Adverse drug reactions of angiotensin converting enzyme inhibitors:

Towards precision medicine

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Adverse drug reactions of angiotensin converting enzyme inhibitors:

Towards precision medicine

Bijwerkingen van angiotensine I converterend enzym (ACE)-remmers:

Op weg naar geïndividualiseerde farmacotherapie

(met een samenvatting in het Nederlands)

Proefschrift

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To my family

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Chapter 1 | General introduction

History of angiotensin converting enzyme inhibitors (ACEIs)

In the mid-1950s the angiotensin converting enzyme (ACE) was identified to be responsible for the conversion of angiotensin I into angiotensin II. Angiotensin II is an active vasoconstrictor substance [1]. In 1968, John R. Vane (a Nobel Prize winner) showed that peptides from the Brazilian viper's venom inhibited the activity of ACE in the lung of a dog [2]. Afterwards the US pharmaceutical company, "ER Squibb and Sons" (now part of Bristol Myers Squibb), started the ACE inhibitor programme to develop synthetic ACE inhibitors that were orally active. After testing thousands of chemical structures for ACE inhibitor activity, they finally discovered captopril, and early clinical studies confirmed its antihypertensive effects. The first ACEI was launched on the market in 1981 [3].

Mechanism of action

The renin-angiotensin-aldosterone system (RAAS) is an important regulator of the hemodynamic stability in the body by controlling circulating volume and electrolyte balances. This function is conducted through regulating extracellular fluid volume, sodium balance and cardiovascular function via direct and indirect effects on several organ systems and also interactions with the autonomic nervous system [4–6]. The physiological importance of the RAAS is the compensation of hypovolemia, hyponatremia and hypotension. In people with normal blood pressure and a balanced salt homeostasis, the RAAS is not activated. Decrease in the perfusion of the juxtaglomerular apparatus, increases the production of renin from the kidney. Subsequently a cascade of hormones is triggered by the release of renin [7,8]. Angiotensinogen is a protein precursor produced in the liver and is cleaved by renin to form an inactive peptide angiotensin I, which is converted to the active octapeptide angiotensin II by the ACE. ACE is a zinc metalloprotease, mainly generated by the lungs, the cell membranes of the kidneys and the endothelial cells of the vasculature [8]. Therefore, the serum concentrations of ACE determine the levels of angiotensin II, which is the active metabolite of the system through which the RAAS mediates its main effects. There are at least two classes of angiotensin II receptors (AT1 and AT2); most of the vascular effects of angiotensin II are mediated by AT1 receptors. AT1 receptors are serpentine receptors coupled by a G-protein to phospholipase C, and angiotensin II increases the cytosolic free Ca2+ level. AT1 receptors are distributed in both vascular smooth muscle and the adrenal cortex, AT2 receptors are more present in foetal and neonatal life, but they persist in the brain and other organs in adults [9]. Angiotensin II produces arteriolar constriction and a rise in systolic and diastolic blood pressure, it also acts directly on the adrenal cortex to increase the secretion of aldosterone, and the renin-angiotensin system is a major regulator of aldosterone secretion. Aldosterone increases sodium and water reabsorption in the renal collecting tubule cells, and causes potassium excretion. This increases the volume of extracellular fluid in the body [10]. Furthermore on the autonomic level, angiotensin II enhances the release of sympathetic transmitter by a presynaptic action, it can also inhibit norepinephrine reuptake.

Excessive stimulation of this cascade causes pathologic changes. For instance, an overactive RAAS is associated with high levels of tissue ACE, and this leads to hypertension, renal injury, atherosclerosis and left ventricular dysfunction [5,11]. On the other hand, blocking of an activated RAAS has become a key therapeutic target in a wide range of diseases, such as hypertension, heart failure, renal disease, and atherosclerotic cardiovascular disease. Currently the most frequently prescribed examples of pharmacologic agents that block the RAAS are the ACEIs. Furthermore, angiotensin receptor blockers (ARBs) act directly on the angiotensin II receptor and antagonise

its action. Direct renin inhibitors are also available now and they block the renin directly from converting angiotensinogen to angiotensin I.

Indications

ACEIs have been approved for several indications. Guidelines for the pharmacologic management of hypertension issued by the World Health Organization and the International Society of Hypertension placed ACEIs with diuretics and beta blockers as first-line therapy in 1997, however the recent Report in 2014 did not recommend beta blockers for the initial treatment of hypertension and instead diuretics and calcium channel blockers are recommended with either ACEIs or ARBs [12,13].

ACEIs are first-line therapy in patients with left ventricular systolic dysfunction. Efficacy has been confirmed in multiple trials and meta-analyses. Decreases in dyspnoea, emergency department visits, hospitalizations, disease progression and death have been shown, as well as increases in ejection fraction and exercise tolerance. Furthermore the need for diuretics can also be decreased by using ACEIs [14–16].

ACEIs are also used post myocardial infarction (MI); a pragmatic approach is to give ACEIs to all patients with acute MI who are clinically stable and to continue that therapy indefinitely in those with anterior myocardial infarction or systolic dysfunction, others should be re-evaluated for continuation of therapy at four to six weeks [17]. Newer guidelines suggest them for patients with anterior infarction, post-MI left ventricular systolic dysfunction (ejection fraction ≤ 0.40) or heart failure [18].

