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New loci and coding variants confer risk for age-related macular degeneration in East Asians

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Age-related macular degeneration (AMD) is a major cause of blindness, but presents differently in Europeans and Asians. Here, we perform a genome-wide and exome-wide association study on 2,119 patients with exudative AMD and 5,691 controls, with independent replication in 4,226 patients and 10,289 controls, all of East Asian descent, as part of The Genetics of AMD in Asians (GAMA) Consortium. We find a strong association between *CETP* Asp442Gly (rs2303790), an East Asian-specific mutation, and increased risk of AMD (odds ratio (OR) = 1.70, $P = 5.60 \times 10^{-22}$). The AMD risk allele (442Gly), known to protect from coronary heart disease, increases HDL cholesterol levels by 0.17 mmol l^{-1} ($P = 5.82 \times 10^{-21}$) in East Asians ($n = 7,102$). We also identify three novel AMD loci: *C6orf223* Ala231Ala (OR = 0.78, $P = 6.19 \times 10^{-18}$), *SLC44A4* Asp47Val (OR = 1.27, $P = 1.08 \times 10^{-11}$) and *FGD6* Gln257Arg (OR = 0.87, $P = 2.85 \times 10^{-8}$). Our findings suggest that some of the genetic loci conferring AMD susceptibility in East Asians are shared with Europeans, yet AMD in East Asians may also have a distinct genetic signature.

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Age-related macular degeneration (AMD) is a progressive, blinding disease affecting millions of elderly individuals worldwide^{1,2}. Several genome-wide association studies (GWAS) have identified common variants associated with AMD in European-ancestry populations^{3–6}, and recently, rare genetic variation at *CFH*, *CFI*, *C3* and *C9* were also shown to strongly associate with AMD in Europeans^{7–10}. However, there are few such studies in Asians¹¹. Importantly, Asians appear to have a distinct clinical presentation of the disease (for example, absence of drusen and minimal fibrous scarring in polypoidal choroidal vasculopathy, a variant of AMD accounting for 20–55% of Asian patients with exudative AMD) and different responses to treatment (for example, poorer response to inhibitors of vascular endothelial growth factor (VEGF) compared with patients of European ancestry)^{12,13}. It remains unclear whether there are differences in underlying genetic characteristics of AMD between patients of Asian versus European ancestry.

Concurrently, previous GWAS studies provide limited coverage of low-frequency coding variants, which may result in the loss of function and are often ethnic-specific. There is thus interest in genetic studies of AMD and other diseases beyond standard-content GWAS to discover potentially causative coding variants in different ethnic groups.

To address these questions, the Genetics of AMD in Asians Consortium perform a genome-wide (GWAS) and exome-wide association study (EWAS) of advanced AMD solely on the exudative (neovascular) disease subtype in East Asians. Compared with standard-content GWAS arrays, the exome array has significantly increased marker density across the coding human exome, thus increasing power to detect disease associations located within the coding frame. EWAS of AMD have not been previously conducted in either Europeans or Asians. In this paper, we present data from eight independent AMD case-control collections enrolled across multiple sites in East Asia, totalling 6,345 exudative AMD cases and 15,980 controls. This is the largest sample, to our knowledge, of East Asians ever assembled for genetic studies of AMD.

Results

Association with previously identified AMD variants. After genotype imputation, synchronization and stringent quality filters were performed, a total of 4,471,719 SNPs were assessed for the GWAS and 120,027 autosomal coding-frame SNPs for EWAS from 2,119 AMD cases and 5,691 controls (Table 1). Overall

genomic inflation was very low ($\lambda_{gc} = 1.031$; Supplementary Fig. 1), suggesting minimal confounding of the disease association analysis by population stratification or other systematic study design biases. Data from the discovery stage analysis confirmed previously identified AMD variants in *ARMS2-HTRA1* rs10490924 ($P = 1.20 \times 10^{-103}$), *CFH* rs10737680 ($P = 7.54 \times 10^{-38}$), *CETP* rs3764261 ($P = 1.66 \times 10^{-12}$), *ADAMTS9* rs6795735 ($P = 1.13 \times 10^{-5}$), *C2-CFB* rs429608 ($P = 1.06 \times 10^{-4}$), as well as *CFI* rs4698775 ($P = 7.5 \times 10^{-4}$; Supplementary Table 1 and Supplementary Fig. 2). Our data also showed nominal evidence of replication in the same direction as the initial study for a further three previously reported variants (*TGFBR1* rs334353, *APOE* rs4420638, and *VEGFA* rs943080; $P < 0.05$ for each). The remaining 8 out of 17 previously described SNPs that were non-monomorphic in our East Asian collections did not show evidence of replication in our study (Supplementary Table 1). A recently described rare, functional and highly penetrant genetic mutation within *CFI* (G119R, rs141853578) shown to confer markedly elevated risk of AMD in Europeans¹⁴ was observed to be non-polymorphic in our East Asian samples (Supplementary Table 1). Similarly, recently described rare mutations in *C3* (K155Q, rs147859257) and *C9* (P167S, rs34882957) were also shown to be non-polymorphic in our East Asian samples (Supplementary Table 1).

Discovery of new SNP variants associated with AMD. Apart from verifying previous observations, our discovery analysis also revealed genome-wide significant association at *C6orf223* (rs2295334 encoding for A231A, $P = 1.41 \times 10^{-8}$; Table 2, Supplementary Table 2 and Supplementary Fig. 2), a novel SNP marker not previously reported to associate with AMD risk. We observed a further 21 independent SNPs from distinct loci not previously implicated with susceptibility to AMD showing evidence of association surpassing $P < 1 \times 10^{-4}$. We then brought forward all the 22 markers (Table 2) for replication genotyping in independent sample collections comprising 4,226 exudative AMD cases and 10,289 controls (Table 1). Replication evidence was compelling for *CETP* rs2303790 (encoding D442G; odds ratio (OR) = 1.73, $P = 2.95 \times 10^{-16}$), as well as for *C6orf223* rs2295334 (A231A; OR = 0.80, $P = 5.25 \times 10^{-11}$), *SLC44A4* rs12661281 (D47V; OR = 1.22, $P = 5.13 \times 10^{-6}$) and *FGD6* rs10507047 (Q257R; OR = 0.88, $P = 7.69 \times 10^{-5}$), leading to genome-wide significant findings in the meta-analysis of all 6,345

Table 1 | Baseline characteristics of exudative age-related macular degeneration cases and controls in the discovery and replication sample collections.

