# Association of the *HtrA1* rs11200638 Polymorphism with Neovascular Age-Related Macular Degeneration in Indonesia

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## ABSTRACT

***Introduction***: The aim of this study was to investigate the association of the *HtrA1* rs11200638 polymorphism with neovascular age-related macular degeneration (nAMD) in Indonesia.

***Methods***: This case–control study included 80 patients with nAMD and 85 controls. Demographic parameters and whole blood were collected from each participant. Genomic DNA was extracted and used to assess the rs11200638 genotype by PCR and restriction enzyme digestion. Associations between the *HtrA1* rs11200638 polymorphism and other risk factors for susceptibility to nAMD were assessed using the logistic regression model.

***Results***: Significant allelic associations between the *HtrA1* polymorphism and nAMD were detected (odds ratio [OR] 8.67; 95% confidence interval [CI] 4.88–15.41; $P < 0.001$). Genotype analysis showed a statistical difference between the nAMD group and the control group ($P < 0.001$). In the multiple adjusted logistic regression model, people with the AA genotype were more likely to have nAMD although there was a wide confidence interval (OR 19.65; 95% CI 4.52–85.38; $P < 0.001$).

***Conclusion***: Our findings show that the risk of nAMD increased in the presence of risk alleles of *HtrA1* rs11200638.

**Keywords**: Age-related macular degeneration; *HtrA1*; Polymorphism

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**Key Summary Points**

The *HtrA1* rs11200638 polymorphism is associated with the risk of neovascular age-related macular degeneration (nAMD) in Indonesian patients.

The presence of hypertension compounds the genetic risk for nAMD.

The results of this study are in accordance with those of other epidemiological studies involving patients of different ethnicity in supporting the hypothesis that *HtrA1* contributes to the risk of nAMD.

## INTRODUCTION

Age-related macular degeneration (AMD) is a progressive degenerative disease affecting the macula and is among the five leading causes of vision loss worldwide [1]. AMD results from a sequence of deterioration processes that occur in photoreceptors, the retinal pigment epithelium (RPE), and Bruch's membrane (BM). These result in an irreversible lesion that manifests clinically as geographic atrophy (dry AMD) or they cause aberrant blood vessels originating from the choroid to leak at the macular region (neovascular AMD [nAMD]). If untreated, these conditions can lead to permanent vision loss. It is notable that that not all aged individuals follow the similar processes and develop AMD, implying a genetic-driven pathophysiology of the disease process.

High-temperature requirement A1 (*HtrA1*) is one of important genetic factors in nAMD etiology, in addition to Age-related maculopathy susceptibility 2 (*ARMS2*) and Complement factor H (CFH). The *HtrA1* genetic variant rs11200638 is located precisely at the *HtrA1* promoter. The three most strongly associated loci identified to date are located on chromosome one (1q31), CFH (rs1061170), chromosome 10 (10q26), *ARMS2* (rs10490924;

del443ins54), and *HtrA1* (rs11200638) [2]. In an earlier study, we have shown that the *ARMS2* rs10490924 and del443ins54 variants show a strong association with nAMD in Indonesia [3]. The position of the rs11200638 polymorphism in the genome is very close to those of the rs10490924 and del443ins54 polymorphisms [4–7]. These single nucleotide polymorphisms segregate together during chromosome crossing-over, resulting in almost all cases having the same status (wildtype, carrier, and mutant homozygote) with each other. Research has also shown that these two loci are highly predictive (i.e., in near perfect linkage disequilibrium [$D' > 0.98$]) for AMD [7]. Therefore, investigation of the presence of the rs11200638 polymorphism in Indonesia will provide a large overview of the epidemiology and pathogenesis of nAMD.

Replication studies on different ethnic populations show consistent results [7–20], but there are no reports on Indonesian populations. In addition to CFH and *ARMS2* [3], no other genetics factors have been examined in Indonesia. The aim of this study was to investigate the associations of *HtrA1* with nAMD in the Indonesian population, of which the majority ethnic group is the Asian Malay group.

## METHODS

This case–control study included participants aged $\geq$ 45 years. Between 2016 and 2018 we recruited 80 patients with nAMD and 85 age-matched control participants into this study. The purpose of the research and the procedures were fully explained prior to the participants signing an informed consent form and undergoing blood collection and comprehensive ophthalmologic examinations. The study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (ethics number: KE/FK/864/EC [5 August 2016]; KE/FK/1109/EC/2017 [12 October 2017]; KE/FK/1108/EC/2018 [18 October 2018]). This study adhered to the ethical standards of the Declaration of Helsinki of 1964 and its later amendments. The inclusion/exclusion criteria

and the criteria for AMD diagnosis were as reported previously [3].

