Materials and Methods

Study Populations

Heredity and Phenotype Intervention (HAPI) Heart Study

The Heredity and Phenotype Intervention (HAPI) Heart Study began in 2003 to identify genes that interact with environmental exposures to influence risk for cardiovascular disease *(S1)*. The study protocol was approved by the Institutional Review Board at the University of Maryland and all subjects gave written informed consent. The genome-wide association study (GWAS) was carried out in 809 Old Order Amish participants (of a total of 868) who completed the high fat feeding intervention and were successfully genotyped using the Affymetrix GeneChip® Human Mapping 500K Array Set. All 809 subjects could be connected to a single 14-generation pedigree, which was constructed using published Amish genealogy sources *(S2, S3)* and by querying the Anabaptist Genealogy Database (AGDB) 4.0 *(S4)* for all common ancestors of and connections among study subjects using the PedHunter software package *(S5)*.

HAPI participants underwent a medical history interview including assessment of CVD risk factors, prescription and nonprescription medication usage and questions about prior history of CVD. Physical examinations were conducted at the Amish Research Clinic in Strasburg, PA and blood samples were obtained following an overnight fast. Those taking lipid-lowering medications at enrollment (1.3%) discontinued usage 7 days prior to examination.

Amish Family Calcification Study

The Amish Family Calcification Study (AFCS) was initiated in 2001 to identify the determinants of vascular calcification and to evaluate the relationship between calcification of bone and vascular tissue *(S6)*. Old Order Amish subjects underwent a detailed medical examination including the measurement of coronary artery calcification (CAC) by electron beam computed tomography (EBCT) and a standard lipid panel as described below. Of 1,033 AFCS participants successfully genotyped for the APOC3 R19X mutation, 335 were also participants in the HAPI Heart high fat feeding intervention and GWAS, and 698 individuals were non-overlapping.

High Fat Feeding Intervention

The high fat challenge, administered to the HAPI Heart subjects, was prepared in the form of a whipping cream milk shake and was standardized to consist of 782 calories per m^2 of body surface with 77.6% of calories from fat, 19.2% from carbohydrate, and 3.1% from protein, and was administered after an overnight fast. Blood was drawn just prior to (time = 0 hours) and 1, 2, 3, 4, and 6 hours after ingestion of the fat load to assess the triglyceride excursion. The subject rested and remained otherwise fasting during the 6 hours post-fat challenge.

Phenotype Measurement

Height and weight were measured using a stadiometer and calibrated scale with shoes removed and in light clothing, and body mass index (BMI) (kg/m^2) was computed. Systolic blood pressure (SBP) (1st phase) and diastolic blood pressure (DBP) (5th phase) were obtained in triplicate using a standard sphygmomanometer with the subject sitting for at least 5 minutes. Fasting (FTG) and post-challenge triglyceride and fasting total cholesterol, HDL-C and glucose levels were measured by Quest Diagnostics (Lancaster, PA). All subjects except three AFCS participants had FTG < 400 mg/dl and LDL-C levels were calculated by the Friedewald equation *(S7)*. Framingham 10 year coronary heart disease (CHD) risk was calculated for each AFCS participant by entering measured lipid levels/ blood pressure and self-reported diabetes/ smoking status into LDL-based risk equations *(S8)*.

Triglyceride total area under the curve (tAUCTG) was calculated in fat challenge participants by the trapezoid method, and incremental area under the curve (iAUCTG) was calculated as tAUCTG – 6 x FTG. Incremental maximum TG (iMAXTG) was calculated as the highest of the six TG levels obtained (MAXTG) – FTG. Lipoprotein subfractions, including those of very low density lipoprotein (VLDL) and intermediate density lipoprotein (VLDL) cholesterol, were measured at zero and four hours by Vertical Auto Profile (VAP) technology (Atherotech, Birmingham, AL). Insulin was measured by radioimmunoassay (coefficient of variation = 4.42%).

Circulating apoC-III serum protein levels were assayed in 20 HAPI Heart participants from four two-generation families and one sib pair using a western blot. Plasma was diluted 1:4 with phosphate-buffered saline (PBS). One microliter of the diluted serum was subjected to 10-20% SDS-PAGE, transferred to immobilon-P (Millipore) membranes, probed with anti-apoC-III polyclonal antibodies (Santa Cruz Biotechnologies), detected with goat anti-mouse HRP (horseradish peroxidase) conjugate, visualized with Femto-West chemiluminescence reagent (Pierce) and quantitated with a Fluorchem 8000 chemiluminescence /fluorescence imager (Alpha Innotech). Each serum sample was run in duplicate and normalized to a standardized pooled serum sample derived from a group of anonymous donors. The intra-subject coefficient of variation was 11.1%.

Coronary artery calcification (CAC) was measured in AFCS participants by electron beam computed tomography in a total of 1,033 Amish individuals, including 335 from the HAPI Heart Study as well as an additional 698 participating in the AFCS *(S6, S9)*. Measurement was performed with an Imatron C-150 scanner (Imatron, Inc.: San Francisco, CA). Scan protocol included 30 to 40 three mm contiguous transverse slices between the aortic root and the apex of the heart, gated to 80% of the R-R interval obtained during a single breath hold. CAC was quantified using the Agatston method which incorporates both density and area *(S10)*. The sum of the Agatson scores in the left main, left anterior descending, circumflex, and right coronary arteries was considered the CAC score. CAC presence was defined as a density >130 Hounsfield units in greater than three contiguous pixels (> 1 mm^2). All scans were scored by an experienced cardiologist and reviewed for extra-cardiac findings by an experienced radiologist. Interscan reproducibility for CAC quantity with the scoring software was previously reported to range from 89% *(S11)* to 94% *(S12)*. The interreader and intrareader reproducibilities were each ~99%.

Genotyping and sequencing

Genome-wide association analysis

GWAS of the 809 HAPI Heart Study samples was carried out using the Affymetrix GeneChip® Human Mapping 500K Array set. The 500K array set consists of two microarray chips (*NspI* and *StyI*), which collectively include a total of 500,568 single nucleotide polymorphisms (SNPs). Total genomic DNA (250 ng) was digested with *NspI* or *StyI* and processed according to the Affymetrix protocol. Genotype calls were made using the BRLMM genotype calling algorithm (http://www.affymetrix.com). A confidence threshold, a measure of the call quality, of 0.5 was used for this analysis. The resulting mean genotype call rate across the 809 samples was 98.1%. SNPs with minor allele frequencies (MAF) < 1% in the overall sample were removed from further analyses. Finally, 381,934 informative autosomal SNPs that passed quality-control (call rate $\geq$ 90%) and Hardy-Weinberg Equilibrium checks ($p > 10^{-5}$) were included in the analysis.

APOC3 gene sequencing and genotyping

Exons 1-3 and the coding region of exon 4 of the *APOC3* gene were amplified in three PCR reactions (primer sequences available upon request) and sequenced in eight rs10892151-A carriers and eight of their non-carrier relatives using an ABI 3730xl sequencer. Sequences were compared to the reference sequence and mutations identified using the Sequencher software package. Upon discovery of the 55C>T (R19X) nonsense mutation in seven of the eight rs10892151-A carriers, a Taqman® assay was designed to genotype all HAPI Heart Study and AFCS participants as well as 996 additional Amish adults participating in studies of complex diseases at the University of Maryland and 214 unrelated Caucasian adults from the Baltimore community serving as healthy controls for several studies at the University of Maryland. Blind replicate concordance rates were 100% and no Mendelian inconsistencies were found.