Sample collection	Ethnicity	No. of cases*	No. of controls*	AMD phenotyping	Genotyping platform
<i>Discovery</i>					
Singapore	Chinese	631	1,967	Dilated funduscopy, FA, ICG & OCT	Illumina OmniExpress, 610 K & Exome Chips
Hong Kong	Chinese	507	2,967	Dilated funduscopy, FA & ICG	Illumina OmniExpress & Exome Chips
Japan	Japanese	981	757	Dilated funduscopy, FA, ICG & OCT	Illumina OmniExpress & Exome Chips
Subtotal		2,119	5,691		
<i>Replication</i>					
Korea	Korean	757	1,829	Dilated funduscopy, FA, ICG & OCT	Sequenom MassArray & Taqman
Japan	Japanese	1,213	4,035	Dilated funduscopy, FA, ICG & OCT	Sequenom MassArray & Taqman
Guangdong, China	Chinese	398	2,478	Dilated funduscopy, FA & ICG	Sequenom MassArray & Taqman
Sichuan, China	Chinese	1,055	1,089	Dilated funduscopy, FA & ICG	SNAPSHOT
Beijing, China	Chinese	803	858	Dilated funduscopy, FA, ICG & OCT	Sequenom MassArray & Taqman
Subtotal		4,226	10,289		
All samples		6,345	15,980		

AMD, age-related macular degeneration; FA, fluorescein angiography; ICG, indocyanine green angiography; OCT, optical coherence tomography.

*No. of samples reflect those passing quality checks.

Table 2 | Summary of results of the genome-wide and exome-wide association study on exudative age-related macular degeneration.

SNP	Chr	Position	Nearest gene	Minor allele	Discovery (2,119/ 5,691) ^a		Replication (4,226/ 10,289) ^a		Combined (6,345/ 15,980) ^a	
					OR	<i>P</i> _{discovery}	OR	<i>P</i> _{replication}	OR	<i>P</i> _{combined}
<i>Loci reaching P_{discovery} < 1 × 10⁻⁴ and P_{combined} < 5 × 10⁻⁸</i>										
rs2303790	16	57,017,292	<i>CETP</i>	G	1.69	3.36 × 10 ⁻⁷	1.73	2.95 × 10 ⁻¹⁶	1.70	5.60 × 10 ^{-22†}
rs2295334	6	43,970,827	<i>C6orf223</i>	A	0.75	1.41 × 10 ⁻⁸	0.80	5.25 × 10 ⁻¹¹	0.78	6.19 × 10 ^{-18‡}
rs12661281	6	31,842,598	<i>SLC44A4</i>	T	1.38	1.23 × 10 ⁻⁷	1.22	5.13 × 10 ⁻⁶	1.27	1.08 × 10 ^{-11§}
rs10507047	12	95,604,290	<i>FGD6</i>	G	0.83	4.75 × 10 ⁻⁵	0.88	7.69 × 10 ⁻⁵	0.87	2.85 × 10 ^{-8‡}
<i>Loci reaching P_{discovery} < 1 × 10⁻⁴ but P_{combined} ≥ 5 × 10⁻⁸</i>										
rs62191056	2	227,779,676	<i>RHBDD1</i>	A	1.33	2.27 × 10 ⁻⁵	1.14	0.046	1.23	1.10 × 10 ⁻⁵
rs7274811	20	32,333,384	<i>ZNF341</i>	T	0.83	5.72 × 10 ⁻⁵	0.92	0.011	0.89	1.26 × 10 ⁻⁵
rs3894326	19	5,843,784	<i>FUT3</i>	T	0.75	6.22 × 10 ⁻⁶	0.91	0.27	0.81	1.85 × 10 ⁻⁵
rs117581914	19	9,236,724	<i>OR7G3</i>	G	2.02	3.27 × 10 ⁻⁵	1.36	0.19	1.78	3.05 × 10 ⁻⁵
rs2287921	19	49,228,272	<i>RASIP1</i>	C	1.74	7.70 × 10 ⁻⁶	1.20	0.13	1.43	3.56 × 10 ⁻⁵
rs17143419	7	70,829,578	<i>WBSCR17</i>	T	1.49	2.28 × 10 ⁻⁵	1.12	0.34	1.34	8.66 × 10 ⁻⁵
rs4280803	4	57,760,424	<i>REST</i>	T	0.79	4.87 × 10 ⁻⁵	0.65	0.76	0.79	4.68 × 10 ⁻⁵
rs202018816	19	10,445,066	<i>ICAM3</i>	A	5.13	1.39 × 10 ⁻⁵	1.42	0.28	2.47	2.46 × 10 ⁻⁴
rs7165901	15	102,021,219	<i>PCSK6</i>	C	0.80	1.46 × 10 ⁻⁵	0.96	0.44	0.88	3.21 × 10 ⁻⁴
rs1891359	10	127,495,153	<i>UROS</i>	G	1.77	5.42 × 10 ⁻⁵	0.95	0.74	1.36	0.0044
rs215736	7	32,443,119	<i>PDE1C-LSM5</i>	T	3.28	3.82 × 10 ⁻⁵	0.71	0.38	1.93	0.0049
rs2221338	2	68,224,745	<i>C1D</i>	A	1.22	2.60 × 10 ⁻⁵	1.02	0.65	1.13	5.83 × 10 ⁻⁴
rs1538240	13	101,968,310	<i>NALCN</i>	T	1.24	2.92 × 10 ⁻⁵	1.03	0.59	1.14	6.54 × 10 ⁻⁴
rs7560053	2	228,220,769	<i>MFF</i>	T	0.83	7.00 × 10 ⁻⁵	0.98	0.76	0.90	0.0023
rs73509026	19	12,059,467	<i>ZNF700</i>	G	1.65	1.25 × 10 ⁻⁵	0.85	0.34	1.32	0.0039
rs1241050	4	141,410,099	<i>LOC152586</i>	T	0.72	3.35 × 10 ⁻⁵	1.02	0.79	0.86	0.0068
rs7612209	3	177,596,989	<i>AK056252</i>	A	1.21	6.45 × 10 ⁻⁵	0.93	0.11	1.06	0.087

Summary of SNPs that exceeded the threshold of $P < 1 \times 10^{-4}$ on the basis of score-based tests using logistic regression in the discovery stage and were brought forward to the replication stage. In the combined meta-analysis of the discovery and replication samples, four new variants (the first four SNPs in the table) reached the threshold of genome-wide significance ($P < 5 \times 10^{-8}$). All of the four new variants are coding variants (*CETP* rs2303790 encoding D442G; *C6orf223* rs2295334 encoding A231A; *SLC44A4* rs12661281 encoding D47V; and *FGD6* rs10507047 encoding Q257R). Physical positions and nearest genes are based on NCBI build 37 of the human genome. SNP, single-nucleotide polymorphism; Chr, chromosome; OR, odds ratio for per copy of the minor allele. ^aNumber in parentheses presents the no. of cases and no. of controls, respectively. [†]Heterogeneity $I^2 = 0\%$ for *CETP* rs2303790 and *FGD6* rs10507047. [‡]Heterogeneity $I^2 = 41.5\%$, random-effects P in the combined analysis = 1.61×10^{-13} . [§]Heterogeneity $I^2 = 23.1\%$, random-effects P in the combined analysis = 6.91×10^{-9} . ^{||}These index SNPs are located in intergenic regions. Other index SNPs are located within the genes.

