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3 # PAI-1 and tPA gene polymorphisms and susceptibility to chronic
4 obstructive pulmonary disease in a sample of Turkish population

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12

13 ## Abstract

14 **Objective:** The aim of this study was to assess the influence of plasminogen
15 activator inhibitor-1 (*PAI-1*) *4G/5G* or tissue plasminogen activator (*tPA*) *I/D*
16 polymorphisms in chronic obstructive pulmonary disease (COPD) cases in a
17 sample of Turkish population.
18 **Methods:** *PAI-1 4G/5G* and *tPA* Alu-repeat *I/D* genetic polymorphisms in 153
19 COPD subjects and 160 controls were investigated using PCR-RFLP and PCR
20 methods, respectively.
21 **Results:** *4G* allele frequency was 0.62 and 0.39 for COPD and control groups,
22 respectively. *4G* allele had an estimated 2.56-fold [95% CI = 1.85–3.53]
23 increased risk of COPD. tPA *I* allele frequency was 0.55 and 0.50, for COPD
24 and control groups, respectively. I allele had an estimated 1.19-fold [95%
25 CI = 0.87–1.62] increased risk of COPD
26 **Conclusions:** PAI-1 4G/4G and 4G/5G genotypes seemed to play a key role in
27 the pathophysiology of COPD in Turkish individuals.

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28 **Keywords:** COPD; Genetic susceptibility; Polymorphisms; Tissue-type
29 plasminogen activator (tPA); Plasminogen activator inhibitor-1 (PAI-1)

30

31 ## Introduction

32 Chronic obstructive pulmonary disease (COPD) is a preventable and treatable
33 disease, characterized by progressive airflow limitation, which might be
34 completely irreversible, with systemic effects, related to inflammatory response
35 due to various harmful particles and gases. Although the exact mechanisms
36 behind the development of COPD are not fully understood, potential
37 mechanisms of the disease are thought to include protease/antiprotease
38 imbalance, inhibition of antiproteases by oxidants, such as tobacco smoke, and
39 oxidant/free radical mediated cellular and tissue damage. Risk factors for COPD
40 include both genetic factors and environmental factors (e.g., cigarette smoking).
41 The primary environmental risk factor is smoking, but other risk factors include
42 history of respiratory infection, air pollution, second-hand smoke, and
43 occupational exposures to certain industrial pollutants [1].

44 Pathological studies have indicated that microthrombosis might occur in the
45 pulmonary vessels of COPD patients and such changes might be a cause of
46 disease exacerbation. Thrombosis could be due to platelet activation or the
47 existence of prothrombotic condition in subjects with vascular and alveolar
48 lesions but this possibility has never been throughly investigated in COPD
49 subjects [2].

50 The degradation of fibrin is dependent on the fibrinolytic or plasminogen
51 activator system. This system basically involves plasminogen activators
52 likewise belonging to the class of serine proteases and inhibitors called serpins.
53 Under physiological conditions, the major plasminogen activator contributing to
54 fibrinolysis is tissue plasminogen activator (tPA). The substrate plasminogen is
55 cleaved at its lysine residues by tPA to form active plasmin. Finally plasmin
56 acts on fibrin resulting in resorption of fibrin dots. Plasminogen activator and

