

# Meta-analysis of ACE inhibitor-induced angioedema identifies novel risk locus



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**Background:** Angioedema is a rare but potentially life-threatening adverse drug reaction in patients receiving angiotensin-converting enzyme inhibitors (ACEis). Research suggests that susceptibility to ACEi-induced angioedema (ACEi-AE) involves both genetic and nongenetic risk factors. Genome- and exome-wide studies of ACEi-AE have identified the first genetic risk loci. However, understanding of the underlying pathophysiology remains limited.

**Objective:** We sought to identify further genetic factors of ACEi-AE to eventually gain a deeper understanding of its pathophysiology.

**Methods:** By combining data from 8 cohorts, a genome-wide association study meta-analysis was performed in more than 1000 European patients with ACEi-AE. Secondary bioinformatic analyses were conducted to fine-map associated loci, identify relevant genes and pathways, and assess the genetic

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overlap between ACEi-AE and other traits. Finally, an exploratory cross-ancestry analysis was performed to assess shared genetic factors in European and African-American patients with ACEi-AE.

**Results:** Three genome-wide significant risk loci were identified. One of these, located on chromosome 20q11.22, has not been implicated previously in ACEi-AE. Integrative secondary analyses highlighted previously reported genes (*BDKRB2* [bradykinin receptor B2] and *F5* [coagulation factor 5]) as well as biologically plausible novel candidate genes (*PROCR* [protein C receptor] and *EDEM2* [endoplasmic reticulum degradation enhancing alpha-mannosidase like protein 2]). Lead variants at the risk loci were found with similar effect sizes and directions in an African-American cohort.

**Conclusions:** The present results contributed to a deeper understanding of the pathophysiology of ACEi-AE by (1) providing further evidence for the involvement of bradykinin signaling and coagulation pathways and (2) suggesting, for the first time, the involvement of the fibrinolysis pathway in this adverse drug reaction. An exploratory cross-ancestry comparison implicated the relevance of the associated risk loci across diverse ancestries. (*J Allergy Clin Immunol* 2024;153:1073-82.)

**Key words:** Genome-wide association study, meta-analysis, angiotensin-converting enzyme inhibitor, angioedema, angiotensin-converting enzyme inhibitor-induced angioedema

Angioedema is a recognized adverse drug reaction of medications that act on the renin-angiotensin system, in particular the angiotensin-converting enzyme inhibitors (ACEis). Although ACEi-induced angioedema (ACEi-AE) is rare,<sup>1,2</sup> a relatively large number of cases occur because of the widespread use of this drug class in antihypertensive therapy. In fact, research suggests that the 12-month prevalence of ACEi-AE is about 0.004% to 0.026%, depending on the population studied,<sup>3</sup> and that approximately one-third of all angioedema cases admitted to an emergency department are caused by an ACEi.<sup>4</sup> The clinical presentation of ACEi-AE is usually mild; however, fatalities secondary to angioedema of the upper airways and subsequent airway obstruction have been reported.<sup>4</sup>

Etiologically, an increase in the level of bradykinin—a consequence of ACEi therapy—is implicated as a key factor in the development of ACEi-AE.<sup>5,6</sup> However, the precise pathophysiological mechanisms remain otherwise unclear, and individual ACEi-AE susceptibility is assumed to be dependent on genetic predisposition and contributing or interacting environmental factors.<sup>7</sup>

Reported risk factors for ACEi-AE include female sex,<sup>1,8,9</sup> advanced age,<sup>10-12</sup> smoking,<sup>8,13</sup> a history of drug rash or seasonal allergies,<sup>10,12</sup> and coronary artery disease.<sup>1,2</sup> In contrast, ACEi-AE is reported to occur less frequently in individuals with diabetes.<sup>1,12,14</sup>

At the genetic level, recent genome-wide association studies (GWASs) have identified 2 loci with a genome-wide significant association with ACEi-AE: the bradykinin receptor B2 (*BDKRB2*) locus on chromosome 14<sup>15</sup> and the *KCNMA1* (potassium calcium-activated channel subfamily M alpha 1) locus on chromosome 10.<sup>16</sup> Moreover, a previous exome-sequencing study reported an association of the coagulation factor 5 (*F5*) gene.<sup>17</sup> However, with the exception of the *BDKRB2* locus, these associations have not yet been replicated in independent studies.

#### Abbreviations used

ACEi:	Angiotensin-converting enzyme inhibitor
ACEi-AE:	Angiotensin-converting enzyme inhibitor-induced angioedema
<i>BDKRB2</i> :	Bradykinin receptor B2
CADD:	Combined annotation-dependent depletion
CHB-CVDC/DBDS:	Copenhagen Hospital Biobank—Cardiovascular Disease Cohort/Danish Blood Donor Study
<i>EDEM2</i> :	Endoplasmic reticulum degradation enhancing alpha-mannosidase like protein 2
<i>EPCR</i> :	Endothelial protein C receptor
eQTL:	Expression quantitative trait locus
EstBB:	Estonian Biobank
<i>F5</i> :	Coagulation factor 5
GWAS:	Genome-wide association study
LD:	Linkage disequilibrium
OR:	Odds ratio
PIP:	Posterior inclusion probability
<i>PROCR</i> :	Protein C receptor
PRS:	Polygenic risk score
QC:	Quality control
SNP:	Single-nucleotide polymorphism
UKB:	UK Biobank

