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ARTICLE

Association of coding and UTR variants in the known regions with wet age-related macular degeneration in Han Chinese population

Lulin Huang[1] · Xiongze Zhang[2] · Pancy O S Tam[3] · Haoyu Chen[4] · Fang Hao[1] · Chi-Pui Pang[3] · Fen Wen[2] · Zhenglin Yang[1,5,6]

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Abstract

Age-related macular degeneration (AMD) is the leading cause worldwide of severe visual impairment among people older than 55 years of age. This study aimed to investigate the genetic association between coding and untranslated region (UTR) variants in previously reported loci and exudative age-related macular degeneration (wet AMD) in a Han Chinese population. Using our previously published whole exome sequencing dataset of 349 wet AMD patients and 1253 controls, we searched for associations between coding and UTR variants of the 72 genes located within the 47 reported wet AMD loci regions. From these, 25 variants in 18 of the 72 genes with $P < 10 \times 10^{-3}$ were selected for the first replication of Sequenom mass-array genotyping in 885 wet AMD subjects and 562 controls. Next, four SNPs were selected for further validation by SNaPshot genotyping in a third Chinese cohort with 456 wet AMD subjects and 211 controls. As a result, we identified two new potential coding and UTR variant SNPs (rs189132250 in *BBX* located in 3q12.1 and rs144351944 in *FILIP1L* located in 3q12.1) that showed weak associations with wet AMD in the Han Chinese population. These findings provide new information regarding the coding and UTR variants of the known wet AMD loci in the studied Chinese cohort.

Introduction

Age-related macular degeneration (AMD) is the leading cause worldwide of severe visual impairment among people older than 55 years of age [1]. Its prevalence is likely to increase with the exponential growth in the aging population [1, 2]. Advanced AMD can be divided into two subtypes based on the nature and extent of the patient's retinal damage: dry AMD (also called geographic atrophy, GA) and wet AMD (also called neovascular AMD). Wet AMD is the main type of advanced AMD in Asia, while GA rarely occurs in the Han Chinese population [2]. Wet AMD causes vision loss due to abnormal blood vessel growth in the choriocapillaris and retina; it leads to more vision loss than the dry form of the disease [3]. Wet AMD can be further classified into choroidal neovascularization (CNV) and polypoidal choroidal vasculopathy (PCV). The latter is markedly more common in Asia than in Europe [2]. Wet AMD is a complex disease caused by a combination of genetic and environmental factors [4]. In 2005 and 2006, complement factor H (*CFH*) [5–7] and *ARMS2/HTRA1* [8, 9] were identified as two significant AMD risk loci by genome wide association analysis (GWAS). Thus far, at least 59 loci have been associated with AMD (Table S1).

The association of coding and untranslated region (UTR) variants with wet AMD is often ethnicity-specific. Some of the coding variants, such as rs9332739 (E318D) in *C2* [10], rs2230199 (R102G) and rs1047286 (P314L) in *C3* [11], and rs4151667 (L9H) and rs641153 (R32Q) in *CFB* [12], have only been validated in Caucasians, whereas rs2303790 (D442G) in *CETP* [13] has only been validated in East Asians. Most of the reported SNPs associated with AMD are not in the coding and UTR gene regions.

The coding and UTR variants in the reported AMD loci have not yet been systematically investigated in Han

These authors contributed equally: Lulin Huang, Xiongze Zhang.

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✉ Fen Wen
wenfeng208@foxmail.com

✉ Zhenglin Yang
zliny@yahoo.com

Extended author information available on the last page of the article

Table 1 Sample collections of the cohorts for wet AMD (PCV and CNV) cases and controls

Stage	Sample information	PCV	CNV	AMD (PCV + CNV)	Controls
Exome sequencing stage (ref14.)	No. of samples	194	155	349	1250
	Age, years	67.7 ± 9.04 (43.0–90.0)	74.9 ± 7.8 (50.0–94.0)	70.9 ± 8.5 (43.0–94.0)	68.5 ± 9.0 (50.0–87.0)
	Percent female (%)	33	45	38	39
First replication stage	No. of samples	523	362	885	572
	Age, years	65 ± 9.0 (42.0–85.0)	67 ± 9.2 (46.0–89.0)	65.8 ± 9.1 (42.0–89.0)	69.2 ± 9.0 (60.0–89.0)
	Percent female (%)	33	37	35	39
Second replication stage	No. of samples	241	215	456	211
	Age, years	63 ± 10.9 (36.0–83.0)	67 ± 10.0 (42.0–90.0)	65.8 ± 9.1 (42.0–89.0)	69.0 ± 8.9 (50.0–93.0)
	Percent female (%)	33	37	35	39
Combined	No. of samples	958	732	1690	2033
	Age, years	65.7 ± 9.3 (36.0–90.0)	69.4 ± 9.0 (42.0–90.4)	67.2 ± 8.9 (42.0–94)	68.7 ± 9.0 (50.0–93.0)
	Percent female (%)	33	39	36	39

Chinese subjects. In our previous study of whole exome sequencing (WES) for wet AMD, we only reported on the SNP/loci that surpassed the genome-wide significance association ($P < 4.24 \times 10^{-7}$) in the discovery phase [14]. Based on our previous WES work for the wet AMD study [14], we systematically investigated the contribution of coding and UTR variants in the 72 genes located within the 47 reported wet AMD genetic susceptibility loci in a Han Chinese population.

Methods

Ethics statement

The study protocol was approved by Sichuan Provincial People's Hospital's Ethics Committee, Sun Yat-sen University's Ethics Committee, Chinese University of Hong Kong's Ethics Committee, the Joint Shantou International Eye Center of Shantou University and Chinese University of Hong Kong's Ethics Committee. Written informed consent was obtained from all participants involved in this study. All the experiments were performed in accordance with the relevant guidelines and regulations, including any relevant details.

Study sample descriptions

All study subjects were Han Chinese from South China. The subjects were unrelated and were recruited from the Department of Ophthalmology of the Sichuan Provincial People's Hospital, Zhongshan Ophthalmic Center, Chinese University of Hong Kong, and the Joint Shantou International Eye Center, as previously described [14]. The patients received complete ophthalmic investigations, including fluorescein angiography and indocyanine green angiography (ICGA). PCV was diagnosed based on the onchoroidal origin of the polypoidal lesions detected with ICGA. CNV and PCV were distinguished using the fluorescein angiography and ICGA findings. The control subjects were ≥50 years old and also received ophthalmic examinations. Those with macular degeneration from any cause were excluded from the study.

During the first stage of the study, we analyzed the genomic sequences from 349 patients with wet AMD (Table 1). There were 194 patients with PCV, and 155 patients with CNV. During the replication stages, we analyzed the genomic sequences from 1341 patients (764 with PCV and 577 with CNV). This study included a higher number of male patients (60–70%), which is compatible with previous studies that have shown that men are more susceptible than women to wet AMD.

WES

WES was performed in the discovery stage as previously described [14].

Inclusion of genes from the previously reported AMD association studies

Through a literature search, we identified 47 reported loci associated with AMD that involved 72 genes (Table S1). Using our previously published WES results, we analyzed

the coding and UTR variants of these 72 genes using the cutoff value of $P < 10 \times 10^{-3}$ for wet AMD.

Genotyping

The Sequenom mass-array system and SNaPshot genotyping were used for the replication studies.

Statistical analysis

Single-variant association analysis for SNPs was performed with case-control association analysis and Fisher's exact test model using PLINK version 1.9. The parameters included minor-allele frequency (MAF) cutoffs >0.01, genotypic success rate >90%, individual call rate >90% and Hardye Weinberg equilibrium $>1 \times 10^{-4}$. The combined meta-analysis power was calculated across the WES discovery and the replication collections using PLINK version 1.9.

Power calculation

The power calculations [15] of the collected data indicated that there was a 99% probability of detecting loci at $P < 1 \times 10^{-3}$ with a MAF of 30% if the per-allele OR was 1.30 and the disease prevalence was 5% in the multiplicative genetic model for the entire sample (1690 wet AMD cases and 2033 controls). For the discovery stage (349 wet AMD cases and 1250 controls), the power calculations indicated that there was 92% probability of detecting loci at $P < 1 \times 10^{-3}$ with a MAF of 30% if the per-allele OR was 1.30 and the disease prevalence was 5% in the multiplicative genetic model.

Results

In the coding and UTR regions, a previously reported SNP (rs10490924, p.A69S in *ARMS2*) at the *ARMS2/HTRA1* loci showed the strongest association with wet AMD, with no significant differences between PCV and CNV ($P = 2.37 \times 10^{-18}$, OR = 2.11 for wet AMD; $P = 2.12 \times 10^{-9}$, OR = 1.93 for PCV; $P = 2.05 \times 10^{-9}$, OR = 2.42 for CNV). The following four previously reported SNPs in the *CFH* gene had strong associations with wet AMD, and again there were no significant differences between PCV and CNV: rs3753396 (p.Q672Q) ($P = 2.60 \times 10^{-10}$, OR = 1.35 for wet AMD; $P = 7.35 \times 10^{-8}$, OR = 1.80 for PCV; and $P = 2.22 \times 10^{-5}$, OR = 1.90 for CNV), rs1065489 (p.E936D) ($P = 4.21 \times 10^{-10}$, OR = 2.53 for wet AMD; $P = 1.21 \times 10^{-7}$, OR = 1.84 for PCV; and $P = 2.71 \times 10^{-5}$, OR = 2.01 for CNV), rs2274700 (p.A473A)($P = 8.21 \times 10^{-10}$, OR = 1.81 for wet AMD; $P = 1.33 \times 10^{-6}$, OR = 1.75 for PCV; and $P = 4.31 \times 10^{-6}$, OR = 1.89 for CNV), and rs800292 (p.V62I) ($P = 1.83 \times 10^{-7}$, OR = 1.74 for wet AMD; $P = 2.32 \times 10^{-5}$, OR = 2.05 for PCV; and $P = 3.11 \times 10^{-5}$, OR = 1.80 for CNV).

