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

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.

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...
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 infarction8 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 (χ² = 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 ($\chi^2 = 5.13$, df = 1, p = 0.024). In AMD patients and controls, 38.7% (of 111) and 21.6% (of 74), respectively, were smokers ($\chi^2 = 5.98$, 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 ($\chi^2 = 0.59$, df = 1, p = 0.441) and AMD patients ($\chi^2 = 0.13$, 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

<table>
<thead>
<tr>
<th>Genotypes/Alleles</th>
<th>Controls</th>
<th>Cases</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ins/Ins</td>
<td>29</td>
<td>20</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ins/Del</td>
<td>50</td>
<td>56</td>
<td>1.62</td>
<td>0.81-3.22</td>
<td>0.166</td>
</tr>
<tr>
<td>Del/Del</td>
<td>29</td>
<td>45</td>
<td>2.25</td>
<td>1.07-4.69</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Homozgyosity 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 ($\chi^2$ 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).

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 (OR = 2.51, 95% CI = 1.01-6.23, p = 0.049).

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 and submacular haemorrhages in AMD. Co-treatment with t-PA and anti-VEGF is effective for submacular haemorrhages and improves visual acuity, according to a recently published meta-analysis. It has been reported that vitrectomy with subretinal injection of t-PA may be an effective procedure in the treatment of submacular haemorrhages. Further 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

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Conflict of interest

None.

References


