

ASSOCIATION STUDIES ARTICLE

A multi-ancestry genome-wide study incorporating gene–smoking interactions identifies multiple new loci for pulse pressure and mean arterial pressure

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Abstract

Elevated blood pressure (BP), a leading cause of global morbidity and mortality, is influenced by both genetic and lifestyle factors. Cigarette smoking is one such lifestyle factor. Across five ancestries, we performed a genome-wide gene–smoking interaction study of mean arterial pressure (MAP) and pulse pressure (PP) in 129 913 individuals in stage 1 and follow-up analysis in 480 178 additional individuals in stage 2. We report here 136 loci significantly associated with MAP and/or PP. Of these, 61 were previously published through main-effect analysis of BP traits, 37 were recently reported by us for systolic BP and/or diastolic BP through gene–smoking interaction analysis and 38 were newly identified ($P < 5 \times 10^{-8}$, false discovery rate < 0.05). We also identified nine new signals near known loci. Of the 136 loci, 8 showed significant interaction with smoking status. They include CSMD1 previously reported for insulin resistance and BP in the spontaneously hypertensive rats. Many of the 38 new loci show biologic plausibility for a role in BP regulation. SLC26A7 encodes a chloride/bicarbonate exchanger expressed in the renal outer medullary collecting duct. AVPR1A is widely expressed, including in vascular smooth

muscle cells, kidney, myocardium and brain. *FHAD1* is a long non-coding RNA overexpressed in heart failure. *TMEM51* was associated with contractile function in cardiomyocytes. *CASP9* plays a central role in cardiomyocyte apoptosis. Identified only in African ancestry were 30 novel loci. Our findings highlight the value of multi-ancestry investigations, particularly in studies of interaction with lifestyle factors, where genomic and lifestyle differences may contribute to novel findings.

Introduction

Elevated blood pressure (BP), a leading cause of morbidity and mortality worldwide, is known to be influenced by both genetic and lifestyle factors. To date genome-wide association studies (GWAS) have identified over 1000 loci associated with BP and hypertension (1–10). The effects of genetic variants on BP may manifest differently depending on lifestyle exposures. Therefore, incorporating gene–environment (G×E) interactions may identify additional loci (11,12). We established the Gene–Lifestyle Interactions Working Group within the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium in order to assess the impact of interactions with multiple lifestyle factors on the genetics of cardiovascular traits (13). Among many lifestyle factors, cigarette smoking influences BP in both acute (14) and chronic (15) fashion, motivating genetic association studies of gene-by-smoking interactions.

Recently we reported findings from a genome-wide association meta-analysis incorporating gene–smoking interactions for systolic BP (SBP) and diastolic BP (DBP) (16). In addition to SBP and DBP, BP can also be characterized as having both steady and pulsatile components, each determined by different physiologic properties of the heart and vasculature and differently related to cardiovascular outcomes. Mean arterial pressure (MAP) reflects the steady component of BP, which is predominantly determined by cardiac output and systemic vascular resistance and regulated by small artery and arteriole tone (17). MAP has been found to be more ‘informative’ than SBP and DBP in predicting mortality from cardiovascular disease including stroke and ischemic heart disease (18,19). Pulse pressure (PP) represents the pulsatile

component of BP and is largely determined by cardiac stroke volume and large artery stiffness (17,20). PP has been found to be predictive of coronary heart disease risk and, in some cases, superior to both SBP and DBP, in particular for older adults (21,22). Thus, while SBP is prioritized as the primary treatment target for hypertension (23), MAP and PP continue to be relevant BP traits for investigation. Understanding their biological underpinnings may lead to discovery of new BP pathways.

In this study, we performed a genome-wide association meta-analysis of MAP and PP incorporating gene–smoking interactions (Fig. 1). The aim is to evaluate whether any of the previously identified BP loci are modified by smoking, whether interactions can be identified using a genome-wide approach and whether additional novel BP loci can be identified by accounting for potential single nucleotide polymorphism (SNP)–smoking interactions. Here, we report our findings through two degrees of freedom (DF) test that jointly evaluates genetic main and interaction effects (24) based on 610 091 individuals across five ancestries.

Results

Overview

Across five ancestries, we performed a genome-wide gene–smoking interaction study of MAP and PP in 129 913 individuals in stage 1 and follow-up analysis in 480 178 additional individuals in stage 2: summary information is in Table 1 (Supplementary Materials, Tables S1–S6). Through genome-wide search in stage 1, we identified 1692 significant ($P \leq 5 \times 10^{-8}$) and 2681 suggestive ($P \leq 10^{-6}$) variants associated with MAP and/or PP.

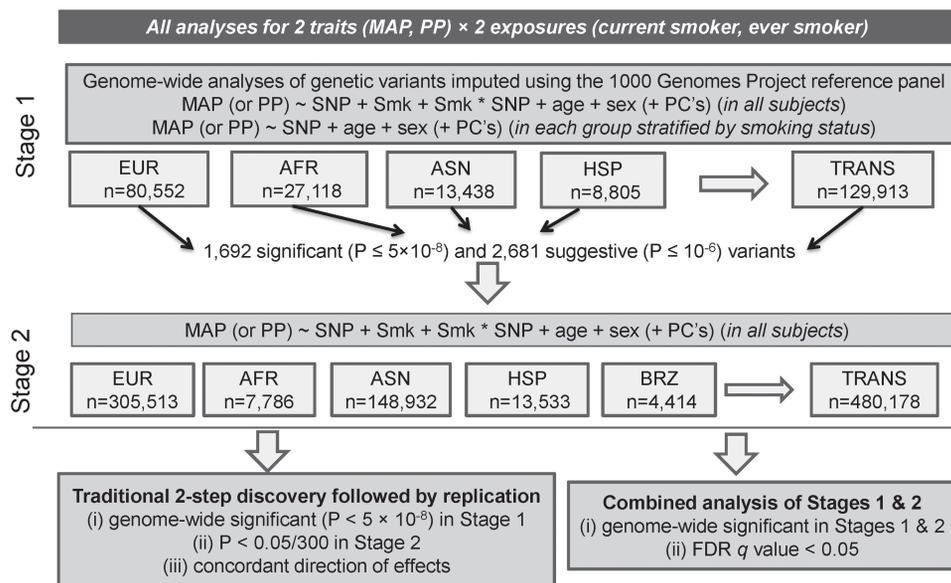


Figure 1. Study design. Summary of data included in this study. Smk: smoking status (considering either current smoking or ever smoking status separately); PC: principal component; EUR: European; AFR: African; ASN: Asian; HIS: Hispanic; BRZ: Brazilian; TRANS: trans-ancestry (i.e. combining all ancestry groups through meta-analysis).

Table 1. Basic characteristics of cohorts in stages 1 and 2 in each ancestry

	Current smoker		Former smoker		Never smoker		Male	HTN	HT meds	Age		MAP		PP	
	N	%	N	%	N	%				Mean	SD	Mean	SD	Mean	SD
Stage 1															
EUR	14 607	18.1	28 409	35.3	37 535	46.6	32.6	38.2	25.4	54.63	8	94.63	12.9	52.02	13.3
AFR	5545	21.5	7185	27.8	13 121	50.8	26.5	55.9	39.5	54.49	9.1	99.96	14.9	54.67	16.4
ASN	2465	18.3	1677	12.5	9296	69.2	51.2	46.9	27	55.42	9.7	98.70	13.4	57.86	15.8
HIS	1068	12.1	2160	24.5	5577	63.3	24.9	43.5	13.3	55.5	11	94.80	13.9	53.55	16.4
Stage 1 total	23 685	18.4	39 431	30.7	65 529	50.9	32.8	43.1	27.7	54.74	8.6	96.17	13.4	53.28	14.4
Stage 2															
EUR	48 198	17	89 597	31.6	145 914	51.4	47.8	44.8	25	55.91	8.6	102.17	13.5	55.29	13.9
AFR	1971	29.8	1579	23.8	3075	46.4	40.9	54.3	42.8	53.66	10.2	101.21	14.7	53.68	14.8
ASN	29 485	19.8	40 850	27.4	78 597	52.8	54.9	50.3	33.1	60.76	12.3	98.31	13.9	54.91	14.0
HIS	2739	20.3	2559	18.9	8231	60.8	41	26.9	16.3	45.86	13.8	91.36	13.7	48.99	13.3
BRZ	998	22.6	514	11.6	2902	65.8	48	15.5	6.3	27.78	3.2	89.75	12.3	45.23	9.8
Stage 2 total	83 391	18.2	135 099	29.6	238 719	52.2	49.7	45.9	27.4	56.84	9.9	100.54	13.7	54.88	13.9
TOTAL	107 076	18.3	174 530	29.8	304 248	51.9	46.1	45.3	27.4	56.4	9.6	99.61	13.6	54.54	14.0

The cell entries for the covariates and BP traits correspond to sample-size-weighted averages across all cohorts in each category. EUR: European; AFR: African; ASN: Asian; HIS: Hispanic; BRZ: Brazilian; ALL: *trans*-ancestry (i.e. combining all ancestry groups through meta-analysis); HTN: hypertension; MAP: mean arterial pressure; PP: pulse pressure.

Of these 4373 variants, 2982 variants were replicated in stage 2 with $P < 0.05/4373$ (to an aggregate replication rate of 68.2%). Of the 1692 significant variants in stage 1, a total of 1449 were replicated in stage 2 with $P < 0.05/1692$ to a replication rate of 85.6%. Among the genome-wide significant variants in stage 1, which resided in 112 loci (defined by physical distance ± 1 Mb), 53 loci were formally replicated in stage 2 using Bonferroni-adjusted significance levels ($P < 0.05/112$). Most of the remaining 59 loci were identified in African or Hispanic ancestries in stage 1, which quite plausibly failed to replicate in stage 2 due to these smaller sample sizes and hence lack of power. For 10 loci, no additional data were available in stage 2, and therefore, it was not possible to check for replication. All of these formally replicated loci had been identified previously: 44 through main effects GWAS (1–8) and 9 through gene–smoking interaction analysis we reported recently for SBP and DBP (16). For these nine formally replicated loci, estimates of the genetic main effects were all consistent between stages 1 and 2; estimates of SNP–smoking interaction effects were not statistically significant (Supplementary Material, Table S7).

We performed meta-analysis combining stages 1 and 2 (Manhattan plots, Supplementary Material, Fig. S1; quantile–quantile, QQ, plots, Supplementary Material, Fig. S2). Through this combined analysis with 610 091 individuals, we identified 136 loci that were associated with MAP and/or PP at genome-wide significance ($P \leq 5 \times 10^{-8}$). Of these, 61 loci were previously published through main effects GWAS for any BP trait (1–8), 37 loci (presented in Supplementary Material, Table S7) were recently reported by us for SBP and/or DBP through gene–smoking interaction analysis (16) and the remaining 38 loci are newly reported here (Table 2).

Among the 136 loci associated with MAP and/or PP, 38 loci are completely new and at least 1 Mb away from any of known BP loci. A total of 16 novel loci passed a more stringent threshold ($P < 6.25 \times 10^{-9}$, adjusted for two smoking exposures, two tests and two BP traits). We also identified nine additional new signals within the known BP loci but not in linkage disequilibrium (LD), $r^2 < 0.1$, with known BP loci (Table 3). Among the nine identified signals, four signals were identified in *trans*-ancestry, and the remaining five were ancestry-specific (two European, two African and one Hispanic signals). The LocusZoom plots

for these completely novel 38 loci and 9 signals are shown in Supplementary Material, Figure S3. As shown in Venn diagram (Fig. 2), among 38 new loci and 9 signals, 38 were newly PP associated and 12 were newly MAP associated (with 3 common between PP and MAP). These were not associated with SBP or DBP. False discovery rate (FDR) q -values provided additional evidence for these newly identified loci (FDR < 0.01 for 43 of the 47 and FDR < 0.05 for all 47 loci or signals).

Supplementary Material, Table S8 presents more detailed results for the lead variants representing the 136 loci and the 9 signals associated with MAP and PP: ancestry-specific and *trans*-ancestry meta-analysis results within each stage (1 and 2) and ancestry-specific and *trans*-ancestry meta-analysis results combining stages 1 and 2. Scatterplots comparing ancestry-specific genetic effects at these variants are presented in Supplementary Material, Figure S4. Genetic effects between European and Hispanic ancestries had the highest correlation (0.79), whereas those between African and Hispanic ancestries had the lowest correlation (0.29).

The role of interactions

Among the 136 loci and 9 new signals associated with MAP and/or PP, variants at 8 loci showed genome-wide significant interactions (1 DF interaction $P < 5 \times 10^{-8}$) with smoking status (Fig. 3). All eight loci were identified with current smoking status; these variants have larger effects in current smokers than in non-current smokers. Of the eight loci, six loci showed increasing effects on BP in current-smokers. Five interactions were newly identified (Table 2), and the other three were previously reported for SBP or DBP (Supplementary Material, Table S7). These variants showing interaction effects were identified only in individuals of African ancestry in stage 1. These variants were not present in stage 2 because of the limited sample size (ranges from 418 to 1993) of stage 2 African ancestry cohorts, and therefore, replication of these interactions was not possible.

BP variance explained

Within each of the smoking strata, we computed the variance of MAP and PP explained by genome-wide results (25) in European

Table 2. Thirty-eight new loci associated with MAP and/or PP that are at least 1 Mb away from any known BP locus

Locus	rsID	Nearest gene	Position	EAF	Race	Trait/exposure	G effect	G StdErr	G×E effect	G×E StdErr	Interaction P	Joint P	FDR q value	N
1	rs115356163	PADI2	1:17466024	0.02	AFR	PP/CS	0.22	0.87	-7.70	1.53	0.04	5.17E-09*	3.63E-05	12 712
2	rs147515295	EYA3; SESN2	1:28389841	0.98	HIS	MAP/ES	2.94	1.04	2.80	1.52	0.10	3.47E-08	0.018721	7287
3	rs11587661	COG2	1:230671208	0.02	AFR	PP/CS	0.44	0.86	-7.63	1.51	1.31E-06	4.95E-08	0.010168	13 888
4	rs138318054	KIAA1804	1:233578559	0.02	AFR	PP/CS	-0.37	0.93	-7.58	1.66	1.40E-05	4.84E-08	0.010095	10 787
5	rs79113694	GALNT14	2:31253799	0.03	AFR	PP/ES	-0.60	0.58	-2.91	0.83	1.98E-04	7.65E-09	5.96E-05	25 557
6	rs183927068	MAP2	2:210288479	0.98	AFR	MAP/CS	-0.60	1.09	11.29	2.02	8.36E-08	2.05E-09*	0.001619	7925
7	rs75875736	STAC	3:36341106	0.02	AFR	PP/ES	-3.49	0.58	3.15	0.94	1.23E-03	1.41E-08	0.000108	21 985
8	rs116199364	CLSTN2	3:139951198	0.02	AFR	PP/CS	1.94	0.92	-10.54	1.88	2.23E-08	1.04E-07	0.000675	10 787
9	rs114619985	BOD1L	4:13599930	0.02	AFR	PP/ES	-2.74	0.78	-1.86	1.13	0.04	2.71E-10*	2.61E-06	18 015
10	rs201223145	PRDM5	4:121706475	0.97	AFR	PP/CS	2.67	0.68	2.91	1.39	0.12	5.91E-09*	0.001905	15 574
11	rs147998309	PCDH10	4:133596832	0.99	AFR	PP/CS	1.61	1.18	12.94	2.64	1.78E-06	2.41E-09*	1.74E-05	7925
12	rs146622638	GPM6A	4:176524533	0.97	AFR	PP/ES	2.76	0.65	0.16	0.98	0.95	4.55E-08	0.000334	21 332
13	rs72723039	IRX2	5:2664169	0.98	AFR	PP/CS	-1.69	1.10	10.76	1.88	2.39E-08	6.55E-09	0.002064	7925
14	rs79205226	CDKAL1	6:21103825	0.02	AFR	PP/CS	1.46	0.68	-7.94	1.30	1.60E-09	3.38E-09*	2.41E-05	15 574
15	rs200495667	ALDH8A1	6:135152480	0.08	ASN	PP/CS	-2.48	0.41	2.63	0.92	3.11E-03	1.50E-08	0.000378	10 110
16	rs19090939	ACTR3B	7:152802243	0.01	AFR	PP/CS	-0.01	1.12	-11.94	2.24	1.86E-07	5.41E-09*	3.79E-05	7925
17	rs140994551	CSMD1	8:4449086	0.01	AFR	PP/CS	0.43	1.07	-11.39	1.89	4.34E-09	2.07E-11*	1.93E-07	7925
18	rs7817784	TNKS	8:9682553	0.57	EUR	MAP/CS	-0.23	0.03	0.05	0.08	0.89	6.93E-13*	2.59E-09	364 584
19	rs12156238	FAM167A	8:11285135	0.19	EUR	MAP/ES	-0.30	0.06	0.10	0.08	0.29	1.03E-08	1.69E-05	349 729
20	MERGED_DEL_2_50178	PKIA	8:92188440	0.01	EUR	MAP/CS	1.60	1.34	-9.18	1.86	6.30E-07	1.25E-08	3.56E-05	9465
21	rs11991823	LRRC69; SLC26A7	8:92188440	0.37	Trans	PP/ES	-0.23	0.03	0.06	0.05	0.43	1.29E-15*	8.89E-11	552 719
22	rs7823377	TRHR	8:110073120	0.63	Trans	PP/CS	-0.15	0.03	0.05	0.06	0.41	3.90E-08	0.000260	583 554
23	rs76209156	KDM4C	9:7423109	0.99	AFR	PP/CS	0.03	1.19	10.43	2.14	1.96E-06	2.94E-08	0.000197	7925
24	rs77548020	FUJ41200; NFIB	9:13480744	0.98	AFR	PP/CS	0.75	0.83	7.27	1.59	1.56E-04	1.91E-08	0.00013	10 787
25	rs75872665	LOC100128811	10:25388468	0.99	AFR	PP/CS	0.08	1.09	8.94	1.88	3.41E-04	2.80E-08	0.000188	10 787
26	rs76497600	BUB3	10:125119610	0.03	AFR	PP/ES	-0.79	0.61	-2.65	0.85	0.01	2.29E-08	0.000173	21 336
27	rs148454833	OR52A4	11:5114798	0.98	AFR	PP/CS	0.34	0.76	7.09	1.47	8.39E-06	2.16E-08	0.000147	13 888
28	rs186331780	FAM19A2	12:61710810	0.02	AFR	PP/CS	-2.43	0.89	-4.88	1.66	0.02	3.15E-08	0.007099	10 787
29	rs146924684	AVPR1A	12:63437286	0.99	AFR	MAP/ES	4.88	0.83	-3.20	1.22	0.18	5.29E-09*	2.62E-05	18 015
30	rs117206641	FBRSL1	12:133086888	0.11	Trans	MAP/CS	0.32	0.05	0.03	0.13	0.70	1.14E-10*	5.71E-07	393 100
31	rs73212161	TDRD3	13:61261485	0.99	AFR	PP/ES	-1.39	1.40	7.80	1.77	1.50E-05	1.68E-08	0.006503	13 888
32	rs78265647	IGFIR	15:99247941	0.98	AFR	PP/CS	-1.71	0.72	7.64	1.28	2.02E-09*	8.86E-09	6.09E-05	15 847
33	rs145181522	TOX3	16:52490106	0.02	AFR	PP/CS	-0.65	0.95	-8.04	1.58	3.67E-05	3.66E-11*	3.32E-07	10 787
34	rs114511313	NUDT7	16:77706251	0.98	AFR	PP/CS	1.67	0.73	4.06	1.28	0.13	1.63E-08	0.000111	15 574
35	rs75129914	RIT2	18:40267945	0.97	AFR	PP/ES	0.32	0.61	3.42	0.85	3.81E-04	2.13E-09*	1.80E-05	21 794
36	rs115134409	MALTI1; NEDD4L	18:56324467	0.02	AFR	PP/CS	-0.31	0.77	-6.46	1.29	3.26E-03	3.64E-10*	2.92E-06	12 890
37	rs78375085	TNFRSF11A	18:60032891	0.98	AFR	PP/ES	4.55	0.77	-5.57	1.21	4.71E-06	1.64E-08	0.000124	17 616
38	rs191056303	PXMP4	20:32306802	0.98	AFR	PP/CS	0.15	0.74	7.41	1.47	5.99E-07	1.77E-08	0.000121	13 888

A new BP locus was defined as a significantly associated variant that is at least 1 Mb away from any previously identified BP locus. Each locus is genome-wide significant ($P < 5 \times 10^{-8}$) in the combined analyses of stages 1 and 2 and had FDR q value < 0.05 . Positions are based on human genome build 37. EAF: effect allele frequency; G effect: the estimate of the genetic main effect (β_G); G×E effect: the estimate of genetic-smoking interaction effect ($\beta_{G \times E}$); Interaction P: P-value for testing the G×E interaction effect with one DF; Joint P: P-value for jointly testing G main and G×E interaction effects with two DF; EUR: European ancestry. Trans: trans-ancestry (i.e. combining all ancestry groups through meta-analysis); MAP: mean arterial pressure; PP: pulse pressure; CS: current-smoking; ES: ever-smoking.

*Findings with an asterisk indicate statistical significance using a stricter P-value threshold, after Bonferroni correction for two smoking traits, two tests, and two BP traits ($5 \times 10^{-8}/8 = 6.25 \times 10^{-9}$).

Table 3. Nine new signals associated with MAP and/or PP that are near known BP loci (but not in LD, $r^2 < 0.1$)

Locus	rsID	Nearest gene	Position	EAF	Race	Trait/ exposure	G effect	G StdErr	G×E effect	G×E StdErr	Interaction P	Joint P	FDR q value
1	rs140881076	KAZN	1:15364113	0.01	AFR	PP/CS	0.45	1.13	-11.95	1.85	2.30E-03	3.29E-14*	4.16E-10
2	rs2071405	AGT	1:230850658	0.13	Trans	MAP/CS	0.28	0.04	-0.18	0.09	0.20	3.02E-12*	1.62E-08
3	rs143802076	C3orf38	3:88646080	0.01	AFR	PP/CS	-0.50	0.90	-8.54	1.68	8.97E-04	1.33E-09*	9.81E-06
4	rs1009382	TNXB	6:32026107	0.71	EUR	PP/CS	0.26	0.04	-0.16	0.08	0.15	4.84E-13*	3.30E-09
5	rs7005363	MSRA	8:10283748	0.54	EUR	MAP/ES	-0.34	0.04	0.15	0.06	0.02	3.13E-17*	1.59E-13
6	rs187148391	TXN	9:112998518	0.99	HIS	MAP/ES	0.09	0.69	4.48	1.03	1.01E-03	1.95E-08	0.013302
7	rs10894198	ADAMTS8	11:130285493	0.38	Trans	PP/CS	0.27	0.03	-0.12	0.07	0.33	1.38E-19*	3.19E-15
8	rs1010064	LOC100506393 PDE3A	12:20000315	0.75	Trans	MAP/ES	0.24	0.04	-0.12	0.06	0.03	5.91E-11*	6.64E-10
9	rs201028933	LOC338758	12:90111249	0.79	Trans	MAP/ES	0.32	0.08	0.16	0.11	0.28	1.73E-11*	9.75E-08

A new signal is defined as a significantly associated variant within 1 Mb of known BP loci but in weak LD $r^2 < 0.1$ with the known BP loci. LD for the *trans*-ancestry signals was based on the entire 1000 Genomes cosmopolitan data, whereas LD for ancestry-specific signals was based on ancestry-specific population (e.g. LD for European signals were based on 1000 Genomes European data). Each locus is genome-wide significant ($P < 5 \times 10^{-8}$) in the combined analyses of stages 1 and 2 and had FDR q value < 0.05 . Positions are based on human genome build 37. EA: effect allele; EAF: effect allele frequency; G effect: the estimate of the genetic main effect (β_G); G×E effect: the estimate of genetic-smoking interaction effect (β_{GE}); Interaction P: P-value for testing the G×E interaction effect with one DF; Joint P: P-value for jointly testing G main and G×E interaction effects with two DF; EUR: European ancestry. Trans: *trans*-ancestry (i.e. combining all ancestry groups through meta-analysis); MAP: mean arterial pressure; PP: pulse pressure; CS: current-smoking; ES: ever-smoking.

*Findings with an asterisk indicate statistical significance using a stricter P-value threshold, after Bonferroni correction for two smoking traits, two tests, and two BP traits ($5 \times 10^{-8}/8 = 6.25 \times 10^{-9}$).

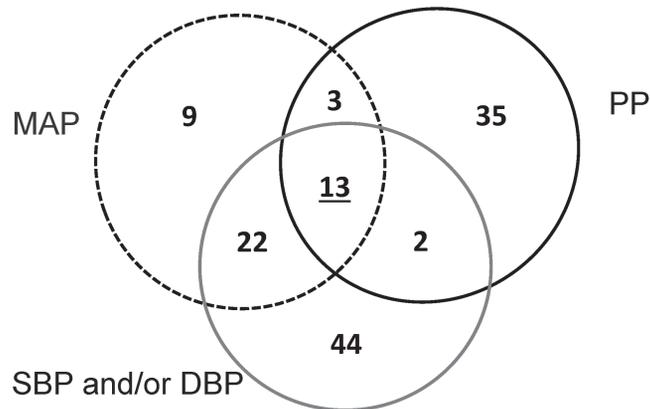


Figure 2. Venn diagram of loci/signals associated with the four BP traits. The diagram shows 133 loci and/or signals that were identified through gene-smoking interactions. In this paper, we newly identified 38 loci (Table 2) and 9 signals near known BP loci (Table 3) that are unique to MAP and/or PP (to a total of 49 new loci/signals). We had reported 81 loci associated with SBP/DBP (16), among which 37 showed association with MAP or PP. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure.

ancestry (Fig. 4). The independent set of variants, 38 for MAP and 12 for PP, with $P \leq 5 \times 10^{-8}$ explained 1.9% of variance in MAP and 0.5% of variance in PP. The difference in explained variance between the smokers and non-smokers was not significant, suggesting that BP variance explained by interaction effects is very small. Similar inference was observed with the results from ever-smoking status (data not shown).

Functional inferences

To obtain functional annotations from HaploReg (26), we focused on the index variants representing the 84 loci (38 novel loci, 9 new signals near known loci and 37 recently reported) that showed association with MAP and/or PP. There was one missense variant, rs1009382. Of the remaining non-coding variants (37 intronic and 51 intergenic), 15 were in promoter histone marks, 47 in enhancer histone marks, 28 in DNase I marks and 8 altered the binding sites of regulatory proteins (Supplementary Material, Table S9). Using GERP (27), five variants were identified as being

conserved among vertebrates, with three variants identified as such using SiPhy (28). For 27 variants, *cis*-expression quantitative trait loci (eQTL) evidence was available with varying degrees of association with expression probes. In particular, 10 of them were identified by GTEx (29) as *cis*-eQTLs across various tissues (Supplementary Material, Table S9). In addition, we obtained information on microarray-based gene and exon expression levels in whole blood from over 5000 individuals of the Framingham Heart Study (30) (Supplementary Material, Table S10). There were 109 variant-transcript pairs (representing 26 variants) with *cis*-eQTL evidence (at $P < 8.9 \times 10^{-5}$, FDR < 0.002). Among 26 variants (Supplementary Material, Table S10), the 3 variants had the most abundant evidence of *cis*-eQTL association: rs112947839, rs1009382 and rs7753826 associated with 21, 18, and 10 transcripts, respectively.

The analyses using data-driven expression prioritized integration for complex traits (DEPICT) prioritized genes (FDR $< 5\%$) at 40 loci, including 16 genes that did not match the nearest gene of the identified lead variant (Supplementary Material, Table S11).

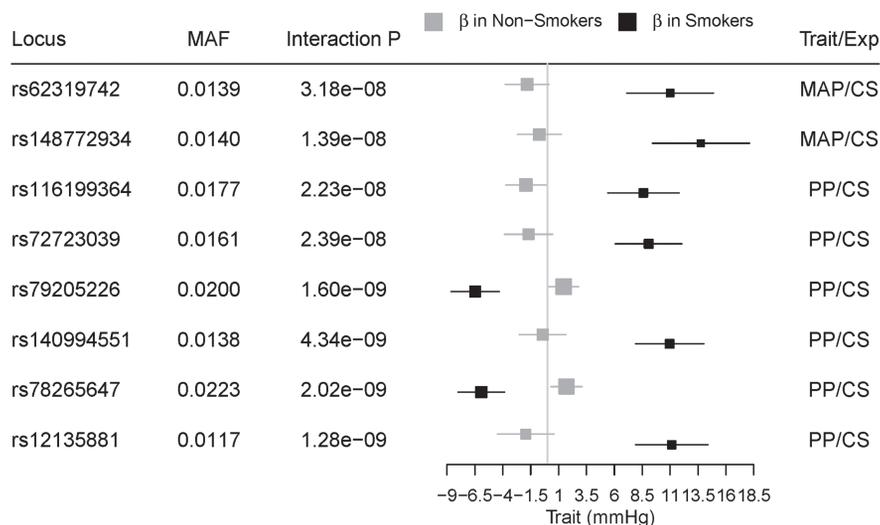


Figure 3. Smoking-specific genetic effect sizes in African ancestry for MAP or PP. Among the 138 loci significantly associated with MAP and/or PP, 8 loci show significant interactions with smoking exposure status in African ancestry. Smoking-specific effect estimates and 95% confidence intervals for variants associated with BP traits are shown as red and blue squares for current-smokers and non-current smokers, respectively. SNP effects between two strata are significantly different (one DF interaction $P < 5 \times 10^{-8}$). These results were based on African-specific results in stage 1. MAP: mean arterial pressure; PP: pulse pressure; CS: current-smoking.

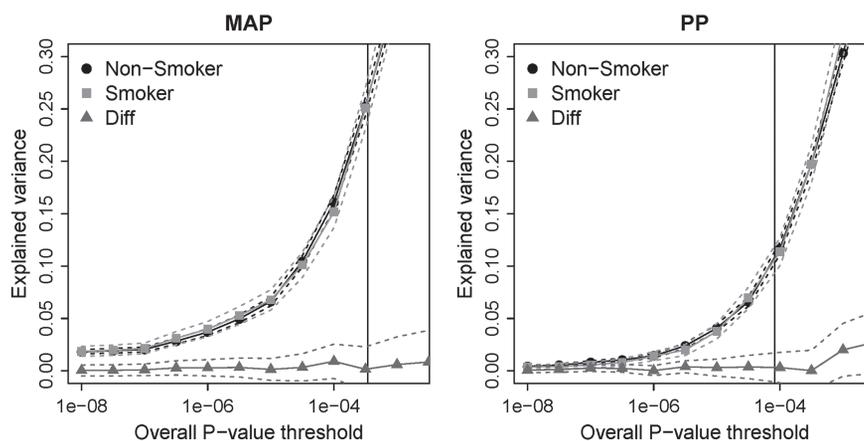


Figure 4. Smoking-specific estimates of variance explained in European ancestry. The variants with $P \leq 5 \times 10^{-8}$ explained 1.9% of variance in MAP and 0.5% of variance in PP, whereas variants with $P \leq 10^{-4}$ explained 16% of variance in MAP and 11% of variance in PP. The vertical line corresponds to FDR = 0.1.

Furthermore, the analyses highlighted 56 significantly ($FDR < 5\%$) enriched gene sets. Many of these highlight cardiovascular mechanisms, such as ‘abnormal blood vessel morphology’, ‘thin myocardium’ or ‘abnormal heart development’ (Supplementary Material, Table S12). We also observed that genome-wide significant MAP and PP loci are enriched for genes expressed in the ileum (Supplementary Material, Table S13).

Associations of BP loci with cardiometabolic traits

We obtained association results of the 84 index variants associated with MAP or PP (representing 38 novel loci, 9 new signals near known loci and 37 recently reported loci) with multiple cardiometabolic traits: coronary artery disease (CAD), stroke, adiposity, diabetes and renal function (Supplementary Materials, Tables S14–S19). For 36 out of 47 scenarios (highlighted in red, Supplementary Material, Table S20), the observed number of variants with nominal evidence of association ($P < 0.05$) was

higher than that expected by chance alone ($P_{\text{Binomial}} < 0.05/11$, corrected for 11 traits used in the lookups). For example, we observed 7 and 11 such associations with CAD and myocardial infarction, respectively, where the expected count is 2.2 for both traits. Corroborating evidence of the multiple cardiometabolic traits were found for the 2 of the 38 new loci: (rs146622638, GPM6A; rs12156238, FAM167A) and the 5 of the 9 new signals near known BP loci (rs2071405, AGT; rs1009382, TNXB; rs7005363, MSRA; rs1010064, LOC100506393; rs201028933, LOC338758). These overlapping signals support that these traits may share a common pathophysiology.

Loci overlapping with previously reported SBP or DBP loci

Among the loci that were reported by us recently as significantly associated with SBP and/or DBP based on gene-by-smoking interaction analysis (16), 37 loci were also associated with MAP

and/or PP (Supplementary Material, Table S7). Among them, nine loci were formally replicated in stage 2 and showed association with all four BP traits. Variants at these nine loci were all also genome-wide significant in the combined analysis of stages 1 and 2 in individuals of European ancestry. For variants at six of the nine loci, there was supporting evidence of association in individuals of non-European ancestry, which resulted in stronger statistical significance from *trans*-ancestry analysis. One such locus was rs351364 (in *WNT2B*), where only *trans*-ancestry analysis reached genome-wide significance in stage 1; the direction of the genetic effect was consistent across all ancestries (with 2DF $P = 2.8 \times 10^{-31}$; Supplementary Material, Table S7).

New signals near known BP loci

Nine new signals were identified near known BP loci (but not in LD, $r^2 < 0.1$). One such signal was rs140881076 (chr1:15364113, 2DF $P = 3.3 \times 10^{-14}$, Fig. 5A) in association with PP in individuals of African ancestry. This signal is 434 kb away and in complete linkage equilibrium with *CELA2A* locus (rs3820068, chr1:15798197) that was recently identified in individuals of European ancestry (7,8). Several nearby genes have been implicated in cardiovascular traits. *FHAD1* is a long non-coding RNA overexpressed in heart failure (31), *TMEM51* has been associated with contractile function in cardiomyocytes (32) and *CASP9* plays a central role in cardiomyocyte apoptosis (33). A candidate gene study identified a missense mutation in *CASP9* as associated with ischemic stroke in Koreans (34). Differential methylation patterns in *TMEM51* have also been described in peripheral blood leukocytes of smokers (35,36).

Through *trans*-ancestry analysis, we identified one locus (rs1010064) associated with both MAP and PP (2DF $P = 5.9 \times 10^{-11}$). This is located approximately 500 kb upstream of, but not in LD with, *PDE3A*, a known BP gene with a role in regulating growth in vascular smooth muscle cells (4,37). Missense mutations in *PDE3A* have been linked with autosomal dominant syndrome characterized by treatment-resistant hypertension and brachydactyly (38,39). SNPs in this locus have also shown suggestive associations with aortic root diameter (40), resistant hypertension (41) and SBP in a SNP–alcohol consumption interaction analysis (42).

Biological relevance of newly identified BP loci

Several genes near the 38 novel loci show biologic plausibility for a role in BP regulation. One such gene is *CSMD1* (rs140994551, chr8:4449086, associated with PP in individuals of African ancestry while considering interaction with current smoking status, 2DF $P = 2.1 \times 10^{-11}$, Fig. 5B). In animal models, variants in *CSMD1* were associated with both insulin resistance and BP in the spontaneously hypertensive rats (SHRs) (43). In humans, there was suggestive evidence of association with hypertension in two Korean cohorts (44), with peripheral artery disease in a Japanese population (45), with waist–hip ratio adjusted for BMI in men (46), with insulin resistance in African Americans (47) and with studies of addiction and related disorders (48). Another new locus is *LRRC69* (rs11991823, chr8:92188440, associated with PP, identified through *trans*-ancestry analysis, 2DF $P = 1.3 \times 10^{-15}$, Fig. 5C). A copy number variant in this gene has been shown to be weakly associated ($P = 0.04$) with BP in a Korean population (49). The nearby gene *SLC26A7* encodes a chloride/bicarbonate exchanger expressed specifically in the renal outer medullary collecting duct (50). Two PP loci include genes involved in the

NF κ B signaling pathway (*TNFRSF11A* and *NFIB*). This inflammatory pathway has been implicated in hypertension-induced renal dysfunction in murine models (51) and with endothelial dysfunction in overweight/obese and older humans (52). There was suggested evidence of association of variants in *TNFRSF11A* with BP traits in Chinese women (53).

A new locus near *AVPR1A* (rs146924684 chr12:63437286, associated with MAP, 2DF $P = 5.3 \times 10^{-9}$, Fig. 5D) also has strong biologic plausibility. Vasopressin is an antidiuretic hormone and a potent vasoconstrictor that exerts its effect through activation of a family of receptors, including the arginine vasopressin receptor subtype 1A (*AVPR1A*) that is widely expressed including in vascular smooth muscle cells, kidney, myocardium and brain (54). In glomerular macula densa cells, *AVPR1A* facilitates activation of the renin–angiotensin–aldosterone system and increases expression of the aquaporin 2 water channel (55). *AVPR1A* stimulation is also necessary for maintaining normal BP; in murine knockout models, basal BP is significantly decreased and the arterial baroreceptor reflex markedly impaired (56). Notably, there are data to support a role for vasopressin not only in the maintenance, but also in the development, of hypertension. Vasopressin receptor 1A blockade in young, still normotensive, SHR attenuates the later development of hypertension in adult SHR despite withdrawal of drug therapy (57).

