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Original article

Copy number variation in *VEGF* gene as a biomarker of susceptibility to age-related macular degeneration

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ABSTRACT

Background: Several studies in various populations have been conducted to determine candidate genes that could contribute to age-related macular degeneration (AMD) pathogenesis.
Objective: The present study was undertaken to determine the association of high temperature requirement A-1 (*HTRA1*), vascular endothelial growth factor (*VEGF*) and very-low-density receptor (*VLDR*) genes with wet AMD subjects in Malaysia.
Methods: A total of 125 subjects with wet AMD and 120 subjects without AMD from the Malaysian population were selected for this study. Genomic DNA was extracted and copy number variations (CNVs) were determined using quantitative real-time Polymerase Chain Reaction (qPCR) and comparison between the two groups was done. The demographic characteristics were also recorded. Statistical analysis was carried out using software where a level of $P < 0.05$ was considered to be statistically significant.
Result: Statistically significant associations of the *VEGF* gene were observed in mean copy differences between case and control subjects ($P < 0.05$). The consistency of both unadjusted and age-adjusted data at Copy Number CN gain (CN = 3 and CN = 4) suggested that *VEGF* could increase the risk of wet AMD disease ($P < 0.05$). None of CNVs of *HTRA1* and *VLDR* genes showed associations with the wet AMD disease based on comparisons of the frequencies of mean ($P > 0.05$).
Conclusion: Observations of an association between CNVs of *VEGF* gene and wet AMD have revealed that the CNVs of *VEGF* gene appears to be a possible contributor to wet AMD subjects in Malaysia.

1. Introduction

Age-related macular degeneration (AMD) is a common form of irreversible vision loss and a leading cause of blindness among the elderly [1]. The prevalence of AMD among Japanese (0.87%) [2], Chinese (0.2%) [3], Taiwanese (1.9%) [4], Singaporean Malays (0.34%) [5] and Indians (1.9%) [6] are expected to increase with the increasing aging population.

Neovascular or wet AMD is a complex multifactorial disease associated with environmental and genetic risk-factors in many populations. Wet AMD is characterized by abnormal blood vessel (choroidal neovascular membrane) growth and leakage in the choroid that results in subretinal bleeding and scar formation [7]. Despite intensive research efforts, the genetic factors for the development of nAMD remain unclear. Genetic variants found in interleukin, the chemokine (C-C motif) ligand 2 (*CCL2*) and the chemokine (C-X3-C motif) ligand 1 (*CX3CL1*) genes are likely to be responsible for the development of AMD among various ethnicities. *CCL2* and *CX3CL1* genes appear to be crucial in monocyte and microglial cell recruitment to the sub-retinal space in AMD [8,9]. Most of the studies revealed highly significant associations of

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various Single Nucleotide Polymorphism (SNPs) and wet AMD [10]. However, recently copy number variations (CNVs) are also linked to various diseases such as systemic lupus erythematosus [11], hypertension-left ventricular hypertrophy [12], and rheumatoid arthritis [13]. CNVs may account for a significant proportion for the development of various eye diseases in many population studies with conflicting results (Table 1).

PCR-based arrays are commonly used to detect CNVs, in particular quantitative PCR (Q-PCR) are used to determine CNVs precisely for specific candidate genes. Q-PCR is considered as a most reliable and cost effective method compared to conventional PCR and Multiplex ligation dependent probe (MLPA) [12]. The occurrences of CNVs in genes are tending to be highly influential for the individuals predicted to be at high risk for the development of wet AMD. Candidate genes such as Complement Factor H (*CFH*), vascular endothelial growth factor (*VEGF*), high temperature requirement A-1 (*HTRA1*) and very-low-density receptor (*VLDR*) with their putative functions are known to be responsible for the development of wet AMD [14].

In the present study, we analysed the polymorphisms of the three candidate genes; *VEGF, HTRA1* and *VLDR* genes which are identified to be associated with both risk of, and protection against, AMD [15,16]. To our knowledge, there are lacks of report on CNVs among wet AMD subjects particularly in Malaysia. This led us to determine whether the CNVs in *VEGF, HTRA1* and *VLDR* genes could be possible contributor for the development of wet AMD or not.