ACEIs slow the onset and progression of diabetic nephropathy in patients with microalbuminuria and diabetes [19]. ACEIs in nondiabetic patients with nephropathy are more effective than other antihypertensives at slowing progression to end-stage renal disease [20].

Adverse drug reactions of ACEIs

The most important adverse drug reactions (ADRs) in users of ACEIs, cough and angioedema are probably caused by the accumulation of bradykinin, which is a substrate of ACE. However, there are still lots of controversies about the real mechanisms of ACEI-induced ADRs. Cough is the most prevalent ADR with a reported incidence ranging from 5 to 35 % which may occur months and even years after ACEI initiation [21,22]. ACEI induced cough is not dose dependent and is more common in women [23]. The persistent and troublesome nature of the cough usually results in discontinuation of ACEIs, after which the adverse reaction will often abate within a few days. Substitution of the ACEI with alternative agents, specifically ARBs, is recommended. However cough can be misdiagnosed or mistreated temporarily by anti-cough agents [24,25]. More rarely, patients can develop potentially life-threatening angioedema that occurs in an estimated 0.1–0.7% of patients [26]. This life-threatening angioedema is the most severe ADR of ACEIs that mostly occurs at the larynx or tongue [27] and less frequently at the gastrointestinal wall [28]. If ACEIs are continued after the first angioedema, the recurrence risk of such angioedema is high and the reaction is even more severe, therefore discontinuation of ACEI treatment after the first angioedema is highly recommended [29].

Personalized medicine

When the efficacy of a drug is proven on a population level, it may still fail to work in individual patients or may cause serious ADRs. Not only patients but also physicians and pharmaceutical

companies will benefit from the possibility to identify which patients are likely to be non-responders as well as patients with an increased risk of ADRs. These patients can be treated with an adjusted dose or an alternative medication to optimize the clinical outcomes. The basis of inter-individual variability in response to the treatment, however, is in most cases complex and multifactorial. Potential causes of differences in medication response include among others age, gender, co-medication, co-morbidities, racial background and genetic polymorphisms. Using biomarkers to prescribe the right medication to the individual patient is called personalized medicine. Pharmacogenetics, investigates the contribution of genetic factors in predicting variability in both efficacy and safety of a drug. In the beginning, studies on pharmacogenetics were hypothesis-driven and focused on single genetic variants (single nucleotide polymorphisms, SNPs) in candidate gene association studies. However it became clear that most of the results from those studies were not replicated in subsequent studies including meta-analyses. One review showed that out of the 166 putative associations which have been studied three or more times, only 6 have been consistently replicated [30]. The association of ACE gene insertion/deletion polymorphism with ACEI-induced cough is an example of conflicting results [31]. After decoding the human genome, expectations of personalized medicine were high. A sharp rise of publications in the field of pharmacogenetics clearly reflects the interest in pharmacogenetics and the increase in technological possibilities [32]. After completion of the human genome project in 2003, in combination with the rapid development of new genotyping technologies, the comprehensive assessment of genetic variation became possible [33]. Systematic cataloguing of common SNPs, in the HapMap project allowed for the design of genome-wide chips [34]. Nowadays more hypothesis-free research is performed using genome-wide association studies (GWAS) which is a less biased approach in genetic studies [35]. Despite the dramatically decreasing trends in the cost per SNP, GWAS arrays are still relatively expensive and because of the large number of SNPs tested in a GWAS, a strict correction for multiple testing is necessary to prevent false positive findings. Assuming relatively modest effect sizes for individual SNP variants, large sample sizes are required to ensure reasonable statistical power to detect an association and these are the main reasons that most of the GWAS are collaborative projects within a consortium [36]. GWAS is generally accepted as the most robust way of investigating common genetic variation (having minor allele frequencies (MAF) greater than 5%), but for rare variations (with MAF <1%) there are 2 options: first is genotype imputation, predicting genotypes that are not directly assayed using special algorithms [37] and the second one is the next-generation (DNA) sequencing, the process of determining the order of all bases in one's genome [38]. There is evidence showing that genetic factors are involved in the risk of developing angioedema and cough due to ACEIs. [39,40]. Angioedema for instance is a very rare ADR of ACEIs that may be potentially caused by the rare genetic variations because the only available GWAS on ACEI-induced angioedema could not find an association with common genetic variations, [41] therefore a well-powered genetic study using the next-generation genotyping method can answer the questions regarding to the contribution of genetic variations to this rare ADR.

Objectives and outline of thesis

The objective of this thesis was to describe the usage of ACEIs and to investigate the occurrence of ACEI-induced ADRs in population based databases; subsequently to study the genetic and non-genetic factors associated with those ADRs. **Chapter 2** of this thesis focuses on the pattern of usage for ACEIs within a large population; **chapter 2.1** gives an overview of how ACEIs are being used in daily practice in the United Kingdom using the large population based clinical

practice research datalink (CPRD), while in **chapter 2.2** the pattern of ACEIs use was studied after the life-threatening angioedema had occurred. In **chapter 3**, a proxy for ACEI-induced ADRs specifically cough was identified to be used in prescription data. In **chapter 4** non-genetic factors associated with ACEI-induced ADRs were investigated using the electronic health records from CPRD. **Chapter 5** focuses on genetic factors associated with ACEI-induced ADRs, in **chapter 5.1** all published genetic association studies on ACEI-induced ADRs were reviewed and meta-analysed where possible. In **chapter 5.2** a genome wide association study (GWAS) on the intolerance of ACEIs was performed in 2 separate white populations and then meta-analysed to search for novel genetic loci associated with ACEI switching to ARBs as a marker of ADRs. Finally, the results of this thesis were summarized and discussed in **chapter 6**. In this chapter the findings of the thesis are translated into recommendations for practice and future research and ends with final conclusions.