We identified several loci with potential relevance to the structure and function of primary cilia, in addition to those we reported recently (16). Three PP-associated loci were near genes implicated with nephronophthisis, including those with mutations linked to Bardet–Biedl Syndrome (*BBS7* and *MYO3A*) and with Joubert Syndrome (*AHI1*). Another PP-associated locus was near *NEDD4L*, which encodes the E3 ubiquitin ligase *NEDD4-2* and has been shown to regulate a renal epithelial sodium channel (*ENaC/SCNN1*) that is critical for maintenance of sodium homeostasis (58). *ENaC* is the channel responsible for the monogenetic disorder of BP regulation, Liddle Syndrome. Loss of *NEDD4-2* in the renal tubules results in increased activity of the *ENaC* channel, resulting in salt-sensitive hypertension (59). Candidate gene studies identified variants in *NEDD4L* as associated with sodium lithium countertransport (60), hypertension (61), treatment response to β -blockers and diuretics in hypertensive patients (61–63).

We identified two additional loci with potential relevance to the dopaminergic system, in addition to those we reported recently (16). Dopamine signaling plays a key role in both central and peripheral BP regulation (64–66). A regulatory subunit (*PPP2R2A*) of the dopamine receptor 2R (*D2R*) was associated with MAP. In murine renal proximal tubule cells, inhibition of this regulatory protein leads to increased expression of markers of renal inflammation and injury (67). A newly identified MAP-associated locus *SESN2* is also related to the dopaminergic system; activation of the *D2R* has been shown to increase the expression of *SESN2*, which protects the kidney against renal oxidative stress (68). *SESN2* also protects endothelial cell lines against angiotensin II-induced endothelial toxicity (69). Two additional loci include genes involved in dopamine signaling: *ATP13A2* (70) and *ARPP21* (71). Activation of dopamine centers of the brain has also been implicated in drug and nicotine abuse (72).

In addition, we found a PP-associated locus near *SDHB*, which encodes the mitochondrial protein succinate dehydrogenase. Variants in this gene have been identified in individuals with carotid body tumors and pheochromocytomas/parangliomas, endocrine tumors that secrete dopamine and/or norepinephrine and can modulate BP regulation even when tumors are not

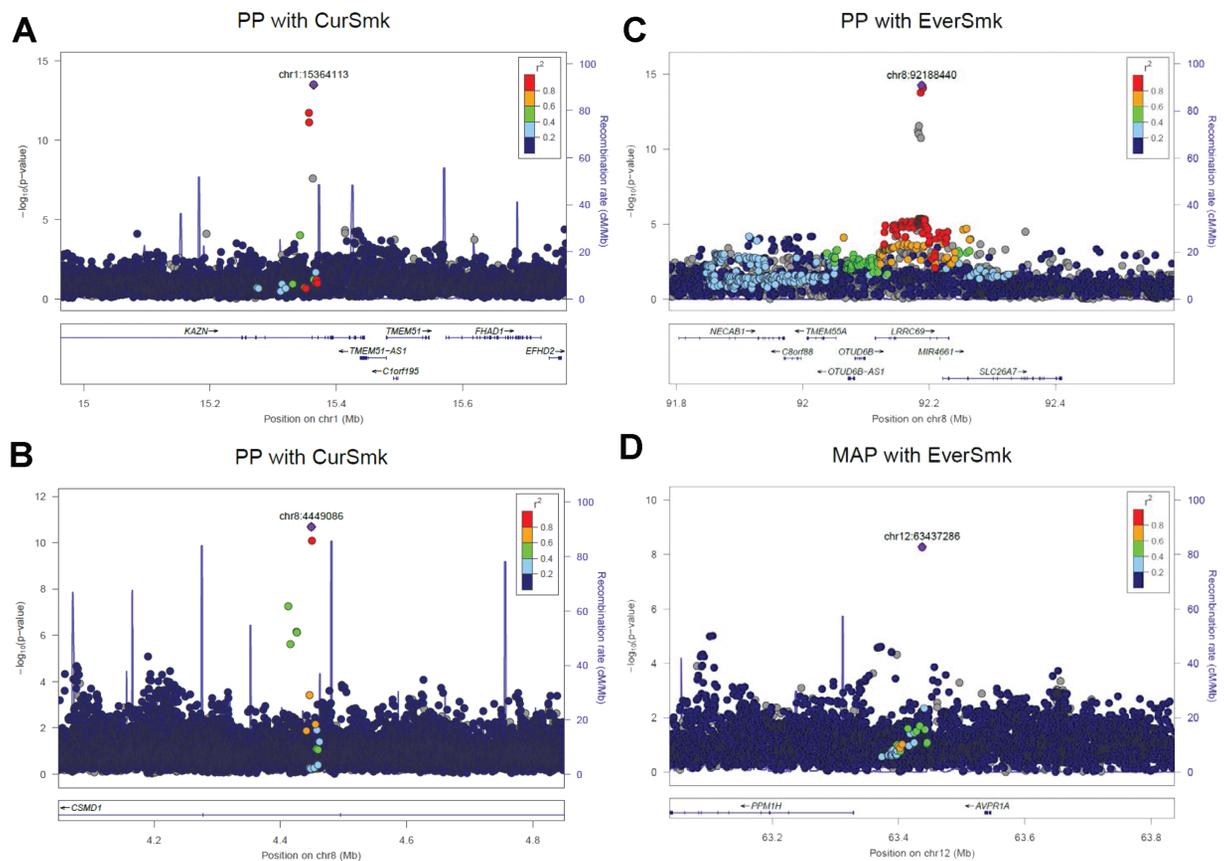


Figure 5. LocusZoom plots for four selected loci associated with MAP and/or PP. (A) rs140881076 (chr1:15364113) was identified in an analysis of individuals of African ancestry and is intronic to *KAZN*; neighboring genes have been implicated in cardiovascular traits. *FHAD1* is a long non-coding RNA overexpressed in heart failure, *TMEM51* has been associated with contractile function in cardiomyocytes and *CASP9* plays a central role in cardiomyocyte apoptosis. (B) rs140994551 (chr8:4449086), intronic to *CSMD1*, shows interaction with current smoking in individuals of African ancestry. *CSMD1* are shown to be associated with insulin resistance and BP in the spontaneously hypertensive rats. *CSMD1* is also suggestively associated with studies of addiction and related disorders. (C) rs11991823 (chr8:92188440) was associated with PP in trans-ancestry analyses and is intronic to *LRRRC69*. The nearby gene *SLC26A7* encodes a chloride/bicarbonate exchanger expressed specifically in the renal outer medullary collecting duct. (D) rs146924684 (chr12:63437286) was associated with MAP in individuals of African ancestry. The nearby gene *AVPR1A* is widely expressed including in vascular smooth muscle cells, kidney, myocardium and brain. CurSmk: current smoking status; EverSmk: ever smoking status; MAP: mean arterial pressure; PP: pulse pressure. The plots were created using LocusZoom (<http://locuszoom.sph.umich.edu/>).

clinically apparent (73,74). Variants near this locus have been marginally associated with DBP in pre-pubertal European children (75). Tyrosinase (with its related protein, *TYRP1*) catalyzes the first rate-limiting step in pathway in the formation of L-Dopa (76). Although variants in *TYRP1* were suggestively associated with SBP by the International Consortium for Blood Pressure (77), we identified this locus as associated with PP at genome-wide significance.

Discussion

MAP measures the steady component, which is a function of the left ventricular contractility, heart rate, small-artery resistance and vascular elasticity averaged over time (17). PP measures the pulsatile component, which is a function of the left ventricular stroke volume, large-artery stiffness, early pulse wave reflectio, and heart rate (19). These BP traits not only differ in their physiologic properties but are also differently related to cardiovascular outcomes (17,19,78,79). Our genome-wide association meta-analysis incorporating gene-smoking interactions identified 136 loci significantly associated with MAP and/or PP: 61 were previously published through main-effect GWAS analysis (1–8),

37 were recently reported by us for SBP and/or DBP through gene-smoking interaction analysis (16) and 38 are newly reported here. Our analysis also identified nine new signals near known BP loci (but not in LD, $r^2 < 0.1$).

Among the loci significantly associated with MAP and/or PP, eight loci showed significant interaction with smoking status from the one DF interaction tests. At these eight loci, the joint two DF *P*-values ranged from 1×10^{-7} to 5×10^{-11} , indicating that loci were identified mostly because of their interaction with smoking status. We observed that the genetic effect at these loci is negligible in non-smokers but larger in smokers. As such, a drug that targets this locus with strong interactions may achieve a greater treatment effect among smokers than non-smokers; elevated BP may be treated in smokers using such a drug, whereas the same drug is unlikely to be effective in non-smokers. Alternatively, physicians may counsel patients on specific antihypertensive drugs that they may obtain greater treatment effect if they modify their exposure (e.g. smoking cessation). While precision medicine interventions are still emerging in cardiovascular care, a consideration of interaction effects lays an important foundation. In addition to drug targeting, a smoking interaction can also help us to identify novel biological mechanisms underlying BP traits.

One such locus showing significant interaction with smoking status is CSMD1. While variants of this gene were previously suggested for addiction and related disorders (48), we identified this locus at genome-wide significance (1DF $P=4.3 \times 10^{-9}$, 2DF $P=2.1 \times 10^{-11}$). In our study, another locus near AHR showed weak evidence of interaction with smoking (1DF $P=1.6 \times 10^{-4}$, 2DF $P=1.7 \times 10^{-9}$ associated with MAP). Variants in AHR are shown to interact with variants in CYP1A1, a detoxifying enzyme, to explain BP differences between smokers and non-smokers (80). AHR encodes a ligand-activated transcription factor, and AHR knock-out mice have increased MAP and ventricular hypertrophy/fibrosis with increased plasma levels of angiotensin II (81). Given the evidence that environmental toxins, including tobacco smoke, activate AHR, it is pertinent to note that AHR, in turn, activates tyrosinase activity, the rate limiting step for L-dopa biosynthesis (76). Activation of the AHR protein represses T-cadherin expression, which functions as a negative growth regulator in vascular smooth muscle cells (82,83). T-cadherin (encoded by CDH13) has been previously identified as a BP susceptibility locus (84). Notably, while the endogenous ligand for AHR remains uncertain (85), exogenous ligands include polycyclic aromatic hydrocarbons that are found in tobacco smoke and other environmental pollutants (86).

We found that most of MAP-associated loci were previously associated with SBP and/or DBP. This is not surprising given that MAP is closely related physiologically to SBP and DBP. In contrast, analysis of PP yielded a greater number of novel significant loci that are unique to PP. Loci associated with PP may be identifying different physiologic processes than loci associated with MAP, SBP and DBP. For example, the steady component of BP can be effectively targeted by β -adrenergic receptor and calcium-channel blockers that both modulate arteriolar tone. Angiotensin converting enzyme inhibitors, which favor remodeling of vascular connective tissue, may impact PP to a greater extent (87). This is a clinically important concept since hypertension is often more effectively treated by combination drug therapy to target different physiologic pathways (23).

We identified 30 loci that were statistically significant only in the meta-analyses of African ancestry individuals (forest plots in [Supplementary Material, Fig. S5](#)). Due to many prior BP GWAS discoveries, mostly based on European or Asian ancestries, identifying new BP loci in European and Asian ancestries may be challenging. There are also more opportunities to identify lower frequency variants in African ancestry individuals because there are more of these variants in this genetically more diverse population (with correspondingly smaller LD blocks, allowing closer identification of multiple underlying causal variants). The observed effect sizes (in African ancestry, [Fig. 3](#)) may be larger than their true values due to winners' curse (88). All identified loci were in low frequency [with minor allele frequency (MAF) ranging from 1.2% to 3.1%] but had good imputation quality scores ranging from 0.62 to 0.95 (presented in [Supplementary Material, Fig. S5](#)). In many of these loci, forest plots show consistent association across the contributing African cohorts. Out of 30, 23 loci were only present in African ancestry, and therefore, these associations could not be effectively evaluated in other ancestry groups as a result of their inter-ancestry differences in MAF. Because of the limited sample sizes available for African ancestry in stage 2, genome-wide significant loci in stage 1 African ancestry could not be formally replicated in stage 2; only the largest African cohort in stage 2 (Health and Retirement Study, $N=1993$) provided association results for a subset of 23 loci ([Supplementary Material, Fig. S5](#)). For the remaining seven loci, we found evidence of association

in African ancestry but not in meta-analyses in other ancestries, despite comparable or higher allele frequencies, such as those observed with rs11587661 (COG2) or rs72723039 (IRX2). We found similar smoking-specific effects on lipid traits that were unique to African ancestry (89). They may relate at least in part to inter-ancestry differences, including preference of menthol cigarettes. Therefore, African-specific loci should be treated cautiously since they require further validation.

This large-scale multi-ancestry study has some limitations. First, because most of the known BP loci were identified in European and Asian ancestries, considerable effort was made to recruit most of the available studies from the other ancestries into stage 1. Although we were able to identify several new loci in African ancestry, the relatively smaller stage 2 sample size of African ancestry ($N=7786$) has limited our ability to replicate these new loci. Second, some of our new loci identified through the 2DF joint test may have been identified due to a main effect because of a larger sample size and more diverse ancestries, not necessarily from gene-smoking interaction. Unfortunately, we are unable to verify this because analysis of main effects alone, without regard to smoking status, was not performed. Third, conditional analysis (such as genome-wide complex trait analysis, GCTA) based on summary statistics was not performed because valid methods do not currently exist for G×E interactions. Therefore, we relied on a relatively more stringent LD threshold ($r^2 < 0.1$) for identifying additional signals within the known BP loci. Fourth, if there is a G×E correlation, a potential confounding of G×E with interaction between covariate and smoking exposure may exist. This can inflate Type I error of the G×E interaction test (90).

In summary, this study identified 38 new loci and 9 new signals near known BP loci that are uniquely associated with MAP and/or PP (and not associated with SBP or DBP), demonstrating the promise of gene-lifestyle interactions for genetic and environmental dissection of BP traits. Of our 38 loci, 10 were within 1 Mb of those recently reported by both Evangelou et al. (9) and Giri et al. (10); 6 loci were African-specific. Additional seven loci (including four African-specific loci) were within 1 Mb of those reported by Evangelou et al. (9). Variants in several loci were identified in individuals of African ancestry, highlighting the importance of genetic studies in diverse populations. Many of these new loci (including CSMD1, TMEM51, SLC26A7, TNFRSF11A and AVPR1A) show biologic plausibility for a role in BP regulation. They include additional loci of potential relevance to the structure and function of primary cilia and the dopaminergic system. Understanding underlying mechanisms for the newly identified loci and biological insights into the genetics of BP traits will require further investigation. Out of 136 significant loci, 8 showed significant interaction with smoking status. Because some interactions may be driven by other lifestyle factors that are correlated with smoking, a follow-up study such as Tyrrell and her colleague (91) that jointly examines multiple lifestyle factors can shed light on further understanding of the nature of the smoking interaction effects on BP. Our findings highlight the value of multi-ancestry investigations, particularly in studies of interaction with lifestyle factors, where genomic and lifestyle differences may contribute to novel findings.

Materials and Methods

Participating studies

Analyses included men and women between 18 and 80 years of age from European (EUR), African (AFR), Asian (ASN),

Hispanic (HIS) and Brazilian (BRZ) ancestries. A total of 48 cohorts consisting of 129 913 individuals (80 552 EUR; 27 118 AFR; 13 438 ASN; 8 805 HSP; [Supplementary Material, Table S1](#)) participated in stage 1 and performed genome-wide analyses. Studies that included data from multiple ancestries (cohorts) contributed multiple analyses, one for each ancestry/cohort. For example, multi-ethnic study of atherosclerosis has four cohorts. A total of 76 additional cohorts consisting of 480 178 individuals (305 513 EUR; 7826 AFR; 148 932 ASN; 13 533 HSP; 4414 BRZ; [Supplementary Material, Table S2](#)) participated in stage 2 and performed association analyses of 4373 variants that were identified in stage 1 as either genome-wide significant ($P < 5 \times 10^{-8}$) or suggestive ($P < 10^{-6}$). ASN participants include both south Asian and east Asians. Stage 1 ASN includes 7873 East Asians and 5566 South Asians, whereas stage 2 ASN includes 136 961 East Asians and 12 481 South Asians. All participating studies are described in the Supplementary Material. Since discoveries of BP loci to date were largely from EUR populations, considerable effort was made for recruiting most of the available non-EUR cohorts into stage 1 (which limited the availability of non-EUR cohorts in stage 2). Each study obtained informed consent from participants and approval from the appropriate institutional review boards.

Phenotypes and lifestyle variables

Resting SBP and DBP were measured using standard clinical procedures that produce comparable measurements (specific methods per study were described more in Supplementary Material). Even with some difference in measurement across studies, the measures were standardized, through previous main effect BP GWAS studies, as much as possible for BP. For individuals on any anti-hypertensive (BP lowering) medications, 15 mmHg and 10 mmHg were added to their SBP and DBP values, respectively (1). PP was computed as SBP minus DBP ($PP = SBP - DBP$), and MAP was computed as the sum of DBP and one-third of PP ($MAP = DBP + PP/3$). To reduce the influence of possible outliers, each BP value was winsorized at six standard deviations (SD) away from the mean (i.e. values greater than six SD away from the mean were set at six SD).

Obtained through interview-based or self-reported questionnaire, varying levels of smoking information were available across studies, some with a simple binary variable and others with repeated data. We considered two of the most widely available smoking variables: 'current smoking' status (CurSmk) and 'ever smoking' status (EverSmk) ([Table 1](#)). Current smoking status was defined as 1 if the individual smoked regularly in past year (and as 0 for non-current smokers, which includes both never and former smokers). Ever smoking status was defined as 1 if the individual smoked at least 100 cigarettes during his/her lifetime (and as 0 for the never smokers). Smoking status was assessed at the time of the BP measurements. Covariates include age, sex, field center (for multi-center studies) and principal components (PCs) (to account for population stratification and admixture). No additional covariates were included. Individuals with missing data for BP, the smoking variable or any covariates were excluded from analysis. Study-specific summary statistics on phenotypes are presented in [Supplementary Materials, Tables S3 and S4](#).

Genotype data

Genotyping was obtained using Illumina (San Diego, CA, USA) or Affymetrix (Santa Clara, CA, USA) genotyping arrays. Each study

performed genotype imputation at SNPs, short insertions and deletions (indels), and larger deletions that were not genotyped directly but are available from the 1000 Genomes Project (92). For imputation, most studies used the 1000 Genomes Project Phase I Integrated Release Version 3 Haplotypes (2010–11 data freeze, 2012–03–14 haplotypes), which contain haplotypes of 1092 individuals of all ancestry backgrounds. Study-specific information on genotyping and imputation is presented in [Supplementary Materials, Tables S5 and S6](#).

Cohort-specific analysis

We identified loci through the two DF test that jointly test the genetic main effect and the gene–smoking interaction jointly. This approach has previously enabled identification of new loci associated with insulin resistance, including how the effect of variants differs with levels of BMI (11). The method is described in detail for single studies in Kraft *et al.* (93) and for implementation in meta-analyses in Manning *et al.* (24).

Participating studies performed association analyses separately within each ancestry for MAP and PP incorporating CurSmk and EverSmk. All studies performed regression analysis using a model with both genetic main and G×E interaction effects (93): $E[Y] = \beta_0 + \beta_E Smk + \beta_G G + \beta_{GE} Smk * G + \beta_C C$.

Y is the medication-adjusted BP value, Smk is the smoking variable (with 0/1 coding for the absence/presence of the smoking exposure), G is the dosage of the imputed genetic variant coded additively (from 0 to 2) and C is the vector of all other covariates, which include age, sex, field center (for multi-center studies) and PCs (to account for population stratification and admixture). No additional cohort-specific covariates were included. From this model, the studies provided the estimated genetic main and interaction effects and a robust estimate of the corresponding covariance matrix. In addition, studies in stage 1 performed regression analyses with the genetic main-effect model, in the exposed ($Smk = 1$) and unexposed strata ($Smk = 0$) separately, and provided estimates of the stratum-specific effects and robust estimates of their standard errors (SE).

Either sandwich (94) or ProbABEL (95) packages were used to obtain robust estimates of covariance matrices and robust SEs for samples of unrelated individuals. Family studies used the generalized estimating equations approach, treating each family as a cluster, or the linear mixed effect model approach with a random polygenic component (for which the covariance matrix depends on the kinship matrix). Robust estimates of covariance matrices and SEs were used to safeguard against misspecification of the mean model and violation of the assumption of constant BP variance across smoking groups (heteroscedasticity) (96,97).

Quality control

Each study performed standard genotype quality control (QC) that includes excluding SNPs with call rate (<95% or higher) and Hardy–Weinberg equilibrium $P < 10^{-6}$. In addition, we performed extensive QC using the R package EasyQC (98) for all cohort-specific results. For GWAS results in stage 1, each cohort applied a preliminary filter on their imputed data excluding variants with $MAF < 1\%$. Variants with imputation quality measure of <0.5 were subsequently excluded. We performed the 'study-level' QC, which included carefully checking the observed allele frequencies against the corresponding ancestry-specific 1000 Genomes Project data and harmonizing marker names to ensure consistencies across cohorts. In addition, in stage 1, we com-

pared results from the joint and stratified models, as explained elsewhere (99). To identify cross-study issues, we then performed the 'meta-level' QC by checking result files across all cohorts for each analysis. This included visually comparing summary statistics (mean, median, inter-quartile range, etc.) on all effect estimates, SEs and *P*-values, and examining SE-N (i.e., inverse of the median standard error versus the square root of the sample size) plots and QQ plots to reveal issues with trait transformation (98) or other analytical problems. Encountered QC problems were communicated and resolved with the individual cohorts. More detailed information about QC is described elsewhere (13,16).

Meta-analyses

After selecting high-quality variants through extensive QC, ~18.8 million SNPs and small indels variants were included in the meta-analysis (the number of variants varied across the ancestry groups). To combine cohort-specific results within each ancestry, we first performed ancestry-specific meta-analyses; the results were then combined through meta-analysis to obtain evidence of 'trans-ancestry' association. Inverse-variance-weighted meta-analysis with METAL (100) was used for the one DF test of interaction effect (with $H_0: \beta_{GE} = 0$). For two DF test of both SNP main and interaction effects (with $H_0: \beta_G = \beta_{GE} = 0$), the joint meta-analysis of Manning *et al.* (24) was used. In the stratified model, we performed meta-analysis using the approach of Randall *et al.* (101) for the one DF test and the approach of Aschard *et al.* (102) for the two DF test using the R package EasyStrata (103). Additional details about the meta-analytic approach are described elsewhere (99).

In stage 1, genomic control correction (104) was applied twice, first for cohort-specific GWAS results if their genomic control lambda value was greater than 1 and again after the meta-analysis. Variants that passed QC were excluded if they were represented in fewer than 5000 samples or fewer than three cohorts. Variants that were genome-wide significant ($P < 5 \times 10^{-8}$) or suggestive ($P < 1 \times 10^{-6}$) in stage 1 were pursued in stage 2. Heterogeneity *P*-values at the selected variants were $> 1 \times 10^{-5}$, indicating limited heterogeneity (data not shown). In stage 2, genomic control correction was not applied to the replication statistics as association analysis was performed only at select variants. Meta-analysis combining results of stages 1 and 2 was also performed. In addition, genome-wide significant variants in stage 1 were tested for formal replication in stage 2 using Bonferroni-corrected significance threshold.

Genome-wide significant variants

We considered a variant with $P < 5 \times 10^{-8}$ (the standard threshold in the field) to be genome-wide significant. We also identified novel loci that pass a more stringent threshold ($P < 6.25 \times 10^{-9}$, $P < 5 \times 10^{-8}$ adjusted for two smoking exposures, two tests and two BP traits, where this correction is somewhat conservative given dependence between the various test statistics). Loci that pass the stricter *P*-value are indicated in main tables. FDR *q*-values were computed using the R function `p.adjust` using the step-up method by Benjamini and Hochberg (105). A new locus was identified if it was 1 Mb away from any previously identified BP locus. A new signal was identified if it is within 1 Mb of known BP loci but not in LD $r^2 < 0.1$ with the known BP loci. Since valid methods do not exist for conditional analysis involving interactions across multi-ancestry studies, we relied on a relatively more stringent LD threshold ($r^2 < 0.1$) for identifying additional signals. For LD reference, ancestry-specific 1000 Genomes Project

data (106) were used for ancestry-specific results, and the entire cosmopolitan data set was used for *trans*-ancestry results.

BP variance explained

We computed BP variance explained by genome-wide results, based on stage 1 stratified results with current-smoking status in European ancestry (25). Within each of the smoking strata, we computed the variance of MAP and PP explained by subsets of variants selected using 15 significance thresholds ranging from 1×10^{-8} to 0.1

Functional inferences

We conducted DEPICT analyses (107) based on genome-wide significant ($P < 5 \times 10^{-8}$) variants from the combined analysis of stages 1 and 2. DEPICT performs three consecutive analyses: i) gene prioritization at the identified loci, ii) gene set enrichment analyses and iii) tissue- and cell-type-specific expression analyses. To obtain input for the analyses, DEPICT applied a combined distance and LD-based threshold (500 kb flanking regions and LD $r^2 > 0.1$) between the identified variants and the 1000 Genomes reference data (106). A further clumping (LD $r^2 > 0.5$ between the non-overlapping variants and known functional coding or cis-acting regulatory variants) was used to obtain a list of genes overlapping with the identified variants. The major histocompatibility complex region on chromosome 6 (25–35 Mb) was removed for further analyses.

For gene prioritization, DEPICT compared functional similarity of genes across identified loci using a gene score, which was adjusted for confounders like gene length. To obtain FDR, the scoring was repeated 50× based on 500 pre-compiled null GWAS. For gene-set enrichment analyses, DEPICT used 14 461 pre-compiled reconstituted gene sets; they include 737 Reactome pathways, 2473 phenotypic gene sets (derived from the Mouse Genetics Initiative), 184 Kyoto Encyclopedia of Genes and Genomes pathways, 5083 Gene Ontology terms and 5984 protein molecular pathways (derived from protein-protein interactions). For tissue- and cell-type enrichment analyses, DEPICT used expression data from the 209 MeSH annotations for 37 427 microarrays of the Affymetrix U133 Plus 2.0 Array platform.

Supplementary Material

Supplementary Material is available at HMG online.

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A multi-ancestry genome-wide study incorporating gene-smoking interactions identifies multiple new loci for pulse pressure and mean arterial pressure: Supplementary Material

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Supplementary Notes

Stage 1 Study Descriptions

Brief descriptions are provided below for each of the discovery studies some of which are based outside the United States.:

AGES (Age Gene/Environment Susceptibility Reykjavik Study): The AGES Reykjavik study originally comprised a random sample of 30,795 men and women born in 1907-1935 and living in Reykjavik in 1967. A total of 19,381 people attended, resulting in a 71% recruitment rate. The study sample was divided into six groups by birth year and birth date within month. One group was designated for longitudinal follow up and was examined in all stages; another was designated as a control group and was not included in examinations until 1991. Other groups were invited to participate in specific stages of the study. Between 2002 and 2006, the AGES Reykjavik study re-examined 5,764 survivors of the original cohort who had participated before in the Reykjavik Study. The midlife data blood pressure measurement was taken from stage 3 of the Reykjavik Study (1974-1979), if available. Half of the cohort attended during this period. Otherwise an observation was selected closest in time to the stage 3 visit. The supine blood pressure was measured twice by a nurse using a mercury sphygmomanometer after 5 minutes rest following World Health Organization recommendations.

ARIC (Atherosclerosis Risk in Communities): The ARIC study is a population-based prospective cohort study of cardiovascular disease sponsored by the National Heart, Lung, and Blood Institute (NHLBI). ARIC included 15,792 individuals, predominantly European American and African American, aged 45-64 years at baseline (1987-89), chosen by probability sampling from four US communities. Cohort members completed three additional triennial follow-up examinations, a fifth exam in 2011-2013, and a sixth exam in 2016-2017. The ARIC study has been described in detail previously (The ARIC Investigators. The Atherosclerosis Risk in Communities (ARIC) study: Design and objectives. *Am J Epidemiol.* 1989;129:687-702).. Blood pressure was measured using a standardized Hawksley random-zero mercury column sphygmomanometer with participants in a sitting position after a resting period of 5 minutes. The size of the cuff was chosen according to the arm circumference. Three sequential recordings for systolic and diastolic blood pressure were obtained; the mean of the last two measurements was used in this analysis, discarding the first reading. Blood pressure lowering medication use was recorded from the medication history.

Baependi Heart Study (Brazil): The Baependi Heart Study, is an ongoing family-based cohort conducted in a rural town of the state of Minas Gerais. The study has enrolled approximate 2,200 individuals (over 10% of the town's adult population) and 10-year follow up period of longitudinal data. Briefly, probands were selected at random across 11 out of the 12 census districts in Baependi. After enrolment, the proband's first-degree (parents, siblings, and offspring), second-degree (half-siblings, grandparents/grandchildren, uncles/aunts, nephews/nieces, and double cousins), and third-degree (first cousins, great uncles/aunts, and great nephews/nieces) relatives, and his/her respective spouse's relatives resident both within Baependi (municipal and rural area) and surrounding towns were invited to participate. Only individuals age 18 and older were eligible to participate in the study. The study is conducted from a clinic/office in an easily accessible sector of the town, where the questionnaires were completed. A broad range of phenotypes ranging from cardiovascular, neurocognitive, psychiatric, imaging, physiologic and several layers of endophenotypes like metabolomics and

lipidomics have been collected throughout the years. Details about follow-up visits and available data can be found in the cohort profile paper (PMID: 18430212). DNA samples were genotyped using the Affymetrix 6.0 genechip. After quality control, the data were prephased using SHAPEIT and imputed using IMPUTE2 based on 1000 Genomes haplotypes.

BioMe Biobank (BioMe Biobank of Institute for Personalized Medicine at Mount Sinai):

The BioMe Biobank, founded in September 2007, is an ongoing, consented electronic medical record (EMR)-linked bio- and data repository that enrolls participants non-selectively from the Mount Sinai Medical Center patient population. The BioMe Biobank currently (Winter 2015) comprises over 31,000 participants from diverse ancestries characterized by a broad spectrum of (longitudinal) biomedical traits. On average 400 new participants are consented each month. BioMe participants represent the broad ancestral, ethnic and socioeconomic diversity with a distinct and population-specific disease burden, characteristic of Northern Manhattan communities served by Mount Sinai Hospital. Enrolled participants consent to be followed throughout their clinical care (past, present, and future) at Mount Sinai in real-time, integrating their genomic information with their electronic health record for discovery research and clinical care implementation. BioMe participants are predominantly of African, Hispanic/Latino, and European ancestry. Participants who self-identify as Hispanic/Latino further report to be of Puerto Rican (39%), Dominican (23%), Central/South American (17%), Mexican (5%) or other Hispanic (16%) ancestry. More than 40% of European ancestry participants are genetically determined to be of Ashkenazi Jewish ancestry.

The IRB-approved BioMe Biobank consent permits use of samples and de-identified linkable past, present and future clinical information from EMRs; re-contacting participants for enrollment in future research; unlimited duration of storage, and access to clinical information from the entire medical records, as well as local and external sharing of specimens and data.

The BioMe Biobank has a longitudinal design as participants consent to make any EMR data from past (dating back as far as 2003), present and future inpatient or outpatient encounters available for research. The median number of clinical encounters per participant is 21, reflecting predominant enrollment of participants with common chronic conditions from primary care facilities. Mount Sinai's system-wide Epic EMR implementation captures a full spectrum of biomedical phenotypes, including clinical outcomes, covariate and exposure data. This clinical information is complemented by detailed information on ancestry, residence history, familial medical history, education, socio-economic status, physical activity, smoking, alcohol use, and weight history being collected in a systematic manner by interview-based questionnaire at time of enrollment. Phenotype harmonization and validation is critical to facilitate consortium-wide analyses. By applying advanced medical informatics and data mining tools, high-quality and validated phenotype data can be culled from Mount Sinai's Epic EMR. Fully-implemented phenotype algorithms include; T2D, CKD, CAD, lipid disorders, peripheral artery disease, resistant hypertension, blood cell traits, abdominal aortic aneurism, venous thromboembolism among others (see also Phenotype KnowledgeBase ([PheKB](http://phekb.org)) of the eMERGE Network (<http://emerge.mc.vanderbilt.edu/emerge-network>)).

A total of 14,017 participants have been genotyped for both GWAS (11,150 Illumina OmniExpress BeadChip, 2,867 Affymetrix Human SNP Array 6.0) and ExomeChip (Illumina

HumanExome v1.0 BeadChip) arrays funded by institutional sources. An additional 16,000 BioMe participants are scheduled for genotyping using the Illumina MEGA Chip (by April 2015), funded by NHGRI through our PAGEII grant (U01HG007417) (n=12,500) and through institutional funds (n=3,500).

CARDIA (Coronary Artery Risk Development in Young Adults): CARDIA is a prospective multicenter study with 5,115 adults Caucasian and African American participants of the age group 18-30 years, recruited from four centers at the baseline examination in 1985-1986. The recruitment was done from the total community in Birmingham, AL, from selected census tracts in Chicago, IL and Minneapolis, MN; and from the Kaiser Permanente health plan membership in Oakland, CA. The details of the study design for the CARDIA study have been previously published¹. Eight examinations have been completed since initiation of the study, respectively in the years 0, 2, 5, 7, 10, 15, 20 and 25. Written informed consent was obtained from participants at each examination and all study protocols were approved by the institutional review boards of the participating institutions. Systolic and diastolic blood pressure was measured in triplicate on the right arm using a random-zero sphygmomanometer with the participant seated and following a 5-min. rest. The average of the second and third measurements was taken as the blood pressure value. Blood pressure medication use was obtained by questionnaire.

Reference: Friedman GD, Cutter GR, Donahue RP, Hughes GH, Hulley SM, Jacobs DR Jr, Liu K, Savage PJ. CARDIA: study design, recruitment, and some characteristics of the examined subjects. *J Clin Epidemiol.* 1988; 41(11):1105-16

CHS (Cardiovascular Health Study): CHS is a population-based cohort study of risk factors for cardiovascular disease in adults 65 years of age or older conducted across four field centers [PMID: 1669507]. The original predominantly European ancestry cohort of 5,201 persons was recruited in 1989-1990 from random samples of the Medicare eligibility lists and an additional predominately African-American cohort of 687 persons was enrolled in 1992-93 for a total sample of 5,888. Research staff with central training in blood pressure measurement assessed repeated right-arm seated systolic and diastolic blood pressure levels at baseline with a Hawksley random-zero sphygmomanometer. Blood samples were drawn from all participants at their baseline examination and DNA was subsequently extracted from available samples. European ancestry participants were excluded from the GWAS study sample due to prevalent coronary heart disease, congestive heart failure, peripheral vascular disease, valvular heart disease, stroke, or transient ischemic attack at baseline. After QC, genotyping was successful for 3271 European ancestry and 823 African-American participants. CHS was approved by institutional review committees at each site and individuals in the present analysis gave informed consent including consent to use of genetic information for the study of cardiovascular disease.

CROATIA-Korcula: The CROATIA-Korcula study is a family-based, cross-sectional study in the isolated island of Korcula that included 965 examinees aged 18-95. Blood samples were collected in 2007 along with many clinical and biochemical measures and lifestyle and health questionnaires.

CROATIA-Vis: The CROATIA-Vis study is a family-based, cross-sectional study in the isolated island of Vis that included 1,056 examinees aged 8-93. Blood samples were collected in 2003 and 2004 along with many clinical and biochemical measures and lifestyle and health questionnaires.

ERF (Erasmus Rucphen Family study): Erasmus Rucphen Family is a family based study that includes inhabitants of a genetically isolated community in the South-West of the Netherlands, studied as part of the Genetic Research in Isolated Population (GRIP) program. The goal of the study is to identify the risk factors in the development of complex disorders. Study population includes approximately 3,000 individuals who are living descendants of 22 couples who lived in the isolate between 1850 and 1900 and had at least six children baptized in the community church. All data were collected between 2002 and 2005. All participants gave informed consent, and the Medical Ethics Committee of the Erasmus University Medical Centre approved the study.

FamHS (Family Heart Study): The NHLBI FamHS study design, collection of phenotypes and covariates as well as clinical examination have been previously described (<https://dsgweb.wustl.edu/fhsc/>; PMID: 8651220). In brief, the FamHS recruited 1,200 families (approximately 6,000 individuals), half randomly sampled, and half selected because of an excess of coronary heart disease (CHD) or risk factor abnormalities as compared with age- and sex-specific population rates. The participants were sampled from four population-based parent studies: the Framingham Heart Study, the Utah Family Tree Study, and two centers for the Atherosclerosis Risk in Communities study (ARIC: Minneapolis, and Forsyth County, NC). These individuals attended a clinic exam (1994-1996) and a broad range of phenotypes were assessed in the general domains of CHD, atherosclerosis, cardiac and vascular function, inflammation and hemostasis, lipids and lipoproteins, blood pressure, diabetes and insulin resistance, pulmonary function, diet, education, socioeconomic status, habitual behavior, physical activity, anthropometry, medical history and medication use. Approximately 8 years later, study participants belonging to the largest pedigrees were invited for a second clinical exam (2002-04). The most important CHD risk factors were measured again, including lipids, parameters of glucose metabolism, blood pressure, anthropometry, and several biochemical and hematologic markers. In addition, a computed tomography examination provided measures of coronary and aortic calcification, and abdominal and liver fat burden. Medical history and medication use was updated. A total of 2,756 European ancestry subjects in 510 extended random and high CHD risk families were studied. Also, 633 African ancestry subjects were recruited at ARIC field center at the University of Alabama in Birmingham. Informed consent was obtained from all participants.