2. Subjects and methods

2.1. Study subjects

Based on clinical findings in this study, we recruited 245 subjects [125 wet AMD and 120 without AMD cases] using an inclusion and exclusion criteria as a basis for selection. Sample size was adequate for the present study, which is a minimum sample of 110 in each group was calculated based on the ratio of 1:1 for case and control groups, was at significance level of $P < 0.05$ at power 80% on the basis of prevalence of minor alleles referred from previous studies [5,17].

Subjects who undergone comprehensive ophthalmic examinations and diagnosed as wet AMD and presence of choroidal neovascularization (CNV) in either or both eyes, were included in this study [18]. The three main ethnicities (Malay, Chinese and Indians), both male and female subjects, were also included in this study. Polypoidal choroidal vasculopathy and the other retinal disorders were excluded by indocyanine green angiography (ICG) method. Control subjects were free of any eye disorders at the time of ascertainment. They were screened for a complete eye examination by the ophthalmologist and/or review of eye clinic charts. Those with significant signs of retinal diseases such as central serous retinopathy, myopia, retinal dystrophies, diabetic retinopathy, vein occlusion, uveitis and dry AMD were excluded from the control group. A questionnaire was prepared in both Malay and English languages and were distributed to all the subjects to assess their demographic data, relevant medical history and smoking history. Both male and female subjects were recruited from the three main ethnic groups: Malay, Chinese and Indian under this study. A total of 5 ml of peripheral blood were drawn by phlebotomists into an EDTA tubes for further analysis. Genomic DNA from peripheral blood was isolated with QIAamp Blood DNA Mini Kit (QIAGEN, Germany). The purity of extracted DNA was quantified by Nanodrop ND-1000 spectrophotometer (Nano-drop Technologies, Wilmington, DE) and qualified using gel electrophoresis. Amplification and annealing temperatures of the PCR products were optimized using thermal cyclers (Thermo Fisher Scientific, Finland) as in Table 2.

In this study, the qPCR method was used to determine the genetic CNVs in VEGF, HTRA1 and VLDR genes. QPCR was performed with the MiniOpticon Real-Time PCR System (Bio-rad Hungary Ltd) based on the following thermal cycling conditions: holding at 95 °C for 1.0 min, 40 cycles for 5 s at 95 °C for denaturation and 20–30 s at 60–65 °C for the amplification of all genes. Individual real-time PCR reactions were carried out in 20 μl volumes in a 48-well plate containing 1.0 uL of genomic DNA, 10.0 uL 2X qPCR BIO SyGreen Mix L0-Rox Master Mix, 0.8 uL of each primer and run simultaneously with a Type-it CNV Reference Primer Assay according to manufacturer's instructions.

TERT (telomerase reverse transcriptase) is a single copy gene that is used as endogenous reference genes for qPCR-based CNVs validation [12]. However, in our study 10% of samples were selected randomly and the assay was carried out on two separate occasions and the results were consistent and identical. CNVs gain is defined as CN higher than 2 (>2 copies), and CN lower than 2 (<2 copies) would be regarded as CNVs loss [19]. In the qPCR assay, the cycle threshold (CT) value is defined as the number of cycles required for the fluorescent signal to cross the threshold exceeds background level. The CT values was generated from equivalent standard curve mass points (target versus reference) which used in the ΔCT calculation (CT target – CT reference, normalized by relative quantitative PCR (ΔΔCT) then followed by CNVs ratio formula calculation ($2 - \Delta\Delta Ct$) with their sensitivity is commonly used to validate the CNVs in association studies [20,21].

2.2. Statistical method

All statistical and analysis data were secured using statistical software (IBM SPSS Statistics version 22, US). Logistic regressions, t-tests and age-adjusted analysis of covariance were performed to compare the frequencies of each copy number category (CN = 0, 1, 2, or 3+) and the mean number of copies of each gene, respectively, between the AMD subjects and controls. All numerical variables that were evaluated show normally distributed after the Kolmogorov-Smirnov test analysis.

