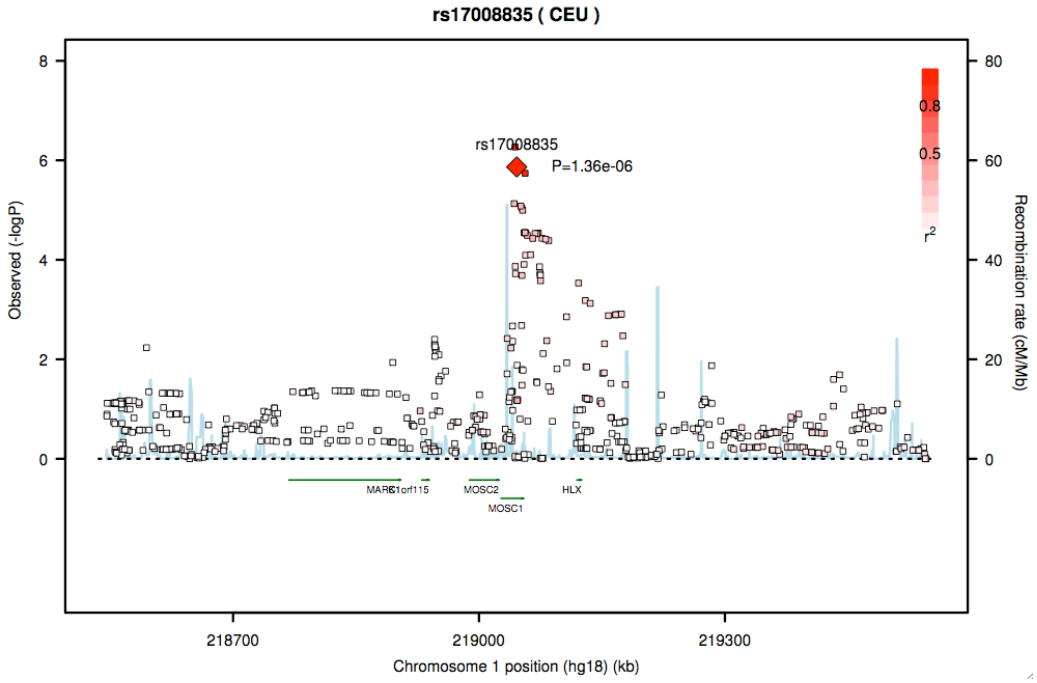
Supplemental Figures Legend

Supplemental Figure 1. Regional plots for top GWAS loci, showing directly measured SNPs (red diamonds) in high LD with imputed SNPs with the lowest p-values (squares with darker red coloring)

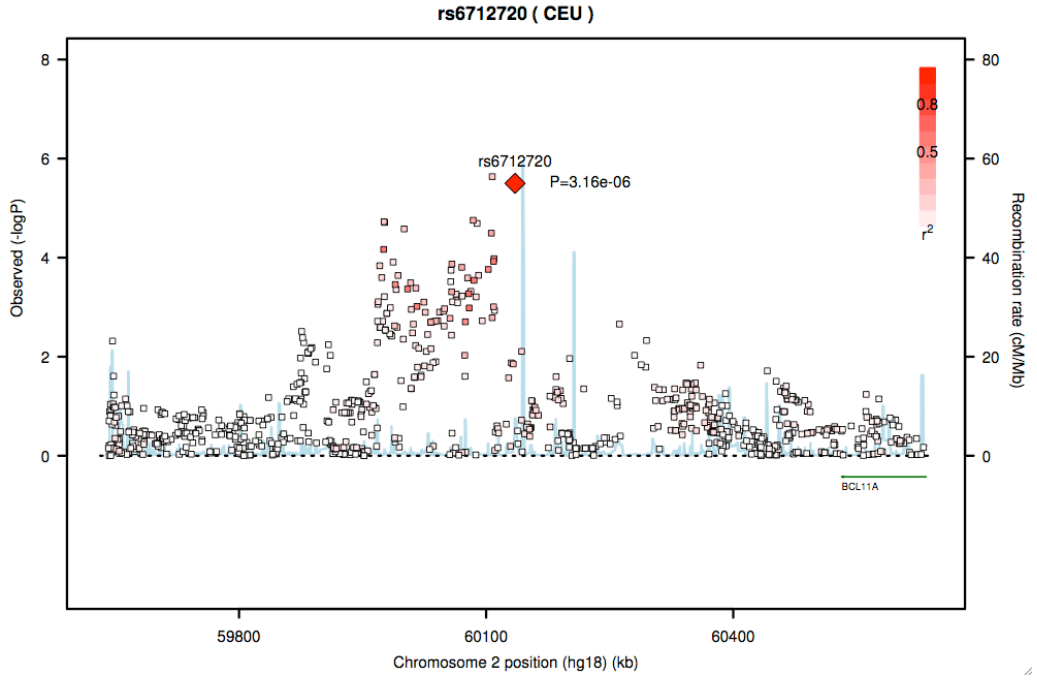
Supplemental Figure 2. Sampling for genetic analyses.

