Supplemental Data

A genome wide association study on lipoprotein(a) levels and coronary artery disease severity in a Chinese population

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Supplemental materials

Comparison of characteristics of patients within the two statins subgroups

The subjects included in this study received atorvastatin(AT) or rosuvastatin(RST). We compared the characteristics of the patients within the two statins subgroups. Data are presented as counts (%) for categorical variables and mean ± standard deviation for continuous variables. The homogeneity of the subgroups was assessed by the Student's t-test or χ^2 test to baseline data. As shown in Supplemental Table S1, the lipoprotein(a)(Lp[a]) level (30.83 ± 33.06 in AT users versus 28.74 ± 28.80 in RST users) and SYNTAX score (17.20 ± 10.32 in AT users versus 17.87 ± 11.12 in RST users) of the two subgroups did not exhibit significant differences ($P > 0.05$). This result indicates a homogeneity between the two subgroups and minimal bias in the subsequent analysis on the overall cohort. Notably, the total cholesterol (TC) and low-density lipoprotein cholesterol (LDLC) levels in RST users were higher than those of the AT users ($P < 0.05$). This result agrees with the effect of medication in clinical treatment because the RST has a stronger TC lowering effect than AT and physicians are likely to prescribe RST to patients with high levels of TC and LDLC.

Quantification of KIV-2 copy number variation

We randomly selected 645 subjects and used real-time fluorescence quota PCR for the relative quantification of the copy number of KIV-2 in the LPA gene by using the Bio-RAD CFX96 system and 96-well formats to evaluate the independence of Lp(a)-associated SNPs from KIV-2 CNV.

First, we synthetized the TaqMan® probe and primer for LPA KIV-2 exon2 in accordance with the study of Kamstrup PR(1). The probe and primers sequence were as follows:

Probe labeled by FAM 5′-CAACCTGACGCAATGC-3′;

Forward primer 5′-ATCCAGATGCTGTGGCAGCT-3′;

Reverse primer 5′-GCGACGGCAGTCCCTTCT-3′.

We used the RNase P (RNAP) control reagent (Part Number 4316844) as an endogenous single-copy control gene in accordance with Matthew B's method(2). The reaction volumes

included 1.25 uL of 20× TaqMan® primer/probe mix for LPA KIV-2; 1.25 uL of 20× TaqMan® RNAP control reagent, which was used as an endogenous single-copy control gene; 12.5 uL of 2× gene expression GX master mix; 5 uL of water; and 4 uL of genomic DNA. The thermocycler conditions were as follows: 95 °C hot-start for 10 min, followed by 40 cycles at 95 °C for 15 s, and 63 °C for 1 min. A disparity of 0.25 or more in CT value between the two duplicate samples was considered an error and required the sample to be rerun. ΔCT values were obtained by subtracting the CT value of the single-copy gene from the CT value of KIV-2 and were used to represent KIV-2 CNV.

Previously reported Lp(a)-associated SNPs in the present study.

We explored the replication of some previously reported SNPs in our data. For example, studies on Caucasian populations reported that rs3798220 and rs7412 in the LPA gene are related to Lp(a) levels. However, we found that they were nominally associated with Lp(a) levels (MAF = 0.09 and P = 0.0039 for rs3798220 and MAF = 0.06 and P = 0.0183 for rs7412). Other published SNPs (e.g., rs10455872 and rs143431368) were also included in our genotyping panel, but these SNPs were all rare in the Chinese population (both with MAF < 0.01 in our cohort and in 1KGP subjects). A full detailed list of reported SNPs associated with Lp(a) levels is shown in Table S4.

Reference

1. Kamstrup, P. R., A. Tybjaerg-Hansen, R. Steffensen, and B. G. Nordestgaard. 2009. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. *Jama* **301**: 2331-2339.

2. Lanktree, M. B., C. Rajakumar, J. H. Brunt, M. L. Koschinsky, P. W. Connelly, and R. A. Hegele. 2009. Determination of lipoprotein(a) kringle repeat number from genomic DNA: copy number variation genotyping using qPCR. *Journal of lipid research* **50**: 768-772.