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57 plasmin inhibitors regulate the conversion of plasminogen to active plasmin and
58 thereby regulate fibrin clearance [3]. A 300 base pair Alu repeat
59 insertion/deletion (I/D) polymorphism in intron of the *tPA* gene at chromosome
60 8p12-q11.2 was described by Ludwig et al [4]. Although tPA is the primary
61 enzyme responsible for dissolving fibrous clots, few studies have evaluated the
62 role of tPA polymorphisms and risk of poor fibrinolysis.
63 Plasminogen activator inhibitor-1 (PAI-1) is a potent and major inhibitor of
64 tPA. The efficacy of fibrinolysis depends on the interactions of the plasminogen
65 activators and inhibitory proteins of the plasminogen activator - plasmin system.
66 Deficient expression of PAI-1 can lead to relatively unrestricted expression of
67 plasmin. This scenario promotes excessive degradation of fibrin and could
68 result in an increased risk of bleeding. Conversely, excessive production of
69 PAI-1 would be expected to limit the generation of plasmin and facilitate
70 persistence of fibrin clots. This is a central event that contributes to the
71 pathogenesis of diverse processes including atherosclerosis, coronary artery
72 disease, intravascular thrombosis, extravascular fibrin deposition associated
73 with tissue inflammation and airway remodeling associated with chronic
74 obstructive pulmonary disease. The gene encoding for *PAI-1* is located at
75 chromosome 7q22. A single guanine insertion/deletion (4G/4G) polymorphism
76 in the promoter region of the *PAI-1* gene, 675 base pairs upstream from the
77 transcriptional start, has been associated with plasma PAI-1 levels. The deletion
78 allele (4G) fails to bind repressor proteins, down-regulating fibrinolysis and up-
79 regulating inflammatory activity [5].
80 *PAI-1* and *tPA* are expressed by numerous cells types in the lung including
81 endothelial cells, epithelial cells, and alveolar macrophages. Then, PAI-l and
82 tPA are involved in plasmin formation and plasmin contributes to extracellular
83 matrix proteolysis [6]. For this reason, *PAI-1* and *tPA* are among the candidate
84 genes that are thought to play a role in the pathogenesis of COPD. The aim of
85 this study was to assess the influence of *PAI-1 4G/5G* or *tPA I/D*

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86 polymorphisms in COPD cases in a sample of Turkish population. To the best
87 of our knowledge, this is the first study that investigated the effect of *PAI-1*
88 *4G/5G* or *tPA I/D* polymorphisms on COPD.

89

90 **Methodology**

91 The study was approved by the Clinical Research Ethics Committee of Yuzuncu
92 Yıl University and all subjects gave written informed consent. Our patient group
93 included 153 COPD subjects who have been treated in Yuzuncu Yıl University,
94 Dursun Odabaş Medical Center, Department of Pulmonary Medicine, Van,
95 Turkey. Diagnosis of COPD was based on symptoms, physical examination,
96 and presence of risk factors. Diagnosis was confirmed by post-bronchodilator
97 spirometry performed 15 min after administration of four doses of salbutamol
98 sulfate. Pre-and post-bronchodilator spirometry was performed according to
99 American Thoracic Society/European Respiratory Society recommendations
100 using a spirometer in all subjects [7]. The diagnosis of COPD and its severity
101 were determined according to GOLD criteria. Patients fulfilling the criteria for
102 COPD were enrolled as cases and those who did not fulfill the standard
103 diagnostic criteria were enrolled as controls. Clinical examination of respiratory
104 system was carried out to document obstructive airway disease and to rule out
105 other forms of pulmonary diseases.

106 The control group consisted of 160 healthy individuals who consulted to the
107 laboratories of Yüzüncü Yıl University, Medical Faculty, Dursun Odabaş
108 Medical Center and do not have any inherited, acquired or chronic illnesses, and
109 airflow limitation. Healthy individuals were in the similar age and sex
110 distribution to the subjects with COPD.

111 Forced expiration (FEV1), FEV1/ Forced vital capacity (FVC), mean platelet
112 volume (MPV), platelet distribution width (PDW), platelet count (PLT) and
113 plateletocrit (PCT) values, Prothrombin Time (PT), activated partial

