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Exome Sequencing Reveals Common and Rare Variants in *F5* Associated With ACE Inhibitor and Angiotensin Receptor Blocker–Induced Angioedema

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Angioedema occurring in the head and neck region is a rare and sometimes life-threatening adverse reaction to angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs). Few studies have investigated the association of common variants with this extreme reaction, but none have explored the combined influence of rare variants yet. Adjudicated cases of ACEI-induced angioedema (ACEI-AE) or ARB-induced angioedema (ARB-AE) and controls were recruited at five different centers. Sequencing of 1,066 samples (408 ACEI-AE, ARB-AE, and 658 controls) was performed using exome-enriched sequence data. A common variant of the *F5* gene that causes an increase in blood clotting (rs6025, p.Arg506Gln, also called factor V Leiden), was significantly associated with both ACEI-AE and ARB-AE (odds ratio: 2.85, 95% confidence interval (CI), 1.89–4.25). A burden test analysis of five rare missense variants in *F5* was also found to be associated with ACEI-AE or ARB-AE, $P = 2.09 \times 10^{-3}$. A combined gene risk score of these variants, and the common variants rs6025 and rs6020, showed that individuals carrying at least one variant had 2.21 (95% CI, 1.49–3.27, $P = 6.30 \times 10^{-9}$) times the odds of having ACEI-AE or ARB-AE. The increased risk due to the common Leiden allele was confirmed in a genome-wide association study from the United States. A high risk of angioedema was also observed for the rs6020 variant that is the main coagulation defect-causing variant in black African and Asian populations. We found that deleterious missense variants in *F5* are associated with an increased risk of ACEI-AE or ARB-AE.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Patients taking drugs acting on the renin-angiotensin system to manage hypertension can develop adverse drug reactions. Few studies have investigated the association of common variants with extreme reaction, such as angioedema of the face, lip, and pharynx, but none have explored the influence of rare variants yet.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ We confirm that genetic variations (common and rare) are involved in this serious adverse drug reaction and report the implication of another gene from the coagulation system (factor V) in the development of angioedema induced by angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) therapy.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This study presents new common and rare variants in the *F5* gene associated with an increased risk of developing an angioedema when under antihypertensive drugs.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ These findings have important clinical implications for the prevention of ACE inhibitor and ARB-induced angioedema. We propose a new genetic marker which may inform personalized antihypertension therapy.

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Hypertension is a widespread health problem that is present in a quarter of the Western adult population. Drugs acting on the renin-angiotensin system are commonly prescribed to manage this condition. Even though angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are generally well tolerated and effective, some patients experience adverse drug reactions (ADRs) during treatment.[1,2] A serious ADR is angioedema of the tongue, cheeks, lips, or pharynx, characterized by swelling of the reticular dermis and subcutaneous skin layers or mucosa. Angioedema induced by ACEIs or ARBs can sometimes be life threatening or even lethal, and it usually occurs during the first months of therapy but may also appear after several years. The incidence of angioedema is estimated to be 0.2–0.7% for ACEIs and 0.03% for ARBs,[3,4] with a higher rate in patients of African descent and in those with affected relatives.[5,6]

To understand the genetic underpinnings of rare ADRs to statins and ACEIs or ARBs, an international multicenter project, Personalization of Treatment In Cardiovascular drugs using next generation sequencing (PREDICTION-ADR) was established in 2013. Recruitment of patients was facilitated by the development of standardized phenotypes for statin myopathy and angioedema induced by ACEIs or ARBs with inputs from experts in the field.[7,8] PREDICTION-ADR has established novel genetic biomarkers in the pharmacogenetics of statin intolerance.[9]

In previous studies, genetic variants in the bradykinin pathway, bradykinin-degrading enzyme aminopeptidase P gene (*XPNPEP2*)[10,11] and type 2 bradykinin receptor gene (*BDKRB2*), have been associated with ACEI-induced and ARB-induced cough and angioedema. Other genes, serpin family E member 1 gene (*SERPINE1*) and the coagulation factor XII gene (*F12*), have been associated with hereditary angioedema. None of them have been significantly replicated in the only two published genome-wide association studies, where intronic variants in the calcium-activated potassium channel subunit alpha-1 (*KCNMA1*) were significantly associated with ACEI-AE in a Swedish cohort (SWEDEGENE study)[12] and a variant of the protein kinase C theta gene (*PRKCQ*) tended to be protective in a European American cohort.[13]

In addition, studies have reported that the risk of ACEI-AE varies according to ethnicity and gender, with women and black Africans being more likely to develop this particular ADR[5,6,14–18].