In 2018, our group initiated the ongoing vARIANCE study with the aim of elucidating genetic and nongenetic risk factors for ACEi-AE susceptibility (<https://variance-studie.info/>).<sup>18</sup> In the present study, genome-wide genotyping was performed in German/Austrian patients with ACEi-AE from the vARIANCE study and in 2 independent ACEi-AE cohorts from Denmark and Sweden, respectively. GWASs were performed for each patient cohort using ethnically matched control data. The obtained data were then combined in a meta-analysis with GWAS data from 5 further case-control studies respectively from the United States, the United Kingdom, Estonia, Sweden, and Denmark, 3 of which have been published previously.<sup>15,16,19</sup> In total, the GWAS meta-analysis included more than 1000 European patients with ACEi-AE. To generate further insights into the associated loci, the meta-analysis was complemented by more in-depth analyses, such as fine-mapping, the integration of expression quantitative trait loci (eQTL) and chromatin interaction data, and gene- and pathway-based analyses. Moreover, linkage disequilibrium (LD) score regression analyses<sup>20</sup> were performed to estimate the heritability of ACEi-AE on the basis of common variants and to investigate the genetic overlap between ACEi-AE and its associated diseases and previously reported risk/protective factors. Finally, to gain initial insights into the extent to which the genetics of ACEi-AE are shared across different ancestries, an exploratory cross-ancestry analysis was conducted using GWAS data from an African-American cohort.

## METHODS

### Sample description

A brief description of the patients and controls included in the present study is provided herein. More detailed information, including the respective phenotype definitions, can be found in the Online Repository at [www.jacionline.org](http://www.jacionline.org). A summary of all meta-analysis cohorts is provided in Table I.

**TABLE I.** GWAS meta-analysis cohorts

Cohort	Origin	Ancestry	No. of cases	No. of controls	Available data level	meta <sub>EUR</sub>	meta <sub>ALL</sub>
vARIANCE	Germany/Austria	European	95	4,135	Genotype data	x	x
Denmark	Denmark	European	45	1,489	Genotype data	x	x
Sweden	Sweden	European	42	975	Genotype data	x	x
VanMar <sub>EUR</sub> *	United States	European	106	321	Genotype data	x	x
UKB*	United Kingdom	European	86	356	Imputed genotype data	x	x
EstBB*	Estonia	European	82	15,787	Summary statistics	x	x
Swedegene*	Sweden	European	142	1,345	Summary statistics	x	x
CHB-CVDC/DBDS*	Denmark	European	462	53,391	Summary statistics	x	x
VanMar <sub>AFR</sub> *	United States	African American	63	149	Genotype data		x

Overall, 78,859 individuals ( $N_{\text{case}}/N_{\text{control}} = 1,060/77,799$ ) were included in the meta<sub>EUR</sub> analysis. The meta<sub>ALL</sub> analysis comprised a total of 79,071 individuals ( $N_{\text{case}}/N_{\text{control}} = 1,123/77,948$ ). The number of cases and controls refers to those available after QC.

\*GWAS data sets with treatment-matched controls.

The patients from the vARIANCE cohort were selected from the ongoing vARIANCE study, a clinically recruited case collection of German/Austrian patients with ACEi-AE and angiotensin-receptor blocker-induced angioedema.<sup>18</sup> Ethnically matched controls were drawn from the German Heinz Nixdorf Recall study.<sup>21</sup> For the Danish cohort, patients with ACEi-AE were recruited from clinical centers, and healthy Danish blood donors were used as controls. For the Swedish cohort, patients with ACEi-AE were selected from the Swedegene database ([www.swedegene.se](http://www.swedegene.se)), and ethnically matched controls were drawn from the Anorexia Nervosa Genetics Initiative Sweden (SE, community) cohort.<sup>22</sup> The 2 VanMar cohorts comprised patients with ACEi-AE and treatment-matched controls from 2 different ancestries who had been recruited from clinical centers within the context of the Pharmacogenomics Research Network (PGRN)-RIKEN study.<sup>19</sup> For the purposes of the present analyses, the study participants were stratified into 2 case-control cohorts of European (VanMar<sub>EUR</sub>) and African-American (VanMar<sub>AFR</sub>) ancestry, respectively. Patients and treatment-matched controls from the UK Biobank (UKB) cohort were drawn from the whole UKB data set using information on *International Classification of Diseases, Tenth Revision* diagnoses and medication intake (see Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The Estonian Biobank (EstBB) cohort included patients with ACEi-AE and treatment-matched controls drawn from the whole EstBB cohort on the basis of *International Classification of Diseases, Tenth Revision* diagnoses and prescription data. The Swedegene cohort included patients with ACEi-AE and treatment-matched controls of Swedish origin and represents an ACEi-AE stratified subcohort of a previously published GWAS.<sup>16</sup> The Copenhagen Hospital Biobank—Cardiovascular Disease Cohort/Danish Blood Donor Study (CHB-CVDC/DBDS) cohort comprised patients with ACEi-AE and treatment-matched controls of Danish origin who were drawn from the CHB-CVDC/DBDS<sup>23</sup> and who were the discovery cohort of a previously reported GWAS.<sup>15</sup>

All studies were approved by the respective institutional ethics committee. Individuals of the CHB-CVDC/DBDS cohort have scientific ethical approval and were informed that their samples would be used for research purposes, while being given the option to opt out. For the remaining cohorts, participants provided written informed consent before inclusion.

### Genome-wide genotyping, quality control, imputation, and association analysis

Individual-level genotype data were available for 6 of the 9 GWAS cohorts (Table I). The analysis of these data is described in detail in the Online Repository. The GWASs of the remaining 3 cohorts (Swedegene, CHB-CVDC/DBDS, and EstBB) were performed externally, and summary statistics were provided for the purposes of the present meta-analysis. Detailed information on these GWASs is provided in the Online Repository (EstBB cohort) or in the original publications (Swedegene<sup>15,16</sup> and CHB-CVDC/DBDS<sup>15,16</sup> cohorts).