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114 thromboplastin time (aPTT) and international normalized ratio (INR) levels
115 were recorded from the patient folder among the COPD subjects.
116 **DNA isolation:** 5 mL peripheral blood was taken from COPD-diagnosed
117 patients and control group individuals to K2-EDTA tubes and stored at + 4 °C
118 until the study day. All studies were carried out in Yüzüncü Yıl University
119 Pharmacy Faculty, Biochemistry Research Laboratory. In addition, the patient
120 follow-up form was used for the detection of laboratory and clinical data of
121 patients with COPD and these forms were filled in order to refer patients'
122 polyclinic and service files. Genomic DNA isolation from whole blood samples
123 was performed according to the Poncz method [8]. In this method firstly, 0.5 ml
124 of human whole blood anticoagulated with EDTA-K_2 at 1 mg/mL blood was
125 mixed with an equal volume of a lysis solution containing 1% Triton X-100 to
126 lyse the cells and the nuclei were isolated as described. Isolated nuclei were
127 suspended in an enzyme reaction solution containing 1% SDS and digested with
128 0.8 mg/ml proteinase K to liberate DNA from nuclear proteins. After 1h
129 incubation, NaI solution was added to the nuclear lysate to the final
130 concentrations of 4.5 M NaI and 0.4% SDS, and was followed by isopropanol
131 addition. The content in the tube was mixed well by inversion until whitish
132 precipitate appeared. The precipitate was collected by centrifugation and
133 washed with the alcohol solutions. If required, contaminant RNA could be
134 removed by pancreatic RNAse treatment prior to the proteolysis [8].
135 **Determination of Genotypes:** Identification of *PAI-1* was assayed with PCR
136 restriction fragment length polymorphism (PCR-RFLP) based methods, as
137 described by Diamanti-Kandarakis *et al,* [5] and for tPA genotyping, PCR based
138 method was performed as described by Ferrari *et al,* [9].
139 For genotyping of *PAI-1 4G/5G* polymorphism; Forward 5′-
140 CACAGAGAGAGTCTGGCCACGT-3′ and Reverse 5′-
141 CCAACAGAGGACTCTTGGTCT-3′ primers (Genbank accession codes:
142 J03836.1) were used. After the PCR amplification, RFLP analysis was

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performed with the restriction enzyme *BslI* to detect the 4G>5G change. The samples carrying 4G genotype were identified as a single band; 99 bp, 5G genotype were identified as double bands; 77 bp and 22 bp, and 4G/5G genotype were identified as three bands; 99 bp, 77 bp and 22 bp (Figure 1). For genotyping of *tPA* Alu-repeat *I/D* polymorphism; Forward 5′-TCCGTAACAGGACAGCTCA-3′ and Reverse 5′-ACCGTGGCTTCAGTCATGGA-3′ primers (Genbank accession codes: X77531.1) were used [9]. Obtained fragment were 967 bp for *II* genotype, 655 bp for *DD* genotype, and 967 bp and 655 bp for *ID* genotype. The primers were provided by PRZ BioTECH (Bilkent, Ankara, Turkey). Sequenced reads were aligned against the human *PAI-1* and tPA genes using CLC Main Workbench Version 7.6.4 (www.clcbio.com) in order to assess for polymorphisms.

Statistical analysis: Assuming a probability of disease of 0.01, a risk genotype frequency in population of 0.6 and an odds ratio (OR) of 1.8 with a two-sided p value of 0.05, and a case–control design with a 1:3 ratio, by means of Power 3.9, we estimated that we would need at least 140 cases to reach a power of more than 95% under a recessive model of inheritance [10]. The distributions of the *PAI-1 4G/5G* or *tPA I/D* polymorphisms were compared by using the Hardy–Weinberg heredity equilibrium by $\chi 2$ tests. Odds ratios (ORs) with 95% confidence intervals (CIs) were also calculated to examine the association between the *PAI-1* and *tPA* genotypes and the risk of COPD. Clinical features are presented as means ± standard deviation. All tests were performed using Statistical Package for the Social Sciences, version 14.0 (SPSS Inc., Chicago, IL, USA). $p<0.05$ was considered significant.

