Ghorbani and Saadat. Scr Med 2023 Sep;54(3):285-8. DOI:10.5937/scriptamed54-45176

ORIGINAL ARTICLE

Association Between *Alu* Insertion/Deletion Polymorphism in Intron 8 of Human Tissue Plasminogen Activator Gene *(PLAT)* and Risk of Age-Related Macular Degeneration

Saghar Ghorbani,[1] Mostafa Saadat[1]

1. Department of Biology, School of Science, Shiraz University, Shiraz, Iran.

Abstract

Background/Aim: Age-related macular degeneration (AMD) is major reason of blindness in human. Plasminogen is converted to plasmin by tissue plasminogen activator protein (*PLAT*, formerly known as TPA). A polymorphism in intron 8 of *PLAT* gene has been reported, either with (insertion) or without (deletion) a 311 bp *Alu* sequence. This polymorphism is associated with plasma levels of glycoprotein t-PA. t-PA is expressed in the retina and is involved in the development of the eye. It can be hypothesised that the *PLAT* polymorphism may be correlated with AMD. Therefore, the current study was conducted.
Methods: A total of 121 AMD patients and 108 healthy subjects were included in the study. Genotyping was performed by PCR. The strength of the association between AMD and polymorphism was expressed by estimating the odds ratio (OR).
Results: There was a significant relationship between the Del/Del genotype and susceptibility to AMD (OR = 2.25, 95 % CI = 1.07-4.69, p = 0.031). After adjusting for various factors such as age, smoking habit and workplace, a similar relationship was obtained (OR = 2.51, 95 % CI = 1.01-6.23, p = 0.049).
Conclusions: The homozygosity of the Del allele was found to increase the susceptibility to AMD. This polymorphism may contribute to the risk of AMD in population.

Key words: AMD; Insertion/Deletion; Polymorphism; *PLAT*.

Correspondence:
MOSTAFA SAADAT
T: +98-71-36137432
E: saadat@shirazu.ac.ir

ARTICLE INFO

Received: 25 June 2023
Revision received: 21 July 2023
Accepted: 23 July 2023

Introduction

One third of blindness in human occurs due to age-related macular degeneration (AMD). AMD results in progressive and irreversible central vision loss. In AMD, bleeding occurs in the macula soon after fibrin clots form, leading to blindness if not treated in time. Genetic and non-genetic factors play a critical role in pathogenesis of AMD.[1]

The tissue plasminogen activator gene (*PLAT*, MIM: 173370, formerly known as *TPA*), located on human chromosome 8p11.21, encodes a single-chain glycoprotein of 562 amino acids called t-PA, which has serine protease activity. Plasminogen is converted to plasmin by t-PA.[2]

The *PLAT* gene has numerous genetic polymorphisms in the human gene pool. A polymorphism in intron 8 of the *PLAT* has been reported throughout the human genome, either with a 311bp *Alu* sequence (Alu^+, insertion) or without (Alu^-, deletion).[3] The *Alu* sequence affects the function of a given gene. This can be negative, by inactivating the gene, or positive, by altering its function.[4] The *Alu* insertion polymorphism is an intronic polymorphism and it does not seem that intronic polymorphisms have a direct impact on release rates of t-PA or protein production. Perhaps the linkage disequilibrium between this polymorphism and other changes that have not yet been

This article should be cited as follows: Ghorbani S, Saadat M. Association between *Alu* insertion/deletion polymorphism in intron 8 of human tissue plasminogen activator gene (*PLAT*) and risk of age-related macular degeneration. Scr Med 2023 Sep;54(3):285-8.

identified is an appropriate justification for the observed association.[5]

This polymorphism has three different genotypes: $Alu^{+/+}$ (Ins/Ins), $Alu^{+/-}$ (Ins/Del), and $Alu^{-/-}$ (Del/Del). This polymorphism may play a role in t-PA plasma levels.[5, 6] It has been reported that subjects homozygous for the Ins allele have a significantly higher rate of t-PA release than both heterozygotes and homozygous subjects for the Del allele.[5] The Del allele is reversibly associated with the risk of several complex diseases, including recurrent pregnancy loss,[6] diabetes mellitus,[7] myocardial infarction[8] and schizophrenia.[9]

It should be noted that t-PA is expressed in retina and plays a key role in the eye development.[10] Therefore, it was hypothesised that a functional polymorphism of *PLAT* may be related with risk of AMD.