Supplemental Tables

Supplemental Table S1: Baseline Characteristics of the subgroups with two different statins medication

Characteristics	Subgroups comparison		
	AT Users (n=1013)	RST Users (n=390)	P value
Demographic data			
Age	63.00 ± 10.07	62.04 ± 10.02	0.111
Sex (male)	807 (79.7)	312 (80.0)	0.889
BMI, kg/m2	24.25 ± 4.81	24.30 ± 3.35	0.854
Comorbidities			
Arrhythmia	91 (9.0)	15 (3.8)	0.001
Diabetes	278 (27.4)	107 (27.4)	0.998
Heart failure	89 (8.8)	31 (7.9)	0.615
Hypertension	609 (60.1)	207 (53.1)	0.017
Hyperlipidemia	117 (11.5)	57 (14.6)	0.119
Baseline biochemical measurements			
ALT, U/L	27.51 ± 13.25	27.76 ± 14.05	0.770
AST, U/L	26.71 ± 10.70	26.74 ± 11.32	0.964
CK, U/L	111.84 ± 112.19	106.82 ± 78.98	0.359
CREA,umol/L	86.52 ± 25.06	85.23 ± 26.06	0.133
CKMB, U/L	7.52 ± 5.97	7.05 ± 4.12	0.397
TC, mmol/L	4.29 ± 1.13	4.54 ± 1.49	0.003
LDLC, mmol/L	2.59 ± 0.93	2.81 ± 1.18	0.001
HDLC, mmol/L	0.97 ± 0.26	0.99 ± 0.24	0.154
TG, mmol/L	1.61 ± 1.13	1.62 ± 1.16	0.845
GLUC, mmol/L	6.72 ± 2.70	6.75 ± 2.69	0.818

Lpa, mg/dL	30.83 ± 33.06	28.74 ± 28.80	0.332
APOA, g/L	1.04 ± 0.28	1.06 ± 0.27	0.406
BNP, pg/mL	775.52 ± 1603.38	980.12 ± 2719.83	0.277
Medication			
β-blockers	904 (89.2)	350 (89.7)	0.821
ACEIs	646 (63.8)	234 (60.0)	0.183
CCBs	291 (28.7)	85 (21.8)	0.008
PPIs	488 (48.2)	191 (49.0)	0.800
SYNTAX score	17.20 ± 10.32	17.87 ± 11.12	0.289

Abbreviation as Table 1

Supplemental Table S2: Association of Lp(a) associated SNPs with CAD severity (as continuous variables)

Severity of CAD	SNP	Univariate analysis		Adjusted analysis	
		Estimates ± Se	P value	Estimates ± Se	P value
SYNTAX score	rs7770628	0.45 ± 0.64	0.4815		
	rs73596816	0.37 ± 0.87	0.6676		
	rs6926458	0.08 ± 0.40	0.8388		
	rs144217738	1.21 ± 0.64	0.0614	0.88 ± 0.71	0.2163
Count of heavy calcified lesions	rs7770628	0.01 ± 0.02	0.6382		
	rs73596816	0.01 ± 0.03	0.7415		
	rs6926458	0.01 ± 0.01	0.5936		
	rs9365171	0.03 ± 0.02	0.2790		
Count of long-range lesions	rs7770628	0.07 ± 0.04	0.0948	0.10 ± 0.05	0.0348
	rs73596816	0.13 ± 0.06	0.0329	0.19 ± 0.07	0.0044
	rs6926458	-0.03 ± 0.03	0.2303		
	rs144217738	0.05 ± 0.04	0.2553		

Supplemental Table S3: Independence of Lp(a) associated SNP from KIV-2 CNV

Locus	SNP	N	Lp(a) associated SNP		ΔCT[a]	
			Beta ± Se	P[b]	Beta ± Se	P[b]
LPA	rs7770628	644	0.64 ± 0.08	7.25E-14	-0.12 ± 0.04	0.0057
LPA	rs73596816	644	0.78 ± 0.11	4.58E-12	-0.13 ± 0.04	0.0040
LPA	rs6926458	644	-0.30 ± 0.06	6.21E-08	-0.13 ± 0.05	0.0041
SLC22A2	rs144217738	636	0.33 ± 0.09	1.36E-04	-0.15 ± 0.05	0.0010

[a] Δ CT values of KIV-2 CNV were included into the original models to evaluated the independence of four Lp(a) associated SNP from KIV-2 CNV.