Results

The frequencies of *PAI-1 4G/4G* genotype in COPD and control group were 73(47.71%) and 51(31.87%), respectively. The frequency of the *PAI-1 4G/5G* genotype in COPD and control group were 45(29.41%) and 24(15.00%),

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172 respectively. The frequency of the *PAI-1 5G/5G* genotype in COPD and control
173 group were 35(22.87%) and 85(53.13%), respectively. According to these data,
174 we could suggest that *PAI-1 -675 4G/4G* genotype increase the COPD risk by
175 3.47-fold [95% CI = 2.04–5.92], and *4G/5G* genotype increase the COPD risk
176 by 4.55-fold [95% CI = 2.42–8.57] ($p<0.05$) (Table 1). *4G* allele frequency was
177 191(62.42%) and 126(39.37%), *5G* allele frequency was 115(37.58%) and
178 194(60.63%) for COPD and control groups, respectively. According to these
179 data, we could suggest that 4G allele had an estimated 2.56-fold [95%
180 CI = 1.85–3.53] increased risk of COPD ($p<0.05$) (Table 1).
181 The frequency of *tPA II* genotype in COPD and control group were 52(33.99%)
182 and 48(30.00%), respectively. The frequency of the *tPA ID* genotype in COPD
183 and control group were 63(41.18%) and 65(40.63%), respectively. The
184 frequency of the *tPA DD* genotype in COPD and control group were
185 38(24.83%) and 47(29.37%), respectively. According to these data, we could
186 suggest that *tPA II* genotype increase the COPD risk by 1.34-fold [95%
187 CI = 0.75–2.39], and *tPA ID* genotype increase the COPD risk by 1.20-fold
188 [95% CI = 0.69–2.08] ($p>0.05$) (Table 1). *tPA I* allele frequency was
189 167(54.58%) and 161(50.31%), *D* allele frequency was 139(45.42%) and
190 159(49.69%) for COPD and control groups, respectively. According to these
191 data, we could suggest that *I* allele had an estimated 1.19-fold [95% CI = 0.87–
192 1.62] increased risk of COPD ($p>0.05$) (Table 1).
193 Patients' FEV1, FVC and FEV1/FVC values, mean platelet volume (MPV),
194 platelet distribution width (PDW), platelet count (PLT) and plateletocrit (PCT)
195 values, PT, aPTT and INR levels were compared according to the genotype and
196 allele distributions of *PAI-1* and *tPA* genes and showed in Table 2 and Table 3,
197 respectively.

198 **Discussion**

199 Susceptibility to develop COPD results from a combination of environmental
200 and genetic factors. Cigarette smoking is undoubtedly the main environmental

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201 risk factor for COPD in the developed world [11]. In addition, genetic factors
202 are influential in the development of COPD. There are good reasons to assume
203 that multiple genes, each with only a modest effect, contribute to the
204 development of COPD. It could also be speculated that multiple predisposing
205 gene variants are interacting with each other and with environmental risk factors
206 [11].

207 PAI-1 and tPA might play a vital role in the pathogenesis of COPD and are
208 excellent candidate genes for a COPD association studies. First, both genes are
209 expressed by numerous cells types in the lung including endothelial cells,
210 epithelial cells, and alveolar macrophages. Second, PAI-1 and tPA are involved
211 in plasmin formation and plasmin contributes to extracellular matrix proteolysis
212 [2]. In addition, plasmin regulates MMP-1 and MMP-9 activities and both
213 proteinases have been implicated in the pathogenesis of COPD. Finally, PAI-1
214 could be induced by inflammatory cytokines such as IL-1 and TNF-α, which are
215 increased in patients with COPD [11]. As far as we know, *PAI-1* and *tPA* have
216 not been previously investigated as candidate genes for COPD until now. This
217 is the first time polymorphisms in these genes have been tested for an
218 association with rate of decline in lung function.

