Differentially Regulated Apolipoproteins and Lipid Profiles as Novel Biomarkers for Polypoidal Choroidal Vasculopathy and Neovascular Age-Related Macular Degeneration

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Lipid dyshomeostasis has been implicated in the pathogenesis of various retinal and choroidal vascular diseases. This study aims to investigate whether apolipoprotein (apo) mediated differential regulation of lipid metabolism contributes to the phenotypes of polypoidal choroidal vasculopathy (PCV) and neovascular age-related macular degeneration (nAMD). This study involved 148 subjects including 53 patients with PCV, 44 patients with nAMD, and 51 age-, sex-matched subjects with normal fundus controls. Routine blood biochemistry profile was evaluated. Apolipoproteins was estimated by Luminex technology. After controlling for age, gender, body mass index, duration of hypertension and type 2 diabetes mellitus, apoB/non-high density lipoprotein cholesterol (HDL-C) \( (p=0.015) \) was an independent risk factor for nAMD, apoB was an independent risk factor for PCV \( (p=0.011) \), compared with control. Low-density lipoprotein cholesterol (LDL-C) was significantly higher in patients with PCV when compared with nAMD \( (p=0.037) \). Furthermore, apoB/non-HDL, LDL-C, triglycerides and were significantly correlated with the pathogenesis of subgroups of PCV and nAMD. We concluded that lipid profiles and apos are differential regulated in PCV, nAMD and their subtypes, indicating different pathogenicity contributed to the different phenotypes of PCV and nAMD. Non-pachy PCV shares pathological similarities with nAMD, which is highly correlated with age-related atherosclerosis.

Keywords: polypoidal choroidal vasculopathy, neovascular age-related macular degeneration, serum lipid profiles, apolipoproteins, regulatory mechanisms
INTRODUCTION

Age-related macular degeneration (AMD) is a major and irreversible blindness in elderly people worldwide (1, 2). Polypoidal choroidal vasculopathy (PCV), which is considered a distinct subtype of neovascular AMD (nAMD), is more prevalent in Asia population than Caucasians although it was first reported by Dr. Yannuzzi in US in the 1990s (3, 4). Both PCV and nAMD are multifactorial disorders and share a variety of characteristics in phenotype, risk factors, natural history, and treatment strategies (5). Recently, several genetic and epigenetic studies raised the hypothesis that PCV is a genetic disorder, AMD-related gene polymorphisms such as ARMS2 A69S and CFH Y402H (6) have been found associated with PCV. On the other hand, the disparity in response to intravitreal anti-VEGF drugs between nAMD and PCV has also received extensive attention. Although the natural history, clinical features, pathological and genetic correlation of the two diseases has been intensively studied (5, 7–9), the pathogenic similarities and differences between PCV and nAMD remain largely unknown.

Dysregulated lipid metabolism has been implicated in the pathogenesis of PCV and nAMD. Lipoproteins are soluble lipid protein complexes that are formed by the interactions between apolipoproteins (apos, amphipathic proteins) and lipids (10). Abnormal expression of genes of lipid metabolism and the reverse cholesterol transport (RCT) pathway, such as Cholesterol Ester Transfer Protein (CETP), Hepatic Lipase (LIPC), ATP-binding Cassette Transporter A1 (ABCA1) and Apolipoprotein E (APOE), has been reported in patients with PCV and nAMD (11–13). Furthermore, dysregulated lipoproteins [triglycerides (TG), total cholesterol (TC), high-density lipoprotein- cholesterol (HDL-C) and low-density lipoprotein (LDL-C)] and apolipoproteins have been reported in both PCV and nAMD (12, 14, 15); however, results from existing studies are controversial and the connections remain elusive (15, 16).

The differential regulation mechanism and the interactions between apop and lipid profiles remain uncertain. Our previous studies have shown that abnormal plasma levels of apolipoproteins and lipid profiles are correlated with variety of retinal vascular disorders including diabetic retinopathy (17–19). It is interesting to determine if apolipoprotein-mediated differential regulation of lipid metabolism is a pathological cause of the clinical phenotypes of nAMD and PCV. In this study, we aim to determine the differential serum levels of lipoproteins, apoproteins and their ratios, and to determine their associations with the pathogenesis of PCV and nAMD.

MATERIALS AND METHODS

Study Subjects

The study subjects were selected from our Retinal and Choroidal Study Cohort (RCSC), a prospective cohort from year of 2016 to 2022 in Beijing Tongren Eye Center. This cohort comprises patients with PCV, nAMD, pachychoroid pigment epitheliopathy, pachychoroid neovasculopathy, focal choroidal excavation, peripapillary pachychoroid syndrome pachydrusen and age-, sex-, duration of the systemic disorders (hypertension and diabetes mellitus) matched controlled subjects (with normal fundus in both eyes). This study was approved by the institutional review board of Beijing Tongren Hospital and adhered to the tenets of the Declaration of Helsinki. All participants signed informed consent form approved by the Ethics Committee of Beijing Tongren Hospital, Capital Medical University before participation.

This nested case-control study compromised 148 subjects, including 53 patients with PCV, 44 patients with nAMD and 51 age-, sex- matched healthy controls from RCSC as described above.

The inclusion criteria were: 1) naïve PCV and nAMD not treated with anti-vascular endothelial growth factor (VEGF) at least within 1 year 2) No history of lipid-lowering medications. PCV was diagnosed by the fundus examination, fundus color photography, Swept Source optical coherent tomography (SS-OCT) and further confirmed by indocyanine green angiography (ICGA) according to EVEREST study report 2, modified EVEREST criteria (19–21) and the 2020 Asia-pacific eye image association PCV expert consensus of the working group (22). nAMD was diagnosed according to the “Expert consensus on macular degeneration” published in 2019 by Spaiide et al. (23).

Exclusion criteria included those with 1) complicated with other fundus diseases such as macular hole, diabetic retinopathy, metastatic carcinoma, melanoma and others 2) history of serious systemic diseases including stroke and myocardial infarction within 6 months or not be able to tolerate eye examination 4) patients with history of metabolic disorders or on lipid lowering therapy. 5) subjects with one eye diagnosed with PCV and the other eye diagnosed with nAMD were also excluded.

Normal fundus control was defined as: 1) normal fundus in both eyes were evaluated by fundus ophthalmoscopy and OCT configuration 2) history of serious systemic diseases including stroke and myocardial infarction within 6 months or not be able to tolerate eye examination 3) patients with history of metabolic disorders or on lipid lowering therapy was excluded 4) subjects (with normal fundus in both eyes) with other systemic disorders were matched with the study groups when enrolled.