Statistical Analysis

Association analyses of quantitative traits were performed using the measured genotype approach that models variation in the trait of interest as a function of measured environmental covariates, measured genotype, and a polygenic component to account for phenotypic correlation due to relatedness. A t-test was used to assess significance of the measured genotype beta coefficient under an additive model. We included sex and sex-specific age and age^2 and in some models body mass index (BMI) as covariates and coded the SNPs using an additive model. The polygenic component was modeled using the relationship matrix derived from the complete 14-generation pedigree structure to properly control for the relatedness of all subjects in the study. These analyses were carried out using software developed in our group *(S13)*. Quantile-quantile plots comparing the observed GWA p-values with those expected under the null are provided in Figure S1. Quantitative traits which were not normally distributed were transformed by their natural logarithm. For CAC, a constant of one was added prior to transformation since some participants had a raw trait level of zero. Results were plotted using Haploview Version 4.0 *(S14)* and are shown in Figure S1.

Odds ratios were estimated and significance of association of the variant with optimal plasma lipid categories, CAC presence and CAC score > 100 Agatston units was evaluated using generalized estimating equations (GEE) under a logistic model as implemented in SAS Version 9.1 with age and sex included as fixed covariates and sibship membership included as a random effect with an exchangeable correlation matrix. Association with CAC score $\geq$ 75th age- and sex-specific percentile as determined in asymptomatic participants in the Multi-Ethnic Study of Atherosclerosis (MESA) *(S15)* was analyzed with GEE adjusted for sibship only.

Linkage disequilibrium was evaluated using Haploview Version 4 *(S14)*.

SOM Text: Origin of the *APOC3* R19X Mutation

A total of 2,503 Amish adults (approximately 15-20% of the total adult Lancaster Amish population) were genotyped for *APOC3* R19X. Of these, 140 (5.6%) are carriers of the R19X mutation. To identify the origin of the mutation, we searched a 14-generation pedigree constructed from the Anabaptist Genealogy Database (AGDB) *(S5, S16)* for common ancestors of these 140 individuals using the ANCESTOR routine in the PEDSYS software package *(S17)*. A couple born near the beginning of the 19th century (1800) emerged as the most likely origin of all present-day copies of the mutation (Figure S3); all 140 individuals with the RX genotype were descended from this couple, whereas only 1,312 of 2,363 (56%) of individuals with the RR genotype were descended from this couple. The pedigree is compatible with either a *de novo* mutation or a founder effect, as the couple was two to five generations removed from original

Amish founders. Extended haplotype analysis (Figure S4) was most consistent with a founder effect, with a single copy of the mutated allele entering the population through a single founder prior to 1800. Only one extended haplotype contained the *APOC3* R19X mutation (Figure S4). . The mutation was not present in 214 non-Amish Caucasian subjects (428 alleles).

Table S1: Characteristics of 809 HAPI Heart study subjects completing the high fat challenge (Mean ± SD or Median [IQR]) and GWAS. Serum traits are fasting values.

Trait	Men	Women
N	443	366
Age (y)	42 ± 14	45 ± 14
BMI (kg/m^2)	25.6 ± 3.2	27.7 ± 5.3
Waist (cm)	90.0 ± 10.0	84.0 ± 11.0
Total Cholesterol (mg/dl)	203 ± 44	214 ± 48
HDL-C (mg/dl)	53 ± 13	59 ± 15
LDL-C (mg/dl)	137 ± 40	140 ± 45
Triglycerides (mg/dl) Median (IQR)	53 (41 – 75)	59 (42 – 89)
Glucose (mg/dl)	86 ± 8	86 ± 9
Insulin (μU/ml) Median (IQR)	7.8 (6.3 – 9.7)	8.5 (6.9 – 10.9)
SBP (mm Hg)	122± 12	121 ± 16
DBP (mm Hg)	78 ± 9	76 ± 8

Table S2: GWAS SNPs with p-values for ln-FTG or ln-iAUCTG $\leq$ 0.0001. SNPs with significant association after Bonferroni correction ($p<10^{-7}$) indicated in bold. SNP closest to *APOC3* indicated by an asterisk. Relative location of APOC5/A4/C3/A1 cluster indicated by a text box.

SNP	Chr	Position	Alleles (A/B)	Allele Freq (B)	Strand	ln-FTG p-value	ln-iAUCTG p-value	Nearby Gene(s)
rs630075	1	9,293,498	C/T	0.34	-	7.0E-05	0.06	*SPSB1*
rs4908842	1	9,309,898	A/G	0.55	-	5.9E-05	0.03	*SPSB1*
rs912962	1	10,271,299	G/T	0.44	-	9.6E-05	0.95	*KIF1B*
rs2745276	1	11,563,870	A/G	0.89	+	2.4E-05	0.02	*FBXO2/ FBXO44*
rs2817644	1	11,570,256	A/G	0.87	-	7.8E-06	0.22	*FBXO2/ FBXO44*
rs10492995	1	18,579,960	G/T	0.97	-	0.005	9.1E-05	*IGSF21/ KLHDC7A*
rs34783763	1	43,530,349	A/G	0.26	-	0.01	4.5E-05	*C1orf210/ TIE1*
rs787503	1	67,846,229	A/G	0.92	+	3.0E-04	7.9E-05	
rs1749517	1	69,837,458	C/G	0.86	+	6.0E-05	0.03	
rs4656414	1	162,511,653	C/T	0.59	-	0.007	8.2E-05	
rs13395978	2	121,104,379	A/G	0.08	-	8.5E-05	0.04	
rs12104997	2	207,547,751	C/T	0.07	-	0.001	6.5E-05	*CPO/ KLF7*
rs28294	3	45,231,195	C/G	0.54	-	0.56	6.6E-05	*CDCP1/ TMEM158*
rs13081218	3	105,942,556	C/T	0.08	+	1.3E-04	3.6E-05	
rs2895287	3	105,946,243	C/T	0.08	-	1.3E-04	3.6E-05	
rs7626000	3	105,956,811	A/T	0.92	+	2.7E-04	1.2E-05	
rs6534543	4	127,465,125	A/C	0.76	-	1.3E-05	0.003	*FAT4*
rs17623445	4	166,114,108	C/T	0.07	-	5.4E-05	0.003	
rs10070815	5	14,010,465	C/G	0.15	+	8.9E-05	0.003	*DNAH5/ TRIO*
rs268731	5	33,400,441	C/T	0.96	+	5.8E-05	0.006	*TARS*
rs17207053	5	85,528,275	C/T	0.03	-	0.01	8.4E-05	
rs6414953	5	88,999,906	A/G	0.98	+	7.6E-06	0.004	*CETN3/ MEF2C*
rs146689	5	96,782,445	A/C	0.67	-	0.04	6.5E-06	
rs953952	5	96,791,184	A/G	0.30	+	0.02	4.6E-05	
rs9314208	5	96,813,918	A/G	0.65	+	0.06	1.1E-05	
rs11135514	5	96,816,018	C/G	0.30	-	0.02	4.3E-05	
rs4869355	5	96,824,371	C/G	0.70	+	0.02	4.0E-05	
rs1485469	5	96,843,284	C/T	0.70	-	0.01	4.5E-05	
rs9384465	6	156,772,914	A/C	0.90	+	2.8E-05	0.04	