Supplemental Figure 3. Sex-specific distribution of CFU measurements in the study sample.

Supplemental Figure 1



Chromosome 1



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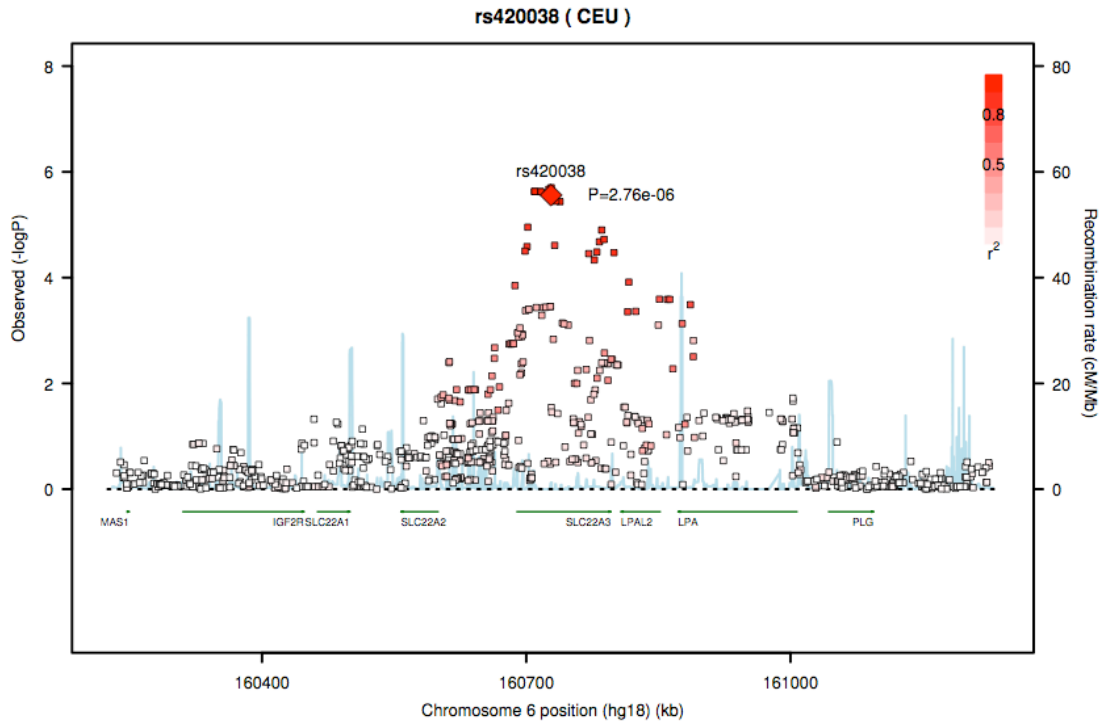