Subgroup Grouping Criteria

The enrolled patients with PCV and nAMD were further categorized to the pachy-PCV/nAMD group or the non-pachy nAMD group respectively, according to the likelihood ratio test
results determined in the same large cohort as we described previously (24). After controlling for age and diopter, the diagnostic cut off value range of pachy-choroid among 20-29 years old was 320-330 μm [likelihood ratio (LR): 1.17]; among 40-59 years old was 330-340 μm (LR: 1.07); among 60 to 79 years old was 250-275 μm (LR: 1.07) and ≥ 80 years old was 200-225 μm (LR: 1.00).

**Eye Examination**

The best-corrected visual acuity (BCVA), slit-lamp microscopic examination (SL-IE Slit Lamp Microscope, Topcon Co., Ltd, Tokyo, Japan), non-contact intraocular pressure (TX20 Automatic Non-contact Tonometer, Canon Co., Ltd, Tokyo, Japan), fundus photography (CR-1 non-mydriatic Fundus Camera, Canon Co., Ltd), OCT, fluorescent angiography and indocyanine green angiography (ICGA) were performed for all the participants. nAMD and PCV were diagnosed by OCT and indocyanine green angiography (ICGA) according to EVEREST criteria (20, 21) and the 2020 Asia-pacific eye image association PCV expert consensus of the working group (22).

**Lipoprotein Profiling and Routine Biochemical Examination**

All participants underwent a standardized assessment of other risk factors including age, gender, duration of diabetes, duration of hypertension (HBP) and body mass index (BMI). The serum levels of TC, TG, LDL-C and HDL-C were tested by routine biochemical examination.

50 μL of serum was utilized to determine the level of apolipoproteins (A-I, A-II, B, C-II, C-III, E) determined by Luminex technology (Luminex 200™ liquid chip detector, Millipore, Boston, Massachusetts, USA), according to the manufacturer’s instructions.

Non-HDL-C, LDL/HDL, (TC-HDL)/HDL, log (TG/HDL), apoA-I/apoA-II, apoB/non-HDL-C, apoB/apoA-I, apoC-III/apoC-II and apo E/apolipoprotein II were also calculated.

**Determination of the Cutoff Value for apoA-I/apoA-II, apoB/non-HDL-C, apoB/apoA-I, apoC-III/apoC-II and apoE/apolipoprotein II Ratios by Receiver Operating Characteristic Curve**

The cut off values for the ratios of apoA-I/apoA-II, apoB/non-HDL-C, apoB/apoA-I, apoC-III/apoC-II, apoE/apolipoprotein II, LDL/HDL, (TC- HDL)/HDL and log (TG/HDL) were determined by ROC curve. The indicators with high sensitivity and specificity (with maximum Youden index) were selected as the cut off values on ROC curve. The control group as stated above in 2.1. The cut off value of the apoB/non-HDL-C ratio was 0.43 [Area under curve (AUC): 0.62, sensitivity= 0.68, specificity=0.60], the apoB/apoA-I ratio was 1.77 (AUC: 0.544, sensitivity = 0.16, specificity = 0.94) in patients with PCV, the cut off value for the apoB/non-HDL-C ratio was 0.45 (AUC:0.71, sensitivity = 0.78, specificity = 0.63) in patients with nAMD. The ratio values (apoA-I/apoA-II, apoC-III/apoC-II and apo E/apolipoprotein II) with lower sensitivity (AUC< 0.50, sensitivity and specificity <0.5) were not considered in this study.

**Sample Size Determination**

Sample size was calculated by the Power Analysis and Sample Size software (PASS 2022, NCSS LLC, Utah, USA) based on our pilot study result. According to the pilot study results, the mean value of LDL-C (which required maximum numbers among all the biochemical parameters) in the study group was 3.32 ± 0.79, in the control group was 3.08 ± 0.87, the minimum number per arm (sample size) was 36 subjects to detect the difference between the four groups with the designed power (1- beta= 90%) at 95% confidence level (alpha=0.05) as we previously described [32]. We increased the sample size to above 40 to in each group considering the variability (even in normal controls) of apop.

**Statistical Analysis**

SPSS software (SPSS, Inc. 25.0, Chicago, IL, USA) was utilized for the statistical analysis. Baseline parameters including the age and gender of participants, duration of diabetes and HBP, BMI, TG, TC, HDL-C, LDL-C, the serum levels of apolipoproteins, LDL/HDL, (TC- HDL)/HDL, log (TG/HDL), the ApoB/ApoA-I ratio and the ApoB/Non-HDL ratio were calculated by means ± standard deviation (mean ± SD). One-way analysis of variance (ANOVA) or the Kruskal–Wallis test was applied according to the data distribution for the group comparisons. ANOVA or the univariate logistic regression was performed to assess the association between the serum levels of lipids, apolipoproteins and the ratios of lipoproteins and apolipoproteins between different groups. Multivariate logistic regression models were applied to evaluate the effects of serum lipids or apolipoproteins among PCV, nAMD and normal control groups. The Odds ratios for group comparison was analyzed using the normal control as the reference, OR > 1 means the variable is an independent risk factor for the study group, OR < 1 means the variable is an independent protective factor for the disease. When the disease sub-group was used as the reference, OR > 1 means that higher level of the independent variable was found in the study group, whereas an OR < 1 means the higher level of the independent variable was in the reference group (25). Statistical significance was defined as p<0.05.

**RESULTS**

**Baseline Demographic and Clinical Characteristics**

A total of 148 subjects (84 males and 64 females, aged 51-94 years old) including 53 patients with PCV (aged 54-81 yrs, 67.49 ± 7.67 yrs), 44 patients with nAMD (aged 51-94 yrs, 71.30 ± 9.46 yrs) and age-, gender-, duration of hypertension and type 2 diabetes mellitus matched 51 subjects with normal fundus in both eyes (aged 51-78 yrs, 66.04 ± 7.91 yrs) were recruited from the outpatient clinic of Beijing Tongren Hospital from September 2016 to September 2021 (Table 1).
There were no statistically significant differences in age or gender between the PCV, nAMD and control groups (\(p_{\text{age}}=0.066, p_{\text{gender}}=0.253\)). No statistically significant difference was found in the duration of DM, HBP and BMI among the PCV, nAMD and healthy control groups (\(p_{\text{duration of DM}}=0.053, p_{\text{duration of HBP}}=0.141, p_{\text{BMI}}=0.390\)). Furthermore, serum levels of TC, LDL, apoB/non-HDL, non-HDL-C was significant differences among the three groups (\(p_{\text{TC}}=0.030, p_{\text{LDL-C}}=0.032, p_{\text{apoB/non-HDL-C}}=0.001, p_{\text{non-HDL-C}}=0.049\)) by one-way ANOVA analysis or Kruskal–Wallis test (Table 1).

**Comparison of the PCV Group and nAMD Group by Univariate Logistic Regression Analysis**

Univariate logistic regression model showed that in comparison with healthy controls, apoB was an independent risk factors for PCV with statistical difference (OR 3.31, 95% CI 1.23-8.92, \(p=0.018\)) (Table 2).