### Meta-analysis (meta<sub>EUR</sub>)

The 8 European GWAS cohorts were meta-analyzed using METAL (V.2011-03-25<sup>24</sup>) under a fixed-effects model by weighting the effect sizes and the inverse of the standard error under genomic control correction. Only single-nucleotide polymorphisms (SNPs) with a minor allele frequency of more than 0.01 and an imputation info score higher than 0.3 were included. Genome-wide significance was set at a  $P$  value of  $5 \times 10^{-8}$ , whereas loci reaching a  $P$  value of  $1 \times 10^{-5}$  were considered suggestive.<sup>25</sup> For all subsequent analyses, only variants analyzed in patients from the CHB-CVDC/DBDS cohort and at least 4 other cohorts were retained. Thereafter, each variant that remained postfiltering was present in at least 67.6% of all patients and 92.5% of all controls.

### Polygenic risk score analysis

Polygenic risk scores (PRSs) were calculated for all individuals for whom genotype data were available. To avoid overfitting, this was performed in a leave-one-out setting, meaning that the PRS for each European cohort was based on effect sizes derived from a meta-analysis that did not include the tested cohort.

PRSs were calculated at 10  $P$ -value thresholds ( $5 \times 10^{-8}$ ,  $1 \times 10^{-6}$ ,  $1 \times 10^{-4}$ , .001, .01, .05, .1, .2, .5, and 1.0) using PRSice-2 (2.3.3 [2020-08-05]<sup>26</sup>), and only those variants that were filtered according to standard quality control (QC) parameters were included.<sup>27</sup> The association with ACEi-AE case-control status was assessed in a logistic regression, which included the same covariates as those used in the association analysis (sex

and principal components 1 to 4). Finally, the proportion of variance explained (Nagelkerke  $R^2$ ) was calculated for each  $P$ -value threshold by comparing the PRS from a full model (covariates and PRS) and a reduced model (covariates only).

### Genomic risk loci, functional annotation, and gene mapping

FUMA (v1.4.1<sup>28</sup>) was used to define independent genomic risk loci, functionally annotate the SNPs, and prioritize the most likely causal genes within these loci.

First, genomic risk loci were defined according to the default FUMA settings using precalculated LD structures from the European 1000 Genomes reference population. To identify suggestive loci, the  $P$ -value thresholds were modified to (1)  $P$  less than  $1 \times 10^{-5}$  for independent significant SNPs and (2)  $P$  less than .05 for candidate SNPs.

Then, all SNPs within the predefined genome-wide and suggestive loci were functionally annotated using ANNOVAR,<sup>29</sup> combined annotation-dependent depletion (CADD) scores,<sup>30</sup> RegulomeDB scores,<sup>31</sup> and chromatin state annotations.<sup>32</sup>

At the genome-wide significant loci, genes were prioritized by mapping the identified lead and candidate SNPs on the basis of (1) their position and suggestive deleteriousness (CADD > 12.37<sup>33,34</sup>); (2) their eQTL effects derived from eQTL data of GTEx v8 tissues<sup>35</sup>; and (3) their 3-dimensional chromatin interaction effects derived from 21 different tissue/cell types.<sup>36</sup> Otherwise, the default settings of FUMA were adopted.

### Gene-based tests, gene-set enrichment, and tissue expression analyses

Gene-based tests, gene-set enrichment, and tissue expression analyses were performed using MAGMA (v1.08<sup>37</sup>), as implemented in FUMA.<sup>28</sup> To ensure the inclusion of regulatory regions in the assignment of SNPs to genes, a window size of 35 kb upstream and 10 kb downstream of a gene was set.<sup>38</sup> After considering the number of tested protein coding genes ( $n = 18,983$ ), the genome-wide significance threshold for the gene-based tests was set at  $P_{\text{bon}} = 2.63 \times 10^{-6}$  (Bonferroni correction). For the gene-set analyses, a total of 15,496 gene sets from MSigDB (v7.0<sup>39,40</sup>) were tested. Bonferroni correction was applied for all tested gene sets, resulting in  $P_{\text{bon}} = 3.23 \times 10^{-6}$ . The tissue expression analysis was performed for 53 GTEx v8 tissues.<sup>35</sup>

### Fine-mapping

Statistical fine-mapping was performed using SuSiE,<sup>41,42</sup> as implemented in PolyFun.<sup>43</sup> For the fine-mapping model, all SNPs within 1 megabase surrounding the lead SNP were considered and the maximum number of causal SNPs was set to 5. The following were used as an LD reference panel: (1) pre-computed LD information from the UKB ( $N = 337,000$  unrelated British-ancestry individuals, as provided by PolyFun) and (2) LD information from the Danish GWAS cohort ( $N = 1,542$ , derived from Plink files using LDstore 2.0), because this ancestry is representative for most of the individuals in the present meta-analysis.

### LD score regression analyses

LD score regression (LDSC, version 1.0.1<sup>20</sup>) was used to assess the SNP-based heritability of ACEi-AE. Here, liability-scale heritability estimates were obtained, taking into account the lower (0.004%) and upper (0.026%) limits of the population prevalence estimates for ACEi-AE.<sup>3</sup>

Furthermore, LDSC was used to assess the genetic correlation between ACEi-AE and selected associated traits. To address the potential influence of hypertension on the obtained association signals and the observed genetic correlations, the analysis was rerun for all traits that showed at least a nominally significant association using a stratified meta<sub>EUR</sub> data set and including only cohorts with treatment-matched controls ( $N_{\text{case}}/N_{\text{control}} = 878/71,200$ ; Table I). In total, genetic correlations with 9 traits were tested, as provided in Table E1 (in the Online Repository available at [www.jacionline.org](http://www.jacionline.org)). For all analyses performed with LDSC, the ACEi-AE meta-analyses were (re-)run without correction for genomic control using METAL, and only high-confidence variants (imputation info score  $\geq 0.8$ ) were considered.

