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BRIEF REPORT

# Thrombotic risk determined by rare and common *SERPINA1* variants in a population-based cohort study

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## Abstract

**Background:** Severe alpha-1-antitrypsin deficiency (AATD), phenotype PiZZ, was associated with venous thromboembolism (VTE) in a case-control study.

**Objectives:** This study aimed to determine the genetic variation in the *SERPINA1* gene and a possible thrombotic risk of these variants in a population-based cohort study.

**Patients/Methods:** The coding sequence of *SERPINA1* was analyzed for the Z (rs28929474), S (rs17580), and other qualifying variants in 28,794 subjects without previous VTE (born 1923–1950, 60% women), who participated in the Malmö Diet and Cancer study (1991–1996). Individuals were followed from baseline until the first event of VTE, death, or 2018.

**Results:** Resequencing the coding sequence of *SERPINA1* identified 84 variants in the total study population, 21 synonymous, 62 missense, and 1 loss-of-function variant. Kaplan-Meier analysis showed that homozygosity for the Z allele increased the risk of VTE whereas heterozygosity showed no effect. The S (rs17580) variant was not associated with VTE. Thirty-one rare variants were qualifying and included in collapsing analysis using the following selection criteria, loss of function, in frame deletion or non-benign (PolyPhen-2) missense variants with minor allele frequency (MAF) <0.1%. Combining the rare qualifying variants with the Z variant showed that carrying two alleles (ZZ or compound heterozygotes) showed increased risk. Cox regression analysis revealed an adjusted hazard ratio of 4.5 (95% confidence interval 2.0–10.0) for combinations of the Z variant and rare qualifying variants. One other variant (rs141620200; MAF = 0.002) showed an increased risk of VTE.

**Conclusions:** The *SERPINA1* ZZ genotype and compound heterozygotes for severe AATD are rare but associated with VTE in a population-based Swedish study.

*Full information for the Regeneron Genetics Center is provided in Appendix S1.

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KEYWORDS
alpha-1-antitrypsin, epidemiology, genetics, *SERPINA1*, venous thromboembolism

## 1 | INTRODUCTION

The serpin (serine protease inhibitor) $\alpha_1$-antitrypsin (AAT) is a glycoprotein produced in the liver. Its main function is to inhibit neutrophil elastase,[1–5] but it also inhibits several other serine proteases, such as the coagulation cascade serine proteases.[4,6] AAT in plasma protects the lung parenchyma from neutrophil elastase.[1–5] The phenotype PiZZ (Glu366Lys) is associated with severe AAT deficiency (AATD) and markedly reduced AAT plasma levels (<35%).[1–5] AATD was described in 1963 to cause emphysema and chronic obstructive pulmonary disease (COPD).[7] AATD may also cause liver cirrhosis due to polymerization and accumulation of mutated AAT molecules in the hepatocytes.[1–5] Cigarette smoking increases risk of early emphysema in patients with severe AATD.[1–5] The common deficiency alleles S (rs17580) and Z (rs28929474) have a prevalence in Caucasian populations between 5–10% and 1–3%, respectively.[4,5] The S allele (Glu288Val) is only associated with mild AATD.

AAT Pittsburgh has a Met382Arg substitution (legacy Met358Arg) at the reactive Met-Ser site of AAT, which enables the protein to act as a potent thrombin inhibitor.[8–10] Patients with AAT Pittsburgh exhibit bleeding tendency. Severe AATD has been reported in cases with venous thromboembolism (VTE).[11,12] Recently, a case-control study by Basil et al. observed an association between PiZZ and VTE with a hazard ratio (HR) of 4.2 (95% confidence interval [CI] 2.9–6.2).[13] There was a high prevalence of COPD (46%) among AATD patients compared to 4% in controls.[13] COPD has been reported to be associated with VTE.[14] Liver disease was also more common among patients than controls (4% vs. 1%).[13] A Danish cohort study confirmed the association between homozygosity for the PiZZ variant and VTE (HR = 2.2, 95% CI 1.3–3.7).[15] However, there exists no information about other *SERPINA1* gene variants and VTE.