Compared with healthy controls, univariate logistic regression analysis showed that age (OR=1.10, 95%CI 1.04-1.16, \(p=0.001\)), apo-B/non-HDL-C (per 0.1) were independent risk factors for nAMD (OR=2.08, 95% CI 1.35-3.21, \(p=0.001\)). Interestingly, TC was found to have a protective effect for patients with nAMD with statistical significance (OR=0.67, 95% CI 0.46-0.98, \(p=0.036\)) (Table 2).

In comparison with nAMD, univariable logistic regression analysis showed that the serum levels of TC, LDL-C and non-HDL-C were significantly higher and associated with increased risk for PCV (OR_{TC}=0.65, 95% CI 0.44-0.95, \(p=0.027\), OR_{LDL-C}=0.55, 95% CI 0.34-0.89, \(p=0.015\), OR_{non-HDL-C} 0.62 95% CI 0.40-0.95, \(p=0.027\), respectively). In addition, age was significantly older in patients with nAMD patients compared with that with PCV patients (OR=1.07, 95% CI 1.01-1.13, \(p=0.015\)) and apo-B/non-HDL-C (per 0.1) were independent risk factors for nAMD compared to PCV patients (OR=1.50, 95% CI 1.08-2.08, \(p=0.016\)) (Table 2).

**Comparisons of the Subgroups of PCV and nAMD by Univariable Logistic Regression Analysis**

To further explore the correlations of the serum levels of apolipoproteins and lipid profiles with PCV and nAMD, patients with PCV and nAMD were subgrouped to pachy (eyes with pachychoroid) and non-pachy groups according to the criteria as we described previously. Serum levels of TG were significantly lower in the pachy-PCV patients when compared with healthy controls (OR_{TG} 6.52, 95% CI 1.29-32.97, \(p=0.043\)). The univariate logistic regression analysis indicated that serum levels of apoA-II (per 0.1mg/dl) and Apo-B were significantly higher in patients with non-pachy PCV (OR_{apoA-II} 1.76, 95% CI 1.08-2.86, \(p=0.024\); OR_{Apo-B} 5.74, 95% CI 1.72-19.12, \(p=0.004\) respectively) (Table 3).

The serum levels of TG, apoA-I (per 0.1mg/dl) and apoA-II (per 0.1mg/dl) were significantly higher in non-pachy PCV than those in pachy-PCV (OR_{TG} 5.62, 95% CI 1.29-32.97, \(p=0.023\); OR_{apoA-I} 1.41, 95% CI 1.01-1.98, \(p=0.044\); OR_{apoA-II} 1.88, 95% CI 1.02-3.44, \(p=0.043\), respectively) (Table 3).

Compared to the healthy control group, the level of TG, TC, LDL-C and non-HDL-C were significantly lower in the pachy- nAMD group (OR_{TG} 10, 95% CI 0.02-0.61, \(p=0.013\); OR_{TC} 0.38, 95% CI 0.18-0.81, \(p=0.012\); OR_{LDL-C} 0.40, 95% CI 0.17-0.97, \(p=0.044\); OR_{non-HDL-C} 0.35, 95% CI 0.15-0.82, \(p=0.015\), respectively). Additionally, the apo-B/non-HDL-C ratio (per 0.1)
| (a) PCV vs. N<sup>‡</sup> | Factor | OR     | 95% CI    | p value |
|-------------------------|--------|--------|-----------|---------|
| Gender, ref: male       | 1.31   | (0.60, 2.85) | 0.493    |
| Age                     | 1.03   | (0.98, 1.08) | 0.310    |
| BMI                     | 1.07   | (0.94, 1.23) | 0.300    |
| Duration of hypertension| 1.01   | (0.96, 1.05) | 0.760    |
| Duration of DM          | 1.80   | (0.64, 5.01) | 0.264    |
| TG                      | 0.63   | (0.32, 1.24) | 0.180    |
| TC                      | 1.03   | (0.69, 1.53) | 0.894    |
| LDL-C                   | 1.32   | (0.84, 2.08) | 0.228    |
| HDL-C                   | 0.59   | (0.19, 1.84) | 0.362    |
| LDL/HDL                 | 1.38   | (0.88, 2.16) | 0.156    |
| (TC- HDL)/HDL           | 1.17   | (0.81, 1.68) | 0.408    |
| log (TG/HDL), per 0.1   | 0.96   | (0.82, 1.12) | 0.607    |
| apo A-I, per 0.1        | 1.11   | (0.89, 1.39) | 0.354    |
| apo C-III, per 0.1      | 1.06   | (0.77, 1.47) | 0.717    |
| apo E, per 0.01         | 1.07   | (0.76, 1.51) | 0.711    |
| apo A-II, per 0.1       | 1.31   | (0.88, 1.96) | 0.182    |
| apo B                   | 3.31   | (1.23, 8.92) | 0.018    |
| apo C-II, per 0.1       | 1.08   | (0.60, 1.97) | 0.794    |
| apo B/non-HDL-C, per 0.1 | 1.17 | (0.83, 1.63) | 0.368    |
| apo B/apo AI            | 1.21   | (0.83, 1.76) | 0.315    |
| non-HDL-C               | 1.11   | (0.73, 1.68) | 0.632    |

| (b) nAMD vs. N<sup>‡</sup> | Factor         | OR     | 95% CI     | p value |
|---------------------------|----------------|--------|------------|---------|
| Gender, ref: male         | 1.87           | (0.81, 4.32) | 0.142   |
| Age                       | 1.10           | (1.04, 1.16) | 0.001   |
| BMI                       | 1.08           | (0.94, 1.25) | 0.260   |
| Duration of hypertension  | 1.04           | (1.00, 1.08) | 0.068   |
| Duration of DM            | 1.78           | (0.64, 4.96) | 0.273   |
| TG                        | 0.61           | (0.32, 1.18) | 0.141   |
| TC                        | 0.67           | (0.46, 0.98) | 0.036   |
| LDL-C                     | 0.70           | (0.44, 1.12) | 0.137   |
| HDL-C                     | 0.39           | (0.11, 1.30) | 0.124   |
| LDL/HDL                   | 0.89           | (0.55, 1.44) | 0.627   |
| (TC- HDL)/HDL             | 0.87           | (0.59, 1.29) | 0.483   |
| log (TG/HDL), per 0.1     | 0.96           | (0.83, 1.12) | 0.597   |
| apo A-I, per 0.1          | 1.13           | (0.86, 1.49) | 0.387   |
| apo C-III, per 0.1        | 1.01           | (0.74, 1.37) | 0.962   |
| apo E, per 0.01           | 1.10           | (0.81, 1.50) | 0.529   |
| apo A-II, per 0.1         | 1.09           | (0.71, 1.68) | 0.689   |
| apo B                     | 1.76           | (0.78, 3.94) | 0.172   |
| apo C-II, per 0.1         | 0.97           | (0.49, 1.89) | 0.918   |
| apo B/non-HDL-C, per 0.1  | 2.08           | (1.35, 3.21) | 0.001   |
| apo B/apo A-I             | 0.96           | (0.61, 1.50) | 0.843   |
| non-HDL-C                 | 0.68           | (0.45, 1.03) | 0.071   |

