Novel Genetic Loci Associated with Retinal Microvascular Diameter

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Abstract

Background—There is increasing evidence that retinal microvascular diameters are associated with cardio- and cerebrovascular conditions. The shared genetic effects of these associations are currently unknown. The aim of this study was to increase our understanding of the genetic factors that mediate retinal vessel size.

Methods and Results—This study extends previous genome-wide association study results using 24,000+ multi-ethnic participants from 7 discovery and 5,000+ subjects of European ancestry from 2 replication cohorts. Using the Illumina HumanExome BeadChip, we investigate the association of single nucleotide polymorphisms (SNPs) and variants collectively across genes with summary measures of retinal vessel diameters, referred to as the central retinal venule equivalent (CRVE) and the central retinal arteriole equivalent (CRAE). We report 4 new loci associated with CRVE, one of which is also associated with CRAE. The 4 SNPs are rs7926971 in *TEAD1* ($p=3.1\times10^{-11}$, minor allele frequency (MAF)=0.43), rs201259422 in *TSPAN10* ($p=4.4\times10^{-9}$, MAF=0.27), rs5442 in *GNB3* ($p=7.0\times10^{-10}$, MAF=0.05) and rs1800407 in *OCA2* ($p=3.4\times10^{-8}$, MAF=0.05). The latter SNP, rs1800407, was also associated with CRAE ($p=6.5\times10^{-12}$). Results from the gene-based burden tests were null. In phenotype look-ups, SNP rs201255422 was associated with both systolic ($p=0.001$) and diastolic blood pressure ($p=8.3\times10^{-04}$).

Conclusions—Our study expands the understanding of genetic factors influencing the size of the retinal microvasculature. These findings may also provide insight into the relationship between retinal and systemic microvascular disease.

Keywords

genetics; microcirculation; meta-analysis; exome; retina

Microvascular disease, affecting blood vessels 100–300 μm in size, plays a significant role in many conditions such as diabetes, stroke, hypertension, coronary artery disease and cognitive decline. It is difficult to study the microvasculature in organs such as the heart and brain and it requires invasive methods. However, retinal venules and arterioles can be photographed non-invasively and their characteristics quantified using computer software.[1] In addition, the physiology and embryology of the brain and retinal microvasculature is similar.[2] Since evidence suggests changes in retinal vessels may provide an indirect indicator of similar changes in the brain,[3] heart[4] and kidneys,[5] a better understanding of the

genes associated the retinal blood vessels may provide insight into microvascular morbidity elsewhere in the body.

Current evidence suggests retinal vessel diameters are associated with a wide array of diseases, reflecting the role and contribution of microvascular disease processes in these conditions.[6–8] For instance, narrower retinal arterioles is a sign of hypertension and remarkably, the association between blood pressure and smaller retinal arteriole diameter can be seen with current,[9] past[10] and incident hypertension.[11]

Based on previous studies, retinal venule diameter is associated with different pathophysiologic processes than retinal arterioles. Wider retinal venules are associated with hyperglycemia,[12] dyslipidemia,[13] measures of inflammation,[13] obesity,[14] stroke,[15] subclinical cerebrovascular disease,[16] coronary heart disease,[15] cardiovascular mortality in younger participants,[17] cognitive impairment[18] and renal dysfunction in diabetics.[19]

Even as the evidence that retinal microvascular diameter is a marker of more generalized microvascular changes in other organs increases, our understanding of the genetic factors that control retinal vessel size is limited. Previous studies have shown retinal vessel diameters are more highly correlated among related than unrelated individuals. These values range from 0.23 (95% CI: 0.16, 0.31) in retinal venule diameter and 0.20 (95% CI: 0.12, 0.28) in retinal arteriole diameter of siblings compared with 0.03 and 0.04 in spouses.[20] In a twin study, the heritability of retinal vessels diameters was estimated at 83% (95% CI: 73%– 89%) for retinal venule diameter and 70% (95% CI: 54%–80%) for retinal arteriole diameter.[21] A genome-wide linkage study estimated the heritability at 48–51% for retinal vessel diameter[22] and showed evidence that linkage regions overlapped with regions previously associated with hypertension, the eNOS-related pathway, coronary heart disease and vasculogenesis. Results from our first genome-wide association studies found evidence for four novel loci associated with retinal venule diameter[23] and one associated with retinal arteriole diameter.[24] These studies only included Caucasians and were both smaller with 15,358 and 18,722 participants respectively.

Nevertheless, much of the heritability of retinal vessel diameters remains to be explained. It is theorized that rare single nucleotide polymorphisms (SNPs), those with a minor allele frequency (MAF) < 1%, account for some of this unexplained heritability. Recently, the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium cohorts were genotyped with the Illumina HumanExome BeadChip.[25] In this study we combine 24,000+ participants from four ethnic groups (Caucasians, African-Americans, Asians and Hispanics) to investigate the association of single-variant SNPs and SNP variants collectively across genes with summary measures of retinal venule and arteriole diameters, referred to as the central retinal venule equivalent (CRVE) and the central retinal arteriole equivalent (CRAE).

Methods

Participants

The discovery phase included four members of the CHARGE Consortium;[26] the Age, Gene, Environment, Susceptibility Study – Reykjavik (AGES),[27] the Atherosclerosis Risk in Communities Study (ARIC),[28] the Cardiovascular Health Study (CHS)[29] and the Multi-Ethnic Study of Atherosclerosis (MESA).[30] Three additional Asian cohorts also contributed data, the Singapore Chinese Eye Study (SCES),[31], the Singapore Malay Eye Study (SiMES)[32] and the Singapore Indian Eye Study (SINDI).[31] The overall mix of ethnic backgrounds included European ancestry (60%), African Americans (14%), Asians (21%) and Hispanics (4%), Table 1.