## Genotyping

The procedures followed for extraction of the genomic DNA and gene amplification were as described previously [3]. The primer sequences were: forward 5′-TTCCCATCTGAGACCGCT-3′ and reverse 5′-GGAAAGTTCCTGCAAATCG-3′ [17]. The PCR cycling conditions were: 1 cycle of 95 °C for 10 min, followed by 35 cycles of 95 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s, with a final cycle of 72 °C for 5 min. For genotype discrimination, the 400-bp amplicon was digested with 5 U of EagI restriction enzyme (New England BioLabs, Ipswich. MA, USA) by an overnight incubation at 37 °C. Following electrophoresis in 2% agarose gels, the DNA bands were visualized with fluorosafe DNA staining (1st Base Asia, Selangor, Malaysia; Cat. No. BIO-5170-1 ml). The undigested samples were determined to be the AA genotype, whereas the partially or totally digested samples were designated the AG or GG genotype. Sanger sequencing was used to verify each genotype (AA; GA; GG) in the nAMD patient and control groups.

## Statistical Analysis

A complete case analysis of those with complete covariate and genetic data was performed. Statistical analysis, including the Hardy–Weinberg equilibrium (HWE) test and multivariable logistic regression, were performed using STATA® version 16 (StataCorp, College Station, TX, USA) as reported previously [3].

# RESULTS

Blood samples were collected from 80 patients with nAMD and 85 controls, all of whom were Indonesian. The cases and controls were similar in terms of demographic data (Table 1). The average age of the patient and control groups was 67.3 and 68.2 years, respectively; 50.0 and 54.0% of the patient and control groups were

**Table 1** Demographic characteristics of study participants

| Demographic characteristics | Case group (*n* = 80) | Control group (*n* = 85) | *P* |
|---|---|---|---|
| Age, years | | | 0.49 |
| Range (median) | 45–82 (67.5) | 49–99 (69) | |
| Mean ± SD | 67.3 ± 8.4 | 68.2 ± 7.8 | |
| Sex | | | 0.55 |
| Male | 33 (41.0%) | 39 (46.0%) | |
| Female | 47 (59.0%) | 46 (54.0%) | |
| BMI (kg/m$^2$) | | | 0.02 |
| Range (median) | 16.7–37.1 (23.3) | 15.2–36.8 (21.5) | |
| Mean ± SD | 23.6 ± 3.5 | 22.2 ± 4.2 | |
| Sunlight exposure | | | 0.92 |
| Indoor workplace | 58 (72.0%) | 61 (72.0%) | |
| Outdoor workplace | 22 (28.0%) | 24 (28.0%) | |
| Smoking | | | 0.75 |
| Never | 61 (76.0%) | 63 (74.0%) | |
| Ever | 19 (24.0%) | 22 (26.0%) | |
| Blood pressure | | | < 0.001 |
| Normal blood pressure | 44 (55.0%) | 68 (80.0%) | |
| High blood pressure | 36 (45.0%) | 17 (20.0%) | |

*BMI* Body mass index, *nAMD* neovascular age-related macular degeneration, *SD* standard deviation

female; and the mean body mass index (BMI) for AMD cases and controls was 23.6 and 22.2 kg/m$^2$, respectively. Most participants were predominantly working indoors with less exposure to sunlight (72% for both AMD cases and controls). The number of AMD patients

with high blood pressure was significantly higher in the nAMD group than in the controls.

The allele and genotype distributions of the *HtrA1* rs11200638 polymorphism are summarized in Table 2. Comparison of the cases and controls revealed a statistically significant difference in the allele or genotype distributions of *HtrA1* rs11200638 ($P < 0.001$). The A allele frequency was 88.7% in nAMD patients and 42.2% in controls. There was a higher percentage of AA genotypes in the nAMD group (77.4%) than in the control group (22.6%), and the opposite was true for AG genotypes, which were less frequent in the nAMD group (21.8%) than in the control group (76.2%). The unconditional logistic regression analysis showed that the A allele was associated with an increased risk of nAMD (odds ratio [OR] 8.67; 95% confidence Interval [CI] 4.88–15.41; $P < 0.001$). Similarly, an association was found in adjusted logistic regression model with a per-A-allele OR of 3.73 (95% CI 1.11–12.56; $P = 0.034$).