[b] $P < 0.0125 = 0.05/4$ were consider significant.

Supplemental Table S4: Full list of 61 Lp(a) significantly associated SNPs in GWAS

SNP	EA[a]	N	Beta	Se	P value	Dependent SNP	LD[b] (r2)
rs4708876	C	1145	0.73	0.06	2.01E-30	rs7770628	1
rs7770628	C	1147	0.73	0.06	2.29E-30	-	-
rs56393506	T	1121	0.72	0.06	3.82E-28	rs7770628	1
rs7454595	C	1110	0.70	0.07	2.82E-23	rs7770628	0.94
rs73596816	A	1147	0.86	0.09	1.27E-22	-	-
rs62442784	C	1129	-0.34	0.04	2.81E-16	rs6926458	1
rs7770685	T	1129	-0.34	0.04	2.81E-16	rs6926458	1
rs1406889	C	1128	-0.34	0.04	2.94E-16	rs6926458	1
rs7761377	G	1115	-0.34	0.04	3.65E-16	rs6926458	0.99
rs13202636	C	1139	-0.34	0.04	3.78E-16	rs6926458	1
rs1830522	T	1126	-0.34	0.04	3.96E-16	rs6926458	1
rs6926896	G	1140	-0.34	0.04	4.10E-16	rs6926458	1
rs35600881	A	1141	-0.34	0.04	4.18E-16	rs6926458	1
rs6940254	G	1141	-0.34	0.04	4.18E-16	rs6926458	1
rs9355297	G	1141	-0.34	0.04	4.18E-16	rs6926458	1
rs6926458	G	1147	-0.34	0.04	4.59E-16	-	-
rs12175867	C	1142	-0.34	0.04	4.93E-16	rs6926458	1
rs113727842	G	1129	-0.33	0.04	1.33E-15	rs6926458	0.99
rs6455696	C	1129	-0.33	0.04	1.33E-15	rs6926458	0.99
rs36115561	T	1128	-0.33	0.04	1.45E-15	rs6926458	0.99
rs6905073	T	1128	-0.33	0.04	1.45E-15	rs6926458	0.99
rs6905422	C	1128	-0.33	0.04	1.45E-15	rs6926458	0.99
rs6933576	G	1128	-0.33	0.04	1.45E-15	rs6926458	0.99
rs6929299	C	1133	-0.33	0.04	1.54E-15	rs6926458	0.99
rs10945682	A	1129	-0.33	0.04	1.71E-15	rs6926458	0.99
rs10945683	C	1129	-0.33	0.04	1.71E-15	rs6926458	0.99

rs1569933	T	1129	-0.33	0.04	1.71E-15	rs6926458	0.99
rs2983236	G	1129	-0.33	0.04	1.71E-15	rs6926458	0.99
rs7760585	G	1129	-0.33	0.04	1.71E-15	rs6926458	0.99
rs9295130	A	1129	-0.33	0.04	1.71E-15	rs6926458	0.99
rs1950563	C	1128	-0.33	0.04	1.78E-15	rs6926458	0.99
rs1950564	T	1128	-0.33	0.04	1.78E-15	rs6926458	0.99
rs2144726	A	1128	-0.33	0.04	1.78E-15	rs6926458	0.99
rs9355815	G	1134	-0.33	0.04	1.86E-15	rs6926458	0.99
rs6455695	C	1138	-0.33	0.04	1.94E-15	rs6926458	0.99
rs6919497	G	1138	-0.33	0.04	1.94E-15	rs6926458	0.99
rs6923917	T	1138	-0.33	0.04	1.94E-15	rs6926458	0.99
rs6937879	A	1138	-0.33	0.04	1.94E-15	rs6926458	0.99
rs9355813	C	1138	-0.33	0.04	1.94E-15	rs6926458	0.99
rs9355814	T	1138	-0.33	0.04	1.94E-15	rs6926458	0.99
rs6415085	G	1139	-0.33	0.04	1.98E-15	rs6926458	0.99
rs6455691	A	1139	-0.33	0.04	1.98E-15	rs6926458	0.99
rs6455692	A	1139	-0.33	0.04	1.98E-15	rs6926458	0.99
rs6455693	T	1139	-0.33	0.04	1.98E-15	rs6926458	0.99
rs6902102	A	1139	-0.33	0.04	1.98E-15	rs6926458	0.99
rs6930342	G	1139	-0.33	0.04	1.98E-15	rs6926458	0.99
rs6932014	G	1139	-0.33	0.04	1.98E-15	rs6926458	0.99
rs9365179	T	1139	-0.33	0.04	1.98E-15	rs6926458	0.99
rs6913833	C	1139	-0.33	0.04	2.12E-15	rs6926458	0.99
rs7771129	G	1139	-0.33	0.04	2.12E-15	rs6926458	1
rs9456552	T	1138	-0.33	0.04	2.17E-15	rs6926458	0.99
rs13192132	C	1140	-0.33	0.04	2.31E-15	rs6926458	0.99
rs7752408	C	1140	-0.33	0.04	2.31E-15	rs6926458	0.99
rs2092931	G	1126	-0.33	0.04	2.39E-15	rs6926458	0.99

