Meta-analysis of genome-wide association studies on the intolerance of angiotensin-converting enzyme inhibitors

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Objectives **To identify single nucleotide polymorphisms (SNPs) associated with switching from an angiotensin-converting enzyme (ACE)-inhibitor to an angiotensin receptor blocker.**

Methods **Two cohorts of patients starting ACE-inhibitors were identified within the Rotterdam Study in the Netherlands and the Genetics of Diabetes Audit and Research in Tayside Scotland study in Scotland. Cases were intolerant patients who switched from an ACE-inhibitor to an angiotensin receptor blocker and controls were individuals who used ACE-inhibitors continuously for at least 2 years and did not switch. Genome-wide association study (GWAS) using an additive model was run in these sets and the results were meta-analysed using Genome-Wide Association Meta Analysis software.**

Results **A total of 972 cases out of 5161 ACE-inhibitor starters were identified. Eight SNPs within four genes reached the genome-wide association study significance level ($P < 5 \times 10^{-8}$) in the meta-analysis [RNA binding protein, Fox-1 homolog (*Caenorhabditis elegans*), γ-aminobutyric acid receptor subunit γ-2, sarcoma (Src) homology 2 (SH2) B adaptor protein 1 and membrane bound O-acyltransferase domain containing 1]. The strongest associated SNP was located in an intron of RNA binding protein, Fox-1 homolog (*Caenorhabditis elegans*), which contains an RNA binding protein [rs2061538: minor allele frequency = 0.16, odds ratio = 1.52 (95% confidence interval: 1.32–1.76), $P = 6.2 \times 10^{-9}$].**

Conclusion **These results indicate that genetic variation in the above-mentioned genes may increase the risk of ACE-inhibitor-induced adverse reactions.** ***Pharmacogenetics and Genomics*** **27:112–119**

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Keywords: angiotensin-converting enzyme inhibitors, angiotensin-converting enzyme-inhibitor intolerance, adverse drug reaction, angio-oedema, cough, genome-wide association study

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Introduction

Angiotensin-converting enzyme inhibitors (ACE-inhibitors) are one of the most frequently prescribed groups of medications for the management of high blood pressure, heart failure and renal disease [1]. Although ACE-inhibitors are generally prescribed for lifetime treatment, a cohort study showed that 32.4% of patients halted their medication likely because of adverse drug reactions (ADRs) within a median 336 days of follow-up [2]. The most common ACE-inhibitor-induced ADR is a persistent, dry cough and the most severe one is life-threatening angio-oedema of the lips, tongue and upper airway [3]. There is evidence suggesting genetic predisposition to these ADRs; ACE-inhibitor-induced cough occurs with a higher incidence in East Asian patients (23%) compared with Caucasians (5–11%) [4,5]. The ACE-inhibitor-induced angio-oedema rate is higher in black patients than in white patients and angio-oedema patients often have affected relatives [6,7].

The mechanism of ACE-inhibitor-induced cough and angio-oedema is not completely understood. ACE-

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inhibitors inhibit ACE-I that cleaves several target proteins including angiotensin I and proinflammatory kinins. The blood pressure modification occurs through angiotensin I [8]. Accumulation of these inflammatory kinins is hypothesized to be the main reason of ACE-inhibitor-induced angio-oedema and cough [9,10]. For two decades, multiple candidate genes studies have tested the associations between ACE-inhibitor-induced cough and genetic variation in ACE and bradykinin pathways, of which the insertion/deletion (I/D) variation in the ACE gene has been investigated most frequently [11–14]. A meta-analysis of 12 such studies did not find a statistically significant association for the ACE I/D polymorphism [15]. Studies on ACE-inhibitor-induced angio-oedema have also been carried out using the same approach; three of these found a statistically significant association between ACE-inhibitor-induced angio-oedema and single nucleotide polymorphisms (SNPs) in the *XPNPEP2* gene [16–18]. One study showed that the bradykinin receptor2 (B2) $-9/+9$ polymorphism is associated with both ACE-inhibitor-induced cough and angio-oedema [19]. However, generally, most of the candidate gene approach studies have been difficult to replicate and their results should be interpreted with caution [20]. The only genome-wide association study (GWAS) on 175 ACE-inhibitor-induced angio-oedema cases and 489 controls that also used ACE-inhibitors found no genome-wide association, which might be because of the small sample size [21]. For ACE-inhibitor-induced cough, the only GWAS with 1595 cases and 5485 controls identified genome-wide significant associations in the Kv Channel Interacting Protein 4 gene at chromosome 4 (rs145489027, $P = 1.0 \times 10^{-8}$), which was replicated in two independent populations [22].