FHS (Framingham Heart Study): FHS began in 1948 with the recruitment of an original cohort of 5,209 men and women (mean age 44 years; 55 percent women). In 1971 a second generation of study participants was enrolled; this cohort (mean age 37 years; 52% women) consisted of 5,124 children and spouses of children of the original cohort. A third generation cohort of 4,095 children of offspring cohort participants (mean age 40 years; 53 percent women) was enrolled in 2002-2005 and are seen every 4 to 8 years. Details of study designs for the three cohorts are summarized elsewhere. At each clinic visit, a medical history was obtained with a focus on cardiovascular content, and participants underwent a physical examination including measurement of height and weight from which BMI was calculated. Systolic and diastolic blood pressures were measured twice by a physician on the left arm of the resting and seated participant using a mercury column sphygmomanometer. Blood pressures were recorded to the nearest even number. The means of two separate systolic and diastolic blood pressure readings at each clinic examination were used for statistical analyses.

GENOA (Genetic Epidemiology Network of Arteriopathy): GENOA is one of four networks in the NHLBI Family-Blood Pressure Program (FBPP). [The FBPP Investigators, 2002; Daniels et al., 2004] GENOA's long-term objective is to elucidate the genetics of target organ complications of hypertension, including both atherosclerotic and arteriolosclerotic complications involving the heart, brain, kidneys, and peripheral arteries. The longitudinal GENOA Study recruited European-American and African-American sibships with at least 2 individuals with clinically diagnosed essential hypertension before age 60 years. All other members of the sibship were invited to participate regardless of their hypertension status. Participants were diagnosed with hypertension if they had either 1) a previous clinical diagnosis of hypertension by a physician with current anti-hypertensive treatment, or 2) an average systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg based on the second and third readings at the time of their clinic visit. Exclusion criteria were secondary hypertension, alcoholism or drug abuse, pregnancy, insulin-dependent diabetes mellitus, or active malignancy. During the first exam (1995-2000), 1,583 European Americans from Rochester, MN and 1,854 African Americans from Jackson, MS were examined. Between 2000 and 2005, 1,241 of the European Americans and 1,482 of the African Americans returned for a second examination. Because African-American probands for GENOA were recruited through the Atherosclerosis Risk in Communities (ARIC) Jackson field center participants, we excluded ARIC participants from analyses.

References: The FBPP Investigators. Multi-center genetic study of hypertension: The Family Blood Pressure Program (FBPP). *Hypertension* 2002;39:3-9. PubMed PMID: 11799070.

Daniels PR, Kardia SL, Hanis CL, Brown CA, Hutchinson R, Boerwinkle E, Turner ST; Genetic Epidemiology Network of Arteriopathy study. Familial aggregation of hypertension treatment and control in the Genetic Epidemiology Network of Arteriopathy (GENOA) study. *Am J Med*. 2004;116:676-81. PubMed PMID: 15121494.

GenSalt (Genetic Epidemiology Network of Salt Sensitivity): GenSalt is a multi-center, family based study designed to identify, through dietary sodium and potassium intervention, salt-sensitivity susceptibility genes which may underlie essential hypertension in rural Han Chinese families. Approximately 629 families with at least one 'proband' with high blood pressure were recruited and tested for a wide variety of physiological, metabolic and biochemical measures at baseline and at multiple times during the 3-week intervention. The intervention consisted of one week on a low sodium diet, followed by one week on a high sodium diet, and finally one week on a high sodium diet with a potassium supplement.

GOLDN (Genetics of Diet and Lipid Lowering Network): GOLDN is a multi-center family pharmacogenetic study that is investigating gene- environment interactions on lipid profiles. 1,200 subjects in extended pedigrees were measured before and after two environmental exposures: 1) a dietary fat challenge to assess genetic regulators of fat uptake and clearance and 2) a 3 week clinical trial of fenofibrate to assess pharmacogenetic influences on response to treatment. The goals of the study are to identify and characterize genetic loci that predict the lipid profile treatment responses. <https://dsgweb.wustl.edu/PROJECTS/MP5.html>

GS:SFHS: The Generation Scotland (www.generationscotland.org) Scottish Family Health Study (GS:SFHS) is a family-based genetic epidemiology cohort with DNA, other biological samples (serum, urine and cryopreserved whole blood) and socio-demographic and clinical data from approximately 24,000 volunteers, aged 18-98 years, in ~7,000 family groups. An important

feature of GS:SFHS is the breadth of phenotype information, including detailed data on cognitive function, personality traits and mental health. Although data collection was cross-sectional, GS:SFHS becomes a longitudinal cohort as a result of the ability to link to routine NHS data, using the community health index (CHI) number.

HANDLS (Healthy Aging in Neighborhoods of Diversity across the Life Span): HANDLS is a community-based, longitudinal epidemiologic study examining the influences of race and socioeconomic status (SES) on the development of age-related health disparities among a sample of socioeconomically diverse African Americans and whites. This unique study will assess over a 20-year period physical parameters and also evaluate genetic, biologic, demographic, and psychosocial, parameters of African American and white participants in higher and lower SES to understand the driving factors behind persistent black-white health disparities in overall longevity, cardiovascular disease, and cognitive decline. The study recruited 3,722 participants from Baltimore, MD with a mean age of 47.7 years, 2,200 African Americans and 1,522 whites, with 41% reporting household incomes below the 125% poverty delimiter.

Genotyping was done on a subset of self-reporting African American participants by the Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health (NIH). A larger genotyping effort included a small subset of self-reporting European ancestry samples. This research was supported by the Intramural Research Program of the NIH, NIA and the National Center on Minority Health and Health Disparities.

Health ABC (Health, Aging, and Body Composition): Cohort description: The Health ABC study is a prospective cohort study investigating the associations between body composition, weight-related health conditions, and incident functional limitation in older adults. Health ABC enrolled well-functioning, community-dwelling black (n=1281) and white (n=1794) men and women aged 70-79 years between April 1997 and June 1998. Participants were recruited from a random sample of white and all black Medicare eligible residents in the Pittsburgh, PA, and Memphis, TN, metropolitan areas. Participants have undergone annual exams and semi-annual phone interviews. The current study sample consists of 1559 white participants who attended the second exam in 1998-1999 with available genotyping data.

Genotyping: Genotyping was performed by the Center for Inherited Disease Research (CIDR) using the Illumina Human1M-Duo BeadChip system. Samples were excluded from the dataset for the reasons of sample failure, genotypic sex mismatch, and first-degree relative of an included individual based on genotype data. Genotyping was successful in 1663 Caucasians. Analysis was restricted to SNPs with minor allele frequency $\geq 1\%$, call rate $\geq 97\%$ and HWE $p \geq 10^{-6}$. Genotypes were available on 914,263 high quality SNPs for imputation based on the HapMap CEU (release 22, build 36) using the MACH software (version 1.0.16). A total of 2,543,888 imputed SNPs were analyzed for association with vitamin D levels.

Association analysis: Linear regression models were used to generate cohort-specific residuals of naturally log transformed vitamin D levels adjusted for age, sex, BMI and season defined as summer (June-August), fall (September-November), winter (December to February) and spring (March to May) standardized to have mean 0 and variance of 1. Association between the additively coded SNP genotypes and the vitamin D residuals standardized was assessed using linear regression models. For imputed SNPs, expected number of minor alleles (i.e. dosage) was used in assessing association with the vitamin D residuals.

HERITAGE (Health, Risk Factors, Exercise Training and Genetics): The HERITAGE is the only known family-based study of exercise intervention to evaluate the role of genes and sequence variants involved in the response to a physically active lifestyle. The current study is based on the data collected at baseline of the study from 99 White families (244 males, 255 females). All subjects were required to be sedentary and free of chronic diseases at baseline. There are over 18 trait domains (e.g. dietary, lipids and lipoproteins, glucose and insulin metabolism [fasting and IVGTT], steroids, body composition and body fat distribution, cardiorespiratory fitness), for a grand total of over one thousand variables. Moreover, most of the outcome traits were measured twice on two separate days both at baseline and after exercise training was completed. Marker data include a genome-wide linkage scan and GWAS, in addition to a large number of candidate genes.

HUFS (Howard University Family Study): HUFS followed a population-based selection strategy designed to be representative of African American families living in the Washington, DC metropolitan area. The major objectives of the HUFS were to study the genetic and environmental basis of common complex diseases including hypertension, obesity and associated phenotypes. Participants were sought through door-to-door canvassing, advertisements in local print media and at health fairs and other community gatherings. In order to maximize the utility of this cohort for the study of multiple common traits, families were not ascertained based on any phenotype. During a clinical examination, demographic information was collected by interview.

HyperGEN (Hypertension Genetic Epidemiology Network): HyperGEN is a family-based study that looks at the genetic causes of hypertension and related conditions in EA and AA subjects. HyperGEN recruited hypertensive sibships, along with their normotensive adult offspring, and an age-matched random sample. HyperGEN has collected data on 2,471 Caucasian-American subjects and 2,300 African-American subjects, from five field centers in Alabama, Massachusetts, Minnesota, North Carolina, and Utah.

JHS (Jackson Heart Study): The Jackson Heart Study is a longitudinal, community-based observational cohort study investigating the role of environmental and genetic factors in the development of cardiovascular disease in African Americans. Between 2000 and 2004, a total of 5,306 participants were recruited from a tri-county area (Hinds, Madison, and Rankin Counties) that encompasses Jackson, MS. Details of the design and recruitment for the Jackson Heart Study cohort has been previously published.¹⁻³ Briefly, approximately 30% of participants were former members of the Atherosclerosis Risk in Communities (ARIC) study. The remainder were recruited by either 1) random selection from the Accudata list, 2) commercial listing, 3) a constrained volunteer sample, in which recruitment was distributed among defined demographic cells in proportions designed to mirror those in the overall population, or through the Jackson Heart Study Family Study.

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2. Taylor HA, Jr., Wilson JG, Jones DW, et al. Toward resolution of cardiovascular health disparities in African Americans: design and methods of the Jackson Heart Study. *Ethn Dis* 2005; 15:S6-17.

3. Fuqua SR, Wyatt SB, Andrew ME, et al. Recruiting African-American research participation in the Jackson Heart Study: methods, response rates, and sample description. *Ethn Dis* 2005; 15:S6-29.

Maywood-Loyola Study: Participants were self-identified African Americans from a working class suburb of Chicago, Illinois, USA who were enrolled in studies of BP at the Loyola University Medical Center in Maywood, Illinois, USA as part of the International Collaborative Study on Hypertension in Blacks (ICSHIB) which is described in detail elsewhere (**PMID: 9103091**). Briefly, nuclear families were identified through middle-aged probands who were not ascertained based on any phenotype. Thereafter all available first-degree relatives 18 years old and above were enrolled into the study cohort of families. A screening exam was completed by trained and certified research staff using a standardized protocol (**PMID: 9103091 & 10234089**). Information was obtained on medical history, age, body weight and height. Protocols were reviewed and approved by the IRB at the Loyola University Chicago Stritch School of Medicine prior to recruitment activities. This present study included unrelated adults sampled and for whom information on anthropometrics, BP and use of antihypertensive medication was available. BP measurements were obtained using an oscillometric device, previously evaluated in our field settings (**PMID: 10234089**). Three measurements were taken three minutes apart and the average of the final two was used in the analysis. Individuals with SBP ≥ 140 mmHg, DBP ≥ 90 mmHg or on anti-hypertensive medication at time of exam were defined as hypertensive. Participants with hypertension were offered treatment after detection at the screening exam.

Maywood-Nigeria Study: The sampling frame for the Nigeria cohort was also provided by the International Collaborative Study on Hypertension in Blacks (ICSHIB) as described in detail elsewhere (**PMID: 9103091**). Study participants were recruited from Igbo-Ora and Ibadan in southwest Nigeria as part of a long-term study on the environmental and genetic factors underlying hypertension. The base cohort consists of over 15,000 participants with information available on anthropometrics, BP and use of antihypertensive medication. BP measurements followed the same protocol described in the Loyola-Maywood study. This present study included unrelated adults samples from the cohort and some hypertensive participants who were recruited as controls in the Africa-America Diabetes Mellitus (AADM) Study recruited from Ibadan in similar neighborhoods (**PMID: 11164120**). Both projects were reviewed and approved by the sponsoring US institutions (Loyola University Chicago and Howard University) and the University of Ibadan. All participants signed informed consent administered in either English or Yoruba. BP measurements were obtained using an oscillometric device, previously evaluated in our field settings (**PMID: 10234089**). Three measurements were taken three minutes apart and the average of the final two was used in the analysis. Individuals with SBP ≥ 140 mmHg, DBP ≥ 90 mmHg or on anti-hypertensive medication at time of exam were defined as hypertensive. Participants with hypertension were offered treatment after detection at the screening exam.

MESA (Multi-Ethnic Study of Atherosclerosis): The Multi-Ethnic Study of Atherosclerosis (MESA) is a study of the characteristics of subclinical cardiovascular disease and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease. MESA consisted of a diverse, population-based sample of an initial 6,814 asymptomatic men and women aged 45-84. 38 percent of the recruited participants were white, 28 percent African American, 22 percent Hispanic, and 12 percent Asian, predominantly of Chinese descent. Participants were recruited from six field centers across the United States:

Wake Forest University, Columbia University, Johns Hopkins University, University of Minnesota, Northwestern University and University of California - Los Angeles. Participants are being followed for identification and characterization of cardiovascular disease events, including acute myocardial infarction and other forms of coronary heart disease (CHD), stroke, and congestive heart failure; for cardiovascular disease interventions; and for mortality. The first examination took place over two years, from July 2000 - July 2002. It was followed by four examination periods that were 17-20 months in length. Participants have been contacted every 9 to 12 months throughout the study to assess clinical morbidity and mortality.

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NEO (The Netherlands Epidemiology of Obesity study): The NEO was designed for extensive phenotyping to investigate pathways that lead to obesity-related diseases. The NEO study is a population-based, prospective cohort study that includes 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. At baseline, information on demography, lifestyle, and medical history have been collected by questionnaires. In addition, samples of 24-h urine, fasting and postprandial blood plasma and serum, and DNA were collected. Genotyping was performed using the Illumina HumanCoreExome chip, which was subsequently imputed to the 1000 genome reference panel. Participants underwent an extensive physical examination, including anthropometry, electrocardiography, spirometry, and measurement of the carotid artery intima-media thickness by ultrasonography. In random subsamples of participants, magnetic resonance imaging of abdominal fat, pulse wave velocity of the aorta, heart, and brain, magnetic resonance spectroscopy of the liver, indirect calorimetry, dual energy X-ray absorptiometry, or accelerometry measurements were performed. The collection of data started in September 2008 and completed at the end of September 2012. Participants are currently being followed for the incidence of obesity-related diseases and mortality.

Pelotas Birth Cohort Study (The 1982 Pelotas Birth Cohort Study, Brazil): The maternity hospitals in Pelotas, a southern Brazilian city (current population ~330,000), were visited daily in the year of 1982. The 5,914 liveborns whose families lived in the urban area were examined and their mothers interviewed. Information was obtained for more than 99% of the livebirths. These subjects have been followed-up at the following mean ages: 11.3 months (all children born from January to April 1982; n=1457), 19.4 months (entire cohort; n=4934), 43.1 months (entire cohort; n=4742), 13.1 years (random subsample; n=715), 14.7 years (systematic subsample; n=1076); 18.2 (male cohorts attending to compulsory Army recruitment examination; n=2250), 18.9 (systematic subsample; n=1031), 22.8 years (entire cohort; n=4297) and 30.2 years (entire cohort; n=3701). Details about follow-up visits and available data can be found in the two Cohort Profile papers (PMID: 16373375 and 25733577). DNA samples (collected at the mean age of 22.8 years) were genotyped for ~2.5 million of SNPs using the Illumina HumanOmni2.5-8v1 array (which includes autosomal, X and Y chromosomes, and mitochondrial variants). After quality control, the data were prephased using SHAPEIT and imputed using IMPUTE2 based on 1000 Genomes haplotypes.

RS (Rotterdam Study): The Rotterdam Study is a prospective, population-based cohort study among individuals living in the well-defined Ommoord district in the city of Rotterdam in The

Netherlands. The aim of the study is to determine the occurrence of cardiovascular, neurological, ophthalmic, endocrine, hepatic, respiratory, and psychiatric diseases in elderly people. The cohort was initially defined in 1990 among approximately 7,900 persons, aged 55 years and older, who underwent a home interview and extensive physical examination at the baseline and during follow-up rounds every 3-4 years (RS-I). Cohort was extended in 2000/2001 (RS-II, 3,011 individuals aged 55 years and older) and 2006/2008 (RS-III, 3,932 subjects, aged 45 and older). Written informed consent was obtained from all participants and the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, approved the study.

SCHS-CHD (Singapore Chinese Health Study - Coronary Heart Disease): SCHS-CHD is a case-control study of coronary heart disease that was nested within the Singapore Chinese Health Study (SCHS), a prospective cohort study of 63,257 Singaporean Chinese men and women aged 45-74 years living in Singapore. We selected cases and controls from participants that provided blood samples and were free of coronary heart disease and stroke at the time of blood collection (N=24,454). Cases (N=760) had acute myocardial infarction (AMI) or died of coronary heart disease. AMI was identified through the Singapore Myocardial Infarction Registry or through the nationwide hospital discharge database followed by confirmation of AMI by cardiologists' review of medical records using the Multi-Ethnic Study of Atherosclerosis criteria (available at: <http://www.mesa-nhlbi.org/manuals.aspx>). Coronary heart disease deaths were identified through the Singapore Registry of Births and Deaths (ICD9 410-414 as first stated cause of death). Matched controls (N=1,491) were selected using a risk-set sampling strategy. Controls were participants who were alive and free of coronary heart disease at the time of the diagnosis or death of the index cases and were matched for age, sex, dialect group, year of recruitment and date of blood collection. In-person interviews and phlebotomy were conducted before the onset of disease and non-fasting venous blood was stored at -80°C for extraction of DNA and blood biochemistry.

Singapore: SCES (Singapore Chinese Eye Study): SCES is a population-based, cross-sectional study of Chinese adults aged 40–80+ years residing in the South-Western part of Singapore, which is part of the Singapore Epidemiology of Eye Disease (SEED). Age stratified random sampling was used to select 6,350 eligible participants, of which 3,300 participated in the study (73% response rate). Detailed methodology has been published. Two readings of blood pressure were taken from participants after 5 minutes of rest, seated, using an automated blood pressure monitor (Dinamap Pro100V2; Criticon, Norderstedt, Germany) by trained observers. One of two cuff sizes (regular, large) was chosen on the basis of the circumference of the participant's arm. A third reading was performed if the difference between two readings of either the systolic blood pressure was greater than 10mmHg or the diastolic blood pressure was greater than 5mmHg. The mean values of the closest two readings were calculated. **SiMES (Singapore Malay Eye Study):** SiMES is a population-based cross-sectional epidemiological study of 3,280 individuals from one of the three major ethnic groups residing in Singapore. SiMES is part of the Singapore Epidemiology of Eye Disease (SEED) study. In summary, 5,600 individuals have been selected by an age-stratified sampling strategy. Among these 4,168 individuals are eligible for this study. 3,280 individuals finally participated in the study. All subjects were Malay and aged 40-79 years [PMID: 17365815]. Two readings of blood pressure were taken from participants after 5 minutes of rest, seated, using an automated blood pressure monitor (Dinamap Pro100V2; Criticon, Norderstedt, Germany) by trained observers. One of two cuff sizes (regular, large) was chosen on the basis of the circumference of the participant's arm. A third reading was performed if the difference between two readings of either the systolic blood

pressure was greater than 10mmHg or the diastolic blood pressure was greater than 5mmHg. The mean values of the closest two readings were calculated. **SINDI (Singapore Indian Eye Study):** SINDI is a population-based, cross-sectional study of Asian Indian adults aged 40–80+ years residing in the South-Western part of Singapore, which is part of the Singapore Epidemiology of Eye Disease (SEED). Age stratified random sampling was used to select 6,350 eligible participants, of which 3,400 participated in the study (75.6% response rate). Detailed methodology has been published [PMID: 19995197]. Two readings of blood pressure were taken from participants after 5 minutes of rest, seated, using an automated blood pressure monitor (Dinamap Pro100V2; Criticon, Norderstedt, Germany) by trained observers. One of two cuff sizes (regular, large) was chosen on the basis of the circumference of the participant's arm. A third reading was performed if the difference between two readings of either the systolic blood pressure was greater than 10mmHg or the diastolic blood pressure was greater than 5mmHg. The mean values of the closest two readings were calculated. **SP2 (Singapore Prospective Study Program):** The SP2 is a population-based study of diabetes and cardiovascular disease in Singapore. It first surveyed subjects (Chinese, Malay and Indian) from four cross-sectional studies that were conducted in Singapore between 1982 and 1998. Subjects were between the ages of 24-95 years and represented a random sample of the Singapore population. Subjects were re-visited between 2003 and 2007. Among the 10,747 individuals who were eligible, 5,157 subjects completed a questionnaire and the subsequent clinical examinations. Data from this re-visit were utilized for this study [PMID: 19406920]. Two readings of blood pressure were taken from participants after 5 min of rest, seated, using an automated blood pressure monitor (Dinamap Pro100V2; Criticon, Norderstedt, Germany) by trained observers. One of two cuff sizes (regular, large) was chosen on the basis of the circumference of the participant's arm. A third reading was performed if the difference between two readings of either the systolic blood pressure was greater than 10mmHg or the diastolic blood pressure was greater than 5mmHg. The mean values of the closest two readings were calculated.

WGHS (Women's Genome Health Study): WGHS is a prospective cohort of female North American health care professionals representing participants in the Women's Health Study (WHS) trial who provided a blood sample at baseline and consent for blood-based analyses. Participants in the WHS were 45 years or older at enrollment and free of cardiovascular disease, cancer or other major chronic illness. The current data are derived from 23,294 WGHS participants for whom whole genome genotype information was available at the time of analysis and for whom self-reported European ancestry could be confirmed by multidimensional scaling analysis of 1,443 ancestry informative markers in PLINK v. 1.06. At baseline, BP and lifestyle habits related to smoking, consumption of alcohol, and physical activity as well as other general clinical information were ascertained by a self-reported questionnaire, an approach which has been validated in the WGHS demographic, namely female health care professionals. Questionnaires recorded systolic BP in 9 categories (<110, 110-119, 120-129, 130-139, 140-149, 150-159, 160-169, 170-179, ≥180 mmHg), and diastolic BP in 7 categories (<65, 65-74, 75-84, 85-89, 90-94, 95-104, ≥105 mmHg). All analyses treated these BP responses as quantitative variables representing each category with its midpoint value. Hypertension was defined as one or more of reported physician diagnosis, systolic BP ≥140 mmHg, or diastolic BP ≥90 mmHg.

WHI (Women's Health Initiative): WHI is a long-term national health study that focuses on strategies for preventing common diseases such as heart disease, cancer and fracture in postmenopausal women. A total of 161,838 women aged 50–79 years old were recruited from

40 clinical centers in the US between 1993 and 1998. WHI consists of an observational study, two clinical trials of postmenopausal hormone therapy (HT, estrogen alone or estrogen plus progestin), a calcium and vitamin D supplement trial, and a dietary modification trial¹. Study recruitment and exclusion criteria have been described previously¹. Recruitment was done through mass mailing to age-eligible women obtained from voter registration, driver's license and Health Care Financing Administration or other insurance list, with emphasis on recruitment of minorities and older women². Exclusions included participation in other randomized trials, predicted survival < 3 years, alcoholism, drug dependency, mental illness and dementia. For the CT, women were ineligible if they had a systolic BP > 200 mm Hg or diastolic BP > 105 mm Hg, a history of hypertriglyceridemia or breast cancer. Study protocols and consent forms were approved by the IRB at all participating institutions. Socio-demographic characteristics, lifestyle, medical history and self-reported medications were collected using standardized questionnaires at the screening visit. Physical measures of height, weight and blood pressure were measured at a baseline clinical visit². BP was measured by certified staff using standardized procedures and instruments³. Two BP measures were recorded after 5 minutes rest using a mercury sphygmomanometer. Appropriate cuff bladder size was determined at each visit based on arm circumference. Diastolic BP was taken from the phase V Korotkoff measures. The average of the two measurements, obtained 30 seconds apart, was used in analyses. The genome wide association study (GWAS) non-overlapping samples are composed of a case-control study (WHI Genomics and Randomized Trials Network – GARNET, which included all coronary heart disease, stroke, venous thromboembolic events and selected diabetes cases that happened during the active intervention phase in the WHI HT clinical trials and aged matched controls), women selected to be "representative" of the HT trial (mostly younger white HT subjects that were also enrolled in the WHI memory study - WHIMS) and the WHI SNP Health Association Resource (WHI SHARe), a randomly selected sample of 8,515 African American and 3,642 Hispanic women from WHI. GWAS was performed using Affymetrix 6.0 (WHI-SHARe), HumanOmniExpressExome-8v1_B (WHIMS), Illumina HumanOmni1-Quad v1-0 B (GARNET). Extensive quality control (QC) of the GWAS data included alignment ("flipping") to the same reference panel, imputation to the 1000G data (using the recent reference panel - v3.20101123), identification of genetically related individuals, and computations of principal components (PCs) using methods developed by Price et al. (using EIGENSOFT software 53), and finally the comparison with self-reported ethnicity. After QC and exclusions from analysis protocol, the number of women included in analysis is 4,423 whites for GARNET, 5,202 white for WHIMS, 7,919 for SHARe African American and 3,377 for SHARe Hispanics.

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2. Design of the women's health initiative clinical trial and observational study. The women's health initiative study group. *Control Clin Trials.* 1998;19:61-109
3. Hsia J, Margolis KL, Eaton CB, Wenger NK, Allison M, Wu L, LaCroix AZ, Black HR. Prehypertension and cardiovascular disease risk in the women's health initiative. *Circulation.* 2007;115:855-860

Stage 2 Study Descriptions

Brief descriptions are provided below for each of the replication studies/cohorts:

AA-DHS (African American Diabetes Heart Study): AA-DHS objectives are to improve understanding of ethnic differences in CAC and CP in populations of African and European ancestry. The AA-DHS consists of self-reported African Americans with T2D recruited from two Wake Forest School of Medicine (WFSM) studies: the family-based Diabetes Heart Study (DHS) and unrelated individuals in the AA-DHS. DHS is a cross-sectional study of European American and African American families with siblings concordant for T2D. AA-DHS started after DHS and enrolled unrelated African Americans. The AA-DHS GWAS utilized the Illumina 5M chip with imputation to 1,000 Genomes.

Airwave (The Airwave Health Monitoring Study): The Airwave Health Monitoring Study (22) was established to evaluate possible health risks associated with use of TETRA, a digital communication system used by police forces and other emergency services in Great Britain since 2001. The study has been broadened to investigate more generally the health of the work force. From 2004, participants from each force who agreed to participate were enrolled either with an enrolment questionnaire or a comprehensive health screening performed locally. This includes questionnaire, 7-day food diaries, anthropometry, measurements of cardiovascular and cognitive function, blood chemistry, coagulation and hematology. Systolic and diastolic blood pressures were measured as three consecutive readings using a digital blood pressure monitor (Omron HEM 705-CP digital BP monitor). By March 2015, the study had recruited 53,606 participants, of whom 45,433 had attended the health screening, and 14,002 have genotype data (1000G imputed).

Ref: Elliott, P. et al. The Airwave Health Monitoring Study of police officers and staff in Great Britain: rationale, design and methods. *Environ Res* 134, 280-5 (2014).

ASCOT (Anglo-Scandinavian Cardiac Outcomes Trial): ASCOT is a randomised control clinical trial investigating the cardiac outcomes of blood pressure lowering and lipid lowering treatments. Of 19,342 hypertensive patients (40–79 years of age with at least three other cardiovascular risk factors) who were randomized to one of two antihypertensive regimens in ASCOT (atenolol, Beta-Blocker vs amlodipine, Calcium-Channel-Blocker), 10,305 patients with non-fasting total cholesterol concentrations of 6.5 mmol/l or less (measured at the non-fasting screening visit) had been randomly assigned additional atorvastatin 10 mg or placebo. Only a proportion of United Kingdom, Irish, Sweden, Norway, Finland and Denmark consented to contribute DNA and participate in genetic studies. PMID 11685901

BBJ (Biobank Japan Project): The Biobank Japan (BBJ) Project was established in 2003 with the aim of the implementation of personalized medicine as a leading project of Ministry of Education, Culture, Sports, Science and Technology (MEXT). In collaboration with twelve cooperating institutes, the BBJ has recruited a total of 200,000 people, suffering from at least one of the 47 target common diseases, in the first phase (5-year period). BBJ has collected biospecimens including DNA and serum as well as various clinical and lifestyle information through interview or medical records by using standardized questionnaire. All participants gave written informed consent to this project and this study was approved by ethical committees of RIKEN and participating institutes.

BES (Beijing Eye Study): The Beijing Eye Study is a population-based study that assesses the associated and risk factors of ocular and general diseases in a Chinese population. The study was initialized in 2001 and collected data from 4439 subjects aged ≥ 40 years and living in seven communities in the Beijing area. Three of these communities were located in a rural district and four were located in an urban district. The BES was followed-up in 2006, with 3251 of the original subjects participating, and in 2011, with 2695 subjects returning for the follow-up examination. At the examinations in 2006 and 2011, trained research staffs asked the subjects questions from a standard questionnaire providing information on the family status, level of education, income, quality of life, psychic depression, physical activity, and known major systemic diseases. Fasting blood samples were taken for measurement of concentrations of substances such as blood lipids, glucose, and glycosylated hemoglobin. Individuals were classified as self-reported non-smokers or self-reported current smokers. Alcohol consumption habits based on number of drinks per day were collected. Physical activity was assessed in questions on the number of hours per day and number of days per week spent on intensively or moderately performed sport activities, spent on walking, on riding a bicycle, and spent on sitting. All variables used in analyses were taken from examinations in 2006 or in 2011. The BES subjects were genotyped on two arrays, Illumina Human610-Quad (N = 832) and Illumina OmniExpress (N = 814).

BRIGHT (British Genetics of Hypertension): Participants of the BRIGHT Study are recruited from the Medical Research Council General Practice Framework and other primary care practices in the UK. Each case had a history of hypertension diagnosed prior to 60 years of age with confirmed blood pressure recordings corresponding to seated levels $>150/100$ mmHg (1 reading) or mean of 3 readings $>145/95$ mmHg. BRIGHT is focused on recruitment of hypertensive individuals with BMI <30 . Sample selection for GWAS was based on DNA availability and quantity. PMID 12826435

CAGE-Amagasaki (Cardio-metabolic Genome Epidemiology Network, Amagasaki Study): The Amagasaki Study (CAGE-Amagasaki) is an ongoing population-based cohort study of 5,743 individuals (3,435 males and 2,310 females), aged >18 years and recruited for a baseline examination between September 2002 to August 2003. Participants were interviewed by trained personnel to obtain information on medical and lifestyle variables, and consented to provide DNA for genotyping of molecular variants to investigate genetic susceptibility for so-called lifestyle-related diseases such as hypertension and cardiovascular disorder.

CFS (Cleveland Family Study): The Cleveland Family Study (CFS) is a family-based, longitudinal study designed to characterize the genetic and non-genetic risk factors for sleep apnea. In total, 2534 individuals (46% African American) from 352 families were studied on up to 4 occasions over a period of 16 years (1990-2006). The initial aim of the study was to quantify the familial aggregation of sleep apnea. 632 African Americans were genotyped on the Affymetrix array 6.0 platform through the CARE Consortium with suitable genotyping quality control. A further 122 African-Americans had genotyping based on the Illumina OmniExpress + Exome platform. Genomes were imputed separately for each chip based on a 1000 Genomes Project Phase 3 Version 5 cosmopolitan template using SHAPEIT and IMPUTE2. Participants had three supine BP measurements each performed after lying quietly for 10 minutes, before bed (10:00 P.M.) and upon awakening (7:00 A.M.), and another three sitting at 11 am, following standardized guidelines using a calibrated sphygmomanometer. Cuff size was determined by

the circumference of the upper arm and the appropriate bladder size from a standard chart. BP phenotypes were determined from the average of the nine measurements.

Colaus (Cohorte Lausannoise): The cohort is a random population sample of the city of Lausanne aged 35-75 years. Recruitment began in June 2003 and ended in May 2006, and the first follow-up was conducted between April 2009 and September 2012. The CoLaus study was approved by the Institutional Ethics Committee of the University of Lausanne and informed consent was appropriately obtained by all participants. Both at baseline and follow-up, all participants attended the outpatient clinic of the University Hospital of Lausanne in the morning after an overnight fast. Data were collected by trained field interviewers in a single visit lasting about 60 min.

DESIR (Data from an Epidemiological Study on the Insulin Resistance): The DESIR cohort study aims to: describe and understand the relations between the abnormalities of the syndrome, their evolution, according to age and sex; search for risk factors of insulin resistance, in particular factors associated with the environment, lifestyle and genetic markers; quantify the links between the syndrome and both cardiovascular disease and diabetes; evaluate the frequency of the syndrome in terms of its consequences on public health.

DFTJ (Dongfeng-Tongji Cohort Study): The DFTJ-cohort study includes 27,009 retired employees from a state-owned automobile enterprise in China. This study was launched in 2008 and will be followed up every 5 years. In 2013 we conducted the first follow-up. By using semi-structural questionnaire and health examination, those having cancer or severe diseases were excluded. Fasting blood samples and detailed epidemiology data were collected. The main goal of the cohort was to identify the environmental and genetic risk factors and the gene-environment interactions on chronic diseases, and to find novel biomarkers for chronic disease and mortality prediction. Finally, 1,461 included in the present study with GWAS data. All of the participants wrote informed consent and the ethical committees in the Tongji Medical College approved this research project. Detailed information has been described in elsewhere(1).

QC criteria and imputation methods:

We did the GWAS scan on the DFTJ-cohort with Affymetrix Genome-Wide Human SNP Array 6.0 chips. In total, we genotyped 906,703 SNPs among 1,461 subjects. After stringent QC filtering, SNPs with MAF < 0.01, Hardy-Weinberg Equilibrium (HWE) < 0.0001, and SNP call rate < 95% were excluded. Individuals with call rates < 95% were also not included for further analysis. In total, we retained 1,452 subjects with 658,288 autosomal SNPs for statistical analyses, with an overall call rate of 99.68%. We used MACH 1.0 software to impute untyped SNPs using the LD information from the HapMap phase II database (CHB+JPT as a reference set (2007-08_rel22, released 2007-03-02). Imputed SNPs with high genotype information content ($R_{sq} > 0.3$ for MACH) were kept for the further association analysis.

1) Wang, F., Zhu, J., Yao, P., Li, X., He, M., Liu, Y., Yuan, J., Chen, W., Zhou, L., Min, X. et al. (2012) Cohort profile: The Dongfeng-Tongji cohort study of retired workers. *International journal of epidemiology*.

DHS (Diabetes Heart Study): The Diabetes Heart Study (DHS) is an ongoing family-based cohort study investigating the epidemiology and genetics of cardiovascular disease (CVD) in a population-based sample. The DHS recruited T2D-affected siblings without advanced renal insufficiency from 1998 through 2005 in western North Carolina. DHS has collected genetic data

on 1,220 self-described European American (EA) individuals from 475 families. Genotyping was completed using an Affymetrix Genome-Wide Human SNP Array 5.0 with imputation of 1,000 Genomes project SNPs from this array using IMPUTE2 and the Phase I v2, cosmopolitan (integrated) reference panel, build 37.

DR's EXTRA (Dose-Responses to Exercise Training): The Dose-Responses to Exercise Training (DR's EXTRA) Study is a 4-year RCT on the effects of regular physical exercise and healthy diet on endothelial function, atherosclerosis and cognition in a randomly selected population sample (n=3000) of Eastern Finnish men and women, identified from the national population register, aged 55-74 years. Of the eligible sample, 1410 individuals were randomized into one of the 6 groups: aerobic exercise, resistance exercise, diet, combined aerobic exercise and diet, combined resistance exercise and diet, or reference group following baseline assessments. During the four year intervention the drop-out rate was 15%.

EGCUT (Estonian Genome Center - University of Tartu (Estonian Biobank)): The Estonian Biobank is the population-based biobank of the Estonian Genome Center at the University of Tartu (www.biobank.ee; EGCUT). The entire project is conducted according to the Estonian Gene Research Act and all of the participants have signed the broad informed consent. The cohort size is up to 51535 individuals from 18 years of age and up, which closely reflects the age, sex and geographical distribution of the Estonian population. All of the subjects are recruited randomly by general practitioners and physicians in hospitals. A Computer Assisted Personal interview is filled within 1-2 hours at a doctor's office, which includes personal, genealogical, educational, occupational history and lifestyle data. Anthropometric measurements, blood pressure and resting heart rate are measured and venous blood taken during the visit. Medical history and current health status is recorded according to ICD-10 codes.

EPIC (European Prospective Investigation into Cancer and Nutrition): The European Prospective Investigation of Cancer (EPIC) began as a large multi-centre cohort study primarily looking at the connection between diet, lifestyle factors and cancer, although the study was broadened from the outset to include other conditions. The EPIC-Norfolk participants are men and women who were aged between 40 and 79 when they joined the study and who lived in Norwich and the surrounding towns and rural areas. They have been contributing information about their diet, lifestyle and health through questionnaires and health checks over two decades. The Norwich Local Research Ethics Committee granted ethical approval for the study. All participants gave written informed consent.

FENLAND (The Fenland Study): The Fenland study is a population-based cohort study that uses objective measures of disease exposure to investigate the influence of diet, lifestyle and genetic factors on the development of diabetes and obesity. The volunteers are recruited from general practice lists in and around Cambridgeshire (Cambridge, Ely, and Wisbech) in the United Kingdom from birth cohorts from 1950–1975.

