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CLINICAL RESEARCH
Genetics

Polygenic risk score for ACE-inhibitor-associated cough based on the discovery of new genetic loci

Jonas Ghouse [1,2]*, Vinicius Tragante [3], Ayesha Muhammad [4], Gustav Ahlberg [1,2], Morten W. Skov[1,2], Dan M. Roden [4,5], Ingileif Jonsdottir [3,6,7], Laura Andreasen [1,2], Pia Rengtved Lundegaard [1,2], Linea C. Trudsø [1,2], Karina Banasik [9], Søren Brunak[9], Sisse R. Ostrowski [10,17], eMERGE consortium, Christian Torp-Pedersen [11,17], Ole V. Pedersen[12,17], Erik Sørensen[10,17], Lars Køber [13,17], Kasper Iversen[14,17], Unnur Thorsteinsdottir [3,6], Gudmundur Thorgeirsson [3,8], Henrik Ullum [15], Daniel F. Gudbjartsson[3,16], Jonathan D. Mosley [5], Hilma Holm [3], Kari Stefansson [3,6], Henning Bundgaard [13,17†], and Morten Salling Olesen[1,2†]

[1]Laboratory for Molecular Cardiology, Department of Cardiology, Copenhagen University Hospital, Rigshospitalet, Building 9312, Henrik Harpestrengs Vej 4C, 2100 Copenhagen, Denmark; [2]Laboratory for Molecular Cardiology, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; [3]deCODE genetics/Amgen, Inc., Reykjavik, Iceland; [4]Vanderbilt Genetics Institute, Department of Medicine, Vanderbilt University Medical Center, and Vanderbilt Medical Scientist Training Program, Vanderbilt University, USA; [5]Departments of Internal Medicine and Biomedical Informatics, Vanderbilt University Medical Center, Nashville, Tennessee, USA; [6]Faculty of Medicine, University of Iceland, Iceland; [7]Iceland Department of Immunology, Landspitali—The National University Hospital of Iceland, Reykjavik, Iceland; [8]Department of Medicine, Landspitali—The National University Hospital of Iceland, Reykjavik, Iceland; [9]Translational Disease Systems Biology, Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [10]Department of Clinical Immunology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [11]Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark; [12]Department of Clinical Immunology, Næstved Hospital, Næstved, Denmark; [13]Department of Cardiology, Copenhagen University Hospital, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [14]Department of Cardiology, Copenhagen University Hospital, Herlev-Gentofte Hospital, Herlev, Denmark; [15]Statens Serum Institut, Copenhagen, Denmark; [16]School of Engineering and Natural Sciences, University of Iceland, Reykjavik, Iceland; and [17]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

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Abstract

Aims To search for sequence variants associated with ACEi discontinuation and to test their association with ACEi-associated adverse drug reactions (ADRs).

Methods and results A genome-wide association study (GWAS) on ACEi discontinuation was conducted, including 33 959 ACEi-discontinuers and 44 041 controls. Cases were defined as persons who switched from an ACEi treatment to an angiotensin receptor blocker. Controls were defined as persons who continued ACEi treatment for at least 1 year. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were computed for ACEi discontinuation risk by mixed model regression analysis. Summary statistics from the individual cohorts were meta-analyzed with a fixed-effects model. To test for association with specific ACEi-associated ADRs, any genome-wide significant ($P < 5 \times 10\text{-}8$) ACEi discontinuation variants was tested for association with ACEi-associated cough and angioedema. A polygenetic risk score (PRS) based on ACEi discontinuation GWAS data was constructed and tested for association with ACEi-associated cough and angioedema in two population-based samples. In total, seven genetic genome-wide loci were identified, of which six were previously unreported. The strongest association with ACEi

* Corresponding author. Tel: +45 35456734, Fax: +45 3545 6500, Email: jonasghouse@gmail.com
† These authors contributed equally.

discontinuation was at 20q13.3 (*NTSR1*; OR: 1.21; 95% CI: 1.17–1.24; $P = 2.1 \times 10–34$). Five of seven lead variants were associated with ACEi-associated cough, whereas none were associated with ACEi-associated angioedema. The ACEi discontinuation PRS was associated with ACEi-associated cough in a dose–response manner but not with ACEi-associated angioedema. ACEi discontinuation was genetically correlated with important causes for cough, including gastro-esophageal reflux disease, allergic rhinitis, hay fever, and asthma, which indicates partly shared genetic underpinning between these traits.

Conclusion This study showed the advantage of using prescription patterns to discover genetic links with ADRs. In total, seven genetic loci that associated with ACEi discontinuation were identified. There was evidence of a strong association between our ADR phenotype and ACEi-associated cough. Taken together, these findings increase insight into the pathophysiological processes that underlie ACEi-associated ADRs.

Structured Graphical Abstract

Key Question

Can novel genetic associations for ACE-inhibitor (ACEi)-associated adverse drug reactions (ADRs) be detected using ACE prescription patterns embedded in registries?

Are these loci associated with specific ACEi-associated ADRs?

Key Finding

7 genetic loci that associated with ACEi-discontinuation were identified.

The majority of variants did not associate with common causes of chronic cough, suggesting specific roles in ACEi-discontinuation.

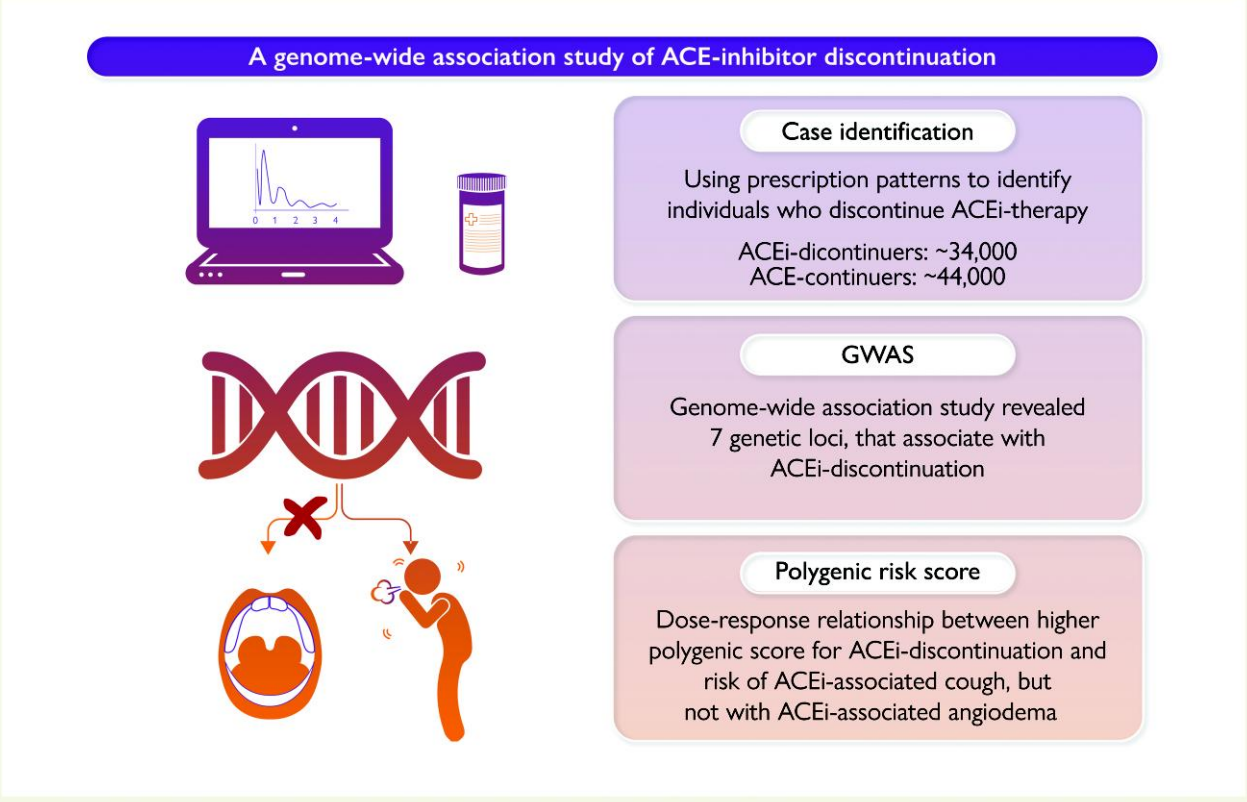
A polygenic risk score showed a dose-response relationship between higher score and risk of ACEi-associated cough.