| (c) nAMD vs. PCV<sup>‡</sup> | Factor         | OR     | 95% CI     | p value |
|-----------------------------|----------------|--------|------------|---------|
| Gender, ref: male           | 1.43           | (0.62, 3.29) | 0.404   |
| Age                         | 1.07           | (1.01, 1.13) | 0.015   |
| BMI                         | 1.01           | (0.87, 1.18) | 0.883   |
| Duration of hypertension    | 1.03           | (0.99, 1.08) | 0.143   |
| Duration of DM              | 0.99           | (0.92, 1.06) | 0.763   |
| TG                          | 0.88           | (0.43, 1.79) | 0.727   |
| TC                          | 0.65           | (0.44, 0.95) | 0.027   |
| LDL-C                       | 0.55           | (0.34, 0.89) | 0.015   |
| HDL-C                       | 0.60           | (0.17, 2.11) | 0.422   |
| LDL/HDL                     | 0.64           | (0.40, 1.04) | 0.074   |
| (TC- HDL)/HDL               | 0.74           | (0.50, 1.10) | 0.159   |
| log (TG/HDL), per 0.1       | 0.99           | (0.83, 1.19) | 0.929   |
| apo A-I, per 0.1            | 0.98           | (0.78, 1.24) | 0.888   |
| apo C-III, per 0.1          | 0.97           | (0.73, 1.28) | 0.610   |
| apo E, per 0.01             | 1.05           | (0.78, 1.41) | 0.740   |
| apo A-II, per 0.1           | 0.86           | (0.60, 1.24) | 0.417   |
| apo B                       | 0.83           | (0.40, 1.73) | 0.617   |

(Continued)
### TABLE 2 | Continued

| (a) PCV vs. N² | Factor | OR   | 95% CI       | p value |
|----------------|--------|------|--------------|---------|
| apo C-II, per 0.1 | 0.91   | (0.50, 1.64) | 0.751 |
| apo B/non-HDL-C, per 0.1 | 1.50   | (1.08, 2.08) | 0.016 |
| apo B/apo A-I | 0.77   | (0.50, 1.19) | 0.238 |
| non-HDL-C-C | 0.62   | (0.40, 0.95) | 0.027 |

*Statistically significant: p ≤ 0.05. ‡ represents for the reference group.

ref: reference; OR, Odd ratio; CI, confidence interval; BMI, body mass index; DM, diabetic retinopathy; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high density lipoprotein-cholesterol; apo, apolipoprotein; PCV, polypoidal choroidal vasculopathy; nAMD, neovascular age-related macular degeneration; N, normal fundus control.

### TABLE 3 | Comparisons of the variables between the sub-groups (pachy and non-pachy) of PCV and control group by univariate logistic regression analysis.

| (c) pachy-PCV vs. N² | Factor | OR   | 95% CI       | p value |
|-----------------------|--------|------|--------------|---------|
| Gender, ref: male | 0.56   | (0.19, 1.66) | 0.295 |
| Age | 0.99   | (0.92, 1.06) | 0.702 |
| BMI | 1.09   | (0.92, 1.28) | 0.326 |
| Duration of hypertension | 0.99   | (0.93, 1.06) | 0.74 |
| Duration of DM | 2.01   | (0.58, 6.94) | 0.27 |
| TG | 0.21   | (0.06, 0.80) | 0.022 |
| TC | 0.94   | (0.53, 1.69) | 0.84 |
| LDL-C | 1.35   | (0.71, 2.58) | 0.358 |
| HDL-C | 0.37   | (0.06, 2.23) | 0.28 |
| LDL/HDL | 1.53   | (0.33, 2.81) | 0.175 |
| (TC- HDL)/HDL | 1.20   | (0.72, 2.00) | 0.48 |
| log (TG/HDL), per 0.1 | 0.88   | (0.72, 1.09) | 0.254 |
| apo A-I, per 0.1 | 0.98   | (0.95, 1.02) | 0.323 |
| apo C-III, per 0.1 | 0.71   | (0.42, 1.19) | 0.197 |
| apo E, per 0.01 | 0.82   | (0.49, 1.38) | 0.453 |
| apo A-II, per 0.1 | 0.78   | (0.43, 1.42) | 0.412 |
| apo B | 1.44   | (0.39, 5.30) | 0.579 |
| apo C-II, per 0.1 | 0.64   | (0.25, 1.65) | 0.354 |
| apo B/non-HDL-C, per 0.1 | 0.79   | (0.47, 1.31) | 0.354 |
| apo B/apo A-I | 1.42   | (0.87, 2.33) | 0.161 |
| non-HDL-C | 1.06   | (0.58, 1.92) | 0.835 |

| (b) non-pachy PCV vs. N² | Factor | OR   | 95% CI       | p value |
|-----------------------|--------|------|--------------|---------|
| Gender, ref: male | 0.86   | (0.36, 2.04) | 0.723 |
| Age | 1.05   | (0.99, 1.11) | 0.128 |
| BMI | 1.04   | (0.90, 1.21) | 0.566 |
| Duration of hypertension | 1.01   | (0.97, 1.06) | 0.58 |
| Duration of DM | 1.97   | (0.51, 7.61) | 0.326 |
| TG | 0.93   | (0.46, 1.89) | 0.837 |
| TC | 1.07   | (0.70, 1.63) | 0.769 |
| LDL-C | 1.30   | (0.80, 2.13) | 0.295 |
| HDL-C | 0.73   | (0.22, 2.45) | 0.612 |
| LDL/HDL | 1.31   | (0.80, 2.16) | 0.281 |
| (TC- HDL)/HDL | 1.15   | (0.77, 1.71) | 0.506 |
| log (TG/HDL), per 0.1 | 1.01   | (0.86, 1.19) | 0.924 |
| apo A-I, per 0.1 | 1.32   | (0.99, 1.74) | 0.065 |
| apo C-III, per 0.1 | 1.31   | (0.89, 1.93) | 0.17 |
| apo E, per 0.01 | 1.22   | (0.83, 1.80) | 0.319 |
| apo A-II, per 0.1 | 1.76   | (1.08, 2.86) | 0.024 |
| apo B | 5.74   | (1.72, 19.12) | 0.004 |
| apo C-II, per 0.1 | 1.40   | (0.70, 2.79) | 0.336 |
| apo B/non-HDL-C, per 0.1 | 1.50   | (0.98, 2.30) | 0.064 |
| apo B/apo A-I | 1.10   | (0.72, 1.67) | 0.657 |
| non-HDL-C | 1.12   | (0.72, 1.78) | 0.815 |

| (c) non-pachy PCV vs. pachy-PCV² | Factor | OR   | 95% CI       | p value |
|-----------------------|--------|------|--------------|---------|
| Gender, ref: male | 1.52   | (0.48, 4.81) | 0.473 |
| Age | 1.06   | (0.99, 1.15) | 0.117 |

