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SERPINE1 -675 4G/5G polymorphism is associated with asthma severity and inhaled corticosteroid response

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ABSTRACT

Asthma is characterized by chronic airway inflammation and remodeling that can be (partially) suppressed by inhaled corticosteroids (ICS). Plasminogen activator inhibitor-1, encoded by the *SERPINE1* gene, is the key inhibitor of the plasminogen activator system that affects tissue repair and remodeling.

We studied associations between a functional *SERPINE1* -675 4G/5G promoter polymorphism and asthma development and severity and response to ICS.

Longitudinal cohorts of 281 asthmatics and their non-asthmatic spouses and the general population (n=1390) were studied. No significant associations were found with asthma development and IgE levels, nor with FEV_1 in non-asthmatic controls. Asthmatic subjects carrying the *SERPINE1* 5G allele had higher IgE and lower lung function levels at follow-up, lower maximally attained lung function level, and faster lung function decline compared to individuals with the 4G/4G genotype. ICS treatment showed an immediate improvement in FEV_1 level in asthmatics carrying the 5G allele. However, these asthmatics still had the fastest rate of FEV_1 decline after initiating ICS treatment. Finally, the 5G allele was associated with a lower prevalence of complete asthma remission at follow-up.

These findings suggest that *SERPINE1* is not an asthma susceptibility gene, but rather affects the severity, progression and long-term ICS response in asthma.

Keywords:

asthma severity; asthma remission; lung function decline; inhaled corticosteriods; PAI-1; genetic

INTRODUCTION

Asthma is characterized by chronic airways inflammation which contributes to characteristic structural changes, referred to as airway remodeling. A proportion of patients with asthma develops persistent airflow limitation[1-4] or shows an accelerated lung function decline[2,5]. Conversely, some individuals outgrow their asthma[6,7]. However, in patients in clinical asthma remission ongoing airway inflammation is present with risk of relapse of symptoms and a small subset of asthmatics may show complete remission later in life[8].

Airway remodeling in asthma is the process in which injured epithelial layers are replaced by extracellular matrix (ECM) instead of parenchymal cells of the same cell type. This pathologic remodeling eventually leads to changes in the airway structure including ECM deposition, subepithelial fibrosis, smooth muscle hypertrophy, and goblet cell hyperplasia[9]. An imbalance between proteases and their inhibitors in response to inflammation contributes to remodelling[10,11]. The plasminogen activator system (PAS) is a regulator of ECM proteolysis, both directly through plasmin formation and indirectly through plasmin-mediated activation of matrix metalloproteinases (MMPs). Plasminogen activator inhibitor-1 (PAI-1) is a key regulator of PAS, inhibiting both fibrinolysis and MMP in the lungs. Serum and sputum PAI-1 levels are higher in asthmatic than in healthy subjects[12,13]. Increased activity of PAI-1 has been associated with lung fibrosis in murine models[14,15] and PAI-1 is thought to play an essential role in tissue repair and remodeling[14,16,17]. PAI-1 is synthesized by many cells relevant to asthma, and human mast cells release functionally active PAI-1 when stimulated by immunoglobulin E (IgE) receptor cross-linking[18].

We have previously shown in a genome-wide screen that elevated IgE is linked to chromosome 7q21 in a Dutch asthma family study[19]. A number of candidate genes are located in this region, one of these being *SERPINE1* that encodes PAI-1. Elevated serum PAI-1 levels and the *SERPINE1* -675 4G/5G promoter polymorphism[20,21] have previously been

associated with elevated serum IgE levels in allergic diseases and allergic asthma[12,22]. The 4G/5G polymorphism has also been associated with both the development of asthma[12,23,24], and asthma severity (lower lung function and increased airway hyperresponsiveness (AHR))[12]. Of importance, the *SERPINE1* 4G/5G polymorphism influences PAI-1 expression, the 4G allele being associated with higher PAI-1 levels[20,21].