The replication cohorts included the Rotterdam Study (RS)[33] and a subset of the Blue Mountain Eye Study (BMES)[34] that had exome chip data. Additional details of each participating study are provided in the Data Supplement. All participating cohorts secured approval from their respective institutional review boards, and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Retinal Phenotypes

The central retinal venule equivalent (CRVE) and central retinal arteriole equivalent (CRAE) are summary measures of the six largest retinal microvascular diameters. Standardized protocols and software were developed at the University of Wisconsin.[35] Retinal photographs were centered on the optic nerve. Photographs were obtained through pharmacologically dilated (AGES, BMES, RS) or un-dilated pupils of one (ARIC, CHS) or both eyes. Photographs were digitized using a high-resolution scanner (ARIC, CHS, RS) or digitally captured. All digital retinal images were analyzed with a semi-automated measurement system and the calibers of all retinal venules and arterioles were measured in the area located between half and one disc-diameter from the optic disc margin. Trunk vessels were measured using the Parr-Hubbard-Knudtson formulas[36] and measures are expressed in micrometers (μm).

Exome Chip

Cohort participants (AGES, ARIC, CHS, MESA, RS, SCES, SINDI) were genotyped with the Illumina HumanExome BeadChip v1.0 (Illumina, Inc., San Diego, CA) and v1.1 for SiMES. It covers putative functional exonic variants selected from over 12,000 individual exome and whole-genome sequences. It consists of 247,870 markers comprised primarily of 219,621 non-synonymous single nucleotide polymorphisms (SNPs) representing European, African, Chinese and Hispanic populations. Additional markers include splice-site variation, stop codons, promoter region SNPs, GWAS tag markers, ancestry informative markers, X / Y / mitochondrial variants, SNPs in the extended major histocompatibility complex region, human leukocyte antigen tag variants, identical by descent markers and insertion-deletions. (Exome-array design: http://genome.sph.umich.edu/wiki/Exome_Chip_Design)

Genotyping and Quality Control

Approximately 62,000 ethnically diverse samples from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium were genotyped with the Illumina HumanExome BeadChip. The raw data files for the samples were assembled into a single project for joint calling and are described in detail elsewhere.[25] Briefly, Illumina's GenTrain version 2.0 clustering algorithm in GenomeStudio or zCall[37] was used for genotype calling. A total of 8,997 variants failed quality control measures and were eliminated from all analyses. Additional details regarding genotyping and QC performed centrally and by each study are summarized in the Data Supplement. Annotation of the v1.0 exome chip variants are expressed with dbNSFP v2.0.

Statistical Analysis

The discovery phase included single variant tests and gene-based association analyses in which CRVE and CRAE were analyzed separately. Where applicable, each of the seven cohorts, (AGES, ARIC, CHS, MESA, SCES, SiMES and SINDI) analyzed their participants by ethnicity, Caucasian, African-American, Asian or Hispanic. This resulted in 12 ethnic specific groups that were combined for meta-analysis to elucidate any ethnic specific differences in the results. For the single variant tests, genome wide significance was set at $p=2.0\times10^{-7}$ (0.05/~250,000 variants). Significance for the gene-based tests was set at 2.5×10^{-6} (0.05/20,000 genes). To validate findings from the discovery process, a replication phase was performed using two independent cohorts (BMES and RS). Replication was considered significant at $p<0.05$ and the direction of the effect was consistent with discovery.

Diabetes was defined as fasting blood glucose 126 mg/dL [7.0 mmol/L], current use of insulin or other hypoglycemic agent. If fasting blood glucose was not available then a random glucose>198 mg/dL [11.0 mmol/L] was used. Hypertension was defined as current use of blood pressure medication or systolic/diastolic blood pressure (SBP/DBP) 140/90 mm Hg.

Residuals were analyzed because results using untransformed CRVE & CRAE led to inflation of our findings due to extreme phenotypes in the adjusted analysis and small minor-allele frequencies of many of the SNPs, Figures 1SA & 2SA. Residuals were derived by linear regression for each outcome adjusting for age (years), sex (male–yes/no), BMI (kg/m^2), SBP (Hg mm), diabetes (yes/no), hypertension (yes/no), the first 5 ethnic and study-specific principle components and study site (where applicable). An inverse normal transformation of the residuals was performed. Each cohort then analyzed the transformed residuals using the R package seqMeta or SkatMeta (http://www.r-project.org) with linear regression adjusting for the SNP using an additive genetic model, the first 5 ethnic and study-specific principle components and study site. Adjusting for population stratification in the calculation of the residuals alone would not be enough to correct for any similar stratification which may occur in our primary analysis induced by the inclusion of SNP data. Model based standard errors were used in the analysis.

Inverse variance weighted fixed-effects meta-analysis was performed by ethnic group for single variant tests and gene-based association analyses including the Sequence Kernel Association Test (SKAT)[38] and the T1 Count Test[39] using the R package seqMeta. Gene-based burden tests were included to aggregate evidence from multiple rare/low-frequency variants. We used a minor allele frequency threshold of 0.01 in the T1 Test and 0.05 in the SKAT Test.

The Data Supplement contains additional information on the choice of p-value for replication, choice of minor allele frequency threshold for the burden tests, information regarding forest and regional association plots, and details of the the *in-silico* algorithms used to predict whether SNPs would be damaging.

Rotterdam did not genotype all of their subjects using the exome chip. For those that were not genotyped they imputed the SNPs for the remaining subjects. For clarity we analyzed the two groups separately. Details of the imputation are supplied in the Data Supplement. Replication of gene based results was carried out using Rotterdam data only.

The CHARGE Exome Chip Blood Pressure Consortium provided phenotype look-ups for 4 newly and 4 previously identified SNPs associated with CRVE or CRAE. Blood pressure phenotypes included SBP, DBP and hypertension.

Results

Table 1 displays the demographic and covariate characteristics stratified by ethnic group within participating cohorts where available. A total of 24,342 participants had measures of CRVE and 24,345 had measures of CRAE. After marker-level and sample-level quality control 223,566 variants were available for analyses of CRVE and 240,931 for CRAE.