rs2872764	G	1126	-0.33	0.04	2.39E-15	rs6926458	0.99
rs1321196	C	1121	-0.33	0.04	3.02E-15	rs6926458	0.99
rs1740428	A	1125	-0.33	0.04	3.57E-15	rs6926458	0.99
rs138400966	C	1106	-0.33	0.04	5.53E-15	rs6926458	0.99
rs7769720	T	1120	-0.32	0.04	1.06E-14	rs6926458	0.99
rs9365171	A	1147	-0.31	0.04	1.23E-12	rs6926458	0.64
rs144217738	G	1136	0.34	0.06	6.90E-08	-	-

[a]EA: effect allele

[b]LD: linkage disequilibrium

Supplemental Table S5: Full list of reported Lp(a) associated SNPs

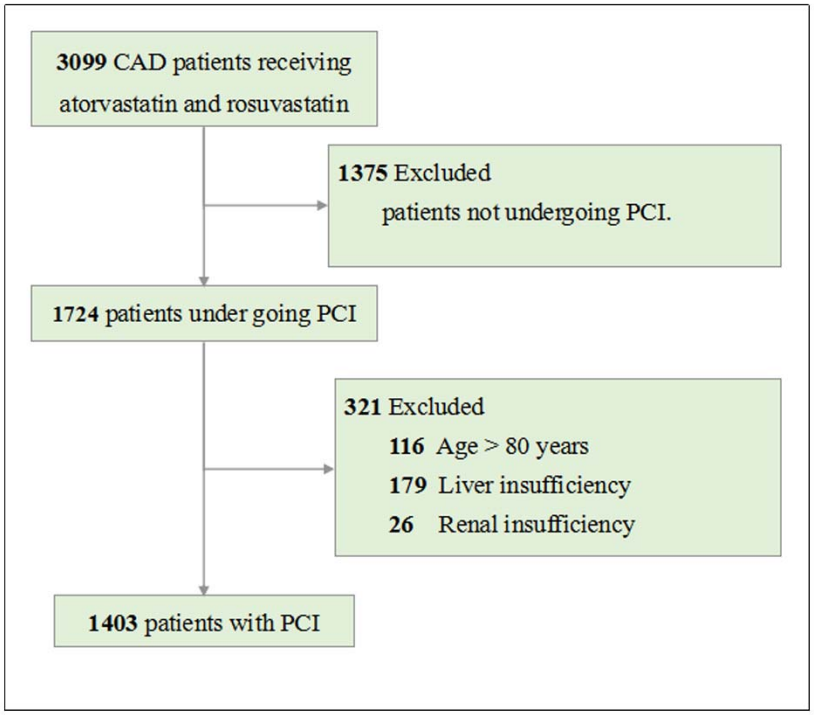
CHR:BP	SNP	EA	N	Beta	Se	P	Referrence (PMID)
6:160894878	rs1510224	C	1126	-0.26	0.08	0.0011	28512139
6:160961137	rs3798220	C	1147	-0.22	0.08	0.0039	28566218
6:161082695	rs1367211	T	1147	-0.14	0.05	0.0121	26377243
6:154896235	rs17539620	T	1111	0.13	0.05	0.0161	26377243
19:45412079	rs7412	T	1147	-0.20	0.08	0.0183	29875488;29777097
6:160767905	rs520829	G	1111	-0.10	0.05	0.0408	28512139
6:159335251	rs9457516	T	1147	0.10	0.05	0.0445	25575512
6:161586840	rs729986	T	1147	-0.10	0.05	0.0505	19124843
6:159721945	rs10806706	A	1123	-0.09	0.05	0.0572	19124843
6:154005613	rs2186140	C	1092	-0.07	0.04	0.119	19124843
6:160741622	rs3120139	A	1147	0.08	0.05	0.1488	21900290
6:161137779	rs14224	C	1121	0.06	0.04	0.1709	19124843
6:161137990	rs783147	G	1147	0.05	0.04	0.2112	21900290
14:89375249	rs10484009	T	1144	0.06	0.05	0.2525	17903299
6:156916766	rs9384478	A	1147	-0.05	0.05	0.2612	25575512
8:107812675	rs2930485	G	1110	-0.06	0.06	0.2761	17903299
6:159436594	rs2057061	G	1119	-0.05	0.04	0.2862	19124843
6:162996263	rs9458611	T	1119	-0.06	0.05	0.2875	19124843
6:159723503	rs4708818	G	1119	0.05	0.05	0.3124	19124843
6:159167285	rs9456350	G	1136	0.06	0.07	0.3259	19124843
6:156628874	rs9397922	C	1147	-0.04	0.04	0.3475	19124843
6:157443353	rs6917698	G	1103	0.05	0.05	0.3572	19124843
6:159425707	rs2016588	C	1101	-0.04	0.04	0.3631	19124843
6:161917337	rs6937817	C	1109	-0.05	0.06	0.3691	19124843
6:159105413	rs1979541	G	1143	0.06	0.07	0.4103	26377243
6:161916248	rs10755582	T	1143	-0.05	0.06	0.4147	19124843