(Continued)
TABLE 3 | Continued

| (c) pachy-PCV vs. N² | Factor | OR     | 95% CI    | p value |
|----------------------|--------|--------|-----------|---------|
|                      |        | BMI    | 0.95      | (0.79, 1.15) | 0.600   |
|                      |        | Duration of hypertension | 1.03 | (0.96, 1.10) | 0.456   |
|                      |        | Duration of DM | 0.93 | (0.84, 1.03) | 0.144   |
|                      |        | TG     | 6.52      | (1.29, 32.97) | 0.023   |
|                      |        | TC     | 1.14      | (0.62, 2.08) | 0.675   |
|                      |        | LDL-C  | 1.00      | (0.52, 1.90) | 0.987   |
|                      |        | HDL-C  | 1.91      | (0.30, 12.36) | 0.498   |
|                      |        | LDL/HDL | 0.87 | (0.48, 1.60) | 0.659   |
|                      |        | (TC/HDL)/HDL | 0.96 | (0.57, 1.63) | 0.889   |
|                      |        | log (TG/HDL), per 0.1 | 1.23 | (0.93, 1.63) | 0.155   |
|                      |        | apo A-I, per 0.1 | 1.41 | (1.01, 1.98) | 0.044   |
|                      |        | apo C-III, per 0.1 | 0.99 | (0.76, 1.30) | 0.964   |
|                      |        | apo E, per 0.01 | 1.46 | (0.84, 2.52) | 0.176   |
|                      |        | apo A-II, per 0.1 | 1.88 | (1.02, 3.44) | 0.043   |
|                      |        | apo B | 0.28      | (0.07, 1.20) | 0.086   |
|                      |        | apo C-II, per 0.1 | 1.88 | (0.76, 4.66) | 0.171   |
|                      |        | apo B/non-HDL-C, per 0.1 | 1.52 | (0.95, 2.43) | 0.082   |
|                      |        | apo B/apo A-I | 0.79 | (0.48, 1.30) | 0.361   |
|                      |        | non-HDL-C | 1.07 | (0.57, 2.00) | 0.856   |

*Statistically significant: p ≤ 0.05. ‡ represents for the reference group.

was significantly higher in the pachy-nAMD group than that in the healthy control group (ORapo-B/non-HDL-C 2.25, 95% CI 1.21-4.19, p=0.011) (Table 4). Age and apo-B/non-HDL-C ratio (per 0.1) were also significantly higher in the non-pachy nAMD group than that in the healthy control group (ORage 1.09, 95% CI 1.03-1.16, p=0.004; ORapo-B/non-HDL-C 2.06, 95% CI 1.29-3.27, p=0.002, respectively) (Table 4). In comparison with the pachy-nAMD group, TG was significantly higher in the non-pachy nAMD group (OR_TG 8.85, 95% CI 1.22-64.32, p=0.031) (Table 4).

Comparisons in the Lipid Profiles and Apolipoproteins Between PCV and nAMD by Multivariable Logistic Regression Analysis

After adjusting for age, gender, BMI and duration of HBP, the results suggested that apo-B was an independent risk factors for PCV when compared to normal control (OR_Apo-B 4.06, 95% CI 1.38-11.96, p=0.011) (Table 5). The analysis also showed that the apo-B/non-HDL-C ratio (per 0.1) was an independent risk factor for nAMD, which is consistent with the univariate logistic analysis (OR 1.80, 95% CI 1.12-2.90, p=0.015) (Table 5). The results indicated that age is a risk factor specifically for nAMD compared with PCV (OR_age 1.07, 95% CI 1.01-1.14, p=0.033) (Table 5).

Comparisons in the Lipid Profiles and Apolipoproteins Between Subgroups of PCV and nAMD by Multivariable Logistic Regression Analysis

To identify specific lipid metabolic dysfunction biomarkers, after adjusting for age, gender, BMI and duration of HBP, we compared nAMD and PCV, pachy and non-pachy PCV and nAMD by multivariate logistic regression analysis.

LDL-C was found to be a specific risk factor for patients with pachy-PCV, whereas lower level of TG was found in non-pachy PCV patients (OR_LDL-C 3.44, 95% CI 1.13-10.47, p=0.030; OR_TG 0.94, 95% CI 0.00-0.37, p=0.005, respectively) (Table 6). Furthermore, significantly increased levels of apoA-II (per 0.1mg/dl) and apoB were associated with increased risk of non-pachy PCV (OR OR apoA-II 1.94, 95% CI 1.03-3.68, p=0.042; OR apoB 6.20, 95% CI 1.37-28.02, p=0.018, respectively) (Table 6). In addition, higher level of apo-B/non-HDL-C (per 0.1) was associated with higher risk for non-pachy PCV in comparison with pachy-PCV (OR apo-B/non-HDL-C 2.64, 95% CI 1.05-6.59, p=0.038) (Table 6).

Compared with healthy controls, age and the apo-B/non-HDL-C ratio (per 0.1) were strong risk factors for pachy-nAMD (OR_age 1.22, 95% CI 1.01-1.47, p=0.036; OR apo-B/non-HDL-C 2.78, 95% CI 1.16-6.68, p=0.022, respectively) (Table 7). The apo-B/non-HDL-C ratio (per 0.1) was specific risk factor for non-pachy nAMD (OR apo-B/non-HDL-C 1.76, 95% CI 1.08-2.87, p=0.023) (Table 7). The serum levels of apoproteins showed no differences between pachy-nAMD and non-pachy nAMD patients (Table 7).

DISCUSSION

In this study, we found that the apoB/non-HDL-C ratio was an independent risk factor for nAMD, whereas apoB was an independent risk factor for PCV. In addition, age was a specific risk factor for nAMD compared with PCV. In the subgroup analysis for PCV and nAMD, it was found that apoB/non-HDL-C was an independent risk factor for both pachy- and non-pachy nAMD compared with control. apoB/non-HDL-C was also an independent risk factor for non-pachy PCV, indicating that non-pachy PCV shares pathological...
# TABLE 4 | Comparisons of the variables between the sub-groups (pachy- and nonpachy-) of nAMD and control groups by univariate logistic regression analysis.