Table 2 contains the results from the discovery phase of our meta-analysis for the single variant tests. Three CRVE SNPs reached exome-wide significance, rs7926971 ($p=7.0\times10^{-9}$) in *TEAD1*, rs201259422 ($p=8.0\times10^{-9}$) in *TSPAN10* and rs5442 ($p=5.8\times10^{-8}$) in *GNB3*. Two other single SNPs of interest were carried forward for replication, rs1800407 ($p=2.1x\ 10^{-7}$) in *OCA2* and rs2306765 ($p=6.9\times10^{-6}$) in *GSG1*. One CRAE SNP reached exome-wide significance, rs1800407 ($p=4.5\times10^{-10}$) in *OCA2*. During replication, 3 CRVE SNPs (rs7926971 in *TEAD1,* rs5442 in *GNB3,* rs1800407 in *OCA2*) and 1 CRAE SNP (rs1800407 in *OCA2)* all reached nominal significance ($p<0.05$) or better. SNPs rs201259422 in *TSPAN10* and rs2306765 in *GSG1* did not replicate, but the direction and effect size for rs201259422 in *TSPAN10* was consistent in the replication sample, and overall increased the statistical significance in the combined sample. Figures 1 – 6, show the regional association plots for each of the SNPs carried forward for replication. No additional ancestry specific SNPs reached exome-wide significance for CRVE or CRAE.

Data Supplement Table 1S contains a more detailed summary of the top SNPs associated with CRVE including the four SNPs we have previously reported[23] and the other SNPs in high linkage disequilibrium with those four SNPs. Similarly, Data Supplement Table 2S contains a more detailed summary of the top SNPs associated with CRAE including a SNP in high linkage disequilibrium with the SNP we have previously reported.[24] All of the

previously reported GWAS findings reached exome-wide significance in the present study. These tables include the annotation of the v1.0 exome chip variants.

Results from the gene-based T1 burden tests were null for both CRVE and CRAE. In the SKAT test, *GSG1* was associated with CRVE (p=1.3×10⁻⁶) and *OCA2* was associated with CRAE (p=2.1×10⁻⁸). In *GSG1*, there were 9 SNPs with cumulative minor allele frequency (MAF) of 0.06. We repeated gene-based testing by excluding rs2306765 after which the gene was not associated with CRVE (cumulative MAF of 0.01 and p=0.01). For CRAE in *OCA2*, there were 38 SNPs included with cumulative MAF of 0.09. Similarly, the gene was not associated with CRAE after excluding rs1800407 (cumulative MAF of 0.04 and p=0.03). Neither gene, *GSG1* for CRVE or *OCA2* in CRAE reached nominal significance in replication.

Data Supplement Figures 3S – 8S show the direction of effects across all studies. These Figures showed no overall heterogeneity in the meta-analysis results any of the SNPs There was evidence the effect size in MESA Hispanics for rs7926971 (*TEAD1*) differed from other ethnic groups (p=0.01), Figure 3S, and that the effect size in MESA Asians for rs201259422 (*TSPAN10*) differed from other Asian cohorts (p=0.02), Figure 4S.

A look-up of the 4 newly and 4 previously identified SNPs associated with CRVE or CRAE was performed by the CHARGE Exome Chip Blood Pressure Consortium, Table 3S. Of the 8 SNPs, 5 SNPs were either associated with SBP, DBP, hypertension or a combination of the three phenotypes. However, after correction for multiple testing, (p=0.05/24=0.002), only 2 SNPs remained associated with blood pressure phenotypes, rs201259422 in *TSPAN10* and rs10774625 in *ATXN2*.

Discussion

Our collaboration has identified four new SNPs associated with retinal venule diameter and one new SNP associated with retinal arteriole diameter bringing the total to eight SNPs now associated with venule diameter and two with arteriole diameter. After adjusting for age, sex, BMI, diabetes (Y/N), hypertension (Y/N) and SBP, the new variants explained 1% of the total variability in CRVE (4 SNPs) and <½% for CRAE (1 SNP). This increased to 2% for CRVE (8 SNPs) and ½% for CRAE (2 SNPs) with all known variants.

Four SNPs identified in our discovery cohort showed evidence of replication in our study; three SNPs for CRVE in *TEAD1, GNB3* and *OCA2* and one SNP for CRAE in *OCA2*. The SNP in *TSPAN10* for CRVE did not replicate, but the direction of the association and the combined discovery and replication results provide some support for its association with CRVE. Our inability to replicate outright may have been due in part to the small size of the replication cohort and smaller effect sizes compared to those seen in some of the other SNPs. The SNP in *GSG1* did show a significant p-value in replication but the association was in the opposite direction from what was observed in discovery. It is of note; none of the new findings were low frequency SNPs. The SNPs in *TSPAN10* and *OCA2* were not genotyped or imputed in our previous GWAS. The SNP in *TEAD1* was included as an imputed SNP. The SNP in *GNB3* was genotyped but results did not pass quality control. It is

unclear if genotyping, covariate adjustment, smaller effect sizes or a combination of these factors resulted in the conflicting results in our GWAS and the present study.

SNP rs5442 in *GNB3* is a predicted damaging non-synonymous SNP on chromosome 12. Each copy of the minor allele decreased CRVE 2.3 ± 0.4 μm. (This decrease and the changes cited below are the observed effects on CRVE and CRAE in the untransformed analyses, not the beta and standard errors from the inverse-quantile normalized traits.) Heterotrimeric guanine nucleotide-binding proteins (G proteins), integrate signals between receptors and effector proteins, are composed of an alpha, a beta, and a gamma subunit and are expressed in all tissues.[40] A separate SNP (C825T) in this gene is associated with essential hypertension, obesity and dyspepsia.[40, 41]

SNP rs1800407 in *OCA2* is a predicted damaging non-synonymous SNP on chromosome 15. Each copy of the minor allele decreased CRAE and CRVE, 1.7 ± 0.3 and 2.2 ± 0.4 μm respectively. *OCA2* encodes the human homolog of the mouse p (pink-eyed dilution) gene.[42] The encoded protein is believed to be an integral membrane protein involved in small molecule transport, specifically tyrosine, which is a precursor to melanin synthesis. It is involved in mammalian pigmentation, where it may control skin color variation and act as a determinant of brown or blue eye color. Mutations in this gene result in type 2 oculocutaneous albinism.[43]