On the basis of the probable similar mechanism of ACE-inhibitor-induced ADRs (cough and angio-oedema), this study aims to use a GWAS approach to identify SNPs associated with intolerance of ACE-inhibitors defined as switching of an ACE-inhibitor to an angiotensin receptor blocker (ARB) as a marker for ADRs [23].

Methods

Study population

This study was carried out in two separate European populations:

(1) The Rotterdam study in the Netherlands has been described in detail previously [24,25]. In summary, it is an ongoing cohort, composed of three different subcohorts (RS1–RS3), started in 1990 in Ommoord, a suburb of Rotterdam that has included 14 926 individuals aged 45 years or older (72.0% of 20 744 eligible invited individuals). The Rotterdam Study has been approved by the medical ethics committee according to the Wet Bevolkingsonderzoek: ERGO (Population Study Act: Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants provided informed consent to participate in the study and to obtain information from treating physicians and pharmacies separately.

(2) The [Genetics of Diabetes Audit and Research in Tayside Scotland (Go-DARTS) study] is a genetic substudy of The DARTS that has been described and validated in previous publications [26]. In summary, this project was based on linking clinical records by a patient-specific identifier, enabling the creation and maintenance of sophisticated regional health informatics systems. The DARTS project electronically followed all residents in Tayside since January 1996 ($n = 391\,274$ including 7596 individuals with diabetes) through linking the clinical datasets with a high degree of reliability and accuracy. Collection and analysis of data in DARTS and Go-DARTS were approved by the East of Scotland Research and Ethics Committee, in compliance with the declaration of Helsinki.

Phenotype

For both study populations, similar phenotype definitions were applied for the selection of cases and control:

Cases: Patients who switched to an ARB during ACEI treatment.

Controls: Patients, who started ACE-inhibitors, and continued treatment for at least 2 years. They did not discontinue or switch their ACE-inhibitors during the follow-up.

To define continuation, discontinuation or switching, a maximum gap of 6 months between two prescription periods was considered. These definitions were validated in our previous study as the best marker of ACE-inhibitor-induced ADRs within the prescription databases [23].

Genotyping

Within the Rotterdam study, a total of 12 453 individuals were genotyped with Illumina 500(+ duo) (Illumina Inc., San Diego, California, USA) and Illumina 610 quad and 11 496 individuals passed genotyping quality control. Exclusion criteria for SNPs were a call rate less than 98%, Hardy–Weinberg P-value less than 1×10^{-6}, minor allele frequency less than 0.01%, excess autosomal heterozygosity more than 0.336, sex mismatch and outlying identity-by-state clustering estimates. Data were imputed with the 1000-Genomes reference panel (phase 1, version 3) using MACH version 1.0.15/1.0.16.

Within the Go-DARTS study, individuals were genotyped on the Affymetrix 6.0 (Affymetrix, Santa Clara, California, USA) or Illumina HumanOmniExpress (Illumina, San Diego, California, USA) platforms. Both

Table 1 General characteristics of the angiotensin-converting enzyme-inhibitors starters included

	Go-DARTS (%)			Rotterdam study (%)		
	Case ($n=710$)	Control ($n=3599$)	*P*-value	Case ($n=262$)	Control ($n=590$)	*P*-value
Sex						
Male	51.4	59.8	<0.001	33.59	53.2	<0.001
Female	48.6	40.2		66.41	46.8	
Age [mean (SD)] (years)	62.77 (9.98)	62.45 (10.84)	0.4631	64.47 (6.79)	65.15 (7.69)	0.2177

Go-DARTS, Genetics of Diabetes Audit and Research in Tayside Scotland.

platforms were imputed using IMPUTE2 and the 1000-Genomes reference panel [27]. Individuals were excluded if they fulfilled any of the following criteria: SNPs call rate less than 95%, sample call rate less than 95%, outliers identified by identity-by-state clustering analysis and sex discordant individuals. SNPs deviating from Hardy–Weinberg equation ($P<1\times10^{-6}$) or with an Info Score less than 0.4 were excluded.