Take Home Message

Use of prescription patterns are useful in the discovery of genetic links with ADRs.

The underlying ACEi-ADR phenotype is ACEi-associated cough.

Genetic loci involve processes that affect neuronal excitability, bradykinin metabolism and airway inflammation.



A genome-wide association study on ACEi discontinuation, using prescription data from three population-based cohorts. We identified seven genetic loci that associated with ACEi discontinuation, of which six were novel. Using a polygenic risk score approach, we found a dose–response relationship between higher score and risk of ACEi-associated cough but not with ACEi-associated angioedema.

Keywords ACE inhibitors • ACE-inhibitor associated cough • Adverse drug reaction • ADR • Drug discontinuation • GWAS • Genome-wide association study

Introduction

Current guidelines recommend angiotensin-converting enzyme inhibitors (ACEis) as the standard of therapy for hypertension, chronic heart failure, diabetes, and chronic nephropathy.[1–5] At present, up to 40 million people are treated with ACEi worldwide. Although generally considered well-tolerated, 14–30% of patients discontinue treatment, mainly due to adverse drug reactions (ADRs).[6,7] These ACEi-associated ADRs include a persistent dry cough (11%),[8] and more rarely, a potentially life-threatening angioedema (<1%).[9] Currently, clinicians have no way of predicting who will develop side effects prior to drug initiation, and trial-and-error switching is the only option in clinical practice. Clearly, better methods of patient–drug matching to minimize side effects would improve treatment.

Previous studies have indicated that genetics may play a role in susceptibility to ACEi-associated ADRs. Candidate gene studies have focused on genes purported to be involved in the therapeutic action of ACEi, but replicable results have been few and far between. Five genome-wide association studies (GWAS) on ACEi-associated ADRs have been published to date.[10–14] However, these studies only yielded one significant variant around *KCNIP4*,[10] that associated with ACEi-induced cough, and another near *BDKRB2*, which associated with ACEi-induced angioedema.[11] This modest yield from GWAS can be ascribed to small sample size, which is inherent to the fact that severe ACEi-related ADRs are often rare, whereas frequent, albeit mild-to-moderately severe ADRs, are grossly underreported.[15] As such, collection of well-powered samples to study ACEi-associated ADRs is challenging.

According to clinical guidelines, patients who develop ACEi-associated ADRs are advised to switch to angiotensin receptor blockers (ARBs), often leading to complete resolution of the ADRs.[16] Accordingly, a recent study showed that a switch from an ACEi to an ARB is a better marker of ADRs, than a switch to different antihypertensive drug class, or abrupt discontinuation.[17] To overcome the difficulties inherent to smaller sample sizes, we explored whether information on ADRs may be contained in prescription patterns. We utilized prescription data from three population-based cohorts, to identify individuals who switched from ACEi treatment to an ARB, serving as a proxy for ADR. We further determined whether associated variants were associated with either ACEi-associated cough, ACEi-associated angioedema, or both.

Methods

Samples and study design

We meta-analyzed results from three independent cohorts, UK Biobank (UKB), the Copenhagen Hospital Biobank (CHB), and deCODE genetics. The cohorts are described in more detail in Supplementary Appendix. A switch from ACEi to ARB has previously been shown to be a sensitive marker for ACEi-related ADRs.[17] Using this validated method, we ascertained cases as persons switching from an ACEi to an ARB. Cases were included only if they had been treated with ACEi continuously and not previously received ARBs before ACEi treatment initiation. Controls were defined as persons with at least 1 year of continuous ACEi treatment and no history of an ACEi-to-ARB switch (see Supplementary material online, *Figure S1*). Continuous treatment was defined as a maximum of 180 days between successive prescription renewals. Data sources used to define cases and controls are summarized in Supplementary Appendix.

Genotyping and imputation

Genetic data for the UKB study participants were provided by UKB and the imputation and quality control procedures have been fully described elsewhere.[18] Briefly, genotypic data were obtained through either UKB Axiom or UK BiLEVE Axiom arrays. Standard quality control procedures were applied prior to imputation using the Haplotype Reference Consortium (HRC) and UK10K haplotype reference panels. Samples were excluded for the following reasons: inferred sex did not match reported sex, kinship was not inferred, putative sex chromosome aneuploidy or excessive heterozygosity or missingness, based on centralized sample quality control performed by UKB.[18] European ancestry was determined by first selecting self-reported ancestry of British, Irish, or other white. Then we excluded population outliers among these, by defining means and standard deviations (SDs) on principal component (PC) 1–5. Individuals with a sum total of more than 6 SDs in PC 1–5 were excluded. We applied additional quality control filters to select high quality single nucleotide polymorphisms (SNPs) including minor allele frequency (MAF) >0.01, imputation score (INFO) >0.7.

Copenhagen Hospital Biobank samples were genotyped using the Global Screening Array by Illumina. Quality control on the genotyped data was performed according to standard protocol.[19] The genotyped data were long-range phased using Eagle2[20] and imputed using a reference panel backbone consisting of whole-genome sequence data from 8429 Danes along with 7146 samples from North-Western Europe.[19] Whole-genome sequencing, chip-genotyping, and the subsequent imputation from which the data for this analysis were generated were performed by deCODE genetics. Additional post-imputation filters included MAF >0.01 and INFO >0.7.

For the deCODE genetics data, the genome of the Icelandic population was characterized by whole-genome sequencing of 49 708 Icelanders using Illumina standard TruSeq methodology to a mean depth of 35× (SD 8×) with subsequent long-range phasing[21] and imputing the information into 166 281 individuals chipped by multiple Illumina platforms.[22] Using genealogic information, sequence variants were imputed into 285 664 relatives of the genotyped individuals. Additional post-imputation filters included MAF >0.01 and INFO >0.8.

Association analysis

In UKB, association testing was carried out using a linear mixed model regression, adjusting for age, sex, and the first 10 PCs, genotyping array and assessment centres. In CHB, we adjusted our model for age, sex, and 10 PCs. For deCODE, regression analyses were adjusted for sex, county of origin, and year of birth as covariates, under an additive model. We used BOLT-LMM, which accounts for population substructure and

sample relatedness.[23] Odds ratios (ORs) from BOLT-LMM were calculated by $OR = e^{\beta/(\mu \times (1-\mu))}$, where μ = case fraction, and standard errors were divided by $(\mu \times (1-\mu))$ to deduce confidence intervals.