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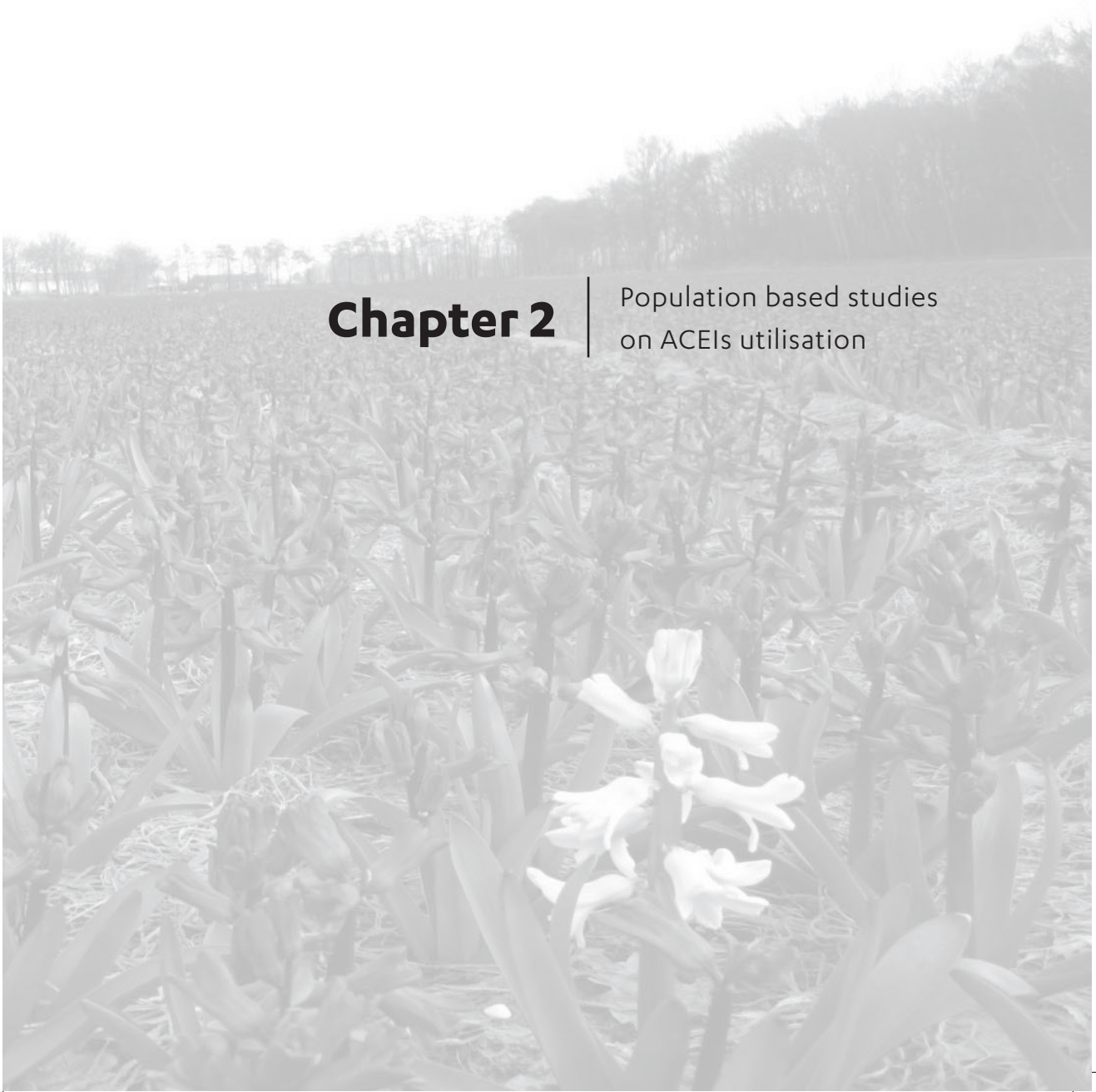

Chapter 2

Population based studies
on ACEIs utilisation

Chapter 2.1

Patterns of angiotensin converting enzyme inhibitors prescribing for different indications: a population based study

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Submitted for publication

ABSTRACT

Objective

To investigate usage patterns for different indications of angiotensin converting enzyme inhibitors (ACE-inhibitors).

2.1

Methods

Patients older than 45 years who started ACE-inhibitor treatment between 2007 and 2014 were selected from the Clinical Practice Research Datalink. Indications for ACE-inhibitor treatment were retrieved from the medical records. Stratified by indication we distinguished between persistent use and non-persistent use, considering a 6 months' time interval between two prescription periods as a maximum for persistent use. Five-year persistence rates among the different indications were calculated using the Kaplan-Meier method and compared by the log-rank test. Non-persistent users were subdivided into stop, restart and switch to an angiotensin II-receptor blocker (ARBs) groups. Furthermore for the patients with the indication of hypertension, switching to other antihypertensive categories was investigated.