### Exploratory cross-ancestry analysis

For the African-American individuals, a PRS was calculated as described previously, using effect size estimates derived from the meta<sub>EUR</sub> analysis. The African-American cohort (VanMar<sub>AFR</sub>) was then meta-analyzed with the meta<sub>EUR</sub> data under a fixed-effects model using METAL. This resulted in a total sample of 1,123 patients with ACEi-AE and 77,948 controls (meta<sub>ALL</sub>) and approximately 7 million markers without evidence of inflation of association  $P$  values ( $\lambda_{\text{GC}} = 0.988$ ; see Fig E2, B, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Finally, the effect estimates and effect allele frequencies of the 3 genome-wide significant SNPs identified in the meta<sub>EUR</sub> analysis were compared between the European cohorts and the African-American cohort using the Pearson correlation coefficient.

## RESULTS

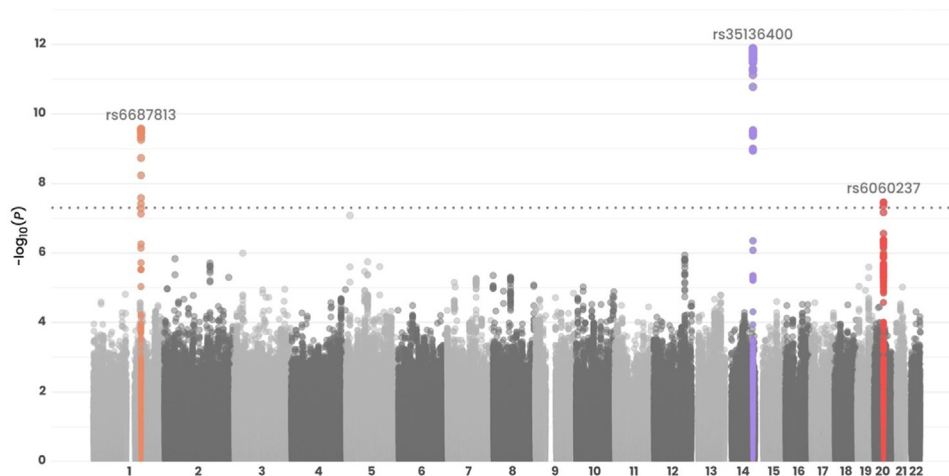
### ACEi-AE GWAS meta-analysis: Single-marker results

The GWAS meta-analysis comprised the data of 1,060 patients with ACEi-AE and 77,799 controls of European ancestry (meta<sub>EUR</sub>) and analyzed approximately 6.9 million post-QC markers that showed no inflation of association  $P$  values ( $\lambda_{\text{GC}} = 0.985$ ; Fig E2, A).

Overall, 3 independent genome-wide significant loci were identified (Fig 1; Table II), including a novel risk locus on chromosome 20q11.22. The other 2 loci have been described previously; however, they were characterized by a different lead SNP in the present study. The 1q24.2 locus was first reported by Maroteau et al<sup>17</sup> albeit at the level of exome-wide significance only ( $P < 1 \times 10^6$ ). The 14q32.2 locus was identified as genome-wide significant ( $P < 5 \times 10^{-8}$ ) in the GWAS by Ghouse et al,<sup>15</sup> which was part of the present meta-analysis.

The lowest  $P$  value was identified at chromosome 14q32.2. The lead SNP (rs35136400;  $P = 1.28 \times 10^{-12}$ ; odds ratio [OR] = 1.50) was located around 50 kb upstream of the *BDKRB2* gene (see Fig E3, A, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)) and was found in near-perfect LD with rs34485356 ( $r^2 = 0.971$ ), the lead SNP in the GWAS in which this locus was first reported.<sup>15</sup>





**FIG 1.** Manhattan plot of the meta<sub>EUR</sub> analysis. The  $-\log_{10}$  association  $P$  values (vertical axis) for all variants of the meta<sub>EUR</sub> analysis against their genomic position (horizontal axis) are displayed. The dotted gray line indicates the threshold for genome-wide significance ( $P = 5 \times 10^{-8}$ ). Loci reaching genome-wide significance are highlighted (annotated lead SNP  $\pm 500$  kb) in red (novel locus), orange (previous exome-wide significant locus), or purple (previous genome-wide significant locus).

**TABLE II.** Genome-wide significant risk loci

Lead SNP	Chr	Pos	A1/A2	FreqA1	OR (effect allele)	95% CI	$P$	Het $P^2$	Het $P$
rs6687813	1	169477574	A/C	0.083	1.70	1.54-1.87	$2.67 \times 10^{-10}$	0	.723
rs35136400	14	96619480	A/G	0.774	1.50	1.39-1.61	$1.28 \times 10^{-12}$	56.5	.024
rs6060237	20	33694210	A/G	0.855	0.70	0.57-0.83	$3.47 \times 10^{-8}$	28.5	.201

A1/A2, Effect allele/other allele; Chr, chromosome; FreqA1, effect allele frequency in the combined case-control cohort; Het  $P^2$ , heterogeneity  $P^2$ ; Het  $P$ , heterogeneity  $P$  value; Pos, genomic position (hg19).

The lead SNP at the 1q24.2 locus was rs6687813 ( $P = 2.67 \times 10^{-10}$ ; OR = 1.70), which is an intergenic variant located approximately 6 kb downstream of the *F5* gene (Fig E3, B). The genome-wide significant SNPs in the present study included the coding variant rs6025 (“factor V Leiden”;  $P = 5.81 \times 10^{-9}$ ). This was reported as the top SNP at this locus in the previous exome-sequencing study<sup>17</sup> and represents a variant that is largely independent of rs6687813 ( $r^2 = 0.172$ ).