Logistic regression was used to compare the odds of wet AMD among the various copy number levels. Analysis of covariance was performed to compare the mean copy numbers for both categorical and continuous covariates. Statistical significance was deemed to have been attained for all analysis when the p-value was lower than 0.05 ($P < 0.05$).

Ethical approval

Ethical approval was obtained from the Ethics Committee of Universiti Kebangsaan Malaysia (Reference: FF-2014-206) and the University Ethics Committee for Research Involving Human Subjects (Reference: MREC15, P008) under the Putra Grant, UPM Project (No. 9409800) followed by the National Medical Research Register (Reference: NMRR-14-1176-21475). This work also has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in human. An informed consent was obtained from all the subjects.

3. Results

3.1. Analysis of CNVS

Fig. 1 shows the amplification plots for quantitative copy numbers which were determined using differences in the amplification

Table 1
CNVs in AMD on various population studies.

Gene	Disease	Population	CNV	Methodology	Reference
HTRA1	AMD	Korea	loss of 1 copy;<2	Q- PCR (SYBR)	[14]
HTRA1	AMD	Caucasian	gain of 1 copy;>2	Duplex Real time PCR	[20]
VLDR	AMD	Korea	loss of 1 copy;<2	Q- PCR (SYBR)	[14]
VEGF	AMD	Korea	loss of 1 copy;<2	Q- PCR (SYBR)	[14]
VEGF	AMD	Caucasian	gain of 1 copy;>2	Duplex Real time PCR	[20]

Table 2
Primer for the Quantization of the target Gene Copy Number Real Time PCR.

Gene	PCR Primer	Assay Cytoband	Chromosome	Amplicon Size (bp)	Annealing temperature (°C)
HTRA 1	Forward primer 5′-TGTCGAATAAGCACGTTTTCATAA-3′ Reverse primer 5′-CTCCAGCCACAACAATATGC-3′	10q26.3	10	122	61.2
VEGF	Forward primer 5′-CGCACTGAAACTTTTCGTCCAA-3′ Reverse primer 5′-CTCTCCTCTTCCTTCTCTTCTTCC-3′	6p12	6	43	61.7
VLDR	Forward primer 5′-CCTTCCCGGTGACGTTTCC-3′ Reverse primer 5′-GCCCTTCCCAACAAGACAGG-3′	9p24	9	84	57.7

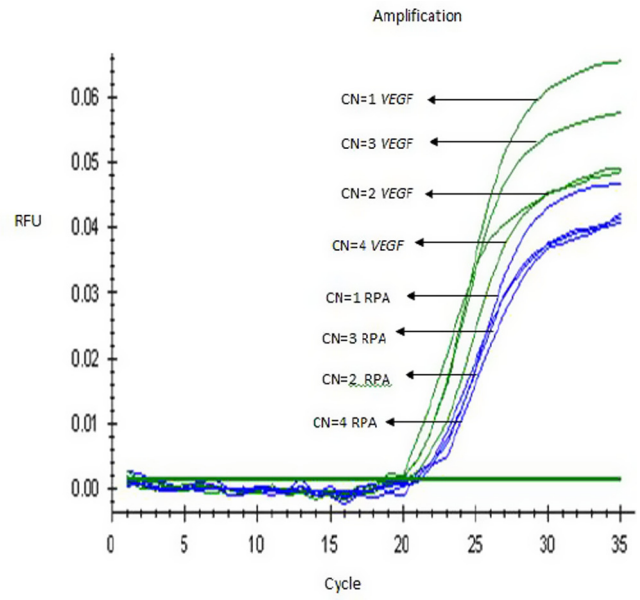

Fig. 1. The amplification plot for quantitative copy number were determined using differences in the amplification of the gene of in interest (VEGF) relative to the Reference primer Essay (RPA) Representative data show amplification plots for participants of CN = 1, CN = 2, CN = 3 and CN = 4 for *VEGF*.

of *VEGF* gene relative to the Reference primer assay (RPA). The consistent amplification observed assured that equal amounts of genomic DNA were used in each assay and amplification efficiencies were similar to *VEGF* gene and the internal control *TERT* gene [22].

