Supplementary Online Content

Tosto G, Vardarajan B, Sariya S, et al. Association of variants in *PINX1* and *TREM2* with late-onset Alzheimer disease per a gene-based, transethnic meta-analysis. *JAMA Neurol*. Published online May 6, 2019. doi:10.1001/jamaneurol.2019.1066

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethod 1. Whole-exome sequencing quality control.

Quality control (QC) procedures excluded monomorphic variants, VQSR non-"PASS" variants with call rates <80%, and variants with low average depth of data (DP) and genotype quality (GQ) (8<DP>500 reads and GQ <20, corresponding to a >1% likelihood of being an incorrect genotype call) [1]. "DP" values represented the number of reads passing QC used to calculate the genotype at a specific site in a specific sample, with higher values for DP generally leading to more accurate genotype calls. "GQ" is a Phred-scaled value representing the confidence that the called genotype is the true genotype. Again, higher values reflected more accurate genotype calls. These thresholds were chosen according to studies of concordance between sequencing experiments and genotyping arrays in order to achieve a 99% genotype likelihood. Because these simulations were focused on SNV only, for INDELS we additionally applied GATK-reccomanded hard filter (QualByDepth (QD) >2.0; FisherStrand (FS) <200.0; ReadPosRankSumTest (ReadPosRankSum) > 20.0) (https://software.broadinstitute.org/gatk/documentation/article?id=2806). We handled multi-allelic site by splitting the alternative alleles in multiple biallelic sites using *bcftools* utility [2]. After this step, we normalized variants by applying parsimonial representation (i.e. coding the variant in as few nucleotides as possible without reducing the length of any allele to 0) and left-alignment (i.e. shifting the start position of that variant to the left till it is no longer possible) [3]. Variants showing strong departure from Hardy Weinberg equilibrium (HWE, p<1-e07) in controls were also filtered out. Principal components (PCs) were estimated using WES data, and filtering out single nucleotide variant (SNV) with minor allele frequency (MAF) ≤5%, call rate <95%. We used the KING software to detect duplicates and cryptic relatedness, and output the first 10 PCs, separately for each ethnic group. We then excluded outliers that deviated more than six standard deviation from the mean.

eMethod 2. Replication cohorts' description.

1. Alzheimer Disease Sequencing Project (ADSP). Individuals were aged 60 years or older and met NINCDS-ADRDA criteria [4] for possible, probable or definite AD based on clinical assessment, or had presence of LOAD (moderate or high likelihood) upon neuropathology examination. Healthy controls were similar in age and either judged to be cognitively normal or did not meet pathological criteria for LOAD following brain autopsy [5]. We did not include the Caribbean Hispanic part of the ADSP dataset because they overlapped with those already present in our WHICAP WES data.
2. The ROS/MAP study. The Religious Orders Study (ROS) is a longitudinal cohort study of aging and Alzheimer's disease from Rush University recruiting Individuals from more than 40 groups of religious orders across the US. Subjects were included at baseline if dementia was not present. The Memory and Aging Project (MAP) is a longitudinal, epidemiologic clinical-pathologic cohort study based at Rush University of aging and risk of LOAD that began in 1997. This study was designed to complement the ROS study by enrolling individuals with a wider range of life experiences and socioeconomic status. The study enrolls older individuals without any signs of dementia, primarily recruiting from continuous care retirement communities throughout northeastern Illinois, USA. LOAD status was determined by an actuarial decision tree that incorporated a computer algorithm and clinical judgment made in series by a neuropsychologist and a clinician who ultimately reviewed all cases. Details of the ROS and MAP study has been described in detail elsewhere [6]. Participants were deemed "non-demented" (healthy or MCI) or LOAD. Other dementia types were excluded from analyses.
3. ADSP Family Study. Whole exome data from 67 families of Caribbean Hispanic ancestry (N=358 participants) were selected from the ADSP family dataset (Table 1). No individuals with known early-onset disease mutations (*APP*, *PSEN1*, *PSEN2*, *GRN*, or *MAPT*) were included. All probands were part of families with three or more affected individuals recruited as part of the Estudio Familiar de Influencia Genetica en Alzheimer (EFIGA) study. Detailed description of this cohort has been published elsewhere [7].
4. We used single-marker summary statistics from the International Genomics of Alzheimer's Project (IGAP), a large two-stage genome-wide association study (GWAS) on individuals of European ancestry (http://web.pasteur-lille.fr/en/recherche/u744/igap/igap_download.php - N=54,162) [8]. In stage 1, IGAP employed 7,055,881 genotyped and imputed single nucleotide polymorphisms to meta-analyses four previously-published GWAS datasets (The European Alzheimer's disease Initiative (EADI); the Alzheimer Disease Genetics Consortium (ADGC); The Cohorts for Heart and Aging Research in Genomic Epidemiology consortium (CHARGE) The Genetic and Environmental Risk in AD consortium (GERAD)). In stage 2, top hits from Stage 1 were genotyped and tested for association in an independent set of 8,572 LOAD cases and 11,312 controls and then again meta-analyzed. We used stage 1 summary results in order to include all SNPs available within each gene.