In summary, there is linkage of IgE to a region on chromosome 7 that harbours the *SERPINE1 (PAI-1)* gene[19]. There is evidence for elevated PAI-1 levels in asthma[12] and a role of PAI-1 in cell migration and tissue repair[14,16,25-27], airway remodeling[17], and *SERPINE1* polymorphisms are associated with asthma development and severity[12,23,24].Therefore, we investigated associations of *SERPINE1* with asthma, IgE, and airway remodelling, as reflected by asthma remission and progression, including effects on FEV_1 level and decline in asthma patients. Since inhaled corticosteroids (ICS) can reduce airway inflammation and lung function decline[5], we also assessed the interaction between *SERPINE1* and ICS use. To this aim we analyzed a unique longitudinal population of patients with moderate to severe asthma and their spouses and a large independent longitudinal population based cohort as a control group.

METHODS

Study populations

Asthma population: A cohort of 281 patients diagnosed with symptomatic asthma that was initially studied in 1962-75 at Beatrixoord Hospital, Haren, The Netherlands, a regional referral center for patients with obstructive airways disease. At that time, all were younger than 45 years and had AHR to histamine (30 seconds method; $PC_{20} \leq 32$ mg/mL)[28]. Between 1990 and 1999, the patients were extensively re-examined as well as 200 spouses of these asthmatic probands[7,29]. At the time of testing, all participants had no exacerbation. Maintenance asthma and allergy medication was stopped during the previous 2 weeks except for oral corticosteroid use[7].

General population: A sample of 1,390 people was selected from 2,467 subjects participating in the last survey in 1989/1990 of the Vlagtwedde-Vlaardingen cohort study[30]. This general population-based cohort study of exclusively Caucasian individuals of Dutch descent started in 1965. Participants have been followed up for 25 years with surveys being performed every 3 years (maximum of 7 surveys per participant)[31]. We genotyped DNA samples of subjects with more than 1,500 ng of isolated DNA available (n = 1,390). There were no differences in characteristics at the last survey between the selected and not-selected groups[30].

The Medical Ethics Committee of the University Medical Centre Groningen approved all studies and all participants gave their written informed consent.

Clinical evaluation

Asthma population: Probands underwent a standardised, comprehensive evaluation for asthma at initial testing, including lung function, AHR to histamine and a symptom questionnaire[7,29]. After initial testing, the asthmatic probands had routine check-ups for

their asthma at least once a year. Data on lung function and corticosteroid use during check-ups were extracted from the medical records. Lung function data during hospital stays because of asthma exacerbations or during pregnancies were not used[5]. In the re-examination between 1991 and 1999 the same standardised methodology was used and spouses were also tested using this methodology. In addition, serum total IgE levels were measured and blood samples for DNA isolation were taken.

General population: During all surveys information on respiratory symptoms and smoking status was collected using a questionnaire[32,33] and lung function testing was performed[34]. In addition, at the last survey in 1989-1990, serum total IgE was measured and blood samples for DNA isolation were taken.

DNA extraction and genotyping

DNA was isolated from peripheral blood leukocytes using standard methods. All subjects were genotyped for the *SERPINE1* -675 4G/5G polymorphism (rs1799889). See online depository for detailed description of methods.

Statistical analyses

The Chi^2 test was used to test for Hardy-Weinberg equilibrium.

Case-control analyses (Chi^2 test) were performed on the association between the *SERPINE1* 4G/5G polymorphism and the presence of asthma according to the algorithm previously published by Panhuysen et al.[29]. (see online depository) Genotype distribution of the asthmatic probands and asthmatic spouses of the asthma population were compared to genotype distribution of 1) the non-asthmatic spouses of the asthma population and 2) the non-asthmatic subjects from the general population. The non-asthmatic spouses are a suitable control group as they shared environmental factors with the asthma cases, therefore excluding

this confounder. In the general population cohort the asthma phenotype according to the algorithm could not be established and therefore asthma was defined as ever having experienced an asthma attack, as reported on the questionnaire.

Associations between the *SERPINE1* 4G/5G polymorphism and serum total IgE were determined within the patient and control groups using one-way analysis of variance. Linear regression analysis was used to evaluate the effect of the *SERPINE1* polymorphism on FEV_1, adjusted for age, height, sex, pack years smoking, and steroid use.

The maximally attained level of FEV_1 during young adulthood (i.e. the plateau phase[35]) was determined as the highest level of FEV_1 reached between the age of 20 and 35 years and could only be determined in the probands of the asthma population (see online depository). A linear regression analysis was used to determine the effect of the *SERPINE1* polymorphism on the maximally attained level, adjusted for age at maximum, sex, height, and pack years of smoking.