In addition to these known SNPs, another 25 SNPs showed an association with wet AMD ($P < 10 \times 10^{-3}$) in the coding and UTR regions; these 25 SNPs have not been previously reported in Chinese and/or Asian populations (Table 2). Using the Sequenom mass-array method, we searched for these SNPs in another Chinese cohort of 885 wet AMD patients and 572 controls (Table 1) whose members were recruited from the Guangdong Province. Table 3 presents the results of this replication. In this stage, rs2736911 (c.C112T, p.R38X) in *ARMS2* showed the strongest association with wet AMD ($P = 9.95 \times 10^{-4}$, OR = 0.69). Interestingly, CNV ($P = 2.69 \times 10^{-5}$, OR = 0.54), not PCV ($P = 0.12$, OR = 0.82), was the main contributor to this association (Table 3). SNPs in *FILIP1L* and *BBX* also showed nominal associations.

When we combined the findings from both the discovery and replication stages (Table 4), rs2736911, located in *ARMS2*, was still strongly associated with wet AMD ($P = 2.34 \times 10^{-6}$, OR = 0.66), and there was a stronger association with the CNV subtype ($P = 8.51 \times 10^{-6}$, OR = 0.66) than the PCV subtype ($P = 0.011$, OR = 0.77). Four other novel SNPs in three loci, including rs189132250 in *BBX* (3q12.1), rs6078 in *LIPC* (15q21.3), rs144351944 in *FILIP1L* (3q12.1) and rs1800978 in *ABCA1* (9q31.1) showed a weak association with wet AMD (Table 4).

For the four newly-discovered SNPs in these three loci (rs189132250, rs6078, rs144351944, and rs1800978), we performed a second replication in another independent Chinese cohort from Shantou in the Guangdong Province (456 wet AMD patients, including 241 PCV cases and 215 CNV cases, and 211 controls). The results are shown in Table 6. The rs189132250 variant in *BBX* and the rs1800978 variant in *ABCA1* showed weak associations with wet AMD (Table 5).

Combining the results of the discovery, first replication and second replication stages (Table 6) revealed that rs189132250 in *BBX* (c.A1329G, p.A443A) was associated with wet AMD ($P = 8.76 \times 10^{-5}$, OR = 6.0) and that it had a stronger association with CNV ($P = 8.56 \times 10^{-7}$, OR = 9.8) than with PCV ($P = 0.02$, OR = 3.8). The rs144351944 variant in the UTR5 of *FILIP1L* was associated with wet AMD, with PCV, not CNV, being the main contributor to the association ($P = 8.44 \times 10^{-4}$, OR = 1.34 for wet AMD; $P = 4.91 \times 10^{-5}$, OR = 1.52 for PCV; and $P = 0.45$, OR = 1.26 for CNV). Similarly, the rs1800978 variant in the UTR5 of *ABCA1* was also associated with wet AMD, with PCV, not CNV, being the main contributor to the association ($P = 4.14 \times 10^{-4}$, OR = 1.26 for wet AMD; $P = 1.85 \times 10^{-4}$, OR = 1.33 for PCV; and $P = 0.015$,

Table 2 Allelic association of the selected genes in the discovery stage (Exome Sequencing stage) ($P < 10^{-3}$ for wet AMD)

Gene	Chr	BP (hg19)	SNP	Region	Changes	A1	A2	Disease	F_A	F_U	*P*	OR	95% CI	FDR	Case (A1/A2)[a]	Control (A1/A2)[b]
CFH	1	196621093	1:196621093	UTR5	NA	T	A	PCV	0.015	0.043	8.50×10^{-3}	0.35	0.15–0.77	1.12×10^{-2}	6/382	108/2392
CFH	1	196621093	1:196621093	UTR5	NA	T	A	CNV	0.024	0.043	0.16	0.53	0.20–1.30	0.16	8/302	108/2392
CFH	1	196621093	1:196621093	UTR5	NA	T	A	AMD	0.021	0.043	7.60×10^{-3}	0.48	0.27–0.81	1.06×10^{-2}	14/684	108/2392
CR1	1	207753621	rs2274567	Exonic	c.A4973G,p.H1658R	G	A	PCV	0.307	0.207	1.06×10^{-4}	1.69	1.28–2.40	2.75×10^{-4}	120/268	518/1982
CR1	1	207753621	rs2274567	Exonic	c.A4973G,p.H1658R	G	A	CNV	0.358	0.207	3.08×10^{-6}	2.14	1.35–3.15	1.93×10^{-5}	110/200	518/1982
CR1	1	207753621	rs2274567	Exonic	c.A4973G,p.H1658R	G	A	AMD	0.308	0.207	2.01×10^{-6}	1.7	1.36–2.23	1.68×10^{-5}	214/484	518/1982
CR1	1	207782931	rs6691117	Exonic	c.A6193G,p.I2065V	G	A	PCV	0.317	0.23	1.80×10^{-4}	1.56	1.20–2.24	4.09×10^{-4}	122/266	576/1926
CR1	1	207782931	rs6691117	Exonic	c.A6193G,p.I2065V	G	A	CNV	0.368	0.23	6.30×10^{-6}	1.95	1.21–2.84	3.15×10^{-5}	114/196	576/1926
CR1	1	207782931	rs6691117	Exonic	c.A6193G,p.I2065V	G	A	AMD	0.319	0.23	1.29×10^{-6}	1.57	1.25–2.05	1.61×10^{-5}	222/476	576/1926
CR1	1	207790088	rs3811381	Exonic	c.C6830G,p.P2277R	G	C	PCV	0.304	0.204	9.28×10^{-4}	1.7	1.33–2.49	1.78×10^{-3}	118/270	510/1990
CR1	1	207790088	rs3811381	Exonic	c.C6830G,p.P2277R	G	C	CNV	0.363	0.204	6.96×10^{-5}	2.22	1.35–3.20	2.49×10^{-4}	112/198	510/1990
CR1	1	207790088	rs3811381	Exonic	c.C6830G,p.P2277R	G	C	AMD	0.307	0.204	1.14×10^{-6}	1.72	1.37–2.26	1.61×10^{-5}	214/484	510/1990
FILIP1L	3	64672474	rs793440	Exonic	c.G503A,p.R168H	T	A	PCV	0.054	0.088	2.50×10^{-2}	0.59	1.02–1.96	2.98×10^{-2}	20/368	220/2280
FILIP1L	3	64672474	rs793440	Exonic	c.G503A,p.R168H	T	A	CNV	0.042	0.088	2.20×10^{-2}	0.46	1.09–2.48	2.75×10^{-2}	14/296	220/2280
FILIP1L	3	64672474	rs793440	Exonic	c.G503A,p.R168H	T	A	AMD	0.05	0.088	1.10×10^{-3}	0.55	0.98–1.64	1.83×10^{-3}	34/664	220/2280
FILIP1L	3	99643176	rs144351944	UTR5	NA	T	C	PCV	0.245	0.171	4.10×10^{-4}	1.57	1.17–3.19	8.54×10^{-4}	96/292	428/2072
FILIP1L	3	99643176	rs144351944	UTR5	NA	T	C	CNV	0.226	0.171	4.10×10^{-2}	1.42	1.01–3.65	4.66×10^{-2}	70/240	428/2072
FILIP1L	3	99643176	rs144351944	UTR5	NA	T	C	AMD	0.221	0.171	2.50×10^{-3}	1.37	1.22–2.77	3.91×10^{-3}	154/544	428/2072
BBX	3	99832939	rs189132250	Exonic	c.A1329G,p.A443A	G	T	PCV	0.005	0.002	0.15	3.24	0.59–17.88	0.16	2/386	6/2496
BBX	3	99832939	rs189132250	Exonic	c.A1329G,p.A443A	G	T	CNV	0.019	0.002	8.29×10^{-5}	12.03	3.02–49.68	2.59×10^{-4}	6/304	6/2496
BBX	3	99832939	rs189132250	Exonic	c.A1329G,p.A443A	G	T	AMD	0.01	0.002	1.10×10^{-3}	6.32	1.53–19.47	1.83×10^{-3}	6/692	6/2496
ADAMTS9	3	107491897	rs36115950	Exonic	c.T286A,p.S96T,	G	A	PCV	0.005	0.002	0.15	3.24	0.41–1.06	0.16	2/386	6/2496
ADAMTS9	3	107491897	rs36115950	Exonic	c.T286A,p.S96T,	G	A	CNV	0.019	0.002	8.29×10^{-6}	12.03	0.25–1.01	3.45×10^{-5}	6/304	6/2496
ADAMTS9	3	107491897	rs36115950	Exonic	c.T286A,p.S96T,	G	A	AMD	0.009	0.002	3.30×10^{-3}	5.42	0.41–0.88	4.85×10^{-3}	6/692	6/2496
PRLR	5	35060054	rs112461	UTR3	NA	G	C	PCV	0.183	0.276	1.10×10^{-4}	0.59	0.48–0.92	2.75×10^{-4}	72/316	690/1810
PRLR	5	35060054	rs112461	UTR3	NA	G	C	CNV	0.208	0.276	3.10×10^{-2}	0.69	0.49–1.14	4.08×10^{-2}	64/246	690/1810
PRLR	5	35060054	rs112461	UTR3	NA	G	C	AMD	0.202	0.276	7.90×10^{-5}	0.66	0.58–0.97	9.88×10^{-4}	140/558	690/1810
PRLR	5	35063292	rs139097019	UTR3	NA	T	A	PCV	0.17	0.256	2.80×10^{-4}	0.6	1.11–6.65	1.75×10^{-3}	66/322	640/1860
PRLR	5	35063292	rs139097019	UTR3	NA	T	A	CNV	0.193	0.256	4.40×10^{-2}	0.7	1.68–11.31	5.50×10^{-2}	60/250	640/1860
PRLR	5	35063292	rs139097019	UTR3	NA	T	A	AMD	0.188	0.256	1.90×10^{-4}	0.67	1.35–5.85	1.58×10^{-3}	132/566	640/1860
SPEF2	5	35063836	rs34708521	Exonic	c.G1498A,p.D500N	T	C	PCV	0.018	0.007	2.20×10^{-2}	2.69	0.90–1.72	3.06×10^{-2}	6/382	18/2482
SPEF2	5	35063836	rs34708521	Exonic	c.G1498A,p.D500N	T	C	CNV	0.028	0.007	1.00×10^{-3}	4.26	0.78–1.83	4.17×10^{-3}	8/302	18/2482
SPEF2	5	35063836	rs34708521	Exonic	c.G1498A,p.D500N	T	C	AMD	0.019	0.007	4.00×10^{-3}	2.78	0.90–1.49	7.69×10^{-3}	14/684	18/2482
SPEF2	5	35670303	rs185752523	Exonic	c.G2524A,p.D842N	A	G	PCV	0.343	0.271	3.30×10^{-3}	1.4	0.72–10.90	7.50×10^{-3}	134/254	678/1822