Meta-analysis

To maximize the statistical power to detect associated sequence variants with small effect sizes, the three cohorts were meta-analyzed. Prior to meta-analysis, individual GWAS association results were assessed using the R package EasyQC (v9.2), and duplicates and monomorphic SNPs were excluded.[24] The absence of population stratification was controlled for based on the genomic inflation factor ($\lambda < 1.10$ for all cohorts). Variants were only included if they were present in at least two of three data sets. Meta-analysis was performed in METAL using the fixed-effect inverse variance-weighted method.[25]

Functional annotation

Functional annotation of GWAS results was performed in FUMA (version 1.3.6).[26] FUMA is an online platform that takes GWAS summary statistics as input, and subsequently annotates, prioritizes, and visualizes the results. Within each locus, the lead SNPs were defined as the variant with the lowest association *P*-value. Genetic risk loci were defined by combining lead SNPs within a 250 kb window and all SNPs in LD of $r^2 \geq 0.8$ with the lead SNPs. The 1000Genomes phase III reference panel was employed to infer LD. The functional consequences of SNPs identified in this study were evaluated by matching each SNP's chromosome location, base-pair position, reference, and alternate alleles to databases containing known functional annotations, including Annotate Variation (ANNOVAR)[27] categories, Combined Annotation Dependent Depletion (CADD) scores,[28] RegulomeDB[29] scores, and chromatin state.[30,31] More information on these databases is available in the Supplementary Appendix.

Gene mapping

Loci obtained from the GWAS meta-analysis were mapped to genes using three different approaches:

(1) *Positional mapping*: Maps SNPs to genes based on physical distance ($\pm$ 50 kb) from known protein-coding genes in the human reference assembly (GRCh37/hg19).
(2) *Expression quantitative trait loci (eQTL) mapping*: We assessed whether any of the ACEi discontinuation lead variants were related to gene expression using data on all available tissue types from the GTEx (https://gtexportal.org/home/), version 8 repository.[32] If the variant at a locus was not available in GTEx, we used proxy variants ($r^2 > 0.8$). To account for multiple testing, we used a false discovery rate of <0.05 to define significant eQTL associations.
(3) *Colocalization analysis:* To prioritize among candidate genes identified through the listed gene mapping strategies, we estimated the posterior probability (PPa) for a common causal variant underlying association with gene expression and ACEi discontinuation, by conducting pairwise Bayesian colocalization analysis, implemented in the *coloc* R package v.3.1.[33] We tested genes with significant *cis*-eQTL association by analyzing all variants within a $\pm$ 250 kilobase (kb) window around our risk locus using eQTL and ACEi discontinuation summary data. We used the default priors supplied by the *coloc* package. We report the posterior probability that the association with gene expression and ACEi discontinuation risk is driven by a single causal variant. We consider a PPa $\geq$ 0.7 as supporting evidence for a causal role for the gene as a mediator of ACEi discontinuation.

Phenome-wide association analysis

We performed phenome-wide association analysis (PheWAS) for the seven ACEi discontinuation lead SNPs using FinnGen R6 (https://r6.finngen.fi/) GWAS summary statistics, with data on 260 405 individuals and 2861 phenotypes. To claim significance, we used a Bonferroni-corrected significance threshold of $P < 2.5 \times 10^{-6}$, Bonferroni-corrected for the number of lead SNPs multiplied by 2861 phenotypes.

Heritability

Single nucleotide polymorphism heritability (h_g^2) of ACEi discontinuation was estimated using BOLT-REML[34] and LD score regression.[35] BOLT-REML applies variance components analysis to estimate h_g^2 and was performed on individual-level phenotype and genotyped data. Our model SNPs consisted of hard called genotypes with a MAF >1%, call rate >95% and LD <0.9. LD score regression uses GWAS summary statistics and assesses h_g^2 based on the expected relationship between LD of neighbouring SNPs and strength of association under a polygenic model. Pre-calculated LD scores from the 1000-Genomes European reference population were from https://data.broadinstitute.org/alkesgroup/LDSCORE/.

Genetic correlation and bi-directional Mendelian randomization analyses

We calculated genetic correlations between ACEi discontinuation and 19 pre-defined cardiometabolic, neuropsychiatric, pulmonary traits, as well as known differential diagnoses to ACEi-associated ADRs, using LDSC on HapMap 3 SNPs only.[35] Genetic correlations were corrected for multiple testing based on the total number of correlations [19 traits; 12 from published GWAS and 7 obtained from the Neale lab (URL: http://www.nealelab.is/uk-biobank)] by applying a Bonferroni-corrected threshold of $P < 0.05/19 = 0.003$. To understand causal–consequential relationships between ACEi discontinuation and chronic cough, allergic disease and gastro-esophageal reflux disease (GERD), we performed bi-directional Mendelian randomization (MR) analyses. We selected independent ($r^2 < 0.01$) genome-wide significant variants to serve as instrumental variables (IVs) for our MR analyses. We applied three different MR methods: IVW using the random-effects model, MR-Egger, and weighted median. MR-Egger-intercept was used to test for pleiotropy. We applied MR-PRESSO to detect outlier IVs.[36] To avoid overfitting due to sample overlap between the exposure and outcome datasets, weights for ACEi discontinuation were derived from meta-analysis of the CHB and deCODE data. MR analyses were conducted in R using the TwoSampleMR package.[37] A detailed description of employed MR methods is available in the Supplementary Appendix. The sources of the summary statistics files (DOI or unpublished results) used in LDSC and MR analyses are provided in Supplementary material online, *Table S1*.

Evaluation of the underlying ADR phenotype

We conducted polygenic risk score (PRS) analyses to determine whether our GWAS on ACEi discontinuation shares genetic etiology with ACEi-associated cough and angioedema. We summed sets of alleles, weighted by their log OR from the ACEi discontinuation GWAS, into PRSs for each individual in the two test data sets (eMERGE and CHB). The eMERGE data set contained 1346 individuals who had experienced ACEi-associated cough and 4662 individuals with at least 6 months of ACEi treatment and no cough (more information in Supplementary Appendix). The CHB data set comprised 201 individuals with an history of ACEi-associated angioedema and 24 394 ACEi-treated individuals without a history angioedema (more information in Supplementary Appendix).[11] Cases and controls used in the ACEi discontinuation GWAS were removed from the ACEi-associated angioedema GWAS to avoid overfitting of PRS models. The PRSice-2

Table 1 Variants associated with ACE-inhibitor discontinuation at genome-wide significance

Lead SNP	Chr location	Alleles		EAF	Gene context	OR (95% CI)	*P*-value
		EA	Non-EA				
rs7526729	1:111087058	A	G	0.69	[*KCNA2*]	1.13 (1.09–1.15)	6.1×10^{-14}
rs1544730	2:45588067	A	G	0.22	*SRBD1*	1.10 (1.07–1.13)	5.8×10^{-13}
rs16870989	4:21386764	A	T	0.33	[*KCNIP4*]	1.12 (1.09–1.14)	2.0×10^{-22}
rs12210271	6:105776312	T	C	0.21	[*PREP*]	1.13 (1.10–1.16)	1.3×10^{-19}
rs360206	9:127795842	C	T	0.19	[*SCAI*]	1.10 (1.07–1.13)	2.0×10^{-11}
rs8097200	18:6309334	A	G	0.83	[*L3MBTL4*]	1.12 (1.09–1.15)	8.6×10^{-15}
rs6062847	20:61322018	T	C	0.14	*SLCO4A1/NTSR1*	1.21 (1.17–1.24)	2.1×10^{-34}