Results

In total 254,002 patients initiating ACE-inhibitors were identified with the following indications: hypertension (57.6%), myocardial infarction (4.2%), renal disease (3.7%), heart failure (1.5%), combinations of them (17.2%) and none of the above (15.8%). Five-year persistence rates ranged from 43.2% for renal disease to 68.2% for myocardial infarction ($p<0.0001$). Renal disease and heart failure patients used ACE-inhibitors for the shortest period of time (average 23.6 and 25.0 months, respectively). Within the non-persistent group the percentages of switchers to ARBs ranged from 27.6% for renal disease to 42.2% for myocardial infarction and for the restarter group ranged from 15.0% for heart failure to 18.1% for the group without indication retrieved.

Conclusion

Dependent on the indication there are different rates of ACE-inhibitor non-persistence. Patients with renal disease are most likely to discontinue treatment.

Keywords

ACE-Inhibitors, prescription pattern, medication persistence, hypertension, heart failure, myocardial infarction, renal disease, angiotensin II receptor blocker.

INTRODUCTION

ACE-inhibitors are one of the most frequently prescribed groups of medication; ramipril for instance was the first antihypertensive medication in 2013 with more than 24 million prescriptions dispensed in community pharmacies in the United Kingdom [1]. ACE-inhibitors are commonly used in the treatment of hypertension, heart failure, myocardial infarction and renal disease. It has been demonstrated that these drugs decrease morbidity and mortality of cardiovascular disease especially in patients with hypertension and heart failure [2–4]. Studies on the use of antihypertensive medications consistently showed that after angiotensin II-receptor blockers (ARBs), ACE-inhibitors had the lowest risk of discontinuation among all groups of antihypertensive medications [5–9]. Nonetheless, a substantial number of patients discontinue therapy with ACE-inhibitors. An American cohort study of more than 2200 outpatients, who received ACE-inhibitors for the first time with a median follow up time of 336 days, showed that 19% of the patients discontinued ACE-inhibitors because of adverse drug reactions (ADRs) [10]. In another study on ACE-inhibitors utilisation in a pharmacy drug dispensing database, Vegter *et al* reported that approximately 24% of ACE-inhibitor starters switched their therapy within the first 3 years and 75% of them switched to ARBs [11]. With a longer follow up time compared to Vegter's study, the percentage of ACE-inhibitor switchers increased to more than 40% in a large population based cohort of newly diagnosed hypertensive patients in the UK which contained a subgroup of more than 36,000 ACE-inhibitor starters with a maximum of 9 years follow up [12].

No study has investigated whether the persistence to ACE-inhibitors differs between indications. A prior study did show a different pattern of beta blocker therapy in such a way that patients with myocardial infarction were less likely to stop beta blockers than patients with heart failure or angina pectoris [13]. The aim of this study is to investigate whether the pattern of ACE-inhibitors use depends on indication in terms of persistence rate, stop, restart and switching to ARBs.

METHODS

Setting

The data used for this study were obtained from the Clinical Practice Research Datalink (CPRD) formerly known as General Practice Research Database (GPRD) which contains the computerized information entered by almost 700 primary care practices in the UK and at the time of this study CPRD covered clinical records of almost 12 million patients. Validity and detailed description of available data in CPRD has been described before [14, 15].

The protocol for this study was reviewed and approved by the independent scientific advisory committee (ISAC) in the UK with the protocol number: 14_030R

Study cohort

A descriptive retrospective cohort study was conducted comprising patients aged 45 years and older who newly started ACE-inhibitor therapy between 1 January 2007 and 1 January 2014. These patients had at least 12 months of valid prescription history before starting the ACE-inhibitor and at least 6 months of valid prescription data after that in order to be able to assess persistence to ACE-inhibitors.

Follow up

Subjects were followed until the end of study period (1 January 2014), time of death or date of move of the patient outside of the practice area. The index date was the date of the first ACE-inhibitor prescription and baseline characteristics were recorded. In order to categorize patients according to the indication for ACE-inhibitor treatment initiation, we assessed whether patients had a diagnosis (based on relevant read codes) of hypertension, heart failure, myocardial infarction or renal disease any time prior to the index date or in the first year thereafter. Patients with more than one indication and patients, for whom we could not retrieve any of the above indications within that period, were classified in separate categories.

Prescription pattern:

According to the prescription data, starters with ACE-inhibitors were divided into 2 main categories, described below:

1. Persistent group: patients who started ACE-inhibitors and continued until the end of follow up.
2. Non-persistent group: patients who stopped receiving ACE-inhibitors prescriptions for at least 6 months after the theoretical end date of the previous ACE-inhibitor prescription. The discontinuation date was defined as the theoretical end date of the last ACE-inhibitor prescription which was calculated by dividing the quantity of medications of the prescription by the number of daily dose. The non-persistent group was divided into 3 mutually exclusive subgroups according to the treatment pattern after the discontinuation of ACE-inhibitor (Figure 1).