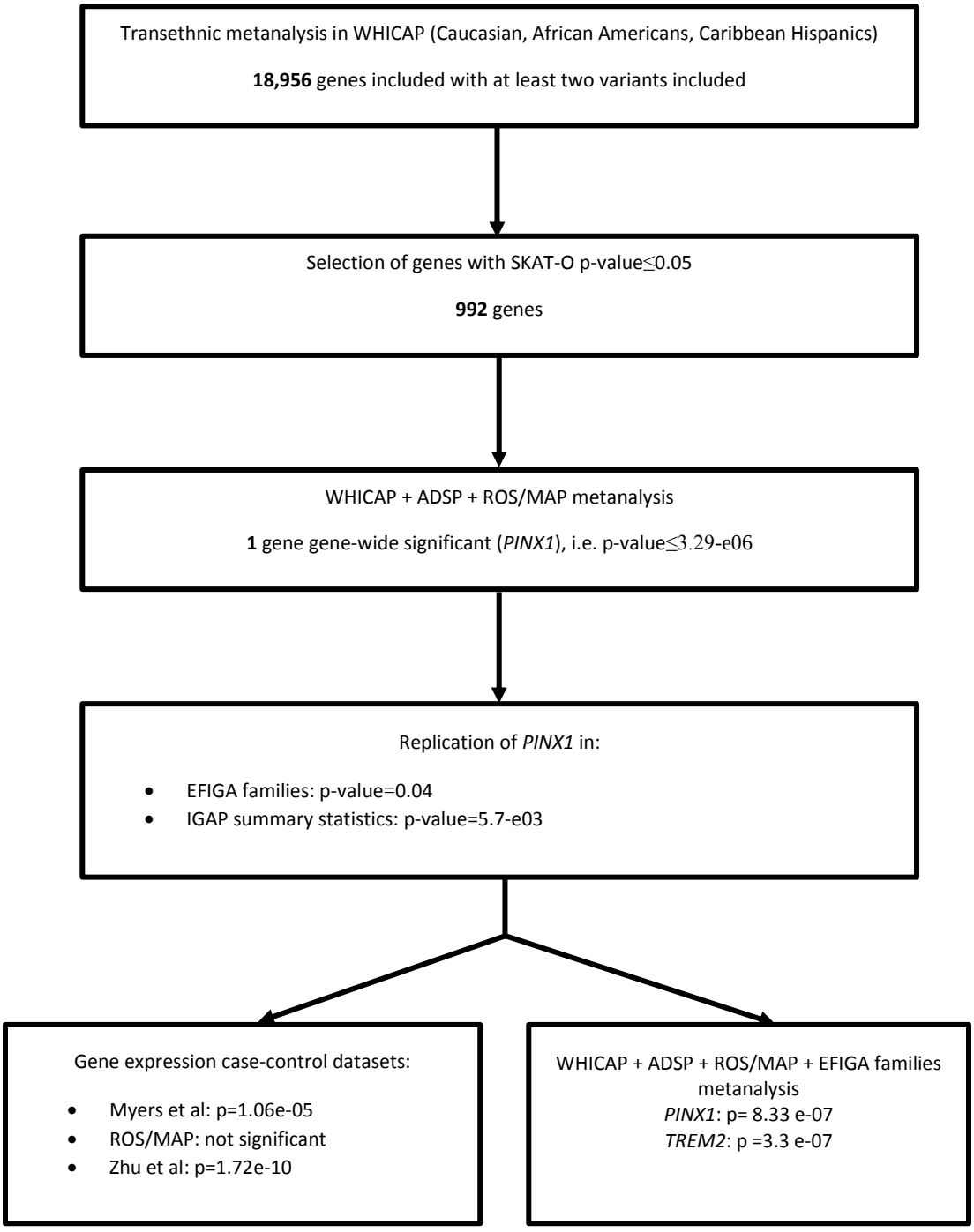
eMethod 3. Expression data description and analyses methods.

1. Description. The Myers and colleagues [9] neocortical transcriptome data. Briefly, a set of 188 individuals without dementia and 176 autopsy confirmed LOAD. Data were downloaded from the NCBI GEO archive (ID: GSE15222). All data were generated using the Illumina HumanRef-8 expression BeadChip (GPL2700) v2 Rev0. Methods. Data were analyzed in R using residual corrected profiles for each individual and for each transcript. Expression measures were corrected for sex, *APOE* status, age at death, cortical region, day of expression hybridization, study cohort, post-mortem interval and transcript detection rate. One-way ANOVA compared expression profile between affected brain sand normal control brains for genes prioritized in genetic analyses.

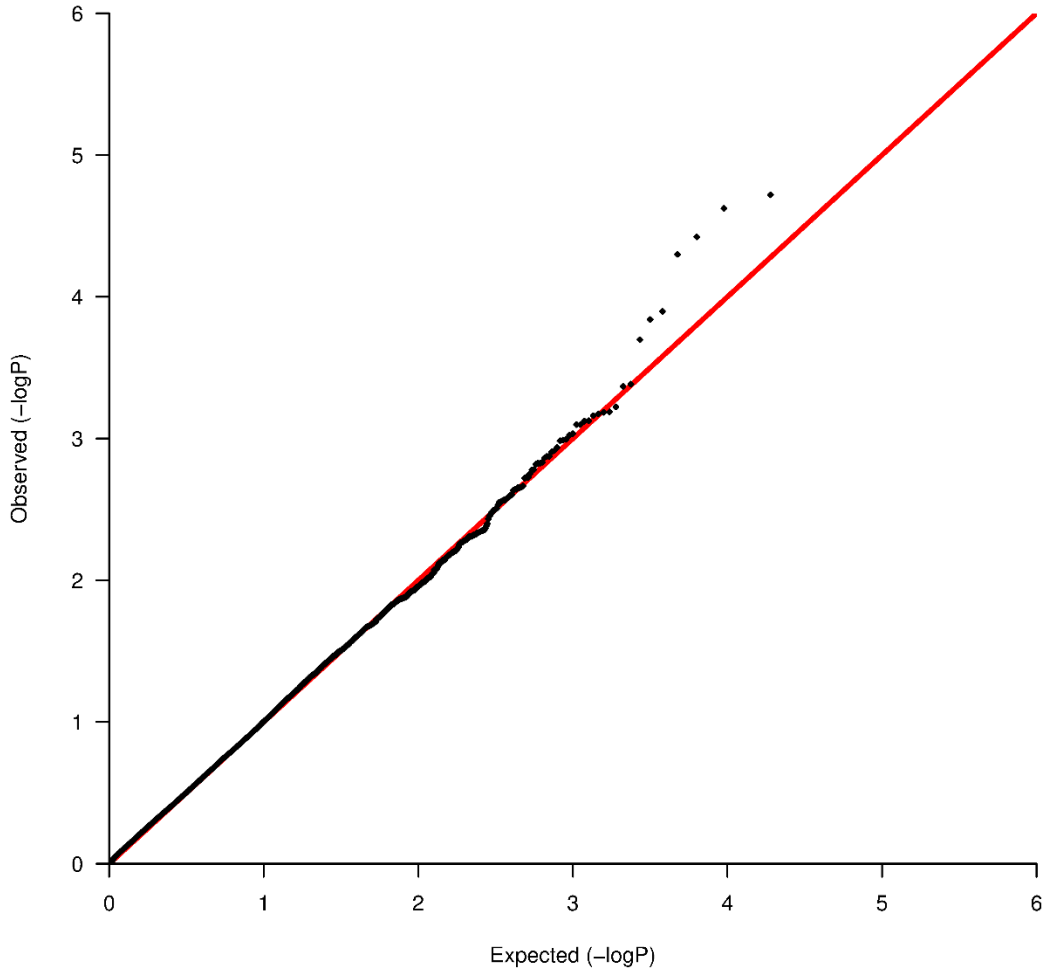
2. Description. ROS/MAP Next-generation RNA sequencing (RNA-seq) data. Briefly, RNA was purified from frozen dorsolateral prefrontal cortex tissue of ROS-MAP participants with the miRNeasy Mini Kit and RNase-Free DNase Set (Qiagen, Germantown, MD). RNA concentration and quality were measured with a Nanodrop (Thermo Fisher Scientific, Wilmington, DE) and Bioanalyzer (Agilent Technologies, Santa Clara, CA). A RIN score of >5 was required for library construction, which was assembled using the strand-specific dUTP method. Sequencing was performed with Illumina HiSeq with 101 base pair paired-end reads and a goal coverage of >85 million paired-end reads as previously described [10]. After QC, 508 subjects were available to analyze. Fragments per kb of transcript per million fragments mapped (FPKM) were quantile-normalized, correcting for batch effect with Combat [11]. Methods. Linear regressions were applied with neuropathological measure as the dependent variables, Combat-adjusted FPKM values as the independent variable, and technical factors as covariates (RNA integrity score, log2[total aligned reads], postmortem interval, and number of ribosomal bases) as detailed elsewhere [12].