The results of the GWAS meta-analysis of ACE-inhibitor discontinuation (33 959 cases and 44 041 controls) are shown, in which seven genome-wide significant loci were detected. EA and non-EA refers to effect allele and non-effect allele. The odds ratio (OR) and 95% CI are shown for the association between effect allele (EA) and the phenotype. EAF refers to effect allele frequency. The meta-analysis was restricted to variants with MAF ≥0.01 and information quality (INFO) score ≥0.7. All loci were confirmed with consistent direction of effect (see Supplementary material online, *Table S2*). The gene context column provides the nearest gene(s). Brackets indicate that the lead SNP or those in LD ($r^2 > 0.8$) are located within the specified genes.

software[38] was used to generate PRSs, according to standard protocol. Briefly, to extract independent SNPs, clumping was applied using a LD $r^2 < 0.05$ and a 1500 kb sliding window. PRS were calculated using six increasingly liberal P-value thresholds [$\leq 5 \times 10^{-8}$, $\leq 5 \times 10^{-7}$, $\leq 5 \times 10^{-6}$, $\leq 5 \times 10^{-5}$, $\leq 5 \times 10^{-4}$, and the full model including all SNPs ($P \leq 1$)]. We used logistic regression with the covariates age, sex and PCs (10 first PCs in CHB and 3 first PCs in eMERGE). We report associations on a linear scale, with ORs per SD increase in PRS. The PRSs were also split into quintiles, and ORs for the tested ADRs were calculated in each quintile, with the first decile set as reference. Pseudo-R^2 as per Lee *et al.*[39] was used to calculate the proportion of phenotypic variance explained on the liability scale, according to disease prevalence (0.7% for ACEi-associated angioedema and 11% for ACEi-associated cough).

Replication

As criteria for sentinel SNP validation, we used a $P < 0.05$ and concordant direction of effect in at least two of the three cohorts. To test the more global robustness of our genetic associations, we calculated an ACEi discontinuation PRS based on the SNP effect sizes of the leave-one-out meta-analyses, from which one of three cohorts were excluded and reserved for score validation. We used the same clumping and thresholding approach as described previously. The PRS leave-one-out analyses provide evidence for the aggregate effects of our genetic signal, by demonstrating a higher burden of common risk variants associated with ACEi discontinuation compared with controls, across all the cohorts. Finally, to further replicate and investigate links with specific ACEi-associated ADRs, we also queried the sentinel SNPs in two available GWAS summary data for ACEi-associated cough[10] and angioedema.[11] We used a Bonferroni-corrected *P*-value threshold of 0.004 (0.05/[2 traits × 7 SNPs]).

Results

Meta-analysis identifies seven loci associated with ACE-inhibitor discontinuation

We performed a fixed-effects inverse variance-weighted meta-analysis linking ∼10.1 million common and low-frequency variants to ACEi discontinuation. Our study sample was based on three population-based cohorts, and comprised 33 959 cases who discontinued their ACEi treatment and 44 041 controls, who continued ACEi treatment (see Supplementary material online, *Table S2*). A summary of the participant selection process is illustrated in Supplementary material online, *Figure S2*. We identified seven loci that exceeded genome-wide significance ($P < 5 \times 10^{-8}$; *Table 1* for loci; *Figure 1* for Manhattan plot; Supplementary material online, *Figure S3* for QQ-plots; Supplementary material online, *Figure S4* for regional plots, respectively), of which six were previously unreported. All lead SNPs were located in non-coding regions (see Supplementary material online, *Table S3*). Therefore, we investigated whether any of the lead SNPs were in LD ($r^2 > 0.8$) with non-synonymous variants with predicted deleterious effects. We found one missense variant (rs12192054; $r^2 = 1$ with lead SNP rs12210271; p.Leu285Val) in the *PREP* gene with a CADD score of 15, which supports a deleterious effect (*Figure 1* and Supplementary material online, *Table S3*). All lead SNPs showed concordant direction of effect in all cohorts (see Supplementary material online, *Table S2*), and all were nominally significant ($P < 0.05$) in at least two cohorts. We also conducted a leave-one-out polygenic prediction analysis, and found that PRS derived from either of our cohorts significantly associated with ACEi discontinuation in the remaining validation cohorts (see Supplementary material online, *Table S4*). The estimated overall SNP heritability for the meta-analysis using LDSC was 3.8% (95% CI: 2.8–5.3%). For the individual data sets, SNP-based heritability was concordant between the two methods and ranged from 4.4 to 5.8% using LDSC and from 3.6 to 7.3% using BOLT-REML (see Supplementary material online, *Table S5*). The proportion of variance explained by the sentinel SNPs ranged from 6.8 to 20.3% across data sets (see Supplementary material online, *Table S5*).

Prioritization of genes through expression analyses

Although GWAS findings are informative in linking genomic regions to a trait, finding strong support about their connections to specific genes is less straightforward. Therefore, we looked at