FUSION (Finland-United States Investigation of NIDDM Genetics): The Finland-United States Investigation of NIDDM Genetics (FUSION) study is a long-term effort to identify genetic variants that predispose to type 2 diabetes (T2D) or that impact the variability of T2D-related quantitative traits. The FUSION GWAS sample consists of 1,161 Finnish T2D cases and 1,174 Finnish normal glucose-tolerant (NGT) controls (Scott et al. Science 2007). Cases are defined by fasting plasma glucose ≥ 7.0 mmol/l or 2-h plasma glucose ≥ 11.1 mmol/l, by report of

diabetes medication use, or based on medical record review. 789 FUSION cases each reported at least one T2D sibling; 372 Finrisk 2002 T2D cases came from a Finnish population-based risk factor survey. NGT controls are defined by fasting glucose < 6.1 mmol/l and 2-h glucose < 7.8 mmol/l. FUSION controls include 119 subjects from Vantaa, Finland who were NGT at ages 65 and 70 years, 304 NGT spouses from FUSION families, and 651 Finrisk 2002 subjects. The controls were approximately frequency matched to the cases by age, sex, and birth province. Smoking and alcohol data are only available in the FUSION subset of our GWAS samples.

Scott, L.J. et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316, 1341–1345, 2007.

GeneSTAR (Genetic Studies of Atherosclerosis Risk): GeneSTAR is a family-based prospective study of more than 4000 participants begun in 1983 to determine phenotypic and genetic causes of premature cardiovascular disease. Families were identified from 1983-2006 from probands with a premature coronary disease event prior to 60 years of age who were identified at the time of hospitalization in any of 10 hospitals in the Baltimore, Maryland area. Their apparently healthy 30-59 year old siblings without known coronary disease were recruited and screened between 1983 and 2006. From 2003-2006, adult offspring over 21 years of age of all participating siblings and probands, as well as the coparents of the offspring were recruited and screened. Genotyping was performed in 3,232 participants on the Illumina 1Mv1_c platform.

GLACIER (Gene x Lifestyle Interactions and Complex Traits Involved in Elevated Disease Risk): The Gene-Lifestyle interactions And Complex traits Involved in Elevated disease Risk (GLACIER) Study is nested within the Västerbotten Intervention Programme, which is part of the Northern Sweden Health and Disease Study, a population-based prospective cohort study from northern Sweden. Participants were genotyped with Illumina CardioMetaboChip array. This array contains ~200,000 variants, the majority being common variants. Systolic and diastolic blood pressures were measured once following a period of five minutes rest with the participant in the supine position using a mercury-gauge sphygmomanometer. Analysis of serum lipids (HDL-C, triglycerides and total cholesterol) were undertaken at the Department of Clinical Chemistry at Umeå University Hospital using routine methods. LDL-C was determined using the Friedewald formula. All participants completed a detailed, optically readable, health and lifestyle questionnaire including questions about smoking status and alcohol intake (FFQ). Cohort description - PMID: 25396097

GRAPHIC (Genetic Regulation of Arterial Pressure of Humans in the Community): The GRAPHIC Study comprises 2024 individuals from 520 nuclear families recruited from the general population in Leicestershire, UK between 2003-2005 for the purpose of investigating the genetic determinants of blood pressure and related cardiovascular traits. A detailed medical history was obtained from study subjects by standardized questionnaires and clinical examination was performed by research nurses following standard procedures. Measurements obtained included height, weight, waist-hip ratio, clinic and ambulatory blood pressure and a 12-lead ECG.

HCHS/SOL (Hispanic Community Health Study/ Study of Latinos): The HCHS/SOL is a community-based cohort study of 16,415 self-identified Hispanic/Latino persons aged 18–74 years and selected from households in predefined census-block groups across four US field centers (in Chicago, Miami, the Bronx, and San Diego). The census-block groups were chosen

to provide diversity among cohort participants with regard to socioeconomic status and national origin or background. The HCHS/SOL cohort includes participants who self-identified as having a Hispanic/Latino background; the largest groups are Central American (n = 1,730), Cuban (n = 2,348), Dominican (n = 1,460), Mexican (n = 6,471), Puerto Rican (n = 2,728), and South American (n = 1,068). The HCHS/SOL baseline clinical examination occurred between 2008 and 2011 and included comprehensive biological, behavioral, and sociodemographic assessments. Consenting HCHS/SOL subjects were genotyped at Illumina on the HCHS/SOL custom 15041502 B3 array. The custom array comprised the Illumina Omni 2.5M array (HumanOmni2.5-8v.1-1) ancestry-informative markers, known GWAS hits and drug absorption, distribution, metabolism, and excretion (ADME) markers, and additional custom content including ~150,000 SNPs selected from the CLM (Colombian in Medellin, Colombia), MXL (Mexican Ancestry in Los Angeles, California), and PUR (Puerto Rican in Puerto Rico) samples in the 1000Genomes phase 1 data to capture a greater amount of Amerindian genetic variation. QA/QC procedures yielded a total of 12,803 unique study participants for imputation and downstream association analyses.

HRS (Health & Retirement Study): The Health and Retirement Study (HRS) is a longitudinal survey of a representative sample of Americans over the age of 50. The current sample is over 26,000 persons in 17,000 households. Respondents are interviewed every two years about income and wealth, health and use of health services, work and retirement, and family connections. DNA was extracted from saliva collected during a face-to-face interview in the respondents' homes. These data represent respondents who provided DNA samples and signed consent forms in 2006, 2008, and 2010. Respondents were removed if they had missing genotype or phenotype data.

Juster, F. T., Suzman, R. (1995). An Overview of the Health and Retirement Study, *Journal of Human Resources* 30:Suppl: S7-S56.

Sonnega A, Faul JD, Ofstedal MB, Langa KM, Phillips JWR, Weir DR. Cohort Profile: the Health and Retirement Study (HRS). *Int. J. Epidemiol.* 2014; 43 (2): 576-585. PMID: 24671021

Crimmins, E.M., Guyer H., Langa K.M., Ofstedal M.B., Wallace R.B., and Weir D.R. (2008). Documentation of Physical Measures, Anthropometrics and Blood Pressure in the Health and Retirement Study. HRS Documentation Report DR-011. <http://hrsonline.isr.umich.edu/sitedocs/userg/dr-011.pdf>

HyperGEN-AXIOM (Hypertension Genetic Epidemiology Network): HyperGEN is a family-based study that investigates the genetic causes of hypertension and related conditions in EA and AA subjects. HyperGEN recruited hypertensive sibships, along with their normotensive adult offspring, and an age-matched random sample. HyperGEN has collected data on 2,471 Caucasian-American subjects and 2,300 African-American subjects, from five field centers in Alabama, Massachusetts, Minnesota, North Carolina, and Utah. HyperGEN participates as a discovery study using GWAS available in a large subset of the samples. The remaining AA subjects without GWAS data were genotyped on the Affymetrix Axiom chip as part of a HyperGEN admixture mapping ancillary study. After excluding subjects already included in the original HyperGEN (or with family members included), this subset of approximately 450 AA subjects are included in the HyperGEN-AXIOM study which participates in replications.

INGI-CARL and INGI-FVG (Italian Network Genetic Isolates): INGI-FVG and INGI-CARL studies include samples coming from isolated populations and belong to the ITALIAN

NETWORK OF GENETIC ISOLATES (INGI). INGI-CARL examined about 1000 subjects between 1998 and 2005 coming from a small village of the South of Italy situated in the extreme northern part of Puglia Region, while INGI FVG involved about 1700 subjects between 2008 and 2011 coming from six different villages located in the North-East of Italy in Friuli Venezia Giulia region. A questionnaire was administered to each participant to obtain socio-demographic information, as well as data on professional activity, family history, eating habits and lifestyle, such as smoking, coffee and alcohol consumption, physical activity. Furthermore, a medical screening, including anamnesis, blood pressure, drugs and clinical chemistry evaluation (blood count and different biochemical parameters, such as lipids) were made. All participants gave their written informed consent.

InterAct (The EPIC-InterAct Case-Cohort Study): The large prospective InterAct type 2 diabetes case-cohort study is coordinated by the MRC Epidemiology Unit in Cambridge and nested within the European Prospective Investigation into Cancer and Nutrition (EPIC). EPIC was initiated in the late 1980s and involves collaboration between 23 research institutions across Europe in 10 countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden and the United Kingdom). The majority of EPIC cohorts were recruited from the general population, with some exceptions. French cohorts included women who were members of a health insurance scheme for school and university employees; Turin and Ragusa (Italy) and the Spanish centres included some blood donors. Participants from Utrecht (Netherlands) and Florence (Italy) were recruited via a breast cancer screening program. The majority of participants recruited by the EPIC Oxford (UK) centre consisted of vegetarian and “health conscious” volunteers from England, Wales, Scotland, and Northern Ireland.

IRAS (Insulin Resistance Atherosclerosis Study): The Insulin Resistance Atherosclerosis Study (IRAS) was an epidemiologic cohort study designed to examine the relationship between insulin resistance and carotid atherosclerosis across a range of glucose tolerance. Individuals of self-reported Mexican-American ethnicity were recruited in San Antonio, TX and San Luis Valley, CO. Recruitment was balanced across age and glucose tolerance status. Inclusion of IRAS data is limited to 194 normoglycemic individuals with genotype data from the Illumina OmniExpress and Omni 1S arrays and imputation to the 1000 Genome Integrated Reference Panel (phase I).

IRAS Family Study (Insulin Resistance Atherosclerosis Study): The IRASFS was a family study designed to examine the genetic and epidemiologic basis of glucose homeostasis traits and abdominal adiposity. Briefly, self-reported Mexican pedigrees were recruited in San Antonio, TX and San Luis Valley, CO. Probands with large families were recruited from the initial non-family-based IRAS, which was modestly enriched for impaired glucose tolerance and T2D. Inclusion of IRASFS data is limited to 1040 normoglycemic individuals in 88 pedigrees with genotype data from the Illumina OmniExpress and Omni 1S arrays and imputation to the 1000 Genome Integrated Reference Panel (phase I).

JUPITER (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin): Genetic analysis was performed in a sub-population from JUPITER (Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin), an international, randomized, placebo-controlled trial of rosuvastatin (20mg/day) in the primary prevention of cardiovascular disease conducted among apparently healthy men and women with LDL-C < 130 mg/dL and hsCRP \geq 2 mg/L (1, 2). Individuals with diabetes or triglyceride concentration >500mg/dL were excluded. The present analysis includes only individuals who

provided consent for genetic analysis, had successfully collected genotype information, and who had either verified European or verified South African black ancestry.

1) Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ; JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* 2008 Nov 20; 359(21):2195-207

2) Chasman DI, Giulianini F, MacFadyen J, Barratt BJ, Nyberg F, Ridker PM. Genetic determinants of statin-induced low-density lipoprotein cholesterol reduction: the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial. *Circ Cardiovasc Genet*. 2012 Apr 1;5(2):257-64. doi: 10.1161/CIRCGENETICS.111.961144. Epub 2012 Feb 13. Erratum in: *Circ Cardiovasc Genet*. 2012 Jun;5(3):e27. PubMed PMID: 22331829.

KORA (Cooperative Health Research in the Augsburg Region): The KORA study is a series of independent population-based epidemiological surveys of participants living in the region of Augsburg, Southern Germany. All survey participants are residents of German nationality identified through the registration office and were examined in 1994/95 (KORA S3) and 1999/2001 (KORA S4). In the KORA S3 and S4 studies 4,856 and 4,261 subjects have been examined implying response rates of 75% and 67%, respectively. 3,006 subjects participated in a 10-year follow-up examination of S3 in 2004/05 (KORA F3), and 3080 of S4 in 2006/2008 (KORA F4). The age range of the participants was 25 to 74 years at recruitment. Informed consent has been given by all participants. The study has been approved by the local ethics committee. Individuals for genotyping in KORA F3 and KORA F4 were randomly selected and these genotypes are taken for the analysis of the phenotypes in KORA S3 and KORA S4.

LBC1921 (Lothian Birth Cohort 1921): LBC1921 consists of 550 (234 male) relatively healthy individuals, assessed on cognitive and medical traits at a mean age of 79.1 years (SD = 0.6). They were born in 1921, most took part in the Scottish Mental Survey of 1932, and almost all lived independently in the Lothian region (Edinburgh City and surrounding area) of Scotland.¹

LBC1936 (Lothian Birth Cohort 1936): LBC1936 consists of 1091 (548 male) relatively healthy individuals who underwent cognitive and medical testing at a mean age of 69.6 years (SD = 0.8). They were born in 1936, most took part in the Scottish Mental Survey of 1947, and almost all lived independently in the Lothian region of Scotland.¹

(1) Deary IJ, Gow AJ, Pattie A, Starr JM. Cohort profile: the Lothian Birth Cohorts of 1921 and 1936. *Int J Epidemiol* 2012;41:1576-1584.

Lifelines (Netherlands Biobank): Lifelines (<https://lifelines.nl/>) is a multi-disciplinary prospective population-based cohort study using a unique three-generation design to examine the health and health-related behaviors of 165,000 persons living in the North East region of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multimorbidity. In addition, the Lifelines project comprises a number of cross-sectional sub-studies which investigate specific age-related conditions. These include investigations into metabolic and hormonal diseases, including obesity, cardiovascular and renal diseases, pulmonary diseases and allergy, cognitive function and depression, and musculoskeletal conditions. All survey

participants are between 18 and 90 years old at the time of enrollment. Recruitment has been going on since the end of 2006, and over 130,000 participants had been included by April 2013. At the baseline examination, the participants in the study were asked to fill in a questionnaire (on paper or online) before the first visit. During the first and second visit, the first or second part of the questionnaire, respectively, are checked for completeness, a number of investigations are conducted, and blood and urine samples are taken. Lifelines is a facility that is open for all researchers. Information on application and data access procedure is summarized on www.lifelines.nl.

Scholtens S, Smidt N, Swertz MA, Bakker SJ, Dotinga A, Vonk JM, et al. Cohort Profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol*. 2014 Dec 14.

LLFS (The Long Life Family Study): LLFS is a family-based cohort study, including four clinical centers: Boston University Medical Center in Boston, MA, USA, Columbia College of Physicians and Surgeons in New York City, NY, USA, the University of Pittsburgh in Pittsburgh PA, USA, and University of Southern Denmark, Denmark. The study characteristics, recruitment, eligibility and enrollment have been previously described (Pedersen et al., 2006, PMID: 17150149; Sebastiani et al., 2009, PMID: 19910380; Newman et al., 2011, PMID: 21258136). In brief, the LLFS was designed to determine genetic, behavioral, and environmental factors related to families of exceptionally healthy, elderly individuals. Phase 1 was conducted between 2006 and 2009 recruiting 4,953 individuals from 539 families. The probands were at least 79 years old in the USA centers, and 90 years old or above in Denmark. The families were selected to participate in the study based on The Family Longevity Selection Score (FLoSS) (Sebastiani et al., 2009, PMID: 19910380), a score generated according to birth-year cohort survival probabilities of the proband and siblings; probands and their families with FLoSS score of 7 or higher, at least one living sibling, and at least one living offspring (minimum family size of 3), who were able to give informed consent and willing to participate were recruited. The individuals were genotyped using ~2.3 million SNPs from the Illumina Omni chip, and then imputed on phased 1000 Genomes with Cosmopolitan data as a reference using MACH and MINIMAC. After excluding participants with 80 years and older, ~3,200 individuals have been included in the analyses for replication.

LOLIPOP (London Life Sciences Prospective Population Study): LOLIPOP is a population based prospective study of about 28K Indian Asian and European men and women, recruited from the lists of 58 General Practitioners in West London, United Kingdom between 2003 and 2008 [1]. Indian Asians had all four grandparents born on the Indian subcontinent. Europeans were of self-reported white ancestry. At enrolment all participants completed an interviewer-administered questionnaire for demographic data, medical history, and smoking and alcohol drinking habits. Anthropometric data were collected and blood pressure measured using an Omron 705CP with the mean of three measurements recorded. Blood samples were collected for the measurement of lipid profile after an overnight fasting of at least 8 hours. Aliquots of whole blood were stored at -80C for extraction of genomic DNA. The LOLIPOP study is approved by the local Research Ethics Committees and all participants provided written informed consent.

Loyola GxE (Kingston Gene-by-environment; subset of International Collaborative Study of Hypertension in Blacks (ICSHIB)): The Kingston GxE cohort was obtained from a survey conducted in Kingston, Jamaica as part of a larger project to examine gene by environment interactions in the determination of blood pressure among adults 25-74 years [PMID: 9103091].

The principal criterion for eligibility was a body mass index in either the top or bottom third of BMI for the Jamaican population. Participants were identified principally from the records of the Heart Foundation of Jamaica, a non-governmental organization based in Kingston, which provides low-cost screening services (height and weight, blood pressure, glucose, cholesterol) to the general public. Other participants were identified from among participants in family studies of blood pressure at the Tropical Metabolism Research Unit (TMRU) and from among staff members at the University of the West Indies, Mona.

Loyola SPT (Spanish Town; subset of International Collaborative Study of Hypertension in Blacks (ICSHIB)): Participants were recruited from Spanish Town, a stable, residential urban area neighboring the capital city of Kingston, Jamaica as part of the ICSHIB [PMID: 9103091]. A stratified random sampling scheme was used to recruit adult males and females aged 25–74 years from the general population. Spanish Town was chosen because its demographic make-up was broadly representative of Jamaica as a whole.

METSIM (Metabolic Syndrome In Men): The METSIM Study includes 10,197 men, aged from 45 to 73 years at recruitment, randomly selected from the population register of the Kuopio town, Eastern Finland, and examined in 2005-2010 (Stancakova A, et al. Diabetes 2009). The aim of the study is to investigate genetic and non-genetic factors associated with type 2 diabetes and cardiovascular disease and its risk factors.

Stancakova A, Javorsky M, Kuulasmaa T, Haffner SM, Kuusisto J, Laakso M: Changes in insulin sensitivity and insulin release in relation to glycemia and glucose tolerance in 6416 Finnish men. Diabetes 58:1212-1221, 2009.

NESDA (Netherlands Study of Depression and Anxiety): NESDA is a multi-center study designed to examine the long-term course and consequences of depressive and anxiety disorders (<http://www.nesda.nl>)¹. NESDA included both individuals with depressive and/or anxiety disorders and controls without psychiatric conditions. Inclusion criteria were age 18-65 years and self-reported western European ancestry while exclusion criteria were not being fluent in Dutch and having a primary diagnosis of another psychiatric condition (psychotic disorder, obsessive compulsive disorder, bipolar disorder, or severe substance use disorder).

Reference: Penninx, B.W. et al. The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res 17, 121-40 (2008).

OBA (French obese cases): Study of the genetic of obesity in adults.

PREVEND(The Prevention of RENal and Vascular ENd stage Disease study): The PREVEND study is an ongoing prospective study investigating the natural course of increased levels of urinary albumin excretion and its relation to renal and cardiovascular disease. Inhabitants 28 to 75 years of age (n=85,421) in the city of Groningen, The Netherlands, were asked to complete a short questionnaire, 47% responded, and individuals were then selected with a urinary albumin concentration of at least 10 mg/L (n = 7,768) and a randomly selected control group with a urinary albumin concentration less than 10 mg/L (n = 3,395). Details of the protocol have been described elsewhere (Hillege HL et al. Circulation 2002;106:1777-82).

PROCARDIS (Precocious Coronary Artery Disease): The PROCARDIS (European collaborative study of the genetics of precocious coronary artery disease) study is a multi-centre case-control study in which CAD cases and controls were recruited from the United Kingdom,

Italy, Sweden and Germany. Cases were defined as symptomatic CAD before age 66 years and 80% of cases also had a sibling in whom CAD had been diagnosed before age 66 years. CAD was defined as clinically documented evidence of myocardial infarction (MI) (80%), coronary artery bypass graft (CABG) (10%), acute coronary syndrome (ACS) (6%), coronary angioplasty (CA) (1%) or stable angina (hospitalization for angina or documented obstructive coronary disease) (3%). The cases included 2,136 cases who were half or full siblings. PROCARDIS controls had no personal or sibling history of CAD before age 66 years.

RHS (Ragama Health Study): The Ragama Health Study (RHS) is a population-based study of South Asian men and women aged 35-64yrs living in the Ragama Medical Officer of Health (MOH) area, near Colombo, Sri Lanka.* Consenting adults attended a clinic after a 12-h fast with available health records, and were interviewed by trained personnel to obtain information on medical, sociodemographic, and lifestyle variables. A 10-mL sample of venous blood was obtained from each subject. The concurrent study was performed in two tea plantation estates in the Lindula MOH area, near Nuwara Eliya (180 km from Colombo), to investigate the gene-environment interaction in a community with differing lifestyles (e.g., physical activity and diet). BP was measured using the Omron 750CP (Omron Co., Japan) in the seated position. The average of two readings was used for the analysis. The RHS is a collaborative effort between the Faculty of Medicine, University of Kelaniya and the National Center for Global Health and Medicine, Japan.

*Reference: Dassanayake, A.S. et al. Prevalence and risk factors for non-alcoholic fatty liver disease among adults in an urban Sri Lankan population. *J Gastroenterol Hepatol* 24, 1284-8 (2009).

SHEEP (Stockholm Heart Epidemiology Project): The SHEEP is a population based case-control study of risk factors for first episode of acute myocardial infarction. The study base comprised all Swedish citizens resident in the Stockholm county 1992-1994 who were 45-70 years of age and were free of previous clinically diagnosed myocardial infarction.

Cases were identified using three different sources: 1) coronary units and internal medicine wards for acute care in all Stockholm hospitals; 2) the National Patient Register; and 3) death certificates. For the present study, only cases who survived at least 28 days were considered (n=1213).

First time incident myocardial infarction cases (n=1213) were identified during a 2-year period (1992-1993) for men and during a 3-year period (1992-1994) for women. Controls (n=1561) were randomly recruited from the study population continuously over time within 2 days of the case occurrence and matched to cases on age (5-years interval), sex and hospital catchment area using computerized registers of the population of Stockholm. Five control candidates were sampled simultaneously to be able to replace potential non-respondent controls. Occasionally, because of late response of the initial control, both the first and alternative controls were considered resulting in the inclusion of more controls than cases. Postal questionnaires covering a wide range of exposure areas including occupational exposures, life style factors, social factors and health related factors were distributed to the participants. Clinical investigations were performed at least three months after myocardial infarction of cases and their matched controls. The investigations included blood samplings under fasting conditions with collection of whole blood for DNA extraction, serum and plasma. A biobank was established containing DNA, serum and plasma.

Exposure information based on both the questionnaire and biological data from the health examination was available for 78% of the male and 67% of the female non-fatal cases; the corresponding figures for their controls were 68% and 64%.

SHIP (Study of Health in Pomerania): The Study of Health In Pomerania (SHIP) is a prospective longitudinal population-based cohort study in Mecklenburg-West Pomerania assessing the prevalence and incidence of common diseases and their risk factors (PMID: 20167617). SHIP encompasses the two independent cohorts SHIP and SHIP-TREND. Participants aged 20 to 79 with German citizenship and principal residency in the study area were recruited from a random sample of residents living in the three local cities, 12 towns as well as 17 randomly selected smaller towns. Individuals were randomly selected stratified by age and sex in proportion to population size of the city, town or small towns, respectively. A total of 4,308 participants were recruited between 1997 and 2001 in the SHIP cohort. Between 2008 and 2012 a total of 4,420 participants were recruited in the SHIP-TREND cohort. Individuals were invited to the SHIP study centre for a computer-assisted personal interviews and extensive physical examinations. The study protocol was approved by the medical ethics committee of the University of Greifswald. Oral and written informed consents was obtained from each of the study participants

Genome-wide SNP-typing was performed using the Affymetrix Genome-Wide Human SNP Array 6.0 or the Illumina Human Omni 2.5 array (SHIP-TREND samples). Array processing was carried out in accordance with the manufacturer's standard recommendations. Genotypes were determined using GenomeStudio Genotyping Module v1.0 (GenCall) for SHIP-TREND and the Birdseed2 clustering algorithm for SHIP. Imputation of genotypes in SHIP and SHIP-TREND was performed with the software IMPUTE v2.2.2 based on 1000 Genomes release March 2012.

SWHS/SMHS (Shanghai Women's Health Study/ Shanghai Men's Health Study): The Shanghai Women's Health Study (SWHS) is an ongoing population-based cohort study of approximately 75,000 women who were aged 40-70 years at study enrollment and resided in in urban Shanghai, China; 56,832 (75.8%) provided a blood samples. Recruitment for the SWHS was initiated in 1997 and completed in 2000. The self-administered questionnaire includes information on demographic characteristics, disease and surgery histories, personal habits (such as cigarette smoking, alcohol consumption, tea drinking, and ginseng use), menstrual history, residential history, occupational history, and family history of cancer.

The blood pressure were measured by trained interviewers (retired nurses) with a conventional mercury sphygmomanometer according to a standard protocol, after the participants sat quietly for 5 min at the study recruitment. Included in the current project were 2970 women who had GWAS data and blood pressure measurements at the baseline interview.

The Shanghai Men's Health Study (SMHS) is an ongoing population-based cohort study of 61,480 Chinese men who were aged between 40 and 74 years, were free of cancer at enrollment, and lived in urban Shanghai, China; 45,766 (74.4%) provided a blood samples. Recruitment for the SMHS was initiated in 2002 and completed in 2006. The self-administered questionnaire includes information on demographic characteristics, disease and surgery histories, personal habits (such as cigarette smoking, alcohol consumption, tea drinking, and ginseng use), residential history, occupational history, and family history of cancer. The blood pressure were measured by trained interviewers (retired nurses) with a conventional mercury sphygmomanometer according to a standard protocol, after the participants sat quietly for 5 min

at the study recruitment. Included in the current project were 892 men who had GWAS data and blood pressure measurements at the baseline interview or 298 men who had GWAS data and lipids data.

Genotyping and imputation: Genomic DNA was extracted from buffy coats by using a Qiagen DNA purification kit (Valencia, CA) or Puregene DNA purification kit (Minneapolis, MN) according to the manufacturers' instructions and then used for genotyping assays. The GWAS genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affy6.0) platform or Illumina 660, following manufacturers' protocols. After sample quality control, we exclude SNPs with 1) MAF <0.01; 2) call rate <95%; 2) bad genotyping cluster; and 3) concordance rate <95% among duplicated QC samples. Genotypes were imputed using the program MACH (<http://www.sph.umich.edu/csg/abecasis/MACH/download/>), which determines the probable distribution of missing genotypes conditional on a set of known haplotypes, while simultaneously estimating the fine-scale recombination map. Phased autosome SNP data from HapMap Phase II Asians (release 22) were used as the reference. To test for associations between the imputed SNP data with BMI, linear regression (additive model) was used, in which SNPs were represented by the expected allele count, an approach that takes into account the degree of uncertainty of genotype imputation (<http://www.sph.umich.edu/csg/abecasis/MACH/download/>).

The lipid profiles were measured at Vanderbilt Lipid Laboratory. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (TG) were measured using an ACE Clinical Chemistry System (Alfa Wassermann, Inc, West Caldwell, NJ). Low-density lipoprotein (LDL) cholesterol levels were calculated by using the Friedwald equation. The levels of LDL cholesterol were directly measured using an ACE Clinical Chemistry System for subjects with TG levels \geq 400 mg/dL. Fasting status was defined as an interval between the last meal and blood draw of 8 hours or longer.

TAICHI-G: The TaiChi consortium consists of 7 studies that collaborated initially in a large scale metabochip study, and became an ongoing consortium for studies of cardiometabolic disease in the Chinese population in Taiwan. The seven studies included the following: 1) HALST (Healthy Aging Longitudinal Study in Taiwan), a population based epidemiologic study of older adults living in all major geographic regions of Taiwan established by the Taiwan National Health Research Institutes (NHRI); 2) SAPPHIRe (Stanford-Asian Pacific Program in Hypertension and Insulin Resistance), a family based study established in 1995 with an initial goal of identifying major genetic loci underlying hypertension and insulin resistance in East Asian populations, with Taiwan subjects participating in the TaiChi consortium; 3) TCAGEN (Taiwan Coronary Artery Disease GENetic), a cohort study that enrolled patients undergoing coronary angiography or percutaneous intervention at the National Taiwan University Hospital (NTUH) in the setting of either stable angina pectoris or prior myocardial infarction; 4) TACT (TAiwan Coronary and Transcatheter intervention), a cohort study enrolled patients with angina pectoris and objective documentation of myocardial ischemia who underwent diagnostic coronary angiography and/or revascularization any time after October 2000 at the National Taiwan University Hospital (NTUH) (similar to TCAGEN but recruitment was independent of TCAGEN); 5) Taiwan DRAGON (Taiwan Diabetes and RelAted Genetic COmplicationN), a cohort study of Type 2 diabetes at Taichung Veterans General Hospital (Taichung VGH) in Taiwan, with participants including individuals with either newly diagnosed or established diabetes (subjects with hyperglycemia who did not meet diagnostic criteria for Type 2 DM were not included); 6)

TCAD (Taichung CAD study), includes patients with a variety of cardiovascular diseases who received care at the Taichung Veterans General Hospital (Taichung VGH), i.e. specifically individuals who were hospitalized for diagnostic and interventional coronary angiography examinations and treatment; 7) TUDR (Taiwan US Diabetic Retinopathy) enrolled subjects with Type 2 diabetes who received care at Taichung Veteran General Hospital (Taichung VGH), and a small number of subjects from Taipei Tri-Service General Hospital (TSGH); TUDR subjects underwent a complete ophthalmic and fundus examination to carefully document the presence and extent of retinopathy. From these 7 studies, samples for over 1,800 subjects were selected based on completeness of standard metabolic phenotyping and knowledge of cardiac disease status, to undergo GWAS genotyping with an Illumina human-omni 'chip' specific for Asian population (Illumina, San Diego, CA; cat. No. 20004337), hence TAICHI-G.

THR (Taiwan study of Hypertensives Rare Variants): THR proposed to identify rare and low frequency genetic variants for blood pressure and hypertension through whole exome sequencing of a subset of highly enriched Taiwan Chinese hypertensive families and as many matched controls. The Taiwan Chinese families (approximately N=1,200 subjects) were previously recruited as part of the NHLBI-sponsored SAPPHIRe Network which is part of the Family Blood Pressure Program (FBPP). The SAPPHIRe families were recruited to have multiple hypertensive sibs and some of them also included one normotensive/hypotensive sib. The matched controls (N=1,200) were selected from the large population-based HALST Study and a Hospital-based population, both in Taipei, Taiwan.

TRAILS (Tracking Adolescents' Individual Lives Survey): TRAILS is a prospective cohort study of Dutch adolescents and young adults, with bi- or triennial measurements from age 11 onwards, which started in 2001. TRAILS consists of a general population and a clinical cohort (<https://www.trails.nl/en/home>). In the population cohort, six assessment waves have been completed to date, at mean ages 11.1 (SD = 0.6), 13.6 (SD = 0.5), 16.3 (SD = 0.7), 19.1 (SD = 0.6), 22.3 (SD = 0.6), and 25.8 (SD = 0.6). Data for the present study were collected in the population cohort only, during the third assessment wave. The study was approved by the Dutch Central Committee on Research Involving Human Subjects.

TUDR (Taiwan-US Diabetic Retinopathy): 2009 to present, is a cohort that enrolled subjects with Type 2 diabetes receiving care at Taichung Veteran General Hospital (Taichung VGH), and a small number of subjects from Taipei Tri-Service General Hospital. All TUDR subjects underwent a complete ophthalmic and fundus examination to carefully document the presence and extent of retinopathy.

TWINGENE (TwinGene of the Swedish Twin Registry): The aim of the TwinGene project has been to systematically transform the oldest cohorts of the Swedish Twin Registry (STR) into a molecular-genetic resource. Beginning in 2004, about 200 twins were contacted each month until the data collection was completed in 2008. A total of 21 500 twins were contacted where of 12 600 participated. Invitations to the study contained information of the study and its purpose. Along with the invitations consent forms and health questionnaire were sent to the subjects. When the signed consent forms were returned, the subjects were sent blood sampling equipment and asked to contact a local health facility for blood sampling. The study population was recruited among twins participating in the Screening Across the Lifespan Twin Study (SALT) which was a telephone interview study conducted in 1998-2002. Other inclusion criteria were that both twins in the pair had to be alive and living in Sweden. Subjects were excluded from the study if they preciously declined participation in future studies or if they had been

enrolled in other STR DNA sampling projects. The subjects were asked to make an appointment for a health check-up at their local health-care facility on the morning Monday to Thursday and not the day before a national holiday, this to ensure that the sample would reach the KI biobank the following morning by overnight mail. The subjects were instructed to fast from 20.00 the previous night. By venipuncture a total of 50 ml of blood was drawn from each subject. Tubes with serum and blood for biobanking as well as for clinical chemistry tests were sent to KI by overnight mail. One 7ml EDTA tube of whole blood is stored in -80°C while a second 7ml EDTA tube of blood is used for DNA extraction using Puregene extraction kit (Gentra systems, Minneapolis, USA). After excluding subjects in which the DNA concentration in the stock-solution was below 20ng/µl as well as subset of 302 female monozygous twin pairs participating in a previous genome wide effort DNA from 9896 individual subjects was sent to SNP&SEQ Technology Platform Uppsala, Sweden for genome wide genotyping with Illumina OmniExpress bead chip (all available dizygous twins + one twin from each available MZ twin pair).

UKB (United Kingdom Biobank, www.ukbiobank.ac.uk): UK Biobank is a major national health resource with the aim of improving the prevention, diagnosis and treatment of a wide range of serious and life-threatening illnesses. UK Biobank includes data from 502,682 individuals (94% of self-reported European ancestry), with extensive health and lifestyle questionnaire data, physical measures and genetic data. A total of 152,249 participants had genetic and phenotypic (blood pressure) data. Central genotyping quality control (QC) had been performed by UK Biobank [The UK Biobank. UK Biobank Genotyping QC documentation. (2015)]. Further QC was also performed locally.

UKHLS (Understanding Society / The UK Household Longitudinal Study): The United Kingdom Household Longitudinal Study, also known as Understanding Society (<https://www.understandingsociety.ac.uk>) is a longitudinal panel survey of 40.000 UK households (England, Scotland, Wales and Northern Ireland) representative of the UK population. Participants are surveyed annually since 2009 and contribute information relating to their socioeconomic circumstances, attitudes, and behaviours via a computer assisted interview. The study includes phenotypical data for a representative sample of participants for a wide range of social and economic indicators as well as a biological sample collection encompassing biometric, physiological, biochemical, and haematological measurements and self-reported medical history and medication use. The United Kingdom Household Longitudinal Study has been approved by the University of Essex Ethics Committee and informed consent was obtained from every participant.

YFS (The Cardiovascular Risk in Young Finns Study): The YFS is a population-based follow up-study started in 1980. The main aim of the YFS is to determine the contribution made by childhood lifestyle, biological and psychological measures to the risk of cardiovascular diseases in adulthood. In 1980, over 3,500 children and adolescents all around Finland participated in the baseline study. The follow-up studies have been conducted mainly with 3-year intervals. The latest 30-year follow-up study was conducted in 2010-11 (ages 33-49 years) with 2,063 participants. The study was approved by the local ethics committees (University Hospitals of Helsinki, Turku, Tampere, Kuopio and Oulu) and was conducted following the guidelines of the Declaration of Helsinki. All participants gave their written informed consent.

NOTE: Baependi, NEO, Pelotas, and WHI (EA) also participated in replications since they did not contribute to Smoking-BP discovery analysis.

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an application system for genetics data and all use of the data should be approved by them. The application form is at:

<https://www.understandingsociety.ac.uk/about/health/data>.