3. Description. The Narayanan and colleagues dataset[12] comprised DLPFC (BA9) brain tissues of 624 individual (AD patients, HD patients and non-demented controls samples), obtained from Harvard Brain tissue resource center (HBTRC). The HBTRC samples were primarily of Caucasian ancestry, as only eight non-Caucasian outliers were identified, and therefore excluded for further analysis. Post-mortem interval (PMI) was 17.8+8.3 hours (mean ± standard deviation), sample pH was 6.4±0.3 and RNA integrity number (RIN) was 6.8±0.8 for the average sample in the overall cohort. Tissues were profiled on a custom-made Agilent 44K array (GPL4372). 310 LOAD cases and 157 controls were included in the analyses.

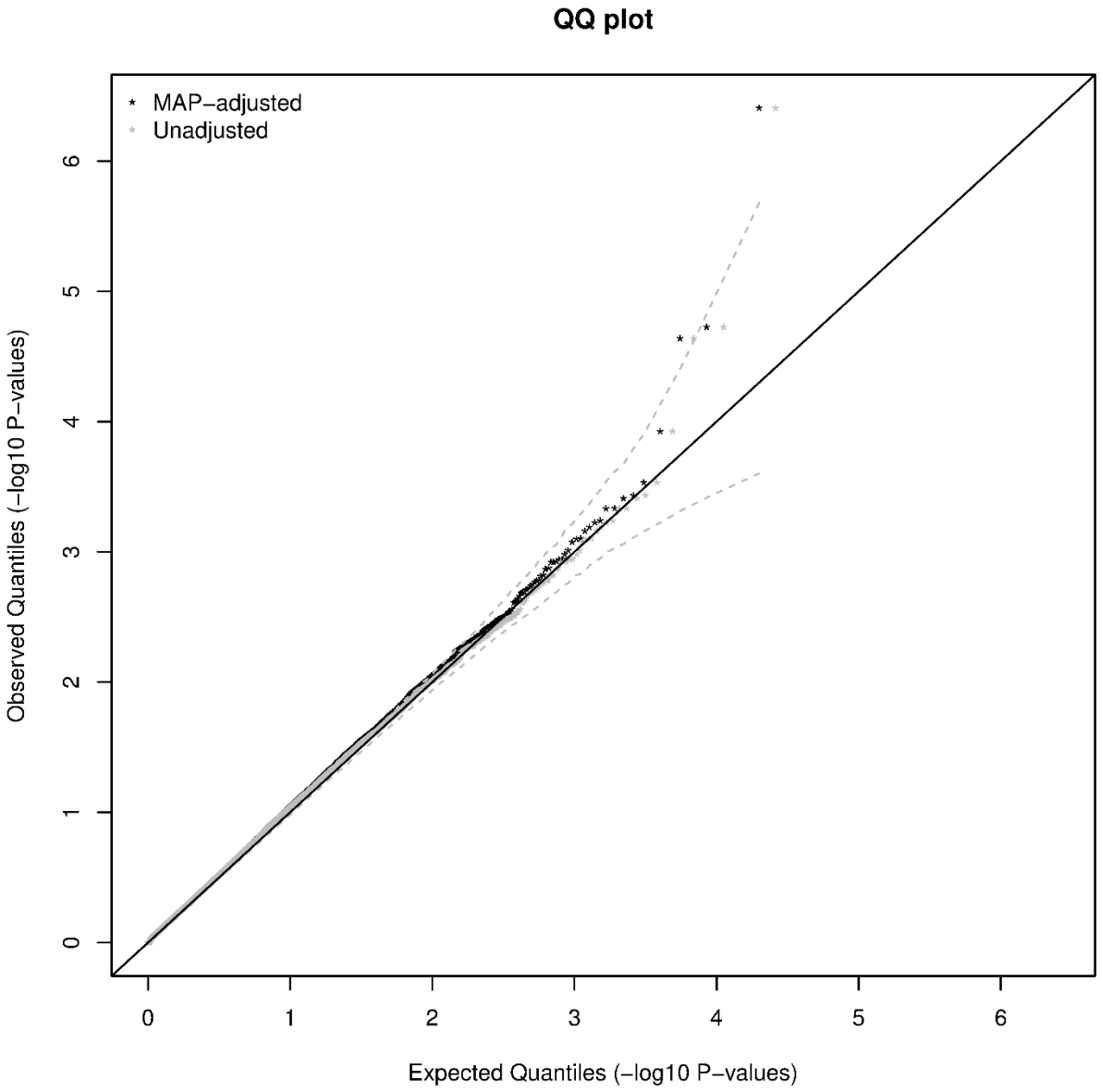
eFigure 1. Flowchart of the study for the MODERATE-HIGH VEP annotation model.