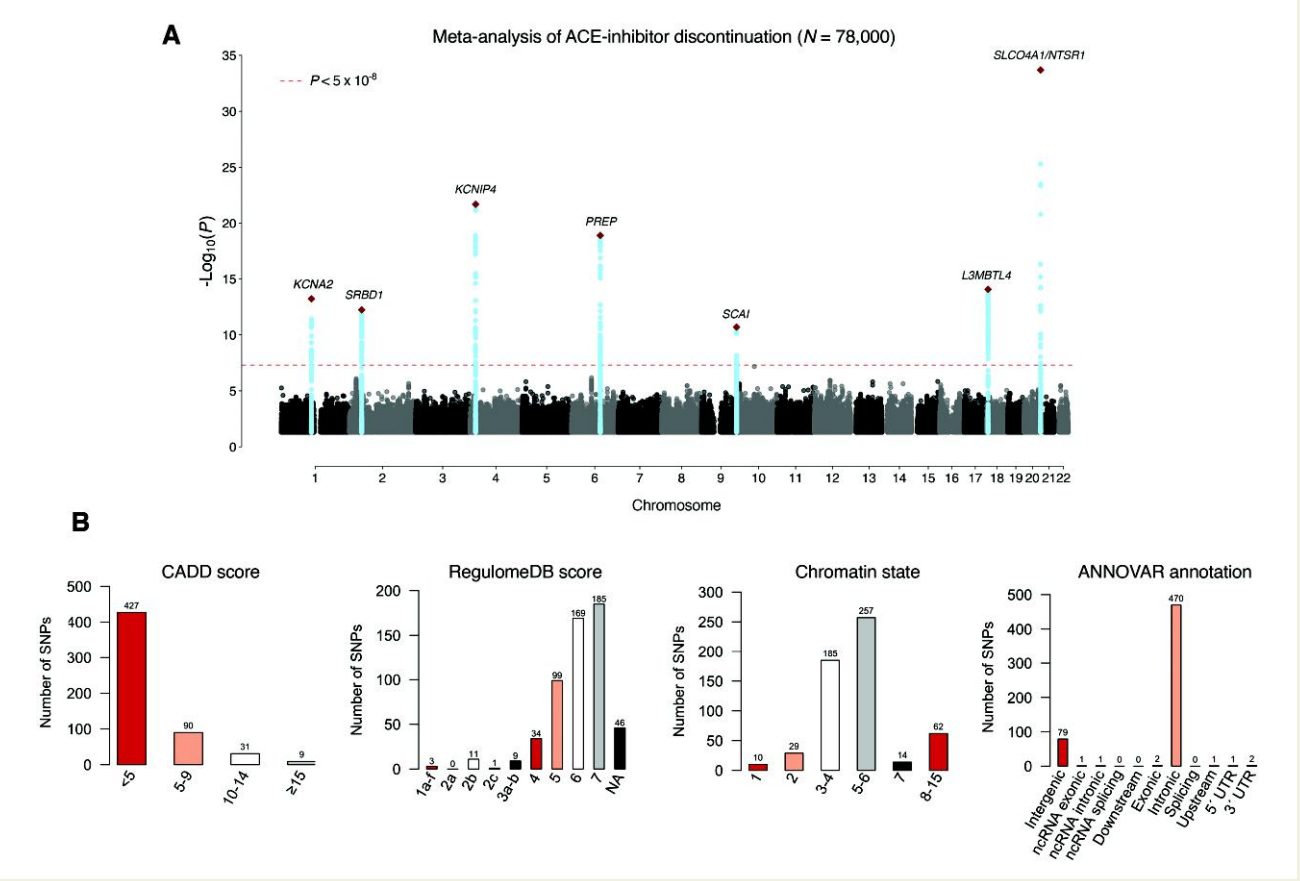


Figure 1 Single nucleotide polymorphism (SNP)-based results from the genome-wide association analysis (GWAS) on ACE-inhibitor discontinuation in 78 000 individuals. (*A*). Manhattan plot of the results from the GWAS meta-analysis of ACE-inhibitor discontinuation. Lead SNPs in the seven genome-wide significant loci are highlighted with a diamond. Independent genome-wide significant variants are annotated to the nearest gene(s). The *y* axis represents –log10 of the two-sided *P*-values for association of variants with ACE-inhibitor discontinuation, from meta-analysis using an inverse variance-weighted fixed-effects model and a total sample size of 78 000. The *x*-axis represents the genome in physical order. The dashed line represents the threshold for genome-wide significance. (*B*). Distribution of CADD scores, RegulomeDB categories, chromatin state and functional consequences of all annotated SNPs (ANNOVAR) in linkage disequilibrium of $r^2 \geq 0.8$ with the lead SNPs. CADD scores predict how deleterious the effect of a SNP is likely to be for protein structure/function, with higher scores referring to higher deleteriousness. A CADD score above 12.37 is considered potentially pathogenic.[28] RegulomeDB categories annotate SNPs in genomic risk loci, with a low score indicating a higher likelihood of a SNP having a regulatory function.[29] Chromatin state corresponds to the minimum chromatin state across 127 tissue and cell types for SNPs in the genomic risk loci, with lower states indicating higher accessibility and states 1–7 referring to open chromatin states.[31] ANNOVAR categories identify the variant genic position (e.g. intronic, exonic, intergenic) and associated function.[27]

association of candidate genes to risk loci by their effects on gene expression. Five of seven sentinel variants were significantly associated with the expression of one or more genes in at least one tissue (see Supplementary material online, *Table S6*). To further prioritize among candidate genes identified in our eQTL analysis, we estimated the posterior probability for a common causal variant underlying the associations with gene expression and ACEi discontinuation at each locus, by conducting pairwise Bayesian colocalization analysis. We found evidence for colocalization for *KCNA2, SRBD1* and *RABEPK*. Moreover, the locus on chromosome 6 colocalized with multiple nearby genes (*PREP, BVES* and *POPDC3*). The nearest gene was concordant with results from colocalization in all but one locus, i.e. chromosome 9, where we found evidence for colocalization with *RABEPK*, whereas the closest gene was *SCAI*.

Association between ACEi discontinuation and other phenotypes

We found that ACEi discontinuation had a significant genetic correlation with five pre-defined traits ($P < 0.002$). ACEi discontinuation had the highest genetic correlation with 'Cough on most days' ($r_G = 0.37$, $P = 4.0 \times 10^{-4}$, *Figure 2*), whereas the most significant association was observed with GERD ($r_G = 0.27$, $P = 4.4 \times 10^{-7}$). In addition, allergic disease ($r_G = 0.26$, $P = 6.2 \times 10^{-6}$), chronic pain

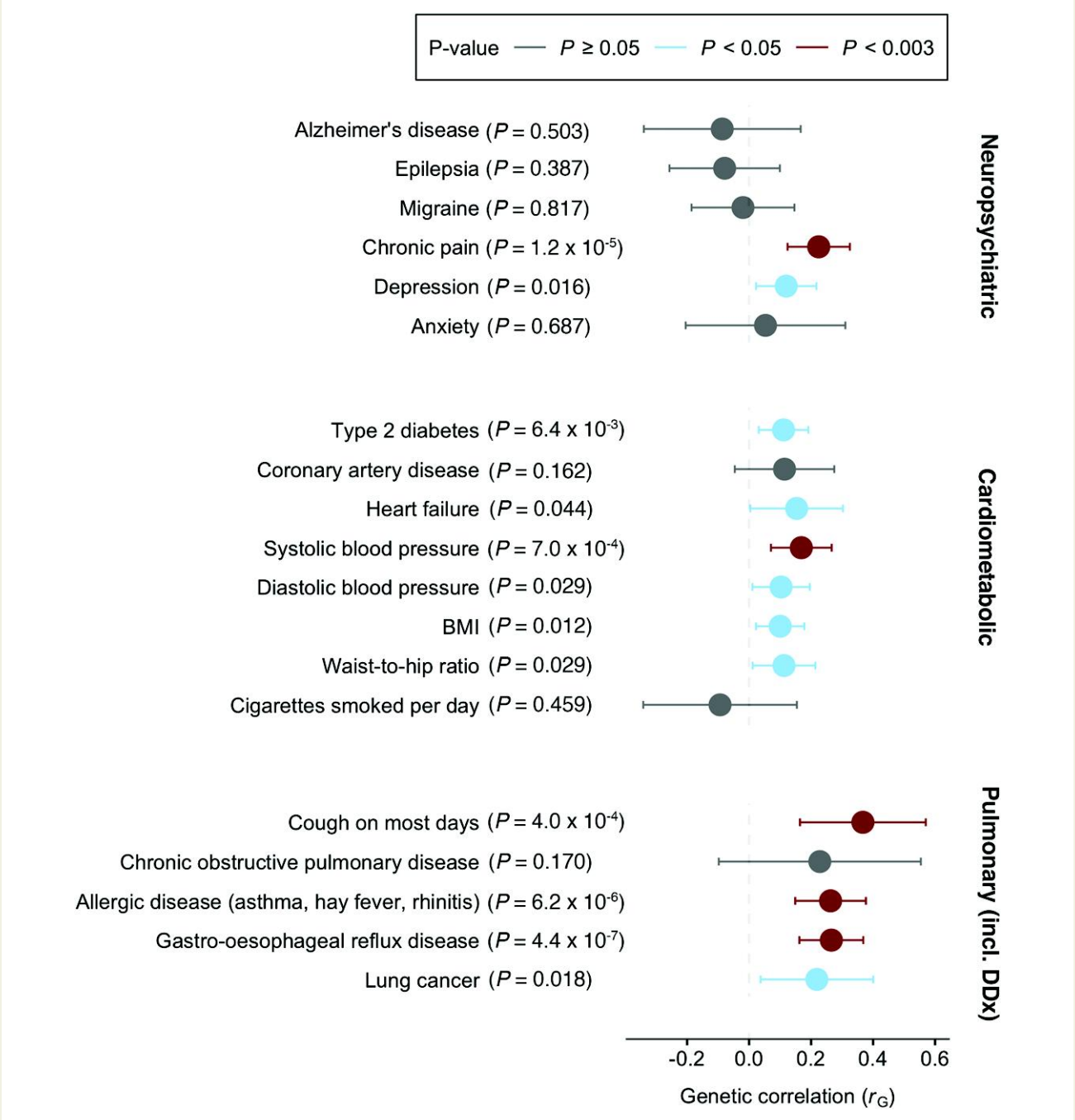


Figure 2 Genetic correlations of ACE-inhibitor discontinuation with selected neuropsychiatric, cardiometabolic and pulmonary traits. Genetic correlations and two-sided *P*-values were calculated using linkage disequilibrium score regression. Genetic correlation is presented as a dot and error bars indicate 95% confidence limits. The threshold for significance was set at $P < 0.003$ (0.05/19 tested traits).