6:161915982	rs992421	T	1143	-0.05	0.06	0.4147	19124843
2:227621831	rs10498211	A	1140	-0.07	0.08	0.4303	17903299
6:162979147	rs4636000	C	1140	0.04	0.05	0.4352	19124843
6:162994639	rs2155510	T	1138	0.04	0.05	0.453	19124843
6:162994752	rs1012424	G	1132	0.03	0.05	0.4911	19124843
6:160796058	rs1018234	T	1136	-0.03	0.04	0.532	28512139
6:162209898	rs3019433	A	1145	0.06	0.09	0.5335	19124843
2:222505204	rs1356702	G	1130	-0.03	0.04	0.5385	17903299
6:162210934	rs3016557	T	1144	0.05	0.09	0.5408	19124843
6:158263456	rs9346930	G	1147	0.04	0.07	0.5525	25575512
6:158371084	rs9347800	C	1147	0.04	0.06	0.579	25575512
6:162474893	rs9456734	A	1133	-0.02	0.04	0.5966	19124843
6:156969316	rs1246182	G	1126	0.02	0.04	0.6047	19124843
6:159801024	rs1544167	G	1147	0.02	0.05	0.6179	25575512
6:156886834	rs288945	A	1120	-0.04	0.08	0.6491	19124843
6:162192994	rs9458363	T	1118	-0.02	0.04	0.6636	19124843
6:158219013	rs9356009	C	1147	0.03	0.06	0.674	25575512
6:152820395	rs2623963	C	1106	-0.02	0.05	0.7066	19124843
6:156150733	rs17086702	T	1147	-0.03	0.09	0.71	25575512
6:152852284	rs7745725	G	1139	-0.02	0.05	0.7243	19124843
6:160745751	rs384156	C	1091	-0.01	0.04	0.7674	19124843
6:163636248	rs2206256	T	1142	-0.02	0.05	0.7747	19124843
6:159340672	rs9365009	A	1147	0.01	0.05	0.776	19124843
6:162248169	rs2022991	C	1147	-0.01	0.04	0.7866	19124843
6:163639850	rs11966842	C	1127	-0.01	0.05	0.7928	19124843
6:153065487	rs633596	G	1147	-0.01	0.05	0.8003	19124843
6:159155998	rs2129209	C	1147	0.01	0.05	0.8196	19124843
6:159146871	rs6455600	T	1147	0.01	0.05	0.831	19124843