SNP	Chr	Position	Alleles (A/B)	Allele Freq (B)	Strand	ln-FTG p-value	ln-iAUCTG p-value	Nearby Gene(s)
rs10227912	7	38,018,111	C/T	0.36	+	5.7E-05	8.2E-05	*EPDR1/ STARD3NL*
rs7784959	7	38,027,068	A/C	0.35	+	3.7E-06	1.1E-06	*EPDR1/ STARD3NL*
rs10954663	7	81,584,556	G/T	0.21	-	7.7E-05	0.24	*CACNA2D1*
rs10486942	7	81,596,318	A/G	0.21	+	9.5E-05	0.22	*CACNA2D1*
rs7786701	7	88,102,149	C/T	0.35	+	0.05	9.3E-05	*MGC26647/ ZNF804B*
rs3807971	7	115,655,129	C/T	0.15	-	0.11	8.4E-05	*TES*
rs1035205	7	117,332,711	C/T	0.62	-	0.34	8.5E-05	*CTTNBP2/ LSM8*
rs1548459	7	117,354,473	A/C	0.62	+	0.35	6.5E-05	*CTTNBP2/ LSM8*
rs2949761	7	142,922,064	A/G	0.04	+	3.8E-05	0.04	
rs2384844	8	123,875,421	C/T	0.22	-	6.5E-06	0.03	*ZHX2*
rs10758849	9	7,258,068	A/G	0.41	-	2.8E-05	0.04	*JMJD2C*
rs2148189	10	4,018,083	C/T	0.49	-	7.4E-05	0.40	*KLF6*
rs7917417	10	26,234,425	A/G	0.85	+	0.002	3.7E-05	
rs12259899	10	26,274,617	A/G	0.86	-	0.002	3.6E-05	*MYO3A*
rs7910887	10	33,730,587	C/T	0.42	+	6.8E-05	0.001	*NRP1/ PARD3*
rs1334469	10	116,101,018	A/G	0.52	+	0.006	1.8E-05	*AFAP1L2*
rs12287008	11	114,166,676	C/T	0.88	-	1.9E-05	0.002	*FAM55B*
rs7122693	11	114,809,573	A/G	0.36	+	6.1E-05	0.01	*CADM1*
rs681524*	11	116,253,524	A/G	0.06	-	1.1E-05	0.003	*KIAA0999*
rs7949670	11	117,036,145	A/C	0.87	+	4.6E-06	0.006	*DSCAML1*
rs10892151	**11**	**117,036,941**	**A/G**	**0.97**	**-**	**3.8E-14**	**2.8E-10**	***DSCAML1***
rs11607985	11	117,535,008	C/G	0.12	+	8.9E-07	8.4E-04	*SCN4B*
rs11608072	**11**	**117,535,296**	**C/G**	**0.86**	**+**	**8.4E-08**	**6.9E-05**	***SCN4B***
rs11600901	11	117,537,196	C/T	0.10	-	2.7E-05	9.6E-04	*SCN4B*
rs10892287	11	118,148,209	C/T	0.76	+	4.6E-05	0.004	*DDX6*
rs10892306	11	118,256,536	A/T	0.08	-	6.6E-05	3.4E-04	*BLR1*
rs11217695	11	119,482,835	A/G	0.93	-	8.5E-06	2.4E-05	*PVRL1/ TRIM29*
rs6589804	11	119,666,851	A/G	0.90	+	3.6E-05	7.2E-04	*POU2F3*
rs12278532	11	119,986,983	A/G	0.37	-	5.5E-05	0.02	*GRIK4*
rs7925270	11	120,068,508	A/G	0.49	-	2.0E-05	0.001	*GRIK4*
rs11217989	11	120,089,193	A/G	0.18	-	1.3E-05	0.009	*GRIK4*
rs11218513	11	121,436,070	A/G	0.93	+	2.4E-06	8.5E-04	
rs492085	11	121,499,910	A/C	0.07	-	4.4E-05	0.005	*BLID*

APOA5/A4/C3/A1 Cluster Location:
116,165,296 - 116,213,548 bp

SNP	Chr	Position	Alleles (A/B)	Allele Freq (B)	Strand	ln-FTG p-value	ln-iAUCTG p-value	Nearby Gene(s)
rs687928	11	121,501,471	C/T	0.50	+	3.5E-05	0.002	*BLID*
rs678586	11	121,507,814	A/G	0.50	-	3.2E-05	0.002	*BLID*
rs594358	11	121,510,335	A/T	0.50	+	4.2E-05	0.003	*BLID*
rs6589937	11	122,006,372	G/T	0.96	+	2.5E-06	1.7E-04	*UBASH3B*
rs7951825	**11**	**123,239,793**	**A/G**	**0.04**	**-**	**2.1E-09**	**4.3E-06**	***OR6M1/ PMP22CD***
rs4575285	11	124,327,379	C/G	0.35	+	4.9E-06	2.9E-04	*CCDC15/ HEPACAM/ HEPN1*
rs1002777	11	124,949,814	C/T	0.46	+	6.5E-05	4.2E-04	*EI24*
rs519297	11	125,140,621	C/G	0.99	-	9.7E-05	0.002	*C11orf38/ PATE*
rs10893450	11	125,323,964	C/G	0.77	-	9.9E-06	7.2E-05	*CDON/ DDX25*
rs11220301	11	125,357,483	C/T	0.16	+	4.9E-05	0.003	*CDON*
rs17136789	11	126,996,515	A/T	0.01	+	5.7E-05	0.003	*ETS1*
rs10894845	11	133,953,680	C/T	0.02	+	1.0E-05	0.005	*B3GAT1*
rs11043458	12	17,479,817	A/C	0.90	-	0.03	6.0E-05	
rs11044361	12	18,977,067	A/T	0.56	-	0.01	1.8E-05	*CAPZA3*
rs7311139	12	30,204,772	A/C	0.60	-	6.5E-05	7.4E-04	*TMTC1*
rs1948032	12	30,220,301	C/T	0.38	+	3.6E-05	0.004	*TMTC1*
rs1879423	12	30,226,589	A/G	0.39	+	9.0E-05	0.006	*TMTC1*
rs7955747	12	120,409,487	C/T	0.24	+	0.006	9.5E-05	*FBXL10*
rs7491873	13	36,849,949	A/G	0.64	+	9.6E-05	2.4E-04	*POSTN*
rs7981543	13	53,211,477	A/G	0.91	+	9.3E-05	7.7E-05	
rs2182665	13	73,360,923	G/T	0.15	+	9.9E-05	0.01	*KLF12*
rs9602209	13	82,998,931	A/G	0.37	+	6.7E-05	0.03	*SLITRK1*
rs1046146	15	72,493,996	A/G	0.94	+	4.4E-05	0.005	*SEMA7A*
rs11073029	15	80,132,145	C/G	0.67	+	0.05	4.3E-05	*EFTUD1/ MEX3B*
rs17670098	16	7,149,248	A/C	0.93	+	0.004	5.6E-05	*A2BP1*
rs16962399	16	55,404,305	A/G	0.88	-	5.1E-05	5.4E-04	*NUP93*
rs16962767	16	55,431,290	A/G	0.11	-	7.5E-05	2.7E-04	*NUP93*
rs11861237	16	63,697,996	C/T	0.20	+	9.4E-05	0.002	*CDH11*
rs1471379	16	80,068,200	C/T	0.40	+	0.89	6.5E-05	*CMIP*
rs1471152	16	80,070,137	G/T	0.40	+	0.96	8.7E-05	*CMIP*
rs2125202	16	80,078,795	C/G	0.49	-	0.40	6.4E-05	*CMIP*
rs17808998	17	8,919,071	A/G	0.75	-	0.44	1.8E-05	*NTN1*
rs9303273	17	11,094,564	A/G	0.89	+	0.06	3.9E-05	