Data analyses

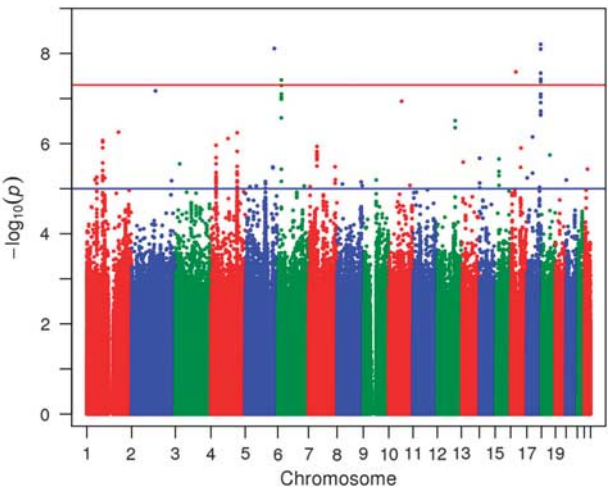
The primary single SNP tests of association were performed using logistic regression assuming an additive genetic model, adjusting for age and sex. PLINK v1.07 was used for the Dutch cohort [28] and SNPTEST-v2.5-beta was used for the Scottish cohort [29]. Fixed-effect meta-analyses were carried out at both sites using the inverse variance weighting, in the Netherlands using METAL and Scotland using Genome-Wide Association Meta Analysis software (GWAMA) [30,31]. The final SNP list in the Netherlands analysis was filtered on the basis of the index of heterogeneity ($I^2<60$) and the number of cohorts that covered an SNP (>two cohorts) [32]. The final values presented in this study are from the analyses in Scotland because GWAMA provides the odds ratios and does not require further calculations; however, the consistency of the results at both sites was considered for the most significantly associated SNPs. Data of SNPs around the most significant gene were visualized using LocusZoom [33]. All other analyses were carried out using SAS v9.3 (SAS Institute, Cary, North Carolina, USA). R packages were used to plot the graphs. Metafor R package was used for the forest plot [34] and the qqman package was used for Manhattan and the QQ plot [35].

Results

A total of 710 ACE-inhibitor intolerant patients and 3599 tolerant controls in the Go-DARTS population and 262 cases and 590 controls in the population of the Rotterdam study were analysed separately and subsequently meta-analysed. 2004 patients from the Go-DARTS population were genotyped using the Illumina chip (GD1) and the rest (2305 patients) were genotyped using the Affymetrix chip (GD2). Three sub populations within the Rotterdam study, RS1–RS3, included 630, 170 and 52 patients, respectively). In both cohorts, the mean age of the patients included was not statistically significantly different between cases and controls. The proportion of women was significantly higher within cases compared with the controls in both cohorts (Table 1).

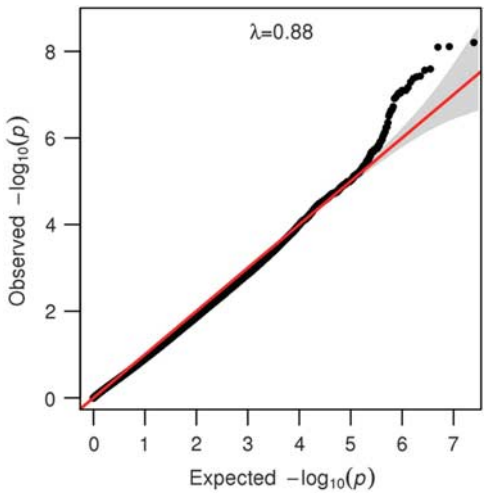
In the meta-analysis of both cohorts using multivariable regression analyses adjusting for sex and age, eight SNPs located on chromosome 5 (one SNP), 6 (one SNP), 16 (one SNP) and 17 (five SNPs) reached a genome-wide significance level ($P<5\times10^{-08}$) (Figs 1 and 2). Table 2 shows the details of the most statistically significantly associated SNPs. From these SNPs, two were only available in the Go-DARTS population (rs192613545 and the I/D polymorphism on chromosome 17 position 77112502). A list of the most significantly associated SNPs that reached a *P*-value of less than 10^{-05} in meta-analysis is available in the supplement in Table 1, Supplemental Digital Content 1, *http://links.lww.com/FPC/B150*. The most significantly associated SNP (rs2061538) was located within the gene RNA binding protein, Fox-1 homolog (*Caenorhabditis elegans*) (*RBFOX3*). There were several other strongly associated SNPs in high linkage disequilibrium with this SNP in that region (Fig. 3a). The second most statistically significant SNP (rs77370934) was located within the gene γ-aminobutyric acid receptor subunit γ-2 (*GABRG2*); however, there were no other

Fig. 1



Manhattan plot of genotyped single nucleotide polymorphisms associated with angiotensin-converting enzyme-inhibitor intolerance using an additive model adjusted for age and sex. The red line indicates the genome-wide significance threshold of $\alpha=5\times10^{-8}$.