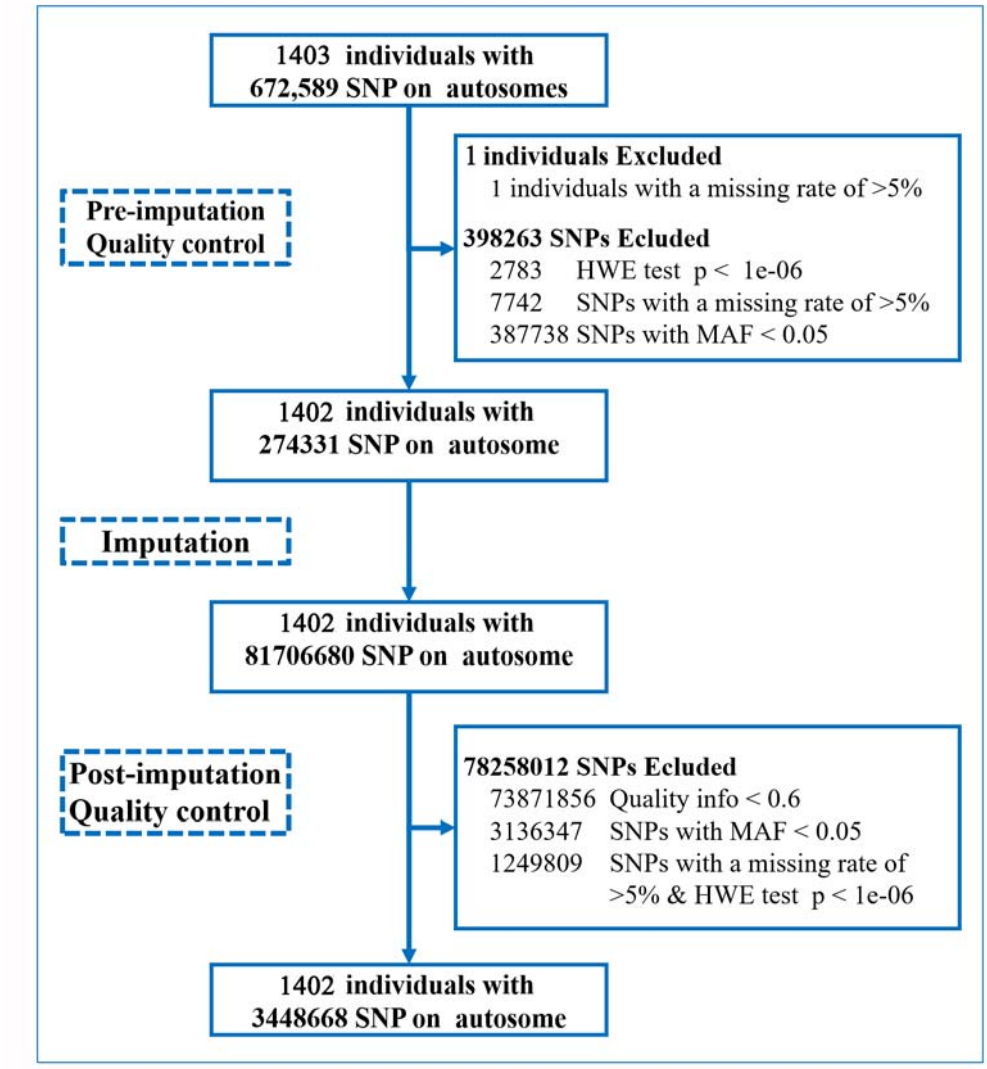
6:160868701	rs2457574	G	1143	0.01	0.04	0.8327	28512139
6:160865645	rs7769879	C	1143	0.01	0.04	0.865	28512139
6:160737581	rs446809	A	1130	0.01	0.04	0.8684	19124843
6:162192874	rs3016562	G	1140	-0.01	0.04	0.872	19124843
7:112969420	rs10500024	G	1099	0.01	0.04	0.8782	17903299
6:160471191	rs8191818	G	1131	0.01	0.05	0.8934	19124843
6:160966347	rs9365169	C	1145	0.01	0.04	0.8957	28512139
6:162192856	rs3016563	C	1147	-0.01	0.04	0.8972	19124843
6:160479478	rs8191829	A	1130	0.0047	0.05	0.9196	19124843
6:161697400	rs3757037	G	1147	0.0040	0.04	0.9251	26377243
6:164982150	rs2201806	T	1110	0.0027	0.04	0.9503	19124843
6:160735746	rs410569	G	1142	-0.0025	0.04	0.9523	19124843
6:165316048	rs6455970	G	1147	-0.0034	0.07	0.9581	19124843
6:161207892	rs2115869	G	1119	-0.0021	0.04	0.9587	19124843
9:5883409	rs10733517	G	1147	-0.0022	0.05	0.9643	17903299
6:159202240	rs744893	C	1101	-0.0037	0.09	0.9688	19124843
6:163640262	rs11966948	C	1123	0.0017	0.04	0.969	19124843
6:158812361	rs705936	A	1094	-0.0025	0.08	0.976	19124843
6:160738920	rs667538	G	1125	0.0004	0.04	0.9917	19124843
6:156886655	rs288944	T	1118	-0.0004	0.07	0.9955	19124843
6:162609719	rs9295184	C	1120	-0.0002	0.05	0.9963	19124843