SNP rs7926971 in *TEAD1* is an intronic SNP on chromosome 11. Each copy of the minor allele increased CRVE 0.5 ± 0.1 μm. TEAD1 encodes a ubiquitous transcriptional enhancer factor that is a member of the TEA/ATTS domain family. This protein directs the transactivation of a wide variety of genes and, in placental cells, also acts as a transcriptional repressor. Mutations in this gene cause Sveinsson's chorioretinal atrophy.[44] The expression of TEAD1 is significantly induced during smooth muscle cell phenotypic modulation and negatively correlates with smooth muscle-specific gene expression. Mechanistically, TEAD1 competes with myocardin for binding to serum response factor (SRF), resulting in disruption of myocardin and SRF interactions and thereby attenuating expression of smooth muscle-specific genes.[45]

SNP rs201259422 in *TSPAN10 (*tetraspanin 10) is a predicted damaging SNP on chromosome 17. Each copy of the minor allele decreased CRVE 1.0 ± 0.2 μm. *TSPAN10* encodes a transcript that is expressed in human RPE & choroid.[46] Tetraspanins are widespread, numerous and largely mysterious in function but are involved in diverse processes such as cell activation and proliferation, adhesion and motility, differentiation, and cancer.[47] Two related tetraspan proteins of the retina, *ROM1* and *RDS*, are both involved in retinal degenerations.[48]

The SNP in *OCA2* and the previously reported SNP rs17421627 in *LINC00461* adjacent to *MEF2C*[23, 24] represent two SNPs now that are associated with both CRAE & CRVE. None of the other previously reported SNPs associated with CRVE were associated with CRAE in our data, nor was the SNP in *TEAD1*, Table 1S. The associations between CRAE and the SNPs in *TSPAN10* and *GNB3* are weaker than for CRVE but not trivial. Despite the strong correlation between CRVE and CRAE it appears that not only is retinal venule diameter

associated with different pathophysiologic processes than retinal arterioles but that the genetic control of retinal venules and retinal arterioles is not identical either.

It is unknown why more genetic loci have been identified for CRVE than CRAE. CRVE has slightly less measurement error than CRAE[35] but is not likely the major reason. Second, hypertension and medications to treat hypertension influence retinal arterioles (CRAE) more than venules (CRVE). Consequently, we adjusted for both systolic blood pressure and medication use in our analyses. Finally, there are anatomical differences in the two types of vessels. Unlike retinal venules, retinal arterioles auto-regulate blood flow in response to changes in perfusion pressure and like the cerebral vasculature are largely independent of extrinsic neurogenic factors.[49] This may make genetic effects on CRAE smaller than the corresponding effects on CRVE and thus may take larger sample sizes to detect, for instance see rs20125942 (*TSPAN10*) in Table 3S.

Two of the eight SNPs associated with CRVE or CRAE were associated with blood pressure phenotypes, rs201259422 on *TSPAN10* and rs10774325 on *ATXN2*. SNP rs10774625 (*ATXN2*) was previously reported to be associated with blood pressure.[50] However, to our knowledge this is the first reported association of rs201259422 (*TSPAN10*) with blood pressure phenotypes. Current thinking is that wider retinal venules and narrower retinal arterioles are associated with increased risk of hypertension,[51] but that relationship does not hold for rs201259422 (*TSPAN10*) & rs10774625 (*ATXN2*) in our analyses, Table 3S. Other SNPs, like rs1800407 (*OCA2*) and rs17421627 (*MEF2C*) had relatively large effects on retinal vessel caliber. However, there was no obvious relationship with blood pressure phenotypes. These findings may reflect developmental differences independent of cumulative or secondary effects occurring over an individual's lifetime. SNPs rs7926971 (*TEAD1*) and rs2287921 (*RASIP1*) show an association with CRVE but not CRAE or blood pressure phenotypes. The significance of these finding remains to be determined.

Strengths of this study include a large sample size, an ethnically diverse population-based sample, quantitative phenotypes assessed in a similar manner, and genotyping performed on the exome array enriched for coding variants with many cohorts involved in joint calling. Weaknesses include a small replication cohort, cross-sectional measures and a phenotype that is a summary measure of the six largest retinal arteriole and venule diameters.

In summary, this study increases our understanding about genes associated with retinal venule and arteriole diameter. However, considerable work remains to be done to determine how these genes act, if their effects vary over time, whether these genes interact or are subject to epigenetic factors and ultimately whether these genes contribute to the risk of microvascular disease in other organs or are associated with major conditions such as diabetes, stroke, hypertension, coronary disease and cognitive decline.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Clinical Perspective