Table 2 (continued)

Gene	Chr	BP (hg19)	SNP	Region	Changes	A1	A2	Disease	F_A	F_U	*P*	OR	95% CI	FDR	Case (A1/A2)[a]	Control (A1/A2)[b]
SPEF2	5	35670303	rs185752523	Exonic	c.G2524A,p.D842N	A	G	CNV	0.344	0.271	2.10×10^{-2}	1.41	3.52–32.40	3.06×10^{-2}	106/204	678/1822
SPEF2	5	35670303	rs185752523	Exonic	c.G2524A,p.D842N	A	G	AMD	0.324	0.271	6.10×10^{-3}	1.29	1.74–12.74	1.02×10^{-2}	226/472	678/1822
ADAM19	5	35705769	rs1422795	Exonic	c.A850G,p.S284G	A	G	PCV	0.008	0.003	0.12	2.78	0.44–0.96	0.14	4/384	8/2492
ADAM19	5	35705769	rs1422795	Exonic	c.A850G,p.S284G	A	G	CNV	0.028	0.003	2.35×10^{-7}	10.4	0.61–1.53	5.88×10^{-6}	8/302	8/2492
ADAM19	5	35705769	rs1422795	Exonic	c.A850G,p.S284G	A	G	AMD	0.013	0.003	8.10×10^{-4}	4.66	0.54–0.98	4.05×10^{-3}	10/688	8/2492
PRLR	5	156936364	rs249524	UTR3	NA	C	T	PCV	0.095	0.148	5.50×10^{-3}	0.61	0.48–0.91	9.82×10^{-3}	36/352	370/2130
PRLR	5	156936364	rs249524	UTR3	NA	C	T	CNV	0.132	0.148	0.52	0.88	0.40–0.97	0.52	40/270	370/2130
PRLR	5	156936364	rs249524	UTR3	NA	C	T	AMD	0.105	0.148	3.20×10^{-3}	0.67	0.52–0.87	7.50×10^{-3}	74/624	370/2130
COL10A1	6	116440811	rs189601123	UTR3	NA	G	A	PCV	0.018	0.004	1.40×10^{-3}	4.17	1.62–11.04	5.00×10^{-3}	6/382	10/2490
COL10A1	6	116440811	rs189601123	UTR3	NA	G	A	CNV	0.014	0.004	5.60×10^{-2}	3.26	0.90–11.97	6.67×10^{-2}	4/306	10/2490
COL10A1	6	116440811	rs189601123	UTR3	NA	G	A	AMD	0.014	0.004	4.00×10^{-3}	3.3	1.40–7.91	7.69×10^{-3}	10/688	10/2490
PILRA	7	99971313	rs2405442	Exonic	c.T34C,p.L12L	C	T	PCV	0.325	0.406	2.30×10^{-3}	0.7	0.47–0.89	6.39×10^{-3}	126/262	1016/1486
PILRA	7	99971313	rs2405442	Exonic	c.T34C,p.L12L	C	T	CNV	0.373	0.406	0.34	0.87	0.44–1.05	0.35	116/194	1016/1486
PILRA	7	99971313	rs2405442	Exonic	c.T34C,p.L12L	C	T	AMD	0.35	0.406	7.10×10^{-3}	0.79	0.53–0.89	1.11×10^{-2}	244/454	1016/1486
PILRA	7	99971834	rs1859788	Exonic	c.A232G,p.R78G	G	A	PCV	0.325	0.407	2.00×10^{-3}	0.7	0.46–0.87	6.25×10^{-3}	126/262	1018/1482
PILRA	7	99971834	rs1859788	Exonic	c.A232G,p.R78G	G	A	CNV	0.373	0.407	0.32	0.87	0.43–1.03	0.35	116/194	1018/1482
PILRA	7	99971834	rs1859788	Exonic	c.A232G,p.R78G	G	A	AMD	0.352	0.407	9.00×10^{-3}	0.79	0.51–0.86	1.58×10^{-2}	246/452	1018/1482
ABCA1	9	107544685	rs4149340	UTR3	NA	A	G	PCV	0.268	0.174	1.10×10^{-5}	1.73	1.25–3.37	2.75×10^{-4}	104/284	436/2066
ABCA1	9	107544685	rs4149340	UTR3	NA	A	G	CNV	0.231	0.174	3.80×10^{-2}	1.42	1.04–2.39	5.00×10^{-2}	72/238	436/2066
ABCA1	9	107544685	rs4149340	UTR3	NA	A	G	AMD	0.244	0.174	3.70×10^{-5}	1.52	1.19–1.98	4.63×10^{-4}	170/528	436/2066
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	PCV	0.209	0.138	2.80×10^{-4}	1.64	1.32–2.55	2.33×10^{-3}	82/306	346/2156
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	CNV	0.184	0.138	6.80×10^{-2}	1.4	1.00–2.39	8.07×10^{-2}	58/252	346/2156
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	AMD	0.188	0.138	1.20×10^{-3}	1.44	1.20–2.04	6.00×10^{-3}	132/566	346/2156
KIAA1958	9	115421749	rs139390384	Exonic	c.G1551C,p.L517L	C	G	PCV	0.01	0.002	6.20×10^{-3}	5.21	1.40–19.74	1.19×10^{-2}	4/384	6/2496
KIAA1958	9	115421749	rs139390384	Exonic	c.G1551C,p.L517L	C	G	CNV	0.009	0.002	4.00×10^{-2}	4.76	0.92–25.04	5.00×10^{-2}	2/308	6/2496
KIAA1958	9	115421749	rs139390384	Exonic	c.G1551C,p.L517L	C	G	AMD	0.01	0.002	2.10×10^{-3}	5.07	1.61–16.20	6.94×10^{-3}	6/692	6/2496
ARMS2	10	124214355	rs2736911	Exonic	c.C112T,p.R38X	T	C	PCV	0.093	0.133	2.70×10^{-2}	0.67	0.40–0.89	3.97×10^{-2}	36/352	332/2168
ARMS2	10	124214355	rs2736911	Exonic	c.C112T,p.R38X	T	C	CNV	0.09	0.133	7.10×10^{-2}	0.64	0.42–1.16	8.07×10^{-2}	28/282	332/2168
ARMS2	10	124214355	rs2736911	Exonic	c.C112T,p.R38X	T	C	AMD	0.085	0.133	5.60×10^{-4}	0.6	0.42–0.79	3.50×10^{-3}	60/638	332/2168
STXBP6	14	25281843	rs712491	UTR3	NA	T	C	PCV	0.358	0.306	3.90×10^{-2}	1.27	1.02–1.95	5.00×10^{-2}	138/250	766/1736
STXBP6	14	25281843	rs712491	UTR3	NA	T	C	CNV	0.41	0.306	1.60×10^{-3}	1.58	0.85–2.07	6.67×10^{-3}	128/182	766/1736
STXBP6	14	25281843	rs712491	UTR3	NA	T	C	AMD	0.362	0.306	4.60×10^{-3}	1.29	1.02–1.69	1.15×10^{-2}	252/446	766/1736
LIPC	15	58833993	rs6078	Exonic	c.G283A,p.V95M	A	G	PCV	0.379	0.315	0.12	1.33	0.91–2.75	0.13	148/240	788/1712
LIPC	15	58833993	rs6078	Exonic	c.G283A,p.V95M	A	G	CNV	0.363	0.315	1.20×10^{-2}	1.85	0.63–2.50	1.88×10^{-2}	112/198	788/1712