Aim of this case-control study was to investigate the association between the Ins/Del 311 bp *Alu* sequence polymorphism in the *PLAT* gene and susceptibility to AMD.

Methods

Participants

This study was a case-control study. The samples used in this study were nearly identical to those used previously,[11] with some minor differences as follows: one patient was added to the patient samples and the genotype of 10 samples from the healthy group could not be determined. Therefore, 121 AMD patients (46 females, 75 males) and 108 normal subjects (45 females, 63 males) were included in this study. The subjects of both the groups were Muslims/Persians living in Shiraz and belonging to Caucasian ethnicity.

The two study groups were gender matched with each other (χ^2 = 0.31, df = 1, p = 0.573). The age range of the case group was 34-90 years and that of the control group was 31-85 years. The mean age (SD) of the AMD and controls was 69.5 (9.6) and 63.9 (10.0) years, respectively, which was significantly different from each other (t = 4.26, df = 227, p < 0.001).

The participants were divided into two groups according to their exposure to light. In the first group were those who were less exposed to sunlight, such as teachers and housewives who spend most of their time in closed and indoor environments. In the second group, the participants were more exposed to sunlight, such as farmers and drivers.

This study was accepted by the local research Ethics Committee (No SU-DB-9831493) and conducted in accordance with the tenets of the Helsinki Declaration. Patients and healthy volunteers provided informed consent.

Genotyping

The extraction of genomic DNA was carried out using the boiling method.[12] Genotyping was performed by simple PCR. Specific primers for the *PLAT* polymorphism were used as previously described.[13] PCR conditions consisted of 10 minutes of initial denaturation at 95 °C, followed by 28 cycles of 30 seconds at 95 °C, 30 seconds at 63 °C, and 60 seconds at 72 °C and a final extension step of 72 °C for 5 minutes. Note that the PCR products for the Del and Ins allele were different in length. The Ins and Del alleles were 424 bp and 113 bp, respectively (Figure 1).

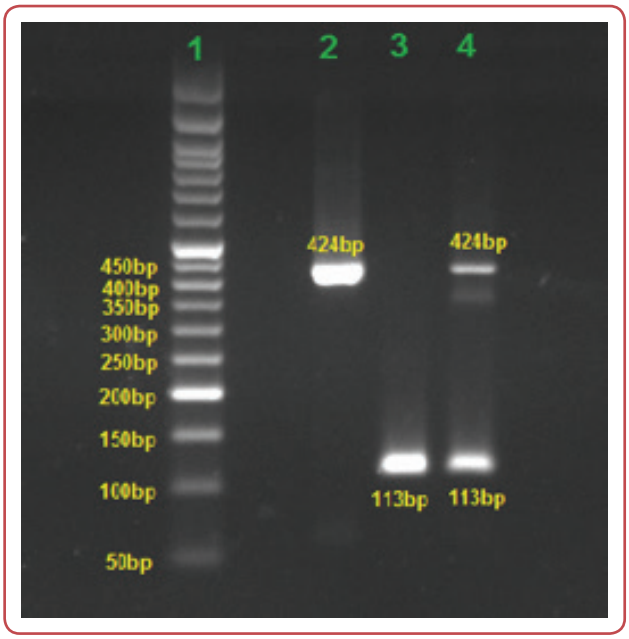

Figure 1: Determination of triple genotypes of insertion/deletion of an Alu sequence in intron 8 of the PLAT gene after gel electrophoresis of the PCR products. Lanes 1 to 4 are 50 bp DNA leader, Ins/Ins, Del/Del and Ins/Del genotypes, respectively

Statistical analysis

Chi-square test was used to compare sex, smoking habits and workplace between study groups. The Hardy-Weinberg equilibrium (HWE) has been checked for the genotypes of the *PLAT* polymor-

phism. The strength of association between genotypes and AMD risk was expressed as odds ratios (ORs). To examine the statistical significance of a given OR, its 95 % confidence interval (CI) was calculated. Multivariable logistic regression was used to estimate the OR after adjustment for age, smoking habit and workplace. The Ins/Ins genotypes were taken as the reference genotype (OR = 1). The SPSS software (version 25) was used for statistical comparisons. Significance was defined as a probability of less than 0.05.

Results

Among study subjects, 45.5 % (of 121) and 29.7 % (of 84) of patients and controls, respectively, worked outdoors. The difference was significant (χ^2 = 5.13, df = 1, p = 0.024). In AMD patients and controls, 38.7 % (of 111) and 21.6 % (of 74), respectively, were smokers (χ^2 = 5.98, df = 1, p = 0.014).