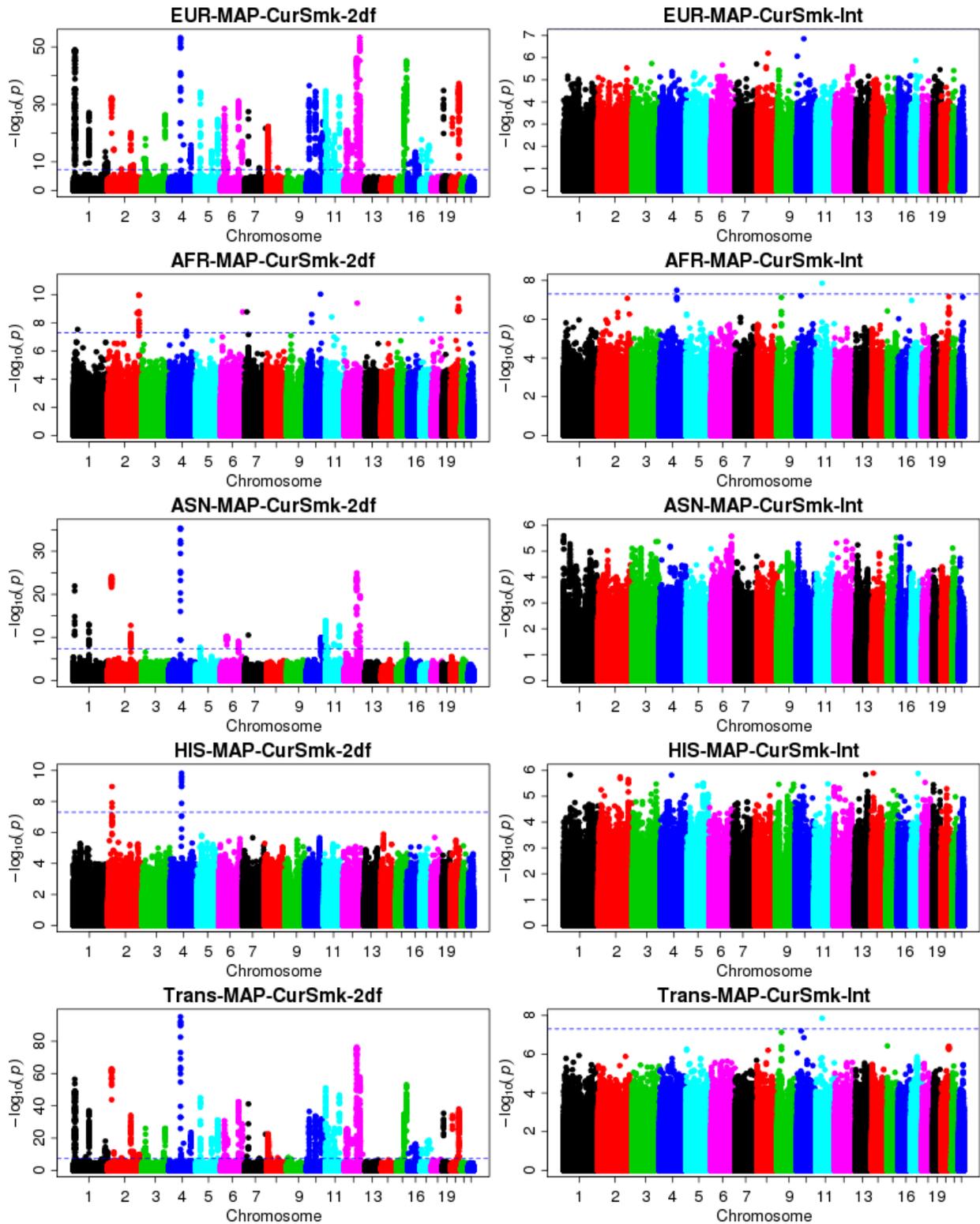
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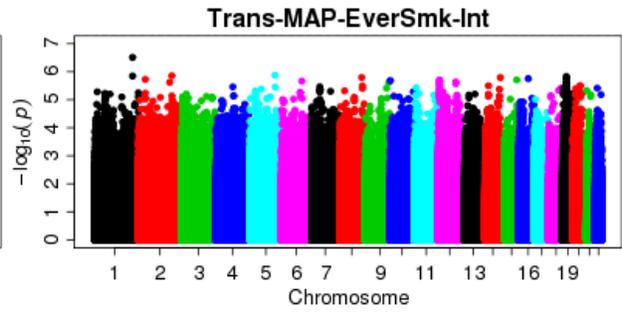
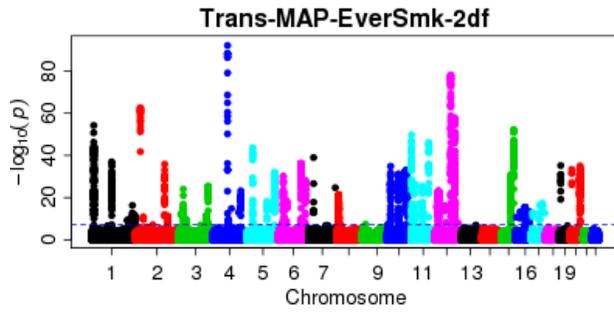
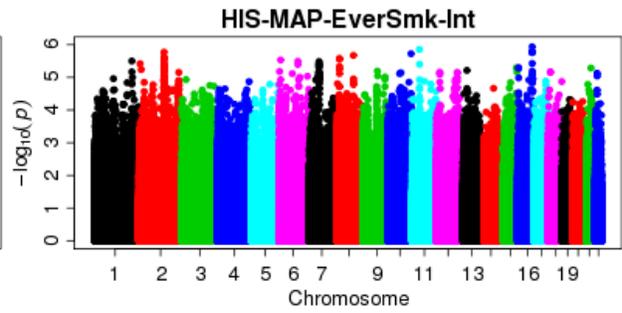
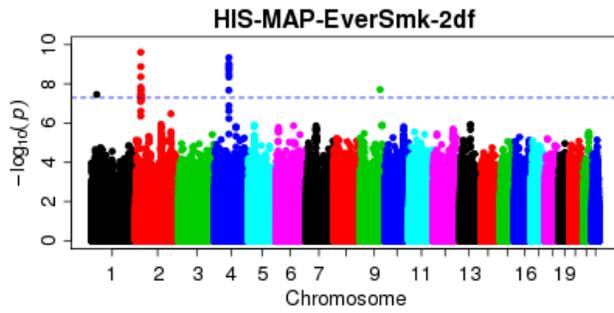
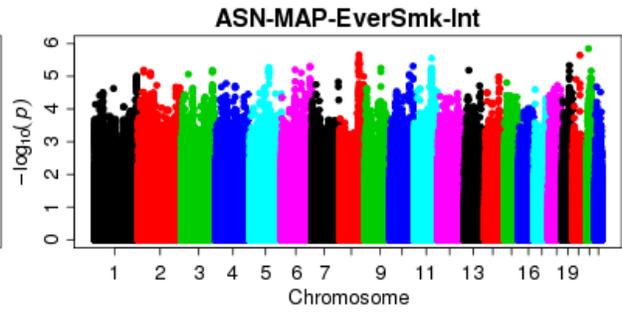
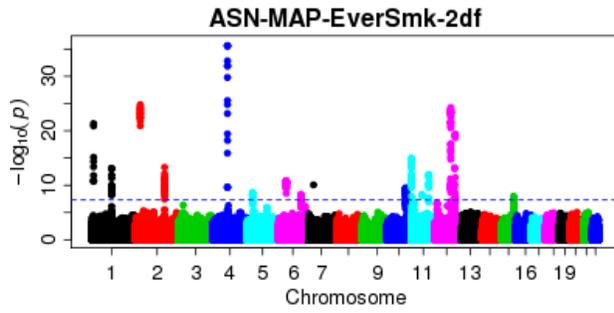
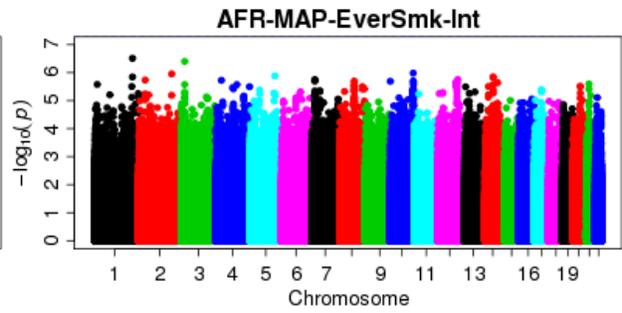
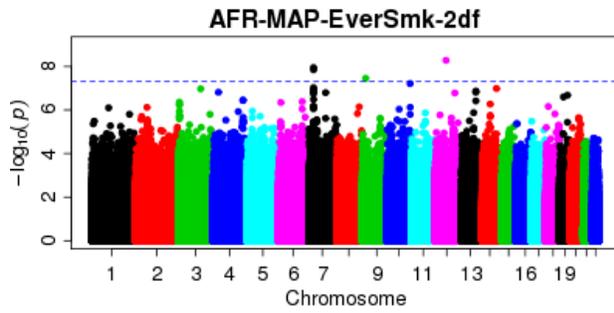
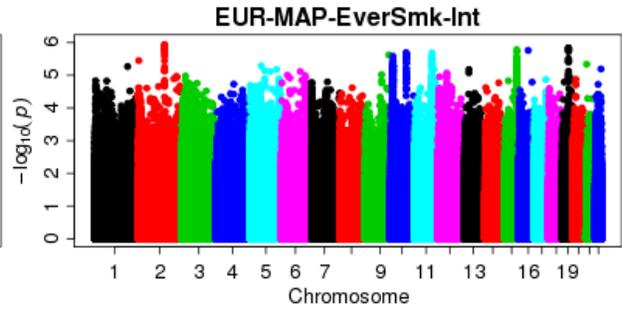
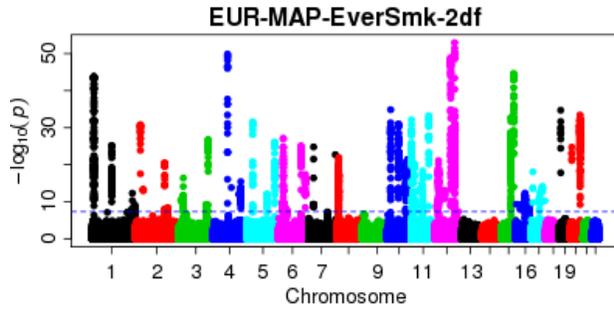
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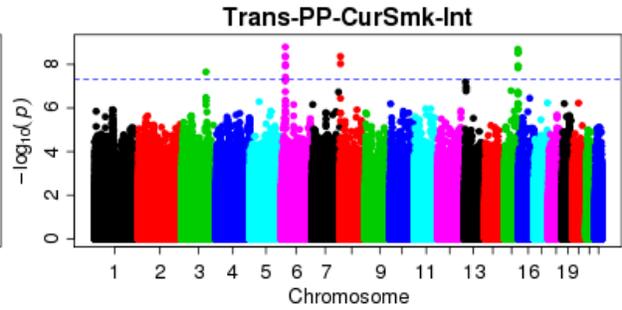
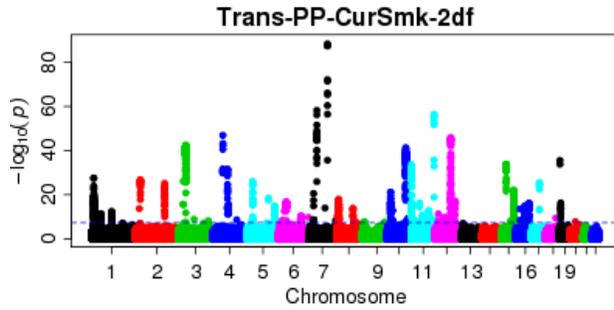
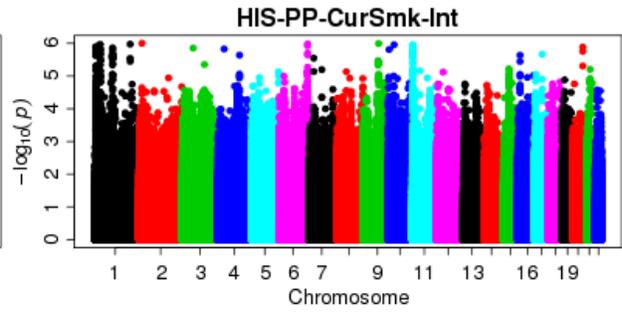
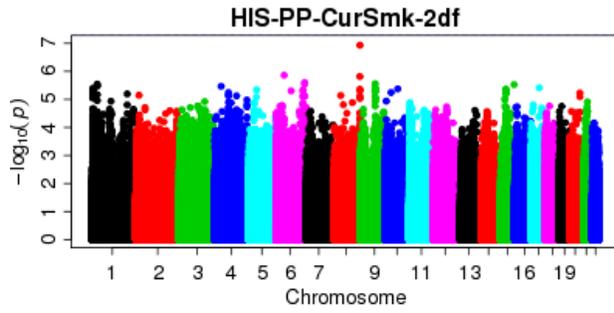
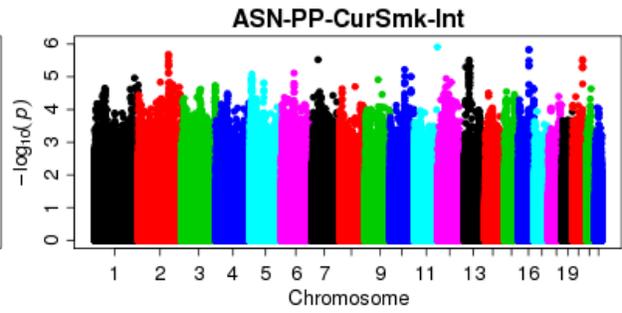
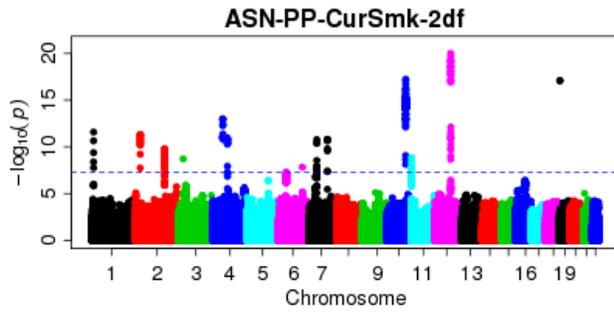
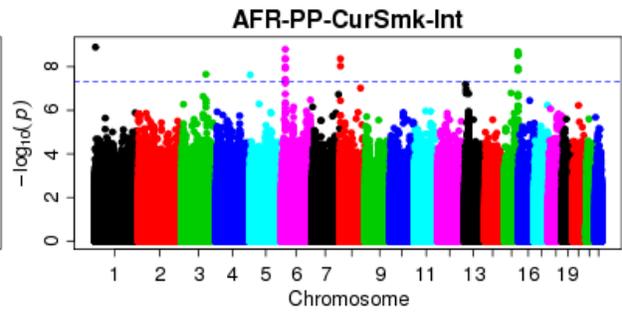
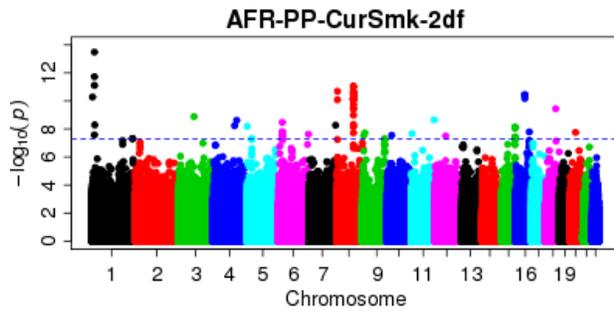
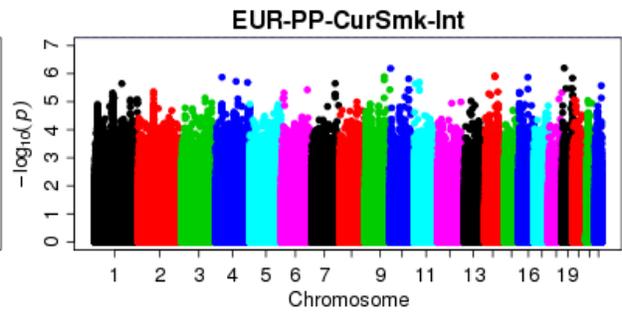
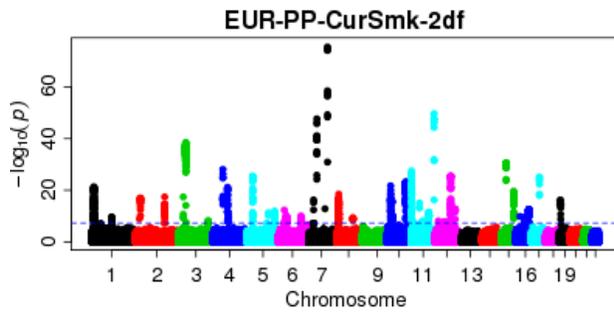
NOTE: Baependi, NEO, Pelotas, and WHI (EA) also participated in replications since they did not contribute to Smoking-BP discovery analysis.

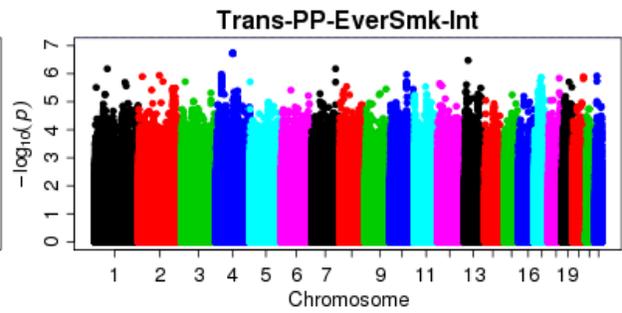
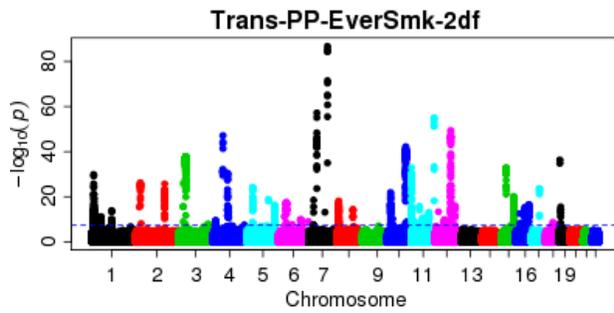
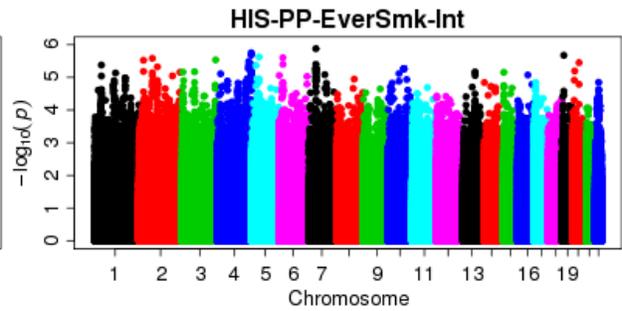
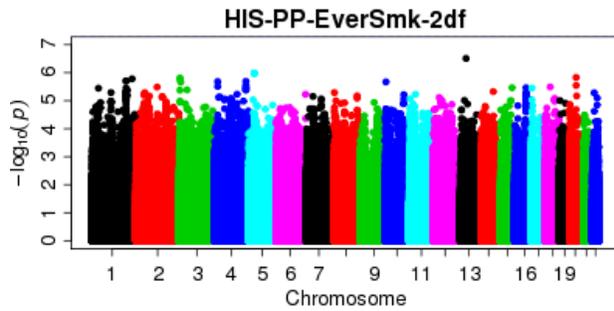
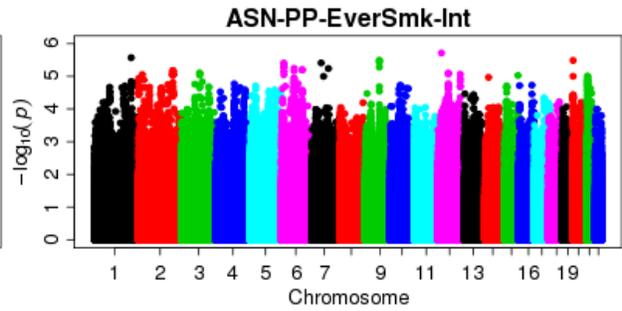
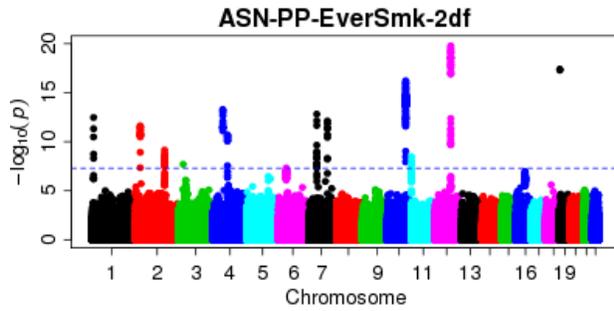
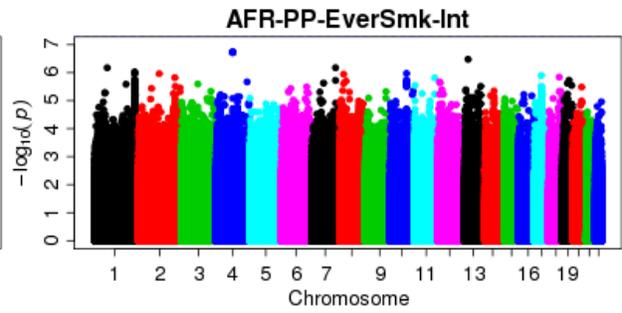
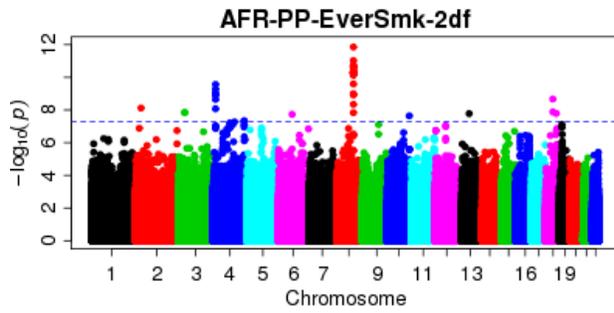
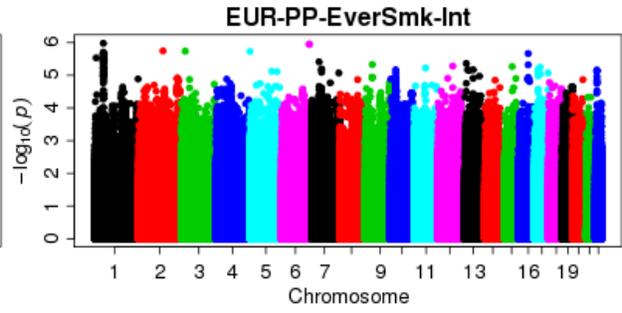
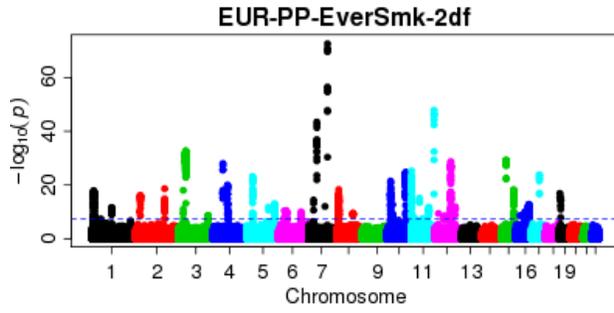
Supplementary Figures

Supplementary Figure 1: Manhattan plots of the combined analyses of Stages 1 and 2

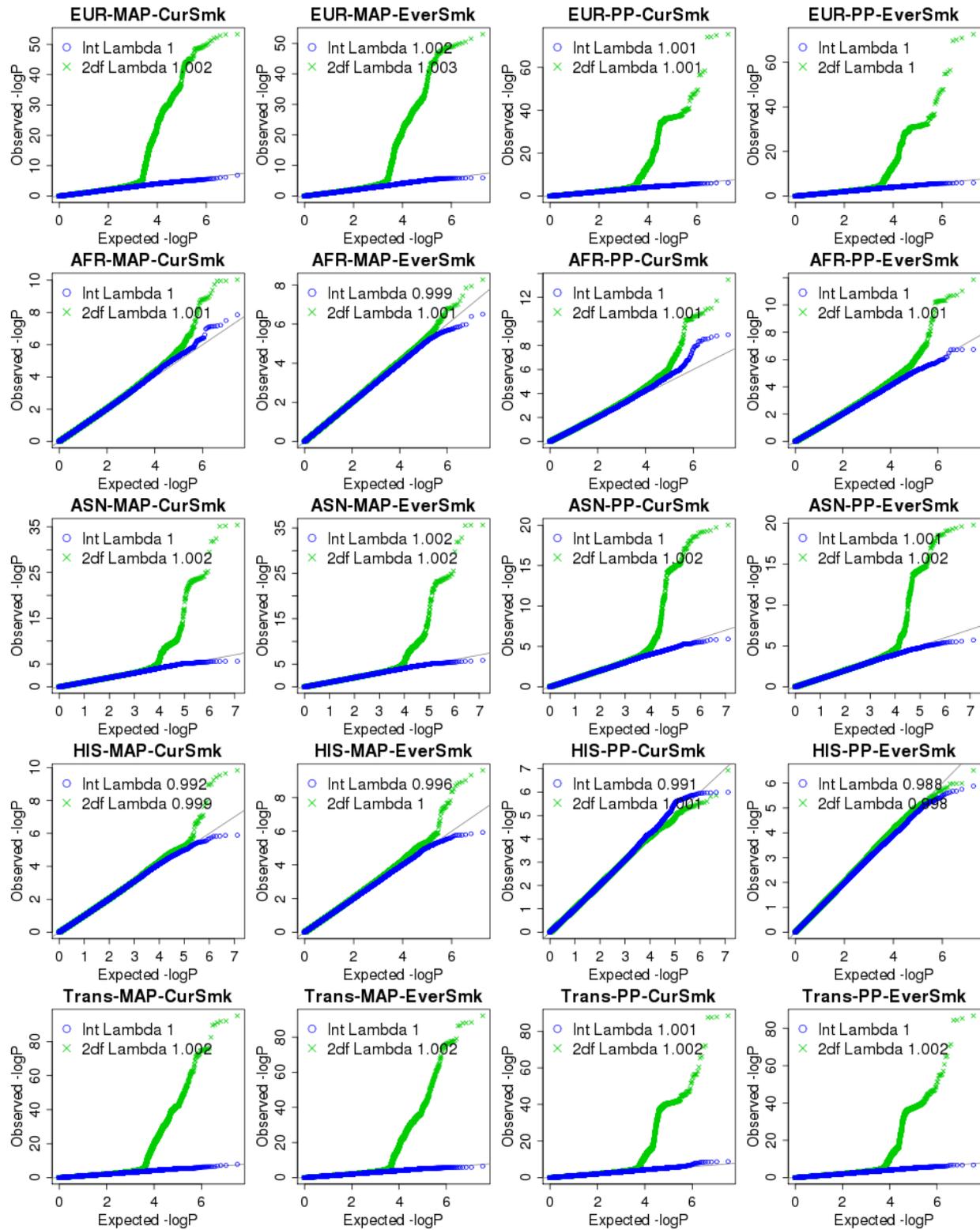






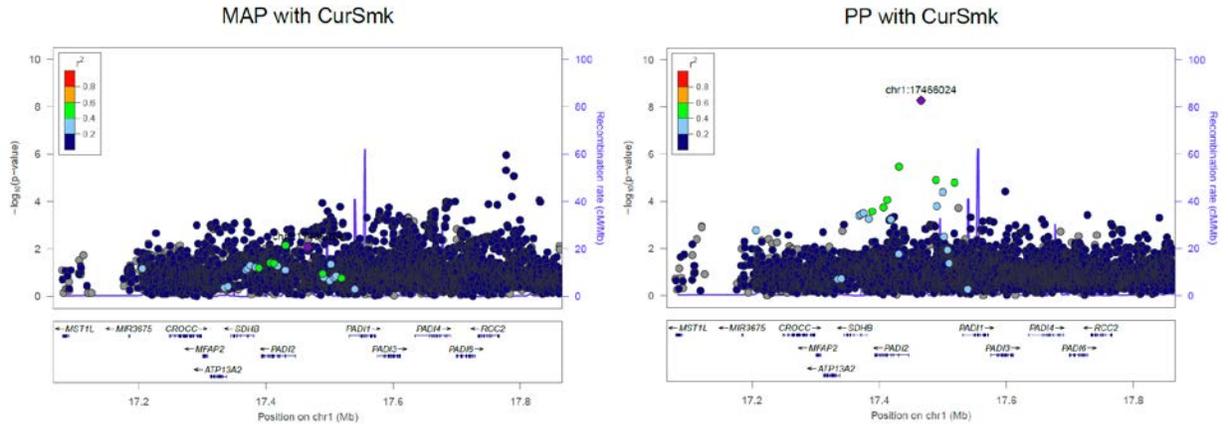


Supplementary Figure 2: QQ plots of the combined analyses of Stages 1 and 2

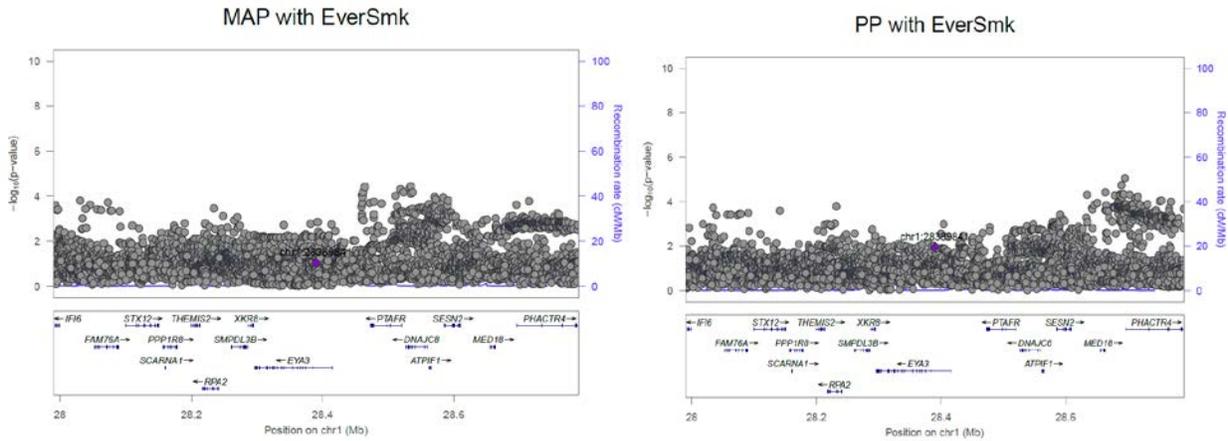


Supplementary Figure 3: LocusZoom plots for the 38 new loci (Table 2) and 9 new signals near known BP loci (Table 3)

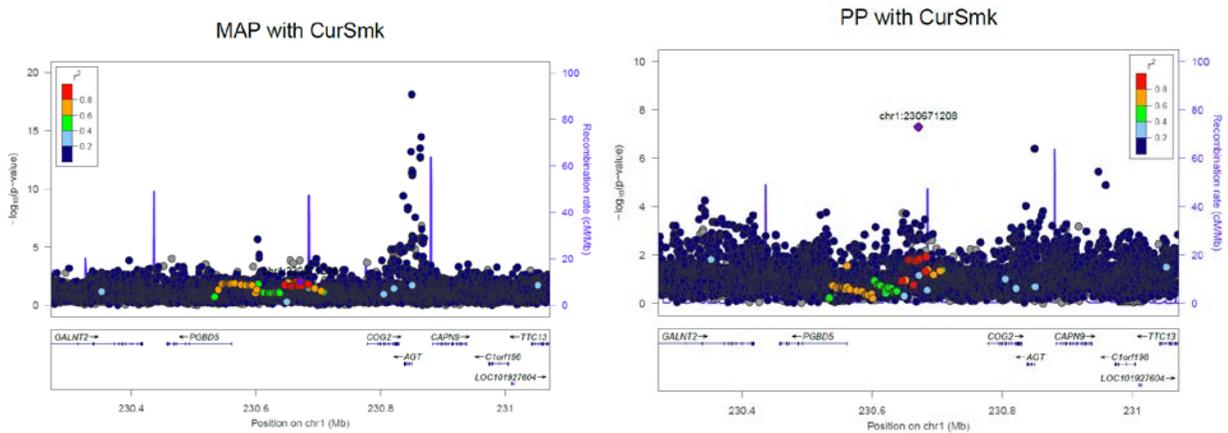
LOCUS 1



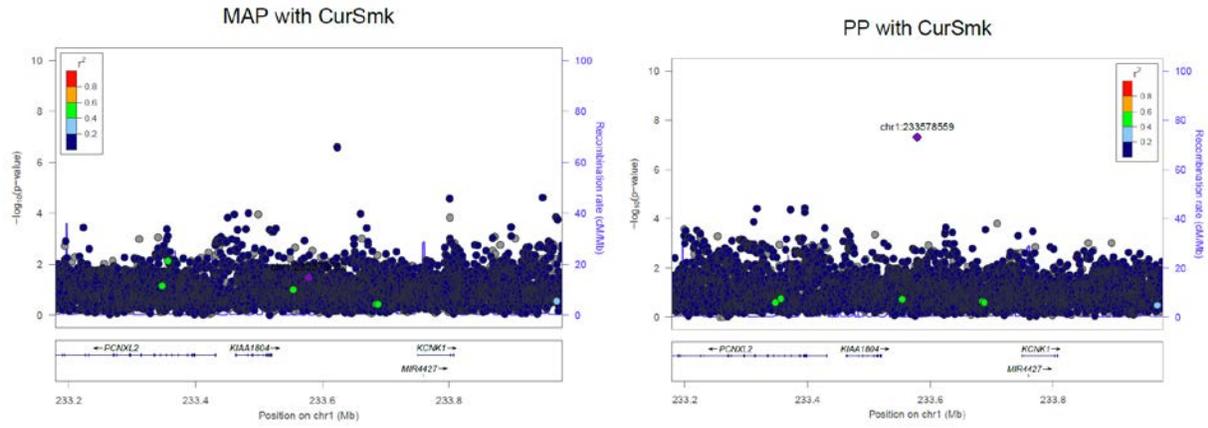
LOCUS 2



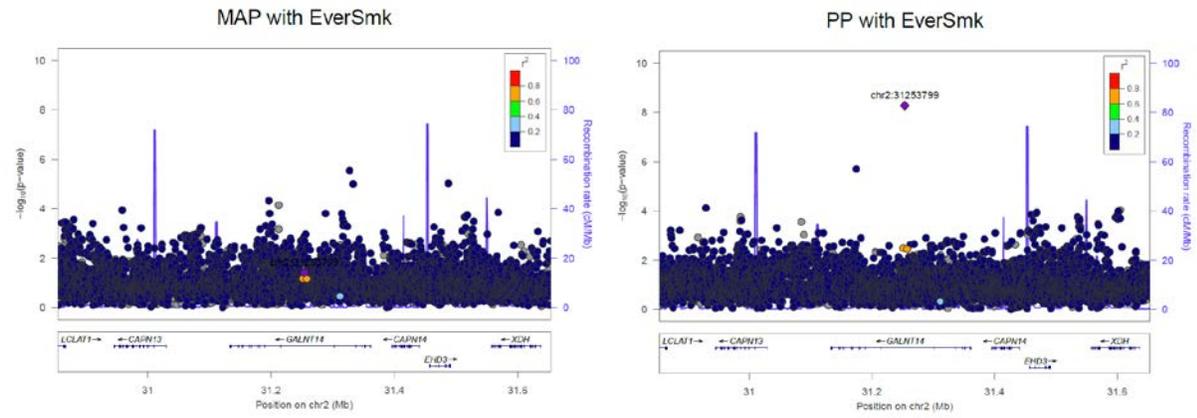
LOCUS 3



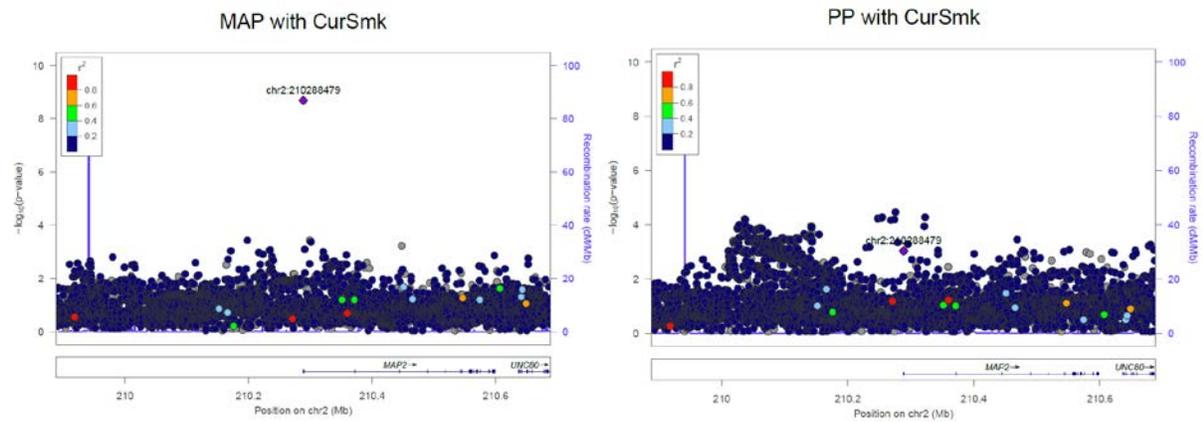
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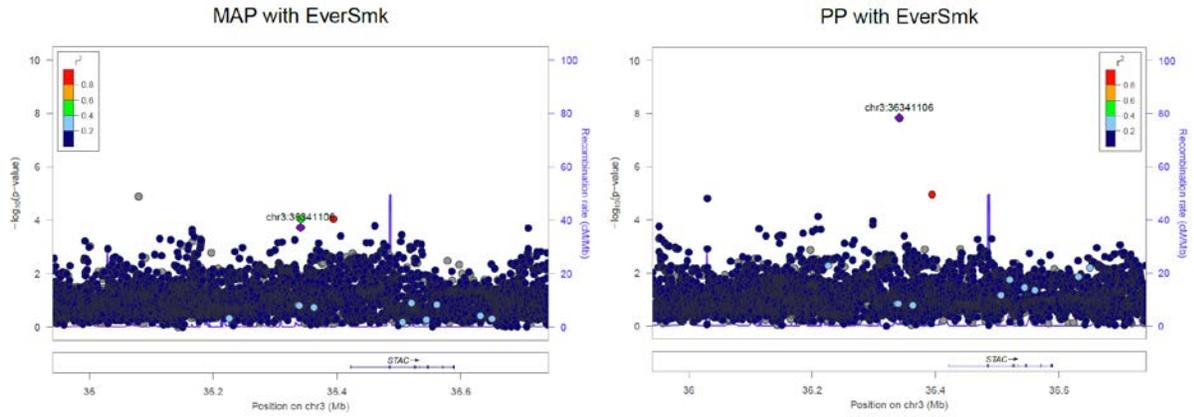
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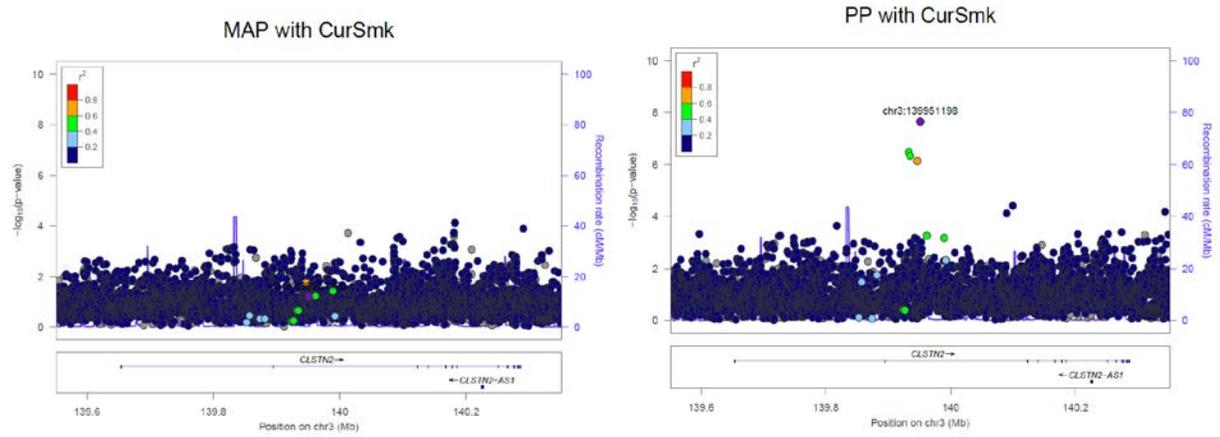
LOCUS 6