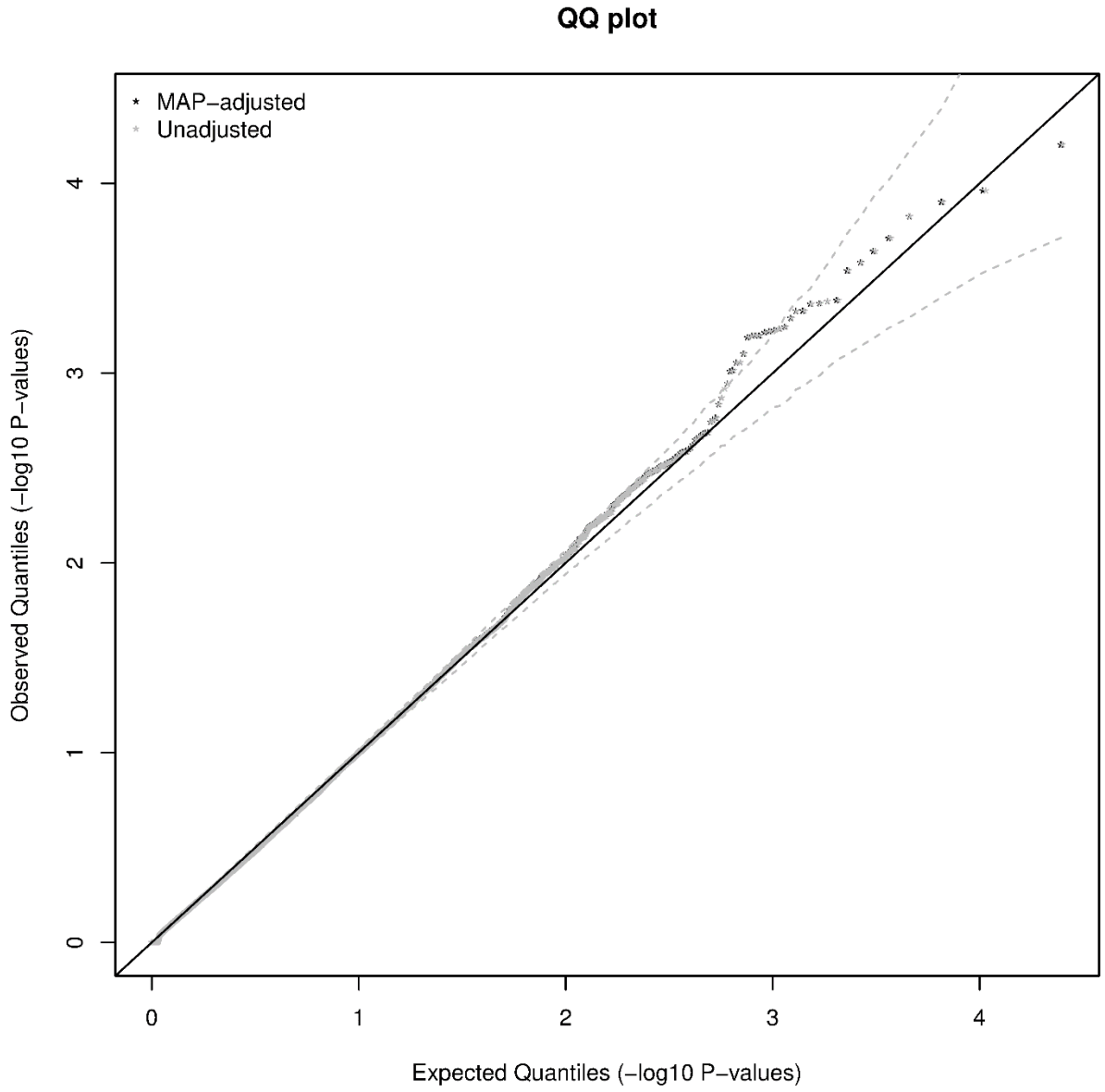
eFigure 2. Quantile-quantile plot for the moderate-high SKAT-O model in the WHICAP meta-analysis.



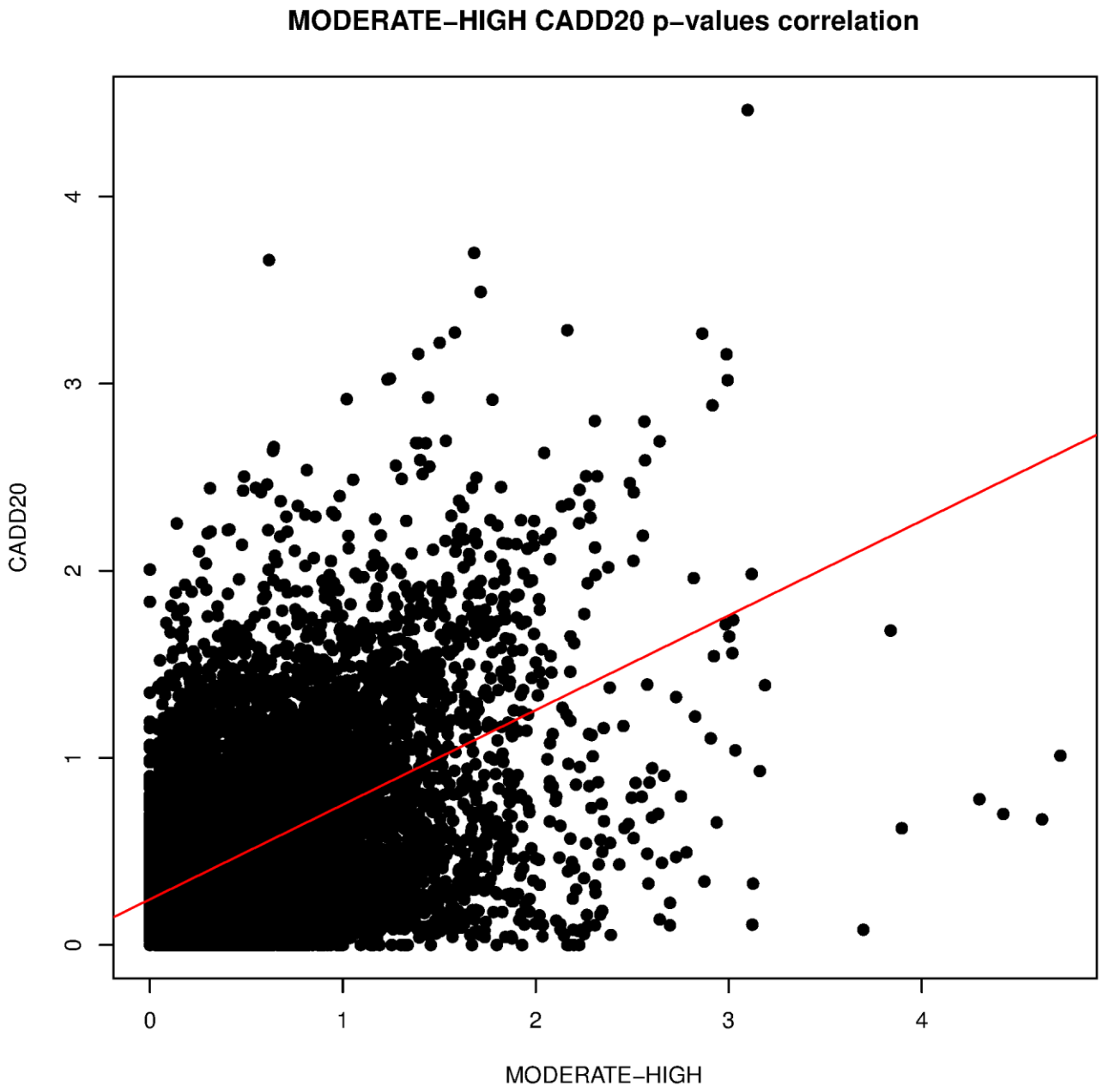
eFigure 3. Quantile-quantile plot for the moderate-high SKAT-O model in the ROS/MAP (unadjusted and adjusted by minimum achievable p-values – MAP).