Table 2 (continued)

Gene	Chr	BP (hg19)	SNP	Region	Changes	A1	A2	Disease	F_A	F_U	*P*	OR	95% CI	FDR	Case (A1/A2)[a]	Control (A1/A2)[b]
LIPC	15	58833993	rs6078	Exonic	c.G283A,p.V95M	A	G	AMD	0.375	0.315	2.50×10^{-3}	1.87	0.92–2.53	6.94×10^{-3}	262/436	788/1712
TMEM97	17	26654350	17:26654350	UTR3	NA	G	T	PCV	0.031	0.072	2.40×10^{-3}	0.41	0.21–0.72	6.94×10^{-3}	12/376	180/2320
TMEM97	17	26654350	17:26654350	UTR3	NA	G	T	CNV	0.071	0.072	0.93	0.98	0.55–1.72	0.93	22/288	180/2320
TMEM97	17	26654350	17:26654350	UTR3	NA	G	T	AMD	0.043	0.072	5.80×10^{-3}	0.58	0.37–0.84	1.19×10^{-2}	30/668	180/2320
EMID1	22	29655416	rs9537	UTR3	NA	T	C	PCV	0.162	0.225	5.50×10^{-3}	0.67	0.50–1.00	1.19×10^{-2}	62/326	562/1938
EMID1	22	29655416	rs9537	UTR3	NA	T	C	CNV	0.189	0.225	0.22	0.8	0.61–1.44	0.23	58/252	562/1938
EMID1	22	29655416	rs9537	UTR3	NA	T	C	AMD	0.179	0.225	9.50×10^{-3}	0.75	0.61–1.03	1.58×10^{-2}	124/574	562/1938

CHR chromosome, *SNP* SNP ID, *BP* physical position (base-pair), *A1* minor allele name (based on whole sample), *F_A* frequency of this allele in cases, *F_U* frequency of this allele in controls, *A2* major allele name, *P* Fisher's exact test p-value, *OR* estimated odds ratio (for A1), *FDR* Benjamini and Hochberg method controls the False Discovery Rate (FDR) using sequential modified Bonferroni correction for multiple hypothesis testing of P, *OR* odds ratio, *95% CI* 95% confidence interval

[a]Allele count in cases

[b]Allele count in controls

OR = 1.23 for CNV; Table 6). The association of rs6078 variant in *LIPC* with wetAMD is $P = 2.27 \times 10{-}4$, OR = 1.23. However, the random-effects meta-analysis *p*-value P(R) for wetAMD is only 0.403 and the heterogeneity I2 is 94.73 that indicate the inconsistent results between these three cohorts. This result (Table 6) suggested that rs6078 variant in *LIPC* is not associated with wetAMD, CNV or PCV. Supplementary Figure 1 shows the forest plots of the four SNPs from each of the independently analyzed cohorts. According to the ORs, the rs189132250 variant in *BBX* carried a high risk for wet AMD. We also performed a rare variant burden assay for these four genes (with a MAF < 0.01 cut-off for the SNPs) using the SKAT R package; however, no significant signals were found in these genes (Table S2).

We then evaluated the associations between wet AMD and the four SNPs in the three previously reported loci. Using the SNaPshot genotyping method, this analysis identified three new potential SNPs: rs13095226 (3q12.1) [16], rs1883025 (9q31.1) [10], rs493258 [17], and rs10468017 (15q21.3) [10]. No significant associations were identified for these SNPs (Table S3). However, among these four SNPs, the rs1883025 variant in *ABCA1* had an LD (r^2) = 0.7 with the rs1800978 variant (Table S4) and showed a consistent trend of association with PCV ($P = 0.08$, OR = 1.24), as did rs1800978 ($P = 3.0 \times 10^{-4}$, OR = 1.33).

Discussion

The currently available data indicate that genetic influences are responsible for up to 70% of a patient's AMD risk [18]. Consequently, research should focus on the functional effects of AMD-associated gene variants, particularly the effects of coding and UTR variants, in AMD progression. Our wet AMD studies in China have demonstrated the association of wet AMD with SNPs in the coding and UTR regions of the GWAS-reported loci. This study validated the wet AMD associations of the five previously reported SNPs (rs10490924 in the *ARMS2/HTRA1* region [14, 19] and rs3753396 [14, 20], rs1065489 [14, 21], rs2274700 [14, 22], and rs800292 [14, 23] in *CFH*). The rs77466370 variant in the *FGD6* gene was associated with wet AMD due to the PCV contribution ($P = 7.75 \times 10^{-6}$, OR = 1.55), as previously reported [14]. Another SNP, rs2303790 in *CETP*, had a *P* value larger than 1×10^{-3} in the discovery cohort; we confirmed that this SNP was associated with wet AMD in a previous study of a Han Chinese population [13].

In this study, the rs2736911 variant, which encodes a stopgap substitution in *ARMS2* (p.R38X) and is independent of rs10490924, showed an association with wet

Table 3 Allelic association of the selected genes in the replication stage

Gene	Chr	BP (hg19)	SNP	Region	Changes	A1	A2	Disease	F_A	F_U	*P*	OR	FDR	95% CI	Allele count in case (A1/A2)[a]	Allele count in control (A1/A2)[b]
CFH	1	196621093	1:196621093	UTR5	NA	0	A	PCV	0	0	NA	NA	NA	NA	0/1046	0/1144
CFH	1	196621093	1:196621093	UTR5	NA	0	A	CNV	0	0	NA	NA	NA	NA	0/724	0/1144
CFH	1	196621093	1:196621093	UTR5	NA	0	A	AMD	0	0	NA	NA	NA	NA	0/1770	0/1144
CR1	1	207753621	rs2274567	Exonic	c.A4973G, p. H1658R	G	A	PCV	0.33	0.36	0.14	0.87	0.32	0.73–1.05	345/701	412/732
CR1	1	207753621	rs2274567	Exonic	c.A4973G, p. H1658R	G	A	CNV	0.343	0.36	0.42	0.93	0.53	0.77–1.12	248/476	412/732
CR1	1	207753621	rs2274567	Exonic	c.A4973G, p. H1658R	G	A	AMD	0.336	0.36	0.17	0.9	0.33	0.77–1.05	595/1175	412/732
CR1	1	207782931	rs6691117	Exonic	c.A6193G, p. I2065V	G	A	PCV	0.352	0.373	0.31	0.91	0.43	0.77–1.09	368/678	427/717
CR1	1	207782931	rs6691117	Exonic	c.A6193G, p. I2065V	G	A	CNV	0.357	0.373	0.48	0.94	0.57	0.78–1.13	258/466	427/717
CR1	1	207782931	rs6691117	Exonic	c.A6193G, p. I2065V	G	A	AMD	0.354	0.373	0.31	0.92	0.43	0.79–1.08	627/1143	427/717
CR1	1	207790088	rs3811381	Exonic	c.C6830G, p. P2277R	G	C	PCV	0.329	0.352	0.25	0.9	0.42	0.75–1.08	344/702	403/741
CR1	1	207790088	rs3811381	Exonic	c.C6830G, p. P2277R	G	C	CNV	0.34	0.352	0.58	0.95	0.66	0.78–1.15	246/478	403/741
CR1	1	207790088	rs3811381	Exonic	c.C6830G, p. P2277R	G	C	AMD	0.334	0.352	0.3	0.92	0.43	0.79–1.08	591/1179	403/741
ADAMTS9	3	64672474	rs36115950	Exonic	c.T286A, p. S96T,	T	A	PCV	0.067	0.051	0.11	1.34	0.32	0.93–1.93	70/976	58/1086
ADAMTS9	3	64672474	rs36115950	Exonic	c.T286A, p. S96T,	T	A	CNV	0.067	0.051	0.12	1.35	0.32	0.92–1.97	49/675	58/1086
ADAMTS9	3	64672474	rs36115950	Exonic	c.T286A, p. S96T,	T	A	AMD	0.067	0.051	0.072	1.34	0.26	0.97–1.86	119/1651	58/1086
FILIP1L	3	99643176	rs793440	Exonic	c.G503A, p. R168H	T	C	PCV	0.207	0.205	0.92	1.01	0.92	0.82–1.25	217/829	235/909
FILIP1L	3	99643176	rs793440	Exonic	c.G503A, p. R168H	T	C	CNV	0.231	0.205	0.17	1.16	0.33	0.93–1.44	167/557	235/909
FILIP1L	3	99643176	rs793440	Exonic	c.G503A, p. R168H	T	C	AMD	0.217	0.205	0.42	1.08	0.53	0.9–1.29	384/1386	235/909
FILIP1L	3	99832939	rs144351944	UTR5	NA	G	T	PCV	0.062	0.042	0.035	1.51	0.18	1.03–2.23	65/981	48/1096
FILIP1L	3	99832939	rs144351944	UTR5	NA	G	T	CNV	0.04	0.042	0.86	0.96	0.90	0.61–1.51	29/695	48/1096
FILIP1L	3	99832939	rs144351944	UTR5	NA	G	T	AMD	0.052	0.042	0.19	1.26	0.34	0.88–1.81	92/1678	48/1096
BBX	3	107491897	rs189132250	Exonic		G	A	PCV	0.004	0.001	0.14	4.43	0.32	0.49–39.7	4/1042	1/1143