Fig. 2



A QQ plot for SNP associations from a meta-analysis of GWAS of ACE-inhibitor intolerance using an additive model adjusted for age and sex ($\lambda = 0.88$). ACE, angiotensin-converting enzyme; GWAS, genome-wide association study; SNPs, single nucleotide polymorphisms.

SNPs with a high level of linkage disequilibrium in that locus (Fig. 3b).

There were also genome-wide statistically significant SNPs within the membrane bound O-acyltransferase domain containing 1 gene (*MBOAT1*) and the sarcoma (Src) homology 2 (SH2) B adaptor protein 1 gene (*SH2B1*).

Figure 4 presents the odds ratio and the 95% confidence interval for the two most statistically significantly associated SNPs for the different sub studies of the Rotterdam study and the Go-DARTS population. Except for the RS3, which is the smallest subpopulation, the effect directions were concordant between the populations.

A high level of consistency was observed for the results of meta-analyses from both sites using the GWAMA and METAL, particularly for the most significantly associated SNPs.

Discussion

Our study describes a large GWAS study investigating SNP variants associated with switching of an ACE-inhibitor to an ARB as a marker for ACE-inhibitor-induced ADRs. All phenotype data for this study were derived from clinical settings that incorporate either the prescription data system (Go-DARTS) or the pharmacy drug-dispensing database (Rotterdam study). We found statistically significant associations with SNPs located within the genes *RBFOX3*, *GABRG2*, *SH2B1* and *MBOAT1*. These are novel candidate genes that may play a role in the ADRs to ACE-inhibitors.

The SNPs showing the strongest association with the phenotype are located on chromosome 17 within the gene *RBFOX3*. This is a member of the *RBFOX* family that in mammals consists of three members: *RBFOX1*, *RBFOX2* and *RBFOX3*. *RBFOX3* is expressed specifically in neuronal cells. This protein contains an RNA recognition motif that binds specifically to an RNA element, UGCAUG, and regulates alternative pre-mRNA splicing. Alternative splicing of pre-mRNA is an important mechanism for post-transcriptional regulation of gene expression and has increasingly been appreciated as a major mechanism to generate a diversity of gene products in higher eukaryotes [36,37].