Although previous studies assessed common variants in the association between ACEI and angioedema,[12,13] they have had limited success in identifying a genetic basis for this ADR. This study used next-generation sequencing technology to assess the influence of low frequency variants with intermediate penetrance and higher relative risks and combined association with common variants with ACEI-AE and ARB-AE, using cases and controls recruited in Scotland, Sweden, Denmark, the Netherlands, and England. We report novel results from both analyses of common and rare variants. Replication was performed in a cohort from the United States, and we developed a gene risk score (GRS) for angioedema using common and rare variants of *F5*.

METHODS

Study population and phenotype definitions

PREDICTION-ADR. In the PREDICTION-ADR project, cases of ACEI-AE and ARB-AE and controls were recruited at five different centers: Uppsala (Sweden), Dundee (Scotland), Utrecht (the Netherlands), Liverpool (England) and Copenhagen (Denmark). Details are available in the **Supplementary Materials** (**Table S1**). All study sites obtained approval from their respective institutional review board or ethics committee. All the recruited cases were adjudicated by a specialist in allergology. Individuals failing adjudication were excluded.

Cases. Cases of ACEI-AE and ARB-AE were defined according to the phenotype standardization published by Wadelius *et al*., i.e., swelling in the head and neck region without any other likely cause (e.g., allergy or hereditary angioedema).[7]

In Sweden, 252 patients with ACEI-AE or ARB-AE were selected from the Swedegene biobank,[19] which is a nationwide collection of ADR cases. Most cases had been recruited among patients reported to the ADR registry at the Swedish Medical Products Agency, but a minority were referred directly from collaborating clinicians. In Denmark, 63 patients with ACEI-induced or ARB-induced angioedema were referred by physicians from seven hospitals and by a collaborating general practitioner.

In Scotland, 15 cases were recruited via electronic medical records with the Scottish Health Registry (SHARE),[20] as well as the Genetics of Scottish Health Registry for Adverse Drug Reactions (GoSHARE-ADR) website. Individuals who had either stopped using ACEIs or had switched from an ACEI to an ARB with an antihistamine prescription in a 30-day window from the last ACEI prescription were selected. Each case was then individually examined for the most accurate summary of events and adjudicated as being "highly probable" candidates for ACEI-AE. In England, 18 cases were recruited using electronic medical records via the UK clinical Practice Research Datalink (CPRD), following the same steps as previously described for Scotland.

Finally, in the Netherlands, 72 cases were selected from the Dutch PHACE study.

Controls. ACEI-tolerant controls presented no relevant adverse effects while on therapy. Controls were matched for age and sex with cases of the same study site whenever possible.

In total 271 Swedish population-controls were selected from the TwinGene Biobank.[21] The controls had all collected at least five prescriptions for an ACEI over at least 1 year according to the Swedish Prescribed

Drug Register. They had never been diagnosed with angioedema or larynx edema according to the Swedish National Patient Register. Using the same criteria, 20 ACEI-treated control subjects were enrolled by collaborating physicians in Denmark. Seventy-two Dutch controls were selected from the Utrecht Cardiovascular Pharmacogenetics study (UCP) using the same criteria as above. One hundred fifteen Scottish and 199 English patients were selected if they were ACEI users for over 2 years with no switching to ARB or discontinuation, via the same databases as the cases.

Replication cohort: the Vanderbilt study. Genome-wide association study results from the Vanderbilt study were used for replication.[22] Data were available for 438 Europeans and 214 black Americans. Population demographic of the genotyped Vanderbilt population has been previously described.

Patients with angioedema were selected by searching electronic medical records followed by their primary physician confirmations or by direct referral from physicians. Patients with hereditary angioedema were discarded. Cases were defined as presenting with swelling of the lips, pharynx, larynx, or face while taking an ACEI therapy. Controls were defined as patients who had been treated with an ACEI for at least six months without developing angioedema. Cases and controls were matched with respect to sex, race, and smoking status. To confirm their medical histories, all patients were interviewed by a research nurse or a physician. The study was approved by Vanderbilt's Institutional Review Board, and informed consent was obtained from all participants. Only Europeans were considered for replication in our study.