## (c) pachy-nAMD vs. N

| Factor                        | OR   | 95% CI          | p value |
|-------------------------------|------|-----------------|---------|
| Gender, ref: male             | 0.18 | (0.04, 0.87)    | 0.033   |
| Age                           | 1.04 | (0.96, 1.13)    | 0.327   |
| BMI                           | 1.15 | (0.94, 1.41)    | 0.169   |
| Duration of hypertension      | 1.01 | (0.94, 1.08)    | 0.830   |
| Duration of DM                | 1.73 | (0.61, 4.85)    | 0.300   |
| TG                            | 0.10 | (0.02, 0.61)    | 0.013   |
| TC                            | 0.38 | (0.18, 0.81)    | 0.012   |
| LDL-C                         | 0.40 | (0.17, 0.97)    | 0.044   |
| HDL-C                         | 0.50 | (0.08, 3.04)    | 0.451   |
| LDL/HDL                       | 0.67 | (0.31, 1.48)    | 0.314   |
| (TC-HDL)/HDL                  | 0.66 | (0.35, 1.25)    | 0.204   |
| log (TG/HDL), per 0.1         | 0.81 | (0.64, 1.04)    | 0.099   |
| apo A-I, per 0.1              | 1.14 | (0.73, 1.79)    | 0.562   |
| apo C-III, per 0.1            | 0.93 | (0.54, 1.61)    | 0.805   |
| apo E, per 0.01               | 1.17 | (0.72, 1.92)    | 0.524   |
| apo A-II, per 0.1             | 0.63 | (0.33, 1.20)    | 0.16    |
| apo B                         | 0.96 | (0.19, 4.79)    | 0.964   |
| apo C-II, per 0.1             | 0.87 | (0.29, 2.58)    | 0.794   |
| apo B/non-HDL-C, per 0.1      | 2.25 | (1.21, 4.19)    | 0.011   |
| apo B/apo A-I                 | 0.71 | (0.31, 1.60)    | 0.405   |
| non-HDL-C, per 0.1            | 0.35 | (0.15, 0.82)    | 0.015   |

## (b) non-pachy nAMD vs. N

| Factor                        | OR   | 95% CI          | p value |
|-------------------------------|------|-----------------|---------|
| Gender, ref: male             | 0.69 | (0.28, 1.71)    | 0.427   |
| Age                           | 1.09 | (1.03, 1.16)    | 0.004   |
| BMI                           | 1.07 | (0.91, 1.25)    | 0.426   |
| Duration of hypertension      | 1.04 | (1.00, 1.08)    | 0.079   |
| Duration of DM                | 1.89 | (0.36, 9.98)    | 0.455   |
| TG                            | 0.87 | (0.44, 1.72)    | 0.686   |
| TC                            | 0.76 | (0.51, 1.13)    | 0.178   |
| LDL-C                         | 0.82 | (0.50, 1.34)    | 0.425   |
| HDL-C                         | 0.35 | (0.09, 1.37)    | 0.129   |
| LDL/HDL                       | 0.99 | (0.58, 1.69)    | 0.968   |
| (TC-HDL)/HDL                  | 0.97 | (0.63, 1.49)    | 0.879   |
| log (TG/HDL), per 0.1         | 1.03 | (0.87, 1.21)    | 0.763   |
| apo A-I, per 0.1              | 1.12 | (0.84, 1.50)    | 0.445   |
| apo C-III, per 0.1            | 1.04 | (0.73, 1.48)    | 0.851   |
| apo E, per 0.01               | 1.10 | (0.77, 1.57)    | 0.615   |
| apo A-II, per 0.1             | 1.47 | (0.85, 2.53)    | 0.188   |
| apo B                         | 0.48 | (0.20, 1.16)    | 0.102   |
| apo C-II, per 0.1             | 1.01 | (0.46, 2.18)    | 0.991   |
| apo B/non-HDL-C, per 0.1      | 2.06 | (1.29, 3.27)    | 0.002   |
| apo B/apo A-I                 | 1.05 | (0.65, 1.68)    | 0.849   |
| non-HDL-C, per 0.1            | 0.81 | (0.52, 1.25)    | 0.332   |

## (c) non-pachy nAMD vs. pachy-nAMD

| Factor                        | OR   | 95% CI          | p value |
|-------------------------------|------|-----------------|---------|
| Gender, ref: male             | 3.97 | (0.75, 21.04)   | 0.105   |
| Age                           | 1.05 | (0.97, 1.14)    | 0.203   |
| BMI                           | 0.85 | (0.63, 1.14)    | 0.280   |
| Duration of hypertension      | 1.04 | (0.96, 1.12)    | 0.342   |
| Duration of DM                | 1.01 | (0.90, 1.13)    | 0.904   |
| TG                            | 8.85 | (1.22, 64.32)   | 0.031   |
| TC                            | 1.38 | (0.80, 2.39)    | 0.242   |
| LDL-C                         | 1.54 | (0.75, 3.17)    | 0.236   |
| HDL-C                         | 0.67 | (0.09, 4.90)    | 0.697   |
| LDL/HDL                       | 1.49 | (0.65, 3.42)    | 0.352   |
| (TC-HDL)/HDL                  | 1.51 | (0.74, 3.09)    | 0.256   |
| log (TG/HDL), per 0.1         | 1.38 | (0.99, 1.91)    | 0.057   |
| apo A-I, per 0.1              | 0.99 | (0.62, 1.58)    | 0.977   |
| apo C-III, per 0.1            | 1.06 | (0.68, 1.65)    | 0.783   |
| apo E, per 0.01               | 0.96 | (0.63, 1.46)    | 0.833   |
| apo A-II, per 0.1             | 1.91 | (0.91, 4.00)    | 0.088   |
| apo B                         | 0.59 | (0.18, 1.95)    | 0.391   |

(Continued)
similarities with nAMD. LDL-C was an independent risk factor for pachy-PCV. These results above indicated differential regulated lipid profiles and apolipoproteins contributed to the phenotypes of PCV and nAMD.

Increasing evidence indicates that dysfunction of lipid metabolism plays a vital role in the pathogenesis of PCV and nAMD. The reverse cholesterol transport pathway regulated by ABCA1 gene has been implicated in the development of choroidal neovascularization (26) and basal laminal deposits in mice on high-fat diets (27, 28). Genetic studies demonstrated that genetic variants associated with high-density lipoprotein (HDL) pathway, such as LIPC, CETP and APOE, are associated with the pathogenesis of nAMD or PCV (12, 29).

In this study, we determined the differential levels of apolipoproteins in plasma, lipid profiles and their ratios, and reported the associations of apolipoprotein-mediated lipoproteins formation with PCV and nAMD. ApoB, especially apoB-100 is involved in lipid metabolism and is the main component of lipoproteins. ApoB provides structural integrity to lipoproteins and shields the water-repellent lipids at its center. Compared with LDL-C, apoB level has been shown to be a better indicator for cardiovascular disease (30). In this study, we found that apoB and LDL-C were independent risk factors for PCV, indicating PCV is a lipid dysfunction associated disease.