AMD cases and 15,980 controls ($P < 5.0 \times 10^{-8}$ for each of the four loci; Table 2 and Supplementary Table 2). Genotyping clusters were directly visualized for the top SNPs and confirmed to be of good quality (Supplementary Fig. 3).

Of note, we did not observe any substantial difference in the association signals of the most significant SNPs in the subgroup analysis of our AMD cases by typical neovascular AMD ($n = 1,083$ cases) and polypoidal choroidal vasculopathy ($n = 1,015$ cases; Supplementary Table 3). The effect size for each of the top SNPs was similar between the two AMD subgroups.

Conditional analysis. The presence of the mutant *CETP* 442G (rs2303790) allele is seen only in East Asians (for example, Chinese, Japanese and Koreans; minor allele frequency (MAF) $< 5\%$ in our controls) and not in South Asians, Europeans or Africans. This mutation is independent from all previously described common, non-coding polymorphisms near the *CETP* locus ($r^2 < 0.1$; Supplementary Fig. 4). Regional association analysis conditioning on other known common AMD variants in *CETP* confirmed the independence of D442G from other nearby common variants (Supplementary Tables 4 and 5). We also genotyped and assessed multiple, rare, protein-changing mutations at *CETP*, including Y74Stop, G331S, N358S and A390P (Fig. 1). None of them showed association with AMD (Table 3). Mutational load and haplotypic analysis considering all amino-acid changes within *CETP* confirmed that the D442G mutation drove all signals of association between *CETP* and AMD (Table 4).

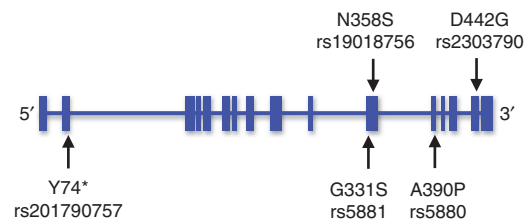


Figure 1 | Genomic organization of the *CETP* gene. The position of five rare (minor allele frequency < 0.05) amino-acid changes observed in the discovery samples are shown as indicated by the arrows. Horizontal bars represent the position of exons in the *CETP* gene.

C6orf223 is located $\sim 220,000$ base pairs downstream of *VEGFA* and $\sim 150,000$ base pairs from rs943080, a marker previously shown to strongly associate with AMD in Europeans^{5,6}. In this study of AMD in East Asians, the evidence of association for *VEGFA* rs943080 was only nominally significant ($P = 0.041$ in the discovery stage; Supplementary Table 1). Linkage disequilibrium analysis revealed no correlation between *C6orf223* rs2295334 and *VEGFA* rs943080 ($r^2 = 0.0$), with both markers separated by significant recombination events (Fig. 2a). Logistic regression adjusting for the allele dosage at *VEGFA* rs943080 did not reveal any attenuation of the association signal for *C6orf223* rs2295334 ($P = 1.66 \times 10^{-8}$; Supplementary Table 6). Regional association

Table 3 | Single variant analysis (score-based tests using logistic regression) of the five observed *CETP* rare variants with exudative age-related macular degeneration in the discovery stage.

Sample collections	SNP	Amino-acid change	A1	A2	Genotype*		MAF		OR	P value
					Cases	Controls	Cases	Controls		
Hong Kong	rs201790757	Y74*	C	A	0/0/507	0/3/2,962	0	0.0005	N/A	1
Hong Kong	rs5881	G331S	A	G	0/5/502	12/1/52	0.0049	0.0024	1.94	0.18
Japan	rs5881	G331S	A	G	0/3/970	0/4/759	0.0015	0.0026	0.59	0.49
Singapore	rs5881	G331S	A	G	0/5/635	0/21/1,935	0.0039	0.0054	0.73	0.52
Hong Kong	rs190187567	N358S	G	A	0/0/507	0/1/2,964	0	0.0002	N/A	1
Japan	rs190187567	N358S	G	A	0/1/972	0/0/763	0.0005	0	N/A	1
Hong Kong	rs5880	A390P	C	G	0/5/502	0/36/2,926	0.0049	0.0061	0.81	0.66
Singapore	rs5880	A390P	C	G	0/13/627	0/28/1,925	0.0102	0.0072	1.43	0.30
Hong Kong	rs2303790	D442G	G	A	4/45/458	2/172/2,791	0.0523	0.0297	1.80	2.8×10^{-4}
Japan	rs2303790	D442G	G	A	4/84/885	0/41/722	0.0473	0.0269	1.79	0.0025
Singapore	rs2303790	D442G	G	A	1/44/595	0/98/1,858	0.0359	0.0251	1.48	0.034

SNP, single-nucleotide polymorphism; A1, minor allele; A2, major allele; MAF, minor allele frequency; OR, odds ratio for per copy of the minor allele.

*Data are number of genotypes A1A1/A1A2/A2A2.

Table 4 | Results of association of the five observed *CETP* rare variants with exudative age-related macular degeneration using gene-based tests in 2,119 cases and 5,691 controls in the discovery stage.

Gene	No. of variants	Variants (minor allele counts)	Unconditional analysis*	Conditional analysis*
<i>CETP</i>	5	Tyr74*(3), Gly331Ser (56), Asn358Ser (2), Ala390Pro (81), Asp442Gly (536)	$P = 5.38 \times 10^{-6}$	$P = 0.96$

*Gene-based tests on mutational load (additive allele based using Burden tests) at *CETP*, unconditioned and conditioned for *CETP* Asp442Gly.

analysis including all markers from the genome and exome array data conditioning on *C6orf223* rs2295334 also did not reveal any secondary signal of association within its 1 Mb flanking region (Supplementary Table 7), thus pointing to *C6orf223* rs2295334 as a novel and uncharacterized genetic risk factor for exudative AMD in East Asians.

SLC44A4 is located ~116,000 base pairs away from a previously reported AMD locus, *C2-CFB* (rs429608)⁶. Nevertheless, *SLC44A4* rs12661281 has no correlation with *C2-CFB* rs429608 ($r^2 = 0.01$) and showed the strongest evidence of association with AMD within the genomic region (Fig. 2b), suggesting it to be also a new and uncharacterized risk factor for AMD. Logistic regression analysis adjusting for allele dosage at *C2-CFB* rs429608 did not result in any significant change in magnitude of the association either at *SLC44A4* rs12661281, testifying to their mutual independence ($OR_{unconditioned} = 1.38$, $P_{unconditioned} = 1.10 \times 10^{-7}$; $OR_{conditioned}$ for rs429608 = 1.35, $P_{conditioned}$ for rs429608 = 1.49×10^{-6} ; Supplementary Table 8).