Table 3 shows the copy number frequencies in wet AMD subjects and controls along with odds ratio and 95% confidence interval. Quantitative copy numbers (CN = 0, 1, 2, 3 or 4) were determined for the three AMD associated genes in each sample. CNVs existed in both wet AMD and control groups. CN = 2 was the predominant copy number genotype [20]. The unadjusted and the age adjusted logistic regressions were performed to compare CNVs frequencies between wet AMD and control subjects. There was no significant differences observed in *HTRA1* and *VLDR* genes between the wet AMD subjects and the controls (P > 0.05).

For *VEGF* gene, both the unadjusted and age-adjusted data showed associations with AMD. CN gain, CN = 3 (OR = 1.26; 95% CI, 1.15–2.44: P = 0.036) and CN = 4 (OR = 0.39; 95% CI, 0.29–1.87: P = 0.028) (P < 0.05). This showed CN gain, CN = 3 and CN = 4 was a risk factor for wet AMD. The patient with CN gain of the *VEGF* gene had wet AMD in the right eye (Fig. 2A–F).

The mean number of copies of each gene was calculated to determine the trend towards increased or decreased copy numbers among the subjects (Table 4). Comparisons between wet AMD and controls were performed using two tail t-tests and age-adjusted analysis of covariance. Both the unadjusted and age-adjusted data showed that there was no association with wet AMD subjects for *HTRA1* and *VLDR* genes (P > 0.05). In contrast, a significant association was observed between wet AMD subjects and controls (P < 0.05) for the *VEGF* gene.

A total of 245 subjects of cases and controls (in Tables 5 and 6) were divided into a higher age group (>60 years) and a lower age group (<60 years) and the relationship between age and gender was evaluated by conventional chi-square tests. All genes did not show any significant association in age and gender except for the *VEGF* case in terms of age (P < 0.05).

Clinical characteristics of all subjects: Table 7 summarizes the demographic and the clinical parameters of all the subjects. Significant differences were observed within ethnic groups with 61.0% for Chinese, 5.5% for Indians and 33.5% for Malays in case subjects as compared to 36.0% for Chinese, 19.9% for Indians and 44.1% for Malays which were observed for controls. There were significant differences observed for ethnicity, hypertension, hypercholesterolemia, smoking and others between wet AMD subjects and controls (P < 0.05). However, gender and diabetes mellitus did not differ significantly (P > 0.05).

4. Discussion

Unravelling the genetics of AMD has been difficult. However, recently a study showed that the innate immunity proved that activation of the complement cascade plays a pivotal role in AMD pathogenesis [23]. Moreover, extensive genetic studies of

Table 3
Copy number frequencies in AMD patients and controls.

Gene	CNV	AMD n (%)	Control n (%)	Unadjusted OR (95% CI)	p-value	Age-Adjusted OR (95% CI)	p-value
HTRA1	0	6(4.8)	2(1.7)	0.34(0.28–1.73)	0.19	0.36(0.71–1.88)	0.22
	1	4(3.2)	5(4.2)	1.28(0.33–4.90)	0.71	1.18(0.29–4.69)	0.81
	2	113(90.4)	110(91.6)	1.0	–	1.0	–
	3	2(1.6)	1(0.8)	1.94(0.24–2.78)	0.58	1.64(0.14–3.56)	0.68
	4	0(0)	2(1.7)	–	–	–	–
	Total	125(1 0 0)	120(1 0 0)	–	–	–	–
VEGF	0	2(1.6)	5(4.2)	1.42(0.46–2.80)	0.29	2.55(0.47–3.74)	0.27
	1	2(1.6)	8(6.7)	2.12(0.80–6.73)	0.091	2.79(0.55–9.19)	0.21
	2	102(81.6)	105(87.5)	1.0	–	1.0	–
	3	9(7.2)	1(0.8)	1.26(1.15–2.44)	0.036*	1.86(1.08–2.65)	0.042*
	4	10(8.0)	1(0.8)	0.39(0.29–1.87)	0.028*	0.89(0.22–2.29)	0.032*
	Total	125(1 0 0)	120(1 0 0)	–	–	–	–
VLDR	0	10(8.0)	7(5.8)	0.68(0.25–1.87)	0.46	0.70(0.25–1.96)	0.5
	1	8(6.4)	6(5.0)	0.73(0.24–2.19)	0.58	0.78(0.25–2.38)	0.66
	2	102(81.6)	104(86.7)	1.0	–	1.0	–
	3	3(2.4)	2(1.7)	1.52(0.25–9.34)	0.64	1.26(0.20–7.80)	0.8
	4	2(1.6)	1(0.8)	2.03(0.18–4.84)	0.56	3.03(0.23–5.93)	0.39
	Total	125(100)	120(100)	–	–	–	–