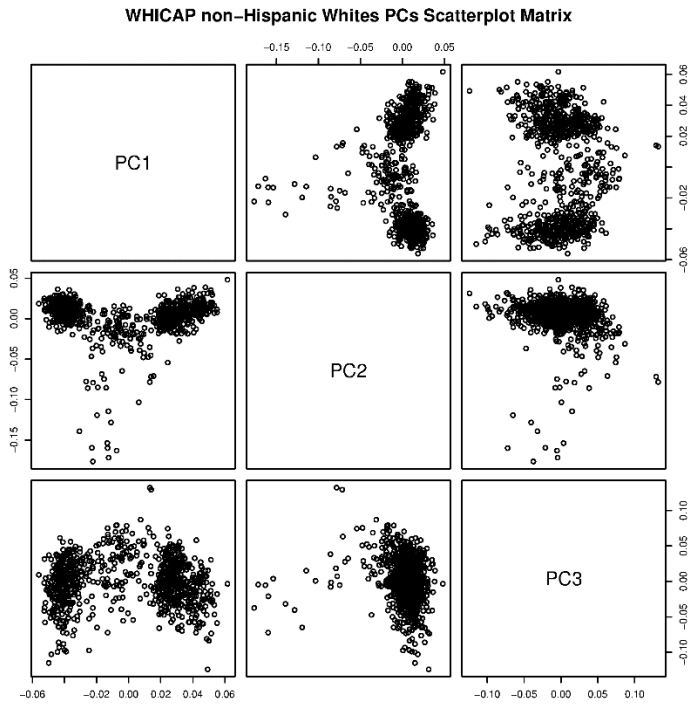
eFigure 4. Quantile-quantile plot for the moderate-high SKAT-O model in the ADSP (unadjusted and adjusted by minimum achievable p-values – MAP).

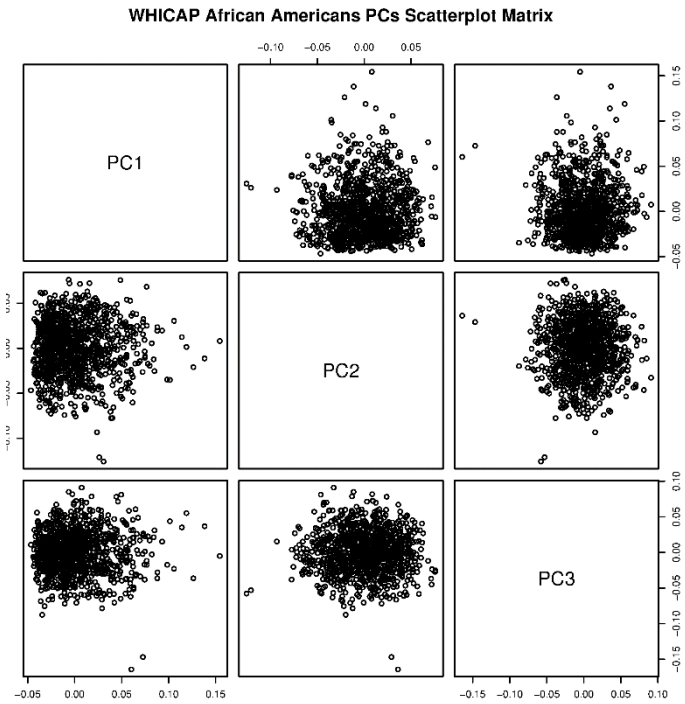


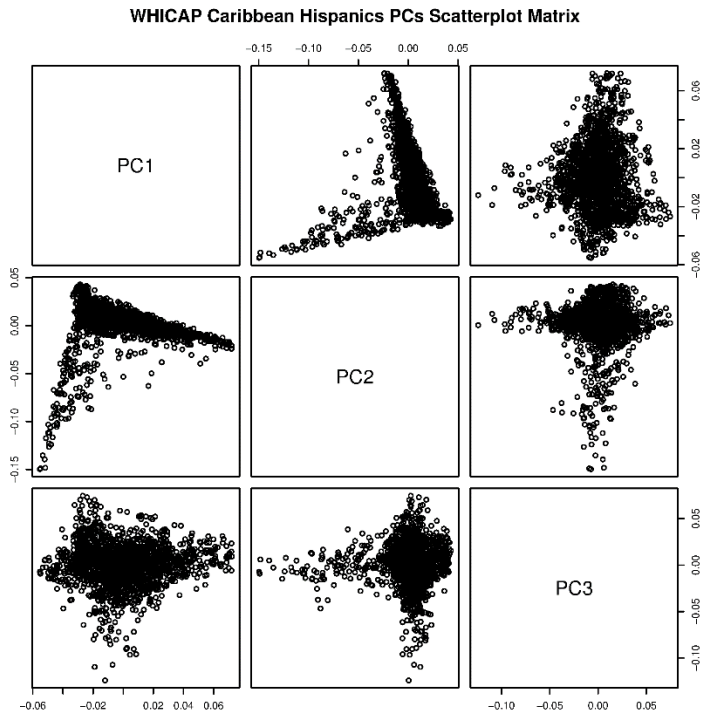
eFigure 5. Correlation between WHICAP meta-analysis SKAT-O p-values according to annotation models, i.e. VEP Moderate-high and CADD20 (Spearman coefficient was =0.49, p-values <0.001).