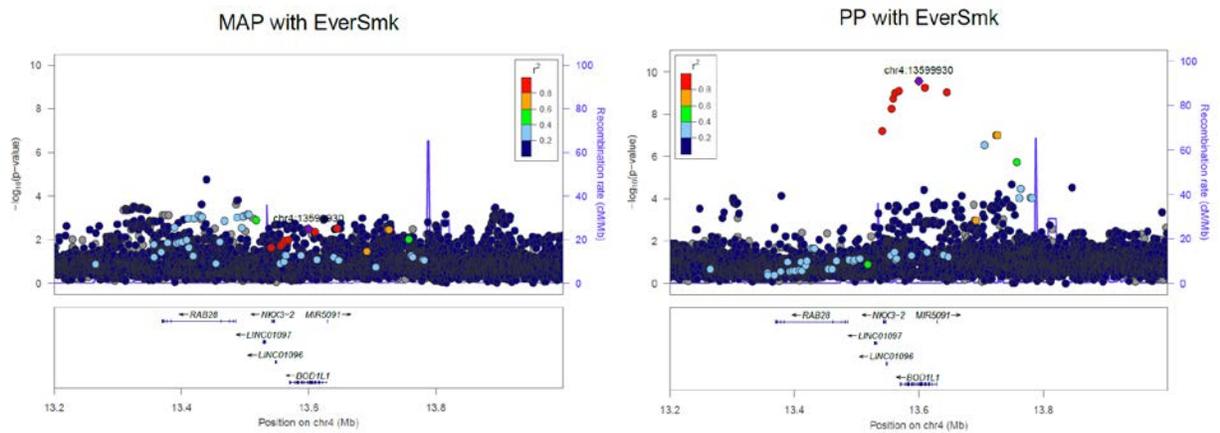
LOCUS 7



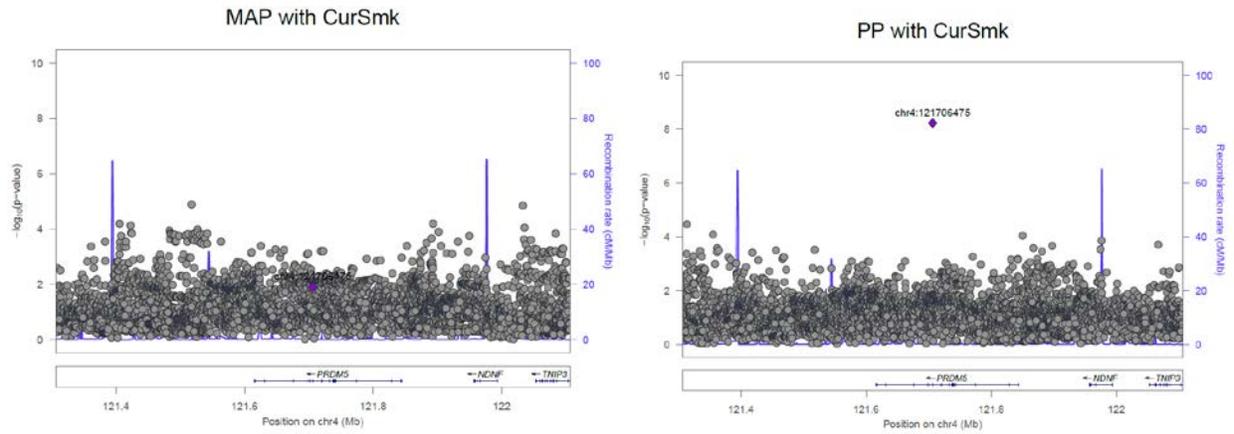
LOCUS 8



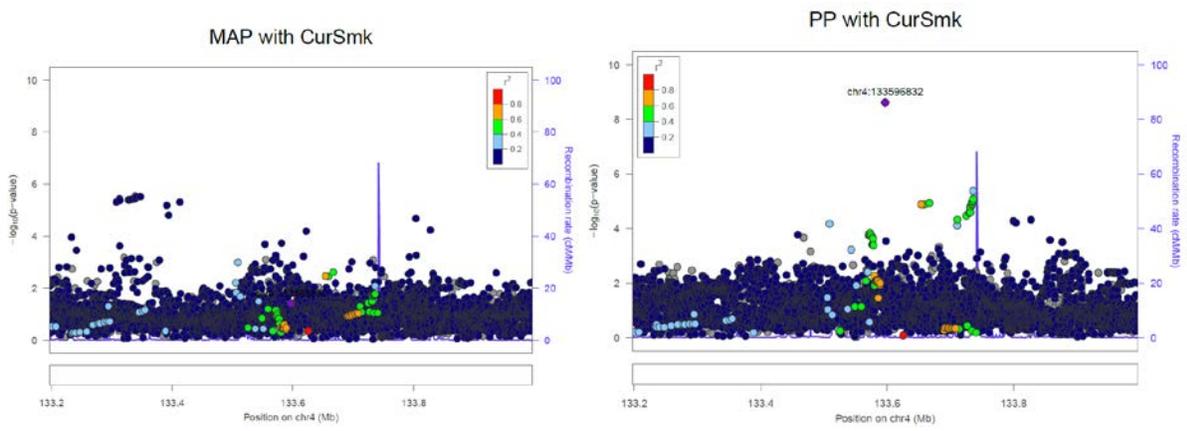
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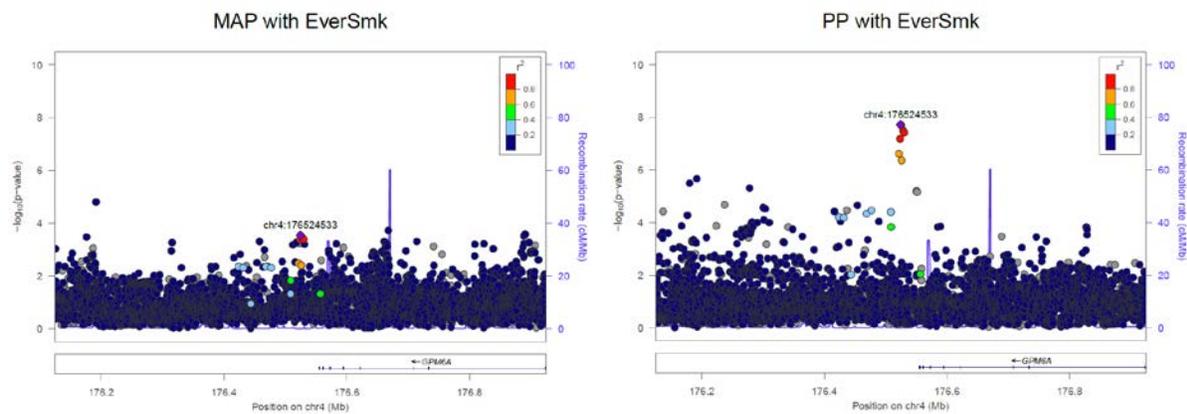
LOCUS 10



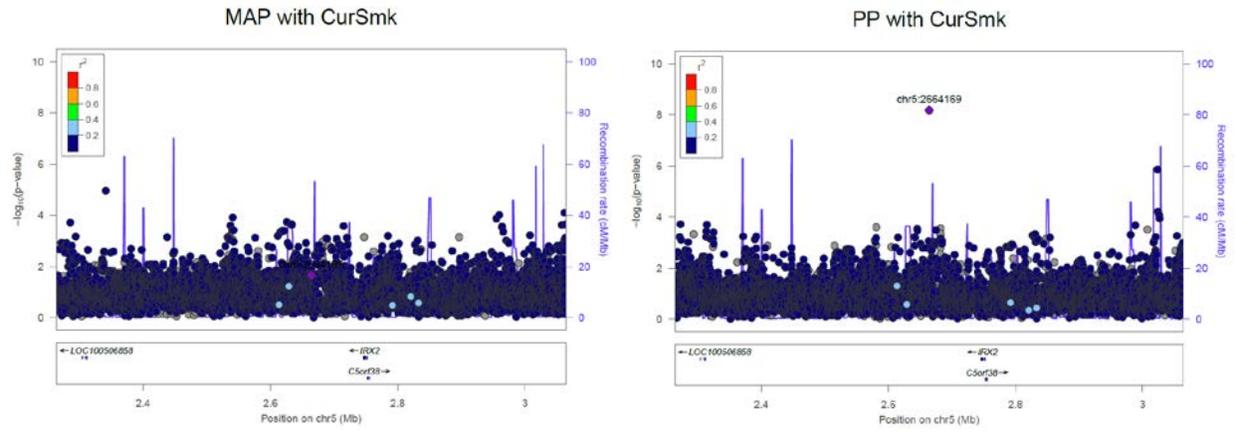
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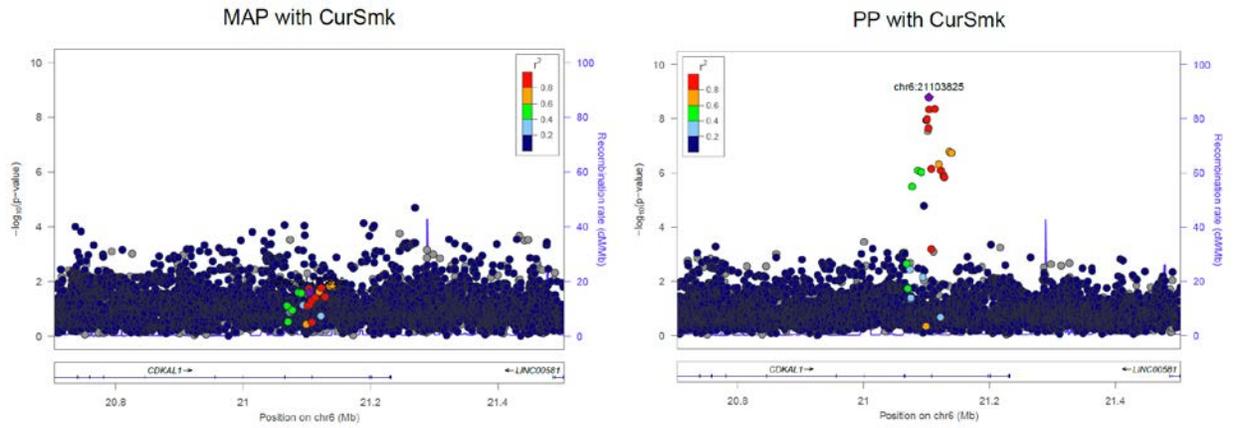
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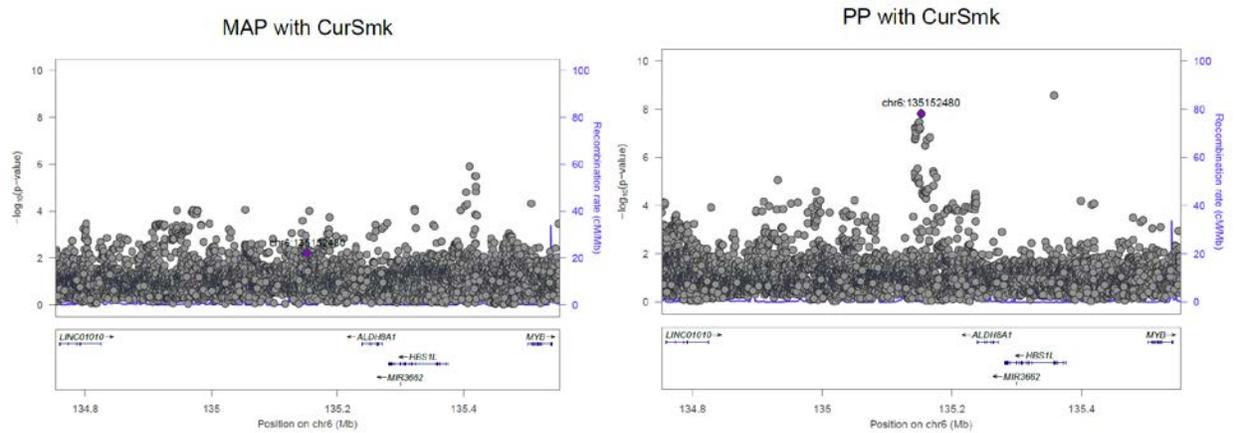
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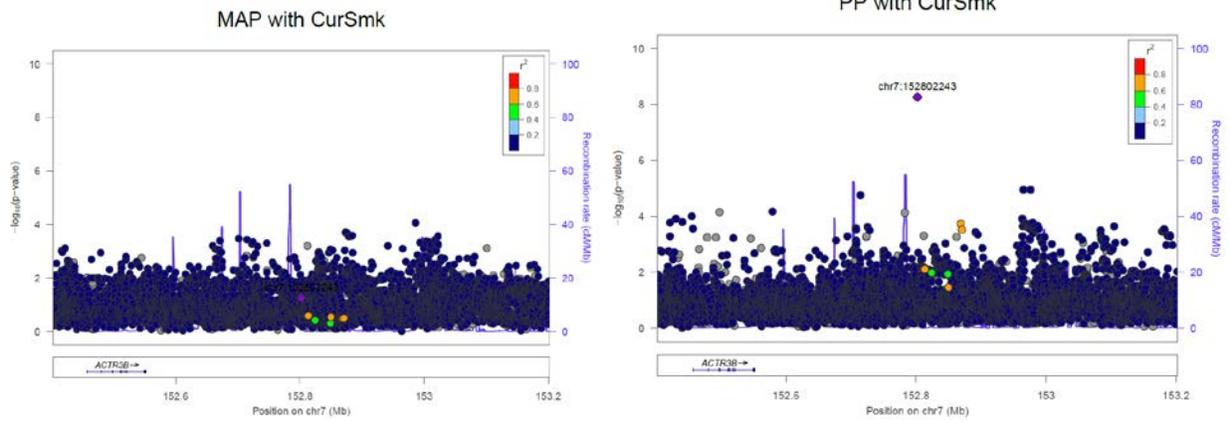
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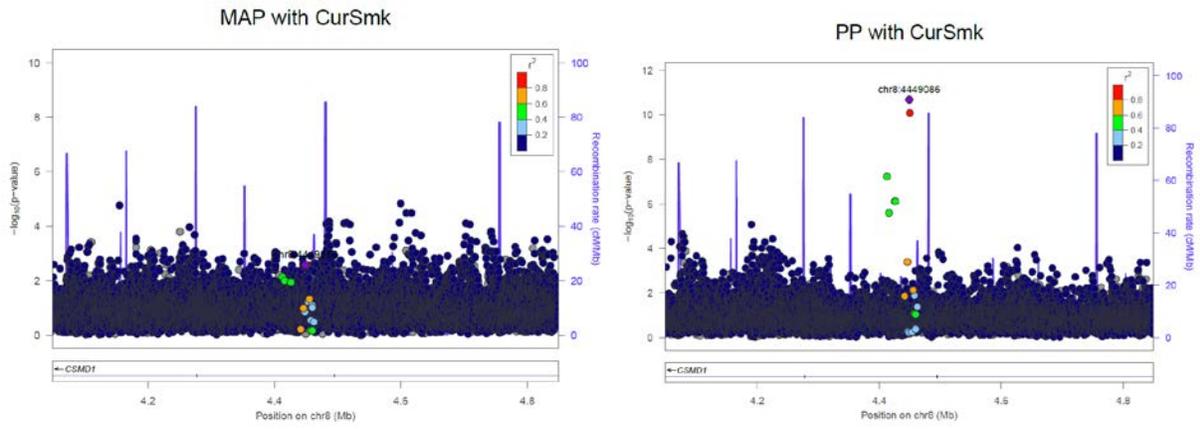
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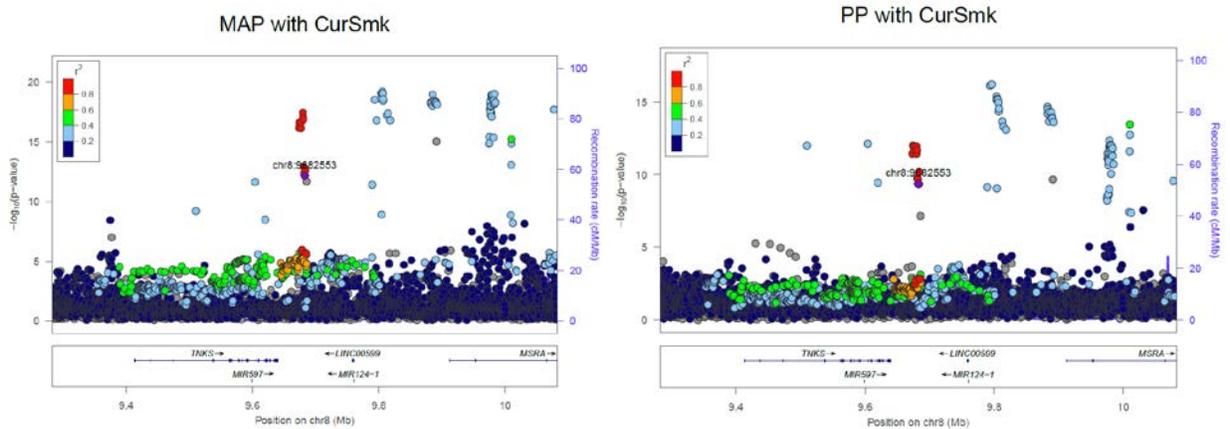
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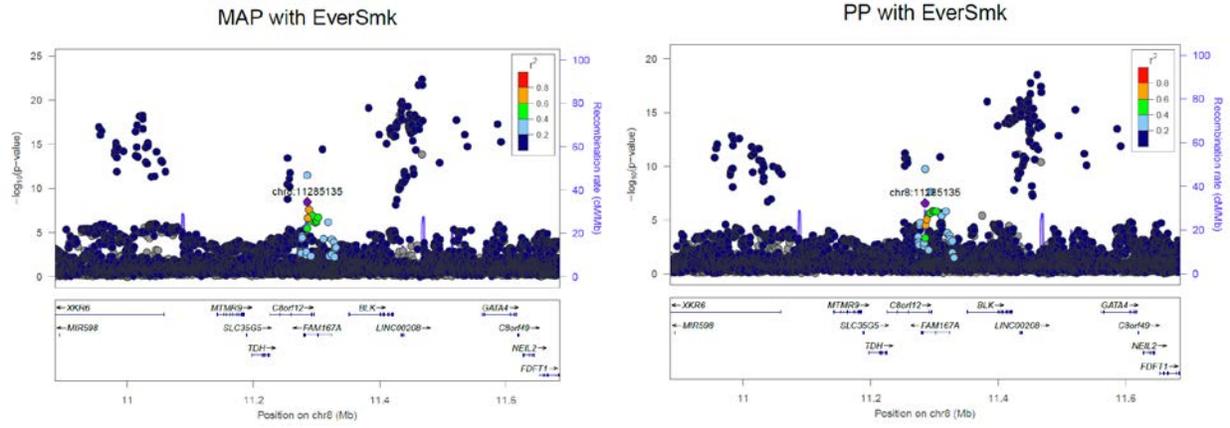
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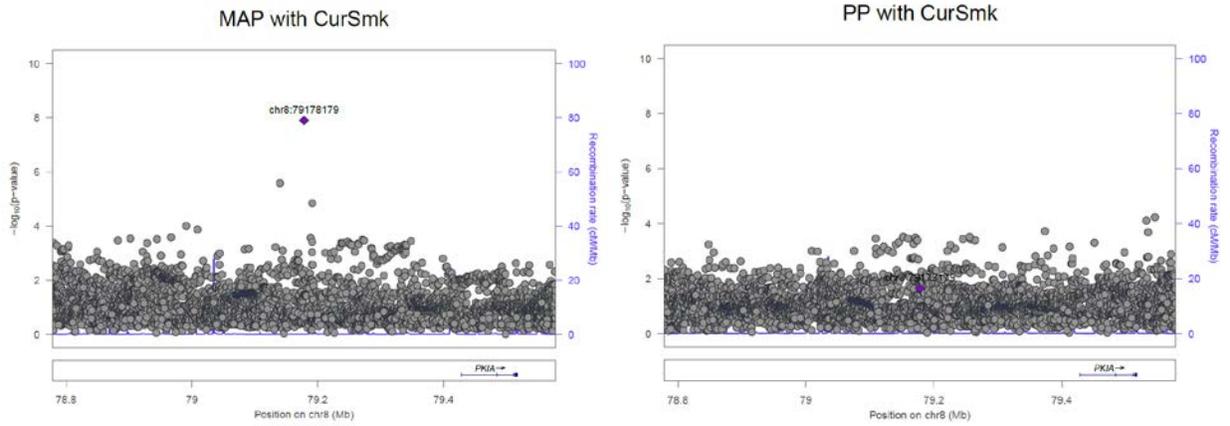
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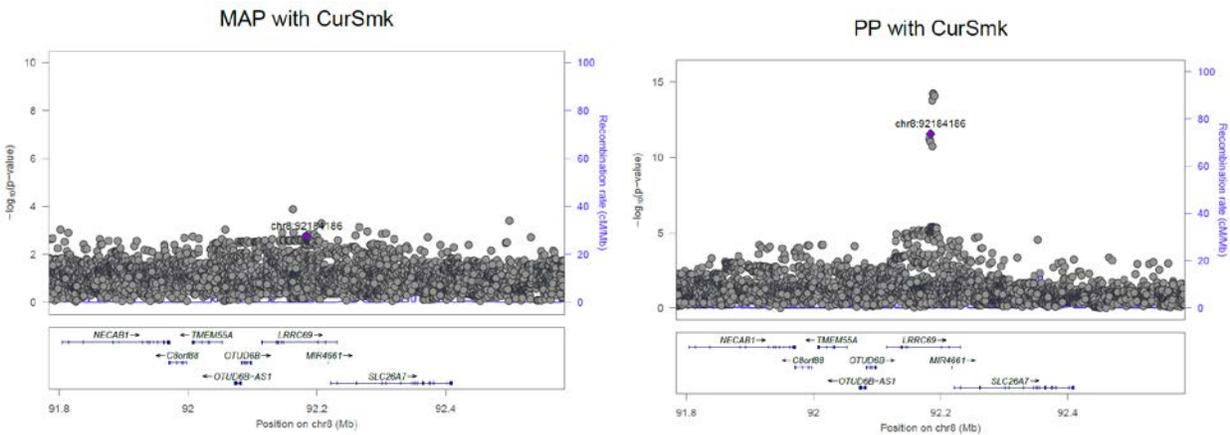
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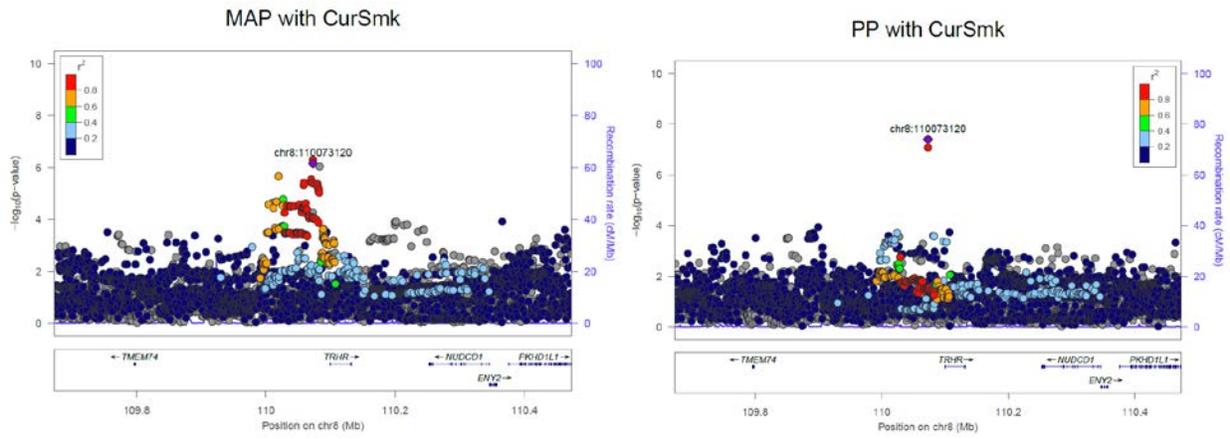
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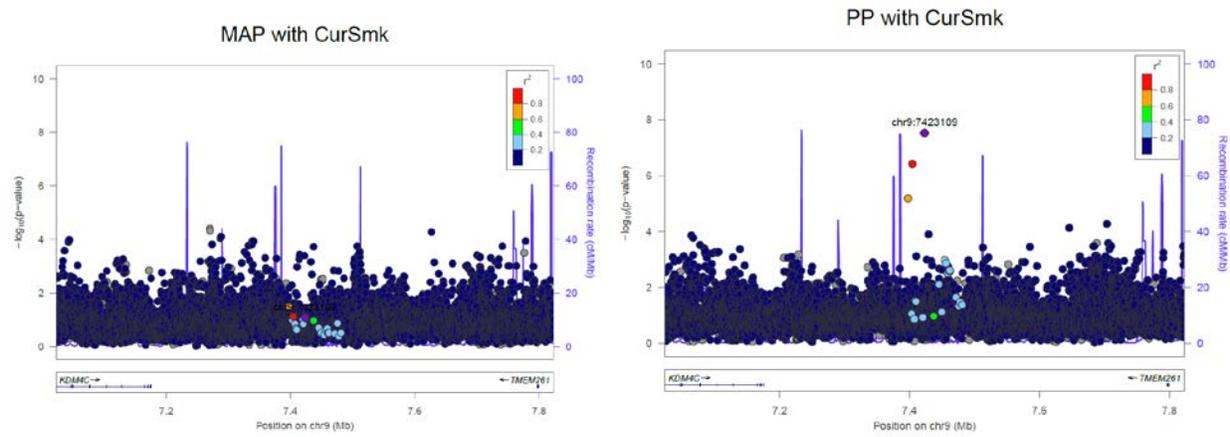
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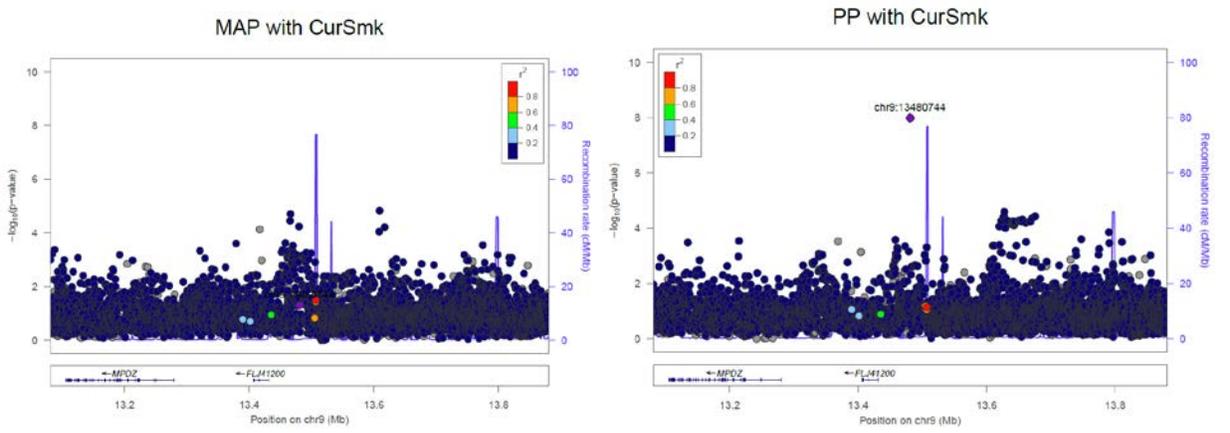
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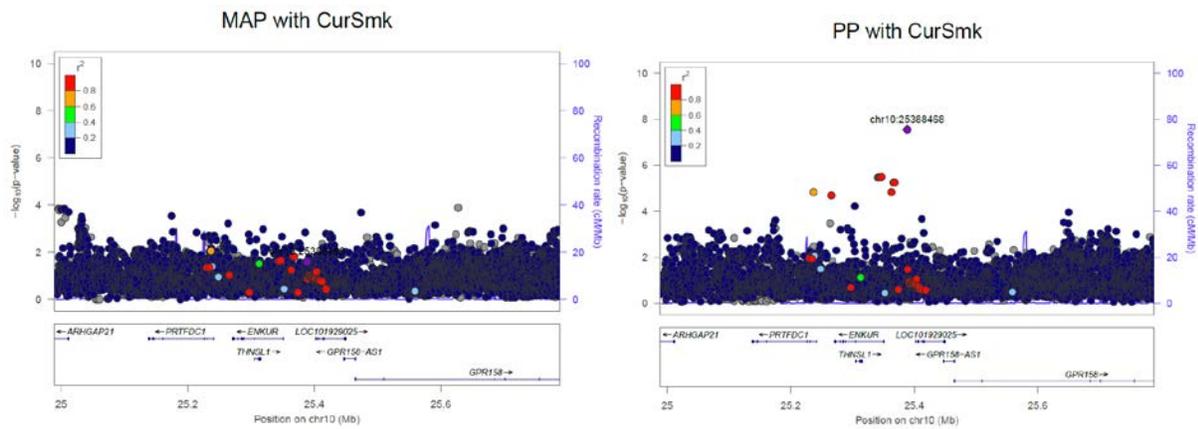
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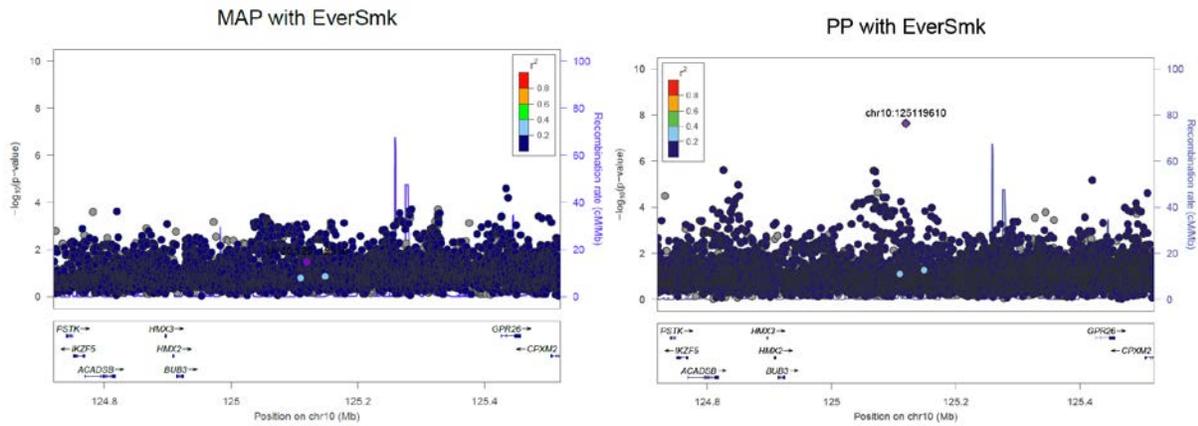
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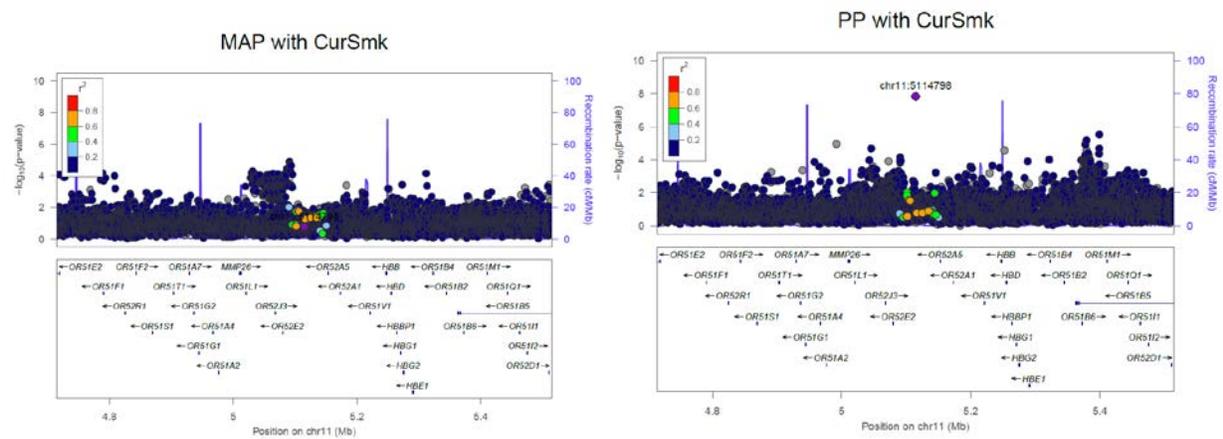
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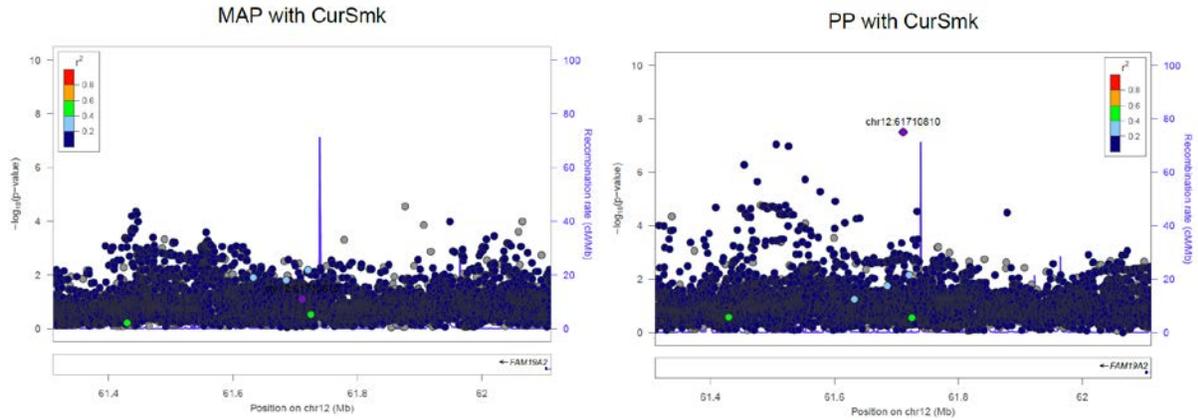
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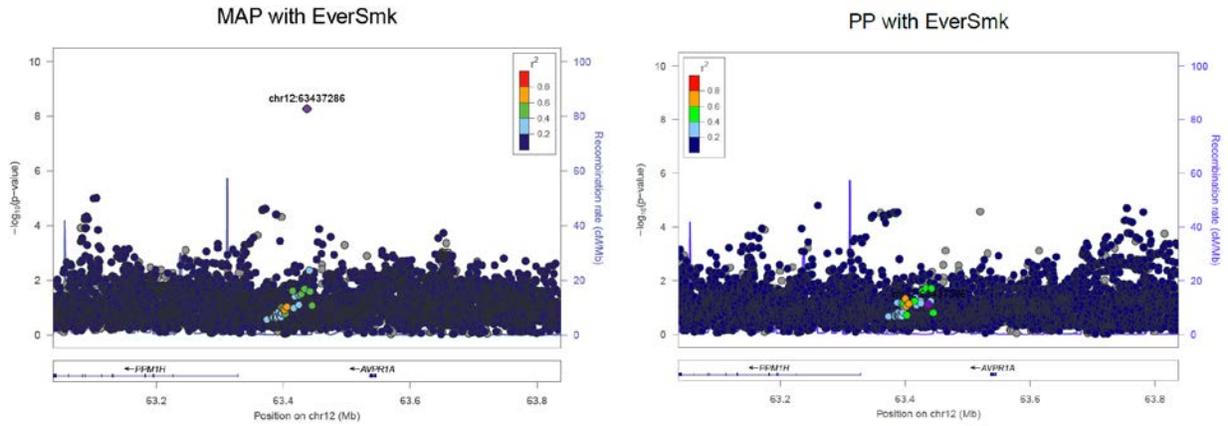
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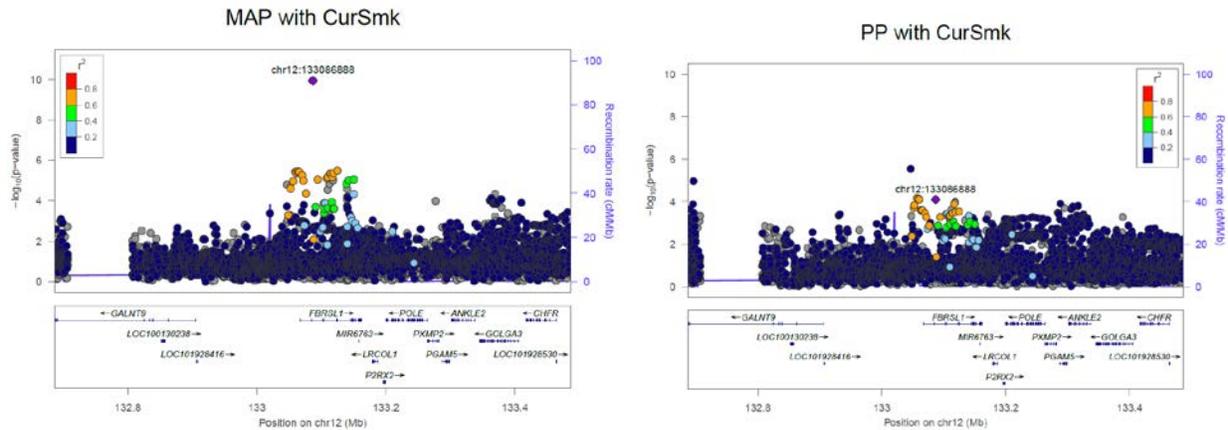
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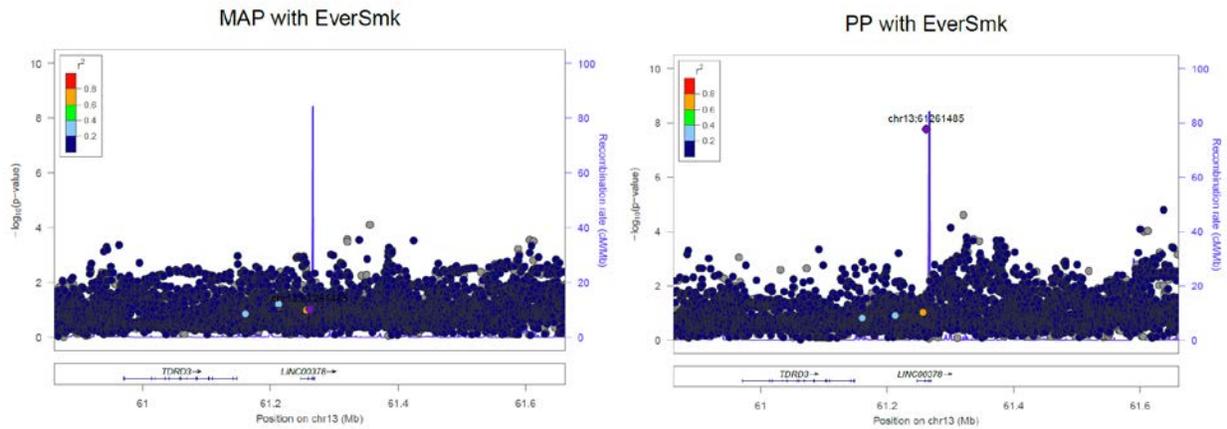
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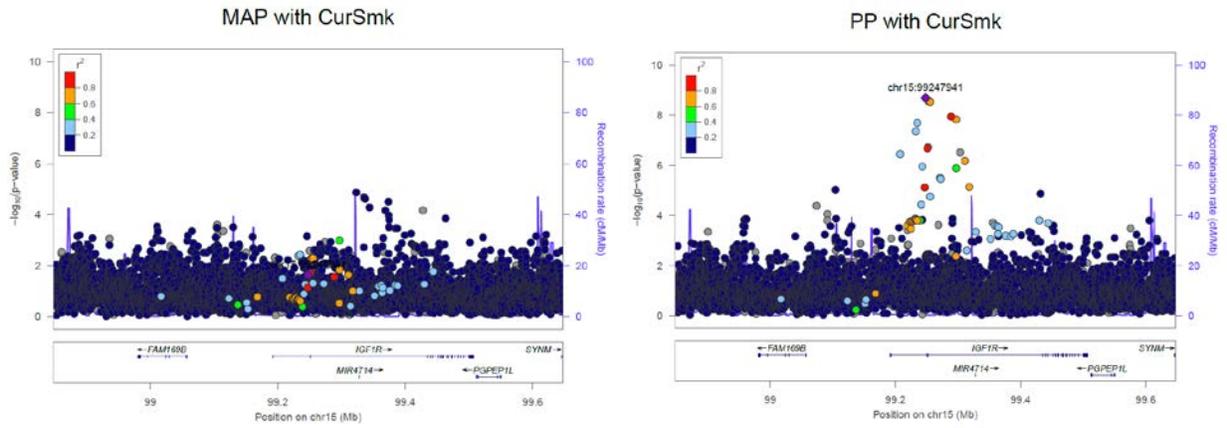
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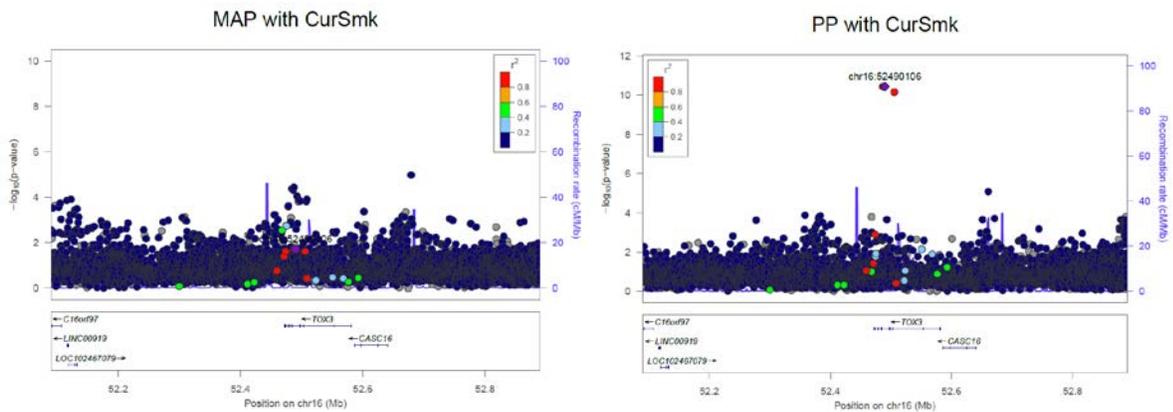
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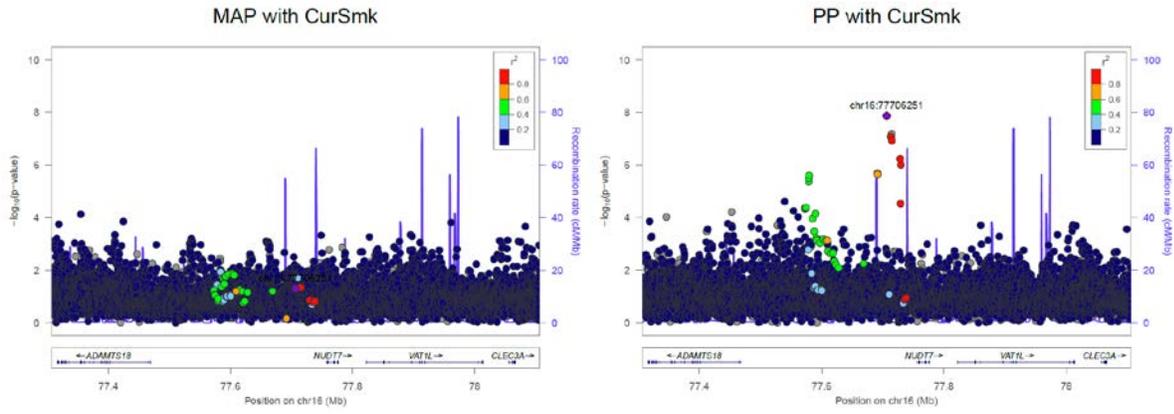
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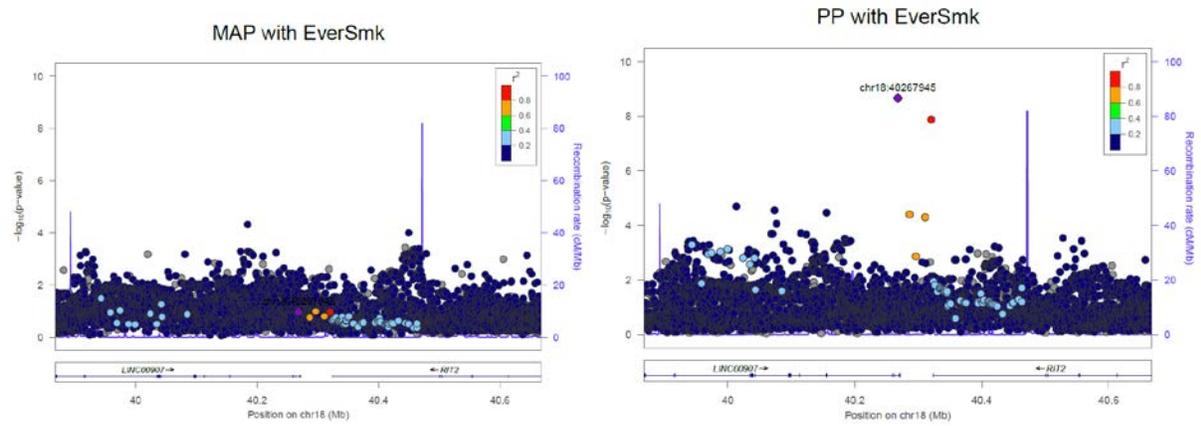
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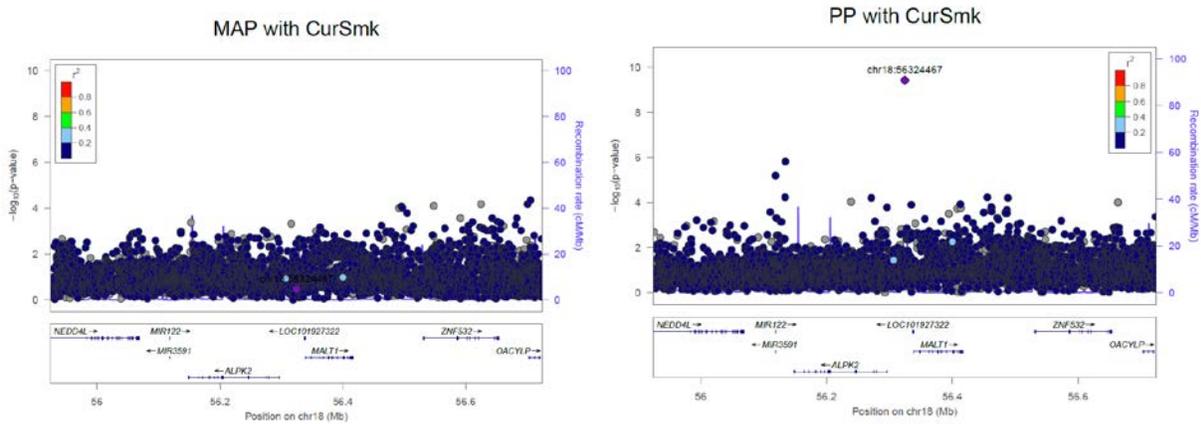
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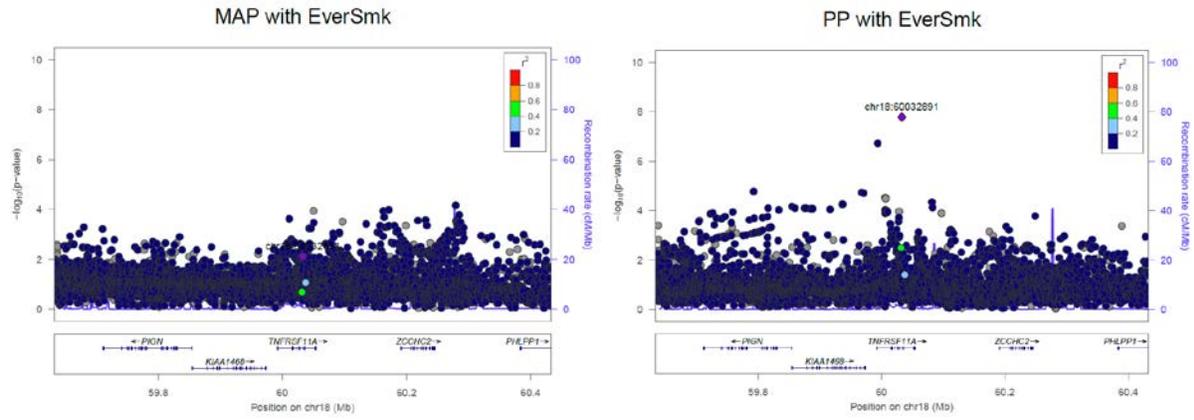
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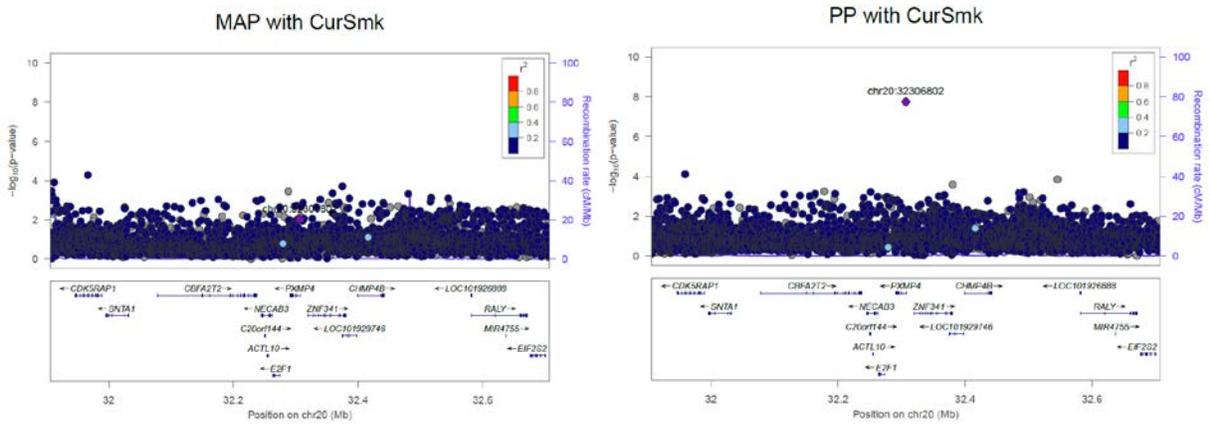
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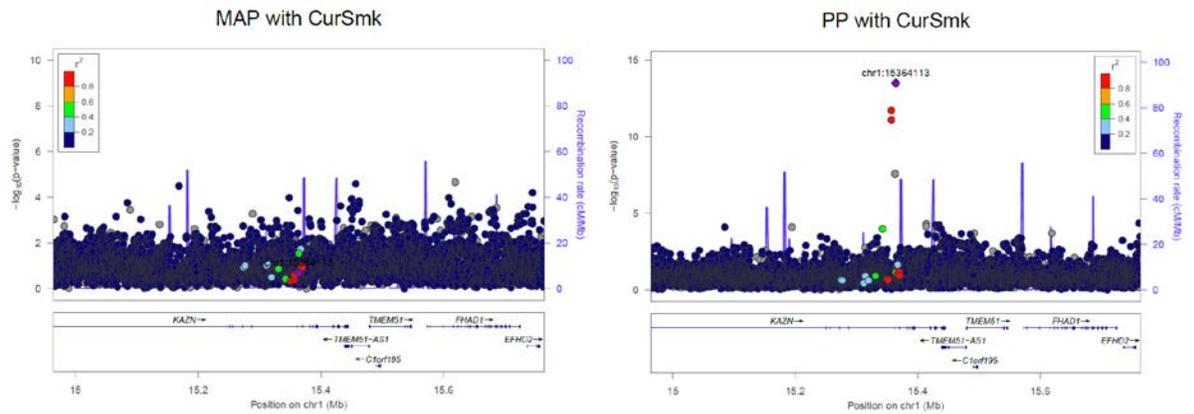
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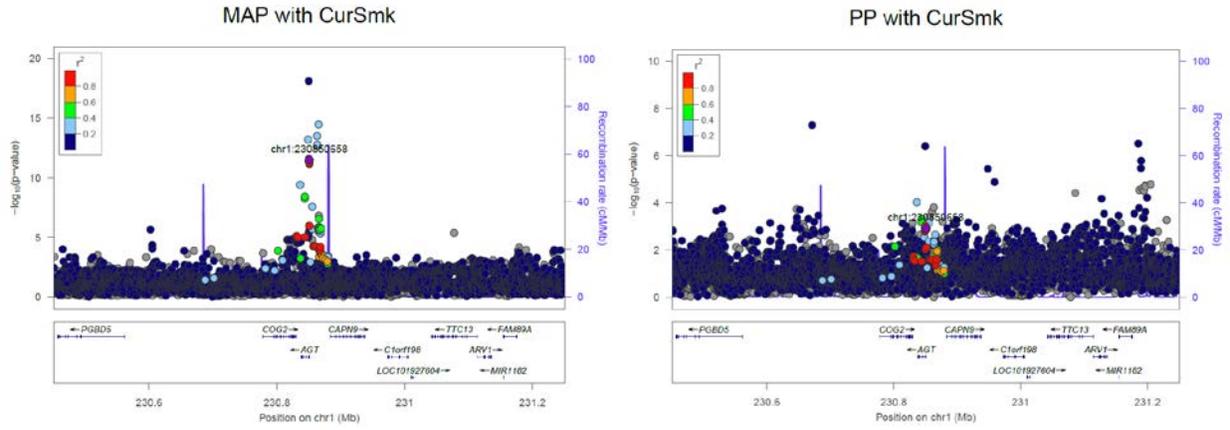
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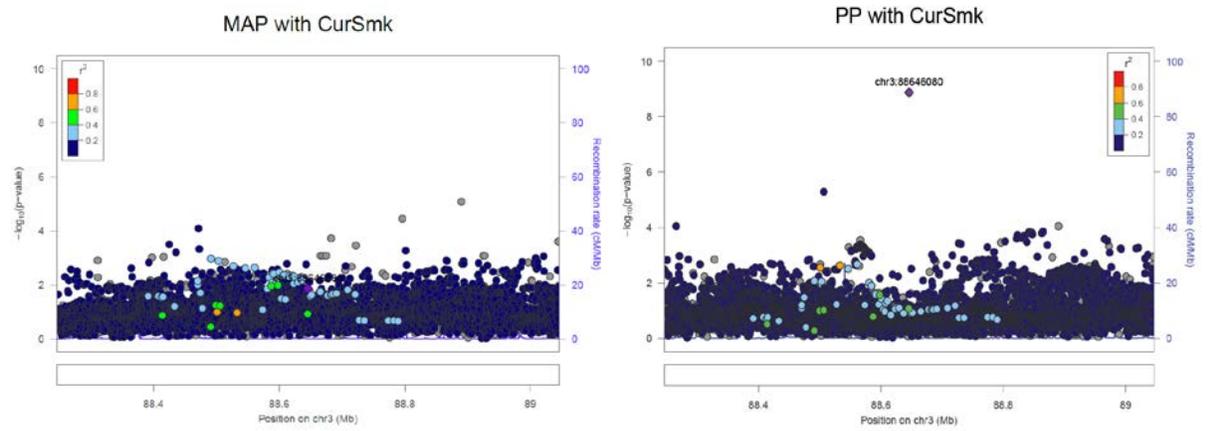
Signal 1