This exome sequence study aimed to determine the genetic variation in the *SERPINA1* gene and a possible thrombotic risk in a population-based study. We analyzed the exome sequence in 28,794 individuals in the large Malmö Diet and Cancer cohort (MDC).[16–19]

**Essentials**

- Severe alpha-1-antitrypsin deficiency (AATD) has been suggested to be linked to venous thromboembolism (VTE).
- The study was a population-based cohort study of middle-aged and older individuals.
- Hazard ratio for VTE was 4.1 (95% confidence interval 1.9–8.6) for carriers of the Z variant and rare qualifying *SERPINA1* variants.
- Increased risk of VTE was associated with homozygosity for the Z variant and rare qualifying variants in the *SERPINA1* gene.

## 2 | METHODS

### 2.1 | Participants

The MDC is a population-based cohort study from Malmö, Sweden, as previously described.[16–19] Participants underwent a medical history, physical examination, and laboratory assessment at baseline (1991–1996).[16–19] The MDC population has 12% admixture from foreign-born individuals. Among foreign-born individuals 1% were non-European. A total of 30,446 individuals—men ($n$ = 12,120, born 1923–1945) and women ($n$ = 18,326, born 1923–1950)—attended a baseline examination between 1991 and 1996. Clinical data and DNA were available for 29,387 subjects sampled at baseline. Of these individuals 593 (2.0%; 315 women, 278 men) were affected by VTE between 1970 and baseline and were excluded. The final study population was 28,794 individuals. The study was conducted according to the principles of the Declaration of Helsinki. The Regional Ethics Review Board at Lund University, Lund, Sweden, approved the study (LU 51/90) and all participants provided informed written consent.

### 2.2 | Clinical endpoints

Venous thromboembolism events were identified from the Swedish National Patient Registry (SNHDR) during follow-up until 2018. The SNHDR had a 100% coverage for inpatients in Malmö during the whole follow-up time and for outpatients from 2001. VTE was based on the International Classification of Diseases 7th, 8th, 9th, and 10th Revisions codes. Diagnosis of VTE in the SNHDR has accuracy of 95%,[20] whereas the overall validity of the SNHDR is 87%.[21]

### 2.3 | Genetic and statistical analysis

Whole exome sequencing (WES) was performed by Regeneron Genetics Center,[16,19,22] such that >85% of targeted bases were covered at a read depth of >20×. ANNOVAR was used for variant annotation, allele frequencies (AF), and *in silico* predictions of deleteriousness.[23] Principal components analysis (PCA) was performed as described.[16,24] The reference genomes were obtained from the 1000 Genomes Project.[24,25] The principal components were first obtained from the reference genomes and then projected individuals from the MDC onto the principal component space via PLINK2.[22,26]

Cox proportional hazards regression was used to examine the association between genotype and incident VTE. A crude model and an age, sex, COPD, and ancestry-adjusted model were calculated. In a multivariable model, the known genetic risk factors rs6025 and rs1799963, in addition to potential cardiovascular risk factors, that is, body mass index (BMI), smoking status, blood pressure (systolic), and high alcohol consumption (>30 g/day women, >40 g/day men), were added to reduce the statistical noise for these factors. There were no significant interactions. The top two eigenvectors from the PCA were included as covariates in the Cox proportional hazard regression models to control for population stratification (ancestry). The fit of the proportional hazards model was checked visually by plotting the incidence rates over time and by calculating Schoenfeld (partial) residuals.[17,18] The proportional hazards assumption was not violated. Subjects were categorized according to genotype and Kaplan-Meier plots were calculated for VTE. R (version 4.0.0) was used for all statistical analyses.