Gene-based tests on mutational load. We next proceeded to conduct gene-based tests on mutational load to further investigate the role of low-frequency variants in exudative AMD for all the patient collections in the discovery stage. Gene-based tests are an alternative to single-marker tests for association, which are often underpowered to detect association with rare variants. We performed our tests as previously described¹⁵. To more directly address the impact of low-frequency, non-synonymous genetic variants, we considered only 109,296 such variants with MAF <5%. As a result, we were able to assess a total of 10,736 genes having at least two such variants using the sequence kernel association optimal (SKAT-O) test¹⁶. We did not detect significant evidence of association ($P < 5 \times 10^{-8}$) between mutational load and AMD at any of the 10,736 genes tested, which are consistent across all three discovery sample collections. Nonetheless, while

looking up on previously reported 22 genes within 17 distinct loci (Supplementary Table 1) associated with AMD in European populations, we note nominal evidence of association between genetic load at *CETP* ($P_{unconditioned} = 5.38 \times 10^{-6}$), whereby the association was almost entirely driven by D442G ($P_{conditioned}$ for D442G = 0.96; Table 4) as well as C2 and AMD ($P = 1.83 \times 10^{-6}$, Supplementary Table 9). All the observations exceeding $P < 1 \times 10^{-4}$ for gene-based tests on mutational load summarized across the three discovery collections are appended as Supplementary Table 9.

***CETP* 442G, HDL and coronary heart disease.** The mutant *CETP* 442G allele was shown to result in an abnormally functioning *CETP* protein¹⁷. As *CETP* is a critical component of the pathways that regulate high-density lipoprotein cholesterol (HDL-c)¹⁸, we assessed this mutation for associations with serum HDL-c levels using linear regression, with adjustment for age, gender and body mass index, in three population-based cohorts of Singaporean Chinese^{19–21} and Japanese²² ($n = 7,102$, see details on the study cohorts in Supplementary Methods) where GWAS data were available. We noted a strong association between 442G allele and increased HDL-c levels ($\beta = 0.174 \text{ mmol l}^{-1}$ per copy of 442G allele; reflecting an ~10% shift within the normal HDL range, $P = 5.82 \times 10^{-21}$; Table 5). This effect size is at least twice that observed for other *CETP* variants reported in European populations (Supplementary Table 10)^{23,24}.

Due to its strong effect on serum HDL-c, we assessed whether the mutant *CETP* 442G allele conferred any effect on individual susceptibility to coronary heart disease (CHD) in East Asians. Using 683 CHD cases and 1,281 controls from the Singapore Chinese Health Study (Supplementary Methods)²⁵, we noted some degree of enrichment of the HDL-increasing, mutant 442G allele in the controls (2.89%) compared with the cases (2.42%,

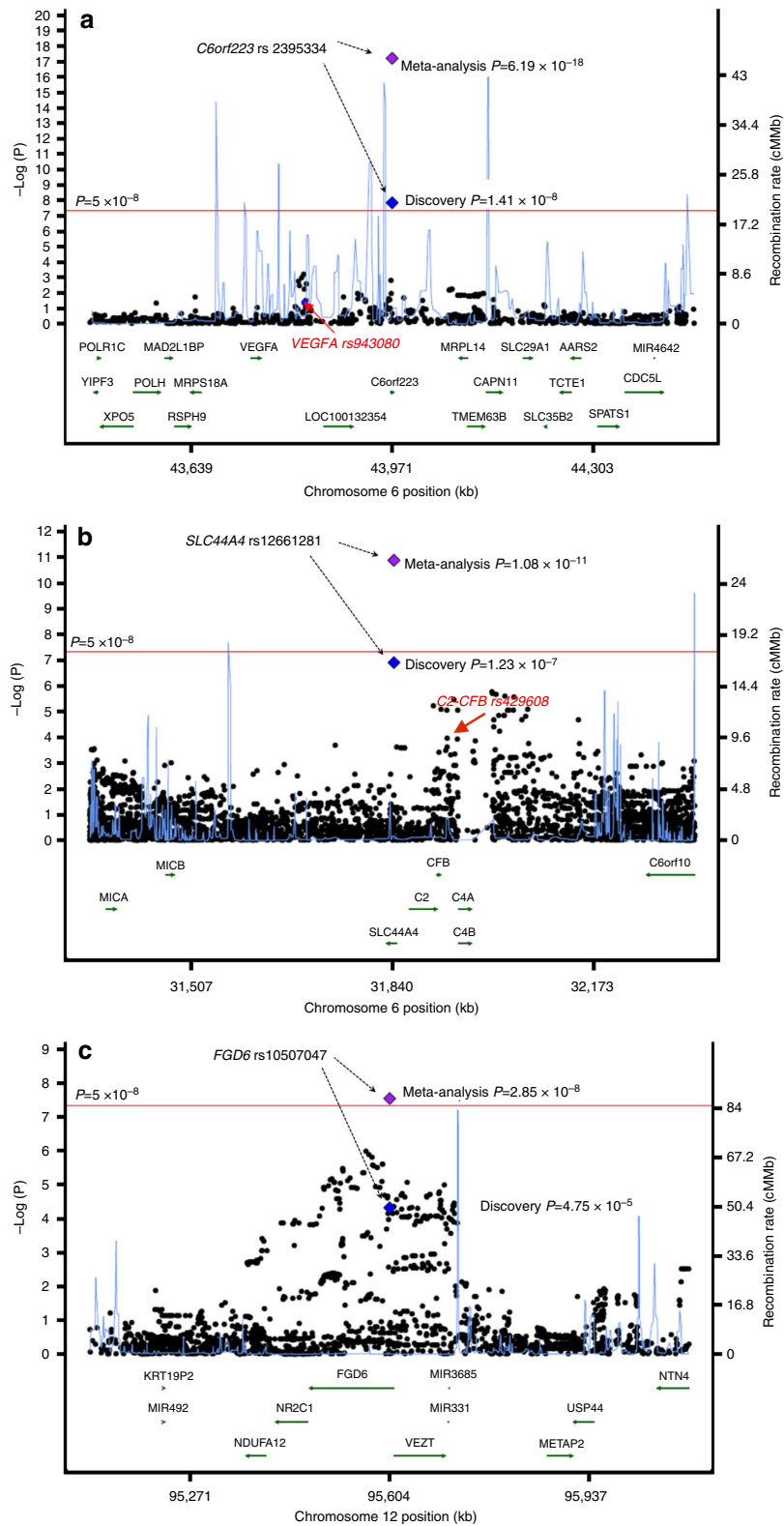


Figure 2 | Regional association plots at the three risk loci for exudative age-related macular degeneration. (a) The *C6orf223* locus (rs2295334): *VEGFA* rs943080, which is located in the intergenic, non-coding region, was very strongly associated with AMD in Europeans, but this study of exudative AMD in East Asians could not reveal significant association with it ($P=0.041$ by score-based tests using logistic regression in 2,119 cases and 5,691 controls). The two markers are independent from each other ($r^2=0.0$), as well as separated by strong recombination events in Asians. **(b)** The *SLC44A4* locus (rs12661281): *C2-CFB* rs429608 was very strongly associated with AMD in Europeans, but revealed modest evidence of association in our discovery stage ($P=1.06 \times 10^{-4}$). The two markers are independent from one another ($r^2=0.01$). **(c)** The *FGD6* locus (rs10507047).