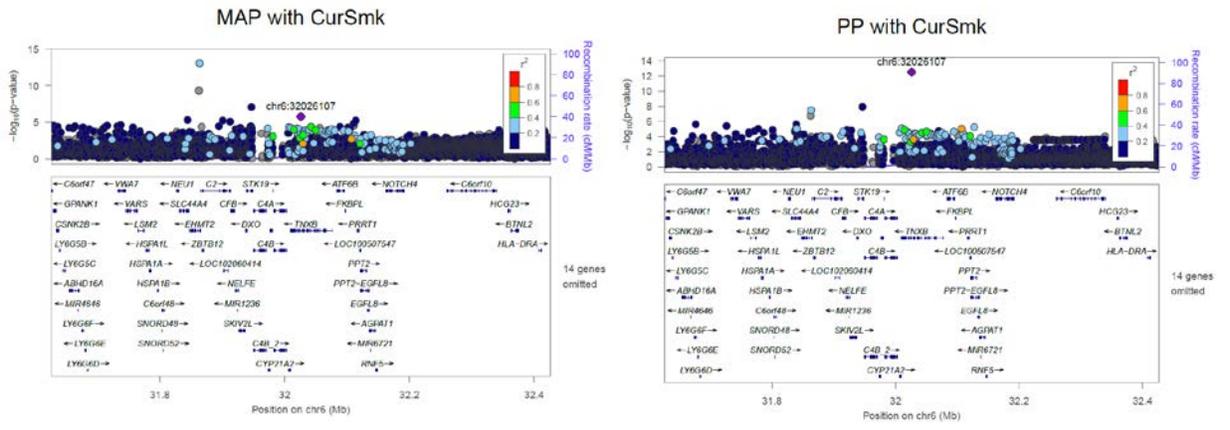
Signal 2



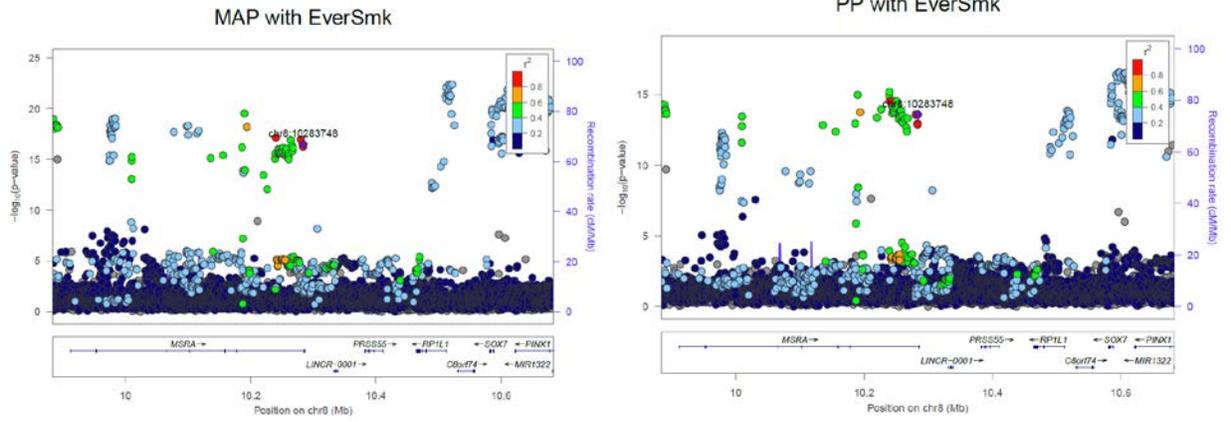
Signal 3



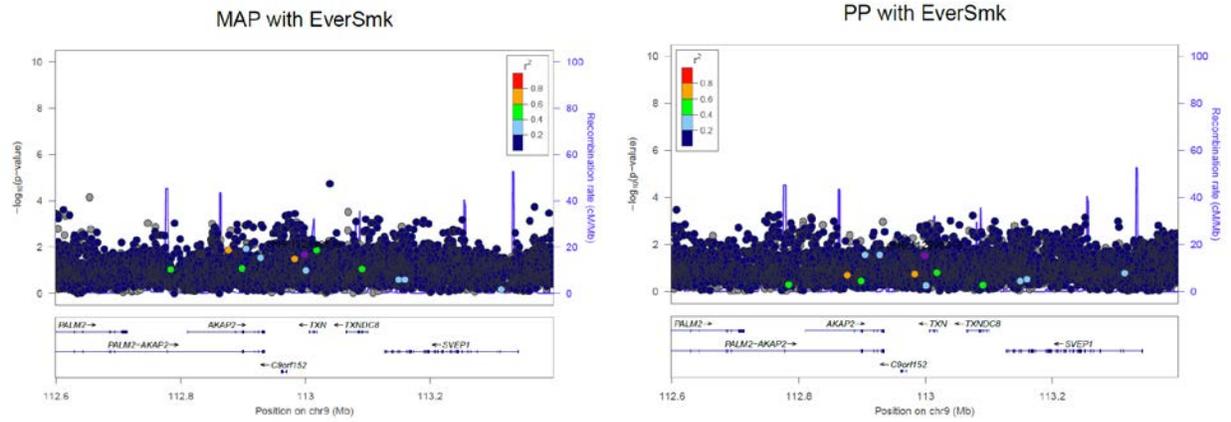
Signal 4



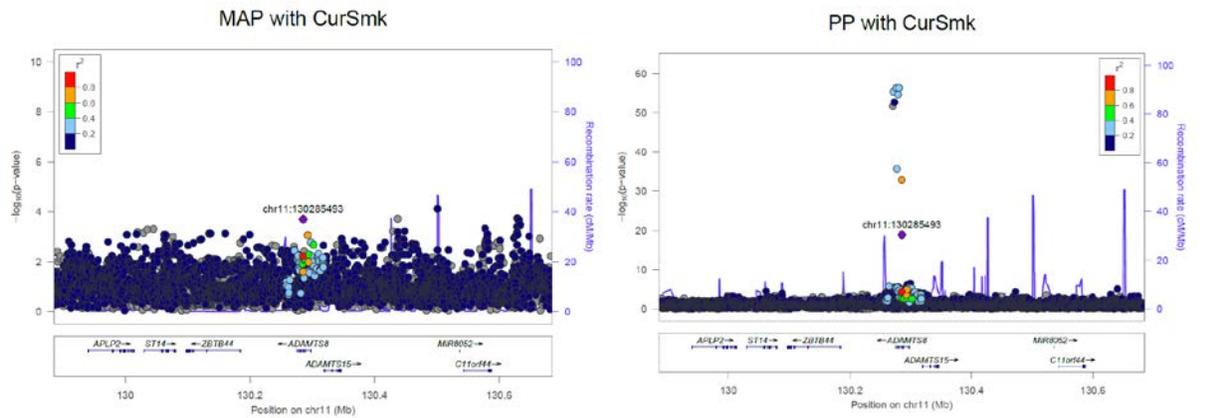
Signal 5



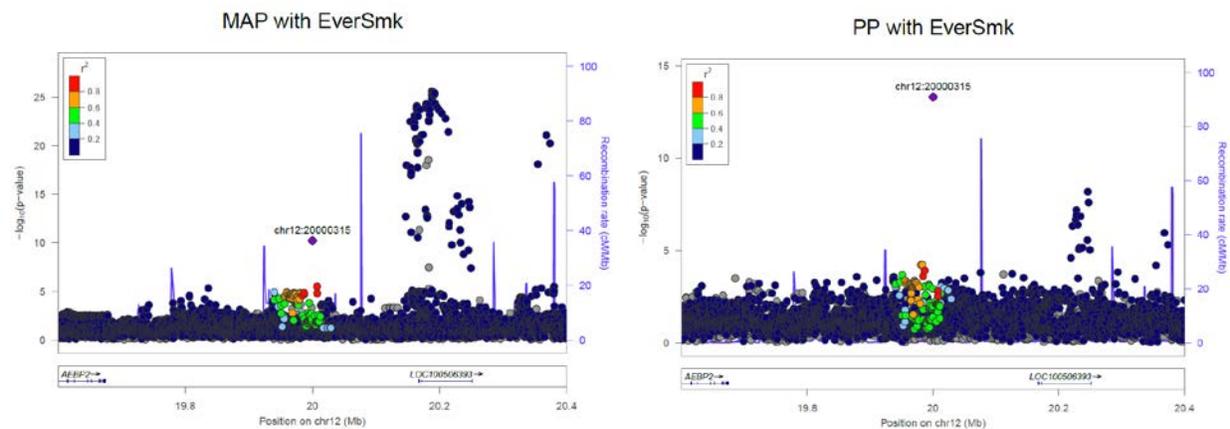
Signal 6



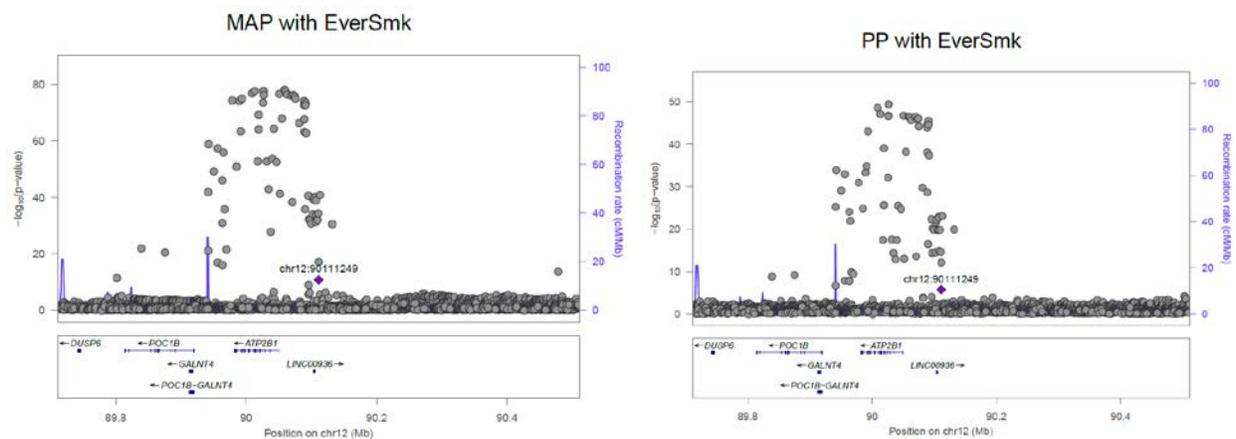
Signal 7



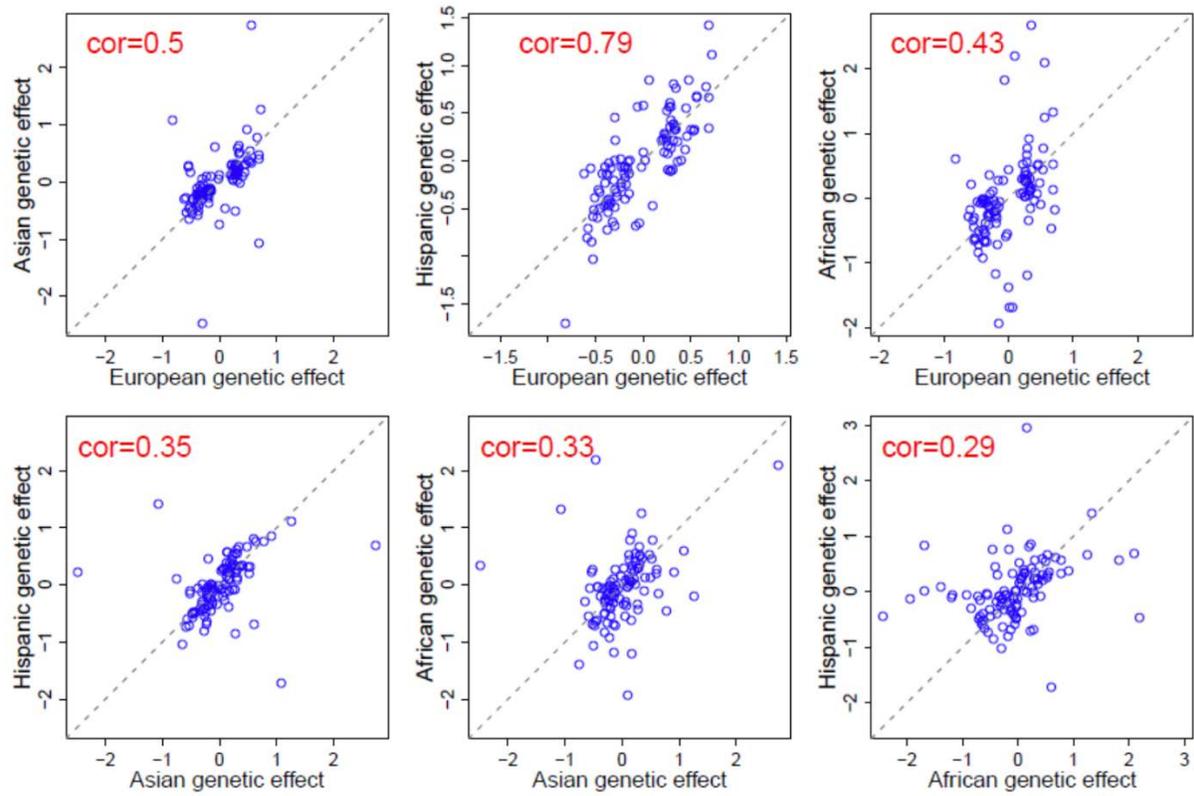
Signal 8



Signal 9



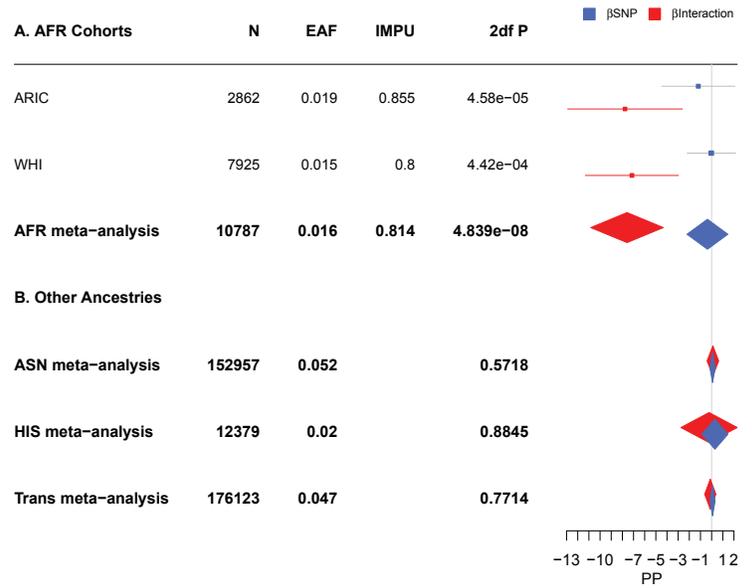
Supplementary Figure 4: Scatterplots comparing ancestry-specific genetic effects of the 136 loci and the 9 signals.



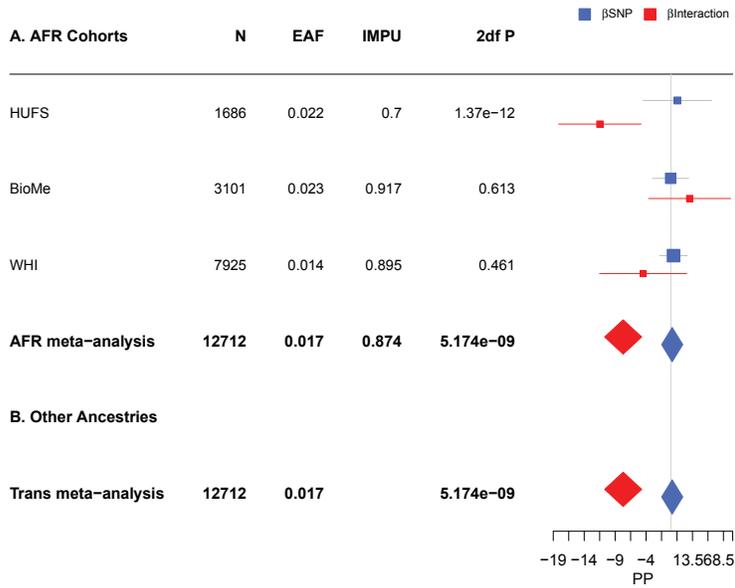
Supplementary Figure5: Forest plots that examine consistencies across African cohorts at 30 African-specific loci (from Table 2).

These 30 loci were statistically significant only in the meta-analyses of African ancestry individuals.