## 3 | RESULTS AND DISCUSSION

A total of 28,794 individuals from the MDC cohort were available for analysis. During a median follow-up of 23 years (interquartile range 17–25 years) until 2018, a total of 2584 (9%) incident VTE events occurred (1030 men, 1554 women) among individuals without prevalent VTE. The sum of the follow-up time was 587,992.7 years, corresponding to a VTE incidence rate of 4.4 (95% CI 4.2–4.6) per 1000 person years. Resequencing identified 84 *SERPINA1* variants in the total study population: 21 synonymous, 62 missense, and one loss-of-function (LoF) variant. Of the 84 variants, 43 were detected in single individuals and the 10 variants lacking an rs-number were only found among individuals without VTE. For the S variant (rs17580), the number of heterozygotes among individuals with and without VTE was 122 (4.7%) versus 1215 (4.6%), and the number of homozygotes was 1 (0.039%) versus 14 (0.053%). For this variant, no overrepresentation in cases was observed. For the Z variant (rs28929474) the number of heterozygotes among individuals with and without VTE was 151 (5.8%) versus 1461 (5.6%), and the number of homozygotes was 5 (0.19%) versus 16 (0.061%). Thus, an overrepresentation of homozygotes was observed among VTE patients. The thrombosis-free survival curves using Kaplan-Meier analysis are presented in Figure 1A,B for individuals heterozygous and homozygous for the Z allele, respectively. Homozygosity for the Z allele increased VTE risk, whereas heterozygosity showed no effect. Thirty-one variants were classified as qualifying and included in collapsing analysis using the following selection criteria: LoF or non-benign (PolyPhen-2) missense variants with minor allele frequency (MAF) <0.1%[19] (Table 1). The total prevalence of these variants in the population was 0.6%. Seventeen (0.66%) individuals with VTE compared to 164 (0.63%) individuals without VTE carried one qualifying variant. Combining the rare qualifying variants with the Z variant showed that carrying one allele did not increase VTE risk, whereas carrying two alleles (either ZZ or compound heterozygotes carrying a combination