The allelic and genotypic frequencies in the study groups are shown in Table 1. The frequency of the Del allele was 0.500, and 0.603 in the control and patient groups, respectively. In both controls (χ^2 = 0.59, df = 1, p = 0.441) and AMD patients (χ^2 = 0.13, df = 1, p = 0.715), the genotype frequencies were consistent with the expected HWE values.

Table 1: Association between the Alu Ins/Del polymorphism in intron 8 of the PLAT gene and the risk of age-related macular degeneration

Genotypes/ Alleles	Controls	Cases	OR	95 % CI	p
Ins/Ins	29	20	1.00	-	-
Ins/Del	50	56	1.62	0.81-3.22	0.166
Del/Del	29	45	2.25	1.07-4.69	0.031
Alleles					
Ins	108	96	1.00	-	-
Del	108	146	1.52	1.05-2.02	0.027

OR: odds ratio; CI: confidence interval; Ins: insertion; Del: deletion; PLAT gene: plasminogen activator gene;

Homozygosity for the Del allele showed a significant association with increased risk of AMD in the statistical analysis (OR = 2.25, 95 % CI = 1.07-4.69, p = 0.031). Although the frequency of heterozygote was higher in the cases of AMD compared to the controls, the difference did not achieve statistical significance (Table 1). The risk of AMD was a function of the number of Del allele (χ^2 for linear trend = 4.61, df = 1, p = 0.032). Comparison of the alleles of the *PLAT* polymorphism between cases and controls revealed that the Del allele significantly increased the susceptibility to AMD (OR = 1.52, 95 % CI = 1.05-2.20, p = 0.027).

Table 2: Association between the Alu Ins/Del polymorphism in intron 8 of the PLAT gene and the risk of age-related macular degeneration after adjusted for age, smoking habit and workplace

Genotypes/Alleles	OR	95 % CI	p
Ins/Ins	1.00	-	-
Ins/Del	1.62	0.69-3.81	0.264
Del/Del	2.51	1.01-6.27	0.049

OR: odds ratio; CI: confidence interval; Ins: insertion; Del: deletion; PLAT gene: plasminogen activator gene;

As mentioned above, study groups showed significant differences in age distribution, smoking habits, and workplace of the participants. To exclude the confounding effects of these variables on the association between the genotypes and susceptibility to AMD, multivariable analysis was used. The results of the multivariable analysis are summarised in Table 2. After adjustment for age, smoking habit, and workplace, homozygosity for the Del allele increased susceptibility to AMD (OR = 2.51, 95 % CI = 1.01-6.23, p = 0.049).

Discussion

The current study showed an increased frequency of the Del/Del genotype in AMD patients. Previous studies have shown that higher levels of t-PA enzyme activity result in excessive fibrinolysis, which can lead to bleeding. On the other hand, low activity causes thrombosis. One study showed that Ins/Ins individuals have a higher release rate compared to the other two genotypes.[5] Presented results showed that the Del/Del genotype increased the susceptibility to AMD by about 2.25. The Del/Del genotype can cause a lower rate of t-PA release in human serum, which can lead to thrombosis, subretinal blood and formation of clot within the macula, which can cause vision loss in patients with AMD.

In AMD patients and controls, 38.7 % and 21.6 %, respectively, were smokers. These associations are consistent with authors' previous report.[10] Several epidemiological studies have shown that AMD is strongly associated with cigarette smoking and outdoor work.[14, 15]

There are some reports that support present findings. Exogenous intravitreal t-PA has been shown to be effective for central retinal vein occlusion[16] and submacular haemorrhages in AMD.[17] Co-treatment with t-PA and anti-VEGF is effective for submacular haemorrhages and improves visual acuity, according to a recently published meta-analysis.[18] It has been reported that vitrectomy with subretinal injection of t-PA may be an effective procedure in the treatment of submacular haemorrhages teatement.[19]

Further larger studies in other populations are needed to confirm the present findings and to draw definitive conclusions regarding the contribution of the *PLAT* polymorphism in AMD, given the small number of participants in this study.

Conclusion

This study represents a genetic association study to investigate the relationship between insertion/deletion of an *Alu* sequence in *PLAT* as a novel candidate gene and susceptibility to AMD. The homozygosity of the Del allele was found to increase the susceptibility to AMD. This polymorphism may contribute to the risk of AMD in the Iranian population. Further studies in other populations are needed to confirm the present findings.