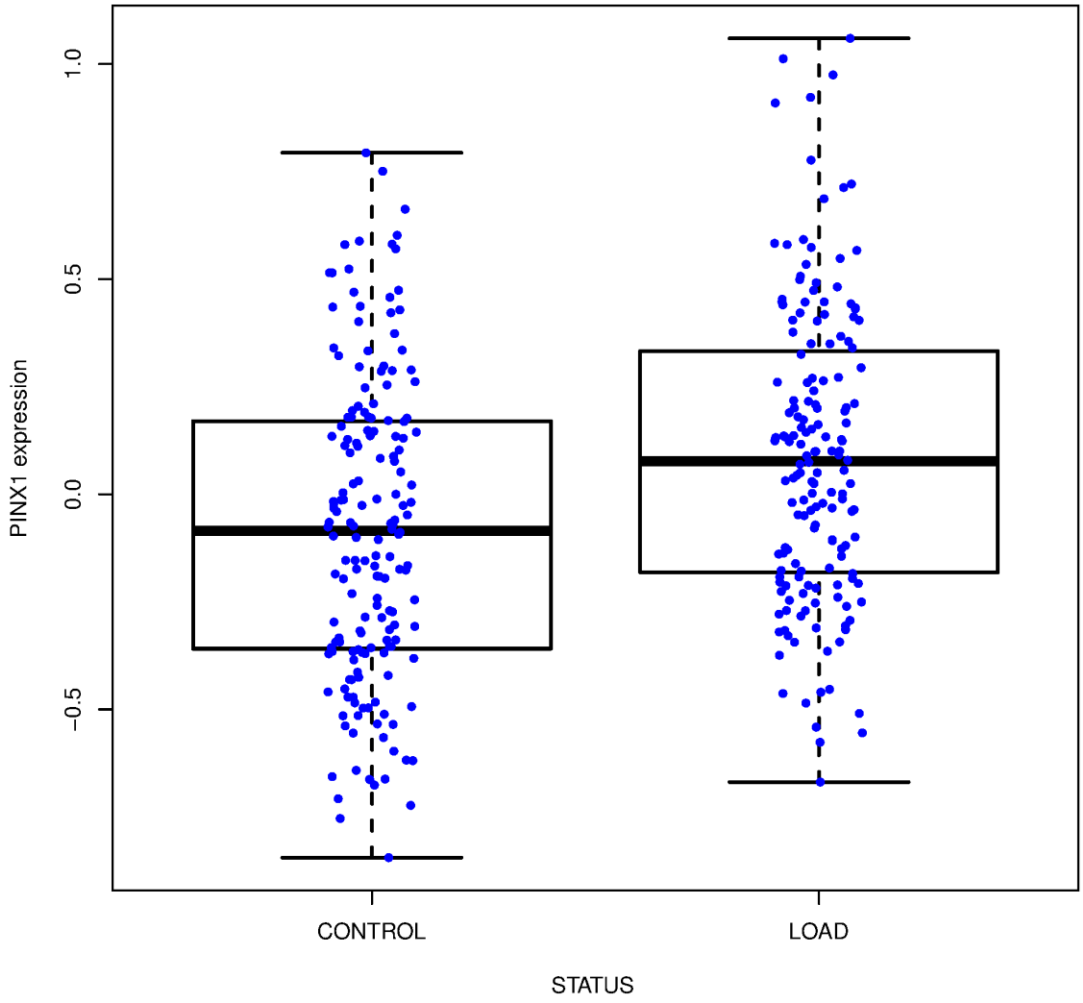
eFigure 6. Principal components (PC) scatterplot matrix for each ethnic group of the WHICAP dataset. We included the PCs #1,#2,#3 in each statistical model.







eFigure 7. Diffierential expression boxplot between LOAD cases and normal controls in Myers et al. dataset for *PINX1*. For LOAD brains, mean standardized pinx1 expression= 0.087; for control brains, mean standardized pinx1 expression= -0.085.



eTable 1. *PINX1* variants included in the VEP MODERATE-HIGH analyses. Table shows base pair (BP, minor allele frequency (MAF), missing rate, major and minor allele, p-value for single-marker analysis (in bold significant p-values) along with effect size (beta) and standard error (s.e.), CADD score. LoF variants are highlighted in red.