SNP	Chr	Position	Alleles (A/B)	Allele Freq (B)	Strand	ln-FTG p-value	ln-iAUCTG p-value	Nearby Gene(s)
rs11078890	17	11,095,333	C/T	0.11	-	0.10	9.0E-05	
rs8069247	17	11,097,192	C/T	0.11	-	0.12	6.9E-05	
rs8069599	17	11,097,447	C/T	0.11	-	0.10	8.0E-05	
rs16947073	17	59,187,050	A/G	0.96	+	5.4E-05	0.21	*CCDC47*
rs4798097	18	3,498,295	C/T	0.56	+	0.005	1.5E-05	*DLGAP1*
rs16966092	18	18,171,472	G/T	0.15	-	1.2E-04	7.6E-05	
rs807969	19	7,744,484	G/T	0.09	+	1.1E-05	0.02	*CLEC4M*
rs4808199	19	19,406,099	C/T	0.20	-	3.2E-05	0.002	*GATAD2A*
rs1621688	19	20,079,226	C/T	0.40	-	0.001	9.2E-05	*ZNF253*
rs2198895	19	36,061,034	A/G	0.96	+	4.7E-05	0.04	*ZNF536*
rs2756260	20	4,657,986	A/G	0.89	-	0.01	4.1E-05	*PRND/ PRNT*
rs2422958	20	4,697,289	G/T	0.26	-	7.3E-05	0.13	*PRNT/ RASSF2*
rs35574319	22	33,181,133	A/G	0.35	-	9.4E-05	0.009	
rs5995078	22	33,928,379	A/T	0.83	+	3.9E-05	0.02	*ISX*
rs932290	22	34,002,183	A/G	0.30	+	5.4E-05	0.03	*HMG2L1*
rs5755683	22	34,005,537	A/G	0.30	+	1.0E-04	0.03	*HMG2L1*

Table S3: Triglyceride levels in mg/dl (or mg/dl x 6 hours for tAUCTG and iAUCTG) during the high fat challenge by rs10892151 genotype and p-values for analyses adjusted for sex and sex-specific age and age^2 and BMI.

Trait	GG Median (IQR)	AG Median (IQR)	p-value
N	763	46	--
Fasting TG	57 (42 – 81)	32 (25 – 46)	3.8×10^{-14}
Hour 1 TG	76 (57 – 108)	48 (33 – 67)	8.1×10^{-12}
Hour 2 TG	122 (89 – 170)	73 (49 – 93)	3.1×10^{-13}
Hour 3 TG	155 (109 – 220)	86 (65 – 121)	8.8×10^{-14}
Hour 4 TG	159 (111 – 238)	88 (66 – 109)	1.3×10^{-13}
Hour 6 TG	137 (92 – 220)	77 (52 – 108)	1.1×10^{-9}
MAXTG	184 (130 – 265)	105 (78 – 140)	5.8×10^{-13}
iMAXTG	122 (83 – 184)	66 (52 – 99)	8.3×10^{-10}
tAUCTG	770 (560 – 1102)	425 (327 – 581)	1.6×10^{-14}
iAUCTG	413 (276 – 610)	216 (163 – 337)	2.8×10^{-10}

Table S4: Triglyceride levels in mg/dl (or mg/dl x 6 hours for tAUCTG and iAUCTG) during the high fat challenge by *APOC3* R19X genotype. Analyses are of ln-transformed traits adjusted for sex- and sex-specific age and age^2 in addition to the covariates indicated.

Trait	RR (CC) Median (IQR)	RX (CT) Median (IQR)	p-value	p-value (BMI-adjusted)	p-value (BMI and FTG adjusted)
N	763	39	--	--	--
Fasting TG	57 (42 – 81)	31 (25 – 48)	4.2×10^{-11}	4.1×10^{-13}	--
Hour 1 TG	76 (57 - 107)	47 (33 – 66)	2.9×10^{-10}	5.4×10^{-12}	0.12
Hour 2 TG	122 (88 – 170)	73 (49 – 93)	2.0×10^{-11}	5.8×10^{-13}	0.008
Hour 3 TG	154 (109 – 217)	75 (64 – 119)	1.5×10^{-12}	3.5×10^{-14}	0.002
Hour 4 TG	159 (110 – 237)	85 (65 – 112)	1.5×10^{-10}	4.1×10^{-12}	0.03
Hour 6 TG	136 (92 – 219)	76 (52 – 105)	1.3×10^{-8}	2.0×10^{-9}	0.59
MAXTG	183 (130 – 265)	104 (95 – 115)	4.8×10^{-11}	1.5×10^{-12}	0.02
iMAXTG	121 (83 – 183)	60 (51 – 90)	6.5×10^{-9}	1.0×10^{-9}	0.03
tAUCTG	761 (556 – 1098)	411 (322 – 581)	4.1×10^{-12}	5.0×10^{-14}	0.006
iAUCTG	410 (276 – 608)	214 (154 – 338)	2.0×10^{-9}	2.7×10^{-10}	0.01

Table S5: Association of *APOC3* R19X mutation with lipid and calcification levels in the Amish Family Calcification Study. All analyses adjusted for BMI (except ln[CAC+1]), sex and sex-specific age and age^2 as well as residual familial covariance. Shown is mean ± SD for traits analyzed directly and mean (interquartile range) for traits analyzed after natural log transformation.

	AFCS Subset not included in HAPI			All AFCS		
Trait	RR (n = 656)	RX (n = 42)	p-value	RR (n = 974)	RX (n = 59)	p-value
Age (years)	60 ± 13	57 ± 11	0.28	57 ± 13	55 ± 11	0.10
Total Cholesterol (mg/dl)	215 ± 44	206 ± 41	0.51	215 ± 42	204 ± 39	0.12
TG (mg/dl)	83 (60 – 120)	44 (36 – 57)	**1.9×10^{-22}**	78 (56 – 112)	43 (34 – 53)	**2.9×10^{-29}**
HDL-C (mg/dl)	54.9 ± 15.0	69.2 ± 14.9	**1.3×10^{-14}**	56.0 ± 15.0	69.3 ± 14.4	**1.3×10^{-17}**
non-HDL-C (mg/dl)	160 ± 44	136 ± 37	**0.001**	159 ± 42	135 ± 34	**7.7×10^{-6}**
LDL-C (mg/dl)	140 ± 40	127 ± 36	0.15	140 ± 39	126 ± 33	**0.01**
ln(CAC score +1)	2.9 ± 2.8	1.9 ± 2.7	**0.03**	2.5 ± 2.7	1.6 ± 2.5	**0.005**