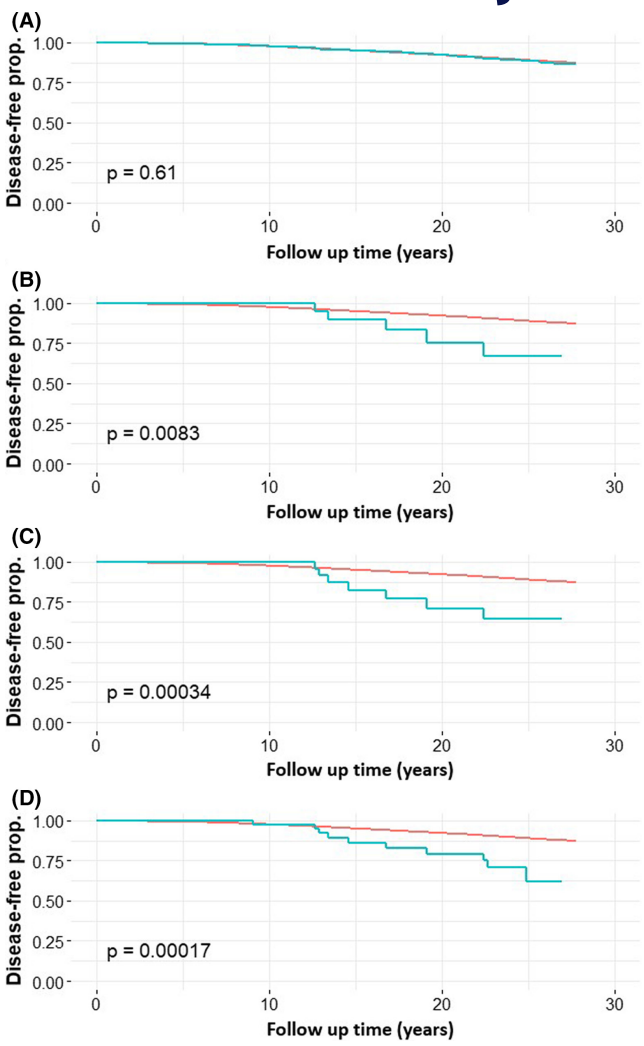


FIGURE 1 Kaplan-Meier curves for thrombosis-free survival for: (A) individuals heterozygous for the Z-allele (blue curve) compared to those without the Z-allele (red curve), (B) individuals homozygous for the Z-allele (blue curve) compared to those without the Z-allele (red curve), (C) individuals homozygous or compound heterozygous for combinations of Z and any of 31 qualifying rare variants (blue curve) compared to those without any variants (red curve), (D) individuals homozygous or compound heterozygous for rs141620200 in addition to the variants described in C (blue curve) compared to those without any variants (red curve)

of Z and any other variant) showed increased risk (Figure 1C). A total of 26 individuals carried two alleles, 21 had the ZZ genotype, whereas the remaining five were compound heterozygotes with one Z allele and either rs199422209 (PiMHeerlen; three individuals),[27] rs111850950 (one individual), and chr14:94382826:T>G (one individual). PiMHeerlen (p.Pro393Leu) is known to be associated with severe AATD.[27]

There were three *SERPINA1* variants with high MAFs: rs1303 (MAF = 0.28), rs6647 (0.25), and rs709932 (0.16). All three were benign according to ClinVar and were not associated with VTE. All remaining variants detected in the total population were compared for their MAFs in individuals with and without VTE in Figure 2A. The 25 most common *SERPINA1* variants detected in the MDC cohort were

**TABLE 1** Rare qualifying variants in the *SERPINA1* gene in the Malmö Diet and Cancer study (MKC), that is, loss of function variants (LOF) or missense variants with pathogenic Polyphen-2 score with minor alle frequencies (MAF) <0.1% according to gnomAD

| | | | | | | | Heterozygotes | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Location (GRCh38) | Consequence | Codon | Protein position | aa | PolyPhen-2 | ACMG | VTE | No VTE | HGMD | rsID |
| 14:94378460 | Missense | Acc/Ccc | 416 | T/P | Possibly damaging | US | 0 | 1 | – | rs3191200 |
| 14:94378528 | Missense | cCc/cTc | 393 | P/L | Probably damaging | P | 4 | 32 | CM890098 | rs199422209 |
| 14:94378529 | Missense | Ccc/Tcc | 393 | P/S | Probably damaging | P | 2 | 24 | HM971366 | rs61761869 |
| 14:94378547–94378548 | Frameshift | -/C | 386–387 | -/X | NA | P | 0 | 1 | – | rs764325655 |
| 14:94378608 | Missense | gaG/gaC | 366 | E/D | Possibly damaging | US/LP | 0 | 1 | – | – |