The frequencies of all CNV categories were compared using unadjusted and age-adjusted logistic regression, with CN = 2 as CN reference.
* $P < 0.05$.

AMD have identified several DNA variants that alter AMD risk. It is estimated that known genetic variants account for more than half of the heritability of AMD [24].

In addition, several genome-wide association studies (GWAS) and meta-analysis studies showed a strong relationship between underlying genetic variants for AMD and their risk factors [1,19]. Meanwhile, CNVs are quite common in the human genome although it is less abundant than SNP, which can have dramatic phenotypic consequences as a result of altering gene dosage, disrupting coding sequences or perturbing long-range gene regulation [25].

Association studies on CNVs of genes involved in a specific function might contribute to AMD, has resulted in conflicting results. Even though the association studies approach is widespread and the lists of CNVs are increasing, still the complexity of AMD is unclear. In this study, we examined the relationship between CNVs of *HTRA1, VEGF* and *VLDR* genes and wet AMD subjects in Malaysia.

VEGF gene has been linked as a key mediator and involved in angiogenesis and experimental choroidal neovascularization in the retina that has consistently demonstrated as risk factor in AMD disease [26]. Eyetech Study reported that *VEGF* gene plays an important role in vascular growth as it is considered as a target for inhibition therapy for AMD [27]. *HTRA1* located at chromosome 10q26 and one of major locus that is associated with AMD. Irregulation of *HTRA1* gene may contribute to disturbance in the Bruch's membrane and retinal pigment epithelium (RPE) thus will lead to AMD pathogenesis [28,29]. In addition, *VLDR* gene is a member of the LDL receptor gene family and has a widespread expression in many tissues. *VLDR* also involved in lipid transportation and chronic inflammation through the Wnt pathway that lead to choroidal neovascularization [30,31].

Recent work suggests that *VEGF* expression levels are significantly increased in carriers of the risk haplotype associated with AMD [32]. An association was found in this study between the CN gain (CN = 3 and CN = 4) in the *VEGF* gene. This concurs well with a study conducted in Korea [14]. However, there was no significant association ($P > 0.05$) between *HTRA1* and the *VLDR* gene with the development of wet AMD in Malaysia. Similarly, a study among the Caucasians of Anglo-Celtic ethnicity reported that there was no significant association ($P > 0.05$) of *HTRA1* gene in the development of wet AMD [33]. The contradictory findings of *HTRA1* and *VLDR* genes could be due to regional complexity in the human genome. In addition, sampling bias, environmental factors, other gene interactions and ethnic differences could be possible explanations for the contradictory findings.

In this study, CN gain; CN = 3 and CN = 4 of the *VEGF* gene was a risk for the development of wet AMD among Malaysian subjects. These findings are well in accordance with the study conducted among Korean population [14]. The deletion or CN loss (CN = 0 & 1) of CNVs in genes could protect against wet AMD and the duplication; CN gain (CN = 3 & 4) could be a risk for AMD [34,35]. We demonstrated the results of *HTRA1, VEGF* and the *VLDR* gene with age using logistic regression analysis (Table 3), analysis of covariance to determine mean copy numbers for all subjects (Table 4) and chi-square tests (Tables 5 and 6). For gender, the trend tended to increase with age between 40 and 70 years in both males and females. However, the frequency of all genes in the highest age group showed a decrease in both genders (Table 6).