Figure S1

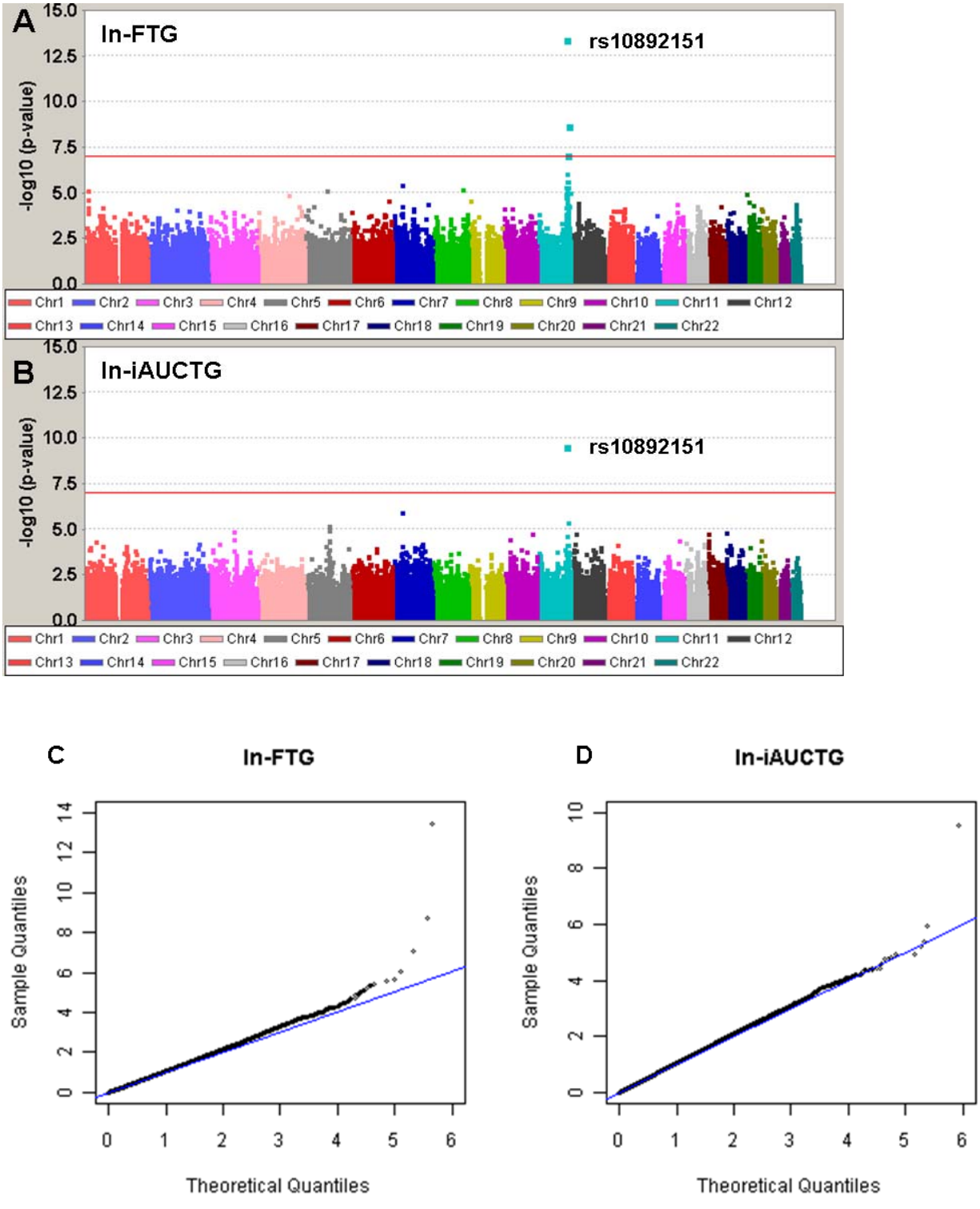


Figure S1: GWAS results for ln-FTG (A) and ln-iAUCTG (B) plotted using Haploview Version 4.1 *(S14)* and associated quantile-quantile plots (C and D).

Figure S2

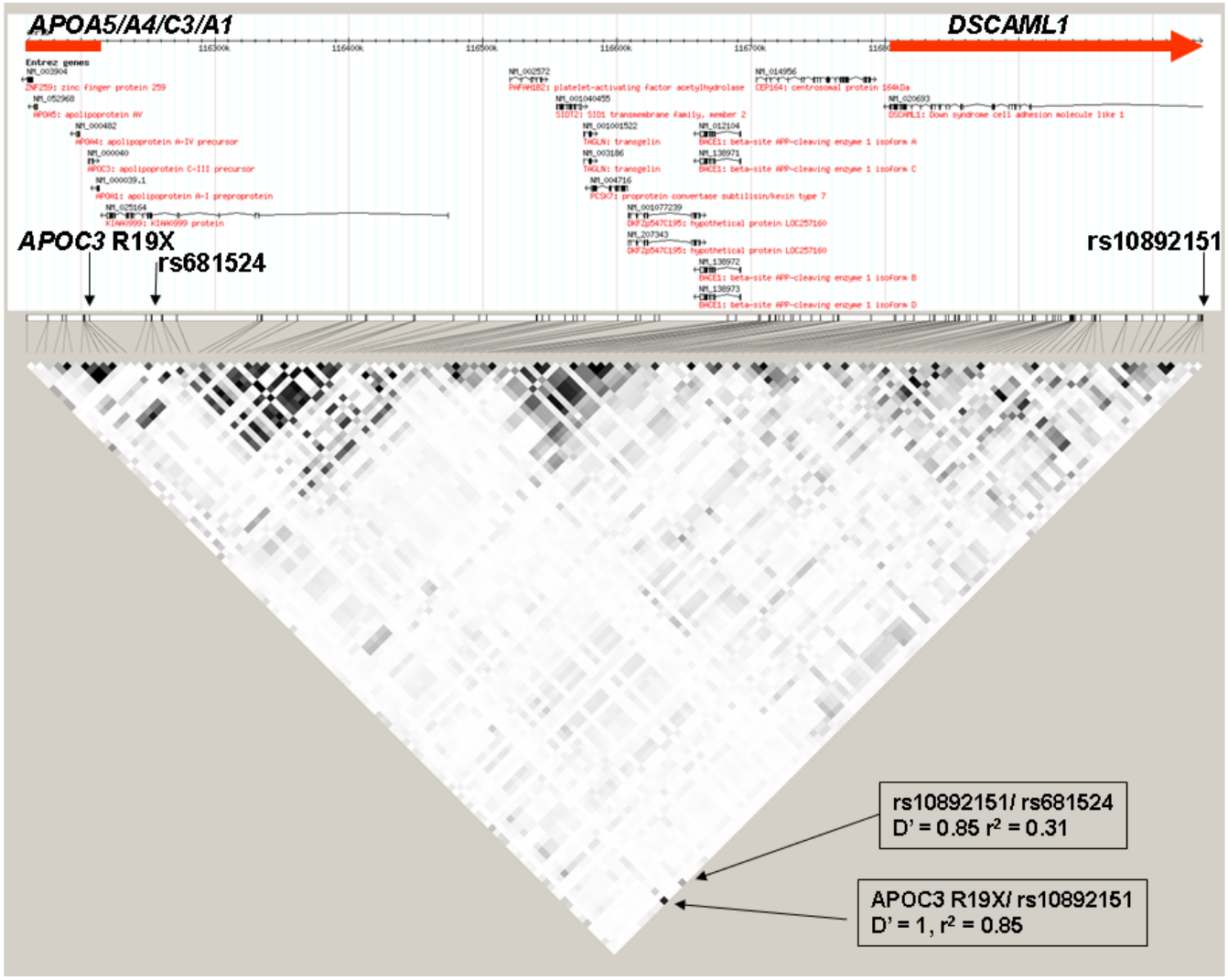


Figure S2: Linkage disequilibrium structure in the Amish of the 872 kb region from the *APOA5/A4/C3/A1* cluster to the GWAS SNP most highly associated with ln-FTG and ln-IAUCTG, located in the *DSCAML1* gene.