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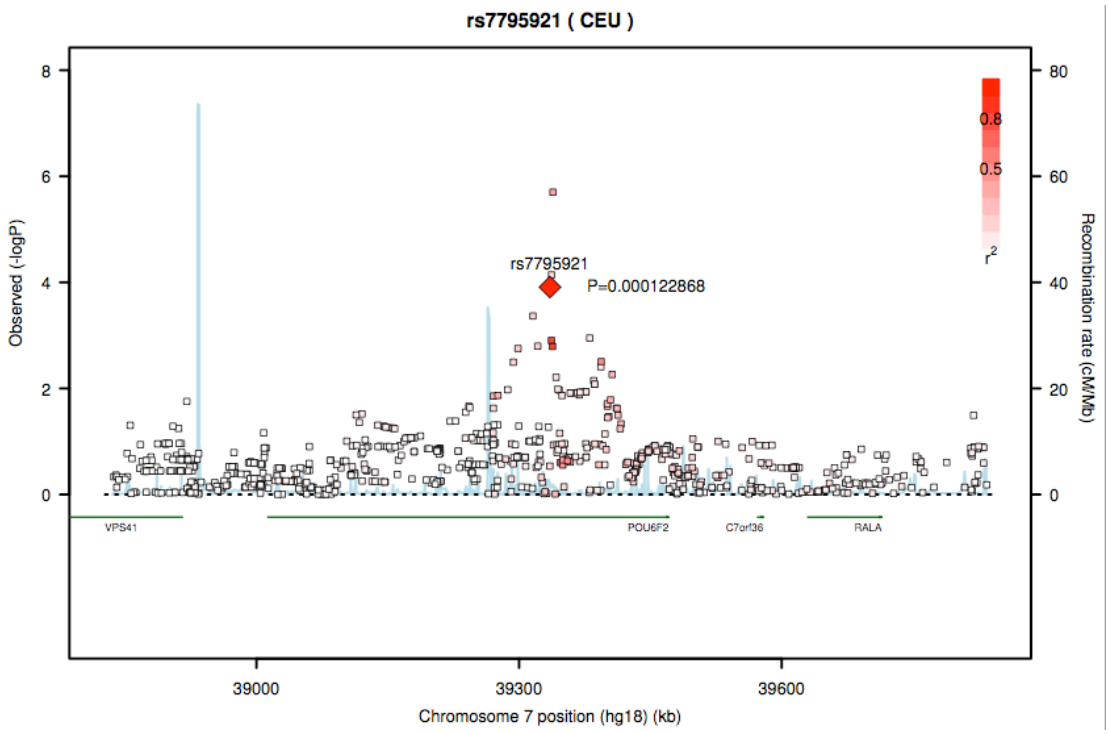