Whole-exome sequencing

Whole-exome sequencing was undertaken in laboratories in Liverpool and Dundee, UK and Uppsala, Sweden. DNA was extracted from blood or saliva samples.

Library preparation and capture. Sequencing of 1,066 samples (408 ACEI-AE, 658 controls) was performed using exome-enriched sequence data. SureSelect QXT, XT, and XT2 reagents (Agilent Technologies, Wokingham, UK) were used to perform fragmentation, end-repair, A-addition, and adaptor ligation reactions to generate Illumina-compatible sequencing libraries. Hybridization capture enrichment of whole-genome libraries was performed using the SureSelect v5 all-exon probe set, according to the manufacturer's recommendations. Genomic DNA was quantified using a Qubit double-stranded DNA high sensitivity assay kit and Qubit Fluorometer (Life Technologies, Carlsbad, CA), using 750 ng for both XT and XT2, and 50 ng for QXT of genomic DNA as input material. The size of the final pool was assessed on a Bioanalyzer high sensitivity DNA chip, and the DNA concentration was determined by Qubit double-stranded DNA high sensitivity assay.

Sequencing. Sequencing was conducted on an Illumina (San Diego, CA) Nextseq500 (Dundee) and a Hiseq2500 (Uppsala and Liverpool). Equimolar aliquots of 8–12 postenrichment libraries (mixing cases and controls) were pooled before sequencing using version 2 TruSeq chemistry (Illumina) on a Nextseq500 and 8–96 libraries on version 4 for Hiseq2500. Respectively 28, 12 and four sequencing runs were carried out in Dundee, Liverpool and Upsala, with 8–12 samples per lane generating 2 × 151 base pair (bp) (Nextseq500) or 2 × 215 bp (Hiseq2500) paired-end reads. Paired-end sequence reads were analyzed through an in-house pipeline (University of Dundee), which gathers preprocessing, demultiplexing, alignment, duplicate removal, variant discovery, and annotation (pipeline description provided in the **Supplementary Materials**).

A presequencing of eight controls was done previously on the three different sequencers to ensure good concordance. Details of sequencing quality can be found **Tables S2 and S3**.

Statistical analysis

Variant filtering and principal component analysis. Quality control filtering was performed on the variant call format using VCFtools.[23] Only high-quality single nucleotide polymorphisms (SNPs) (at least 20-fold coverage, quality score of at least 60, a genotype quality score of at least 20, a quality by depth score of at least 2, a mapping quality score of at least 40, a read rank sum score > −3, and a mapping quality rank of score greater than −10), with a call rate of > 98% as well as not showing deviation from Hardy-Weinberg equilibrium ($P < 1 \times 10^{-6}$ for deviation) and present in at least one individual were kept for the analysis. Each sample was similarly checked, and those with a poor sequencing success rate (< 95%) or having outlying genetic ancestry (defined by being more than