Figure S3

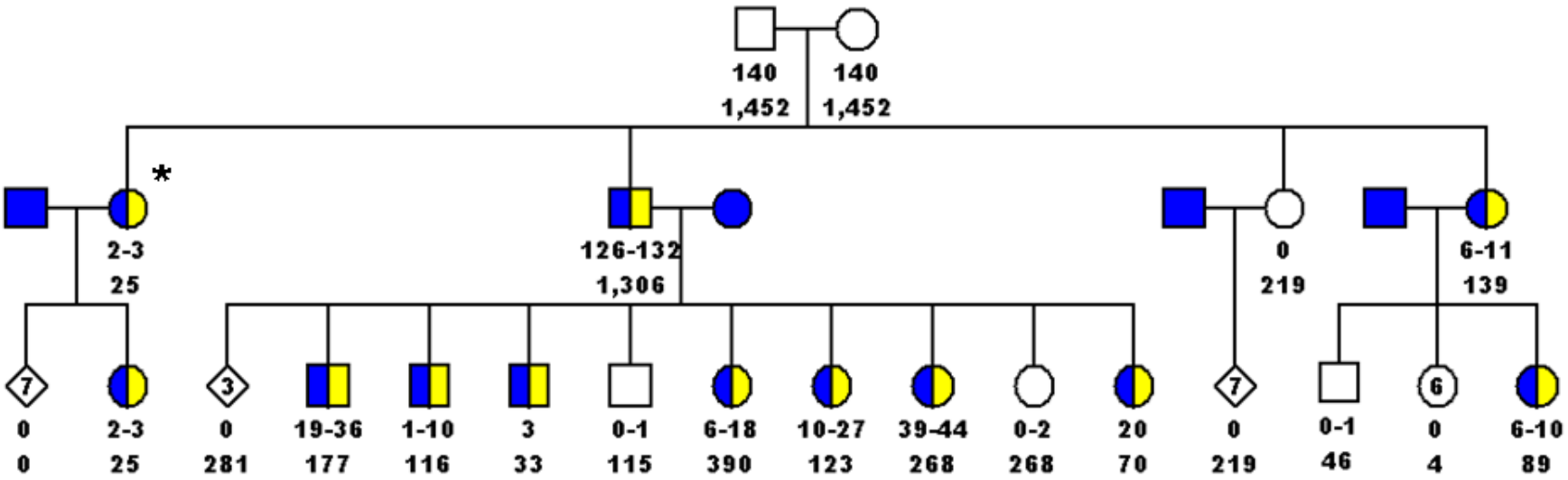
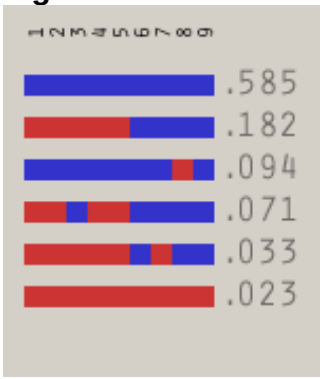


Figure S3: Pedigree showing the first two generations of transmission of the *APOC3* R19X mutation from the most recent common ancestor couple, born in the early 19th century. Solid blue symbols indicate non-descendants inferred to have the RR genotype. Blue/yellow symbols indicate obligate R19X carriers. Open symbols have unknown genotypes. Numbers within symbols indicate the number of individuals. The first line of text below each symbol indicates the number of genotyped descendants receiving the 19X allele from that individual and the second line indicates the number of descendants genotyped. A range given for the first line indicates that some descendant 19X alleles may have come from a different progenitor. For example, the individual with the asterisk is a direct ancestor of 25 genotyped individuals. Of those 25, two descendants received the 19X allele from her and a third descendant received the allele either from her or her brother.

Figure S4



SNP	Name	Position (bp)	MAF	Genes	Relative Location	ln-FTG p-value	Ln-iAUCTG p-value
1	rs2727789	116,202,097	0.32	*APOA4* *APOC3*	2.9 kb downstream 3.7 kb upstream	0.03	0.05
2	rs2849176	116,202,131	0.32	*APOA4* *APOC3*	2.9 kb downstream 3.7 kb upstream	0.04	0.05
3	rs2849174	116,202,276	0.24	*APOA4* *APOC3*	3.1 kb downstream 3.6 kb upstream	0.007	0.03
4	rs2071523	116,202,554	0.32	*APOA4* *APOC3*	3.3 kb downstream 3.3 kb upstream	0.04	0.05
5	rs2071521	116,203,058	0.32	*APOA4* *APOC3*	3.8 kb downstream 2.8 kb upstream	0.04	0.06
6	**R19X**	**116,206,563**	**0.02**	***APOC3***	**Exon 2**	**4.1×10^{-13}**	**2.0×10^{-10}**
7	rs681524	116,253,524	0.06	*KIAA0999*	Intron 6	1.1×10^{-5}	0.003
8	rs7949670	117,036,145	0.12	*DSCAML1*	Intron 3	4.6×10^{-6}	0.006
9	rs10892151	117,036,941	0.03	*DSCAML1*	Intron 3	3.8×10^{-14}	2.8×10^{-10}

Figure S4: Haplotype structure in the Amish of the *APOA5/C3/A4/A1* cluster and selected additional SNPs in the 835 kb region containing both *APOC3* and rs10892151, the SNP in *DSCAML1* originally identifying the association. SNPs 1 – 5 comprise all SNPs in the *APOA5/C3/A4/A1* cluster contained on the Affymetrix 500K gene chip, and SNPs 7 – 9 are additional SNPs on the chip. SNP 6 is the *APOC3* R19X mutation identified by sequencing and is found on a single extended haplotype (solid red bar).