Supplemental Figure

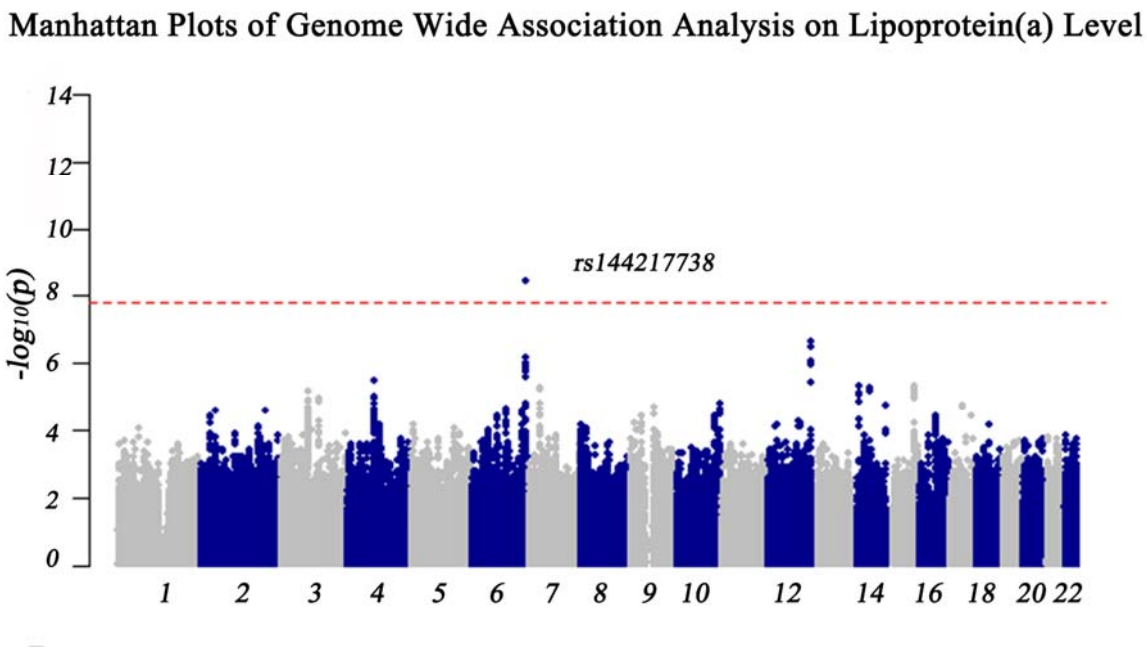
Supplemental Fig. S1 Flow chart of screening study subjects.



Supplemental Fig. S2 Flow chart of genotype data processing



Supplemental Fig. S3 Manhattan plots of further genome wide association analysis on Lp(a) level.



Supplemental Fig. S4 Correlation plot of KIV-2 and Lp(a) levels