Microvascular disease, affecting blood vessels 100–300 μm in size, plays a significant role in many conditions such as diabetes, stroke, hypertension, coronary artery disease and cognitive decline. It is difficult to study the microvasculature in organs such as the heart and brain and it requires invasive methods. However, retinal venules and arterioles can be photographed non-invasively and their characteristics quantified using computer software. In addition, the physiology and embryology of the brain and retinal microvasculature is similar. Since evidence suggests changes in retinal vessels may provide an indirect indicator of similar changes in the brain, heart and kidneys, a better understanding of the genes associated the retinal blood vessels may provide insight into microvascular morbidity elsewhere in the body.
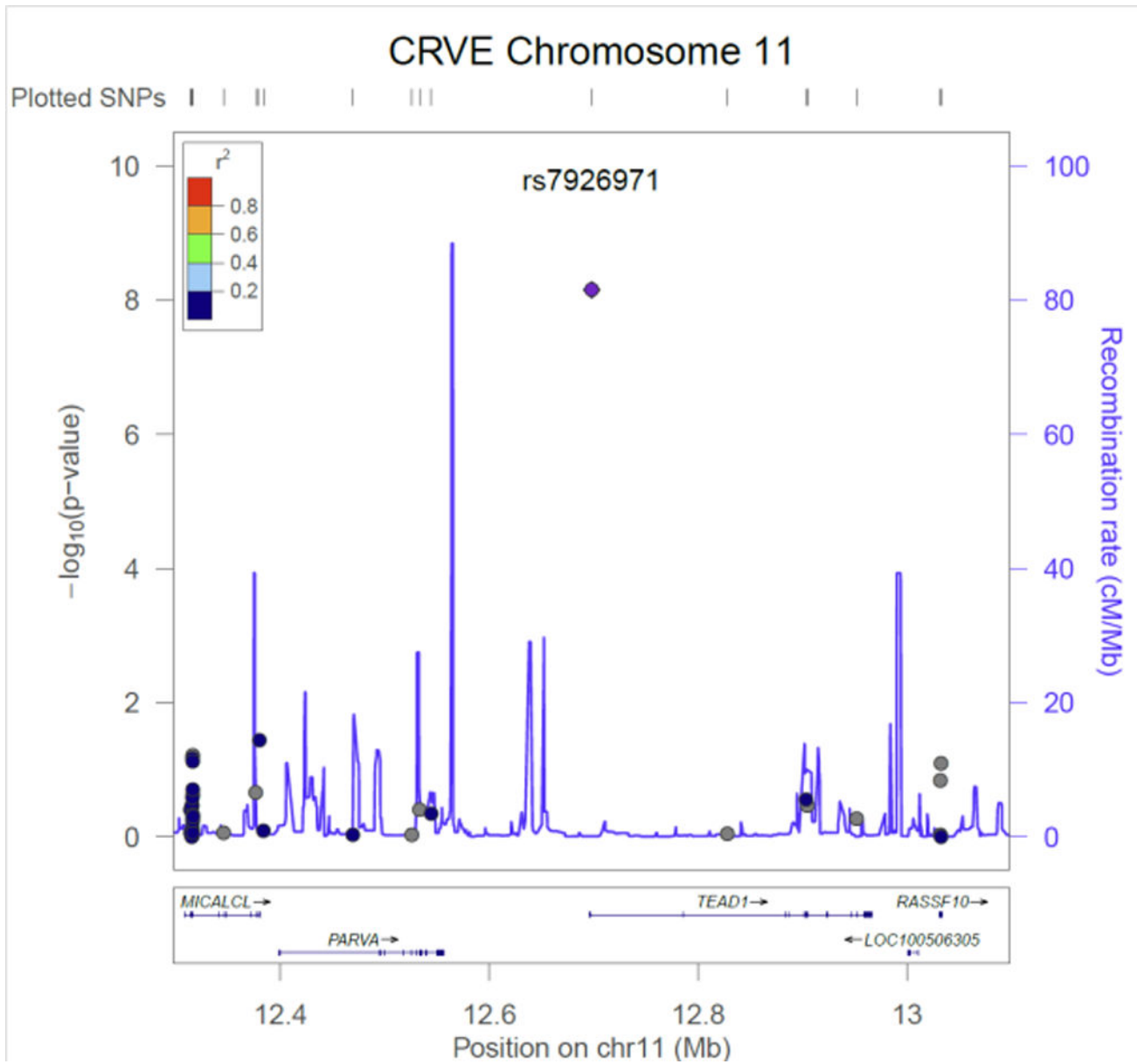
Figure 1.
CRVE regional association plot for SNP rs7926971 in *TEAD1*.

Figure 2.
CRVE regional association plot for SNP rs201259422in *TSPAN10*.

Figure 3.
CRVE regional association plot for SNP rs5442in *GNB3*.