Linear mixed effect models were used to investigate the effect of the *SERPINE1* 4G/5G polymorphism on FEV_1[5,36] decline in the probands of the asthma population and in the general population. The age of 30 years was the starting point of analyses, because at that age the maximum lung function level generally is achieved and lung function starts to decline[35] (online depository).

Finally, the association between complete asthma remission at the follow-up visit and the *SERPINE1* genotype was determined in the asthma population. Complete asthma remission was defined as the absence of wheezing and asthma attacks, presence of normal lung function (FEV_1 post bronchodilator >90% predicted), absence of AHR ($PC_{20} \geq 32$ mg/ml) and no use of inhaled or oral corticosteroids.

As our analyses are hypothesis driven and the outcome variables are not independent from each other (e.g. the different lung function parameters are correlated), we did not apply a

sequential, classical, Bonferroni multiple testing correction. Rather, we used an α of 0.025 and results with a P value between 0.025 and 0.05 are regarded as borderline significant.

Linear mixed effect models on FEV_1 decline were conducted with S-plus 7.0 (Insightful Corp, Seattle, Wash). All other analyses were conducted with SPSS (version 16; SPSS Inc, Chicago, Ill).

RESULTS

The clinical characteristics and the genotype distribution of the study populations are shown in Table 1. The *SERPINE1* 4G/5G polymorphism was in Hardy-Weinberg equilibrium. Twenty of the 200 spouses of the probands had asthma themselves and were included in the analyses on asthma and asthma phenotypes. In the general population, 143 of the 1390 (10.3%) included subjects ever had an asthma attack.

Association with asthma and IgE

The case-control analyses did not show statistically significant differences in genotype distribution between asthma cases and non-asthmatic controls (online depository Table E1).

In probands and spouses with asthma, patients with the 5G allele (4G/5G or 5G/5G) had a higher serum total IgE level; (geometric mean IgE, IU/l (%standard deviation)) for 4G/4G: 58.9 (4.1) and for 4G/5G & 5G/5G: 97.1 (4.6) (P=.019). There were no significant associations between the genotypes and IgE levels in non-asthmatic spouses or in asthmatic or non-asthmatic subjects from the general population.

Association with lung function

Regression analyses showed that the 5G allele was significantly associated with a lower FEV_1 level in asthma probands and affected spouses (n=301) after correction for confounding variables (240 subjects had data on *SERPINE1*; Table 2). A trend for the same association was observed in asthma subjects from the general population (online depository Table E2). No associations between *SERPINE1* genotype and lung function level were found in the control populations (online depository Table E2).

The 5G allele was associated with a lower maximally attained FEV_1 level, i.e. asthmatic probands with the 4G/5G or 5G/5G genotype had a 30.4 cl (95% CI; 1.8 – 59.0) lower FEV_1 level compared to those with 4G/4G genotype (borderline significant: P=0.04).

In the asthma probands, the mean FEV_1 decline was substantially faster in subjects with the 4G/5G or 5G/5G genotype (47.7 ml/year (31.1 – 64.2)) than the 4G/4G genotype (32.4 ml/year (13.4 – 51.3)). Figure 1; difference of 15.3 ml/year (2.1 – 28.6), P=0.024 (online depository Table E3).

As expected, there was an immediate improvement in the FEV_1 level after the start of ICS[37]. However, this was only present in individuals having the 4G/5G or 5G/5G genotypes: FEV_1 increased by a mean of 189.9 ml (30.5 – 349.2). This contrasts to asthmatics with the 4G/4G genotype, i.e. a fall of -63.1 ml (-298.3 – 172.1). Figure 2; difference of 253.0 ml (7.9 – 498.0), borderline significant (P=0.044).

ICS use also slowed down the rate of FEV_1 decline over time by 31.2 ml/yr (12.8-49.5). This effect was comparable between the genotypes (4G/4G vs. 4G/5G or 5G/5G; P=0.28).

In the general population *SERPINE1* genotypes were not significantly associated with FEV_1 decline over time (4G/4G vs. 4G/5G or 5G/5G: -0.8 ml/year (-3.7 – 2.0) faster decline in non-asthmatics and 0.2 ml/year (-9.1 – 9.5) less fast decline in asthmatics).