We have developed a simple and reliable real-time qPCR assay for determining CNVs for *VEGF, HTRA1* and *VLDR* genes. This method is designed to detect all larger deletions and duplications affecting candidate genes and the regulatory region [36]. SYBR Green real-time qPCR has been used for analysis of copy number variation. SYBR Green emits strong fluorescence on excitation when binding double stranded DNA. Compared to TaqMan, the SYBR Green labelling is inexpensive although its detection is prone to lack of specificity [37].

AMD is the most common diagnosis for subjects aged between 50–80 years and this is reflected in other population studies such as America [20] and Taiwan [4]. Prevalence increases substantially with increasing age [35]. Age plays an important role in the development of AMD which is common in adults [38]. The majority of the wet AMD subjects in this study were Chinese followed by Malays and Indians (Table 7). This scenario was similar in another study where the study population consisted of 60.2% Chinese compared to 20.3% Malays and 19.5% Indians [39]. The smoking factor was highly associated with AMD as reported by Spencer et al. [40] and this finding was consistent with the present study where smoking interacted significantly with wet AMD compared to the control subjects ($P < 0.01$). Smoking could increase oxidative stress to tissues which could in turn increase the susceptibility of AMD [41]. According to Donaldson et al. [42], high cholesterol has also been associated with wet AMD. In the present study, hypercholesterolemia showed an association among wet AMD subjects