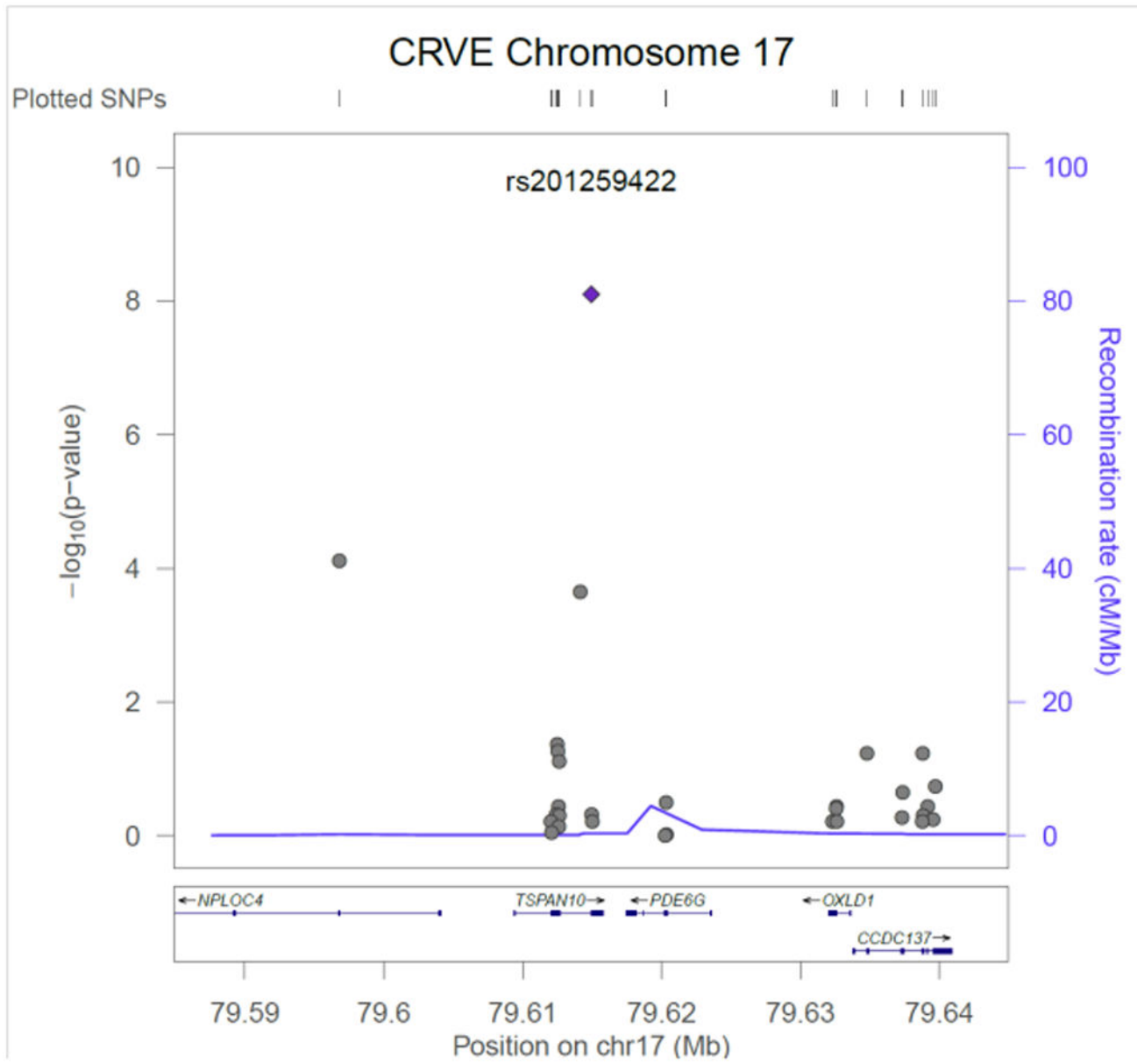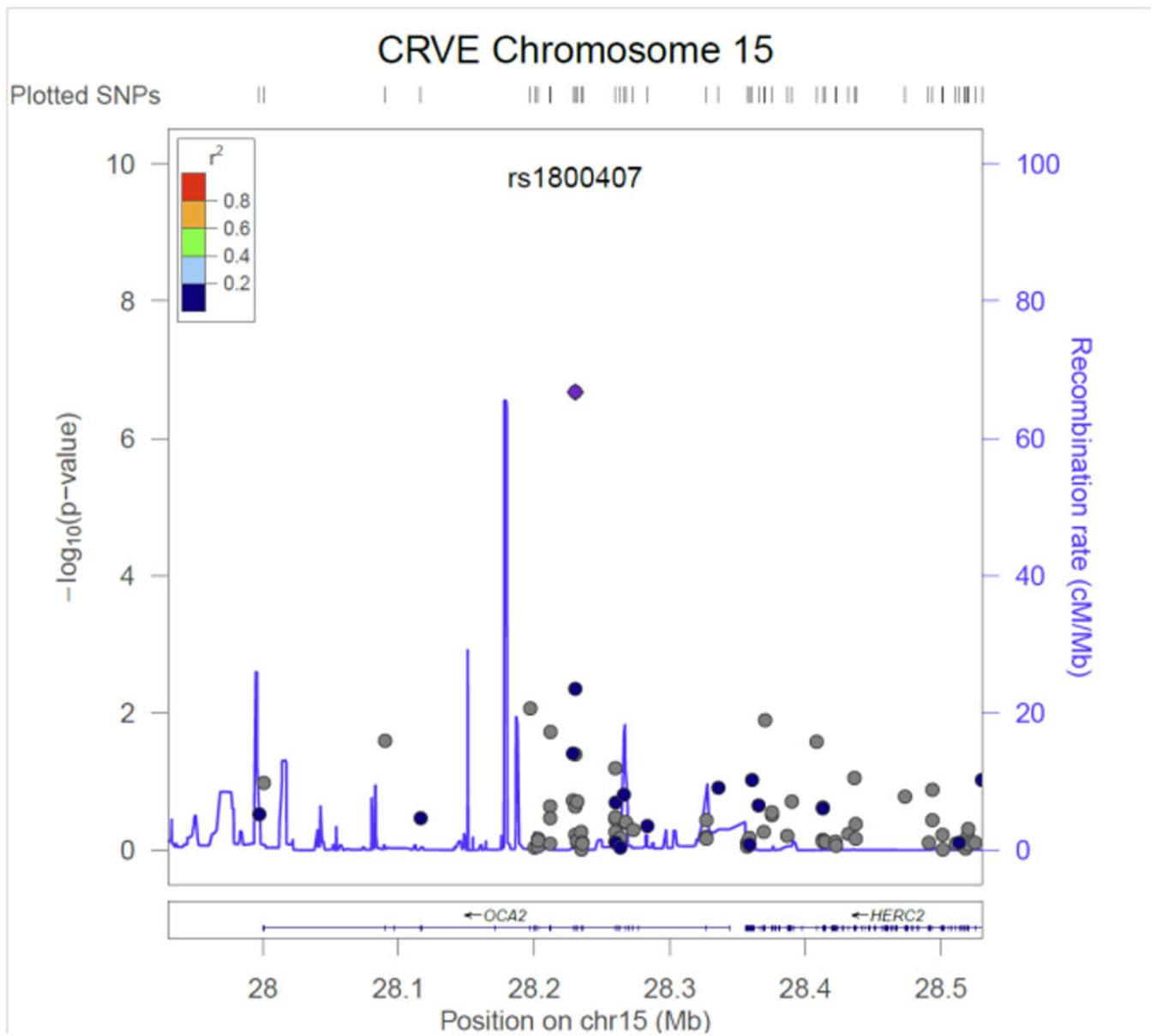**Figure 4.**
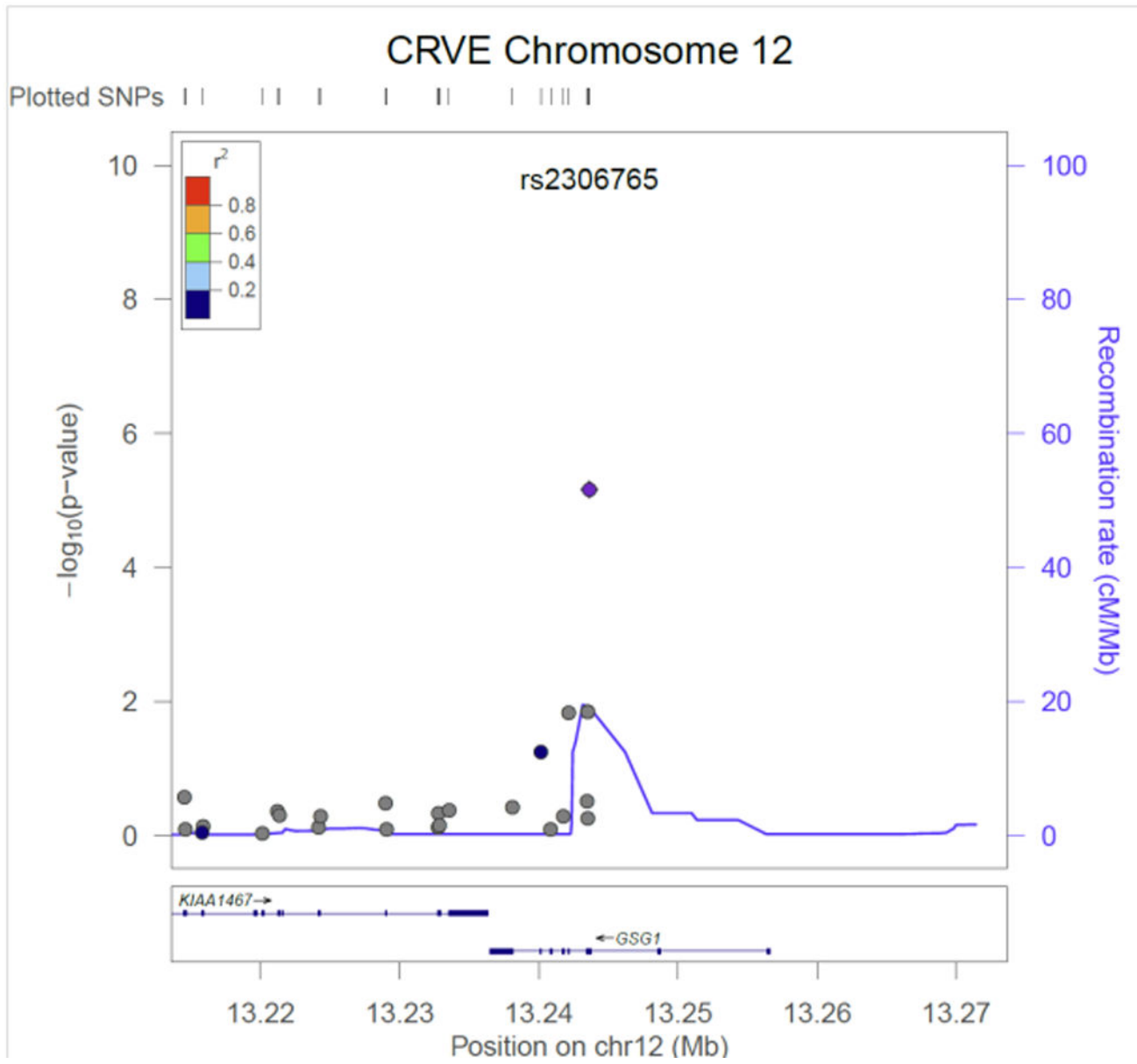CRVE regional association plot for SNP rs1800407in *OCA2*.