Association with complete asthma remission

The prevalence of complete asthma remission at follow-up in the 281 asthmatic probands was 11.7% (n=33). Complete asthma remission was more prevalent in subjects with the 4G/4G genotype (20.3%) compared to subjects with the 4G/5G genotype (10.9%) or the 5G/5G genotype (3.8%) (P=0.025).

DISCUSSION

This study suggests that the functional *SERPINE1* 4G/5G polymorphism is associated with the severity but not the presence of asthma. Asthmatics with the 5G allele had a significantly higher serum total IgE level, a lower FEV_1, a lower maximally attained FEV_1 level during young adulthood, and a faster annual FEV_1 decline. Data in the general population confirmed the association between the 5G allele and lower FEV_1 in asthmatics only. Interestingly, treatment with ICS was associated with an immediate FEV_1 improvement in asthmatics with the *SERPINE1* 5G genotype, a finding that was absent in those with the 4G/4G genotype. Of note, we suggest the 5G allele was also associated with a lower prevalence of complete asthma remission at follow-up.

Our genetic findings can be a direct result from differences in PAI-1 activity in airway remodeling. PAI-1 has been described to regulate the tissue response and repair by PAS inhibition[17]. Higher levels of PAI-1 at the inflammatory site in association with pronounced airway remodeling may therefore lead to a lower lung function. The 4G/5G polymorphism directly influences the level and activity of PAI-1 in plasma and individuals with the 4G allele have higher plasma PAI-1 levels and activity[20,21,38,39]. Differences in PAI-1 levels between the genotypes can therefore be involved in the development and progression of asthma. We show that asthmatics with the 4G/5G or 5G/5G genotype had a lower lung function level than those carrying the 4G/4G genotype. Unfortunately, we have no data on serum PAI-1 levels and are thus unable to confirm higher PAI-1 levels in individuals with the 4G allele. Although some studies have described higher PAI-1 plasma levels to be associated with the 4G allele[20,21,38,39], this has not been invariably shown[12,40]. Moreover, Stevens et al.[41] showed that PAI-1 expression and activity was increased in epithelial brushings of children with asthma which was not reflected in plasma. It thus remains to be determined which is the end result of *SERPINE1* genotypes on the in situ activity that may

affect airway wall and lung tissue remodeling. Furthermore, active smoking, alcohol, obesity, high serum triglycerides, male sex and age seem to increase PAI-1 levels, whilst regular exercise has been associated with lower PAI-1 levels[42-46]. This makes interpreting plasma PAI-1 level differences between the genotype groups difficult.

We found no significant difference in genotype distribution between asthma cases and non-asthmatic controls in the analysis. Although this analysis is based on relatively few subjects, the remarkable similarity of the genotype distributions of the asthma cases and non-asthmatic controls which implies that low study power is not driving this non-significance.

Our longitudinal study allowed us to investigate the effect of *SERPINE1* 4G/5G polymorphism on the rate of FEV_1 decline especially in relation to the start of ICS treatment. As expected, we found a significant initial improvement in FEV_1 after the start of ICS treatment. Interestingly, this improvement was largest in asthmatics having the 4G/5G or 5G/5G genotype and absent in the 4G/4G genotype group. This effect could not be driven by the fact that asthmatics carrying the 5G allele had a lower level of lung function than the 4G/4G genotype group, since this was accounted for in the analysis.

In a pathophysiological perspective, the beneficial effects of the 5G genotype in ICS response might be due to differences in PAI-1 levels in the lungs. However, asthmatic subjects carrying the 5G allele had a more rapid annual decline in FEV_1. ICS treatment did not reduce the difference between the genotype groups and subjects carrying the 5G allele still had a significantly more rapid annual FEV_1 decline during ICS treatment compared to those carrying the 4G/4G genotype. This implies that the short-term effects of ICS on FEV_1 may be differentially regulated in a pathological respect than their long-term effects.

