C2 rs547154 polymorphism and polypoidal choroidal vasculopathy susceptibility: a meta-analysis

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Previous studies have indicated the association between C2 rs547154 polymorphism and polypoidal choroidal vasculopathy (PCV) risk, while the results are controversial and inconsistent. Herein, we perform a meta-analysis to gain a precise estimation of the association using 5 eligible studies involving 4076 subjects, of which 1220 were PCV cases, 1073 were age-related macular degeneration (AMD) cases and 1783 were controls. Allelic frequencies of C2 rs547154 polymorphism between PCV and AMD were also compared. Both crude and adjusted odds ratios (OR) with their 95% confidence interval (CI) were included to assess the strength of the association. The pooled OR in random-effect model for allele T versus G was 0.64 (95% CI, 0.52–0.80; p = 0.0001), for genotype TG versus GG was 0.65 (95% CI, 0.52–0.83; p = 0.0004), and for genotype TT + TG versus GG was 0.64 (95% CI, 0.51–0.80; p = 0.0002). No difference in allelic frequency was observed between PCV and AMD (OR, 0.86; 95% CI, 0.64–1.16; p = 0.32). Sensitivity analysis proved the robustness of our data. No significant ethnic divergence was suggested by subgroup analysis, and no publication bias was detected via Egger’s test. In conclusion, our data indicate that C2 rs547154 polymorphism plays a protective role in the development of PCV.

Polypoidal choroidal vasculopathy (PCV) is a hemorrhagic and exudative maculopathy characterized by hyalinization and peripapillary, macular or peripheral sub-retinal pigment epithelium (RPE) polypoidal dilatations of branching choroidal vascular networks in indocyanine green angiography (ICGA) presentations¹⁻³, which may subsequently lead to detachments of RPE, or even neurosensory retina²⁻⁴⁻⁷. Due to the many shared clinical hallmarks with age-related macular degeneration (AMD), including recurrent subretinal hemorrhage, exudation, serous and hemorrhagic RPE detachment, and vitreous hemorrhage, PCV remains unrecognized for a long period until its first identification by Yannuzzi et al in 1990². Recent studies indicate more unique manifestations in PCV that distinguished PCV from AMD¹,⁸⁻⁹.

The pathogenesis of PCV remains elusive, while it is widely accepted that both genetic and environmental factors play important roles in the disease course of PCV. Considering the clinical similarities between AMD and PCV, researchers aim to find out whether AMD and PCV share common pathogenic pathways. Previous meta-analyses have reported that age-related maculopathy susceptibility 2 (ARMS2; MIM: 611313) rs10490924 and complement factor H (CFH; MIM: 134370) rs800292 polymorphisms, variants implicated in AMD etiology, might also increase PCV risk¹⁰⁻¹¹. The complement component 2 (C2; MIM: 613927) rs547154 variant is a G to T substitution located on chromosome 6p21, which shows protective effect against AMD¹²⁻¹³. This variant has been widely investigated for its association with PCV¹⁴⁻¹⁸, while the results are controversial and the sample size in each study is limited. Therefore, we performed this meta-analysis to assess the relationship between the C2 variant and PCV susceptibility.

Results

Literature. The initial literature search yielded 97 articles relevant to the search terms. Five articles, published from 2008 to 2014, embodying 4076 subjects (1220 PCV cases, 1073 AMD cases and 1783 controls) were finally included for the meta-analysis¹⁴⁻¹⁸. Flow chart of literature screening and review was shown in Figure 1. All five were case-control studies in English with their characteristics listed in Table 1. The average ages ranged from
63.80 to 73.00 years in the PCV case group, from 73.60 to 75.59 years in the AMD case group, and from 48.22 to 72.90 years in the control group, while the gender ratios (male/female) in the three groups varied from 1.39 (32/23) to 3.39 (105/31) for PCV cases, 2.64 (330/125) to 2.97 (187/63) for AMD cases, and 0.67 (110/164) to 1.51 (110/73) for controls (Table 1). Of the 5 studies, 3 were in Japan15,17,18, 1 was in Singapore14, and another one was in USA16. Three studies used TaqMan for genotyping15,17,18 and one used Sanger sequencing14, whereas both polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and TaqMan were applied in the study performed by Lima et al16.