The 20q11.22 locus has not yet been reported in relation to ACEi-AE. The lead SNP at this locus was rs6060237 ( $P = 3.47 \times 10^{-8}$ ; OR = 0.70), which is an intergenic variant about 9 kb downstream of the endoplasmic reticulum degradation enhancing alpha-mannosidase like protein 2 (*EDEM2*) gene (Fig E3, C).

Only SNPs at the 14q32.2 locus showed significant cross-study heterogeneity (Het  $P = 0.024$  for rs35136400), which was probably attributable to the opposite effect direction observed in 1 study (Table II; see also Fig E4, B, in this article’s Online Repository at [www.jacionline.org](http://www.jacionline.org)). Besides the 3 genome-wide significant loci, 20 further loci reached a suggestive  $P$  value of  $1 \times 10^{-5}$  (see Table E2 in this article’s Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### Leave-one-out PRS analyses

Leave-one-out PRS analyses revealed a significant prediction of ACEi-AE case-control status in all investigated cohorts, that is, those with available genotype data (Table I). The maximum variance explained by the PRS ranged from 1.10% (vARIANCE) to 5.37% (VanMar<sub>EUR</sub>) (Fig 2, left panel), thereby suggesting

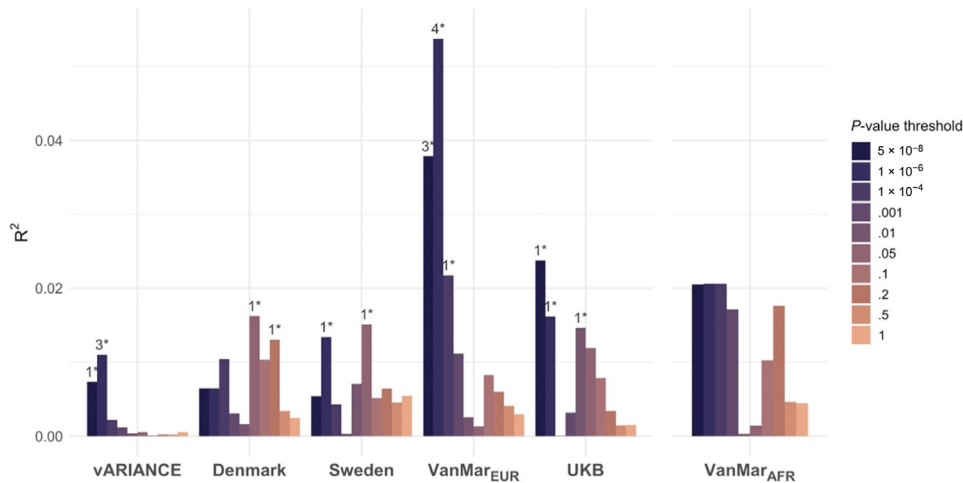
comparable phenotype definitions across the investigated clinical and nonclinical cohorts.

### Fine-mapping analysis

Fine-mapping of the 3 risk loci revealed one 95% credible set for each locus, comprising 15, 40, and 120 variants at the 1q24.2, 14q32.2, and 20q11.22 loci, respectively (see Fig E5, and Table E3 in this article’s Online Repository at [www.jacionline.org](http://www.jacionline.org)). Irrespective of whether the precomputed UKB or the Danish in-sample LD reference was used, the same variants were identified within the credible sets (except for 1 variant each at 20q11.22), with only marginal differences in their derived posterior inclusion probabilities (PIPs). Moreover, the PIPs were relatively low, ranging from 0.1% to a maximum of 12.5%.

### Functional annotation of candidate SNPs and gene prioritization

As is typical for GWAS variants, most of the candidate SNPs ( $P < 1 \times 10^{-5}$  and  $r^2 > 0.6$  relative to one of the lead SNPs) at the genome-wide significant loci were located in noncoding regions of the genome. The low RegulomeDB scores and/or low chromatin state annotations identified for several SNPs suggest potential regulatory effects on transcription factor binding and/or gene regulation at the respective loci. Only 5 of the candidate variants were located in coding regions. Of these, 3 had high CADD scores, indicating a potentially deleterious effect on protein function. These variants were rs6025 (located within



**FIG 2.** PRS results. The leave-one-out PRS results for the 5 European GWAS cohorts (*left panel*) in comparison with the PRS results of the African-American cohort (*right panel*) are shown. For each cohort, the PRS results across each of the 10 tested  $P$ -value thresholds are plotted. The statistical significance of the variance explained ( $R^2$ ) by the PRS is indicated above each bar: 1\* =  $P < .05$ ; 2\* =  $P < .01$ ; 3\* =  $P < .005$ ; 4\* =  $P < 1 \times 10^{-4}$ .

$F5$ ; CADD = 18.92), rs867186 (located within protein C receptor [*PROCR*]; CADD = 16.65), and rs80109502 (located within *MYH7B* [myosin heavy chain 7B]; CADD = 17.02). An overview of the functional annotations of all SNPs within the genome-wide significant loci is provided in [Table E4](#) and [Fig E6](#) (in the Online Repository available at [www.jacionline.org](http://www.jacionline.org)).

Gene prioritization of lead and candidate variants resulted in 84 mapped genes across all 3 risk loci (see [Table E5](#) in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Of these, 21 were supported by at least 2 of the 3 methods ([Fig 3, A](#)).