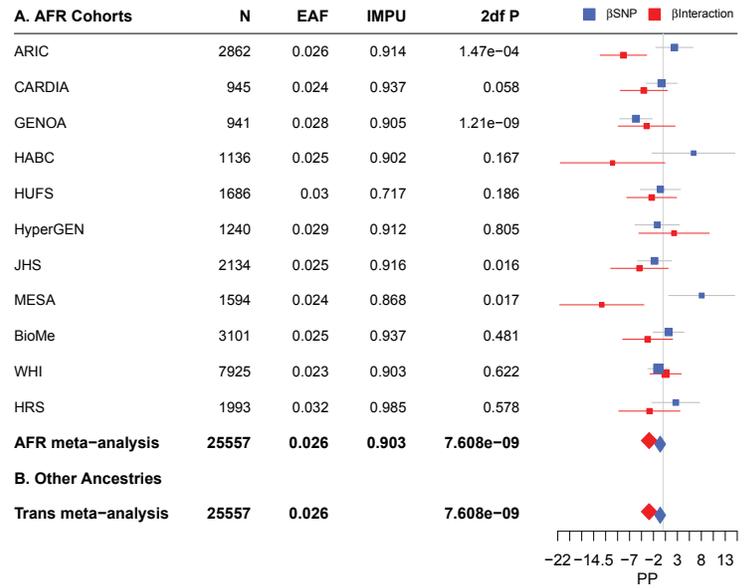
rs138318054 (1:233578559) and CurSmk on PP



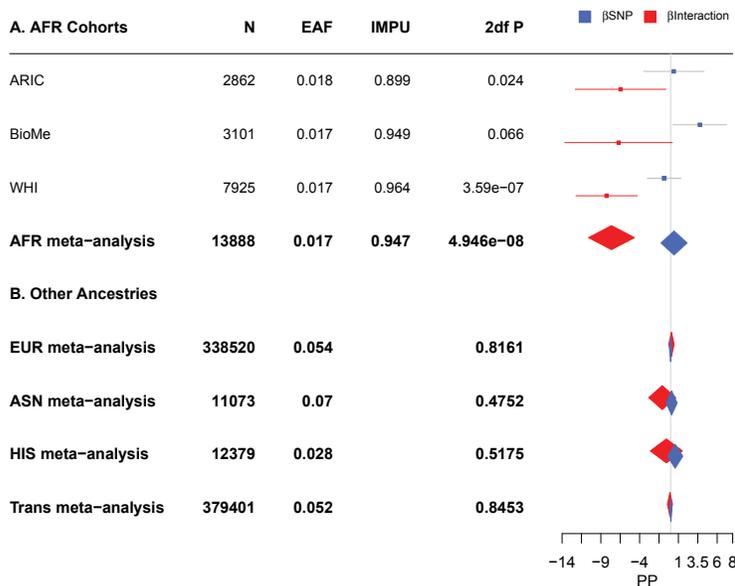
rs115356163 (1:17466024) and CurSmk on PP



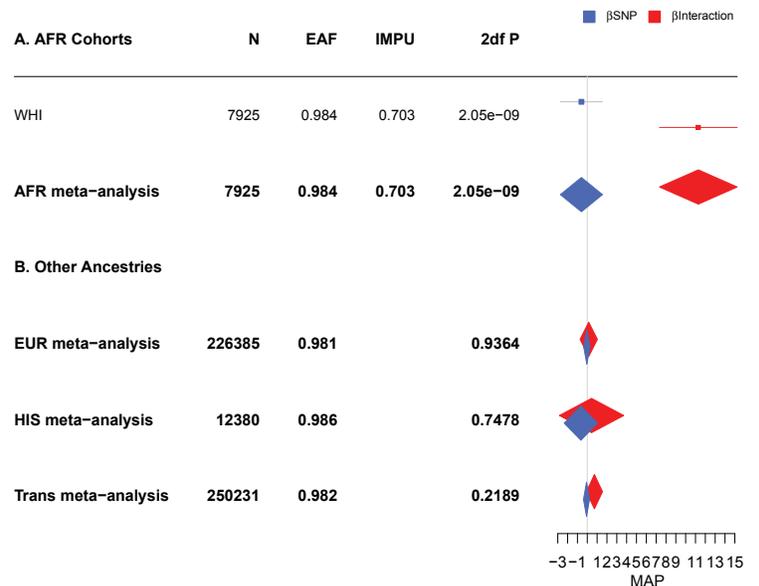
rs79113694 (2:31253799) and EverSmk on PP



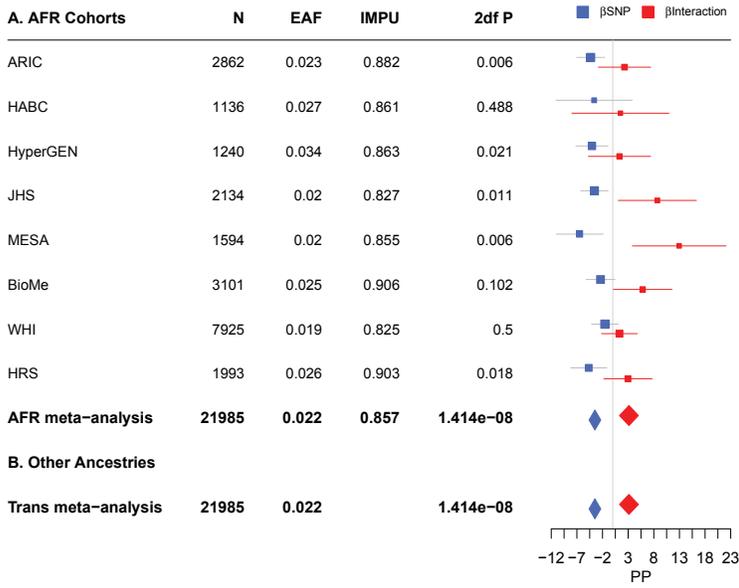
rs11587661 (1:230671208) and CurSmk on PP



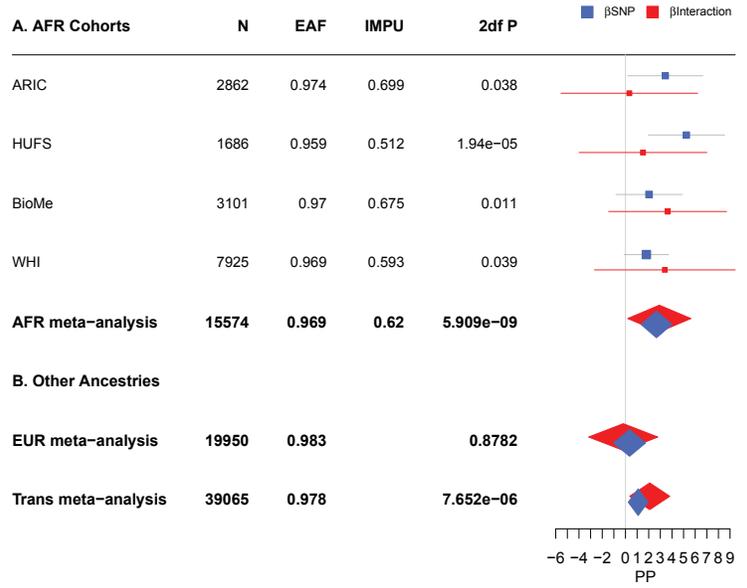
rs183927068 (2:210288479) and CurSmk on MAP



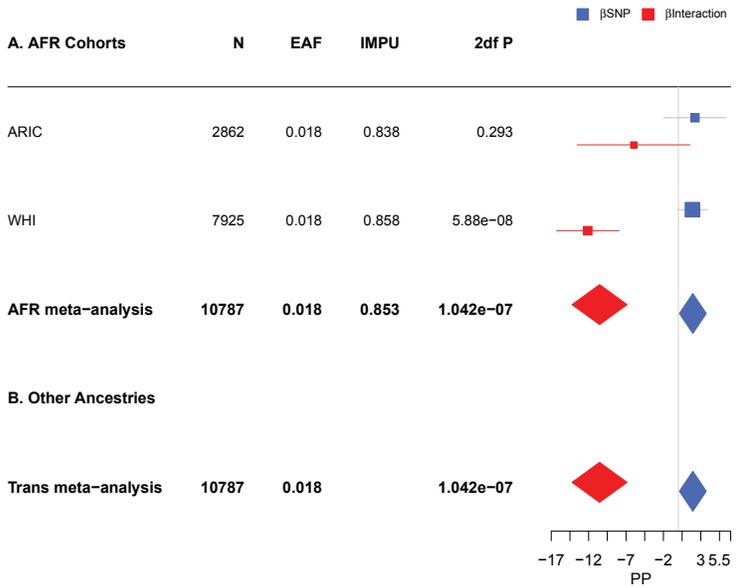
rs75875736 (3:36341106) and EverSmk on PP



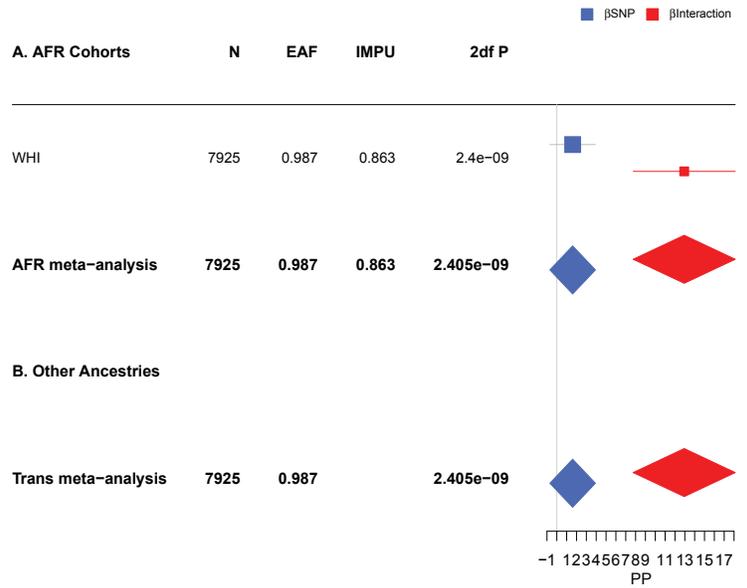
rs201223145 (4:121706475:ID) and CurSmk on PP



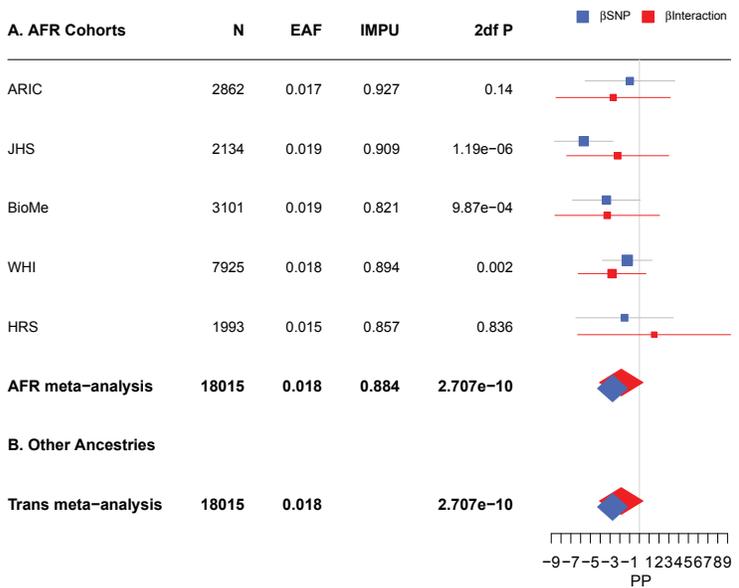
rs116199364 (3:139951198) and CurSmk on PP



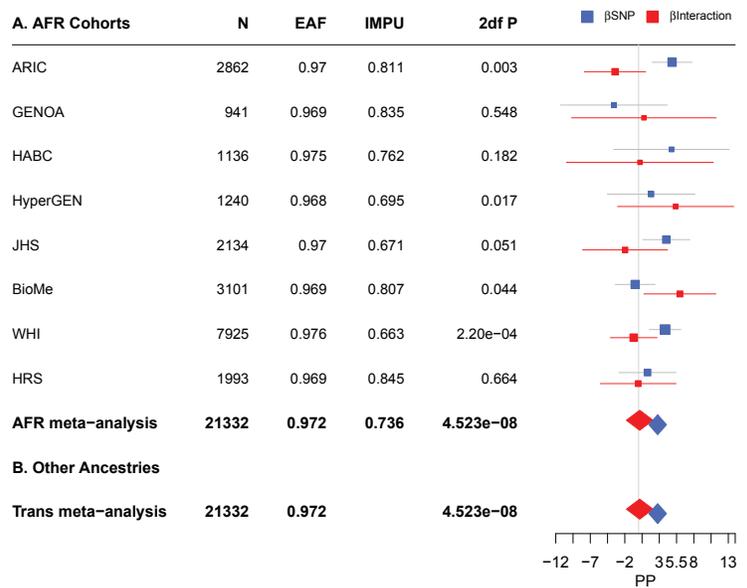
rs147998309 (4:133596832) and CurSmk on PP



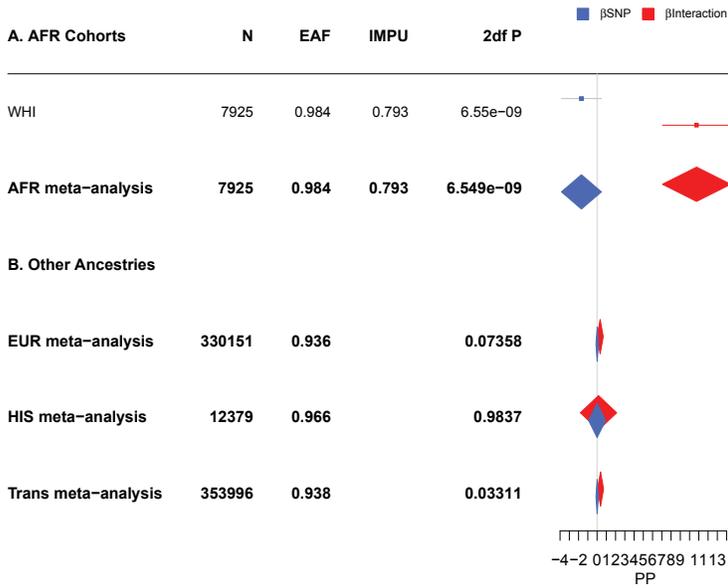
rs114619985 (4:13599930) and EverSmk on PP



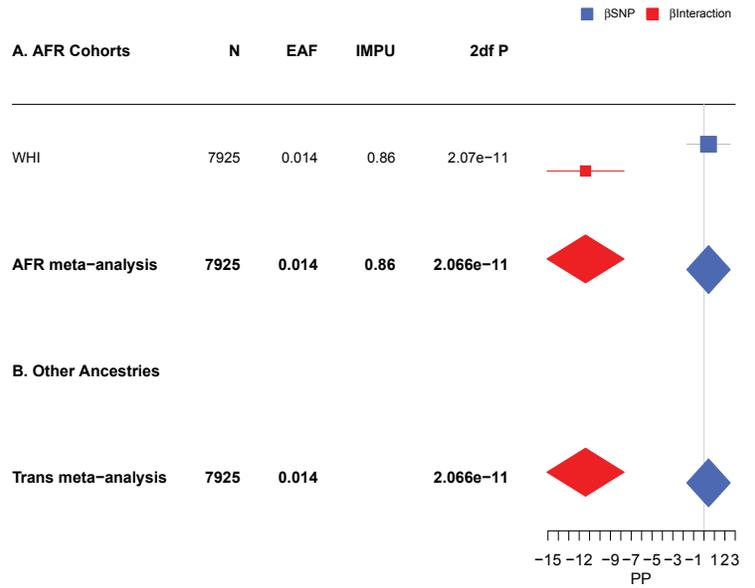
rs146622638 (4:176524533) and EverSmk on PP



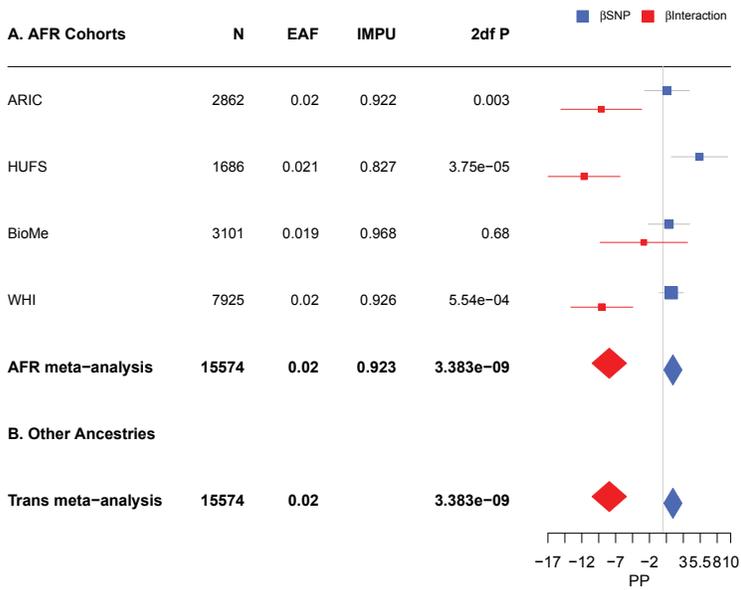
rs72723039 (5:2664169) and CurSmk on PP



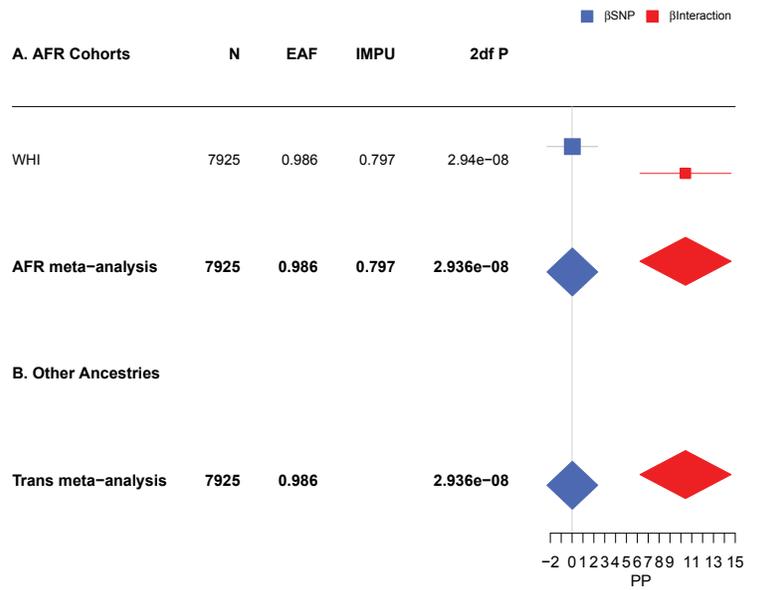
rs140994551 (8:4449086) and CurSmk on PP



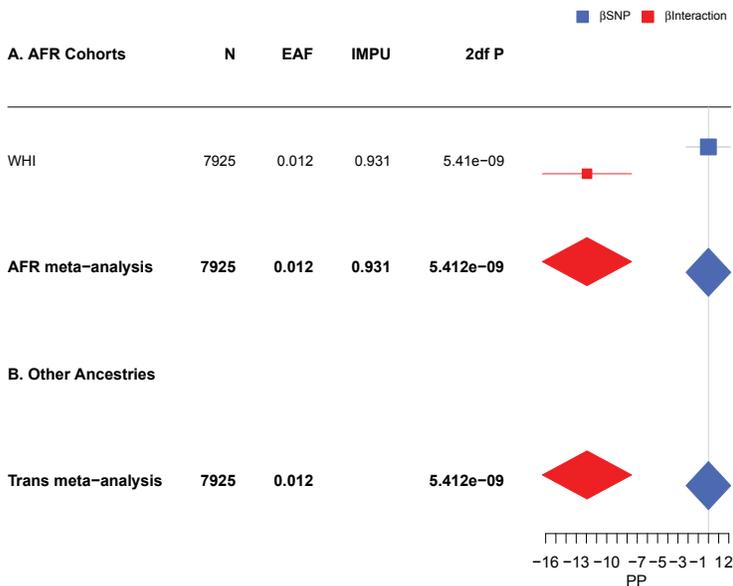
rs79205226 (6:21103825) and CurSmk on PP



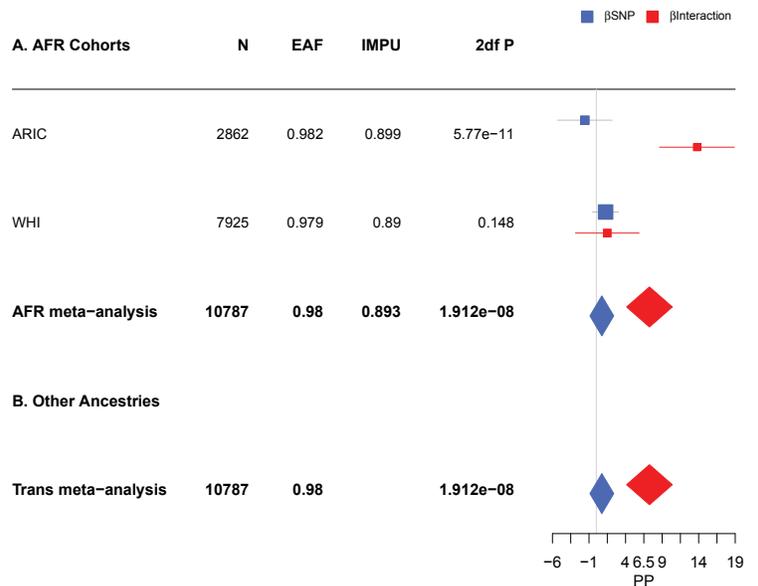
rs76209156 (9:7423109) and CurSmk on PP



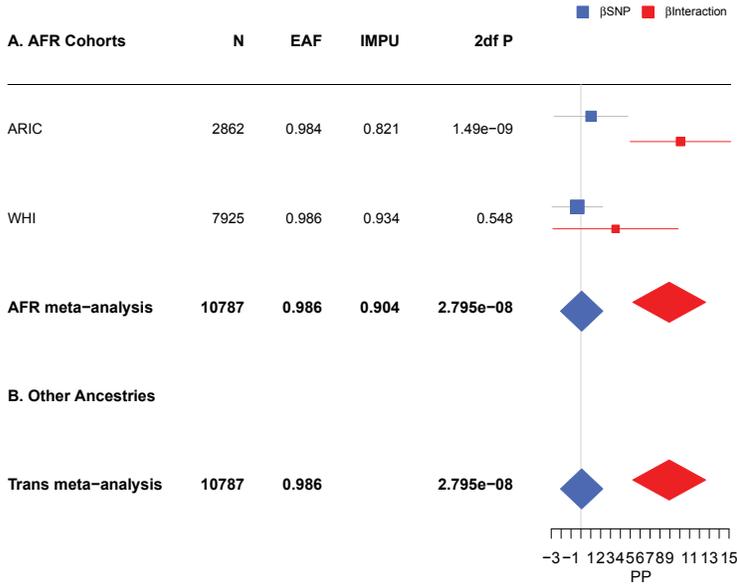
rs190090939 (7:152802243) and CurSmk on PP



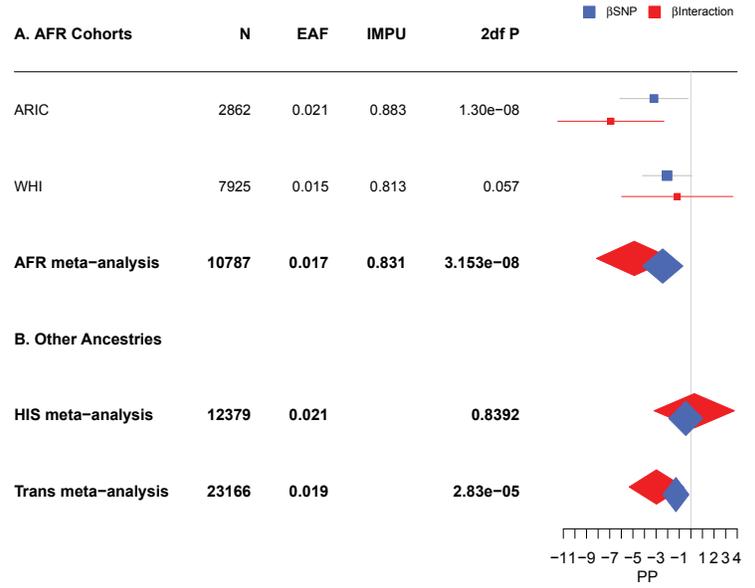
rs77548020 (9:13480744) and CurSmk on PP



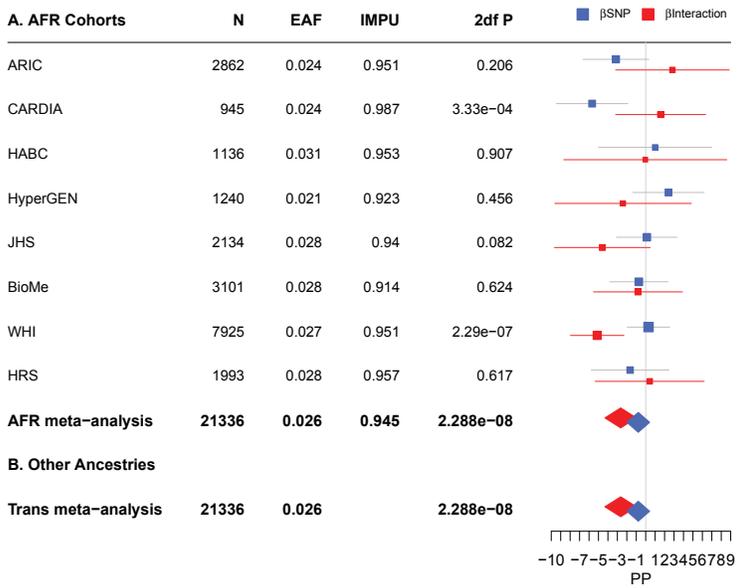
rs75872665 (10:25388468) and CurSmk on PP



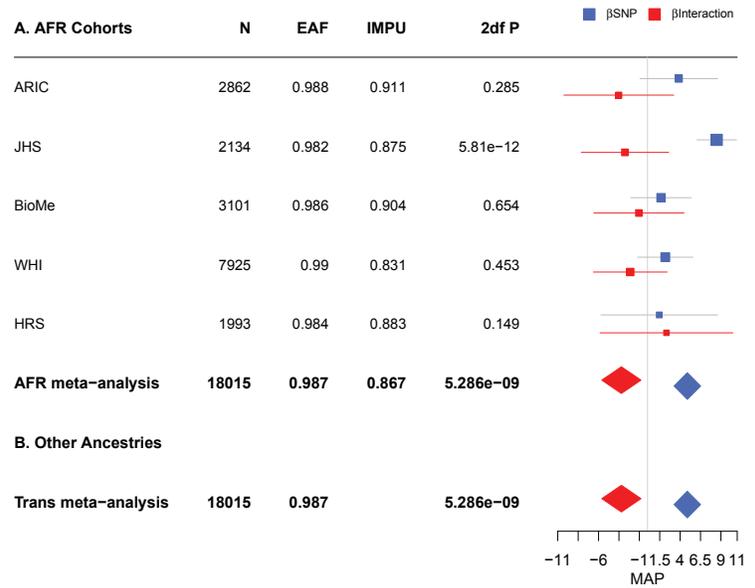
rs186331780 (12:61710810) and CurSmk on PP



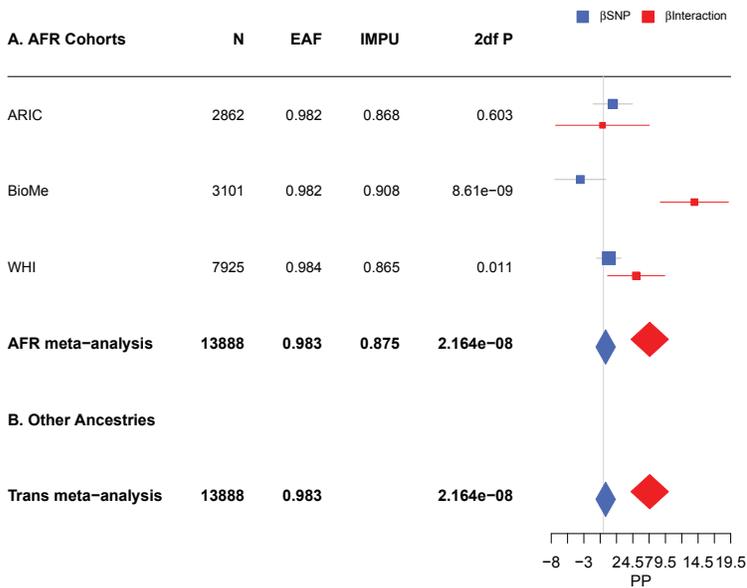
rs76497600 (10:125119610) and EverSmk on PP



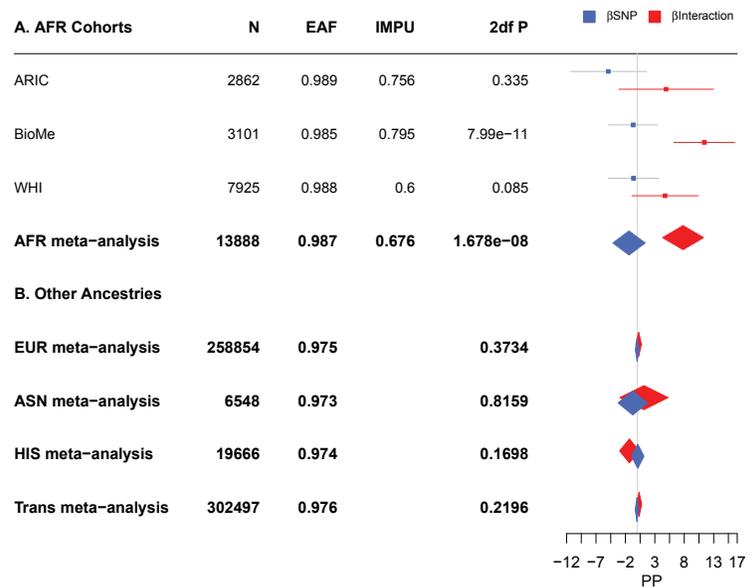
rs146924684 (12:63437286) and EverSmk on MAP



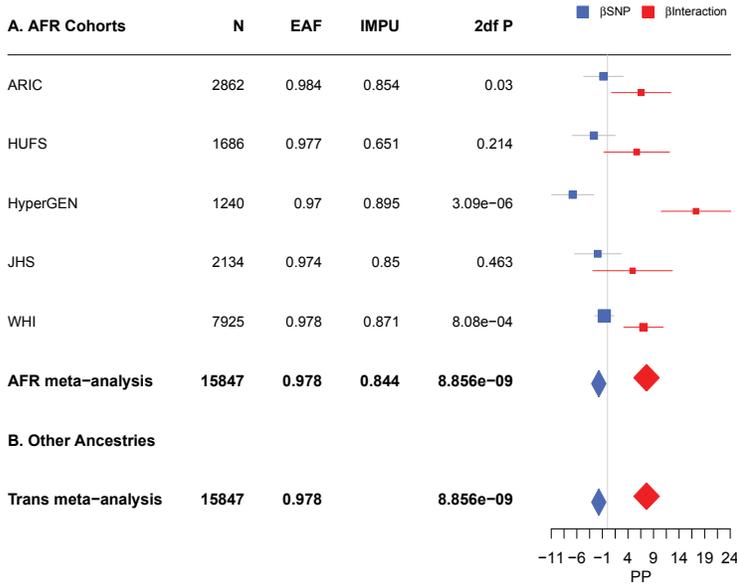
rs148454833 (11:5114798) and CurSmk on PP



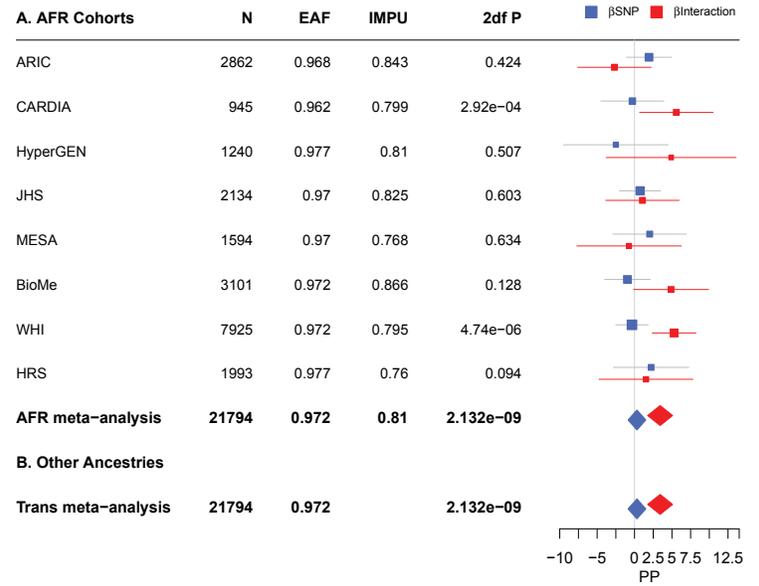
rs73212161 (13:61261485) and EverSmk on PP



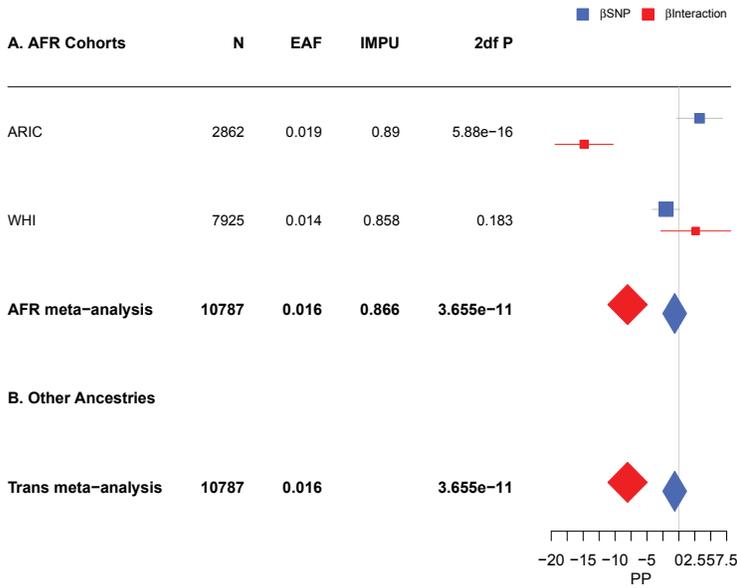
rs78265647 (15:99247941) and CurSmk on PP



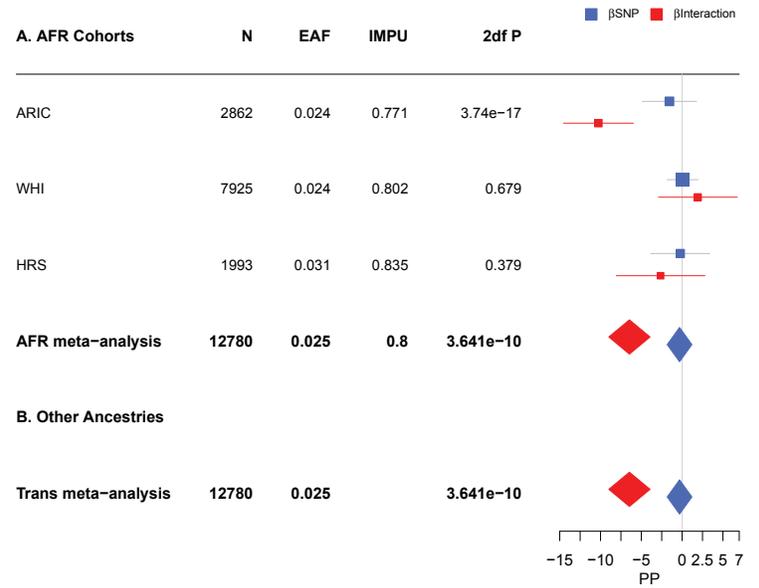
rs75129914 (18:40267945) and EverSmk on PP



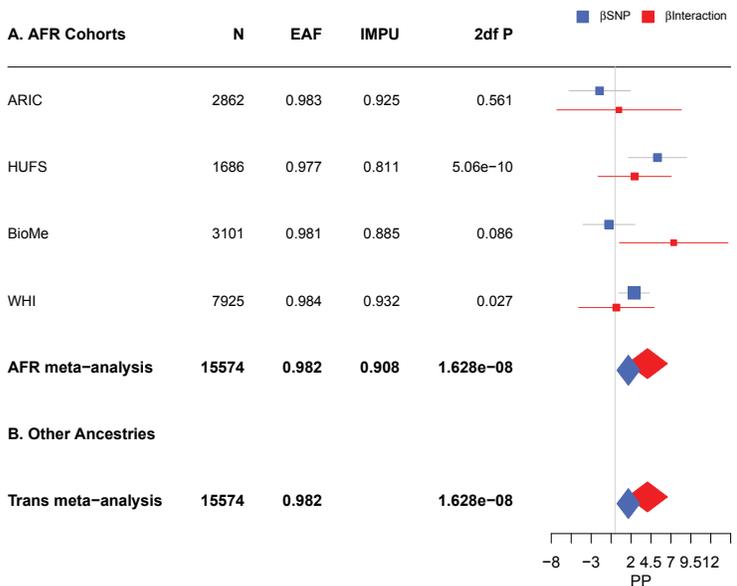
rs145181522 (16:52490106) and CurSmk on PP



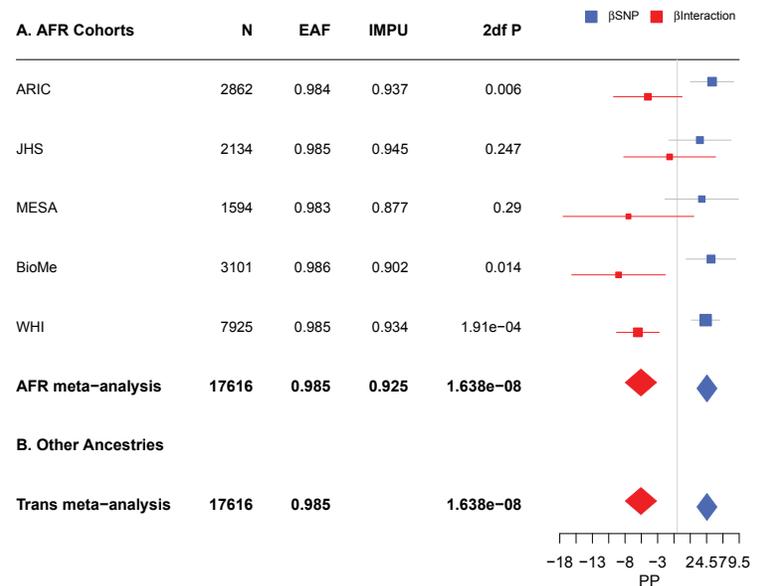
rs115134409 (18:56324467) and CurSmk on PP



rs114511313 (16:77706251) and CurSmk on PP



rs7875085 (18:60032891) and EverSmk on PP



rs191056303 (20:32306802) and CurSmk on PP