As we reported previously (31), the ratios of apolipoproteins or lipoproteins reflect two or three parameters respectively, and serve as better indicators to mirror the metabolic and clinical interactions between lipid fractions. In the current study, we have showed that apoB/non-HDL-C was an independent risk factor for nAMD, pachy-nAMD, non-pachy nAMD, and non-pachy PCV, after adjusting for age, sex, duration of hypertension, BMI, and lipoproteins by multivariable logistic analysis. ApoB-100 binds

| TABLE 5 | Comparisons in the lipid profiles and apolipoproteins between PCV, nAMD and control by multivariable logistic regression analysis.

| (a) PCV vs. N‡ | Factor | OR | 95% CI | p value |
|---|---|---|---|---|
| Gender, ref: male | 0.90 | (0.37, 2.19) | 0.816 |
| Age | 1.00 | (0.94, 1.06) | 0.991 |
| BMI | 1.03 | (0.90, 1.18) | 0.68 |
| Duration of hypertension | 1.02 | (0.97, 1.08) | 0.496 |
| apo B | 4.06 | (1.38, 11.96) | 0.011 |

| (b) nAMD vs. N‡ | Factor | OR | 95% CI | p value |
|---|---|---|---|---|
| Gender, ref: male | 0.88 | (0.29, 2.65) | 0.823 |
| Age | 1.08 | (1.01, 1.15) | 0.029 |
| BMI | 1.07 | (0.89, 1.28) | 0.480 |
| Duration of hypertension | 1.00 | (0.95, 1.05) | 0.962 |
| apo B/non-HDL-C, per 0.1 | 1.80 | (1.12, 2.90) | 0.015 |
| TC | 0.95 | (0.58, 1.56) | 0.840 |

| (c) nAMD vs. PCV‡ | Factor | OR | 95% CI | p value |
|---|---|---|---|---|
| Gender, ref: male | 0.53 | (0.19, 1.44) | 0.213 |
| Age | 1.07 | (1.01, 1.14) | 0.033 |
| BMI | 1.07 | (0.86, 1.23) | 0.777 |
| Duration of hypertension | 1.01 | (0.97, 1.07) | 0.562 |
| apo B/apo A-I | 1.35 | (0.93, 1.97) | 0.112 |
| apo B/non-HDL-C, per 0.1 | OR | 95% CI | p value |
| Gender, ref: male | 0.58 | (0.21, 1.59) | 0.287 |
| Age | 1.06 | (1.00, 1.13) | 0.061 |
| BMI | 1.03 | (0.87, 1.23) | 0.704 |
| Duration of hypertension | 1.01 | (0.97, 1.06) | 0.664 |
| non-HDL-C | 0.60 | (0.35, 1.03) | 0.062 |

*Statistically significant: p ≤ 0.05. ‡represents for the reference group.

ref, reference; OR, Odd ratio; CI, confidence interval; BMI, body mass index; DM, diabetic retinopathy; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high density lipoprotein-cholesterol; apo, apolipoprotein; PCV, Polypoidal choroidal vasculopathy; nAMD, neovascular age-related macular degeneration with pachy-choroid; Non-pachy nAMD, Neovascular age-related macular degeneration with non-pachy choroid.
one VLDL, LDL and lipoprotein (a) (Lpa) to form the lipoprotein particle. Each apoB can only bind one atherosclerotic lipoprotein particle (VLDL, LDL-C, Lpa), thus the serum level of apoB represents the level of atherosclerotic lipoprotein particles. However, the quality of cholesterol carried by apoB varied greatly due to the cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30). On the other hand, non-HDL-C is the arithmetic sum of cholesterol LDL particles (rich or deplete LDL particles) (30).

Biomarker was defined as a “characteristic that is objectively measured and evaluated as an indication of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” by the Biomarker Definition Working Group, supported by the U.S. National Institute of Health (32). A biomarker can be diagnostic, monitoring, safety, and susceptibility/risk biomarkers based on its main clinical applications (33). Serum cytokines including IL-1α, IL-1β, IL-4, IL-5, IL-10, IL-13, and IL-17 were found in patients with AMD, supporting that inflammation contributes to the pathogenesis of AMD. Furthermore, IK-17 and TNF-α are indicators for good responder to anti-VEGF therapy in patients with AMD (34). Serum lipid profile (forty-one discriminating metabolites) identified by mass spectrometry, especially the distinct lipid metabolism activating factor was found to be a distinct indicator for PCV (11). Differently expressed miRNAs including EN5G0000249572, miRNA has-miR-20a-5p in peripheral blood mononuclear cells found were to be predictors for good and poor responders to anti-VEGF therapy in patients with nAMD (35). Furthermore, OCT and OCT angiography characteristics have been used as biomarkers for diagnosis and treatment on PCV and nAMD (36). The height of the pigment epithelium detachment (PED) and reflectivity of the content of the PED may predict polypoidal lesions closure (36). Re-examining the baseline scans for an inverted U-shaped elevation in anti-VEGF resistant cases was helpful for detecting polypoidal lesions closure (33). These cellular, molecular and imaging biomarkers have not only deepened our understanding the pathogenesis of PCV and nAMD, but also provide insight to find novel therapeutic targets.

### TABLE 6 | Comparisons in the lipid profiles and apolipoproteins between the subgroups of PCV (pachy- and non-pachy-) and control by multivariable logistic regression analysis.

| (a) pachy-PCV vs. N | Factor | OR | 95% CI | p value |
|---------------------|--------|----|--------|---------|
| Gender, ref: male   | 0.49   | (0.10, 2.47) | 0.387 |
| Age                 | 0.92   | (0.82, 1.03) | 0.13  |
| BMI                 | 1.15   | (0.91, 1.44) | 0.238 |
| Duration of hypertension | 1.00  | (0.93, 1.09) | 0.939 |
| TG                  | 0.04   | (0.00, 0.57) | 0.005 |
| LDL-C               | 3.44   | (1.13, 10.47) | 0.030 |
| apo B/non-HDL-C, per 0.1 | 1.92 | (0.78, 4.74) | 0.159 |

| (b) non-pachy PCV vs. N | Factor | OR | 95% CI | p value |
|-------------------------|--------|----|--------|---------|
| Gender, ref: male       | 0.74   | (0.24, 2.34) | 0.609 |
| Age                     | 1.01   | (0.94, 1.09) | 0.701 |
| BMI                     | 0.94   | (0.78, 1.14) | 0.519 |
| Duration of hypertension| 1.03   | (0.98, 1.09) | 0.222 |
| apo A-II, per 0.1       | 1.94   | (1.03, 3.68) | 0.042 |
| apo B                   | 6.20   | (1.37, 28.02) | 0.018 |

| (c) non-pachy PCV vs. pachy-PCV | Factor | OR | 95% CI | p value |
|---------------------------------|--------|----|--------|---------|
| Gender, ref: male               | 1.59   | (0.39, 6.41) | 0.518 |
| Age                             | 1.07   | (0.97, 1.18) | 0.155 |
| BMI                             | 1.02   | (0.82, 1.26) | 0.882 |
| Duration of hypertension        | 1.01   | (0.94, 1.10) | 0.715 |
| apo A-II, per 0.1               | 2.64   | (1.05, 6.59) | 0.038 |
| apo C-III, per 0.1              | 0.92   | (0.66, 1.28) | 0.611 |