### Gene-based tests and gene-set and tissue enrichment analyses

The gene-based association analysis using MAGMA revealed 2 genes that were significantly associated with ACEi-AE after correction for multiple testing: *TMEM119* (transmembrane protein 119;  $P = 7.66 \times 10^{-8}$ ) and *EDEM2* ( $P = 2.39 \times 10^{-6}$ ) ([Fig 3, B](#)). The top 50 genes (see [Table E6](#) in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)) included 2 genes with a previously reported association with ACEi-AE: *BDKRB2* ( $P = 8.74 \times 10^{-5}$ ) and *F5* ( $P = 3.08 \times 10^{-4}$ ). Interestingly, the top 50 genes also included a gene with a known pathogenic variant for hereditary forms of angioedema, *KNKI* (kininogen 1;  $P = 1.65 \times 10^{-3}$ ).

The MAGMA gene-set analysis revealed 607 gene sets that showed a nominally significant enrichment (see [Table E7](#) in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). These included biologically plausible pathways, such as “go\_endothelial\_cell\_activation” ( $P = 2.89 \times 10^{-4}$ ).

No significant enrichment was found for any of the 53 GTEx tissue types (see [Fig E7](#) in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### SNP-based heritability

Taking into account the lower and upper estimated population prevalence, the estimated SNP-based heritability for

ACEi-AE (liability scale) ranged from 0.042 ( $\pm 0.026$ ) to 0.052 ( $\pm 0.032$ ).

### Genetic correlation analyses

By investigating the genetic correlation between ACEi-AE and 5 related diseases, as well as 4 reported clinical or lifestyle risk factors ([Table E1](#)), 3 traits—hypertension, asthma, and intake of renin-angiotensin agents—showed a nominally significant positive genetic correlation that did not withstand Bonferroni correction ( $P_{\text{bon}} < 0.05/12 = .0041$ ).

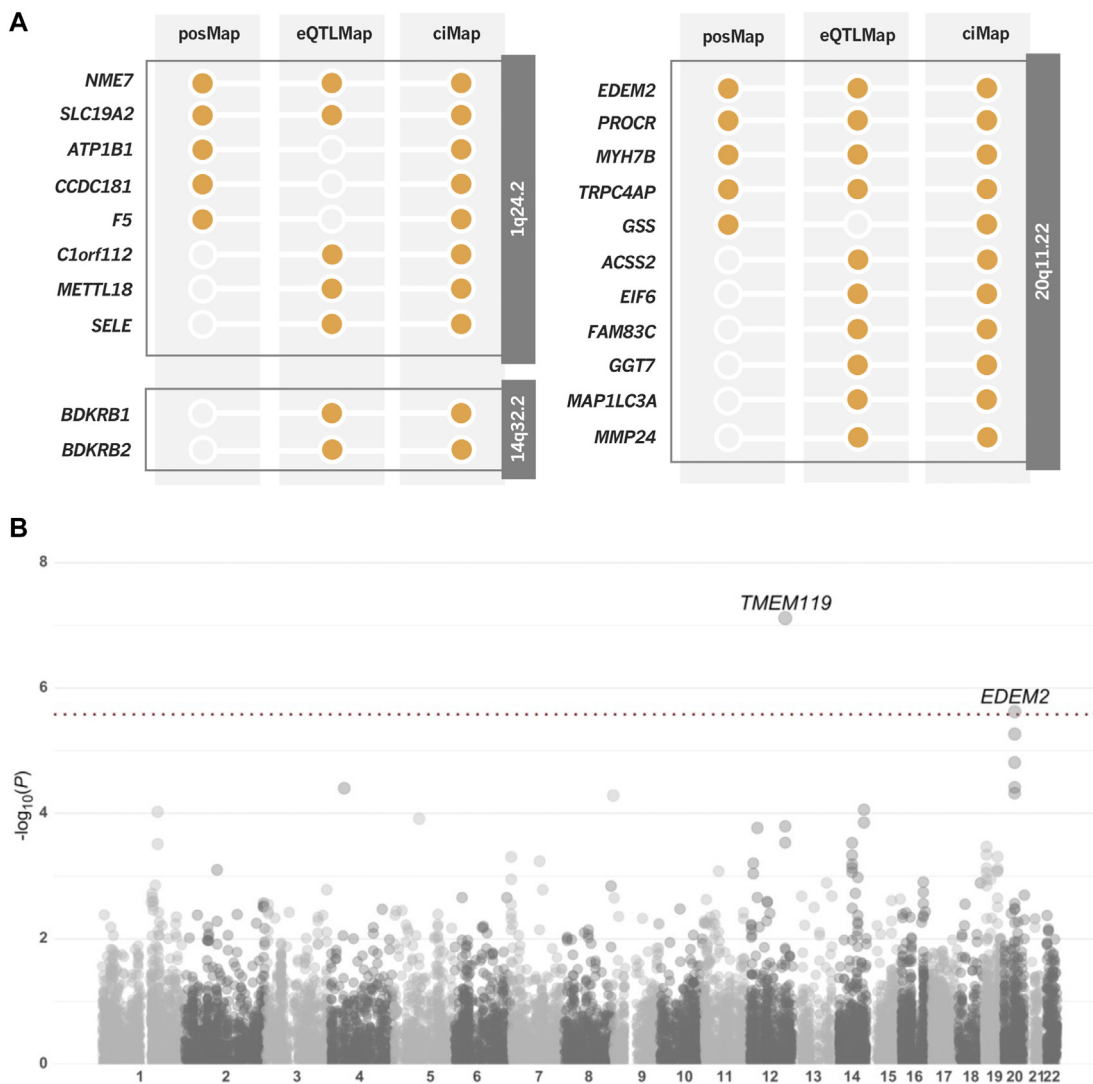
In the reanalysis, using only cohorts with treatment-matched controls, the previously observed nominally significant correlations were no longer significant ([Table III](#); see also [Fig E8](#) in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). For hypertension and intake of renin-angiotensin agents in particular, the genetic correlations were substantially lower, suggesting that these correlations might have been confounded by underlying hypertension-related genetic factors that resulted from the use of population-based controls in the  $\text{meta}_{\text{EUR}}$  analysis (about 8.5% of all controls; [Table I](#)).