SNP	BP	MAF	Missing Rate	MajorAllele	MinorAllele	#SNP in controls	#SNP in cases	beta	s.e.	single-marker pvalue	CADD score	snp138
8:10622923:TTTC:T	10622923	0.006896552	0.000255363	TTTC	T	35	12	-0.788816595	0.306351562	**0.010027668**	.	rs201660183
8:10622930:T:C	10622930	0.004473415	0.00102145	T	C	17	12	0.2291413	0.401678461	0.568366073	17.38	rs61746595
8:10623000:T:TC	10623000	0.000255428	0.000255363	T	TC	0	2	1.918055022	1.43784195	0.182209815	.	.
8:10623027:G:C	10623027	0.000127845	0.001276813	G	C	0	1	2.100124255	2.005695283	0.29506249	0.852	.
8:10623094:C:G	10623094	0.000511117	0.000766088	C	G	2	2	0.204283648	1.01167179	0.83997395	18.77	rs369418829
8:10623104:C:T	10623104	0.000127779	0.000766088	C	T	1	0	-1.678203154	2.045461306	0.411958389	0.047	rs150489215
8:10623134:G:C	10623134	0.000383436	0.00102145	G	C	3	0	-1.721853739	1.183360734	0.14565432	0.002	rs377260866
8:10623135:C:T	10623135	0.000255493	0.000510725	C	T	0	1	1.964770358	2.006240402	0.327417212	5.588	rs202202690
8:10623137:CTCT:C	10623137	0.000127714	0.000255363	CTCT	C	0	1	7.080972646	2.872535284	**0.01369902**	.	.
8:10623141:T:A	10623141	0.000127714	0.000255363	T	A	0	1	7.080972646	2.872535284	**0.01369902**	37	rs201016513
8:10623203:GC:G	10623203	0.000127714	0.000255363	GC	G	1	0	-1.879564594	2.020813157	0.352317703	.	.
8:10623240:T:C	10623240	0.038825032	0.000255363	T	C	192	96	-0.315669347	0.131495673	**0.016367934**	0.001	rs17711777
8:10623254:C:A	10623254	0.01073071	0.000510725	C	A	52	27	-0.380986489	0.235443248	0.105626617	24.2	rs17855458
8:10623280:C:G	10623280	0.009200102	0.000766088	C	G	33	34	0.294594541	0.257680527	0.252932749	0.007	rs35530857
8:10623301:C:A	10623301	0	0.000510725	C	A	0	0	NA	NA	NA	10.82	rs202176931
8:10623309:C:T	10623309	0.000127747	0.000510725	C	T	1	0	-1.373771675	2.262806759	0.543778189	5.326	.
8:10623344:C:T	10623344	0.000127714	0.000255363	C	T	0	1	1.860406497	2.0077784	0.354134506	23.8	rs199675528
8:10623361:C:G	10623361	0.00038324	0.000510725	C	G	2	0	-1.743443751	1.513589574	0.249378482	21.8	rs375581032
8:10623379:G:C	10623379	0.000127714	0.000255363	G	C	0	1	2.218315869	2.014700316	0.270868398	4.485	.
8:10623383:G:T	10623383	0.00038324	0.000510725	G	T	3	0	-1.519737169	1.229890354	0.21658169	13.62	rs202049952
8:10623385:C:T	10623385	0.000638733	0.000510725	C	T	2	2	0.359249756	1.034425968	0.728370585	12.68	rs374083715
8:10623386:G:A	10623386	0.000127714	0.000255363	G	A	1	0	-1.826653777	2.013811482	0.364373511	14.1	rs377311809
8:10623393:C:T	10623393	0.000127714	0.000255363	C	T	1	0	-1.373771675	2.262806759	0.543778189	4.312	rs369880397
8:10623396:TCTC:T	10623396	0.000510856	0.000255363	TCTC	T	3	1	0.143017387	1.219164593	0.906616228	.	.
8:10623407:G:C	10623407	0.000127714	0.000255363	G	C	0	1	3.858862262	2.2865729	**0.091484525**	0.119	.
8:10623423:C:T	10623423	0.001021711	0.000255363	C	T	6	2	-0.508481321	0.809915074	0.530121508	6.407	rs200616748
8:10677710:G:A	10677710	0.000127812	0.00102145	G	A	0	1	2.160866408	2.016170191	0.283824313	13.07	rs189167078
8:10677806:G:C	10677806	0.000255624	0.00102145	G	C	1	1	0.279738036	1.493422653	0.851414938	.	rs368049075
8:10683689:G:T	10683689	0.000127812	0.00102145	G	T	1	0	-1.711168477	2.038053008	0.401127423	32	.
8:10683721:C:G	10683721	0.000127812	0.00102145	C	G	1	0	-1.82932458	2.013645853	0.363633174	26.9	.
8:10683752:A:G	10683752	0.000127812	0.00102145	A	G	0	1	2.370250715	2.030440971	0.243065983	15.88	.
8:10689208:C:T	10689208	0.000127747	0.000510725	C	T	0	1	1.863226453	2.01129488	0.354247733	24.8	rs201784803
8:10689232:C:A	10689232	0	0.000766088	C	A	0	0	NA	NA	NA	32	.
8:10690414:G:GGTA	10690414	0.000127714	0.000255363	G	GGTA	0	1	2.450764209	2.036470665	0.228807245	.	.
8:10690422:C:T	10690422	0.000127714	0.000255363	C	T	1	0	-1.361704771	2.277752881	0.549954612	33	rs377715499
8:10690474:T:G	10690474	0.001405571	0.000766088	T	G	5	5	0.909309109	0.712155695	0.201658626	25.4	rs189959562
8:10690476:G:T	10690476	0.000127779	0.000766088	G	T	1	0	-2.377636724	2.027578635	0.240936855	29	.
8:10692193:C:T	10692193	0.000127747	0.000510725	C	T	1	0	-1.963378374	2.005330672	0.327540645	34	.
8:10692229:C:T	10692229	0.000127714	0.000255363	C	T	1	0	-1.740323036	2.025811988	0.39029952	32	rs142521930
8:10692232:CA:C	10692232	0	0.000255363	CA	C	0	0	NA	NA	NA	.	.
8:10692283:G:A	10692283	0.000127747	0.000510725	G	A	1	0	-1.789379059	2.018317319	0.375310592	26.4	.
8:10697250:T:A	10697250	0.000127747	0.000510725	T	A	0	1	3.19360382	2.1627751	0.139776678	32	.
8:10697260:T:C	10697260	0.000255493	0.000510725	T	C	1	1	-0.016001327	1.4200209	0.991009329	23	rs200886591
8:10697266:T:A	10697266	0.000255493	0.000510725	T	A	0	2	1.991418163	1.418068329	0.160224327	24.4	.

Table 2. *TREM2* variants included in the CADD15/CADD20 analyses. Table shows base pair (BP, minor allele frequency (MAF), missing rate, major and minor allele, p-value for single-marker analysis along with effect size (beta) and standard error (s.e.), CADD score

SNP	MAF	Missing Rate	MajorAllele	MinorAllele	beta	se	single-marker pvalue	CADD score	ExAC_ALL	snp138
6:41127543:G:A	0.004984663	0.00102145	G	A	0.829210707	0.343249645	0.016	23.1	0.0036	rs2234255
6:41127561:C:T	0.000638733	0.000510725	C	T	0.271610307	0.994602461	0.78	23.2	0.0001	rs79011726
6:41129105:G:A	0.000894912	0.001276813	G	A	-0.070395503	0.884520054	0.94	24.3	0.0003	.
6:41130779:A:G	0.000255754	0.001532176	A	G	-2.145244892	1.430444422	0.13	23.6	.	.

eTable 3. Minor allele frequencies for *PINX1* variants in 1000G and ExAC databases ("_ALL"=cumulative, 1000g2015aug_afr= Africans from 1000 Genomes Project; 1000g2015aug_eur = Europeans from 1000 Genomes Project; ExAC_AFR= Africans/African Americans from Exome Aggregation Consortium; ExAC_NFE= Non-Finnish Europeans from Exome Aggregation Consortium).