Individuals with the AA genotype were found to have strong association with nAMD in the unadjusted logistic regression analysis (OR 26.23; 95% CI: 7.10–96.93; $P < 0.001$), age- and sex-adjusted logistic regression analysis (OR 26.85; 95% CI 7.10–101.49), and the multiple variable logistic regression analysis (OR 19.65; 95% CI 4.52–85.38; $P < 0.001$) (Table 3). Additionally, the multiple variable logistic regression analysis showed that hypertension was associated with an increased risk for nAMD (OR 3.4; 95% CI 1.30–8.95; $P = 0.013$). No significant association was found between BMI and smoking with nAMD in the multiple variable logistic regression analysis ($P \geq 0.05$).

## DISCUSSION

We identified an association between the *HtrA1* rs11200638 polymorphism and nAMD in an Indonesian population. However, these results should be interpreted cautiously due to the wide confidence interval, possibly due to the small sample size [21]. The findings from this gender and age-matched case–control study suggest that either A alleles or AA genotypes of the *HtrA1* rs11200638 polymorphism were associated with the onset of nAMD. Patients with the AA genotype were more prone to have AMD by nearly 26-fold. The association remained strong after data adjustment with

**Table 2** Distribution of alleles and genotypes of the *HtrA1* rs11200638 polymorphism

| Allele | Allele distribution, *n* (%) | | *P* | Genotype | Genotype distribution, *n* (%) | | *P* value | *P* (HWE) |
|---|---|---|---|---|---|---|---|---|
| | Case group | Control group | | | Case group | Control group | | |
| G | 18 (11.3%) | 89 (57.8%) | < 0.001 | GG | 3 (11.5%) | 23 (88.5%) | < 0.001 | 0.204 |
| A | 142 (88.7%) | 65 (42.2%) | | GA | 12 (21.8%) | 43 (76.2%) | | |
| | | | | AA | 65 (77.4%) | 19 (22.6%) | | |

*CI* Confidence interval, *HWE* Hardy–Weinberg equilibrium in control group, *OR* odds ratio

**Table 3** Association between the *HtrA1* rs11200638 polymorphism and nAMD in an Indonesian population

| Genotype | Crude OR (95% CI) | *P* | Adjusted OR (95% CI)[a] | *P* | Adjusted OR (95% CI)[b] | *P* |
|---|---|---|---|---|---|---|
| GG | 1.00 (reference) | – | 1.00 (reference) | – | 1.00 (reference) | – |
| GA | 2.14 (0.55–8.36) | 0.274 | 1.80 (0.45–7.22) | 0.409 | 1.37 (0.30–6.21) | 0.683 |
| AA | 26.23 (7.10–96.93) | < 0.001 | 26.85 (7.10–101.49) | < 0.001 | 19.65 (4.52–85.38) | < 0.001 |

[a] Adjusted for age and sex
[b] Adjusted for smoking, body mass index, and blood pressure