Table 5 | Association of serum HDL cholesterol level and *CETP* D442G in East Asian population-based cohorts.

Cohort	No. of samples	D442G genotype	Serum HDL cholesterol mean (s.d.), mmol l ⁻¹	Association of HDL with D442G				
				Effect allele	Effect allele frequency	Effect, [†] mmol l ⁻¹	s.e.m.	P value
SCES	1,922	Gly/Gly	1.46 [†]	442Gly	0.02	0.17	0.04	1.46 × 10 ⁻⁶
		Asp/Gly	1.49 (0.010)					
SP2-set1 [‡]	1,044	Asp/Asp	1.32 (0.009)	442Gly	0.03	0.17	0.04	5.38 × 10 ⁻⁵
		Gly/Gly	— [†]					
		Asp/Gly	1.70 (0.014)					
SP2-set2 [§]	888	Asp/Asp	1.52 (0.011)	442Gly	0.03	0.17	0.05	3.46 × 10 ⁻⁴
		Gly/Gly	— [†]					
		Asp/Gly	1.59 (0.012)					
Nagahama	3,248	Asp/Asp	1.43 (0.012)	442Gly	0.04	0.18	0.03	1.85 × 10 ⁻⁹
		Gly/Gly	2.32 (0.344)					
		Asp/Gly	1.83 (0.030)					
Meta-analysis	7,102	Asp/Asp	1.66 (0.008)	442Gly	0.03	0.174	0.018	5.82 × 10 ⁻²¹
		Gly/Gly	— [†]					

SCES, Singapore Chinese Eye Study; SP2, Singapore Prospective Study Program; Nagahama, Nagahama Prospective Genome Cohort for the Comprehensive Human Bioscience.
[†]Effect per copy of the 442Gly allele on serum HDL-c in mmol l⁻¹.
[‡]Only one individual in SCES was homozygous (Gly/Gly) for the *CETP* 442G mutant allele. No Gly/Gly homozygotes were observed in both SP2 collections.
[§]Genotyped on Illumina 610 K chip.
[¶]Genotyped on Illumina 1M chip.

OR = 0.83), although this did not reach statistical significance ($P = 0.39$). However, our observations were consistent with a recent study from Japan which analysed *CETP* D442G in 4,399 CHD cases and 7,672 controls whereby enrichment of the 442G allele were also found in the controls (3.4%) compared with the CHD cases (2.8%; OR = 0.83, $P = 0.02$)²⁶. We thus performed a meta-analysis of our study and the Japanese study, resulting in a consistent protective effect of this mutation with CHD (OR = 0.83, $P = 0.011$, $I^2 = 0.0\%$; Supplementary Fig. 5).

Discussion

Our studies of exudative AMD in East Asians identified three novel loci (*C6orf233*, *SLC44A4* and *FGD6*), two of which (*SLC44A4* and *FGD6*) harbour coding, non-synonymous variants. These have not been identified in large samples of AMD patients of European descent⁶, thus validating the role of searching for coding variations in diverse ethnic groups to better understand mechanistic basis of complex diseases such as AMD.

Our most interesting findings were the identification of an uncommon East Asian-specific mutation at *CETP* (D442G) associated with exudative AMD. The association is the strongest (per-allele OR = 1.70) observed outside of the classical *CFH* and *ARMS2-HTRA1* loci in East Asians. A common variant (rs3764261) mapping to the intergenic region between *HERPUD1* and *CETP* was previously linked to AMD in Europeans, yet its effect size was modest (OR = 1.15)⁵ and independent from the D442G association. The mutant 442G allele is known to impair *CETP* function with reduction in plasma *CETP* mass and activity^{17,27}, and is associated with elevated HDL-c in Japanese families^{17,27}. This allele is absent in European populations²⁸ and appears to be present only in East Asians, rendering it independent from all other previously described common, non-coding *CETP* polymorphisms. We showed that each copy of the dysfunctional 442G allele confers, on average, a rise in HDL-c levels of 0.174 mmol l⁻¹ and confirmed findings from the earlier Japanese studies. Given the mean serum HDL-c concentration is 1.3 mmol l⁻¹, this is a mutation of considerable effect size even from a population-based perspective. Notably, no other amino-acid substitutions within *CETP* detected by our GWAS and EWAS showed any evidence of association with AMD (Fig. 1

and Table 3), suggesting that the D442G mutation is a possible causative mutation of AMD in East Asians in the *CETP* locus. We are not surprised that *CETP* D442G did not show a clearly significant protective effect against CHD as increasing lines of evidence more directly implicate LDL as the driving force for CHD susceptibility^{29,30}.

C6orf223 is a newly mapped gene with yet unknown functional role. Its A231A synonymous coding change is fivefold rarer in Europeans (MAF = 0.03) as compared with Asians (MAF > 0.15, Supplementary Table 2). It is thus unsurprising that the European GWAS efforts have yet to detect this locus. *VEGFA* rs943080, a marker strongly associated with AMD in European-ancestry populations⁵, is in the vicinity of *C6orf223*, but its association with AMD is much weaker in this study of East Asians (Supplementary Tables 1 and 6), possibly reflecting the differences in therapeutic response to anti-VEGF treatment between Asians and Europeans^{13,31}.

The D47V mutation within *SLC44A4* is not included in most of the routinely used genotyping arrays, but is now included as part of the exome array used in this study. The genomic region around the *SLC44A4* locus is more complex, being located within the broad MHC region on Chromosome 6 between *HLA* class I and class II genes. As this region is very polymorphic, and allele frequency differences between cases and controls could be confounded by even minor population stratification, we thus reassessed the associations with AMD by adjusting for the first 10 principal components (PCs) of genetic ancestry. We did not observe any change in the association signals observed from our standard analysis, which adjusted for the first five PCs (analysis adjusting for the top 10 PCs; OR = 1.39, $P = 2.33 \times 10^{-7}$ in the discovery phase), consistent with previous observations in Asian studies with well-replicated associations within the MHC region^{32,33}. *SLC44A4* encodes for choline transporter protein-4, involved in sodium-dependent choline uptake by cholinergic neurons³⁴. Defects in *SLC44A4* have been linked to sialidosis, which presents with a spectrum of symptoms including eye abnormalities³⁵.

Prior studies on the basis of European patient collections have identified a total of four distinct AMD-associated loci on Chromosome 6 alone⁶. In this light, the burden of proof for the positive identification of *C6orf223* and *SLC44A4* (both are also

located on Chromosome 6) is higher than usual due to the need for appropriate considerations of previously reported variants on the same chromosome. It is reassuring to note that both *C6orf223* and *SLC44A1* showed the strongest two signals of association with AMD outside of *CFH*, *ARMS2-HTRA1* and *CETP*. Our exhaustive analyses using logistic regression adjusting for allele dosages at previously described SNP markers suggest that both *C6orf223* and *SLC44A1* are unrelated to those previously reported, and thus they likely represent Asian-specific genetic associations for AMD.