Acknowledgement

The close cooperation of the AMD patients and healthy controls is gratefully acknowledged by the authors.

Conflict of interest

None.

References

1. Katta S, Kaur I, Chakrabarti S. The molecular genetic basis of age-related macular degeneration: an overview. J Genet 2009;88:425-49.
2. Lijnen HR, Collen D. Mechanisms of physiological fibrinolysis. Baillieres Clin Haematol 1995;8:277-90.
3. Ludwig M, Wohn KD, Schleuning WD, Olek K. Allelic dimorphism in the human tissue-type plasminogen activator (TPA) gene as a result of an Alu insertion/deletion event. Hum Genet 1992;88:388–92.
4. Batzer MA, Deininger PL. Alu repeats and human genomic diversity. Nat Rev Genet 2002;3:370–9.
5. Jern C, Ladenvall P, Wall U, Jern S. Gene polymorphism of t-PA is associated with forearm vascular release rate of t-PA. Arterioscler Thromb Vasc Boil 1999;19:454–9.
6. Cho HY, Park HS, Ahn EH, Ko EJ, Park HW, Kim YR, et al. Association of polymorphisms in plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (tPA), and renin (REN) with recurrent pregnancy loss in Korean women. J Pers Med 2021;11(12):1378. doi: 10.3390/jpm11121378.
7. Yasin SR, AlHawari HH, Alassaf AA, Khadra MM, Al-Mazaydeh ZA, Al-Emerieen AF, et al. Alu DNA polymorphism of human tissue plasminogen activator (tPA) gene in diabetic Jordanian patients. Iran Biomed J 2019;23(6):423-8.
8. van der Bom JG, de Knijff P, Haverkate F, Bots ML, Meijer P, de Jong PT, et al. Tissue plasminogen activator and risk of myocardial infarction. The Rotterdam Study. Circulation 1997;95(12):2623-7.
9. Bordbar M, Saadat M. Association between 15 insertion/deletion genetic polymorphisms and risk of schizophrenia using pooled samples. EXCLI J 2023;22:310-4.
10. Collinge JE, Simirskii VN, Duncan MK. Expression of tissue plasminogen activator during eye development. Exp Eye Res 2005;81(1):90-6.
11. Kalteh S, Saadat M. Lack of association between three common genetic variations of XPC and susceptibility to age-related macular degeneration, a preliminary study. Egypt J Med Hum Genet 2020;21:18. doi: 10.1186/s43042-020-00060-w.
12. Newton CR, Graham A, Heptinstall LE, Powell SJ, Summers C, Kalsheker N, et al. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Res 1989 Apr 11;17(7):2503-16.
13. Valle-Garay E, Montes AH, Corte JR, Meana A, Fierer J, Asensi V. tPA Alu (I/D) polymorphism associates with bacterial osteomyelitis. J Infect Dis 2013;208:218-23.
14. Willeford KT, Rapp J. Smoking and age-related macular degeneration: biochemical mechanisms and patient support. Optom Vis Sci 2012;89:1662-6.
15. Modenese A, Gobba F. Macular degeneration and occupational risk factors: a systematic review. Int Arch Occup Environ Health 2019;92:1-11.
16. Ghazi NG, Noureddine B, Haddad RS, Jurdi FA, Bashshur ZF. Intravitreal tissue plasminogen activator in the management of central retinal vein occlusion. Retina 2003;23:780–4.
17. Schulze SD, Hesse L. Tissue plasminogen activator plus gas injection in patients with subretinal hemorrhage caused by age-related macular degeneration: predictive variables for visual outcome. Graefes Arch Clin Exp Ophthalmol 2002;240:717–20.
18. He X, Cao W, Wang Z, Zhang N, Xu K, Yu L, et al. Efficacy evaluation of tissue plasminogen activator with anti-vascular endothelial growth factor drugs for submacular hemorrhage treatment: A meta-analysis. J Clin Med 2023;12(3):1035. doi: 10.3390/jcm12031035.
19. Ali Said Y, Dewilde E, Stalmans P. Visual outcome after vitrectomy with subretinal tPA injection to treat submacular hemorrhage secondary to age-related macular degeneration or macroaneurysm. J Ophthalmol 2021;2021:3160963. doi: 10.1155/2021/3160963.