There exists an imbalance between metalloproteinases (MMPs) and their inhibitors in asthma that contributes to airway remodeling[10,11,47], an important mechanism of

accelerated lung function decline. Human mast cells are an important source of PAI-1[18], a major inhibitor of MMPs, and pronounced activity of PAI-1 was reported to associate with pulmonary fibrosis[14-17]. Thus PAI-1 may affect airway remodeling in asthma. Cho et al. showed that sensitized mast cells release a considerable amount of PAI-1, and this was associated with blocking of fibrinolysis thereby promoting fibrin and collagen deposition, features of airway remodeling[18]. It is tempting to speculate that ICS use will lead to a reduction of PAI-1 activity since steroids suppress degranulation and cytokine production in mast cells potently in vitro in a time-dependent manner[48-51]. So far conflicting results have been reported on effects of steroids on PAI-1 production by cells in vitro which may largely due to the type of cell under study and time of effect measurements. Incubation for 2 to 8 hours with glucocorticoids resulted in increased PAI-1 levels from keratinocytes and human lung fibroblasts[52-54] and partial suppression of fibrinolytic activity of pulmonary alveolar epithelial cells[55]. In contrast, after an initial increased PAI-1 activity by glucocorticosteroids in human adipose tissue fragments, a significantly reduction in PAI-1 activity and mRNA expression occurred after a further 48-hour incubation[56]. Finally, there is most likely no simple answer to the question in what way ICS use influences PAI-1 levels and activity at the bronchial epithelial level, especially since other anti-inflammatory effects of ICS, like suppression of cytokine release, are also influencing PAI-1 expression and activity. Thus short-term beneficial effects on lung function may result from regulation at different cellular levels than the lack of difference in FEV_1 decline with glucocorticosteroids in relation to PAI-1 genotypes.

Many studies have investigated which factors determine the progression or severity of asthma, especially since severe asthma largely drives the economic costs of asthma management. However, it is also of importance to get insight in the driving factors of asthma remission. We here found that asthma remission, defined as the absence of asthma symptoms,

normal lung function, absence of hyperresponsiveness and no use of asthma treatment, occurred in 11% of our asthma population. We for the first time suggest that remission of asthma may be genetically determined. Asthmatics with the 5G allele in *SERPINE1* had the lowest prevalence of remission and those with the 4G/4G genotype the highest prevalence. Again, this may reflect differences in chronic airway inflammation and remodeling between genotypes.

Higher serum IgE levels have been associated with the 4G allele of the *SERPINE1* 4G/5G polymorphism[12,57]. We found higher IgE levels in those carrying the 5G allele. However, we found no association of the *SERPINE1* 4G/5G polymorphism with serum total IgE in the control groups or with asthma in the case-control analyses. Therefore, we believe *SERPINE1* is not a susceptibility gene of asthma and is neither solely carrying the risk for higher IgE levels in asthma. Thus *SERPINE1* is not the gene by which we can explain our previously found linkage on chromosome 7q21[19].

In accordance with previous studies, the 4G allele was the most prevalent allele in our populations[12,23,24,58]. In our study we found the 5G allele to carry the risk of development of more severe asthma, reflected by lung function impairment, response to ICS use and lower prevalence of asthma remission. Others found that the 4G allele was associated with asthma and with lower lung function in asthmatic patients[12]. Discrepant findings may be due to population stratification. Asthma is a complex disease in which genetic and environmental factors contribute to the asthma phenotype. Gene-environment interactions are extremely complex and not always linear, such that the same genetic variants might be associated with opposite phenotypes in different environments[59]. This might be the case in our population; although we have no direct signal which contributing factors may account for

the conflicting findings. Another explanation may be that the real causal variant in the *SERPINE1* gene is not the polymorphism we genotyped but another polymorphism that is in linkage disequilibrium (LD) with the 4G/5G polymorphism. This LD-structure differs between populations and therefore, we found another risk allele than previous studies.

A recent linkage and association study identified the *plasma urokinase plasminogen activator receptor* (*PLAUR*) gene in chromosome 19q13.1-3 as a potential asthma susceptibility gene[60]. Polymorphisms in the *PLAUR* gene were associated with asthma, AHR, FEV_1 and plasma PLAUR levels (alternative symbols uPAR)[60]. PLAUR interacts with uPA resulting in enhanced activation of cell-bound plasminogen[61] and therefore plays a role in PAS. PLAUR has been implicated in many physiological processes; including cell migration, proliferation, differentiation and tissue fibrosis[62]. These findings further support our findings on *SERPINE1* polymorphism and asthma, implicating that PAS is a candidate pathway for enhanced airway remodeling in asthma.