Neither *FGD6* nor the genes within its vicinity (Fig. 2c) have ever been previously implicated in any ocular disorders. *FGD6* encodes for FYVE, RhoGEF and PH domain-containing protein 6, with its functions yet to be characterized. The Q257R mutation is less than half as common in Europeans (MAF = 0.10) compared with East Asians (MAF = 0.20–0.30), again possibly explaining the ability of our study to pick up this genetic effect.

The identified genes were expressed in human retinal pigment epithelium (Supplementary Table 11). Of the three non-synonymous substitutions, the *CETP* D442G variant was predicted by both PolyPhen³⁶ and SIFT³⁷ to likely be causing damage to the protein structure/function, the *FGD6* Q257R variant was predicted only by PolyPhen to be probably damaging but by SIFT to be tolerated and the *SLC44A4* D47V variant was predicted by both tools to be benign or tolerated. Although the use of both prediction algorithms has been reported to be moderately sensitive, they suffer from lack of specificity³⁸, and thus more evidence should be sought with regards to the *FDG6* and *SLC44A4* non-synonymous variants.

Using HaploReg³⁹, RegulomeDB⁴⁰ and Encyclopaedia of DNA Elements (ENCODE)⁴¹ data, we identified variants within each of the four LD blocks in the 1000 Genomes Project ($r^2 > 0.8$ and < 250 kb from the top SNP) to apply functional annotations relevant to the regulation of transcription (Supplementary Table 12). In addition to their functions on amino-acid substitutions, all of the four identified variants lie within a DNase I hypersensitivity site or in a region where modification of histones is suggestive of promoter, enhancer and other regulatory activity, and/or have an influence on binding of transcription factors or effects on a specific regulatory motif. *C6orf223* A231A (rs2295334) and *FGD6* Q257R could tag genetic variants that lie in potential transcription-factor binding sites (Supplementary Table 13). Examination of a recently available large-scale eQTL mapping database⁴² indicates that out of the four novel genome-wide significant SNPs, markers *SLC44A4* D47V and *FDG6* Q257R could serve as cis-eQTLs. The minor allele at *SLC44A4* D47V (rs12661281) is associated with significantly altered expression of HSPA1B and CSKN2B, which are located within a 210,000 bp region flanking *SLC44A4* D47V. The minor allele at *FDG6* Q257R (rs10507047) is associated with significantly increased expression of the neighbouring *VEZT* gene (Supplementary Table 14). Given that these findings are based on expression in whole blood in European samples, further work will be needed to elucidate their role in retinal tissue and in Asian samples. Nonetheless, these could suggest possible alternate mechanisms whereby both non-synonymous substitutions potentially affect AMD risk apart from directly affecting the protein structure of their parent genes.

Our study examined mainly the exudative subtype of AMD, and therefore cannot be completely compared with other studies looking at advanced AMD including the choroidal neovascularization and geographic atrophy subtypes. We also note substantial differences in inter-ethnic MAF for most of the previously reported loci associated with AMD in European-ancestry populations (Supplementary Table 1). This could represent genuine differences in genetic architecture in AMD between Asians and Europeans, or that the low allele frequency in either

ethnicity could result in insufficient power to replicate genome-wide significant hits initially observed in either Asians or Europeans. Overall, out of 21 previously reported SNPs showing strong evidence of association in Europeans, we were able to replicate 9 of them in our study of East Asians at $P < 0.05$. Taken together with the three novel loci and one novel variant in *CETP* in East Asians discovered in this study, we postulate that the genetic mechanisms of AMD in Asians could, in part, be somewhat distinct from that in Europeans.

In summary, our genome-wide and exome-wide study of AMD provides new insights into the genetic mechanisms of AMD in East Asians. Our study highlights the value of searching for low-frequency, ethnic-specific genetic variants on the coding frame of AMD that may inform pathogenesis. Although some of the genetic loci conferring disease susceptibility in East Asians are shared with Europeans (for example, common variation mapping to *CFH*, *HTRA1* and *CETP*), we identified significant important differences in the fine-scale genetic architecture of AMD, which appear specific to East Asians. Such differences could underpin at least some of the inter-ethnic differences in clinical presentation and response to specific therapies, including the poorer response to anti-VEGF therapy in Asians.

Methods

Study design and phenotyping. We performed a GWAS and EWAS on neovascular AMD in East Asians. In the discovery stage, we included and genotyped 2,119 cases and 5,691 controls from three case-control studies from Singapore, Hong Kong and Japan. For replication, five independent case-control studies were conducted in Korea, Japan, and Guangdong, Sichuan and Beijing in China, totaling 4,226 cases and 10,289 controls. All the studies were performed with the approval of their local Medical Ethics Committee, and written informed consent was obtained from all the participants in accordance with the Declaration of Helsinki.

A detailed description of subject recruitment and phenotyping in each sample collection is provided in Supplementary Methods, and summarized in Table 1. In brief, the diagnosis of exudative AMD was made at each site by retinal specialists, according to standard clinical definitions on the basis of detailed ophthalmic examinations, including dilated fundus photography, fluorescein angiography, indocyanine green angiography and optical coherence tomography (Table 1). Grading of fluorescein angiograms for the presence of choroidal neovascularization were performed using a modification from the Macular Photocoagulation Study⁴³. Indocyanine green angiography was performed to diagnose definitive polypoidal choroidal vasculopathy, a variant of AMD, using the Japanese Study Group guidelines⁴⁴. Cases with other macular diseases such as central serous chorioretinopathy, high myopia and angiod streaks were excluded. Of the 2,119 exudative AMD cases included in the discovery phase, 1,083 (51%) were classified as 'typical neovascular AMD', 1,015 (48%) were polypoidal choroidal vasculopathy and 21 (1%) had one eye with typical neovascular AMD and the other eye with polypoidal choroidal vasculopathy. At each site, controls subjects without any clinical signs of AMD were either recruited from eye clinics or enrolled from population-based studies (Supplementary Methods).

Genotyping and imputation. For the discovery stage, GWAS genotyping was performed using the Illumina Human OmniExpress or Human Hap610-Quad beadchips, and EWAS was done using HumanExome beadchips (Table 1). For replication, genotyping was performed using the MassArray platform (Sequenom), as well as using Taqman allelic discrimination probes (Applied Biosystems).

Stringent quality control filters were used to remove poorly performing samples and SNP markers in both the discovery and replication (de-novo genotyping) phases. For the GWAS, SNPs with a call rate of $< 95\%$, MAF of $< 1\%$, or showing deviation from Hardy-Weinberg Equilibrium ($P < 10^{-6}$) were removed from further statistical analysis. For the EWAS, SNPs with a call rate of $< 99\%$, MAF of $< 0.1\%$ or showing deviation from equilibrium ($P < 10^{-6}$) were removed. The 99% threshold was used as many SNP markers on the exome array had MAF $< 5\%$, and as such, differential genotyping success rates between cases and controls as low as 2% could result in false-positive findings. SNPs which were not monomorphic (whereby at least one heterozygous carrier individual was present) were included for downstream analysis.