**Table 4** Results from studies on the association between the *HtrA1* polymorphism and nAMD

| References | Location | Ethnicity | Sample | OR (95% CI) |
|---|---|---|---|---|
| DeWan et al. [8] | China | Chinese | 96 cases and 130 controls[a] | $OR_{hom}$ = 10 (4.38–22.82) |
| Mori et al. [13] | Japan | East Asian | 123 cases and 133 controls[a] | $OR_{hom}$ = 5.59 (2.66–11.76) |
| Lin et al. [14] | Taiwan | Taiwanese Chinese | 95 cases and 90 controls[b] | $OR_{het}$ = 1.97 (0.81–4.81), $OR_{hom}$ = 8.59, (3.28–22.49) |
| Yan et al. [25] | China | Han Chinese | 109 cases, 150 controls | OR GA + AA = 2.02 (1.20–3.39) |
| Tam et al. [15] | China | Chinese | 163 cases and 183 controls[b] | $OR_{het}$ = 1.88 (0.96–3.66) $OR_{hom}$ = 7.6 (3.94–14.51) |
| Gong et al. [16] | China | Chinese | 99 cases and 73 controls | $OR_{hom}$ = 4.19 (2.28–7.70) |
| Lana et al. [17] | Brazil | South American | 204 cases and 166 controls | $OR_{hom}$ = 25.97 ± 4.42 (16.75–34.32) |
| Abbas and Azzazy [18] | Egypt | Arabic | 26 cases and 20 controls | $OR_{het+hom}$ = 6 (1.4–24.7) |
| Kaur et al. [19] | India | East Asian | 250 cases and 250 controls | $OR_{hom}$ = 6.69 (3.69–12.10) |
| Matušková et al. [24] | Czech Republic | Czech population | 307 cases and 191 controls | OR = 16.02 (5.4–47.54) |
| Francis et al. [20] | Northern Europe | Caucasian | 333 cases and 171 controls[a] | $OR_{hom}$ = 3.973 (2.928–5.390) |
| Gili et al. [9] | Spain | Spanish Caucasian | 187 cases and 196 controls | $OR_{hom}$ = 6.44 (3.62–11.47) |
| Yang et al. [10] | USA | Caucasian | 581 cases and 309 controls | $OR_{het}$ = 1.90 (1.40, 2.58), $OR_{hom}$ = 7.51 (3.75, 15.04) |
| Gibbs et al. [7] | USA | Caucasian | 342 cases and 215 controls[b] | |
| Chen et al. [12] | USA | Caucasian | 774 cases and 294 controls[b]; 192 bilateral nAMD, 278 unilateral nAMD, 234 bilateral GA, 72 unilateral GA | Bil $OR_{hom}$ = 10.95 (5.26–22.77) Uni $OR_{hom}$ = 5.62 (2.65–11.90) |
| DeAngelis et al. [11] | USA | Caucasian | 73 sib pairs[c] | $OR_{hom}$ = 98.41 (13.45–720.08 $P < 10^{-5}$) $OR_{het}$ = 6.05 (2.13–17.21; $P < 10^{-3}$) |

**Table 4** continued

| References | Location | Ethnicity | Sample | OR (95% CI) |
|---|---|---|---|---|
| Mohamad et al. [23] | Malaysia | Asian | 145 cases, 145 controls | $OR_{hom}$ = 1.52 (1.07–2.15) |

*GA* Geographic atrophy
[a] Age-matched controls
[b] Age-matched and sex-matched controls
[c] Control was a sibling of each patient

smoking, BMI, and blood pressure. People with the AA genotype had a 20-fold increased risk of having nAMD than those with the GG genotype. This finding is similar to results from other studies in other ethnic groups [12, 17–19, 22–24].

An association between *HtrA1* and nAMD has also been found in other countries (Table 4). In Southeast Asia, similar results were also reported in Malaysia [23].

The *HtrA1* rs11200638 polymorphism is widely reported to be highly associated with the risk for nAMD worldwide. Epidemiological studies have shown that, compared to people with the GG genotype, those with the AA genotype in the Chinese population have a tenfold increased risk for nAMD [8] and their Caucasian counterparts have a 7.5-fold increased risk [10]. This higher risk of AA genotype for nAMD was reconfirmed in other studies involving Caucasian populations [7, 12]. The A allele and AA genotypes have also associated with the dry forms of AMD in addition to nAMD in Chinese Taiwanese subjects [14]. Another Chinese study showed a 7.6-fold increased risk in subjects with the A allele, with smoking status compounding the risk to 15.7-fold [15]. Studies in Middle East [18], India [19], and Brazil [17] have shown similar results. Individuals with the AG and AA genotype have been found to have a 2.2- and 8.7-fold higher risk of developing AMD, respectively, when compared with those who carry the GG genotype [26].

The *HtrA1* gene encodes a serine protease, and this protease is produced by many tissues, including the RPE [27]. This protein plays an important role in the breakdown of many components of the extracellular matrix (ECM) [27–29]. It has been hypothesized that these breakdowns of ECM proteins is related to development of neovascularization [27, 29].

A high concentration of HtrA1 has been found in the aqueous humor of nAMD patients, with subsequent decrease following intravitreal injections of 0.5 mg ranibizumab [31]. The *HtrA1* polymorphism was also reported to be associated with the AMD onset in the second eye [12]. The association of the *HtrA1* polymorphism to the response to ranibizumab treatment in nAMD has not been consistent in studies [23, 26, 32, 33]

The main limitations of our study are the small sample size and the hospital-based design. The design may have only captured the advanced profiles of AMD patients, thereby masking the true representation of AMD in general population. Future studies should include larger and more diverse sample sizes to allow subanalyses based on ethnic origin in Indonesia. Identifying other target genes related to AMD in the Indonesian population is also warranted as this study is only the second study on the genetic factors associated with nAMD and only the second study on AMD in Indonesia.

## CONCLUSIONS

We identified that the *HtrA1* rs11200638 polymorphism is significantly associated with risk of nAMD albeit with a wide confidence interval.

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