Figure S5

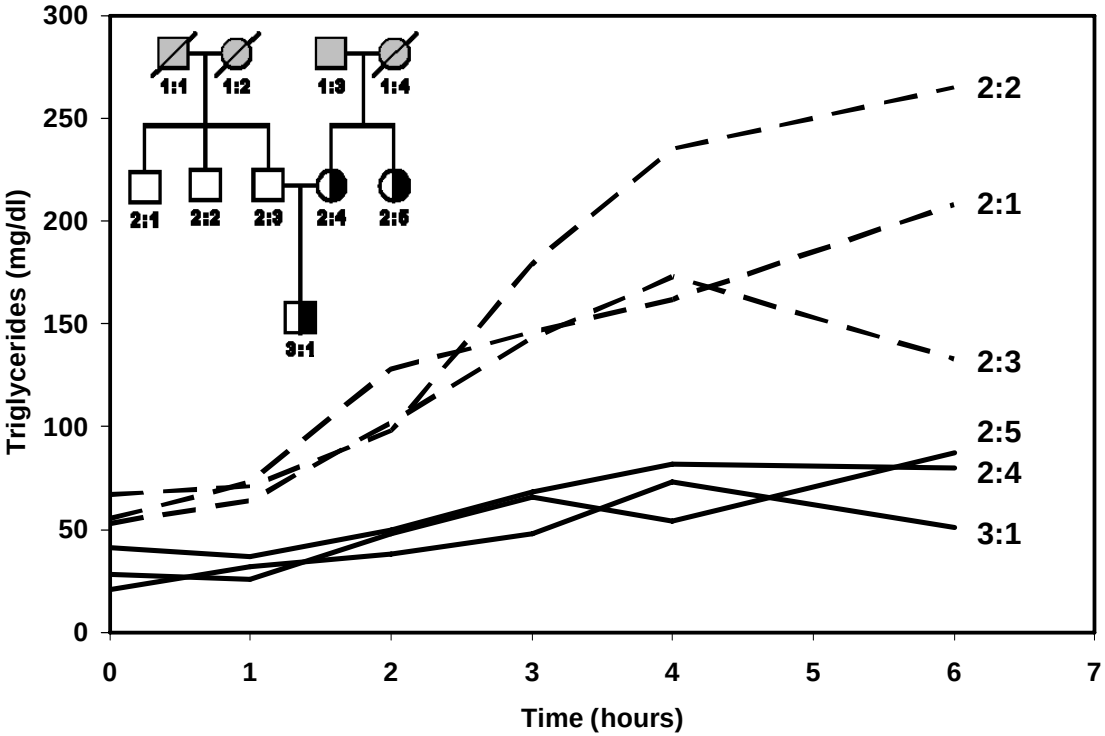


Figure S5: Segregation of the *APOC3* R19X mutation with hypotriglyceridemia and blunted postprandial triglyceride response in a representative family. Square symbols represent males and circles represent females. Individuals carrying the 19X allele are indicated by half-filled pedigree symbols and solid lines; their non-carrier relatives are indicated by open pedigree symbols and broken lines. Grayed individuals were not studied.

Figure S6

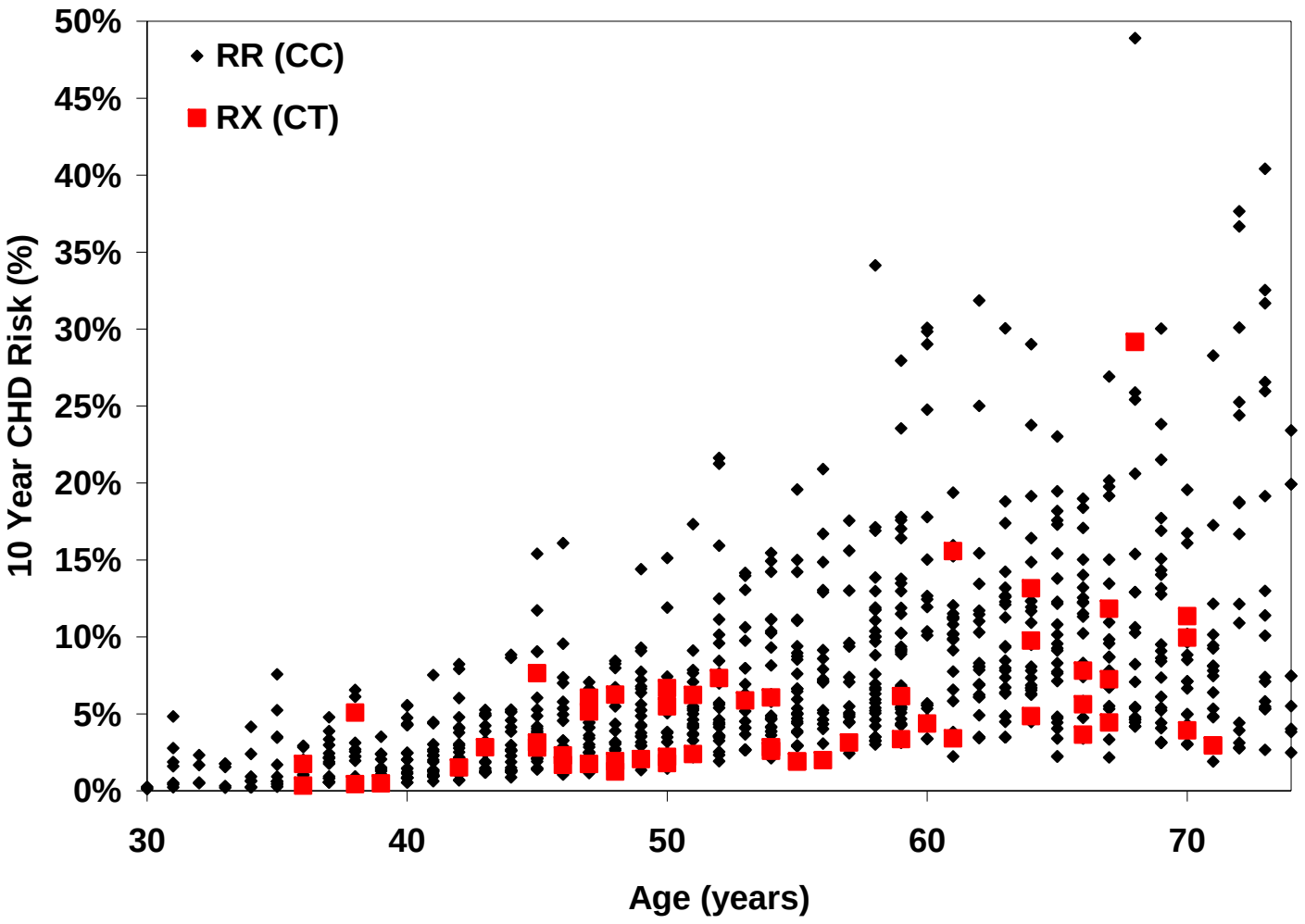


Figure S6: Scatter plot of Framingham 10 year risk of coronary heart disease *(S8)* by age and *APOC3* R19X genotype. Individuals heterozygous for the 19X allele are shown by red squares; those homozygous for the normal APOC3 allele shown with black diamonds.