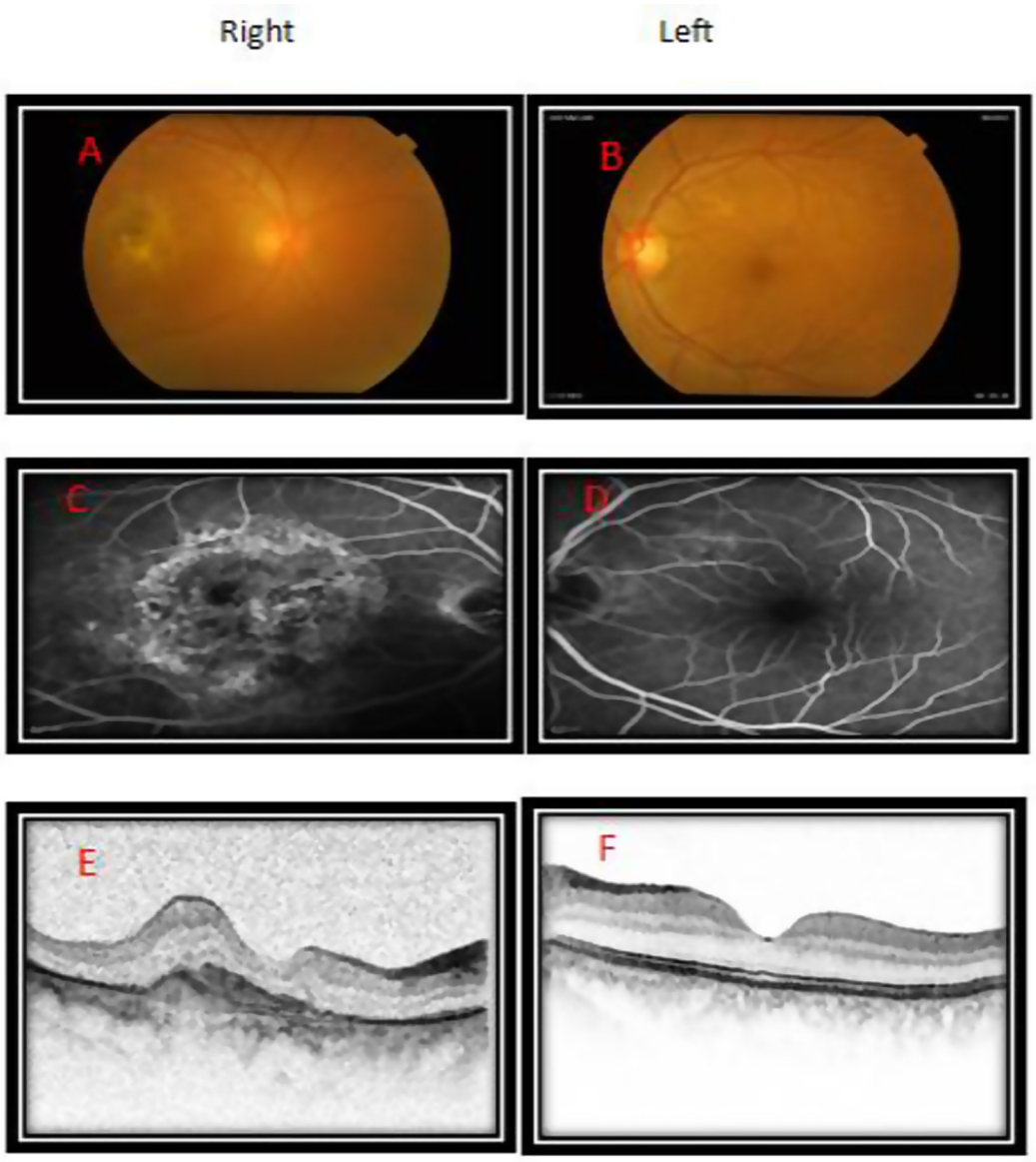

Fig. 2. Fundus photographs in patient with CNV duplication. (A) A 59 year old woman with duplication at *VEGF* gene showed wet AMD with marked leakage at level of the retina in the right eye. (B) The left eye shows no abnormality. (C) Fluorescein angiography (FFA) shows confluent intraretinal fibrosis and retinal oedema of the macula area together with abnormal vascular network in the right eye. (D) The left eye have normal vascular network arrangement. (E) Optical Coherence Tomography (OCT) shows the photoreceptor cell atrophy and focal elevations of retinal pigment epithelial layer in the right eye. (F) For left eye, the image shows junction of photoreceptor looks normal and in a good arrangement.

Table 4
Mean copy numbers in AMD patients and controls.

GENES	Unadjusted			Age-adjusted		
	AMD	Control	P	AMD	Control	P
HTRA1	2.0 ± 0.0	2.03 ± 0.266	0.15	2.0 ± 0.0	2.03 ± 0.266	0.13
VEGF	2.17 ± 0.57	2.01 ± 0.19	0.007*	2.17 ± 0.57	2.01 ± 0.19	0.026*
VLDR	2.03 ± 0.27	2.01 ± 0.19	0.55	2.03 ± 0.27	2.01 ± 0.19	0.49

Data presented as mean ± SD in both unadjusted and age-adjusted results and calculated by using analysis of covariance.
* $P < 0.05$.

($P < 0.05$). Hypertension was associated with AMD in India [6] and Maryland [43] and these finding were consistent with our study where hypertension was highly associated with wet AMD compared to the control subjects ($P < 0.01$). Although relatively few studies reported that diabetes [44] is one of the risk factors for AMD, this study failed to show an association with diabetes among wet AMD subjects ($P > 0.05$).

5. Limitations of the study

Although our study determined the evidence of the association between CNVs of *VEGF* gene and wet AMD, the present study has to be interpreted within the context of its limitations. First, the current sample sizes might have offered insufficient power because of stratification of three ethnicities. Second, the subjects were not

Table 5
Effect of Age on CNVs of AMD.