| 14:94378628 | Missense | Gct/Act | 360 | A/T | Possibly damaging | US | 1 | 0 | CM900185 | rs1802959 |
| 14:94378637 | Missense | Gtg/Atg | 357 | V/M | Possibly damaging | US | 0 | 3 | CM109803 | rs373630097 |
| 14:94379468 | Missense | tCc/tTc | 354 | S/F | Probably damaging | US | 1 | 9 | CM123000 | rs201788603 |
| 14:94379499 | Missense | Ggg/Agg | 344 | G/R | Possibly damaging | US | 0 | 3 | CM1111604 | rs367797069 |
| 14:94379538 | Missense | Ggc/Agc | 331 | G/S | Probably_damaging | US | 0 | 1 | – | rs569455355 |
| 14:94379592 | Missense | Ccc/Tcc | 313 | P/S | Probably damaging | US/LP | 0 | 1 | – | rs779938258 |
| 14:94380871–94380873 | Inframe deletion | agAAGg/agg | 305–306 | RR/R | NA | LP | 1 | 7 | – | rs748777702 |
| 14:94380949 | Missense | gAt/gTt | 280 | D/V | Possibly damaging | US | 1 | 19 | CM890096 | rs121912714 |
| 14:94381043 | Missense | Ggc/Cgc | 249 | G/R | Possibly damaging | US/P | 1 | 0 | CM094671 | rs764220898 |
| 14:94381070 | Missense | Gtg/Atg | 240 | V/M | Probably damaging | US | 0 | 1 | – | rs72552401 |
| 14:94381087 | Missense | gTg/gAg | 234 | V/E | Probably damaging | US | 0 | 4 | CM1311110 | rs746197812 |
| 14:94381088 | Missense | Gtg/Atg | 234 | V/M | Possibly damaging | US | 0 | 1 | – | rs374168370 |
| 14:94382612 | Missense | gTg/gCg | 209 | V/A | Possibly damaging | US | 0 | 2 | – | rs1555368958 |
| 14:94382651 | Missense | tTg/tCg | 196 | L/S | Probably damaging | US | 0 | 1 | – | rs368433503 |
| 14:94382826 | Missense | Acc/Ccc | 138 | T/P | Possibly damaging | US | 1 | 1 | – | – |
| 14:94382912 | Missense | aCg/aTg | 109 | T/M | Possibly damaging | US | 0 | 1 | CM971177 | rs199422213 |
| 14:94382930 | Missense | gGc/gAc | 103 | G/D | Probably damaging | US | 0 | 1 | – | – |
| 14:94382931 | Missense | Ggc/Agc | 103 | G/S | Probably damaging | US | 0 | 1 | – | – |
| 14:94382949 | Missense | Cac/Tac | 97 | H/Y | Probably damaging | US | 0 | 1 | | – |
| 14:94382954 | Missense | gAc/gGc | 95 | D/G | Possibly damaging | US | 0 | 1 | – | – |
| 14:94382988 | Missense | Gcc/Acc | 84 | A/T | Probably damaging | US | 4 | 30 | CM083099 | rs111850950 |
| 14:94382998 | Missense | agC/agA | 80 | S/R | Probably damaging | US | 0 | 2 | – | – |
| 14:94383009–94383011 | Inframe deletion | tTCTcc/tcc | 76–77 | FS/S | NA | P | 0 | 7 | – | rs775982338 |
| 14:94383017 | Missense | aTc/aCc | 74 | I/T | Probably damaging | US | 0 | 3 | – | – |