Figure S7

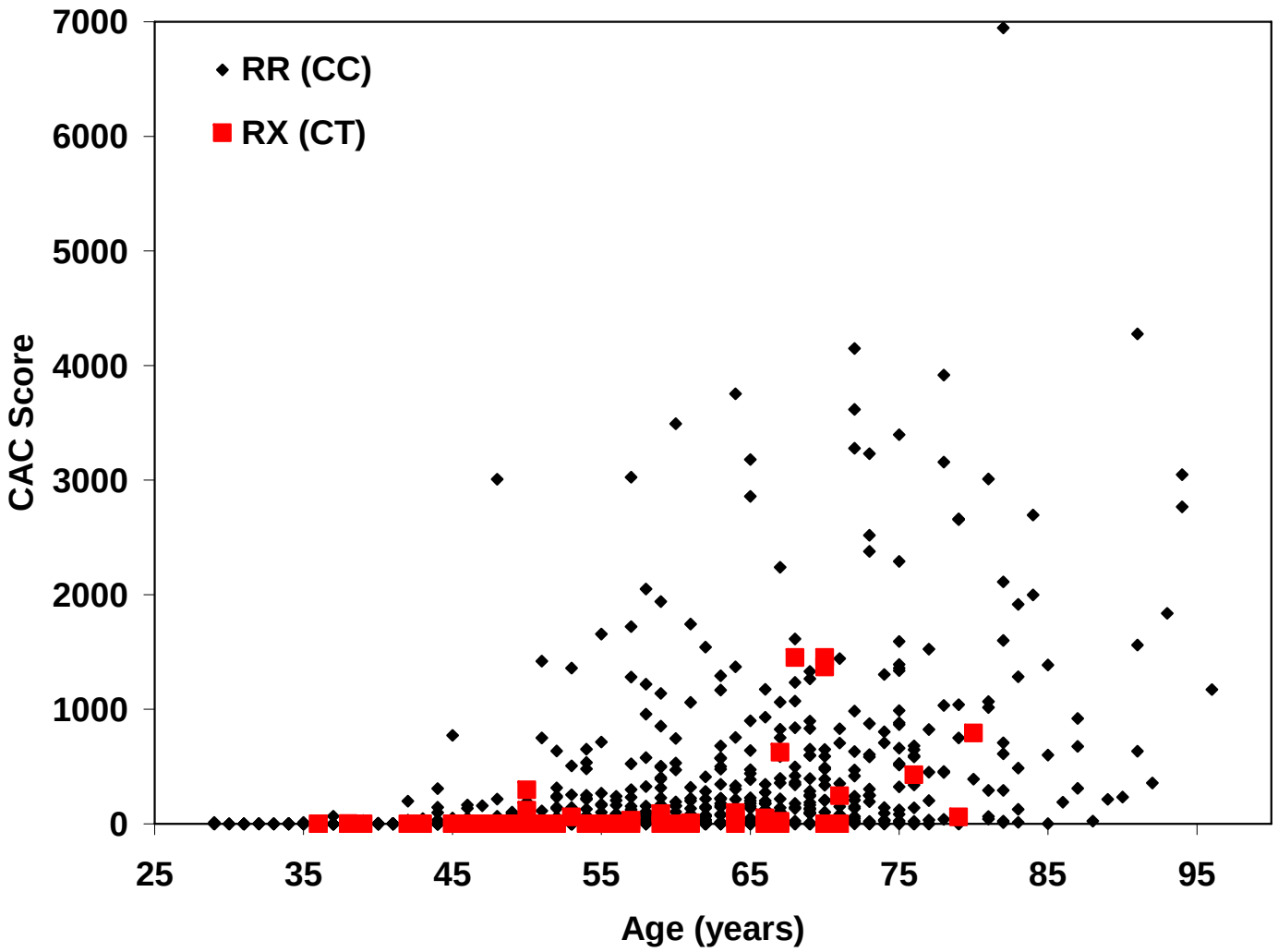


Figure S7: Scatter plot of coronary artery calcification score (CAC) in Agatston units by age and *APOC3* R19X genotype. Individuals heterozygous for the 19X allele are shown by red squares; those homozygous for the normal *APOC3* allele shown with black diamonds.

Figure S8

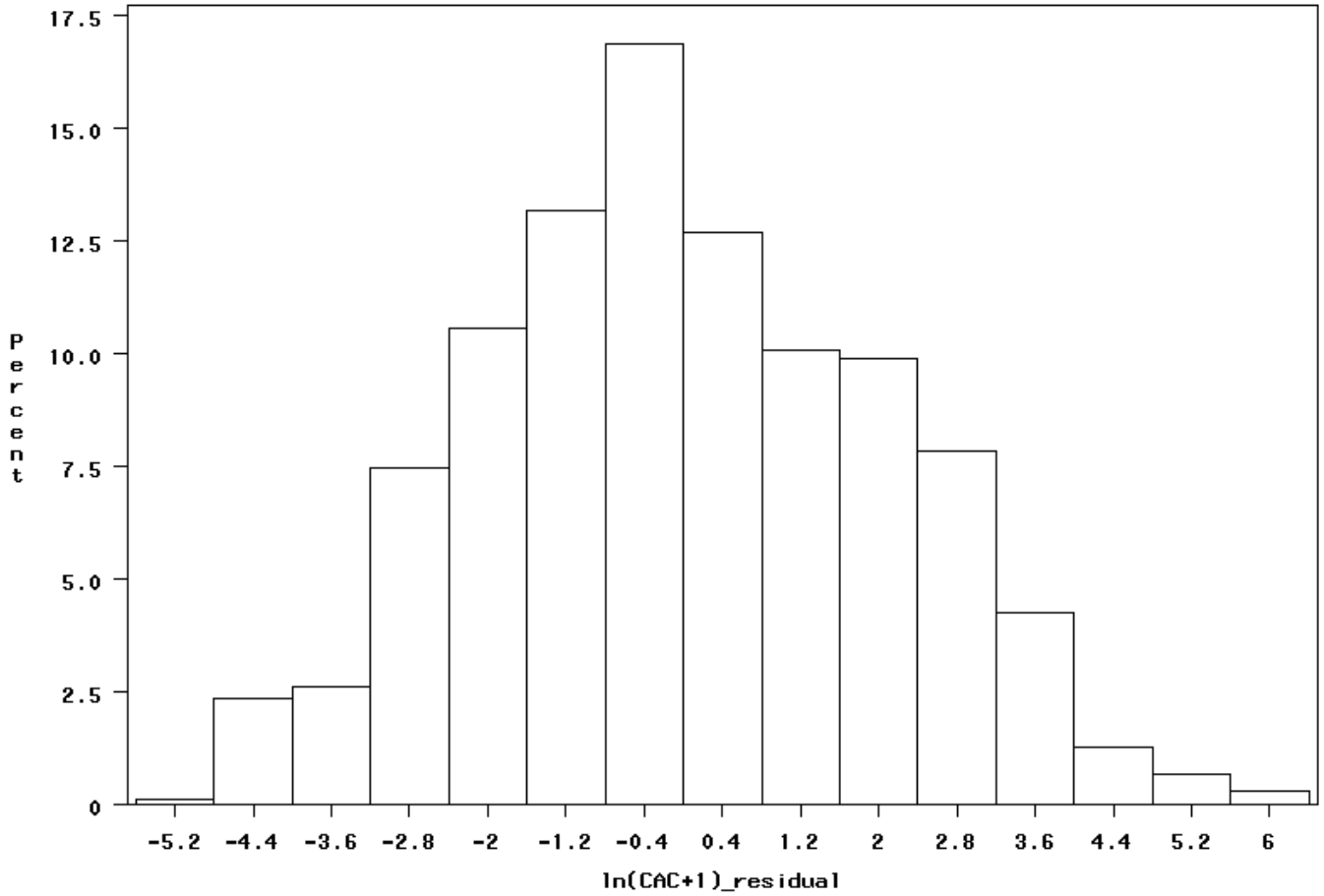


Figure S8: Distribution of residuals of the trait ln(CAC score + 1) adjusted for age and sex in 1,033 participants of the Amish Family Calcification Study.

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