We conclude that our findings may suggest a role of the *SERPINE1* gene in the progression, remission and severity of asthma, likely via the effects of PAI-1 on airway inflammation and remodeling. Clearly, functional studies need to address the exact contribution of PAI-1 in this process and the interaction with ICS treatment.

Table 1: Clinical characteristics and genotype distribution of the study populations

	Asthma population		**General population**
	probands	**spouses**	**controls**
Number, (n)	281	200	1390
Male : female, (%)	60 : 40	38 : 62	51 : 49
Age, (years; median (range))	50 (35 – 75)	50 (33 – 77)	52 (35 – 79)
FEV_1 % predicted pre BD, (%)	70 (23.8)	98 (14.0)	91 (14.6)
FEV_1 % predicted post BD, (%)	82 (22.4)	104 (13.5)	-
FEV_1/VC pre BD, (%)	60 (14.3)	77 (7.3)	74 (8.3)
FEV_1/VC post BD, (%)	65 (13.5)	79 (7.2)	-
Serum total IgE, (IU/l; median (IQR))	81 (31 – 267)	26 (10 – 74)	27 (10 – 66)
Packyears, (years; median (IQR))	3.3 (0 – 14.4)	7.0 (0 – 19.1)	8.0 (0 – 20.5)
Asthma, (%)	100	10	10
Use of inhaled corticosteroids, (%)	51	5	NA
Genotype distribution 4G/5G polymorphism			
4G/4G, n (%)	59 (27)	60 (33)	366 (28)
4G/5G, n (%)	110 (50)	80 (45)	644 (50)
5G/5G, n (%)	52 (23)	39 (22)	292 (22)

Data are presented as mean values (standard deviation) unless stated otherwise. Clinical characteristics of the asthma population are given of the time at re-examination.
IQR = interquartile range; FEV_1 = forced expiratory volume in one second; PC_{20} = concentration histamine at which FEV_1 fell by 20%; IgE = immunoglobulin E; NA=not available

Table 2: Lung function and *SERPINE1* genotypes in asthmatic probands and asthmatic spouses

Genotype (n) *	**FEV_1** (cl)				**FEV_1%VC** (%)			
	before BD	**P-value**	**after BD**	**P-value**	**before BD**	**P-value**	**after BD**	**P-value**
***SERPINE1* 4G/5G**								
4G/4G (70)	Reference		Reference		Reference		Reference	
4G/5G (116)	-28.5 (-47.8 – -9.2)	**0.004**	-28.1 (-45.8 – -10.5)	**0.002**	-4.6 (-8.2 – -1.0)	**0.012**	-4.5 (-7.6 – -1.3)	**0.005**
5G/5G (54)	-25.5 (-48.4 – -2.6)	**0.029**	-25.9 (-46.9 – -5.0)	**0.015**	-4.7 (-9.0 – -0.5)	**0.030**	-4.2 (-7.9 – -0.4)	**0.029**
4G/5G or 5G/5G [#]	-27.5 (-45.6 – -9.5)	**0.003**	-27.4 (-43.9 – -11.0)	**0.001**	-4.7 (-8.0 – -1.3)	**0.007**	-4.4 (-7.3 – -1.4)	**0.004**

* Analyzed with linear regression and presented as regression coefficient (95% confidence interval). Adjusted for age, height, sex, pack years of smoking and steroid use.
[#] P-values given of the analyses on the dominant genetic model (group 4G/4G compared to the combined groups 4G/5G & 5G/5G).
BD = bronchodilator; FEV_1 = forced expiratory volume in one first second; VC = vital capacity

Figure 1: Mean annual change in FEV_1 and the *SERPINE1* 4G/5G polymorphism (dominant genetic model)

Results of linear mixed effect model analyses; Estimates and P-values given of the analyses on the dominant genetic model (group 4G/4G compared to the combined groups 4G/5G & 5G/5G). Corrected for use of inhaled corticosteroids. FEV_1 = forced expiratory volume in one second; *SERPINE1* = serine protease inhibitor type 1