Table 3 (continued)

Gene	Chr	BP (hg19)	SNP	Region	Changes	A1	A2	Disease	F_A	F_U	*P*	OR	FDR	95% CI	Allele count in case (A1/A2)[a]	Allele count in control (A1/A2)[b]
					c.A1329G, p. A443A											
BBX	3	107491897	rs189132250	Exonic	c.A1329G, p. A443A	G	A	CNV	0.007	0.001	0.019	8.28	0.12	0.99–68.89	5/719	1/1143
BBX	3	107491897	rs189132250	Exonic	c.A1329G, p. A443A	G	A	AMD	0.005	0.001	0.048	6.14	0.20	0.79–48.05	9/1761	1/1143
PRLR	5	35060054	rs249524	UTR3	NA	G	C	PCV	0.229	0.222	0.72	1.04	0.78	0.85–1.27	240/806	254/890
PRLR	5	35060054	rs249524	UTR3	NA	G	C	CNV	0.234	0.222	0.54	1.07	0.74	0.86–1.32	169/555	254/890
PRLR	5	35060054	rs249524	UTR3	NA	G	C	AMD	0.231	0.222	0.57	1.05	0.74	0.88–1.26	409/1361	254/890
PRLR	5	35063292	rs112461	UTR3	NA	T	A	PCV	0.216	0.212	0.8	1.03	0.89	0.83–1.26	226/820	243/901
PRLR	5	35063292	rs112461	UTR3	NA	T	A	CNV	0.238	0.212	0.17	1.16	0.55	0.94–1.44	172/552	243/901
PRLR	5	35063292	rs112461	UTR3	NA	T	A	AMD	0.226	0.212	0.37	1.09	0.71	0.91–1.3	400/1370	243/901
PRLR	5	35063836	rs139097019	UTR3	NA	T	C	PCV	0.016	0.023	0.21	0.67	0.55	0.36–1.26	17/1029	26/1118
PRLR	5	35063836	rs139097019	UTR3	NA	T	C	CNV	0.015	0.023	0.18	0.63	0.55	0.31–1.25	11/713	26/1118
PRLR	5	35063836	rs139097019	UTR3	NA	T	C	AMD	0.015	0.023	0.12	0.65	0.55	0.38–1.12	27/1743	26/1118
SPEF2	5	35670303	rs34708521	Exonic	c.G1498A, p. D500N	A	G	PCV	0.322	0.359	0.079	0.85	0.55	0.71–1.02	337/709	411/733
SPEF2	5	35670303	rs34708521	Exonic	c.G1498A, p. D500N	A	G	CNV	0.331	0.359	0.2	0.88	0.55	0.73–1.07	240/484	411/733
SPEF2	5	35670303	rs34708521	Exonic	c.G1498A, p. D500N	A	G	AMD	0.326	0.359	0.071	0.86	0.55	0.74–1.01	577/1193	411/733
SPEF2	5	35705769	rs185752523	Exonic	c.G2524A, p. D842N	A	G	PCV	0.009	0.014	0.24	0.62	0.55	0.27–1.4	9/1037	16/1128
SPEF2	5	35705769	rs185752523	Exonic	c.G2524A, p. D842N	A	G	CNV	0.01	0.014	0.37	0.68	0.71	0.29–1.6	7/717	16/1128
SPEF2	5	35705769	rs185752523	Exonic	c.G2524A, p. D842N	A	G	AMD	0.009	0.014	0.21	0.65	0.55	0.33–1.29	16/1754	16/1128
ADAM19	5	156936364	rs1422795	Exonic	c.A850G, p. S284G	C	T	PCV	0.119	0.111	0.59	1.08	0.74	0.82–1.41	124/922	127/1017
ADAM19	5	156936364	rs1422795	Exonic	c.A850G, p. S284G	C	T	CNV	0.101	0.111	0.48	0.9	0.74	0.67–1.21	73/651	127/1017
ADAM19	5	156936364	rs1422795	Exonic	c.A850G, p. S284G	C	T	AMD	0.111	0.111	0.97	1	0.97	0.78–1.26	196/1574	127/1017
COL10A1	6	116440811	rs189601123	UTR3	NA	G	A	PCV	0.021	0.021	0.91	0.97	0.95	0.53–1.75	22/1024	24/1120
COL10A1	6	116440811	rs189601123	UTR3	NA	G	A	CNV	0.009	0.021	0.025	0.39	0.55	0.17–0.92	7/717	24/1120
COL10A1	6	116440811	rs189601123	UTR3	NA	G	A	AMD	0.015	0.021	0.22	0.71	0.55	0.41–1.23	27/1743	24/1120

Table 3 (continued)

Gene	Chr	BP (hg19)	SNP	Region	Changes	A1	A2	Disease	F_A	F_U	*P*	OR	FDR	95% CI	Allele count in case (A1/A2)[a]	Allele count in control (A1/A2)[b]
PILRA	7	99971313	rs2405442	Exonic	c.T34C, p.L12L	C	T	PCV	0.366	0.382	0.47	0.94	0.74	0.79–1.12	383/663	437/707
PILRA	7	99971313	rs2405442	Exonic	c.T34C, p.L12L	C	T	CNV	0.377	0.382	0.82	0.98	0.89	0.81–1.18	273/451	437/707
PILRA	7	99971313	rs2405442	Exonic	c.T34C, p.L12L	C	T	AMD	0.371	0.382	0.56	0.96	0.74	0.82–1.11	657/1113	437/707
PILRA	7	99971834	rs1859788	Exonic	c.A232G, p.R78G	G	A	PCV	0.367	0.382	0.49	0.94	0.74	0.79–1.12	384/662	437/707
PILRA	7	99971834	rs1859788	Exonic	c.A232G, p.R78G	G	A	CNV	0.374	0.382	0.74	0.97	0.88	0.81–1.17	271/453	437/707
PILRA	7	99971834	rs1859788	Exonic	c.A232G, p.R78G	G	A	AMD	0.371	0.382	0.54	0.95	0.66	0.82–1.11	657/1113	437/707
ABCA1	9	107544685	rs4149340	UTR3	NA	A	G	PCV	0.188	0.226	0.029	0.79	0.10	0.64–0.98	197/849	259/885
ABCA1	9	107544685	rs4149340	UTR3	NA	A	G	CNV	0.236	0.226	0.61	1.06	0.66	0.85–1.31	171/553	259/885
ABCA1	9	107544685	rs4149340	UTR3	NA	A	G	AMD	0.209	0.226	0.27	0.91	0.52	0.76–1.08	370/1400	259/885
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	PCV	0.181	0.168	0.42	1.1	0.62	0.88–1.37	189/857	192/952
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	CNV	0.18	0.168	0.48	1.09	0.66	0.86–1.38	130/594	192/952
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	AMD	0.18	0.168	0.37	1.09	0.61	0.9–1.33	319/1451	192/952
KIAA1958	9	115421749	rs139390384	Exonic	c.G1551C, p.L517L	C	G	PCV	0.003	0.005	0.39	0.55	0.61	0.14–2.2	3/1043	6/1138
KIAA1958	9	115421749	rs139390384	Exonic	c.G1551C, p.L517L	C	G	CNV	0.005	0.005	0.88	0.91	0.88	0.26–3.24	4/720	6/1138
KIAA1958	9	115421749	rs139390384	Exonic	c.G1551C, p.L517L	C	G	AMD	0.004	0.005	0.53	0.71	0.66	0.24–2.12	7/1763	6/1138
ARMS2	10	124214355	rs2736911	Exonic	c.C112T, p.R38X	T	C	PCV	0.127	0.15	0.12	0.82	0.27	0.64–1.05	133/913	172/972
ARMS2	10	124214355	rs2736911	Exonic	c.C112T, p.R38X	T	C	CNV	0.087	0.15	2.69×10^{-5}	0.54	1.68×10^{-4}	0.4–0.72	63/661	172/972
ARMS2	10	124214355	rs2736911	Exonic	c.C112T, p.R38X	T	C	AMD	0.109	0.15	9.95×10^{-4}	0.69	4.98×10^{-3}	0.56–0.86	193/1577	172/972
STXBP6	14	25281843	rs712491	UTR3	NA	T	C	PCV	0.317	0.367	0.016	0.8	0.07	0.67–0.96	332/714	420/724
STXBP6	14	25281843	rs712491	UTR3	NA	T	C	CNV	0.355	0.367	0.59	0.9506	0.66	0.79–1.15	257/467	420/724
STXBP6	14	25281843	rs712491	UTR3	NA	T	C	AMD	0.334	0.367	0.071	0.8669	0.22	0.74–1.01	591/1179	420/724
LIPC	15	58833993	rs6078	Exonic	c.G283A, p.V95M	A	G	PCV	0.352	0.363	0.57	0.9502	0.66	0.80–1.13	368/678	415/729
LIPC	15	58833993	rs6078	Exonic	c.G283A, p.V95M	A	G	CNV	0.326	0.363	0.091	0.8489	0.24	0.71–1.03	236/488	415/729
LIPC	15	58833993	rs6078	Exonic	c.G283A, p.V95M	A	G	AMD	0.34	0.363	0.2	0.9041	0.42	0.77–1.06	602/1168	415/729
TMEM97	17	26654350	17:26654350	UTR3	NA	0	T	PCV	0	0	NA	NA	NA	NA	0/1046	0/1144