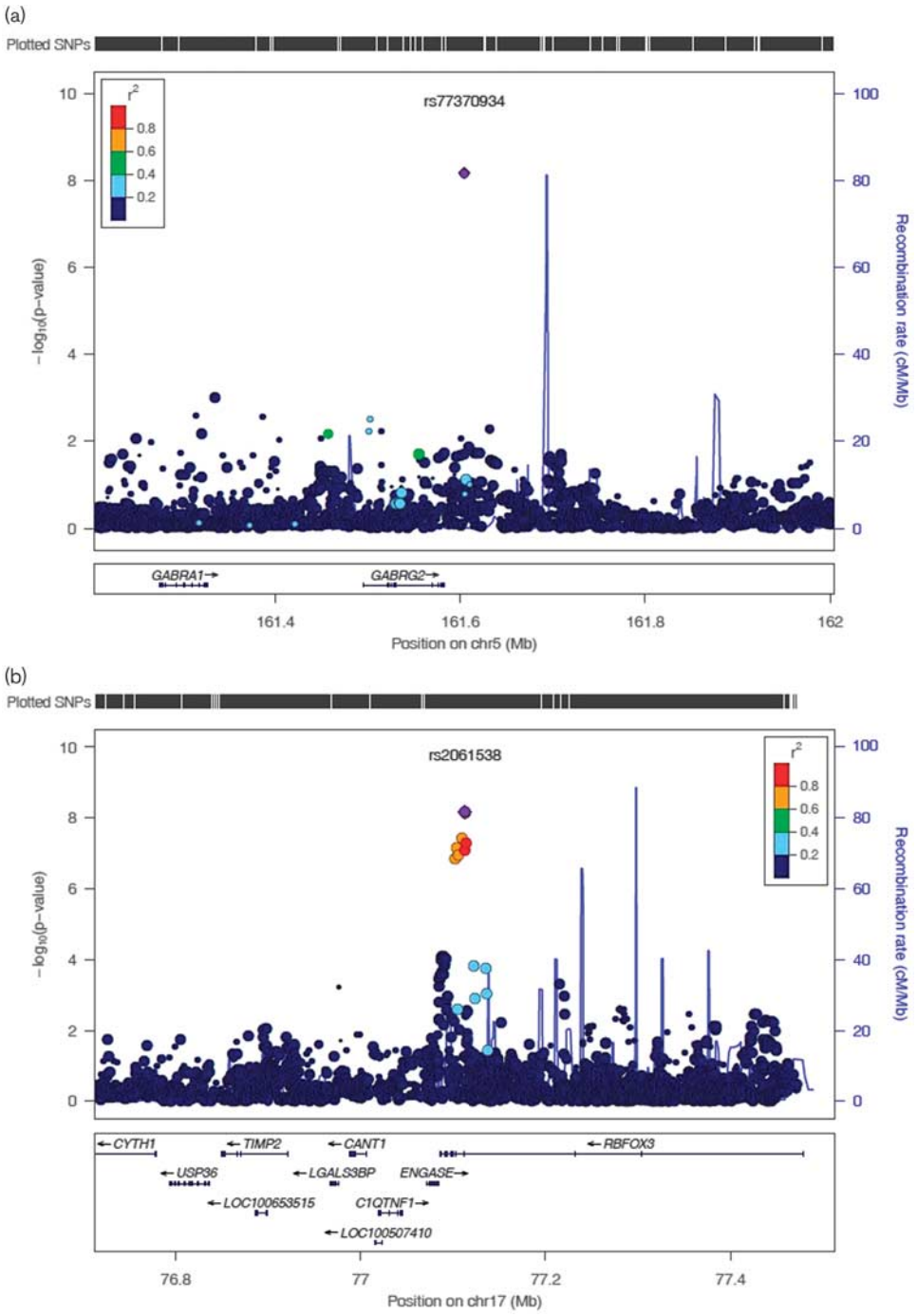
The other most strongly associated SNP was located on chromosome 5 within the gene *GABRG2*, which encodes a γ-aminobutyric acid (GABA) receptor. GABA is the major inhibitory neurotransmitter in the mammalian nervous system, where it acts at GABA-A receptors. GABA-A receptors are pentameric, consisting of proteins from several subunit classes: α, β, γ, δ and ρ [38]. There are several studies proving the effects of GABA receptor agonists in decreasing the sensitivity to cough both in animal models and in humans. This makes them a possible target for cough treatment [39]. Dicpinigaitis *et al.* [40] showed that baclofen (as a GABA receptor agonist) can suppress cough induced by ACE-inhibitors. They also proved in a prospective clinical trial that baclofen can inhibit capsaicin-induced cough [41].

Table 2 Most significantly associated single nucleotide polymorphisms

SNPs	Chr	Position	MA	MAF	OR	95% CI	*P*-value	Genes
rs2061538	17	77112562	G	0.16	1.52	1.3–1.7	6.2×10^{-09}	*RBFOX3*
rs77370934	5	161604254	G	0.03	3.16	2.1–4.6	7.7×10^{-09}	*GABRG2*
rs56209714	17	77113268	G	0.14	1.54	1.3–1.7	7.9×10^{-09}	*RBFOX3*
rs192613545	16	28863901	T	0.07	2.33	1.7–3.1	2.5×10^{-08}	*SH2B1*
Chr17:77112502:I	17	77112502	C	0.14	1.62	1.3–1.9	2.7×10^{-08}	
rs62063838	17	77114028	C	0.17	1.47	1.2–1.6	3.7×10^{-08}	*RBFOX3*
rs10946364	6	20177222	T	0.39	1.34	1.2–1.4	3.8×10^{-08}	*MBOAT1*
rs56044629	17	77109653	G	0.14	1.51	1.3–1.7	4.2×10^{-08}	*RBFOX3*

A list of the most significantly associated SNPs that reached a $P < 10^{-05}$ in meta-analysis is available as supplementary material.
Chr, chromosome; CI, confidence interval; *GABRG2*, γ-aminobutyric acid receptor subunit γ-2; MA, minor allele; MAF, minor allele frequency; *MBOAT1*, membrane bound O-acyltransferase domain containing 1; OR, odds ratio; *RBFOX3*, RNA binding protein, Fox-1 homolog (*Caenorhabditis elegans*); *SH2B1*, sarcoma (Src) homology 2 (SH2) B adaptor protein 1; SNPs, single nucleotide polymorphisms.