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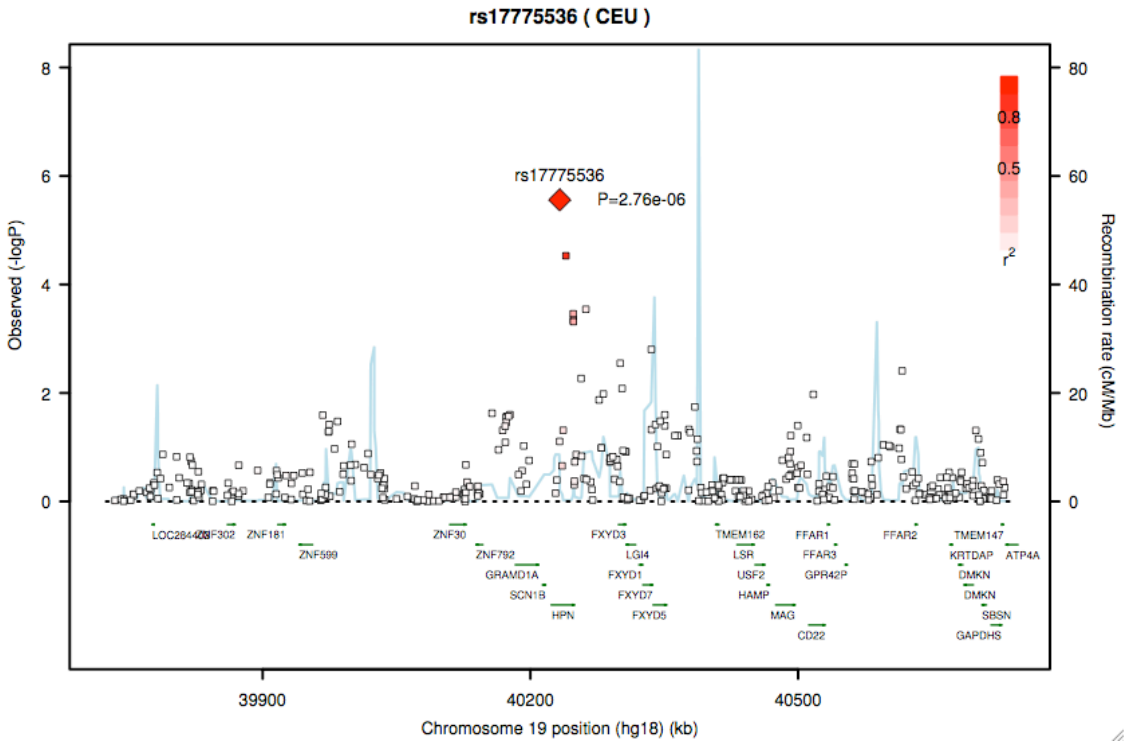
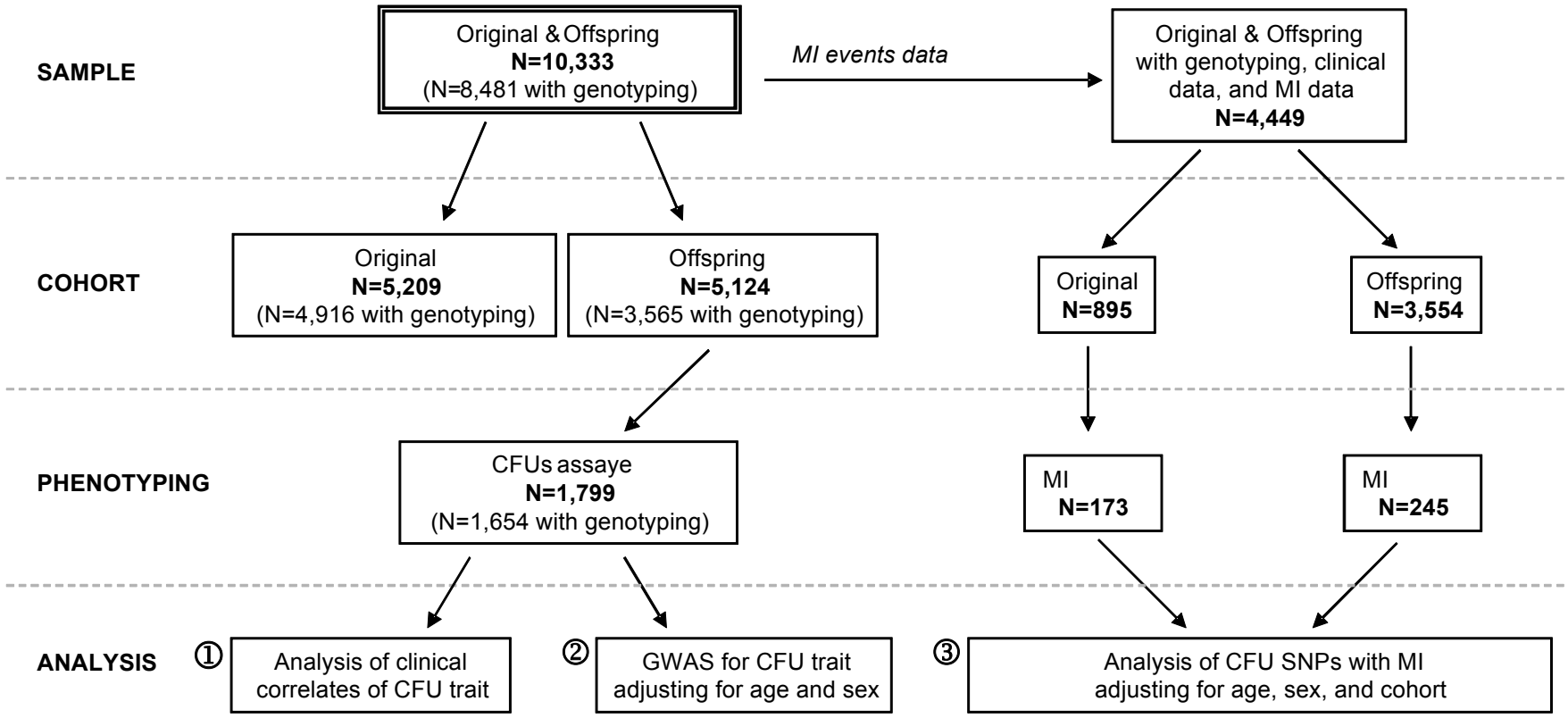


Chromosome 19

Supplemental Figure 2.



Supplemental Figure 3.