Table 3 (continued)

Gene	Chr	BP (hg19)	SNP	Region	Changes	A1	A2	Disease	F_A	F_U	*P*	OR	FDR	95% CI	Allele count in case (A1/A2)[a]	Allele count in control (A1/A2)[b]
TMEM97	17	26654350	17:26654350	UTR3	NA	0	T	CNV	0	0	NA	NA	NA	NA	0/724	0/1144
TMEM97	17	26654350	17:26654350	UTR3	NA	0	T	AMD	0	0	NA	NA	NA	NA	0/1770	0/1144
EMID1	22	29655416	rs9537	UTR3	NA	T	C	PCV	0.301	0.269	0.095	1.17	0.24	0.972–1.416	315/731	308/836
EMID1	22	29655416	rs9537	UTR3	NA	T	C	CNV	0.265	0.269	0.86	0.98	0.88	0.8–1.21	192/532	308/836
EMID1	22	29655416	rs9537	UTR3	NA	T	C	AMD	0.285	0.269	0.33	1.09	0.5893	0.92–1.28	504/1266	308/836

CHR chromosome, *SNP* SNP ID, *BP* physical position (base-pair), *A1* minor allele name (based on whole sample), *F_A* frequency of this allele in cases, *F_U* frequency of this allele in controls, *A2* major allele name, *P* Fisher's exact test p-value, *OR* estimated odds ratio (for A1), *FDR* Benjamini and Hochberg method controls the False Discovery Rate (FDR) using sequential modified Bonferroni correction for multiple hypothesis testing of P, *OR* odds ratio, *95% CI* 95% confidence interval

[a]Allele count in cases

[b]Allele count in controls

AMD. The rs2736911 variant was first reported in a Polish population in 2012 [24]. To date, the association of rs2736911 with wet AMD has only been experimentally validated in Korea [25]. In 2015, Zhan Ye et al. indicated that rs2736911 was weakly associated with wet AMD ($P = 0.0011$, OR = 0.69) using a PheWAS analytical approach [26]. However, a meta-analysis of 23 published AMD studies did not find a strong association between rs2736911 and AMD ($P = 0.122$, OR = 0.77) [27]. In the Chinese cohort of the current study, we confirmed that the rs2736911 variant was associated with wet AMD ($P = 2.90 \times 10^{-6}$, OR = 0.66). By comparing the PCV and CNV subtypes, we found that rs2736911 was more effective in protecting against the CNV subtype ($P = 7.57 \times 10^{-6}$, OR = 0.66) than the PCV subtype ($P = 0.012$, OR = 0.77).

The rare variant rs189132250 in *BBX* (c.A1329G, p.A443A) is located in 3q12.1 near *COL8A1*. In the discovery stage of this study, no SNPs in the coding or UTR regions of *COL8A1* showed an association beyond 10^{-3}. The rs189132250 variant showed an association with wet AMD ($P = 8.75 \times 10^{-5}$) and had a stronger association with the CNV subtype ($P = 8.56 \times 10^{-7}$) than with the PCV subtype ($P = 0.183$). *BBX* is a transcription factor that is necessary for cell cycle progression from the G1 phase to the S phase. In a European ancestry GWAS study, Neil Caporaso et al. reported that this gene region was associated with cigarette smoking [28], an environmental risk factor for AMD [29]. The rs189132250 variant in *BBX* increases the risk of wet AMD, implying an interaction between this gene and the smoking environmental factor.

FILIP1L is also located in 3q12.1 near *COL8A1*. The rs144351944 variant in the UTR5 of *FILIP1L* was nominally associated with wet AMD ($P = 8.44 \times 10^{-4}$ for wet AMD; $P = 4.94 \times 10^{-5}$ for PCV; and $P = 0.45$ for CNV). *FILIP1L* has been predicted to regulate the antiangiogenic activity of endothelial cells; when *FILIP1L* was overexpressed in endothelial cells, it inhibited cell proliferation and migration and increased cell apoptosis [30]. *FILIP1L* was previously reported to be strongly expressed in tumor stroma and colon cancer vasculature (at a protein level) [31]. Previous reports have shown that *FILIP1L* is expressed in ovarian epithelial cells and downregulated in cancer [32, 33]. *FILIP1L* may be involved in wet AMD progression through the VEGF signaling pathway [31].

Except for rs1800978, which is in the reported LD of *ABCA1* [10, 34], the three other newly-identified SNPs in this study were not in the same LD as the SNPs reported in the previous studies. This indicates that our WES analysis detected novel sequences that had been missed by GWAS. We could not evaluate the predicted function of these significant SNPs with respect to pathogenesis because the gene expression regulatory SNPs were not captured by WES. The

Table 4 Combined association of the selected genes in the discovery and replication stages by meta-analysis