**Meta-analysis.** Allelic and genotypic distributions for the C2 rs547154 variant from each individual study are presented in Table 2. The genotypic distribution in the control group was consistent with Hardy-Weinberg equilibrium (HWE) in each study. The adjusted odds ratios (OR) with their 95% confidence interval (CI) and the corresponding adjusted variables were provided by two studies and were listed in Table 217,18. Quality assessments of all included studies with the Newcastle-Ottawa Scale were included in Table S1.

In the random-effect model, the pooled OR for the risk allele T versus the wild type G was 0.64 (95% CI, 0.52–0.80; p < 0.0001) (Figure 2A), pooled OR for genotype TT versus GG was 0.43 (95% CI, 0.15–1.26; p, 0.123), pooled OR for genotype TG versus GG was 0.65 (95% CI, 0.52–0.83; p, 0.0004), pooled OR for genotype TT + TG versus GG was 0.64 (95% CI, 0.51–0.80; p, 0.0002), and pooled OR for genotype TT versus TG + GG was 0.46 (95% CI, 0.16–1.34; p, 0.154) (Table 3). No significant heterogeneities exist within all five comparison groups, and results from the fixed-effect model kept consistent with the random-effect model. To minimize the bias, we used adjusted data for better estimation, and the adjusted OR for T versus G was 0.56 (95% CI, 0.42–0.74; p < 0.0001) (Figure 2B). No difference in allelic frequency was found between the PCV and AMD groups.

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**Table 1: Characteristics of Included Studies**

| Author [Publication Year] | Region [Ethnicity] | Total (N) | Average Age (yrs) | Gender Ratio (M/F) | Genotyping Method |
|---------------------------|-------------------|---------|------------------|-------------------|------------------|
| Lee et al (2008)14        | Singapore (Chinese) | 93      | 63.80 ± 7.60     | NA                | Sanger sequencing |
| Kondo et al (2009)15      | Japan (Japanese)  | 136     | 73.00 ± 6.80     | NA                | TaqMan           |
| Lima et al (2010)16       | USA (Caucasian)   | 55      | 73.00 ± 8.20     | NA                | PCR-RFLP/TaqMan  |
| Nakata et al (2011)17     | Japan (Japanese)  | 581     | 72.59 ± 8.13     | 455/164           | TaqMan           |
| Takahashi et al (2014)18  | Japan (Japanese)  | 376     | 73.90 ± 8.90     | 250/110           | TaqMan           |

**Abbreviations:** RFLP, restriction fragment length polymorphism; PCV, polypoidal choroidal vasculopathy; AMD, age-related macular degeneration; NA, not available; yrs, years; M/F, male/female.
groups (OR, 0.86; 95% CI, 0.64–1.16; p, 0.32) (Figure 2C). Subgroup analysis based on ethnicity revealed that results from both the Asian and the Caucasian groups kept consistent with overall data in all five tested models, suggesting no existed ethnic divergence (Table 3). Sensitivity analysis was applied to estimate the influence of each study on the pooled OR. No individual study was found to affect the result in all comparison groups, which further proved the robustness of our data. Further, no