Routine quality control criteria on a per-sample basis were carried out, and poorly performing samples were removed from further analysis. The remaining samples were then subjected to biological relationship verification by using the principle of variability in allele sharing according to the degree of relationship. Identity-by-state information was derived using the PLINK software⁴⁵. For those pairs of first-degree relatives so identified (for example, parent-offspring, full-

siblings, as well as monozygous twins), we removed the sample with the lower call rate before performing PC analysis.

The imputation was carried out using IMPUTE2 version 2.2.2 with ASN population haplotypes from 1000 Genomes as reference, as described elsewhere^{46–48}. Imputed genotypes were called with an impute probability threshold of 0.9 with all other genotypes classified as missing. Additional quality control filters were applied to remove SNPs with >1% missingness should the SNP have a MAF <5% in either cases or controls. For common SNPs with MAF >5%, the filtering criteria were set at >5% missingness.

Statistical analysis. For the discovery stage, all exudative AMD cases and controls appear well matched when visualized spatially on PC analysis for each sample collection on a per-country basis for Hong Kong, Japan and Singapore and according to self-reported ethnicity (ethnic Chinese for Hong Kong and Singapore, and ethnic Japanese for Japan; Supplementary Fig. 6), using previously reported criteria⁴⁹, indicating that population stratification is unlikely to confound the association results.

For both the discovery and replication stages, analysis of association with exudative AMD was carried out using 1-degree of freedom score-based tests using logistic regression. The tests model for a trend-per-copy effect of the minor allele on disease risk. For the discovery stage, we incorporated the top five PCs of genetic stratification into the logistic regression model to minimize the effect of residual population stratification⁵⁰. We could not adjust for population stratification for the replication stage due to limited number of SNPs tested. Meta-analysis was conducted using inverse variance weights for each sample collection, which calculates an overall Z-statistic, corresponding P value and accompanying per-allele OR for each SNP analysed. Gene-based tests on mutational load was performed using the SKAT-O test¹⁶. The association between CETP D442G and serum HDL-c level was assessed using linear regression assuming an additive model of inheritance as previously described²³ (due to serum HDL-c being distributed normally), with adjustment for age, gender and body mass index.

Regional association and PC plots were analysed and plotted using the R statistical software package.

Power calculations. For the discovery stage (2,119 AMD cases and 5,691 controls), power calculations⁵¹ indicated that there is 80% power of detecting loci at $P < 1 \times 10^{-4}$ (the threshold of association for bringing forward SNPs to the replication stage) at MAF as low as 10% with per-allele OR of 1.30. For rarer variants of higher penetrance, the discovery stage has 80% power of detecting loci at $P < 1 \times 10^{-4}$ at MAF as low as 2% if the per-allele OR is at least 1.70. The entire sample (6,345 AMD cases and 15,980 controls) has 80% power to detect loci at $P < 5.0 \times 10^{-8}$ at MAF as low as 2% if the per-allele OR is at least 1.55 or at MAF as low as 9% with per-allele OR of 1.25, in line with the effect sizes being reported in this study. Supplementary Table 15A shows the power calculations to detect SNPs at the threshold of $P < 1 \times 10^{-4}$ in the discovery stage for bringing forward to the replication stage. Supplementary Table 15B shows the formal power calculations in the context of the combined discovery and replication stages.

References

- Lim, L. S., Mitchell, P., Seddon, J. M., Holz, F. G. & Wong, T. Y. Age-related macular degeneration. *Lancet* **379**, 1728–1738 (2012).
- Wong, W. L. *et al.* Global prevalence and burden of age-related macular degeneration: a meta-analysis and disease burden projection for 2020 and 2040. *Lancet Glob. Health* **2**, e106–e116 (2014).
- Spencer, K. L. *et al.* Protective effect of complement factor B and complement component 2 variants in age-related macular degeneration. *Hum. Mol. Genet.* **16**, 1986–1992 (2007).
- Chen, W. *et al.* Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. *Proc. Natl Acad. Sci. USA* **107**, 7401–7406 (2010).
- Yu, Y. *et al.* Common variants near FRK/COL10A1 and VEGFA are associated with advanced age-related macular degeneration. *Hum. Mol. Genet.* **20**, 3699–3709 (2011).
- Fritsche, L. G. *et al.* Seven new loci associated with age-related macular degeneration. *Nat. Genet.* **45**, 433–439 439e431–432 (2013).
- Raychaudhuri, S. *et al.* A rare penetrant mutation in CFH confers high risk of age-related macular degeneration. *Nat. Genet.* **43**, 1232–1236 (2011).
- Seddon, J. M. *et al.* Rare variants in CFI, C3 and C9 are associated with high risk of advanced age-related macular degeneration. *Nat. Genet.* **45**, 1366–1370 (2013).
- Helgason, H. *et al.* A rare nonsynonymous sequence variant in C3 is associated with high risk of age-related macular degeneration. *Nat. Genet.* **45**, 1371–1374 (2013).
- Zhan, X. *et al.* Identification of a rare coding variant in complement 3 associated with age-related macular degeneration. *Nat. Genet.* **45**, 1375–1379 (2013).
- Arakawa, S. *et al.* Genome-wide association study identifies two susceptibility loci for exudative age-related macular degeneration in the Japanese population. *Nat. Genet.* **43**, 1001–1004 (2011).
- Laude, A. *et al.* Polypoidal choroidal vasculopathy and neovascular age-related macular degeneration: same or different disease? *Prog. Retin. Eye Res.* **29**, 19–29 (2010).
- Cheung, C. M. & Wong, T. Y. Ranibizumab and bevacizumab for AMD. *New Engl. J. Med.* **365**, 2237 author reply 2237 (2011).
- van de Ven, J. P. *et al.* A functional variant in the CFI gene confers a high risk of age-related macular degeneration. *Nat. Genet.* **45**, 813–817 (2013).
- Huuyghe, J. R. *et al.* Exome array analysis identifies new loci and low-frequency variants influencing insulin processing and secretion. *Nat. Genet.* **45**, 197–201 (2013).
- Lee, S., Wu, M. C. & Lin, X. Optimal tests for rare variant effects in sequencing association studies. *Biostatistics* **13**, 762–775 (2012).
- Inazu, A. *et al.* Genetic cholesteryl ester transfer protein deficiency caused by two prevalent mutations as a major determinant of increased levels of high density lipoprotein cholesterol. *J. Clin. Invest.* **94**, 1872–1882 (1994).
- Barter, P. J. *et al.* Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **23**, 160–167 (2003).
- Lavanya, R. *et al.* Methodology of the Singapore Indian Chinese Cohort (SICC) eye study: quantifying ethnic variations in the epidemiology of eye diseases in Asians. *Ophthalmic Epidemiol.* **16**, 325–336 (2009).
- Sabanayagam, C. *et al.* Retinal arteriolar narrowing increases the likelihood of chronic kidney disease in hypertension. *J. Hypertens.* **27**, 2209–2217 (2009).
- Cheung, C. M. *et al.* Prevalence of and risk factors for age-related macular degeneration in a multiethnic Asian cohort. *Arch. Ophthalmol.* **130**, 480–486 (2012).
- Nakata, I. *et al.* Prevalence and characteristics of age-related macular degeneration in the Japanese population: the nagahama study. *Am. J. Ophthalmol.* **156**, 1002–1009 e1002 (2013).
- Kathiresan, S. *et al.* Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. *Nat. Genet.* **40**, 189–197 (2008).
- Kathiresan, S. *et al.* Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat. Genet.* **41**, 56–65 (2009).
- Hankin, J. H. *et al.* Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. *Nutr. Cancer* **39**, 187–195 (2001).
- Takeuchi, F. *et al.* Association of genetic variants influencing lipid levels with coronary artery disease in Japanese individuals. *PLoS ONE* **7**, e46385 (2012).
- Takahashi, K. *et al.* A missense mutation in the cholesteryl ester transfer protein gene with possible dominant effects on plasma high density lipoproteins. *J. Clin. Invest.* **92**, 2060–2064 (1993).
- Freeman, D. J. *et al.* A polymorphism of the cholesteryl ester transfer protein gene predicts cardiovascular events in non-smokers in the West of Scotland Coronary Prevention Study. *Eur. Heart J.* **24**, 1833–1842 (2003).
- Howard, B. V. *et al.* LDL cholesterol as a strong predictor of coronary heart disease in diabetic individuals with insulin resistance and low LDL: The Strong Heart Study. *Arterioscler. Thromb. Vasc. Biol.* **20**, 830–835 (2000).
- Grundy, S. M. Low-density lipoprotein, non-high-density lipoprotein, and apolipoprotein B as targets of lipid-lowering therapy. *Circulation* **106**, 2526–2529 (2002).
- Hara, R. *et al.* Photodynamic therapy alone versus combined with intravitreal bevacizumab for neovascular age-related macular degeneration without polypoidal choroidal vasculopathy in Japanese patients. *Graefes Arch. Clin. Exp. Ophthalmol.* **248**, 931–936 (2010).
- Kumar, V. *et al.* Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. *Nat. Genet.* **43**, 455–458 (2011).
- Su, Z. *et al.* Common variants at the MHC locus and at chromosome 16q24.1 predispose to Barrett's esophagus. *Nat. Genet.* **44**, 1131–1136 (2012).
- O'Regan, S. *et al.* An electric lobe suppressor for a yeast choline transport mutation belongs to a new family of transporter-like proteins. *Proc. Natl Acad. Sci. USA* **97**, 1835–1840 (2000).
- Uhl, J. *et al.* Identification of a CTLA4/Neu1 fusion transcript in a sialidosis patient. *FEBS Lett.* **521**, 19–23 (2002).
- Adzhubei, I. A. *et al.* A method and server for predicting damaging missense mutations. *Nat. Methods* **7**, 248–249 (2010).
- Ng, P. C. & Henikoff, S. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res.* **31**, 3812–3814 (2003).
- Flanagan, S. E., Patch, A. M. & Ellard, S. Using SIFT and PolyPhen to predict loss-of-function and gain-of-function mutations. *Genet. Test. Mol. Biomarkers* **14**, 533–537 (2010).
- Ward, L. D. & Kellis, M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res.* **40**, D930–D934 (2012).