Gene	Chr	BP (hg19)	SNP	Region	Changes	A1	A2	*N*	Disease	*P*	P(R)	FDR	OR	OR(R)	*Q*	I^2
CR1	1	207753621	rs2274567	Exonic	c.A4973G, p.H1658R	G	A	2	PCV	8.37×10^{-2}	0.568	0.113	1.142	1.209	0.000	94.47
CR1	1	207753621	rs2274567	Exonic	c.A4973G, p.H1658R	G	A	2	CNV	2.37×10^{-3}	0.414	9.09×10^{-3}	1.272	1.405	0.000	96.16
CR1	1	207753621	rs2274567	Exonic	c.A4973G, p.H1658R	G	A	2	AMD	1.15×10^{-2}	0.508	2.55×10^{-2}	1.168	1.234	0.000	96.14
CR1	1	207782931	rs6691117	Exonic	c.A6193G, p.I2065V	G	A	2	PCV	0.165	0.530	0.200	1.104	1.185	0.000	92.4
CR1	1	207782931	rs6691117	Exonic	c.A6193G, p.I2065V	G	A	2	CNV	6.14×10^{-3}	0.413	1.57×10^{-2}	1.239	1.348	0.000	95.13
CR1	1	207782931	rs6691117	Exonic	c.A6193G, p.I2065V	G	A	2	AMD	2.26×10^{-2}	0.497	4.33×10^{-2}	1.148	1.199	0.000	94.72
CR1	1	207790088	rs3811381	Exonic	c.C6830G, p.P2277R	G	C	2	PCV	9.34×10^{-2}	0.514	0.119	1.129	1.231	0.000	94.38
CR1	1	207790088	rs3811381	Exonic	c.C6830G, p.P2277R	G	C	2	CNV	6.00×10^{-4}	0.384	3.45×10^{-3}	1.311	1.447	0.000	96.34
CR1	1	207790088	rs3811381	Exonic	c.C6830G, p.P2277R	G	C	2	AMD	4.87×10^{-3}	0.468	1.41×10^{-2}	1.189	1.255	0.000	96.01
ADAMTS9	3	64672474	rs36115950	Exonic	c.T286A, p.S96T,	T	A	2	PCV	0.422	0.416	0.462	0.925	0.806	0.041	76.18
ADAMTS9	3	64672474	rs36115950	Exonic	c.T286A, p.S96T,	T	A	2	CNV	0.877	0.546	0.877	1.017	0.757	0.002	89.16
ADAMTS9	3	64672474	rs36115950	Exonic	c.T286A, p.S96T,	T	A	2	AMD	0.513	0.475	0.536	0.947	0.786	0.001	90.26
FILIP1L	3	99643176	rs793440	Exonic	c.G503A, p.R168H	T	C	2	PCV	4.46×10^{-5}	4.46×10^{-5}	5.13×10^{-4}	1.552	1.552	0.868	0
FILIP1L	3	99643176	rs793440	Exonic	c.G503A, p.R168H	T	C	2	CNV	4.96×10^{-2}	0.290	7.94×10^{-2}	1.278	1.223	0.164	48.51
FILIP1L	3	99643176	rs793440	Exonic	c.G503A, p.R168H	T	C	2	AMD	1.35×10^{-3}	1.35×10^{-3}	6.21×10^{-3}	1.341	1.341	0.691	0
FILIP1L	3	99832939	rs144351944	UTR5	NA	G	T	2	PCV	5.18×10^{-2}	5.18×10^{-2}	7.94×10^{-2}	3.613	3.613	0.821	0
FILIP1L	3	99832939	rs144351944	UTR5	NA	G	T	2	CNV	2.79×10^{-6}	2.79×10^{-6}	6.42×10^{-5}	11.084	11.084	0.763	0
FILIP1L	3	99832939	rs144351944	UTR5	NA	G	T	2	AMD	2.96×10^{-4}	2.96×10^{-4}	2.27×10^{-3}	6.278	6.278	0.981	0
BBX	3	107491897	rs189132250	Exonic	c.A1329G, p.A443A	G	A	2	PCV	6.09×10^{-2}	0.143	8.75×10^{-2}	1.398	1.454	0.292	9.87
BBX	3	107491897	rs189132250	Exonic	c.A1329G, p.A443A	G	A	2	CNV	4.92×10^{-3}	0.225	1.41×10^{-2}	1.702	3.765	0.000	92.11
BBX	3	107491897	rs189132250	Exonic	c.A1329G, p.A443A	G	A	2	AMD	1.22×10^{-2}	0.202	2.55×10^{-2}	1.488	2.414	0.020	81.44
PRLR	5	35060054	rs249524	UTR3	NA	G	C	2	PCV	4.96×10^{-2}	0.404	7.94×10^{-2}	0.851	0.789	0.001	90.84
PRLR	5	35060054	rs249524	UTR3	NA	G	C	2	CNV	0.288	0.518	0.331	0.909	0.868	0.018	82.14
PRLR	5	35060054	rs249524	UTR3	NA	G	C	2	AMD	2.88×10^{-2}	0.437	0.135	0.860	0.835	0.001	91.04
PRLR	5	35063292	rs112461	UTR3	NA	T	A	2	PCV	6.13×10^{-2}	0.391	0.192	0.854	0.793	0.002	89.29
PRLR	5	35063292	rs112461	UTR3	NA	T	A	2	CNV	0.716	0.709	0.788	0.968	0.910	0.007	86.06
PRLR	5	35063292	rs112461	UTR3	NA	T	A	2	AMD	8.37×10^{-2}	0.527	0.233	0.886	0.857	0.001	91.59
PRLR	5	35063836	rs139097019	UTR3	NA	T	C	2	PCV	0.927	0.720	0.966	1.024	1.283	0.015	83.19
PRLR	5	35063836	rs139097019	UTR3	NA	T	C	2	CNV	0.226	0.616	0.514	1.399	1.616	0.001	91.35
PRLR	5	35063836	rs139097019	UTR3	NA	T	C	2	AMD	0.611	0.702	0.788	1.118	1.320	0.001	90.26
SPEF2	5	35670303	rs34708521	Exonic	c.G1498A, p.D500N	A	G	2	PCV	0.725	0.744	0.788	1.025	1.085	0.001	91.34
SPEF2	5	35670303	rs34708521	Exonic	c.G1498A, p.D500N	A	G	2	CNV	0.519	0.668	0.763	1.052	1.106	0.004	88.13
SPEF2	5	35670303	rs34708521	Exonic	c.G1498A, p.D500N	A	G	2	AMD	0.722	0.809	0.788	1.022	1.050	0.001	90.85
SPEF2	5	35705769	rs185752523	Exonic	c.G2524A, p.D842N	A	G	2	PCV	0.995	0.784	0.995	0.998	1.227	0.044	75.4
SPEF2	5	35705769	rs185752523	Exonic	c.G2524A, p.D842N	A	G	2	CNV	1.28×10^{-2}	0.477	0.130	2.321	2.637	0.000	93.79
SPEF2	5	35705769	rs185752523	Exonic	c.G2524A, p.D842N	A	G	2	AMD	0.333	0.591	0.578	1.318	1.697	0.001	90.89
ADAM19	5	156936364	rs1422795	Exonic	c.A850G, p.S284G	C	T	2	PCV	0.264	0.495	0.550	0.886	0.823	0.012	84.12
ADAM19	5	156936364	rs1422795	Exonic	c.A850G, p.S284G	C	T	2	CNV	0.326	0.326	0.578	0.891	0.891	0.924	0
ADAM19	5	156936364	rs1422795	Exonic	c.A850G, p.S284G	C	T	2	AMD	4.77×10^{-2}	0.328	0.170	0.837	0.822	0.027	79.51
COL10A1	6	116440811	rs189601123	UTR3	NA	G	A	2	PCV	0.2	0.381	0.500	1.393	1.891	0.015	83.16
COL10A1	6	116440811	rs189601123	UTR3	NA	G	A	2	CNV	0.551	0.939	0.765	0.812	1.084	0.004	88.01

Table 4 (continued)

Gene	Chr	BP (hg19)	SNP	Region	Changes	A1	A2	*N*	Disease	*P*	P(R)	FDR	OR	OR(R)	*Q*	I^2
COL10A1	6	116440811	rs189601123	UTR3	NA	G	A	2	AMD	0.694	0.615	0.788	1.099	1.471	0.004	88.07
PILRA	7	99971313	rs2405442	Exonic	c.T34C, p.L12L	C	T	2	PCV	1.56×10^{-2}	0.175	0.130	0.844	0.819	0.043	75.61
PILRA	7	99971313	rs2405442	Exonic	c.T34C, p.L12L	C	T	2	CNV	0.391	0.391	0.611	0.936	0.936	0.451	0
PILRA	7	99971313	rs2405442	Exonic	c.T34C, p.L12L	C	T	2	AMD	3.24×10^{-2}	0.170	0.135	0.882	0.875	0.100	63.13
PILRA	7	99971834	rs1859788	Exonic	c.A232G, p.R78G	G	A	2	PCV	1.56×10^{-2}	0.175	0.130	0.844	0.819	0.043	75.61
PILRA	7	99971834	rs1859788	Exonic	c.A232G, p.R78G	G	A	2	CNV	0.347	0.347	0.578	0.930	0.930	0.491	0
PILRA	7	99971834	rs1859788	Exonic	c.A232G, p.R78G	G	A	2	AMD	2.51×10^{-2}	0.133	0.135	0.877	0.871	0.119	58.83
ABCA1	9	107544685	rs4149340	UTR3	NA	A	G	2	PCV	0.272	0.696	0.375	1.093	1.166	0.000	95.59
ABCA1	9	107544685	rs4149340	UTR3	NA	A	G	2	CNV	5.67×10^{-2}	0.191	0.128	1.184	1.210	0.109	61.07
ABCA1	9	107544685	rs4149340	UTR3	NA	A	G	2	AMD	5.20×10^{-2}	0.533	0.128	1.143	1.174	0.000	92.75
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	2	PCV	3.20×10^{-3}	0.150	1.68×10^{-2}	1.293	1.333	0.025	80.23
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	2	CNV	6.09×10^{-2}	0.119	0.128	1.201	1.213	0.212	35.71
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	2	AMD	5.37×10^{-3}	0.113	2.26×10^{-2}	1.232	1.247	0.065	70.64
KIAA1958	9	115421749	rs139390384	Exonic	c.G1551C, p.L517L	C	G	2	PCV	0.191	0.628	0.309	1.870	1.724	0.019	81.78
KIAA1958	9	115421749	rs139390384	Exonic	c.G1551C, p.L517L	C	G	2	CNV	0.286	0.425	0.375	1.719	1.929	0.113	60.21
KIAA1958	9	115421749	rs139390384	Exonic	c.G1551C, p.L517L	C	G	2	AMD	0.133	0.519	0.233	1.830	1.886	0.015	83.27
ARMS2	10	124214355	rs2736911	Exonic	c.C112T, p.R38X	T	C	2	PCV	1.13×10^{-2}	1.13×10^{-2}	3.96×10^{-2}	0.770	0.770	0.364	0
ARMS2	10	124214355	rs2736911	Exonic	c.C112T, p.R38X	T	C	2	CNV	8.51×10^{-6}	8.51×10^{-6}	8.94×10^{-5}	0.574	0.574	0.512	0
ARMS2	10	124214355	rs2736911	Exonic	c.C112T, p.R38X	T	C	2	AMD	2.34×10^{-6}	2.34×10^{-6}	4.91×10^{-5}	0.655	0.655	0.451	0
STXBP6	14	25281843	rs712491	UTR3	NA	T	C	2	PCV	0.502	0.992	0.555	0.954	1.002	0.002	90.02
STXBP6	14	25281843	rs712491	UTR3	NA	T	C	2	CNV	5.32×10^{-2}	0.435	0.128	1.161	1.219	0.001	90.34
STXBP6	14	25281843	rs712491	UTR3	NA	T	C	2	AMD	0.612	0.788	0.643	1.031	1.055	0.001	90.92
LIPC	15	58833993	rs6078	Exonic	c.G283A, p.V95M	A	G	2	PCV	0.267	0.513	0.375	1.081	1.116	0.019	81.7
LIPC	15	58833993	rs6078	Exonic	c.G283A, p.V95M	A	G	2	CNV	7.98×10^{-2}	0.569	0.152	1.147	1.249	0.000	95.74
LIPC	15	58833993	rs6078	Exonic	c.G283A, p.V95M	A	G	2	AMD	2.04×10^{-4}	0.472	1.43×10^{-3}	1.245	1.299	0.000	97.32
EMID1	22	29655416	rs9537	UTR3	NA	T	C	2	PCV	0.928	0.692	0.928	0.993	0.895	0.001	90.14
EMID1	22	29655416	rs9537	UTR3	NA	T	C	2	CNV	0.322	0.342	0.398	0.917	0.912	0.277	15.36
EMID1	22	29655416	rs9537	UTR3	NA	T	C	2	AMD	0.4255	0.614	0.496	0.948	0.910	0.007	86.17