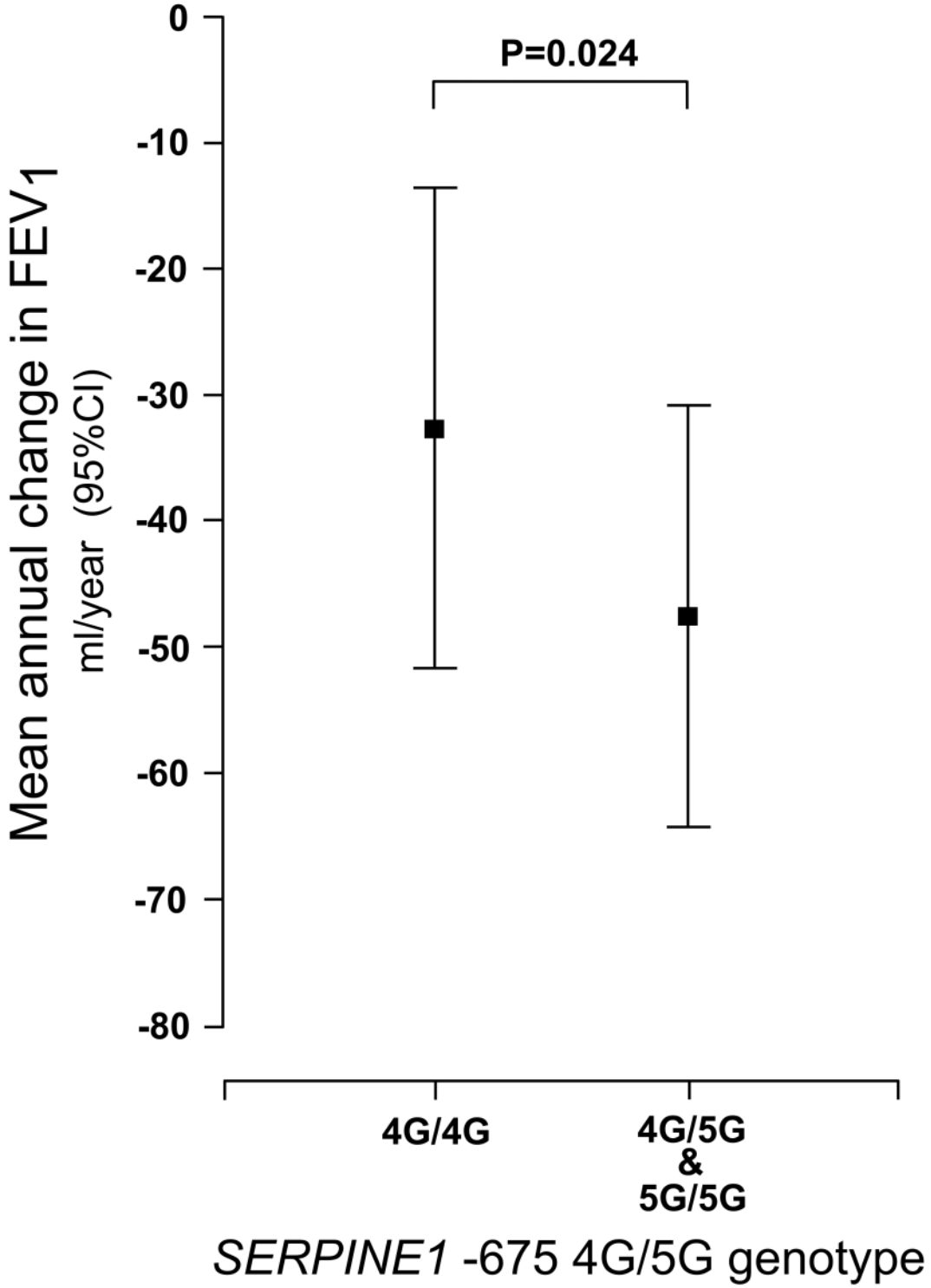
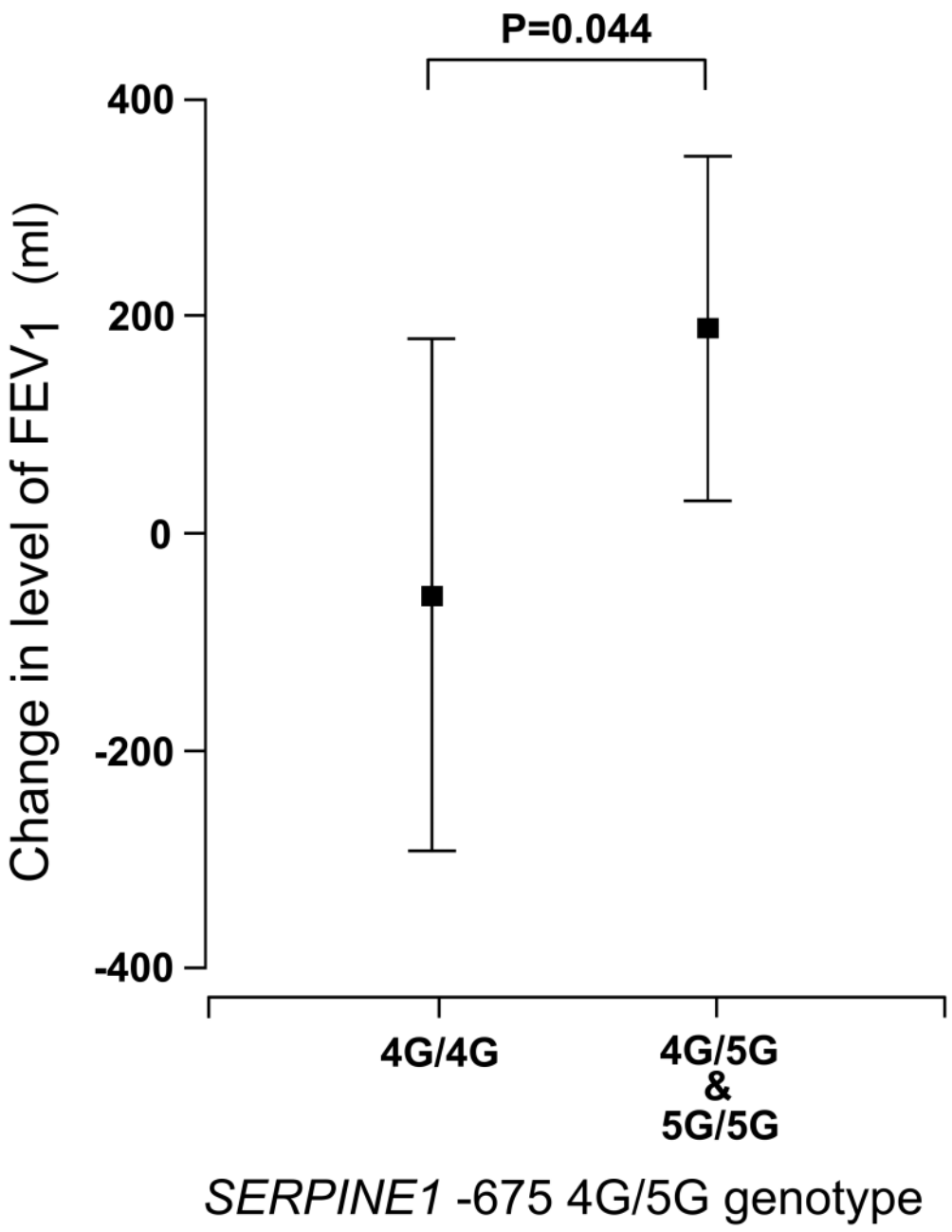

P=0.024
0
-10
-20
-30
-40
-50
-60
-70
-80
Mean annual change in FEV1
ml/year (95%CI)
4G/4G
4G/5G
&
5G/5G
SERPINE1 -675 4G/5G genotype

Figure 2: Change in FEV_1 after starting treatment with inhaled corticosteroids stratified by the *SERPINE1* 4G/5G polymorphism (dominant genetic model)

Results of linear mixed effect model analyses. FEV_1 levels before the start of inhaled corticosteroids were set to zero per genotype-group; Estimates and P-values given of the analyses on the dominant genetic model (group 4G/4G compared to the combined groups 4G/5G & 5G/5G) on the level of FEV_1 before the start of inhaled corticosteroids compared to the level of FEV_1 after the start of inhaled corticosteroids. FEV_1 = forced expiratory volume in one first second; *SERPINE1* = serine protease inhibitor type 1


P=0.044
400
200
0
-200
-400
Change in level of FEV1 (ml)
4G/4G
4G/5G
&
5G/5G
SERPINE1 -675 4G/5G genotype

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