Figure 5.
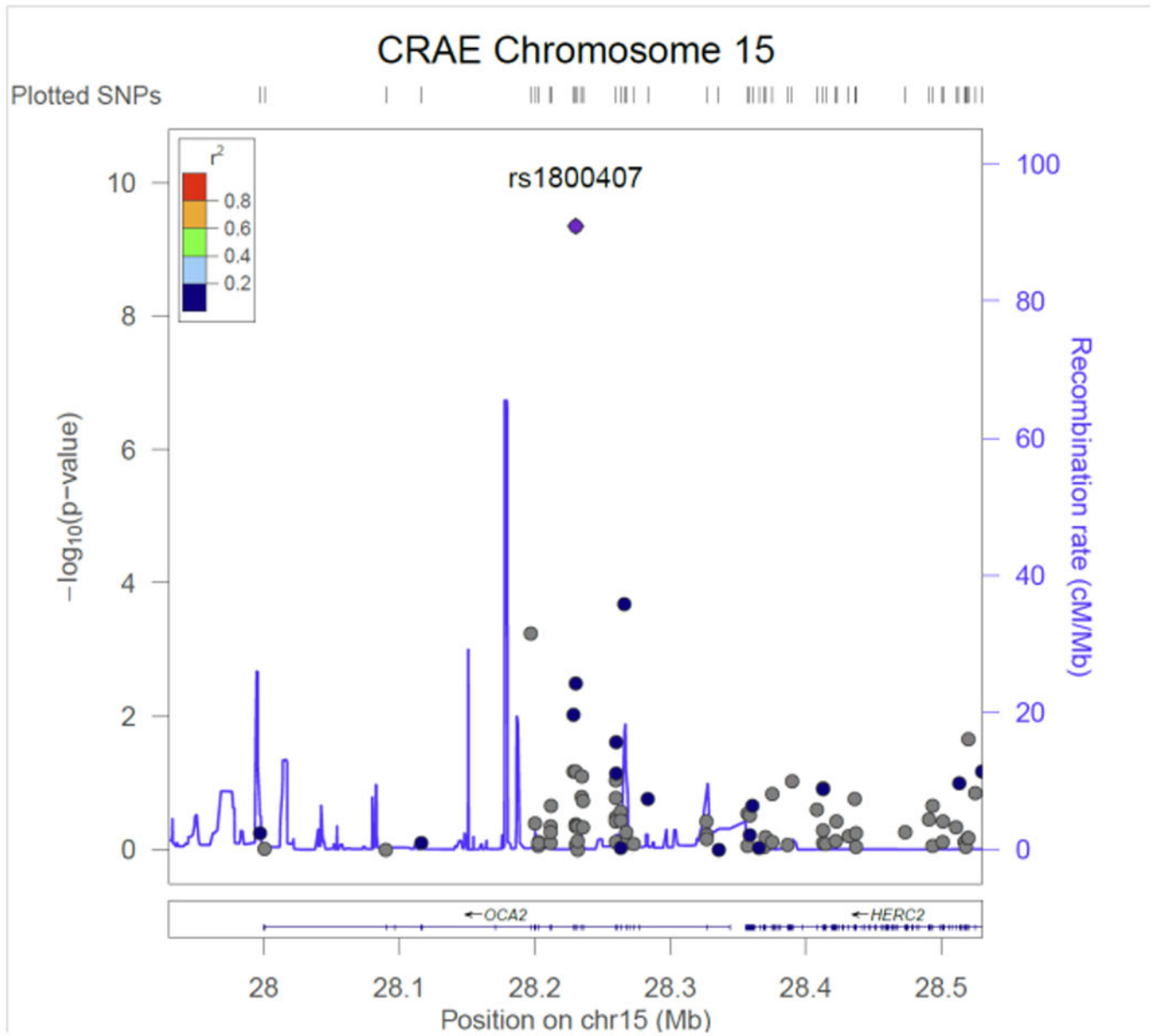CRVE regional association plot for SNP rs2306765 in *GSG1*.