($r_G = 0.22$, $P = 1.2 \times 10^{-5}$), and systolic blood pressure ($r_G = 0.17$, $P = 7.0 \times 10^{-4}$) associated with ACEi discontinuation. To investigate the relationship between ACEi discontinuation and correlated pulmonary traits, we performed bi-directional MR analyses. We found no support for a causal effect of ACEi discontinuation on GERD, allergic disease or 'Cough on most days', nor in the reverse direction (see Supplementary material online, *Table S7*). To further explore whether individual ACEi discontinuation variants associated with blood pressure and cough-related traits, we tested the sentinel SNPs for association with blood pressure indices and eight other chronic cough phenotypes available in the UKB (i.e. asthma, chronic obstructive pulmonary disease, allergic disease, GERD, smoking, bronchiectasis, heart failure and 'Cough on most days'). One sentinel variant, rs6062847 near *NTSR1*, associated with trait 'Cough on most

Table 2 Associations between ACEi discontinuation variants, blood pressure, and eight chronic cough phenotypes in the UK Biobank

		rs7526729 at *KCNA2*		rs1544730 at *SRBD1*		rs16870989 at *KCNIP4*		rs12210271 at *PREP*		rs360206 at *SCAI*		rs8097200 at *L3MBTL4*		rs6062847 at *NTSR1*	
Phenotype	**N**	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
ACEi discontinuation	23 643	1.13 (1.09–1.18)	**2.6E-10**	1.09 (1.06–1.13)	**2.4E-08**	1.11 (1.08–1.14)	**1.4E-12**	1.08 (1.05–1.12)	**1.0E-06**	1.10 (1.07–1.14)	**1.8E-08**	1.08 (1.04–1.12)	**1.3E-05**	1.16 (1.12–1.21)	**8.1E-16**
Cough on most days	15 069	1.00 (0.97–1.03)	0.918	1.04 (1.01–1.08)	0.005	1.02 (1.00–1.15)	0.062	1.04 (1.01–1.07)	0.011	1.03 (1.00–1.06)	0.064	1.01 (0.98–1.05)	0.453	1.11 (1.08–1.15)	**1.0E-09**
Asthma	61 664	1.01 (1.00–1.02)	0.195	0.98 (0.97–1.00)	0.017	1.01 (0.99–1.02)	0.386	1.02 (1.00–1.03)	0.029	1.02 (1.01–1.04)	0.005	0.99 (0.98–1.01)	0.448	1.02 (1.00–1.04)	0.380
Chronic obstructive pulmonary disease	18 809	0.99 (0.97–1.01)	0.393	1.00 (0.97–1.03)	0.999	1.00 (0.98–1.02)	0.956	0.98 (0.95–1.00)	0.071	1.04 (1.02–1.07)	0.002	0.98 (0.96–1.01)	0.182	1.03 (1.00–1.06)	0.081
Allergic disease	74 892	1.01 (1.00–1.03)	0.019	1.01 (1.00–1.02)	0.170	0.99 (0.98–1.00)	0.050	1.01 (0.99–1.02)	0.395	1.00 (0.99–1.02)	0.845	1.00 (0.99–1.02)	0.578	0.99 (0.97–1.01)	0.229
Gastro-esophageal reflux disease	63 268	1.00 (0.99–1.01)	0.859	0.99 (0.98–1.01)	0.430	1.00 (0.98–1.01)	0.700	0.99 (0.97–1.00)	0.080	1.01 (1.00–1.03)	0.143	0.98 (0.97–1.00)	0.060	1.01 (0.99–1.03)	0.280
Smoking (current/ever vs. never)	199 350	0.99 (0.98–1.00)	0.138	1.00 (0.99–1.01)	0.683	1.00 (0.99–1.01)	0.876	1.00 (0.99–1.01)	0.635	1.00 (0.99–1.01)	0.667	0.99 (0.98–1.00)	0.019	1.01 (0.99–1.02)	0.416
Heart failure	13 411	1.00 (0.98–1.03)	0.773	1.00 (0.97–1.03)	0.919	1.01 (0.98–1.04)	0.478	0.98 (0.95–1.01)	0.159	1.03 (1.00–1.06)	0.069	1.01 (0.98–1.04)	0.549	1.02 (0.98–1.06)	0.297
Bronchiectasis	5034	1.00 (0.96–1.04)	0.994	1.00 (0.95–1.05)	0.928	1.01 (0.97–1.05)	0.713	1.01 (0.96–1.06)	0.703	1.03 (0.98–1.08)	0.268	0.98 (0.93–1.03)	0.376	1.04 (0.98–1.10)	0.174
Phenotype	**N**	**Beta, mmHg (95% CI)**	***P***	**Beta, mmHg (95% CI)**	***P***	**Beta, mmHg (95% CI)**	***P***	**Beta, mmHg (95% CI)**	***P***	**Beta, mmHg (95% CI)**	***P***	**Beta, mmHg (95% CI)**	***P***	**Beta, mmHg (95% CI)**	***P***
Systolic blood pressure	376 015	0.02 (–0.07, 0.11)	0.671	0.02 (–0.08, 0.13)	0.664	0.07 (–0.02, 0.16)	0.123	0.00 (–0.10, 0.11)	0.931	0.23 (0.13, 0.34)	1.5E–05	–0.04 (–0.15, 0.07)	0.475	0.12 (–0.00, 0.24)	0.056
Diastolic blood pressure	376 023	0.03 (–0.02, 0.08)	0.260	0.04 (–0.02, 0.10)	0.203	0.02 (–0.03, 0.07)	0.391	0.02 (–0.04, 0.08)	0.442	0.00 (–0.06, 0.06)	0.909	–0.01 (–0.07, 0.05)	0.717	0.05 (–0.02, 0.12)	0.146

P-values below a significance threshold of $P < 0.05/(7 \text{ variants} \times 10 \text{ traits}) = 0.00071$ are bold. Results from meta-analysis of the Copenhagen Hospital Biobank and deCODE for ACEi discontinuation are shown for comparison. UK Biobank data fields used to extract outcomes are listed in Supplementary material online, *Table S11*.