219 In humans, elevated plasma levels of PAI-1 have been associated with
220 myocardial infarction and deep vein thrombosis [12]. Genetically modified mice
221 have provided some insight into the function of PAI-1. Transgenic mice
222 overexpressing PAI-1 develop deep vein thrombosis and vascular fibrinolysis is
223 accelerated in *PAI-1* deficient mice. In addition, PAI-1 is believed to play an
224 important role in a number of plasminogen dependent proteolytic events outside
225 the vasculature. *PAI-1* knockout mice do not develop pulmonary fibrosis after
226 lung injury [13]. Furthermore, evidence suggested that in a murine model of
227 chronic asthma, PAI-1 deficient mice have increased ECM deposition in the
228 airways because of decreased MMP-9 activity and increased fibrinolysis [14].

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229 There is extensive and growing evidence that PAI-1 is involved in ovarian
230 follicular rupture, as well as angiogenesis and tumour invasion [12].
231 Several polymorphisms have been characterized in *PAI-1* gene. A functional
232 polymorphism in the promoter region of the *PAI-1* gene (-675/4G→5G) effects
233 the binding of nuclear proteins regulating transcription and is significantly
234 correlated with the plasma levels of PAI-1 [15]. The 4G allele is associated with
235 increased gene transcription and higher PAI-1 plasma concentrations. The two
236 alleles are almost equally distributed among the Caucasian population [9,16].
237 The 4G allele of this common -675/4G→5G promoter polymorphism is
238 associated with myocardial infarction, coronary artery disease, abdominal aortic
239 aneurysms, stroke, obesity, a poor survival rate after severe trauma,
240 meningococcal disease, and asthma [3,5,9,15,16].
241 We have indicated that the prevalence of PAI-1 4G allele was higher in COPD
242 patients than the control group. Heterozygous or homozygous carriage of 4G
243 allele is thought to play a role in the development of COPD.
244 Up-regulated *PAI-1* expression, because of the *PAI-1 4G* allele, indicate
245 indirectly that COPD subjects may be in a hypercoagulative state [17]. Arboix
246 [18] showed that the presence of COPD was a strong predictor of lacunar
247 stroke. These studies suggested the presence of a hypercoagulative state in
248 systemic circulation in COPD subjects [11,12]. These results showed that the
249 effect of *PAI-1 4G* allele on COPD susceptibility was similar to other diseases.
250 Human tPA is an extracellular serine proteinase produced by numerous cells
251 types in the lung including endothelial cells, epithelial cells, alveolar
252 macrophages, and smooth muscle cells. Endothelial cells are considered the
253 most important source of tPA in vivo. tPA is released from endothelial cells in a
254 constitutive and regulated fashion. The tPA-mediated pathway is thought to be
255 primarily involved in the resolution of blood clots [12]. Studies suggested that
256 high plasma levels of tPA mark an increased risk of atherothrombotic ischemic
257 events such as myocardial infarction and stroke; elevated tPA levels may

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represent the activation of the endogenous fibrinolytic system in response to the existence of preclinical atherosclerosis. Genetic variation at the *tPA* locus has been characterized and extensively studied in association with plasma tPA levels [4]. Ladenvall *et al,* reported an association between SNPs at the tPA locus and vascular tPA release[19]. The Alu-repeat *I/D* polymorphism is associated with vascular tPA release rates [20].

These data showed that *I* allele increases the COPD risk by 1.19-fold. These data showed that *tPA II* genotype increases the COPD risk by 1.34-fold, and *ID* genotype increases the COPD risk by 1.20-fold. Although the prevalence of the *tPA I* allele was higher in COPD subjects compared with those in control group, having the *I* allele, either in heterozygous or homozygous state, didn't have a significant risk factor for the COPD formation. There has been no study that investigate the interaction between *tPA* Alu-repeat *I/D* polymorphism and COPD etiology in the literature, but some reseachers have been indicated that *tPA I* allele has increased the risk of myocardial infarction, stroke and atherosclerosis [20,21].