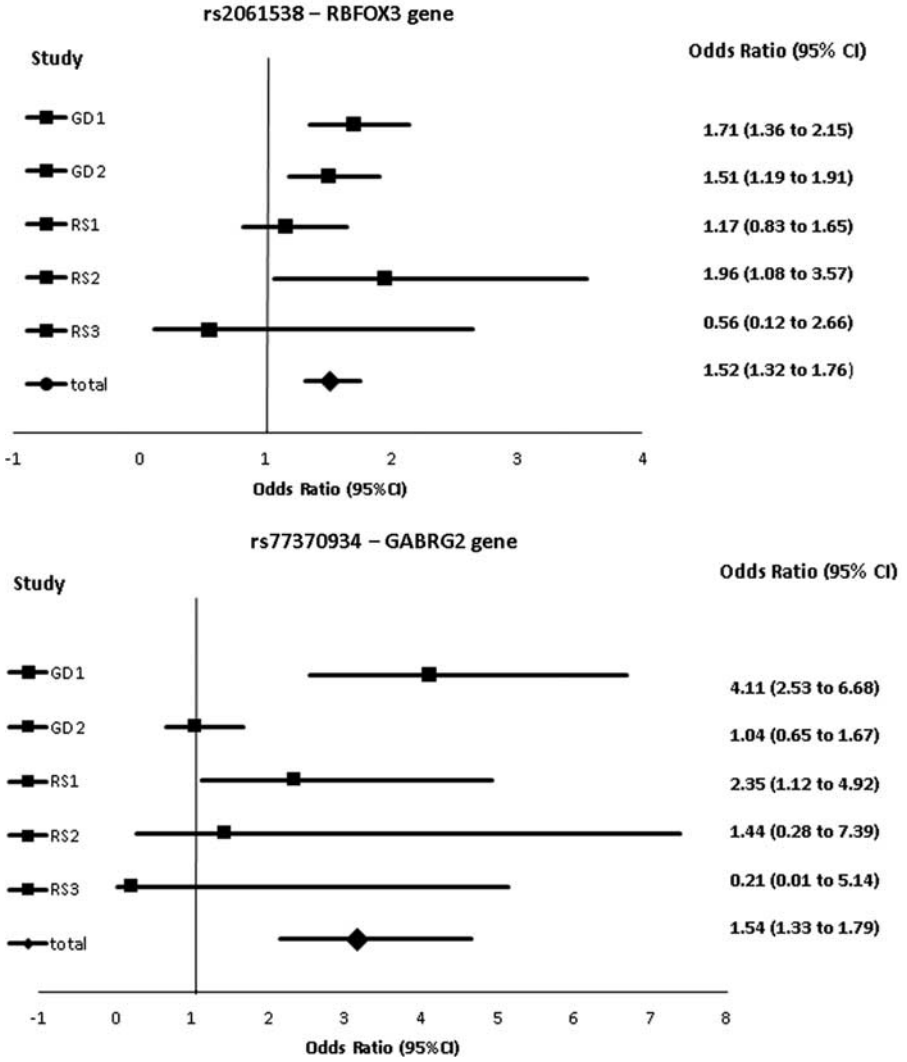
Fig. 3



LocusZoom plot of the most strongly associated SNPs from the meta-analysis located in (a) the region of the most significantly associated genes. (a) The *RBFOX3* (chromosome 17 centred around SNP rs2061538 (shown in purple). Linkage disequilibrium (on the basis of r^2 values) with respect to rs2061538 is based on the CEU reference population. (b) The *GABRG2* (chromosome 5 centred around SNP rs77370934 (shown in purple). Linkage disequilibrium (on the basis of r^2 values) with respect to rs77370934 is based on the CEU reference population. *GABRG2*, γ-aminobutyric acid receptor subunit γ-2; *RBFOX3*, RNA binding protein, Fox-1 homolog (*Caenorhabditis elegans*); SNPs, single nucleotide polymorphisms.