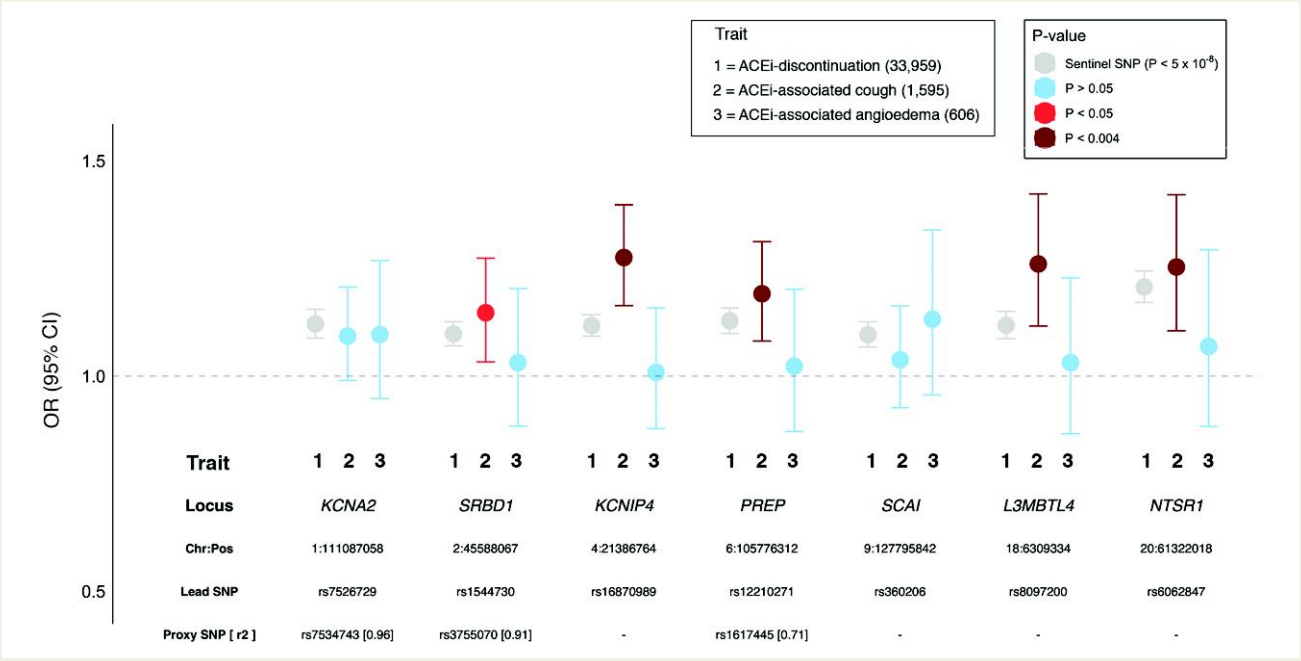


Figure 3 This plot displays the associations between the identified ACE-inhibitor discontinuation loci and ACE-inhibitor associated adverse drug reactions (cough and angioedema), using summary estimates from published GWAS summary data. The points represent the odds ratios (ORs) and lines represent 95% confidence intervals (95% CI). Number in bracket represents number of cases used to derive the GWAS summary statistics. We accounted for multiple testing by Bonferroni correction for the two traits tested per ACE-inhibitor discontinuation locus ($P < 0.004$). The ACE-inhibitor discontinuation lead SNP associations are displayed for comparison. Where a sentinel variant was not available for all traits, a common proxy was selected ($r^2 > 0.7$). Each lead (or proxy) variant is annotated to the nearest gene(s).

days', with a Bonferroni-corrected P-value threshold of $P < 0.05/(7 \times 10) = 7.1 \times 10^{-4}$ (*Table 2*), albeit with smaller effect than with ACEi discontinuation and no consistency across related cough traits. Moreover, the sentinel variant rs360206 near *SCAI* associated with systolic blood pressure ($P = 1.5 \times 10^{-5}$), whereas no sentinel variant associated with diastolic blood pressure ($P < 0.05$).

Discerning the underlying phenotype

To provide further insight into the underlying phenotype of ACEi discontinuation, we performed a PheWAS for the ACEi discontinuation associated loci by testing lead SNPs for association with 2861 traits in FinnGen. There were no associations beyond the threshold for multiple testing. Next, to replicate and to investigate whether the identified genetic loci were associated with the specific ACEi-associated ADRs cough and angioedema, we queried lead SNPs using available GWAS summary data for both traits. We found that four of seven sentinel variants associated with ACEi-associated cough beyond the threshold for multiple testing, and one additional variant was nominally ($P < 0.05$) associated with ACEi-associated cough (*Figure 3*). The strongest association was observed for the *KCNIP4* locus, which constitutes the only previously identified genome-wide significant locus for ACEi-associated cough.[10] None of the loci associated with ACEi-associated angioedema, although the point-estimates tracked in the same direction as the ACEi discontinuation lead SNPs (*Figure 3* and Supplementary material online, *Table S8*). Next, we aimed to examine the contribution of polygenic inheritance of ACEi discontinuation on ACEi-associated cough and angioedema risk. The most significant association between ACEi discontinuation and ACEi-associated cough was observed for a P-value threshold of 5×10^{-8} (see Supplementary material online, *Table S9*). Increasing quintiles of PRS associated with higher OR for ACEi-associated cough compared with the first quintile (*Figure 4* and Supplementary material online, *Table S10*). We found no association between increasing quintiles of PRS and risk of ACEi-associated angioedema (*Figure 4* and Supplementary material online, *Table S10*), nor on the continuous scale (OR: 0.96 per SD increase in PRS; 95% CI: 0.83–1.10; $P = 0.526$) (see Supplementary material online, *Table S9*).

Discussion

To maximize the benefit of antihypertensive or heart failure therapy to the individual patient, not only should the most efficacious drug be prescribed, but also the drug with the least side effects. Understanding differences between individuals in the development of side effects following therapy is therefore essential to personalizing treatment. Using data on 78 000 individuals, we identified seven loci associated with ACEi discontinuation.

To determine whether our ACEi discontinuation phenotype was driven by specific ACEi-associated ADRs, we investigated whether any of the loci also associated with ACEi-associated cough and angioedema using available GWAS summary statistics for both traits. We confirmed the only known ACEi-associated cough locus, *KCNIP4*,[10] and provide data on additional loci that may play a role in ACEi-associated cough. Five of seven loci associated with

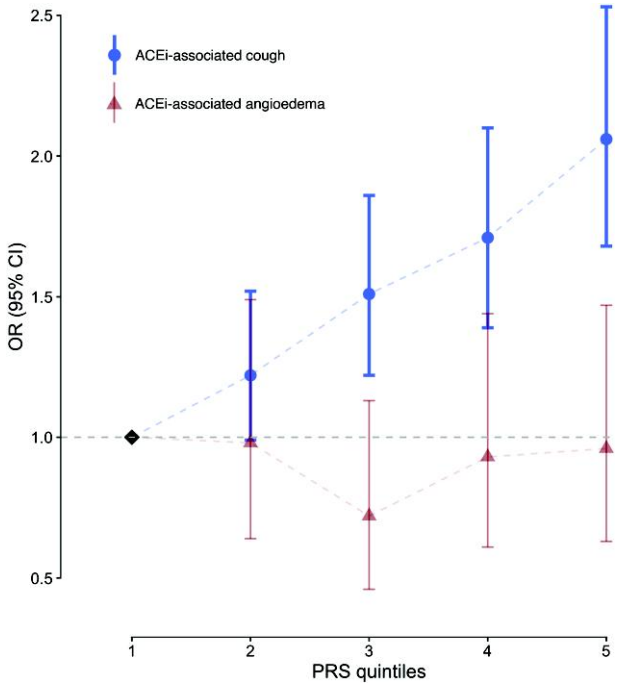


Figure 4 Odds ratio (OR) for ACEi-associated cough and angioedema by quintiles of ACEi discontinuation polygenetic risk score (PRS). Triangles correspond to OR by PRS quintile estimated for 6008 ACEi-treated individuals, of which 1346 constitute individuals with ACEi-associated cough sampled from the eMERGE consortium. Dots represent the OR by PRS quintile for 24 595 ACEi-treated individuals, of which 201 had a history of ACEi-associated angioedema, sampled from the Copenhagen Hospital Biobank. ORs and 95% confidence limits (error bars) were estimated using logistic regression, adjusted for sex, age and principal components. Diamond represents the reference category (1st quintile).