VARIANT	1000g2015aug_all	1000g2015aug_eur	1000g2015aug_afr	ExAC_ALL	ExAC_AFR	ExAC_NFE
8:10622930:T:C	**0.000998403**	**.**	**0.0038**	**0.0012**	**0.0133**	**1.71E-05**
8:10623027:G:C	**.**	**.**	**.**	**.**	**.**	**.**
8:10623094:C:G	**0.000199681**	**.**	**0.0008**	**7.50E-05**	**0.0009**	**0**
8:10623104:C:T	**0.00259585**	**0.001**	**.**	**0.0024**	**0.0001**	**0.0011**
8:10623134:G:C	**.**	**.**	**.**	**3.33E-05**	**0.0004**	**0**
8:10623135:C:T	**.**	**.**	**.**	**5.83E-05**	**0.0006**	**0**
8:10623141:T:A	**.**	**.**	**.**	**0.0001**	**0.0001**	**0.0002**
8:10623240:T:C	**0.0319489**	**0.0487**	**0.003**	**0.0653**	**0.014**	**0.0624**
8:10623254:C:A	**0.0161741**	**0.0119**	**0.0015**	**0.0158**	**0.0034**	**0.0123**
8:10623280:C:G	**0.00738818**	**.**	**0.0272**	**0.002**	**0.023**	**1.50E-05**
8:10623301:C:A	**0.000199681**	**0.001**	**.**	**0.0001**	**0**	**0.0002**
8:10623309:C:T	**.**	**.**	**.**	**.**	**.**	**.**
8:10623344:C:T	**.**	**.**	**.**	**5.80E-05**	**0.0001**	**4.50E-05**
8:10623361:C:G	**.**	**.**	**.**	**4.97E-05**	**0.0003**	**0**
8:10623379:G:C	**.**	**.**	**.**	**4.14E-05**	**0**	**7.50E-05**
8:10623383:G:T	**0.000199681**	**.**	**0.0008**	**8.29E-05**	**0.001**	**0**
8:10623385:C:T	**.**	**.**	**.**	**0.0002**	**0.0021**	**1.50E-05**
8:10623386:G:A	**.**	**.**	**.**	**4.14E-05**	**0.0001**	**6.00E-05**
8:10623393:C:T	**.**	**.**	**.**	**1.66E-05**	**0.0002**	**0**
8:10623407:G:C	**.**	**.**	**.**	**.**	**.**	**.**
8:10623423:C:T	**0.000199681**	**.**	**.**	**0.0009**	**0.0001**	**0.0015**
8:10677710:G:A	**0.000399361**	**.**	**0.0015**	**6.96E-05**	**0.0007**	**0**
8:10677806:G:C	**.**	**.**	**.**	**3.84E-05**	**0.0004**	**0**
8:10683689:G:T	**.**	**.**	**.**	**2.10E-05**	**0**	**3.89E-05**
8:10683721:C:G	**.**	**.**	**.**	**1.05E-05**	**0**	**1.93E-05**
8:10683752:A:G	**.**	**.**	**.**	**1.25E-05**	**0.0001**	**0**
8:10689208:C:T	**0.000199681**	**.**	**.**	**8.82E-05**	**0**	**0.0001**
8:10689232:C:A	**.**	**.**	**.**	**3.30E-05**	**0.0002**	**3.11E-05**
8:10690422:C:T	**.**	**.**	**.**	**2.79E-05**	**0**	**3.29E-05**
8:10690474:T:G	**0.00159744**	**0.006**	**.**	**0.0054**	**0.0006**	**0.0084**
8:10690476:G:T	**.**	**.**	**.**	**.**	**.**	**.**
8:10692193:C:T	**.**	**.**	**.**	**.**	**.**	**.**
8:10692229:C:T	**0.000199681**	**0.001**	**.**	**1.66E-05**	**0**	**1.50E-05**
8:10692283:G:A	**.**	**.**	**.**	**3.40E-05**	**0**	**4.61E-05**
8:10697250:T:A	**.**	**.**	**.**	**1.64E-05**	**0**	**2.99E-05**
8:10697260:T:C	**0.000199681**	**.**	**.**	**6.27E-05**	**0.0004**	**2.85E-05**
8:10697266:T:A	**0.000199681**	**.**	**.**	**1.56E-05**	**0**	**0**
8:10622923:TTTC:T	**0.00778754**	**.**	**0.028**	**0.0026**	**0.0229**	**0.0007**
8:10623000:T:TC	**.**	**.**	**.**	**2.50E-05**	**0.0003**	**0**
8:10623137:CTCT:C	**.**	**.**	**.**	**0.0001**	**0.0001**	**0.0002**
8:10623203:GC:G	**.**	**.**	**.**	**.**	**.**	**.**
8:10623206:T:TAA	**.**	**.**	**.**	**.**	**.**	**.**
8:10623396:TCTC:T	**.**	**.**	**.**	**0.0001**	**0**	**7.50E-05**
8:10690414:G:GGTA	**.**	**.**	**.**	**.**	**.**	**.**
8:10692232:CA:C	**.**	**.**	**.**	**.**	**.**	**.**

eTable 4. LOAD known genes VEP MODERATE-HIGH SKAT-O results (Meta-analysis of WHICAP, ADSP, ROS/MAP). In bold genes that show a p-value<=0.05.

GENE	P.value (WHICAP-ADSP-ROS/MAP MODERATE-HIGH SKATO metanalysis)	symbol
ENSG00000095970	6.10E-05	*TREM2*
ENSG00000140090	0.00254	*SLC24A4*
ENSG00000166961	0.008017	*MS4A15*
ENSG00000137642	0.01175	*SORL1*
ENSG00000086288	0.01686	*NME8*
ENSG00000166928	0.01906	*MS4A14*
ENSG00000166927	0.02066	*MS4A7*
ENSG00000100599	0.0216	*RIN3*
ENSG00000108798	0.02523	*ABI3*
ENSG00000166926	0.06901	*MS4A6E*
ENSG00000130203	0.08161	*APOE*
ENSG00000110077	0.08168	*MS4A6A*
ENSG00000156738	0.09308	*MS4A1*
ENSG00000073921	0.09368	*PICALM*
ENSG00000116032	0.1076	*GRIN3B*
ENSG00000110079	0.1186	*MS4A4A*
ENSG00000105383	0.1314	*CD33*
ENSG00000203710	0.1514	*CR1*
ENSG00000064687	0.1788	*ABCA7*
ENSG00000172689	0.1795	*MS4A10*
ENSG00000183580	0.1854	*FBXL7*
ENSG00000149534	0.1936	*MS4A2*
ENSG00000046604	0.207	*DSG2*
ENSG00000120885	0.2243	*CLU*
ENSG00000073712	0.2399	*FERMT2*
ENSG00000198087	0.2796	*CD2AP*
ENSG00000081189	0.2809	*MEF2C*
ENSG00000197943	0.2825	*PLCG2*
ENSG00000182168	0.3067	*UNC5C*
ENSG00000168918	0.3277	*INPP5D*
ENSG00000166959	0.3386	*MS4A8*
ENSG00000204979	0.3583	*MS4A13*
ENSG00000149516	0.4002	*MS4A3*
ENSG00000149187	0.4011	*CELF1*
ENSG00000166930	0.4132	*MS4A5*
ENSG00000087589	0.4216	*CASS4*
ENSG00000071203	0.4274	*MS4A12*
ENSG00000120899	0.4524	*PTK2B*
ENSG00000078487	0.4723	*ZCWPW1*
ENSG00000136717	0.4788	*BIN1*
ENSG00000198502	0.526	*HLA-DRB5*
ENSG00000146904	0.6476	*EPHA1*
ENSG00000196126	0.7991	*HLA-DRB1*

eAppendix 4. Acknowledgment.

WHICAP and EFIGA
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