### Table 2 | Genotype Distribution and Allele Frequency of Included Studies

| Author          | PCV Control | HWE p Value | M-H Random 95% CI Year | Odds Ratio IV, Fixed 95% CI Year | Odds Ratio IV, Fixed 95% CI Year |
|-----------------|-------------|-------------|-------------------------|---------------------------------|---------------------------------|
| Study or Subgroup | [N] T/TG/GG T/G T [%] | [N] T/TG/GG T/G T [%] |                         |                                 |                                 |
| Lee et al14      | 72 0/5/67  5/139 3.47 | 93 0/9/84 9/177 4.84 | 0.624 NA NA NA            |                                 |                                 |
| Kondo et al15    | 136 1/19/116 21/251 7.72 | 183 2/32/149 36/330 9.84 | 0.848 NA NA NA            |                                 |                                 |
| Lima et al16     | 55 0/4/51  4/106 3.63 | 368 5/7/286 87/649 11.82 | 0.943 NA NA NA            |                                 |                                 |
| Nakata et al17   | 581 2/57/522 61/1101 5.25 | 865 5/121/739 131/1599 7.57 | 0.984 0.54 [0.35–0.84] 0.006 Age, Sex | 0.48 [0.26–0.89] 0.018 Multiple factors |
| Tanaka et al18   | 376 1/40/335 42/710 5.59 | 274 4/41/229 49/499 8.94 | 0.179 0.48 [0.26–0.89] 0.018 Multiple factors |

A {Age, Sex, Hypertension, DM, Smoking.}

Abbreviations: PCV: polypoidal choroidal vasculopathy; HWE: Hardy–Weinberg equilibrium; NA: not available; OR: odds ratio; CI: confidence interval.

Figure 2 | Association between C2 rs547154 polymorphism and polypoidal choroidal vasculopathy (PCV)/age-related macular degeneration (AMD) risk. Forest plots of C2 rs547154 polymorphism (T vs G) and PCV risk based on crude (A) and adjusted data (B). Allelic frequencies of C2 rs547154 polymorphism (T vs G) between PCV and AMD (C).
Discussion
Inconsistencies exist in the association between C2 rs547154 polymorphism and PCV risk, suggesting the necessity to perform an exhaustive review and quantitative analysis on all evidence to determine the effect. In the present study, to assess the association between C2 rs547154 polymorphism and PCV risk, we reviewed a total of 97 published reports and completed an analysis on 4076 subjects from 5 original studies. Our result suggests that C2 rs547154 has protective effect on PCV in all populations. The C2 locus and the CFB locus are located closely together on chromosome 6p19. Primary structure of the C2 gene showed 39% sequence identity with its functionally analogous complement factor B (CFB; MIM: 138470). Component C2, protein encoded by the C2 gene, shows a wide expression in the neural retina, RPE, and choroid. C2 is part of the classical pathway of the complement system. Upon the binding with component C4b, component C2 is cleaved by activated factor component C1 into two fragments, C2b and C2a. The former fragment, C2b, then combines with the complement factor C4b to generate the C3/C5 convertase. Mutations in C2 have been implicated in causing complement component 2 deficiency (C2D; MIM: 217000) and the functional role of C2 in the etiology of PCV and AMD has not been fully elucidated. Thus, more functional investigations are warranted to give a better insight into the relationship between the C2 variant and PCV.

To enhance the reliability of our results, we adopted the quality assessment tool recommended by the Newcastle-Ottawa Scale (NOS) for case-control studies. No language limitation was applied. Only studies published in peer-reviewed journals were included. In addition, we also used adjusted data for better estimation of the association. The association between C2 rs547154 polymorphism and PCV susceptibility has been discussed in a previous report, while they have only included 3 studies and tested the allele model (T vs G)\(^2\). Several limitations of the present study should be acknowledged. The number of included studies was quite limited, and only one report was on Caucasians. Meanwhile, one study was not included due to the lack of allelic and genotypic data\(^3\). Therefore, more investigations are warranted to confirm the conclusion, especially in Caucasians and other populations. Despite our effort to narrow down the influence of other risk factors using adjusted data, the number of studies providing adjusted data, especially multivariate-adjusted data, was quite limited. We only conducted subgroup analysis on ethnicity, while the effects of age and gender were not evaluated due to the incomplete data in the control group.