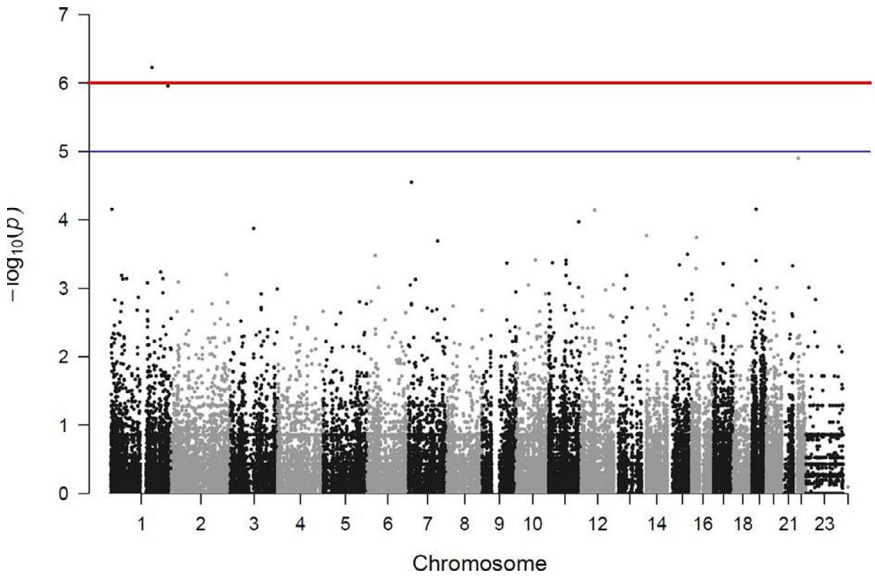

Figure 1 Manhattan plot adjusted by gender and genetic principal components 1–10. The red line corresponds to the genome-wide significance threshold 5.6×10^{-6} (Bonferroni correction). One SNP is above the red line, on chromosome 1, but no clear peak can be seen. SNP, single nucleotide polymorphism.

six standard deviations (SDs) from the mean on principal-component analysis) were excluded from further analysis (**Figures S1 and S2**).

Single variant analysis of common variants minor allele frequency > 1%. Analysis was performed using PLINK/SEQ.[24] Single-variant association was performed on samples using a logistic regression test under the assumption of different genetic models (dominant, additive, and recessive), correcting for the covariates sex, center, sequencing batch, and 1–10 principal components. The exome-wide significance *P* value threshold was set to $P < 1 \times 10^{-6}$ to correct for multiple testing. QQplot and Manhattan plot are presented **Figure S3** and **Figure 1**.

Rare variant analysis (minor allele frequency < 1%). Gene-based associations were performed with RVtest[25] using a sequence kernel association test – optimal (SKAT-O)[26] and burden test,[27] taking into account the same covariates as for the analysis of common variants. Among the 38,642 genes fulfilling all quality control filters, 7,143 genes presenting variants resulting in a stop missense/splicing/gain/loss (13,016 variants) were selected for analyses. The significance *P* value threshold was set to $P < 7 \times 10^{-6}$.

Meta-analysis. A fixed-effects meta-analysis on results from the PREDICTION-ADR and Vanderbilt cohorts was performed using GWAMA.[28] Results are presented in a forest plot using the metafor package in R.[29,30]

Development of the F5 gene score. All nonsynonymous *F5* variants (common and rare) were combined into a gene score computed in SAS 9.3 (SAS Institute, Cary, NC) based on the PREDICTION-ADR data. Variants in the *F5* gene were combined using the weighted GRS calculation method described by Zhou *et al.*[31] Allele frequency differences were assessed for cases and controls. Linear regression was used to test the association between individuals carrying at least one deleterious variant against none and the angioedema phenotype. Center, sequencing batch, and 1–10 principal components, as well as covariates associated with intolerance such as sex and age were added to the model.

RESULTS

Baseline characteristics of ACEI-induced angioedema cases

Baseline characteristics of cases and controls in the PREDICTION-ADR cohort are shown in **Table 1**. Cases and controls were matched for potential risk factors for development of angioedema (time under medication, sex, dose, and use of interacting comedication). The sex distribution was 47% female for ACEI-AE, and 50% for ARB-AE. A majority had presented with a first occurrence of angioedema (72%). The mean time under medication was 3.44 years for ACEI, with doses ranging from 6.11 mg/day to 20.41 mg/day, and the mean time under medication with an ARB was 2.73 years, with doses ranging from 8 mg/day to 125 mg/day. The most commonly suspected ACEI was enalapril (65.8% of all cases), and the most commonly suspected ARB was losartan (6.6% of all cases), **Table 2**. A total of 2% of the ACEI-AE cases and 7% of the ARB-AE cases stated that a family member had experienced angioedema from these medications.

Approximately 23% of the study population had type 2 diabetes, mostly due to the recruitment from the Genetics of Diabetes and Audit Research Tayside Study (GoDARTS) in Scotland.

After removing samples and markers failing quality control, 1,033 individuals (387 cases, 646 controls) remained with 178,995 variants. The average per individual genotyping rate was 98.9%. We then defined two sets of data based on the minor allele frequency (MAF). SNPs with MAF < 1% were used for rare variant analysis (128,157 SNPs), whereas common SNPs with a MAF > 1% were used for single variant analysis (50,838 SNPs).