A. Stop group: Patients who stopped ACE-inhibitors and never restarted ACE-inhibitors until the end of follow up and also did not start ARBs, within 6 months after the theoretical end date of the last ACE-inhibitor prescription.
B. Switch group to ARBs: Patients who stopped ACE-inhibitors and started ARBs, within 6 months after the theoretical end date of the last ACE-inhibitor prescription. Additionally, for the hypertension indication, switches to other antihypertensive medications (beta blockers, diuretics, calcium channel blockers, other antihypertensives which are alpha blockers, vasodilators and centrally acting antihypertensives) were investigated.
C. Restart group: patients who stopped or switched their ACE-inhibitors according to the above definitions but during follow up time they restarted ACE-inhibitor therapy.

Statistical analyses

Baseline characteristics for all ACE-inhibitor starters were reported separately for each indication (hypertension, heart failure, myocardial infarction, renal disease, more than one indication and none of the above). 5-year persistence rates and the time to discontinuation among the different indications were calculated and compared using the Kaplan-Meier method and the log-rank test, respectively. Patients who started an ACE-inhibitor and had a follow up of less than 6 months were excluded. To evaluate the influence of these exclusions we performed a sensitivity analysis in which the included patients were also analysed once considering them all as persistent ACE-inhibitor users and the other time considering them as non-persistent users. All statistical analyses were performed using SPSS 20 (IBM SPSS Statistics for Windows Version 20.0. Armonk, NY: IBM Corp).

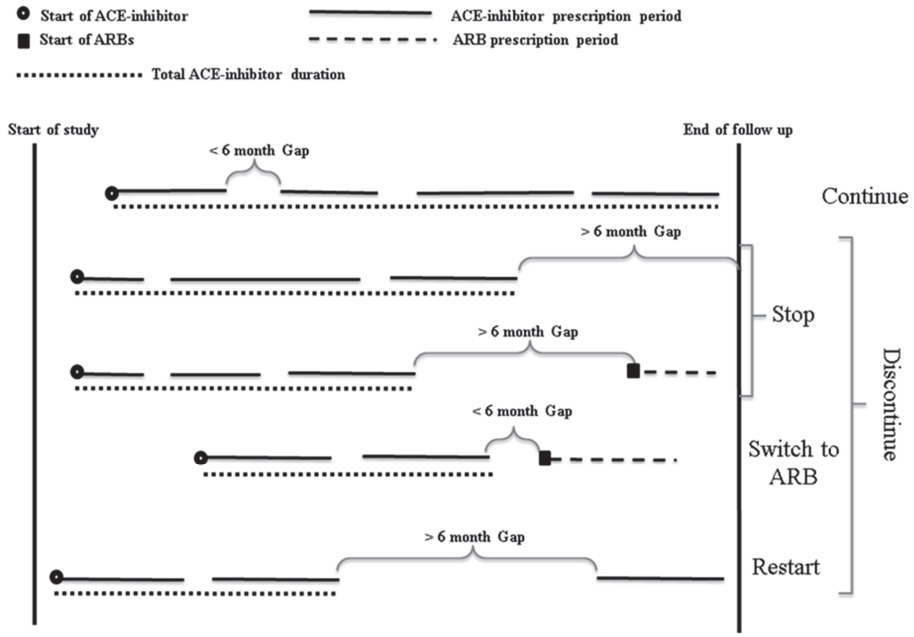

Figure 1. Definition of ACE-inhibitor use pattern

RESULTS

There were 276,973 eligible patients that started with an ACE-inhibitor of which 22,971 patients were excluded from analyses because of less than 6 months follow up time. Table 1 presents the general characteristics of 254,002 patients (51.5% male) at the first date of ACE-inhibitor prescription and the average follow up time, average duration of ACE inhibitor use and proportions of deceased patients during follow up stratified by indication. The majority of included patients started with an ACE-inhibitor because of hypertension (57.6%) and the smallest group was for heart failure (1.5%). The patient group with more than one indication was 17.2% out of which 90.1% had hypertension as one of the indications. Patients who started an ACE-inhibitor because of heart failure and renal disease were on average approximately 9 years older than patients who started an ACE-inhibitor for myocardial infarction or hypertension. The highest percentages of death were in patients with heart failure and more than one indication (21.5% and 15.4%, respectively). The mean duration of ACE-inhibitor use was longest for myocardial infarction patients (30.5 months) and shortest for renal disease and heart failure patients (23.6 and 25.0 months, respectively). (Table 1)

Table 2 shows the patterns of ACE-inhibitors use by indication. In the total study population, 60.3% of ACE-inhibitors starters continued ACE-inhibitors till the end of follow up. From the non-persistent patients, 45.3% stopped, did not switch to ARBs within 6 months and never restarted ACE-inhibitors, 37.1% switched to ARBs and 17.6% restarted ACE-inhibitors after at least 6 months of discontinuation.

Table 1: Demographic and clinical characteristics at the first ACE-inhibitors prescription date for different indications (N= 276,977 patients).