ACEi-associated cough, but none with ACEi-associated angioedema. We found that an incremental increase in polygenetic risk for ACEi discontinuation associated with a dose–response increased risk of ACEi-associated cough but not ACEi-associated angioedema. Altogether, our findings emphasize that the underlying ADR phenotype relates to cough (*Structured Graphical Abstract*).

Cough is mainly thought of as an interplay between cough triggers (e.g. ACEi treatment) and cough sensitization of both peripheral and central neural pathways leading to cough at lower levels of a given stimuli.[40] We found two risk loci near genes which encode potassium ion channels; *KCNA2* (rs7526729) and *KCNIP4* (rs16870989). These two genes are predominantly expressed in neuronal tissue and hold important roles in neuronal excitability,[41,42] suggesting that an innate susceptibility to cough sensitization, due to inborn changes in ion channel activity, may represent one of the contributing mechanisms of this ADR.[43] In fact, inhibition of the voltage-dependent potassium channel blocker encoded by *KCNA2* with dalfampridine, has been shown to evoke action potential discharge in airway vagal sensory nerves and cough in animal models.[44–46] Another main finding in this study relates to the bradykinin pathway. Bradykinin is thought to play a key role in ACEi-induced angioedema and cough, and levels are normally controlled by the action of several kininases, including angiotensin-converting enzyme, carboxypeptidase N, dipeptidyl peptidase-4, aminopeptidase P and prolyl endopeptidase. We found an association between ACEi discontinuation and an intronic variant (rs12210271) of the gene *PREP*. *PREP* encodes a prolyl endopeptidase, responsible for the maturation and breakdown of several vasoactive peptides (in addition to bradykinin), including angiotensin, substance P, vasopressin and neurotensin.[47] Accordingly, impaired breakdown of bradykinin via iatrogenic ACE-inhibition combined with reduced expression of *PREP* may have cumulative effects on cough risk via the bradykinin pathway. We also identified a risk locus near the *NTSR1* gene (rs6062847), which encodes the neurotensin receptor 1.[48] Neurotensin has been shown to cause bronchoconstriction.[49] Activation of the neurotensin receptor 1 has also been shown to potently lead to mast cell degranulation, with release of pro-inflammatory mediators such as histamine and leukotriens.[50] Airway inflammation is thought to play a key role in cough sensitization.[51] We show that genetic susceptibility that affects neuronal excitability, impaired bradykinin metabolism and/or airway inflammation, may explain part of the inter-individual variability observed in ACEi-associated cough.

Observational studies have indicated that the chronicity of both cough and pain may be due to the same neurobiological mechanism.[43] Accordingly, both preclinical and clinical studies have shown that therapeutics purposed for treating pain (e.g. opioids,[52] gabapentin[53,54] and amitriptyline[55]) are effective in treating cough, indicating a shared 'chronic hypersensitization state' or 'neuropathy', involving both peripheral and central sensory nerve pathways. In this study, we observed genetic commonalities between ACEi discontinuation and chronic pain, lending support to hypotheses of shared pathophysiological underpinning.

Our genetic correlation analyses also point toward shared genetic underpinning between GERD, allergic disease and ACEi discontinuation. To investigate whether these genetic correlations reflect a general causal relationship, we performed bi-directional MR analyses. We found no evidence to support a causal role of ACEi discontinuation on these traits, nor in the reverse direction. Moreover, to determine whether our risk loci reflect cough susceptibility loci, rather than *bona fide* ADR loci, we tested for association with blood pressure and eight common causes of chronic cough. These analyses did not support that the identified loci mediate their effect on ACEi discontinuation through general susceptibility for cough or hypertension, because they either did not associate with chronic cough traits or had stronger effects on ACEi discontinuation. Although our sentinel SNPs generally did not associate with common causes of chronic cough, our genetic correlation analysis indicates, that genetic variants beyond the sentinel SNPs reported here, could *partly* share pathophysiological pathways, meaning that genetic susceptibility to ACEi-associated cough could in part be driven by an innate cough sensitization, which progresses into clinically overt cough upon exposure to a trigger (e.g. ACEi treatment, reflux or allergens).

Pharmacogenetic studies on ADRs are limited by the marked underreporting of ADRs, especially mild-to-moderate ADRs which may be transient in nature or do not necessitate hospitalization.[35] This study shows the potential of using information embedded in prescription patterns as a proxy for ADRs to maximize the yield of large biobanks with genetic data.

The goal for healthcare professionals is to deliver pharmacotherapy with the most effective drug with the least side effects. Although ACEi are generally well-tolerated, at least one in five patients will discontinue therapy due to an ADR. Despite its benign nature, ACEi-associated cough constitutes the leading cause for ACEi discontinuation.[56] In this study, we found that ACEi discontinuation polygenic risk prediction can identify people at risk of developing ACEi-associated cough. Considering the low SNP-based heritability (3.8%), environmental factors likely play a larger role than genetics in drug discontinuation. Therefore, if genotyping becomes standard-of-care in healthcare systems, integrating data on both genetic and non-genetic risk factors may facilitate more informed drug selection and improve drug persistence and compliance.

Our study should be interpreted within the context of its limitations. First, UKB prescription data was only available for a subset (~230 000) of the full population. Second, we excluded persons of non-European ancestry to avoid biases due to population stratification. This limits the generalizability of our findings to populations of European ancestry only. Third, given the limited number of available cases with ACEi-associated angioedema, the lack of association with angioedema may reflect limited statistical power and does not imply that the genetic underpinning differs from ACEi-associated cough. Owing to cross-reactivity, a switch from ACEi to ARB is not recommended in the event of angioedema. Moreover, ACEi-induced angioedema is rare, and only a fraction of the total case sample would be attributed to this trait. As such, the *a priori* genetic contribution of ACEi-associated angioedema is likely low.

In conclusion, we found seven risk loci for ACEi discontinuation. Our downstream analyses highlight pathways involved in ACEi-associated cough as the main underlying phenotype. Our results emphasize the relevance of utilizing proxies for ACEi ADRs to provide important insights into the aetiology and genetic architecture of specific ADRs.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Data sharing

Owing to national legislation, no individual-level data will be made available. Summary level data from the GWAS studies will be available after publication from the corresponding author upon request to jonas.ghouse.01@regionh.dk.

Author contributions

J.G., H.H., H.B.U., M.S.O. designed and supervised the study and wrote the manuscript with input from all authors. D.M.R., I.J., K.B., S.B., S.R.O., C.T.P., O.V.P., E.S., L.K., K.I., U.T., G.T., H.U., D.F.G., J.D.M., H.H., KS., and H.B.U. collected the data. J.G., V.T., A.M., M.W.S., G.A., K.B., J.D.M. did the analyses. J.G., V.T., A.M., G.A., I.J., K.B., S.B., U.T., G.T., D.F.G., J.D.M., H.H., H.B.U., and M.S.O. had access to the raw data. All authors reviewed the manuscript, added appropriate revisions, agreed to submission for publication, and

approved the final version. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Ethical approval

The different cohorts had approvals from respective national ethical committees for medical research.

Conflict of interest: The authors who are affiliated with deCODE genetics/Amgen Inc. declare competing financial interests as employees. C.T.P. reports grants from Bayer and grants from Novo Nordisk, outside the submitted work. L.K. reports personal fees and speakers honorarium for Novartis, AstraZeneca, and Boehringer, outside the submitted work. H.B. receives lecture fees from Bristol-Myers Squibb, Merch Sharp and Dohme. S.B. is a board member for Proscion A/S and Intomics A/S. All other authors declare that there is no conflict of interest.

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