Gene	Case Distribution[t]				Control Distribution[t]		
	CNV	<60 Years	>60 Years	P-Value	<60 Years	>60 Years	P-Value
HTRA1	<2	2(5.2)	8(8.6)	0.794	2(3.0)	5(6.5)	0.777
	2	17(94.8)	96(89.7)		34(94.0)	76(91.3)	
	>2	0(0)	2(1.7)		1(3.0)	2(2.2)	
	TOTAL	19(100)	106(100)		37(100)	83(100)	
VEGF	<2	1(4.9)	3(1.8)	0.007*	5(15.4)	8(9.9)	0.597
	2	15(82.6)	87(95.1)		32(84.6)	73(87.4)	
	>2	3(12.5)	16(3.1)		0(0)	2(2.7)	
	TOTAL	19(100)	106(100)		37(100)	83(100)	
VLDR	<2	5(15.5)	13(11.9)	0.413	4(13.3)	9(10.0)	0.274
	2	13(78.8)	89(85.5)		31(83.3)	73(87.8)	
	>2	1(5.7)	4(2.6)		2(3.4)	1(2.2)	
	TOTAL	19(100)	106(100)		37(100)	83(100)	

[t] Values are n (%).
* P < 0.05.

Table 6
Effect of Gender on CNVs of AMD.

Gene	Case Distribution[t]				Control Distribution[t]		
	CNV	Male	Female	P-Value	Male	Female	P-Value
HTRA1	<2	7(10.6)	3(6.8)	0.721	6(6.6)	1(2.9)	0.773
	2	72(88.9)	41(93.2)		79(91.2)	31(94.2)	
	>2	2(0.5)	0(0)		2(2.2)	1(2.9)	
	TOTAL	81(100)	44(100)		87(100)	33(100)	
VEGF	<2	3(3.8)	1(2.2)	0.818	7(7.8)	6(20)	0.061
	2	64(80)	38(84.4)		78(90.0)	27(80)	
	>2	14(16.2)	5(13.4)		2(2.2)	0(0)	
	TOTAL	81(100)	44(100)		87(100)	33(100)	
VLDR	<2	10(10.0)	8(8.9)	0.061	7(10.9)	6(10.7)	0.851
	2	68(86.3)	34(89.0)		78(86.9)	26(85.7)	
	>2	3(3.7)	2(2.1)		2(2.2)	1(3.6)	
	TOTAL	81(100)	44(100)		87(100)	33(100)	

*P < 0.05.
[t] Values are n (%).

Table 7
Demographic and clinical features of study participants.

		AMD (n = 125)	CONTROL (n = 120)	p-value
Age		69.05 ± 8.64	64.45 ± 11.19	<0.01*
Gender	Male	81 (64.8)	87 (71.6)	0.11
	Female	44(35.2)	33(28.4)	
Ethnic	Chinese	77 (61.0)	42(36.0)	<0.01*
	Indian	7(5.5)	25(19.9)	
	Malay	41(33.5)	53(44.1)	
Diabetes mellitus	Yes	37(29.7)	44(36.4)	0.117
	No	88(70.3)	76(63.6)	
Hypertension	Yes	76(59.7)	49(40.7)	<0.01*
	No	49(40.3)	71(59.3)	
Hypercholesterolemia	Yes	18(13.1)	1(1.7)	<0.01*
	No	107(86.9)	119(98.3)	
Smoking	Yes	23(17.8)	47(39.8)	<0.01*
	No	102(82.2)	73(60.2)	
Others (Ischemic heart disease, Stroke)	Yes	28(19.5)	10(7.6)	<0.01*
	No	97(80.5)	110(92.4)	

Data presented as mean ± SD for age and as n (%) for all other type.
* P < 0.05.

age-matched and it is possible that populations with specific genetic backgrounds could affect the results when compared to the western populations. Besides, discrepancies may also be related to study designs and methodologies used. Consequently, there could be other candidate genes were not included as our target genes that may contribute well to the pathogenesis of wet AMD.

6. Conclusion

The duplication or CN gain (CN = 3 and CN = 4) of the *VEGF* gene was associated with the development of wet AMD among Malaysian subjects. In conclusion, CNVs of *VEGF* gene might be a possible genetic marker for the development of wet AMD among Malaysians.

Declaration of conflict of interest

The authors declare no conflict of interests.

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