*Statistically significant: p ≤ 0.05. ‡ represents for the reference group.

ref, reference; OR, Odd ratio; CI, confidence interval; BMI, body mass index; DM, diabetic retinopathy; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high density lipoprotein-cholesterol; apo, apolipoprotein; Pachy-PCV, polypoidal choroidal vasculopathy with pachy-choroid; Non-pachy PCV, polypoidal choroidal vasculopathy with non-pachy choroid.
A sa n o t h e r r i s kf a c t o r f o r a t h erosclerosis, TG was found to play a signifi-

cant protective role in pachy-PCV, which was supported by a meta-analysis (included 9 studies). The results showed that increased TG level of 1 mmol/L would significantly reduce the risk of AMD (relative risk, 0.91; 95% CI, 0.87 to 0.94; $I^2 =2 . 6 % ; p= 0.42$) (37). Several other studies also reported that lower TG level in AMD patients (in comparison with normal control), representing a reverse association between TG and cardiovascular diseases (16, 38).TGs are main from of lipid ester and the main constituent of body fat, containing glycerol and three fatty acids. The underlying mechanism of its protective effects on AMD or PCV merits further in vivo or in vitro studies.

A limitation of this study is that it was a nested case-control study with relatively small sample size. Therefore, further longitudinal follow-up studies of large cohorts are warranted to gain insight into the underlying pathological mechanisms and the causal relationship between apos and PCV/nAMD. Furthermore, as this study only included the subjects without lipid lowering therapy, the subjects with severe hyperglycemia may also be excluded, this group of patients may have distinct phenotype of nAMD or PCV, it is interesting to investigate the correlations between the plasma lipid biomarkers for these patients. Furthermore, although we have demonstrated the difference of the dysregulated apolipoproteins and lipid profile in PCV and nAMD, the present study should also merit consideration in interpreting the findings, which need to be further validated with well-designed, prospective, large cohort clinical studies. Nevertheless, this study provides a basis for future studies in PCV and nAMD subtypes on molecular mechanisms related to lipid metabolism.

### TABLE 7 | Comparisons in the lipid profiles and apolipoproteins between the subgroups of nAMD (pachy- and nonpachy-) and control by multivariable logistic regression analysis.

| (a) pachy nAMD vs. N² | Factor | OR | 95% CI | p value |
|-----------------------|--------|----|--------|---------|
| Gender, ref: male     | 0.35   | (0.04, 3.43) | 0.366 |
| Age                   | 1.22   | (1.01, 1.47) | 0.036 |
| BMI                   | 1.36   | (0.92, 2.02) | 0.121 |
| Duration of hypertension | 0.94  | (0.84, 1.06) | 0.315 |
| apo B/non-HDL-C, per 0.1 | 2.78 | (1.16, 6.68) | 0.022 |

| (b) pachy-pachy nAMD vs. N² | Factor | OR | 95% CI | p value |
|-----------------------------|--------|----|--------|---------|
| Gender, ref: male           | 0.93   | (0.29, 2.96) | 0.904 |
| Age                         | 1.06   | (0.98, 1.13) | 0.137 |
| BMI                         | 1.02   | (0.83, 1.25) | 0.861 |
| Duration of hypertension    | 1.02   | (0.97, 1.07) | 0.536 |
| apo B/non-HDL-C, per 0.1    | 1.76   | (1.08, 2.87) | 0.023 |

| (c) non-pachy nAMD vs. pachy-nAMD² | Factor | OR | 95% CI | p value |
|-----------------------------------|--------|----|--------|---------|
| Gender, ref: male                 | 2.19   | (0.29, 16.59) | 0.449 |
| Age                               | 0.96   | (0.86, 1.07) | 0.425 |
| BMI                               | 0.74   | (0.50, 1.10) | 0.133 |
| Duration of hypertension          | 1.09   | (0.98, 1.21) | 0.113 |
| apo B/non-HDL-C, per 0.1          | 0.71   | (0.41, 1.24) | 0.222 |

*Statistically significant: $p \leq 0.05$. ²represents for the reference group.

ref, reference; OR, Odd ratio; CI, confidence interval; BMI, body mass index; DM, diabetic retinopathy; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high density lipoprotein-cholesterol; apo, apolipoprotein; Pachy-nAMD, Neovascular age-related macular degeneration with pachy-choroid; Non-pachy nAMD, Neovascular age-related macular degeneration with non-pachy-choroid.

As another risk factor for atherosclerosis, TG was found to play a significant protective role in pachy-PCV, which was supported by a meta-analysis (included 9 studies). The results showed that increased TG level of 1 mmol/L would significantly reduce the risk of AMD (relative risk, 0.91; 95% CI, 0.87 to 0.94; $I^2 = 2.6\% ; p = 0.42$) (37). Several other studies also reported that lower TG level in AMD patients (in comparison with normal control), representing a reverse association between TG and cardiovascular diseases (16, 38).TGs are main from of lipid ester and the main constituent of body fat, containing glycerol and three fatty acids. The underlying mechanism of its protective effects on AMD or PCV merits further in vivo or in vitro studies.
In summary, to our knowledge, this is the first study to investigate the associations between the dysfunction of lipid metabolism and the phenotypes of PCV, nAMD, pachy- and non-pachy PCV and nAMD. Our results indicated that different regulatory mechanisms of lipid metabolism are associated with distinct phenotypes of PCV and nAMD. Differentially regulated apolipoproteins and lipid profiles could be used as novel biomarkers for PCV and nAMD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Beijing Tongren Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

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AUTHOR CONTRIBUTIONS

Conceptualization, XZ; methodology, XZ and BQ; formal analysis, XZ and BQ; investigation, XZ and BQ; Statistical analysis: XZ, BQ, ZG, YW. writing—review and editing, XZ and BQ; funding acquisition, XZ subjects enrolment: BQ; YN; XC. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China [Grant 81570850 and 82070988] and the Ministry of Science and Technology Foundation of China [Grant 2016YFC1305604].

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China [Grant 81570850 and 82070988] and the Ministry of Science and Technology Foundation of China [Grant 2016YFC1305604].
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