Table 2 Reported causative drugs in the PREDICTION-ADR angioedema cases

Drugs	Names	Angioedema cases (%)
ACEI	Enalapril	66.8
ACEI	Ramipril	12.1
ARB	Losartan	6.6
ACEI/ARB	Others	14.9

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 1 Characteristics of all angioedema cases and ACEI-treated controls included in the PREDICTION-ADR project

Variables	ACEI-AE (*n* = 343)	ARB-AE (*n* = 44)	ACEI-treated controls (*n* = 646)
Time under medication (years) (mean (SD))	3.44 (3.96)	2.73 (4.01)	NA
Dose (mg/day) (mean (SD))	13.3 (7.15)	63.01 (69.7)	NA
Age at event (years) (mean (SD))	64.9 (9.77)	64.2 (9.34)	NA
BMI (kg/m^2)[a] (mean (SD))	27.4 (4.43)	27.5 (4.95)	30.4 (7.5)
Alcohol consumption (units/week) (mean (SD))	5.56 (19.9)	4.55 (4.89)	NA
Sex: female (%)	47.4	50	38.7
First time of angioedema (%)	71.9	NA	NA
Family angioedema (%)	1.87	6.81	NA
Comedication (%)	95.3	97.7	NA
Diabetes (%)	23.9	22.7	31.7
Smoking (%)	76.06	4.54	NA
Radiation therapy (%)	0.39	0	NA
UV treatment (%)	0.77	0	NA

ACEI, angiotensin-converting enzyme inhibitor; AE, adverse event; ARB, angiotensin receptor blocker; NA, not available; UV, ultraviolet.
[a]Body Mass Index (BMI) only available for about 2/3 of the controls.

Table 3 Distribution of *F5* p.Arg534Gln genotypes by adjudicated ACEI-AE and ARB-AE status

Group	Arg534 only (*n*)	534Gln carriers (*n*)	Total (*n*)	Association
ACEI-AE/ARB-AE	326	61	387	Odds ratio = 2.85
ACEI/ARB controls	609	37[a]	646	95% CI, 1.89–4.25
Total	935	98	1,033	*P* value 1.53×10^{-7}

ACEI, angiotensin-converting enzyme inhibitor; AE, adverse event; ARB, angiotensin receptor blocker.
[a]One homozygous for 534Gln.

Association between ACEI-induced angioedema and factor V Leiden

With a *P* value of 1.53×10^{-7}, *F5* rs6025 (factor V Leiden) passed exome-wide significance in the single variant analysis ($P < 1 \times 10^{-6}$) (**Figure S3** and **Figure 1**). Carriers of the p.534Gln variant had 2.85 (95% confidence interval (CI), 1.89–4.25) times the odds of having angioedema compared with individuals homozygous for p.Arg534 (**Table 3**), in a model adjusted for sex, age, sequencing batch, study center, and 10 principal components. The top 20 hits are available in **Table S4**.

Meta-analysis of results

A large risk effect for the p.534Gln variant was also observed in the Vanderbilt study (odds ratio (OR): 1.68; 95% CI, 0.639–4.40) in a model adjusted for age and sex. While this replication cohort was not individually significant, a meta-analysis of the observed effects across PREDICTION-ADR and the Vanderbilt study revealed that overall, carriers of p.534Gln have 2.63 (95% CI, 1.81–3.83) times the odds of experiencing ACEI-AE compared with p.Arg534 homozygotes (*P* value 3.7×10^{-7}) (**Figure 2**).

Association between ACEI-induced angioedema and *F5* rare variants

The top significant genes found with the burden test analysis are reported in **Table S5**. QQplots for both burden test and SKAT-O are presented in respectively **Figures S4 and S5**.

Five nonsynonymous rare variants were found in the *F5* locus: rs200157005, rs149389480, rs143509841, rs140530655, and chr1:169529903 (**Table 4**). These variants, two deleterious and three protective, were analyzed with a SKAT-O model and found to be nominally significant, *P* value = 0.0021.

F5 GRS

A total of 11 common and rare nonsynonymous variants were found in the *F5* locus (**Table 3**). The variants rs6025, rs200157005, rs149389480, rs143509841, rs140530655, and chr1:169529903 were associated with ACEI-AE and ARB-AE in our study. Using the *F5* genotype risk score we created from those SNPs, we show that angioedema is strongly associated with any *F5* GRS levels against none, with a *P* value of 6.30×10^{-9}.