Characteristics	Heart failure 1.5%	Hypertension 57.6%	Myocardial infarction 4.2%	Renal disease 3.7%	More than one indication 17.2%	None of the mentioned indications 15.8%	Total 100%
Mean age [1] (years) [SD]	72.1 [11.8]	62.7 [11.0]	64.1 [11.1]	72.6 [11.0]	73.4 [10.8]	64.1 [11.5]	65.3 [11.9]
Sex (%male)	60.1%	50.1%	76.2%	43.5%	45.0%	58.0%	51.5%
Mean follow up (months) [SD]	35.2 [20.9]	43.7 [22.2]	39.2 [21.9]	41.8 [22.6]	43.7 [23.0]	39.1 [21.9]	42.6 [22.4]
Mean ACE-inhibitor duration (months) [SD]	25.0 [21.5]	28.8 [25.1]	30.5 [23.3]	23.6 [23.3]	28.1 [25.1]	24.9 [23.2]	27.9 [24.7]
Percentage of death	21.5%	4.2%	7.1%	13.8%	15.4%	7.4%	7.3%

[1]Ages were recorded at the first ACE-inhibitors prescription date
SD: Standard deviation

Patients who started an ACE-inhibitor for myocardial infarction had the highest probability of remaining on initial ACE-inhibitor treatment (73.6%). Patients who started an ACE-inhibitor because of renal disease were most likely to discontinue their ACE-inhibitors use with 49.2% of total ACE-inhibitors starters and 54.5% of those non-persistent patients actually stopped and did not restart ACE-inhibitors or switched to ARBs which was the highest percentage among all indications.

The percentages of switch from ACE-inhibitors to ARBs ranged from 27.6% in renal disease patients to 42.2% in myocardial infarction patients. Within the hypertension indication, 17.2% of those patients who stopped ACE-inhibitor and did not restart or switched to ARBs, actually switched to calcium channel blockers which was the highest percentage, followed by a switch to diuretics (6.3%), combination of antihypertensives (5.0%) or beta blockers (3.6%). The same pattern was observed for patients with hypertension combined with other indications (10.0% switched to calcium channel blockers, 6.3% to diuretics, 3.3% to a combination of antihypertensives or 3.2% to beta blockers)

Kaplan-Meier curves of ACE-inhibitor use for different indications are presented in Figure 2. 5-year persistence rates for different indications were 68.2% for myocardial infarction, 58.6% for heart failure, 56.4% for hypertension, 53.4% for none of the mentioned indication, 53.0% for more than one indication and 43.2% for renal disease (log-rank p-value <0.0001).

Sensitivity analyses, including the 22,971 patients with less than 6 months follow up time changed the percentages for persistent and non-persistent patients for myocardial infarction to 53.1% and 45.1% and for renal disease to 34.4% and 23.6%, respectively.

Table 2. Pattern of ACE-inhibitor use stratified for different indications.

Indication (Numbers)	Pattern(Numbers) Percentage	
Heart failure (n= 3,762)	Persistent (n= 2,507) 66.6%	
	Non-persistent (n= 1,255) 33.4%	Stop (n= 561) 44.7%
		Switch to ARB (n= 506) 40.3%
		Restart (n= 188) 15.0%
Hypertension (n= 146,275)	Persistent (n= 88,632) 60.6%	
	Non-persistent (n= 57,643) 39.4%	Stop [1] (n= 24,206) 42.0%
		Switch to ARB (n= 23,271) 40.4%
		Restart (n= 10,166) 17.6%
Myocardial infarction (n= 10,639)	Persistent (n= 7,826) 73.6%	
	Non-persistent (n= 2,813) 26.4%	Stop (n= 1,200) 42.7%
		Switch to ARB (n= 1,187) 42.2%
		Restart (n= 426) 15.1%
Renal disease (n= 9,299)	Persistent (n= 4727) 50.8%	
	Non-persistent (n= 4,572) 49.2%	Stop (n= 2,493) 54.5%
		Switch to ARB (n= 1,262) 27.6%
		Restart (n= 817) 17.9%
More than one indication (n= 43,753)	Persistent (n= 25,555) 58.4%	
	Non-persistent (n= 18,198) 41.6%	Stop [2] (n= 8,399) 46.2%
		Switch to ARB (n= 6,650) 36.5%
		Restart (n=3,149) 17.3 %

Table 2. Pattern of ACE-inhibitor use stratified for different indications. *(Continued)*

Indication (Numbers)	Pattern(Numbers) Percentage	
None of the mentioned indications (n= 40,274)	Persistent (n= 23,965) 59.5%	
	Non-persistent (n= 16,309) 40.5%	Stop (n= 8,817) 54.1%
		Switch to ARB (n= 4,545) 27.9%
		Restart (n= 2,947) 18.1%
Total (n=254,002)	Persistent (n=153,212) 60.3%	
	Non-persistent (n=100,790) 39.7%	Stop (n= 45,676) 45.3%
		Switch to ARB (n= 37,421) 37.1%
		Restart (n=17,693) 17.6%

[1] 17.2% switched to calcium channel blockers, 6.3% to diuretics, 5.0% to a combination of antihypertensives, 3.6% to beta blockers and 0.1% to other antihypertensives.
[2] This group was divided in 2 subgroups: more than one indication including hypertension (90.5%) and not including hypertension (9.5%). Within the first group, 10.0% switched to calcium channel blockers, 6.3% to diuretics, 3.3% to a combination of antihypertensives, 3.2% to beta blockers and 0.3% switched to other antihypertensives.
CI: confidence interval, ARB: Angiotensin II-receptor blockers.