CHR chromosome code, *BP* basepair position, *SNP* SNP identifier, *A1* first allele code, *A2* second allele code, *N* number of valid studies for this SNP, *P* fixed-effects meta-analysis p-value, *P(R)* random-effects meta-analysis p-value, *FDR* Benjamini and Hochberg method controls the False Discovery Rate (FDR) using sequential modified Bonferroni correction for multiple hypothesis testing of P, *OR* fixed-effects OR estimate, *OR(R)* random-effects OR estimate, *Q* p-value for Cochrane's Q statistic, I^2 heterogeneity index (0–100)

Table 5 Allelic association of the selected genes in the second replication stage

Gene	Chr	BP (hg19)	SNP	Region	Changes	A1	A2	Disease	F_A	F_U	*P*	OR	FDR	95% CI	Allele count in case (A1/A2)[a]	Allele count in control (A1/A2)[b]
FILIP1L	3	99832939	rs144351944	UTR5	NA	G	T	PCV	0.31	0.31	0.6	1.2	0.62	0.6–2.37	14/468	13/409
FILIP1L	3	99832939	rs144351944	UTR5	NA	G	T	CNV	0.37	0.31	0.62	1.18	0.62	0.6–2.32	16/414	13/409
FILIP1L	3	99832939	rs144351944	UTR5	NA	G	T	AMD	0.42	0.31	0.33	1.36	0.62	0.72–2.56	35/877	13/409
BBX	3	107491897	rs189132250	Exonic	c.A1329G,p.A443A	G	A	PCV	0.011	0.003	0.15	4.37	0.6	0.48–39.37	5/477	1/421
BBX	3	107491897	rs189132250	Exonic	c.A1329G,p.A443A	G	A	CNV	0.015	0.003	0.04	5.686	0.07	0.63–51.63	6/424	1/421
BBX	3	107491897	rs189132250	Exonic	c.A1329G,p.A443A	G	A	AMD	0.013	0.003	0.055	4.94	0.07	0.61–39.7	11/901	1/421
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	PCV	0.215	0.151	0.021	1.54	0.07	1.064–2.22	104/378	64/358
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	CNV	0.194	0.151	0.15	1.35	0.15	0.89–2.04	83/347	64/358
ABCA1	9	107665978	rs1800978	UTR5	NA	G	C	AMD	0.206	0.151	0.027	1.464	0.11	1.04–2.06	171/741	64/358
LIPC	15	58833993	rs6078	Exonic	c.G283A,p.V95M	A	G	PCV	0.25	0.228	0.31	1.123	0.47	0.89–1.41	121/361	96/326
LIPC	15	58833993	rs6078	Exonic	c.G283A,p.V95M	A	G	CNV	0.242	0.228	0.54	1.08	0.54	0.85–1.37	104/326	96/326
LIPC	15	58833993	rs6078	Exonic	c.G283A,p.V95M	A	G	AMD	0.246	0.228	0.35	1.1	0.47	0.89–1.36	205/707	96/326

CHR chromosome, *SNP* SNP ID, *BP* physical position (base-pair), *A1* minor allele name (based on whole sample), *F_A* frequency of this allele in cases, *F_U* frequency of this allele in controls, *A2* major allele name, *P* Fisher's exact test *p*-value, *OR* estimated odds ratio (for A1), *FDR* Benjamini and Hochberg method controls the False Discovery Rate (FDR) using sequential modified Bonferroni correction for multiple hypothesis testing of P, *OR* odds ratio, *95% CI* 95% confidence interval

[a]Allele count in cases

[b]Allele count in controls

Table 6 Combined association of the selected four SNPs in the discovery and replication stages by meta analysis

Gene	Chr	BP (hg19)	SNP	Region	Changes	Disease	A1	A2	N	*P*	P(R)	FDR	OR	OR(R)	*Q*	*I*
FILIP1L	3	99832939	rs144351944	UTR5	NA	PCV	G	T	3	4.91×10^{-5}	4.91×10^{-5}	3.42×10^{-4}	1.5237	1.5237	0.8069	0
FILIP1L	3	99832939	rs144351944	UTR5	NA	CNV	G	T	3	4.54×10^{-2}	4.54×10^{-2}	0.982	1.2677	1.2677	0.3713	0
FILIP1L	3	99832939	rs144351944	UTR5	NA	AMD	G	T	3	8.44×10^{-4}	8.44×10^{-4}	1.17×10^{-3}	1.3426	1.3426	0.9233	0
BBX	3	107491897	rs189132250	Exonic	c.A1329G, p.A443A	PCV	G	A	3	1.83×10^{-2}	1.83×10^{-2}	0.844	3.8005	3.8005	0.9642	0
BBX	3	107491897	rs189132250	Exonic	c.A1329G, p.A443A	CNV	G	A	3	8.56×10^{-7}	8.56×10^{-7}	8.28×10^{-6}	9.8043	9.8043	0.8182	0
BBX	3	107491897	rs189132250	Exonic	c.A1329G, p.A443A	AMD	G	A	3	8.75×10^{-5}	8.75×10^{-5}	1.83×10^{-4}	5.9976	5.9976	0.9787	0
ABCA1	9	107665978	rs1800978	UTR5	NA	PCV	G	C	3	1.85×10^{-4}	1.85×10^{-2}	0.183×10^{-3}	1.3388	1.3844	0.0535	65.85
ABCA1	9	107665978	rs1800978	UTR5	NA	CNV	G	C	3	1.53×10^{-2}	1.53×10^{-2}	0.74	1.2327	1.2327	0.3921	0
ABCA1	9	107665978	rs1800978	UTR5	NA	AMD	G	C	3	4.14×10^{-4}	1.24×10^{-2}	0.98	1.2706	1.2961	0.113	54.14
LIPC	15	58833993	rs6078	Exonic	c.G283A, p.V95M	PCV	A	G	3	0.1885	0.3352	0.1885	1.0876	1.1139	0.0635	63.73
LIPC	15	58833993	rs6078	Exonic	c.G283A, p.V95M	CNV	A	G	3	7.44×10^{-2}	0.4894	0.908	1.2225	1.2314	0	94.73
LIPC	15	58833993	rs6078	Exonic	c.G283A, p.V95M	AMD	A	G	3	2.27×10^{-4}	0.4031	9.92×10^{-3}	1.1341	1.1903	0	91.51

CHR chromosome code, *BP* basepair position, *SNP* SNP identifier, *A1* first allele code, *A2* second allele code, *N* number of valid studies for this SNP, *P* fixed-effects meta-analysis p-value, *P(R)* random-effects meta-analysis p-value, *FDR* Benjamini and Hochberg method controls the False Discovery Rate (FDR) using sequential modified Bonferroni correction for multiple hypothesis testing of P, *OR* fixed-effects OR estimate, *OR(R)* random-effects OR estimate, *Q* *p*-value for Cochrane's Q statistic, I^2 heterogeneity index (0–100)

association of these SNPs with wet AMD could be related to the LD of the other SNPs in the gene expression regulatory region.

In summary, we carried out a comprehensive analysis of coding and UTR variants in Chinese cohorts of wet AMD. In addition to the previously reported four coding variants (rs800292, rs2274700, rs3753396, and rs1065489) in *CFH* as well as the rs10490924 and rs2736911 variants in the *ARMS2/HTRA1* region, we identified two novel LDs (located in rs189132250 in *BBX* and rs144351944 in *FILIP1L* regions) that were associated with wet AMD. We also validated the wet AMD association of rs1800978 in *ABCA1*, which is in the same LD as the reported SNPs [10, 34]. These findings provide new information regarding the coding and UTR regions of the known AMD GWAS loci in Chinese cohorts.

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Author contributions ZY designed the study. FW, XZ, POST, HC and CP recruited the participants. LH and FH performed the genotyping. LH wrote the initial draft, with edits from ZY corrected the English spelling and grammar. All authors critically revised and gave final approval of this manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Electronic-database information The URLs for data presented herein are as follows: 1000 Genomes Browser, http://browser.1000genomes.org/index.html; GWAS Integrator database (https://phgkb.cdc.gov/HuGENavigator/gWAHitStartPage.do); UCSC hg19:http://genome.ucsc.edu/; BWA: http://bio-bwa.sourceforge.net/; SAMTOOLS: http://samtools.sourceforge.net/; Annovar: http://annovar.openbioinformatics.org/en/latest/.

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Affiliations

Lulin Huang[1] · Xiongze Zhang[2] · Pancy O S Tam[3] · Haoyu Chen[4] · Fang Hao[1] · Chi-Pui Pang[3] · Fen Wen[2] · Zhenglin Yang[1,5,6]

[1] Sichuan Provincial Key Laboratory for Human Disease Gene Study and the Department of Laboratory Medicine, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, Sichuan, China

[2] State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China

[3] Department of Ophthalmology and Visual Sciences, Chinese University of Hong Kong, Hong Kong, China

[4] Joint Shantou International Eye Center, Shantou University and Chinese University of Hong Kong, Shantou, China

[5] Institute of Chengdu Biology, and Sichuan Translational Medicine Hospital, Chinese Academy of Sciences, Chengdu, Sichuan, China

[6] Center of Information in Biomedicine, University of Electronic Science and Technology of China, Chengdu, Sichuan, China