### Exploratory cross-ancestry comparison and meta-analysis

The PRS analysis for the African-American cohort revealed a positive but nonsignificant signal, with a maximum explained variance that was comparable with those observed for the European cohorts ([Fig 2, right panel](#)).

In the cross-ancestry meta-analysis ( $\text{meta}_{\text{ALL}}$ ), the same 3 genome-wide significant loci that were detected in the  $\text{meta}_{\text{EUR}}$  analysis were identified (see [Table E8](#) and [Fig E9](#) in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). However, 2 of the loci had different lead SNPs: rs12888576 ( $P = 3.53 \times 10^{-13}$ ; OR = 1.50) at the 14q32.2 locus and rs141521143 ( $P = 2.32 \times 10^{-8}$ ; OR = 0.67) at chromosome 20q11.22.

Comparison of the  $\text{meta}_{\text{EUR}}$  lead SNPs in the  $\text{meta}_{\text{EUR}}$  and  $\text{VanMar}_{\text{AFR}}$  data revealed a strong positive correlation between the



**FIG 3.** Results of the gene prioritization and gene-based analyses. **A**, Locus-wise overview of all genes that were prioritized on the basis of at least 2 lines of evidence. All identified lead and candidate SNPs at the 3 risk loci were mapped to genes on the basis of (1) their position and deleteriousness (posMap), (2) their effects on gene expression (eQTLMap), and (3) their 3-dimensional chromatin interactions (ciMap). **B**, Manhattan-like plot of the  $-\log_{10}$  association  $P$  values of the gene-based test (vertical axis) and the genomic position of the respective gene (horizontal axis). The dotted red line indicates the Bonferroni-corrected threshold for genome-wide significance ( $P_{\text{bon}} = 2.63 \times 10^{-6}$ ).

effect estimates ( $R = 0.7$ ; see Fig E10, A, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)) and the effect allele frequencies ( $R = 0.99$ ; Fig E10, B).

## DISCUSSION

To our knowledge, the present study represents the largest GWAS meta-analysis of ACEi-AE to date, having been the first to include more than 1000 patients. Through the investigation of a more than 2-fold higher number of patients than the largest of the previous GWAS,<sup>15</sup> the present analyses identified 3 genome-wide significant loci. Two of these loci (1q24.2 and 14q32.2) have already been associated with ACEi-AE, whereas the present study is the first to identify 20q11.22 as an ACEi-AE risk locus.

ACEi-AE is a form of bradykinin-induced angioedema whose pathogenesis has been suggested to be influenced by dysregulated

endothelial cell permeability, which is regulated among others by the bradykinin 2 receptor.<sup>44,45</sup> The bradykinin 2 receptor locus on 14q32.2 was the first replicated genome-wide significant risk locus for ACEi-AE<sup>15</sup> and showed the strongest association in the present analysis. Consistent with the findings of the previous study,<sup>15</sup> the present results suggest that regulatory effects on *BDKRB2* or *BDKRB1* are the most likely underlying mechanisms for this locus.

Other forms of bradykinin-induced angioedema, such as hereditary angioedema, are caused by mutations in genes involved in the coagulation and fibrinolysis pathways, which ultimately impair bradykinin formation or signaling.<sup>46</sup> By demonstrating an association with variants in the *F5* gene, in particular the factor V Leiden mutation, a recent exome study implicated the coagulation system in ACEi-AE.<sup>17</sup> The present study replicated this locus (1q24.2) at the level of genome-wide

**TABLE III.** Genetic correlation between ACEi-AE, associated diseases, and reported risk/protective factors

Trait	meta <sub>EUR</sub>			Stratified meta <sub>EUR</sub>		
	<i>r<sub>g</sub></i>	SE	<i>P</i> <sub>LDSC</sub>	<i>r<sub>g</sub></i>	SE	<i>P</i> <sub>LDSC</sub>
Hypertension	0.268	0.122	<b>.028</b>	0.160	0.099	.107
Asthma	0.419	0.200	<b>.036</b>	0.409	0.214	.056
Blood clot leg (DVT)	0.028	0.257	.912	x	x	x
Blood clot lung	0.107	0.267	.689	x	x	x
Intake of renin-angiotensin agents	0.281	0.127	<b>.027</b>	0.145	0.105	.168
Coronary artery disease	0.147	0.195	.453	x	x	x
Hay fever/allergic rhinitis	0.080	0.162	.621	x	x	x
Smoking	0.121	0.088	.166	x	x	x
Type 2 diabetes	0.197	0.110	.073	x	x	x

The table presents the results of the genetic correlation analysis obtained from LDSC using the meta<sub>EUR</sub> data ( $N_{\text{case}}/N_{\text{control}} = 1,060/77,799$ ). For all traits with a nominally significant association, the analysis was rerun using a stratified meta<sub>EUR</sub> data set that comprised only cohorts with treatment-matched controls ( $N_{\text{case}}/N_{\text{control}} = 878/71,200$ ). The displayed *P* values are uncorrected and are shown in boldface if nominally significant.

DVT, Deep vein thrombosis; *P*<sub>LDSC</sub>, *P* value obtained from LDSC; *r<sub>g</sub>*, genetic correlation.

significance ( $P = 2.67 \times 10^{-10}$ ). Furthermore, the *F5* gene was ranked as a likely candidate gene at this locus, although our analyses also provided similar evidence for other genes at this locus (Fig 3, A). Although the present analyses identified the factor V Leiden mutation at the level of genome-wide significance (rs6025;  $P = 5.81 \times 10^{-9}$ ; OR = 1.97), it was not prioritized in statistical fine-mapping. However, fine-mapping in general did not reveal distinctively prioritized variants (PIP > 0.5) and was thus not very informative (see study limitations herein). Future functional studies are warranted to clarify the specific role of factor V Leiden in ACEi-AE and to determine whether other variants/genes underlie the association at this GWAS locus.