TABLE 1 (Continued)

| Location (GRCh38) | Consequence | Codon | Protein position | aa | PolyPhen-2 | ACMG | Heterozygotes | | HGMD | rsID |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | VTE | No VTE | | |
| 14:94383032 | Missense | tCc/tTc | 69 | S/F | Probably damaging | US | 1 | 3 | – | rs199687431 |
| 14:94383197 | Missense | gGc/gAc | 14 | G/D | Probably damaging | US | 0 | 1 | – | – |

Abbreviations: aa, amino acid; ACMG, American College of Medical Genetics and Genomics; HGMD, Human Gene Mutation Database http://www.hgmd.cf.ac.uk/ac/index.php; LP, likely pathogenic; P, pathogenic; US, uncertain significance; US/LP, uncertain significance with minor pathogenic evidence; US/P, uncertain significance with some pathogenic evidence; VTE, venous thromboembolism.

detected at frequencies comparable to those observed in gnomAD. In addition to the Z variant only one other variant (rs141620200) showed a difference in MAF between individuals with and without VTE (Figure 2B). To investigate if this variant also added to the risk of VTE this variant was subjected to Kaplan–Meier analysis. Heterozygotes showed no effect, whereas homozygotes or compound heterozygotes with the Z allele and any of the 31 qualifying rare variants showed an effect (Figure 1D). This added a total of 14 individuals to the 26 identified previously: 1 individual homozygous for rs141620200, 12 individuals heterozygous for this variant and the Z variant, and 1 individual heterozygous for this variant and rs199422209 (PiMHeerlen).[27] Out of these 40 homozygous or compound heterozygous individuals, 10 individuals had incident VTE. The results of the Cox proportional regression analysis are shown in Table 2. The Cox multivariable regression analysis showed a fully adjusted HR of 4.5 (95% CI 2.0–10.0) for a combination of the Z variant and the rare qualifying variants. Sensitivity analysis with exclusion of patients who died from liver disease was like those presented in Table 2 (not presented). This estimate is like the study by Basil et al.[13] (HR = 4.2, 95% CI 2.9–6.2). In addition to showing similar effect size, our study describes the spectrum of variants present in both cases and controls. The dominating disease-associated allele was the well-known Z allele (rs28929474) associated in homozygous form with AATD. We detected a total of 21 individuals with the ZZ genotype and 17 individuals who were compound heterozygotes with the Z allele and different rare missense variants. The association with homozygosity for variants associated with severe AATD suggests that even a 50% reduction of plasma-abundant AAT as in heterozygotes is not enough to affect the risk of VTE, though in the Danish study a minor association was observed for PiMZ genotype with VTE (odds ratio = 1.2).[15] This is in line with the high concentration of AAT compared to coagulation enzymes. In the present study heterozygosity for the Z allele did not affect the VTE risk associated with the rs6025 and rs1799963 variants (results not shown).

Chronic obstructive pulmonary disease is associated with VTE and PiZZ may cause COPD.[14] It is possible that latent COPD might contribute to the association with VTE. AATD variants may also be polymerized and retained in the liver.[1–5] The potential effect of such phenomena on coagulation factors, anticoagulants including other serpins such as antithrombin, must be evaluated by further studies.

A limitation is that the present study focuses on a single gene. The observed associations were not genome-wide significant for WES studies ($2.5 \times 10^{-6}$). However, a rs112635299 variant downstream of the *SERPINA1* gene is among the top 297 variants used in a genetic risk score for VTE in a genome wide association study (GWAS) by Klarin et al. (OR = 1.16, *P*-value = .00000665).[28] The rs112635299 is in perfect linkage disequilibrium ($R^2 = 1$) with the rs28929474 variant (https://ldlink.nci.nih.gov). A large study size is necessary for obtaining significance for GWAS studies ($5 \times 10^{-8}$).

Allele frequency comparisons identified one other variant in addition to the Z variant that was associated with VTE. The rs141620200 variant (p.Ala308Ser) showed an increase of approximately 0.4% in VTE individuals compared to controls. The p.Ala308Ser was present

in homozygous form in one VTE individual and in compound heterozygous form in a total of 13 individuals (two with VTE). This variant is described in ClinVar as a variant with conflicting interpretations of pathogenicity and although there is an association with VTE, the numbers are small and further studies are necessary to confirm an association with VTE. Why homozygosity and compound heterozygosity of the rs141620200 variant might be associated with VTE is unclear. We have no access to plasma samples. However, the plasma level of AAT has been reported to be normal (1.43 g/L) in one heterozygote

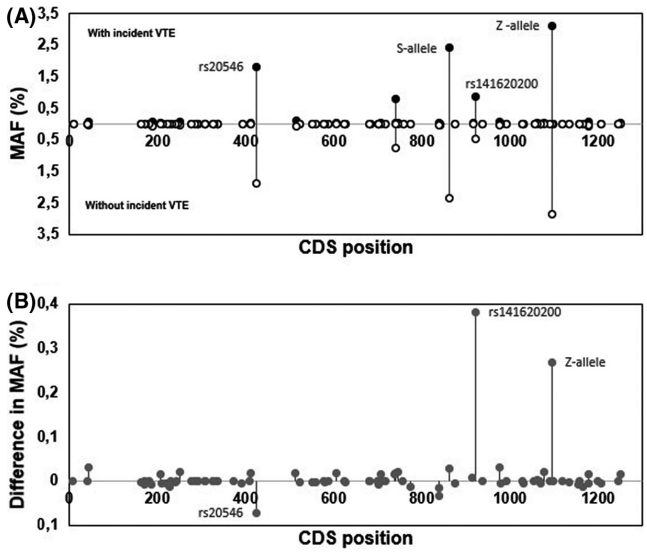


FIGURE 2 A, Minor allele frequencies (MAF) and coding sequence positions for all detected variants except the three most common variants. Closed dots indicate MAF among patients with venous thromboembolism (VTE) and open dots indicate MAF for those without VTE. B, The difference in MAF (closed dots) between individuals with and without incident VTE