Moreover, when considering rs6020, individuals carrying at least one *F5* deleterious variant had 2.21 (95% CI, 1.49–3.27) times the odds of having ACEI-AE or ARB-AE compared with noncarriers in a model adjusted for age, sex, center, batch, and 1–10 principal components (**Table 5**).

DISCUSSION

This is the largest whole-exome sequencing study of ACEI-AE and ARB-AE. We found that common and rare *F5* gene variants were associated with ACEI-AE and ARB-AE. Of these variants, factor V Leiden (rs6025) exhibited the strongest association. Association with angioedema was even higher when rs6025 was combined with rare *F5* variants showing that both common and rare variants influence risk of angioedema. Replication of the GRS association was not possible in the Vanderbilt study due to the absence of rare variants in the genotyping file (no genotype when MAF < 1% or quality of imputation < 0.8). Moreover, considering that ACEI-AE and ARB-AE might be two different diseases, we studied the association without the 44 ARB-AE cases. We found the same association

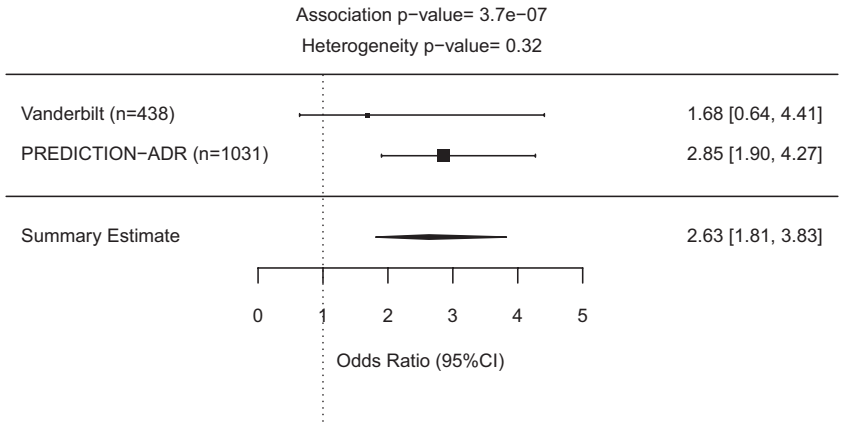

Figure 2 Forest plot representing the meta-analysis association between *F5* p.Arg534Gln and outcomes observed in the Vanderbilt and PREDICTION-ADR studies.

Table 4 Association of nonsynonymous *F5* variants (common and rare) with ACEI-AE and ARB-AE

Chromosome	Position	rsID	REF	ALT	MAF	*P*	OR	Location	AA Changes
1	169519049	rs6025[a]	C	T	0.048	5.96E-07	2.849	exon10	Q534R
1	169511878	rs6018	T	G	0.054	0.228	1.279	exon13	N817T
1	169511755	rs4524	T	C	0.249	0.3177	0.895	exon13	K858R
1	169519112	rs6020[b]	C	T	0.009	0.3278	1.691	exon10	R513K
1	169500232	rs200157005[c]	G	A	0.0005	0.3727	5.054	exon15	P1667L
1	169511576	rs149389480[c]	G	C	0.0005	0.3727	5.054	exon13	P918A
1	169524542	rs143509841[c]	T	G	0.0005	0.3727	5.054	exon7	K332N
1	169511585	rs9332695	T	A	0.015	0.4527	1.691	exon13	T915S
1	169541513	rs6019	C	G	0.051	0.6047	0.718	exon3	D107H
1	169513569	rs140530655[c]	C	T	0.0005	1	0.56	exon12	R647H
1	169529903	chr1:169529903[c]	C	T	0.0005	1	0.56	exon4	G159R

MAF is the minor allele frequency for the combination of cases and controls.
P and OR are from single variant analysis.
AA, amino acid; ACEI, angiotensin-converting enzyme inhibitor; AE, adverse event; ALT, alternative allele; ARB, angiotensin receptor blocker; OR, odds ratio; REF, reference allele; rsID, reference SNP cluster ID; SNP, single nucleotide polymorphism.
[a]Factor V Leiden main causal variant in white European populations for deep vein thrombosis. [b]rs6020 has a 30-fold higher frequency in African and Asian populations (AF = 0.3). These populations are known to be at greater risk of angioedema. [c]Variants are used in the SKAT-O (sequence kernel association test – optimal) analysis ($P = 0.00209$).

with *F5* Leiden (**Table S6**), meaning that the presence of ARB-AE individuals is not diluting the effect.