DISCUSSION

This study showed that patterns of ACE-inhibitors use differ between indications. Patients with renal disease discontinued their ACE-inhibitors therapy more frequently and used ACE-inhibitors for a shorter period compared with patients who started ACE-inhibitors for other indications. 5-year non-persistence rates ranged between 31.8% for myocardial infarction to 56.8% for renal disease.

Hypertension, renal disease and heart failure were the three main indications of ACE-inhibitors that were studied for drug utilisation patterns previously. Within patients using antihypertensive drugs, the risk of drug therapy modification is 3.5 times higher for patients who reported medication problems compared to those who did not report such problems. This resulted in 1.9 times higher risk of uncontrolled blood pressure [16, 17]. Several socio-demographic factors have been shown to be associated with non-persistence to antihypertensive therapy [18] which can eventually result in poor clinical outcome [19]. Additionally when ACE-inhibitor therapy is continued despite putative cough as a medication problem, the compliance of patients was shown to be 20% less, and this non-compliance could ultimately result in non-persistence to therapy; however, the real reason of ACE-inhibitors discontinuation could not be retrieved directly [20]. Within hypertensive patients, two studies using the same source population (égie de l'assurance maladie du Québec administrative database) showed that among all antihypertensive users and specifically among ACE-inhibitor users, those who have more risk factors for cardiovascular events are more persistent with their drug therapy [21, 22]. Patients who start ACE-inhibitors

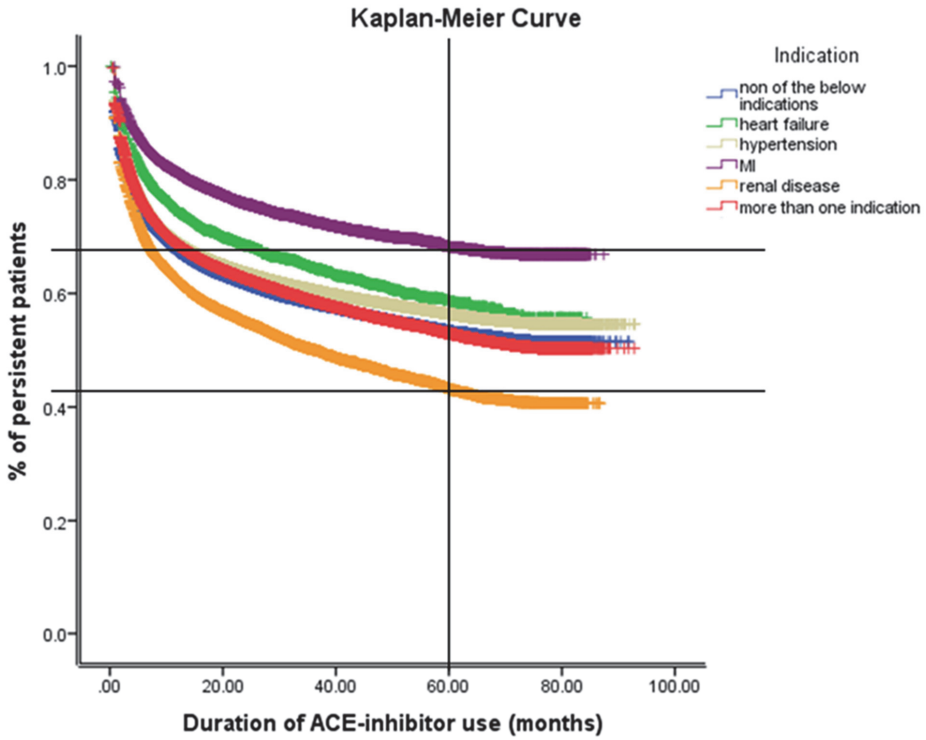

Figure 2: Comparing the non-persistence rates of ACE-inhibitors use between different indications.

for renal disease are -next to the common side effect coughing- more susceptible to adverse effects like renal function deterioration or hyperkalemia because of the combination of both the mechanism of drug action with the complications of the disease. Therefore it is not uncommon for this indication that ACE-inhibitors are recommended to be discontinued either permanently or temporarily [23]. Additionally it has been shown previously that older age in hypertensive patients is associated with a higher risk of non-persistence to ACE-inhibitors [24] and in our study the mean age of patients with renal disease was higher than patients with other indications which can potentially influence the higher non-persistence rate in this group. ACE-inhibitors are one of the main medications in heart failure management and large population based studies demonstrated that drug adherence is significantly associated with increased survival time in heart failure patients [25]. Recently it has been shown that the medication adherence within heart failure patients is already decreasing over the first few months after hospitalization [26]. Another recent study also showed the poor pharmacological management of heart failure in elderly patients [27]; however older age alone is not related to the poor medical management in heart failure patients [28]. In our study, heart failure patients were the third oldest group and had the highest mortality rate which might explain the average short time of ACE-inhibitor use in this group.

Our study shows that patients who start ACE-inhibitors for renal disease and heart failure have a higher probability to stop and should be better followed up and monitored by health care providers to benefit from ACE-inhibitors. Furthermore we suggest the active involvement of either

pharmacists or physicians to contact patients who discontinued relevant medication without clear reason to try to improve persistence and thus patient outcome [29, 30].

More studies are needed to address the issue whether the ACE-inhibitors discontinuations were inevitable or could be managed by adjustments of dose, addition of a new class of medication or other interventions.