In our study, we also considered FEV1 value and FEV1/FVC ratio for verifying the pulmonary function, MPV, PDW, PLT and PCT values for the platelet indice analysis and PT, aPTT and INR levels for coagulation statue among the COPDsubjects according to the genotype distribution of these two genes. We found that mean baseline FEV_1 and FEV1/FVC was significantly lower in subjects carrying the *PAI-1 4G* allele than the *5G* allele. Our study also revealed that mean baseline FEV_1 and FEV1/FVC were significantly lower in subjects with PAI-1 4G/4G genotype than in those with either *4G/5G* or *5G/5G* genotype. We couldn't find a significant differance between PAI-1 *4G/5G* and *5G/5G* genotypes for the mean baseline FEV_1 and FEV1/FVC. According to our study, we could claim that *PAI-1 4G* allele and *4G/4G* genotype could ruin the pulmonary functions and might be a risk factor for COPD formation.

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286 We found that PLT, PCT, PT and INR values was significantly higher in
287 subjects carrying the *PAI-1 4G* allele than the *5G* allele, but there was no
288 differences in PDW, MPV and aPTT values between *PAI-1 4G* and *5G* carriers.
289 Our study also showed that PLT, PCT, PT and INR values was significantly
290 higher insubjects with PAI-1 4G/4G genotype than in those with either *4G/5G*
291 or *5G/5G* genotype. There was no differences in PDW, MPV and aPTT values
292 among three different genotypes. According to our study, we could express that
293 *PAI-1 4G* allele and *4G/4G* genotype could cause to hypercoagulation state and
294 may be a risk factor for COPD formation. Our study also showed that *tPA I* or
295 *D* alleles and *tPA II*, *ID* or *DD* genotypes didn't effect FEV_1 and FVC values,
296 FEV_1/FVC ratio and PLT, PCT, PDW, MPV, PT, aPTT and INR values in
297 COPD subjects. Previous studies indirectly suggested that the presence of a
298 prothrombotic condition in COPD subjects based on changes in the activities of
299 platelets and clotting system [22,23]. Nenci *et al,* demonstrated platelet
300 activation in COPD subjects by detection of high plasma levels of p-
301 thromboglobulin, a substance released from activated platelets[24].

302 In conclusion, it is possible to say that especially *PAI-1 4G/4G* and *4G/5G*
303 genotypes seemed to play a critical role in the progression of COPD. We
304 believe that this result might contribute to the development of new strategies in
305 the treatment of COPD and other fibrinolytic system disorders.

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391 **Table 1.** Genotype/allele frequencies and odds ratios of *PAI-1 4G/5G* and *tPA I/D* polymorphisms in control
392 and COPD cases.

		COPD cases (*n*) (%)	Control group (*n*) (%)	Odds ratio [95% CI] [a]	p value [b]
PAI-1 4G/5G					
Genotype	4G/4G	73 (47.71)	51 (31.87)	3.47 [2.04-5.92]	<0.001
	4G/5G	45 (29.41)	24 (15.00)	4.55 [2.42-8.57]	<0.001
	5G/5G	35 (22.87)	85 (53.13)	1.00 (referance)	
Allele	4G	191 (62.42)	126 (39.37)	2.56 [1.85-3.53]	<0.001
	5G	115 (37.58)	194 (60.63)	1.00 (referance)	
tPA I/D					
Genotype	II	52 (33.99)	48 (30.00)	1.34 [0.75-2.39]	0.323
	ID	63 (41.18)	65 (40.63)	1.20 [0.69-2.08]	0.519
	DD	38 (24.83)	47 (29.37)	1.00 (referance)	
Allele	I	167 (54.58)	161 (50.31)	1.19 [0.87-1.62]	0.286
	D	139 (45.42)	159 (49.69)	1.00 (referance)	

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[a] Crude odds ratio (OR), 95% CI = confidence interval at 95%
[b] *Chi square*
(*n* = number of individual)

Table 2. Clinical features of COPD subjects according to PAI-1 genotype and allele variants