SH2B1 is a member of a family of scaffold proteins implicated in signalling downstream of a variety of receptor tyrosine kinases and cytokine receptors [42]. Variations in this gene have been reported to be associated with obesity [43]; however, its role in the abnormal glucose homeostasis has not been proved [44]. The significant association of this gene with the intolerance of ACE-inhibitors needs to be investigated further

Fig. 4



The forest plot from the meta-analyses of the most strongly associated SNPs. CI, confidence interval; *GABRG2*, γ-aminobutyric acid receptor subunit γ-2; GD, Genetics of Diabetes Audit and Research in Tayside Scotland; RS, Rotterdam study; SNPs, single nucleotide polymorphisms.

because there was no previous report of this gene contributing in cough or angio-oedema.

MBOAT1 belongs to the superfamily of *MBOAT* that transfer organic compounds, usually fatty acids, onto hydroxyl groups of membrane-embedded targets [45]. This trans-membrane protein has been reported to be involved in developmental processes [46].

The main hypothesized mechanism of ACE-inhibitor induced ADRs (mainly cough and angio-oedema) is the stimulation of sensory nerve resulting from the accumulation of inflammatory mediators that are normally cleaved by the ACE [3]. This hypothesis has served as the basis for candidate gene studies that have focused on variations in inflammatory pathways; however, findings of those candidate gene studies were replicated inconsistently and the meta-analyses of loci that had sufficient studies did not find a significant effect for the I/D polymorphism within the ACE gene [15]. Hypothesis-free GWA studies may lead to the finding of novel loci to be associated with ADRs of ACE-inhibitors. The only available large GWAS on ACE-inhibitor-induced cough found an association with Kv Channel Interacting Protein 4, which is predominantly expressed in nervous systems [22]. However, the only available GWAS on the ACE-inhibitor induced angio-oedema with 175 ACE-inhibitor-induced angio-oedema cases and 489 controls could not find any significant association on a genome-wide level, which could be because of the relatively small sample size and lack of power [21]. Our results suggest that an important source of variation may be directly related to the sensory nerves themselves because both *GABRG2* and *RBFOX3* genes

play a role in the central and peripheral nervous systems as well. These findings are in line with the previous GWAS on ACE-inhibitor-induced cough [22].

This study is a large GWAS on the intolerance of ACE-inhibitors within a population of European ancestry. However, the direct relevance of our findings with ACE-inhibitor-induced ADRs is not yet clear and needs to be investigated further; these findings, if replicated in other populations, can improve our understanding of the biological mechanism of ACE-inhibitor-induced ADRs. Furthermore, it will help to identify those patients at high risk of developing ACE-inhibitor-induced ADRs including angio-oedema, which is a life-threatening event. We recently showed that ~50% of ACE-inhibitor users continue using ACE-inhibitors after the first episode of angio-oedema [47]. Identification of those patients at high risk could help physicians guide their treatment choice. ACE-inhibitor-induced cough is not as life-threatening as angio-oedema, but it can be misdiagnosed and mistreated, which significantly decreases the compliance of patients and might finally result in unsuccessful drug therapy [48,49]. Therefore, in the context of precision medicine, the ultimate application of these findings within the clinic would be the prediction of susceptible patients and their treatment with an alternative medication with comparable effect such as ARBs [50].

An important limitation of this study is defining phenotype on the basis of the electronic medical records, which could potentially lead to misclassification of cases and controls. However, in a validation study, the proxy marker for cases showed a positive predictive value of 68.3% for probable ACE-inhibitor-induced ADRs [23]. This study also could not detect associations for rare SNPs (minor allele frequency <0.01%). The study results are restricted to the European ancestor populations.

Conclusion

This study used a GWAS to identify SNP variants associated with ACE-inhibitor intolerance as a marker of ADRs. We identified SNPs in the genes RBFOX3, GABRG2, SH2B1 and MBOAT1 as potential candidates for ACE inhibitor-induced ADRs. Because of the fact that this is a hypothesis-generating study, the functional role of significantly associated genes was not investigated; therefore, future studies are needed to replicate our findings, and epigenetic and molecular studies are also needed to explore the functional roles of variations within genes reported in this study, specifically the *GABRG2* gene, for which several clinical studies have also shown its role in susceptibility to cough [39–41]. The standard clinical criteria have been described for ACE-inhibitor-induced angio-oedema [51] and to enable a combination of results, it would be optimal if new genetic association studies used this standard phenotype in the future.

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Conflicts of interest

There are no conflicts of interest.

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