One of the most highly prioritized genes at the novel risk locus was *PROCR*, which encodes the endothelial protein C receptor (EPCR), and thus appears as a biologically plausible candidate gene. The EPCR enhances the activation of protein C, which plays among others a crucial role in both anticoagulation/fibrinolysis (inactivation of factor Va and VIIa<sup>47</sup>), and stabilization of the endothelial barrier via Tie2 signaling.<sup>48,49</sup> Notably, in addition to being an established risk gene for venous thromboembolism,<sup>50</sup> GWASs have demonstrated that variants in or near *PROCR* affect the plasma levels of protein C.<sup>51-53</sup> A plausible hypothesis therefore is that variation at 20q11.22 interferes with protein C activation, thereby compromising endothelial integrity and ultimately promoting the development of angioedema. This hypothesis is supported by the coding *PROCR* variant (p.Ser219Gly, rs867186), which was among the identified candidate variants, and which has been associated with both venous thromboembolism<sup>54,55</sup> and higher levels of protein C and soluble EPCR.<sup>56,57</sup> Research has shown that soluble EPCR impairs the activation of protein C.<sup>58</sup> Notably, the *EDEM2* gene, which was one of the prioritized genes and was identified in the gene-based tests, has also been shown to influence the level of protein C.<sup>51</sup>

The present analyses determined an SNP-based heritability of 4.2% to 5.2% for ACEi-AE, which contrasts with a previous estimate of about 20%.<sup>15</sup> Although one explanation could be methodological differences (genotype-level vs summary-level estimates), the discrepancy in the heritability estimate could also be due to the use of a generally broader, more heterogeneous phenotype in the present study, as has been observed in meta-analyses of other phenotypes.<sup>59</sup>

Epidemiological studies have reported a higher risk for ACEi-AE in smokers<sup>13</sup> as well as in patients with concomitant hay fever/

allergic rhinitis<sup>10,12</sup> or coronary artery disease,<sup>1</sup> whereas patients with diabetes were less likely to be affected.<sup>1,12,14</sup> In the present study, no such relationships were determined on the genetic level. Moreover, the 3 nominally significant genetic correlations did not withstand a reanalysis using treatment-matched controls only. These results may reflect an absence of genetic correlations or, given the large standard errors, may merely indicate the limited power of our analyses as a result of the still relatively small size of the sample used in the ACEi-AE meta-analyses.<sup>20</sup> Notably, a comparable genetic correlation with asthma was found in both analyses (41.9% vs 40.9%), which may reflect the involvement of bradykinin-related pathways in the pathophysiology of both traits.<sup>60</sup> Future GWAS meta-analyses involving larger sample sizes will eventually provide more robust results in the investigation of genetic correlations and allow the application of innovative methods, such as summary-based Mendelian randomization, to infer the causal relationships that underlie observed genetic correlations.

The combined meta-analysis of the European and African-American GWAS data generated no additional ACEi-AE-associated loci. However, the high correlations observed in the effect sizes of the genome-wide significant SNPs together with the positive, comparable polygenic signal observed in the VanMar<sub>AFR</sub> cohort suggest that these 2 ancestries share common risk variants for ACEi-AE. Future analyses of larger, multiethnic samples will facilitate the discovery of novel loci and will advance the elucidation of risk loci, for example, in terms of fine-mapping.<sup>61</sup>

The present study had several limitations. First, although our GWAS meta-analysis was the largest in the context of ACEi-AE to date, the analyses had limited power in terms of detecting additional risk loci, particularly those with small effect sizes. Similarly, the nonsignificant results obtained in the pathway-based and genetic correlation analyses, for example, probably reflect the relatively small size of the meta-analysis sample. Second, although the functional relevance of our findings was supported by bioinformatic evidence, the present study provides no *in vitro* or *in vivo* evidence concerning biological function. Further studies are warranted to improve understanding of how the identified risk loci contribute to the development of ACEi-AE. Third, because GWAS findings point to genomic regions associated with the trait of interest and do not directly inform about the true causal variant(s) at the respective loci, statistical fine-mapping was performed to identify such variants. However,



as indicated by the rather small PIP ( $PIP_{max} = 0.125$ ), these efforts were limited by the sample size used in the meta-analysis and probably also by the applied LD reference panels that were representative for a large proportion but not all individuals of the present study.

The present GWAS meta-analysis identified a novel risk locus for ACEi-AE, confirmed 2 previously reported loci, and generated further insights into the underlying disease pathophysiology. In addition, the analyses suggest that the identified risk loci are also involved in ACEi-AE risk in individuals of African-American ancestry, thus underscoring their role in the pathophysiology of this adverse drug reaction. Functional studies are now warranted to pinpoint the true causal variants and to elucidate the molecular mechanisms underlying ACEi-AE susceptibility. Such studies, together with further expansion of ideally multi-ancestry GWAS collectives and the identification of additional risk loci, may eventually facilitate the identification of molecular targets that will in turn allow the development of prevention or intervention strategies.

## DISCLOSURE STATEMENT

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## Key messages

- A GWAS meta-analysis of more than 1000 patients with ACEi-AE revealed 3 genome-wide significant risk loci, including a new locus on chromosome 20q11.22.
- The genome-wide associated loci provide further evidence for the involvement of bradykinin signaling and the coagulation and fibrinolysis pathways in ACEi-AE.
- Cross-ancestry analyses provided initial evidence that the identified loci also contribute to ACEi-AE risk in individuals of African-American ancestry.

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