40. Boyle, A. P. *et al.* Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res.* **22**, 1790–1797 (2012).
41. Consortium, E. P. *et al.* An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57–74 (2012).
42. Westra, H. J. *et al.* Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat. Genet.* **45**, 1238–1243 (2013).
43. Laser photocoagulation of subfoveal neovascular lesions in age-related macular degeneration. Results of a randomized clinical trial. Macular Photocoagulation Study Group. *Arch. Ophthalmol.* **109**, 1220–1231 (1991).
44. Japanese Study Group of Polypoidal Choroidal, V. [Criteria for diagnosis of polypoidal choroidal vasculopathy]. *Nihon Ganka Gakkai Zasshi* **109**, 417–427 (2005).
45. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
46. Marchini, J., Howie, B., Myers, S., McVean, G. & Donnelly, P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat. Genet.* **39**, 906–913 (2007).
47. Howie, B. N., Donnelly, P. & Marchini, J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* **5**, e1000529 (2009).
48. Howie, B., Fuchsberger, C., Stephens, M., Marchini, J. & Abecasis, G. R. Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. *Nat. Genet.* **44**, 955–959 (2012).
49. Vithana, E. N. *et al.* Genome-wide association analyses identify three new susceptibility loci for primary angle closure glaucoma. *Nat. Genet.* **44**, 1142–1146 (2012).
50. Khor, C. C. *et al.* Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. *Nat. Genet.* **43**, 1241–1246 (2011).
51. Purcell, S., Cherny, S. S. & Sham, P. C. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* **19**, 149–150 (2003).

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Author contributions

C.-Y.C., K.Y., L.J.C., J.A., K.-H.P., C.P.P., N.Y., T.Y.W. and C.C.K. designed the study. C.-Y.C., C.M.G.C., M.M., P.D.C., I.Y.Y., A.L., R.M., A.H.K., S.Y.L., D.W., C.M.G.C., B.K.L., Y.S., H.N., Y.A.-K., N.G., A.T., K.M., S.Y., Y.S., H.L., T.L., S.H., T.Y.Y.L., H.C., S.T., X.D., F.W., P.Z., B.Z., J.S., J.-M.Y., W.P.K., R.M.v.D., Y.F., N.W., G.S.W.T., S.J.P., M.B., L.G., T.N., P.M., P.Z., S.-M.S., M.O., T.M., Y.K., S.J.W., H.C., H.-G.Y., J.Y.S., D.H.P., I.T.K., W.C., M.S., S.-J.L., H.W.K., J.E.L., C.K.H., T.H.L., S.-K.Y., T.A. and W.T.Y. gathered clinical data. J.P., S.D., I.N., Y.A.-K., F.M., P.O.S.T., F.L., X.Z., Y.S., B.G., R.D., Y.L., M.L.H., J.N.F., C.H.W., X.X., Jinlong Liang, J.M., X.J., Y.L., Jianjun Liu, K.S., E.N.V., J.X.B., Y.X.Z. and C.C.K. generated genetic data. C.-Y.C., K.Y., L.J.C., J.A., Lulin Huang, Lvzhen Huang, K.S.S., P.C., Jiemin Liao, P.G.O., Y.Y.T. and C.C.K. analysed the data. C.-Y.C., K.Y., L.J.C., J.A., Lulin Huang, Lvzhen Huang, E.S.T., X.X.L., Z.Y., K.H.P., C.P.P., N.Y., T.Y.W. and C.C.K. interpreted the data. C.-Y.C., K.Y., L.J.C., J.A., E.S.T., T.Y.W. and C.C.K. drafted the paper. All the authors contributed to revision of the paper.

Additional information

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Corrigendum: New loci and coding variants confer risk for age-related macular degeneration in East Asians

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