for the rs141620200 variant.[29] The Grantham score is 99 and the FATHMM and MetaLR are both signaling a damaging mutation, though Revel score is benign (https://varsome.com/). Speculatively, it could be due to increased inhibitory activity of the Ala308Ser toward activated protein C or plasmin (i.e., gain-of-function variant). Our study showed no association of the S variant (rs17580) with VTE. The S, the rs1416202000, and the Z variants show very low levels of linkage disequilibrium between them, indicating their potentials of acting independently.

In conclusion, homozygosity or compound heterozygosity for variants associated with severe AATD are rare but associated with VTE.

## ACKNOWLEDGMENTS

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## CONFLICTS OF INTEREST

The authors report no conflicts of interest.

## AUTHOR CONTRIBUTIONS

E.M., C.H., and B.Z. conceived and designed the study, analyzed and interpreted data, drafted the manuscript, and gave final approval of the submitted manuscript. All authors interpreted data, critically revised the manuscript for important intellectual content, and gave final approval of the submitted manuscript. Whole exome sequencing was performed by the Regeneron Genetics Center (see the Regeneron Genetics Center Banner Author List and Contribution Statements in Appendix S1). E.M., C.H., and B.Z. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

TABLE 2 Hazard ratios for incident VTE calculated using different combinations of variants in *SERPINA1*

| | No. of carriers of allele combination | | Unadjusted model | | COPD, ancestry, age and sex adjusted model | | Fully adjusted model[a] | |
|---|---|---|---|---|---|---|---|---|
| Allele combination | VTE | No VTE | HR (95% CI) | *P*-value | HR (95% CI) | *P*-value | HR (95% CI) | *P*-value |
| No Z | 2428 | 24,733 | 1 | NA | 1 | NA | 1 | NA |
| Z | 151 | 1461 | 1.0 (0.9–1.2) | .60 | 1.0 (0.9–1.2) | .75 | 1.0 (0.8–1.2) | .85 |
| ZZ | 5 | 16 | 3.1 (1.3–7.4) | .012 | 3.0 (1.2–7.2) | .014 | 4.4 (1.8–10.7) | .0009 |
| No Z or $RV_1$ | 2413 | 24,572 | 1 | NA | 1 | NA | 1 | NA |
| 1 Z or $RV_1$ | 164 | 1619 | 1.0 (0.9–1.2) | .75 | 1.0 (0.9–1.2) | .99 | 1.0 (0.8–1.2) | .98 |
| Z + $RV_1$ | 7 | 19 | 3.6 (1.7–7.4) | .00081 | 3.4 (1.6–7.2) | .0012 | 4.5 (2.0–10.0) | .00025 |
| No Z or $RV_2$ | 2371 | 24,327 | 1 | NA | 1 | NA | 1 | NA |
| 1 Z or $RV_2$ | 203 | 1853 | 1.1 (1.0–1.3) | .16 | 1.1 (0.9–1.2) | .30 | 1.1 (0.9–1.3) | .25 |
| Z + $RV_2$ | 10 | 30 | 3.1 (1.7–5.8) | .00036 | 2.8 (1.5–5.2) | .0011 | 3.4 (1.7–6.5) | .00029 |

*Note:* $RV_1$. Any combination of two or more: Z or non-benign rare missense or loss-of-function alleles.

$RV_2$. Any combination of two or more: Z or non-benign rare missense or loss-of-function or rs141620200 alleles.

Abbreviations: BMI, body mass index; CI, confidence interval; COPD, chronic obstructive pulmonary disorder; HR, hazard ratio; VTE, venous thromboembolism.

[a]Adjusted for prevalent COPD, age, sex, BMI, high alcohol consumption, smoking, ancestry, rs6025, and rs1799963.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.


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