Figure 6.
CRAE regional association plot for SNP rs1800407in *OCA2*.

Table 1

Description of Cohorts

Discovery

Ethnicity	Cohort	n	CRAE (μm)	CRVE (μm)	Age (years)	SBP (mm Hg)	BMI (kg/m²)	FG (mmol/l)	Sex (% male)	HTN (%)	DM (%)
White	AGES	2,755	140 ± 14	202 ± 20	76 ± 5	143 ± 20	27 ± 4	5.8 ± 1.1	43	81	11
N=14,740	ARIC	8,377	135 ± 13	199 ± 19	60 ± 6	122 ± 18	28 ± 5	6.0 ± 1.8	46	40	12
	CHS	1,472	139 ± 14	197 ± 19	79 ± 4	134 ± 21	27 ± 4	5.6 ± 1.6	41	60	14
	MESA	2,136	143 ± 14	207 ± 21	64 ± 10	124 ± 21	28 ± 5	5.3 ± 1.1	49	39	8
AA	ARIC	1,848	137 ± 12	209 ± 19	59 ± 6	130 ± 20	30 ± 7	6.8 ± 3.3	37	61	23
N=3,345	CHS	287	140 ± 13	207 ± 21	76 ± 4	136 ± 22	29 ± 5	6.0 ± 2.3	37	69	21
	MESA	1,300	145 ± 14	222 ± 22	63 ± 10	136 ± 23	30 ± 6	5.7 ± 1.8	47	61	19
Asian	MESA	600	143 ± 14	215 ± 20	63 ± 10	124 ± 22	24 ± 3	5.6 ± 1.5	51	37	15
N=5,113	SCES	1,771	141 ± 16	208 ± 21	58 ± 9	136 ± 19	24 ± 4	*	51	56	13
	SiMES	2,101	140 ± 16	219 ± 22	59 ± 11	147 ± 23	26 ± 5	*	50	69	22
	SINDI	641	143 ± 13	209 ± 18	53 ± 8	128 ± 18	26 ± 5	*	47	32	0
Hispanic	MESA	1,077	146 ± 14	217 ± 21	62 ± 10	129 ± 24	30 ± 5	6.0 ± 2.2	50	45	21

Replication

Ethnicity	Cohort	n	CRAE (μm)	CRVE (μm)	Age (years)	SBP (mm Hg)	BMI (kg/m²)	FG (mmol/l)	Sex (% male)	HTN (%)	DM (%)
White	BMES	448	187 ± 17	224 ± 19	65 ± 7	148 ± 20	28 ± 4	5.5 ± 1.7	47	81	10
White	RS* Genotyped	2,550	147 ± 14	222 ± 21	69 ± 8	139 ± 22	26 ± 4	6.8 ± 2.4	48	59	7
	RS Imputed	2,474	147 ± 15	222 ± 21	67 ± 8	138 ± 22	26 ± 4	6.9 ± 2.7	34	60	7

*
Rotterdam did not genotype all of their subjects using the exome chip. For those that were not genotyped they imputed the SNP for the remaining subjects. For clarity we analyzed the two groups separately.

CRAE-t: central retinal arteriole equivalent-trunk; CRVE: central retinal venule equivalent; SBP: systolic blood pressure; BMI: body mass index; FG: fasting glucose; HTN: hypertension; DM: diabetes mellitus; AA: African-American; AGES: Age, Gene, Environment, Susceptibility Study – Reykjavik; ARIC: Atherosclerosis Risk in Communities Study; CHS: Cardiovascular Health Study; MESA: Multi-Ethnic Study of Atherosclerosis; SCES: Singapore Chinese Eye Study; SiMES: Singapore Malay Eye Study; SINDI: Singapore Indian Eye Study; BMES: Blue Mountain Eye Study; RS: Rotterdam Study

Table 2

Summary of findings

CRVE

| | | | | | effect | | | | Discovery | Replication | | | | Combined | | |
Discovery SNP	gene	function	maf	n	allele	beta*	se	p		n	beta	se	p	beta	se	p
rs7926971	TEAD1	Intron	0.43	24,342	G	0.05	0.01	7.0×10^{-9}		5,472	0.06	0.02	0.001	0.05	0.01	3.1×10^{-11}
rs201259422	TSPAN10	missense	0.27	22,566	A	−0.06	0.01	8.0×10^{-9}		2,998	−0.04	0.03	0.16	−0.06	0.01	4.4×10^{-9}
rs5442	GNB3	missense	0.05	22,566	A	−0.12	0.02	5.8×10^{-8}		5,472	−0.10	0.04	0.003	−0.11	0.02	7.0×10^{-10}
rs1800407	OCA2	missense	0.05	22,566	T	−0.12	0.02	2.1×10^{-7}		5,472	−0.09	0.04	0.05	−0.11	0.02	3.4×10^{-8}
rs2306765	GSG1	missense	0.05	24,342	G	−0.10	0.02	6.9×10^{-6}		5,024	0.14	0.07	0.03	−0.07	0.02	3.5×10^{-4}

CRAE

| rs1800407 | OCA2 | missense | 0.05 | 22,569 | T | −0.14 | 0.02 | 4.5×10^{-10} | | 5,472 | −0.13 | 0.04 | 0.004 | −0.14 | 0.02 | 6.5×10^{-12} |

*
Beta and standard errors represent inverse-quantile normalized traits not direct measures of CRVE and CRAE-t.

CRVE: central retinal venule equivalent; CRAE: central retinal arteriole equivalent; SNP: single nucleotide polymorphisms; maf: minor allele frequency; se: standard error; p: p-value