In conclusion, our study suggests that the C2 rs547154 variant shows a protective effect on the development of PCV. No significant difference was detected between the allelic frequencies in the PCV and the AMD groups. More epidemiological and biological studies are needed to ascertain the association, and to help to understand the molecular basis behind this association.

Methods
Literature Searching and Study Eligibility. This meta-analysis was performed according to the PRISMA guidelines\(^4\). We searched MEDLINE, EMBASE, Web of Science, and the Cochrane Library for all relevant articles using the following three main search terms as indicated previously\(^1\): (1) ("Choroid Diseases"[Mesh] AND "Vascular Diseases"[Mesh]) AND polyfunctional OR (polyfunctional choroidal vasculopathy OR PCV)) AND (complement component 2 OR C2 OR) AND (IVS10 OR rs547154)); (2) ("polyfunctional choroidal vasculopathy" exp OR PCV AND ("complement component 2" OR C2) AND (IVS10 OR rs547154)); and (3) (polyfunctional choroidal vasculopathy) OR PCV) AND (complement component 2 OR C2) AND (IVS10 OR rs547154)). The most recent search was performed on Nov 28, 2016. No language filters were applied. Retrieved records and eligibility status were managed using EndNote X5 software (http://endnote.com/).

Included studies were: (1) studies evaluating the association between C2 rs547154 polymorphism and PCV risk; (2) genome-wide association studies, or studies of case-control, cohort or population-based epidemiologic design; (3) studies using predefined criteria and procedures for PCV and AMD diagnosis; (4) OR have been reported, or present allelic and genotypic distributions of the C2 rs547154 variant in PCV cases and control subjects that enable calculations of the outcomes. Alleles included T and G, and genotypes covered TT, TG and GG. We excluded case reports, reviews, conference proceedings, editorials, reports with incomplete data, and only included those with the latest follow-up information for serial publications from the same research team using overlapped subjects. Our study was approved and reviewed by the institutional ethics committee of The First Affiliated Hospital of Nanjing Medical University and adhered to the tenets of the Declaration of Helsinki.

Data Collection and Quality Evaluation. Two investigators (X.C. and X.K.) independently reviewed all retrieved articles based on title, abstracts, and complete document if necessary. They also extracted data from each study separately with a customized dataset. All data obtained from the two reviewers were compared. Disagreements were resolved through consensus with a senior reviewer (C.Z.). Data collected included: first author, year of publication, country of sample origin, ethnicity, genotyping method, demographics of subjects, average age and gender ratio of each studied group, allelic and genotypic distributions in cases and controls, adjusted OR and adjusted factors. The NOS (accessed via http://www.ncbi.nlm.nih.gov/books/NBK351563/) was applied for evaluation of the risk of biases for included studies.

Data Synthesis and Analysis. Hardy-Weinberg equilibrium was calculated using χ² test to compare the genotypic frequencies of the C2 rs547154 variant among the control subjects. Heterogeneity among studies was assessed using Cochran’s Q.
statistic, and evaluated by the proportion of variation attributable to among-study heterogeneity. F. Heterogeneity was determined as indicated previously.27
The following five ORs and their 95% CI were calculated using both random-effect (the DerSimonian and Laird method)28 and fixed-effect model (the Mantel-Haenszel method):29 T versus G (allele model), TT versus GG (homozygous model), TG versus GG (heterozygous model), TT versus TG (dominant model), and TT versus GG (recessive model). Subgroup analysis was performed by ethnicity. Sensitivity was used to affirm the results by removing one individual study each time. Begg’s funnel plot30 and Egger’s test31 were applied to appraise the publication bias and small-study effects. All analyses were conducted with Review Manager (version 5.2; Cochrane Collaboration, Oxford, UK; http://ims.cochrane.org/revman) and STATA software (version 12.0; StataCorp LP, College Station, TX). Alpha was set to 0.05 for two-sided test.

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