	Genotypes of PAI-1 gene				Alleles of PAI-1 gene		
	4G/4G (n=73)	*4G/5G (n=45)*	*5G/5G (n=35)*	*p values*	*4G (n=191)*	*5G (n=115)*	*p values*
FEV1 (L)	**53.72±6.95**	66.75±9.63	65.17±7.62	*<0.001, **0.325	**55.18±6.14**	65.84±8.64	***<0.000
FEV1/FVC (%)	**42.13±5.63**	54.38±6.34	52.27±6.24	*<0.001, **0.079	**46.59±5.03**	53.97±7.13	***<0.000
MPV (µm³)	7.15±0.81	7.26±0.87	7.33±0.78	*0.233, **0.651	7.2±0.85	7.29±0.87	***0.375
PDW (%)	12.65±2.34	14.58±2.17	14.63±2.1	*0.916, **0.918	13.38±2.36	13.88±2.42	***0.076
PLT (x10³/mm³)	**262.6±31.57**	181.3±22.54	184.5±22.14	*<0.001, **0.527	**220.13±25.69**	183.64±22.69	***<0.000
PCT (%)	**0.18±0.02**	0.14±0.01	0.14±0.02	*<0.001, **1.000	**0.16±0.02**	0.13±0.02	***<0.000
PT (sec.)	**14.15±2.05**	11.37±2.36	11.59±2.06	*<0.001, **0.663	**13.69±1.67**	11.47±2.05	***<0.000
aPTT (sec.)	31.56±4.96	32.34±4.01	31.08±3.65	*0.611, **0.151	32.09±3.97	31.95±4.09	***0.768
INR	**0.92±0.14**	0.71±0.08	0.72±0.09	*<0.001, **0.601	**0.83±0.165**	0.72±0.08	***<0.000

*Shows *p* value between 5G/5G and 4G/4G genotypes
**Shows *p* value between 5G/5G and 4G/5G genotypes
***Shows *p* value between 5G and 4G alleles
Bold data shows the significant differences as compared with *5G/5G* genotype or *5G* allele (*p*<0.05)
(*n* = number of individual)

Table 3. Clinical features of COPD subjects according to tPA genotype and allele variants

	Genotypes of tPA gene				Alleles of tPA gene		
	I/I (n=52)	*I/D (n=63)*	*D/D (n=38)*	*p values*	*I (n=167)*	*D (n=139)*	*p values*
FEV1 (L)	61.78±7.86	62.63±8.62	63.34±8.63	*0.375, **0.689	62.05±7.63	62.97±8.36	***0.315
FEV1/FVC (%)	52.39±6.95	54.67±6.27	53.37±6.51	*0.499, **0.322	53.34±6.54	53.97±6.58	***0.403
MPV (µm³)	7.22±0.86	7.18±0.86	7.21±0.83	*0.956, **0.864	7.22±0.86	7.19±0.85	***0.760
PDW (%)	13.67±2.34	13.78±2.61	13.64±2.36	*0.952, **0.787	13.71±1.52	13.69±1.46	***0.907
PLT (x10³/mm³)	221.64±25.96	219.67±25.94	214.38±24.65	*0.184, **0.314	221.08±24.96	216.94±28.96	***0.180
PCT (%)	160.02±22.64	159.48±18.04	154.56±22.94	*0.264, **0.234	159.62±18.63	155.97±22.34	***0.120
PT (sec.)	12.69±1.34	12.54±1.38	12.08±1.62	*0.054, **0.132	12.87±1.76	12.54±1.57	***0.087
aPTT (sec.)	31.24±3.64	31.98±4.06	31.74±3.85	*0.532, **0.770	31.62±4.52	31.85±3.95	***0.639
INR	0.856±0.95	0.835±0.09	0.863±0.09	*0.964, **0.133	0.845±0.09	0.853±0.15	***0.565

*Shows *p* values between D/D and I/I genotypes
**Shows *p* values between D/D and I/D genotypes
***Shows *p* values between D and I alleles
Bold data shows the significant differences as compared with *D/D* genotype or *D* allele (*p*<0.05)
(*n* = number of individual)

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