Studies have shown evidence for a role of the coagulation system in angioedema. However, no study has previously suggested a role for *F5* variants, possibly due to a lack of statistical power.

The mechanism behind ACEI-AE and ARB-AE is not fully understood, but is presumed to involve an accumulation of the vasoactive mediators bradykinin and substance P. Activation of the kallikrein–kinin system contributes to the pathogenesis of hereditary angioedema with complement C1 esterase inhibitor deficiency.[32] ACE (also called kininase II) catalyzes the formation of angiotensin II from angiotensin I, and is involved in the breakdown of the vasodilator bradykinin. ACEIs potentiate the effect of bradykinin through inhibition of its breakdown. Bradykinin acts on bradykinin receptors 1 and 2 that are important mediators of cardiovascular homeostasis. Stimulation of the bradykinin 2 receptor induces vasodilation and vascular permeability, and also stimulates the release of substance P from sensory nerve endings. Substance P increases vascular permeability and contributes to ACEI-AE in rodent models.[33,34] The coagulation system and genes involved have already been associated with accumulation of bradykinin and angioedema. In a subset of hereditary angioedema patients with normal complement C1 esterase levels and function, *F12* mutations are reported to cause uncontrolled activity of the plasma contact system and massively increased production of bradykinin.[35] It is possible that mutations in other plasma factors involved in coagulation and fibrinolysis may have similar effects. *F5* mutations are known to lead to an accumulation of fibrin. The most well-known example, the *F5* Leiden mutation G1691A in exon 10, results in the conversion of arginine to glycine (p.Arg534Gln). This amino acid exchange is in the site where factor V normally is cleaved by activated protein C. The conversion results in a clotting factor that remains active, since it cannot easily be degraded by activated protein C. This facilitates the overproduction of thrombin that leads to an excess of fibrin, which in turn limits the intercellular flow.[36]

Table 5 Association of combined nonsynonymous *F5* variants (common and rare) with ACEI-AE and ARB-AE

F5 gene score	Odds ratio (95% CI)	*P* value
rs6025, rs200157005, rs149389480, rs143509841, rs140530655 and chr1:169529903	2.64 (1.74–4.01)	8.80×10^{-8}
rs6025, rs6020, rs200157005, rs149389480, rs143509841, rs140530655 and chr1:169529903	2.21 (1.49–3.27)	6.30×10^{-9}

Individuals carrying at least one deleterious variant against none were tested in a model adjusted for age, sex, center, batch, and 1–10 PC.
ACEI, angiotensin-converting enzyme inhibitor; AE, adverse event; ARB, angiotensin receptor blocker; PC, principal components.

Moreover, our GRS results showed an effect of rs6020 on risk of ACEI-AE and ARB-AE. Like *F5* Leiden, this variant is associated with thrombosis.[37,38] The *F5* variant rs6020 is more common in black populations, in whom ACEI-AE is approximately three times more prevalent compared with other populations.[16] This could be related to a difference in bradykinin sensitivity since black Americans have been shown to be more sensitive to increases in vascular permeability caused by bradykinin.

We aimed to replicate our results in the independent Vanderbilt study to confirm positive association signals and exclude possible false-positive results. This was, however, not possible for all associated variants, since some variants were missing (**Figure S6**). In addition, we would have liked to do a separate analysis for ARBs to see if the association signal changes. However, due to the lower incidence of ARB-AE this would have been challenging. Moreover,

we had limited power to detect rare variants with small or moderate effects, or to detect even rarer variants with large effects.

In conclusion, we have identified common and rare genetic variants in the *F5* gene that are associated with an increased risk of developing ACEI-AE or ARB-AE. These findings may have important clinical implications for the prevention of ACEI-AE and safer treatment of hypertension.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

C.M. wrote the manuscript. C.N.A.P., M.W., A.H.M.-v.d.Z., A.A., and P.H. designed the research. C.M., M.K.S., F.C., M.W., A.J.C., E.V.B., E.R.R., A.B., M.K., P.K.E.M., Q.-Y.Y., A.-C.S., and C.v.B. performed the research. C.M., A.V., E.V.B., E.R.R., N.E., K.M.B., and N.J.B. analyzed the data.

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