The main strength of this population based study was the large number of patients which could be considered representative for all ACE-inhibitor starters in the UK.

One of the limitations of this study was that the indications were based on medical records registered by general practitioners, while these diagnoses were not validated, therefore misclassification cannot be ruled out.

In conclusion, this study showed that although ACE-inhibitors are usually initiated for life long treatment, for all indications a high percentage of ACE-inhibitors starters will stop or switch their therapy with the highest risk in renal disease patients and heart failure patients.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Chapter 2.2

Continuation of angiotensin converting enzyme inhibitor therapy, in spite of occurrence of angioedema

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ABSTRACT

Background

Angiotensin converting enzyme inhibitors (ACEis) are one of the most frequently used groups of medication worldwide. The most severe adverse effect of ACEis is angioedema with an incidence of around 0.2%. It is recommended to stop ACEi treatment after the first angioedema. This study aims to evaluate the discontinuation of ACEi use after the occurrence of angioedema and the probability of a recurrent event.

Methods

A cohort of ACEi starters from 2007 to 2014 within the Clinical Practice Research Datalink (CPRD) was defined. In this cohort, patients who developed angioedema for the first time during ACEi use were identified. The proportion of patients that continued ACEi use was calculated. Subsequently the cumulative incidences and Kaplan-Meier curves of the second events that happened either during or after ACEi exposure were calculated.

Results

Out of 267,612 ACEi starters, 425 patients were identified with a first angioedema during ACEi use (cumulative incidence 0.16%). From those patients 205 (48.2%) continued ACEi therapy. Cumulative incidence of recurrent angioedema in patients continuing ACEi was 25.2% and in patients that discontinued ACEi use was 9%. The Kaplan-Meier curves for risk of second angioedema, during ACEi exposure (totally 54 patients) and after ACEi exposure (totally 23 patients) showed the statistically significant difference (p-value<0.001).

Conclusion

There is a need to alert physicians about the serious risks of continuation of ACEi therapy after the occurrence of angioedema

Key words

angiotensin converting enzyme inhibitor; adverse drug reaction; prescribing pattern; drug-induced angioedema.

INTRODUCTION

Angiotensin converting enzyme inhibitors (ACEis) are one of the most frequently used groups of medication worldwide, ramipril for instance was the first antihypertensive medication in 2013 with more than 24 million prescriptions dispensed in community pharmacies in the United Kingdom [1]. Life-threatening angioedema is the most severe adverse effect of ACEis with an incidence of approximately 0.2% which mostly occurs at the larynx or tongue [2] and less frequently at the gastrointestinal wall [3]. In patients continuing ACEi use after the first angioedema, the recurrence risk of ACEi-induced angioedema is high and the reaction is more severe [4]. Therefore, cessation of ACEi treatment after the first angioedema is highly recommended [5,6]. However, angioedema induced by ACEis can be misdiagnosed and there is a lack of systematic studies to evaluate the discontinuation of ACEi use after the occurrence of angioedema and the probability of a recurrent event. We therefore investigated ACEi continuation after a first angioedema and the risk of recurrent angioedema using data from the United Kingdom Clinical Practice Research Datalink (CPRD), a primary care electronic medical record database which covers almost 12 million patients in the UK and has been used in numerous drug safety studies; the details and validity of CPRD data has been published previously [7] .

METHODS

The protocol for this study was approved by the independent scientific advisory committee (ISAC) of CPRD. We defined a cohort of patients older than 45 years who newly started ACEi therapy between the 1st of January 2007 and the 1st of January 2014. Subjects were followed up until end of study, death or moving out of the practice area. The theoretical duration of an ACEi prescription was calculated as the quantity of medication prescribed divided by the number of daily doses extended by 10 percent of the duration of the prescription to take into account non-adherence. The first ever registered angioedema in the medical records for patients was assumed to be an ACEi related angioedema if it was registered during the ACEi use time window; two angioedema events registered within 7 days were classified as the same event. The cumulative incidence with 95% confidence interval (CI) of the first angioedema during ACEi use was calculated. Within the patients with a first ACEi-related angioedema we calculated the proportion of patients that continued ACEi use (received at least one ACEi prescription after the angioedema event) both overall and per year (2007 to 2013). After the first angioedema the cumulative incidences and Kaplan-Meier curves of the second events that happened either during or after ACEi exposure were calculated. All statistical analyses were performed using SPSS 20 (IBM SPSS Statistics for Windows Version 20.0. Armonk, NY: IBM Corp).

RESULTS

The total cohort consisted of 267,612 ACEi starters, with a mean follow up time of 1,197 days (SD 732) and mean ACEi use duration of 826 days (SD 743). There were 425 patients (52.9% male) with a first angioedema during ACEi use: cumulative incidence 0.16% (95%CI 0.15%-0.18%). Of those patients, in total 205 (48.2%) continued ACEis after the first angioedema; this proportion fluctuated between 32.1% and 52.8% from 2007 to 2013 (Figure 1). Out of those 205 patients who continued ACEi therapy, 45 developed recurrent angioedema during ACEi exposure (cumulative incidence 21.9% (95%CI 16.8%-28.1%)) and in 4 patients the second angioedema occurred after discontinuation of the ACEi. In the 220 patients that did not receive a new ACEi prescription after

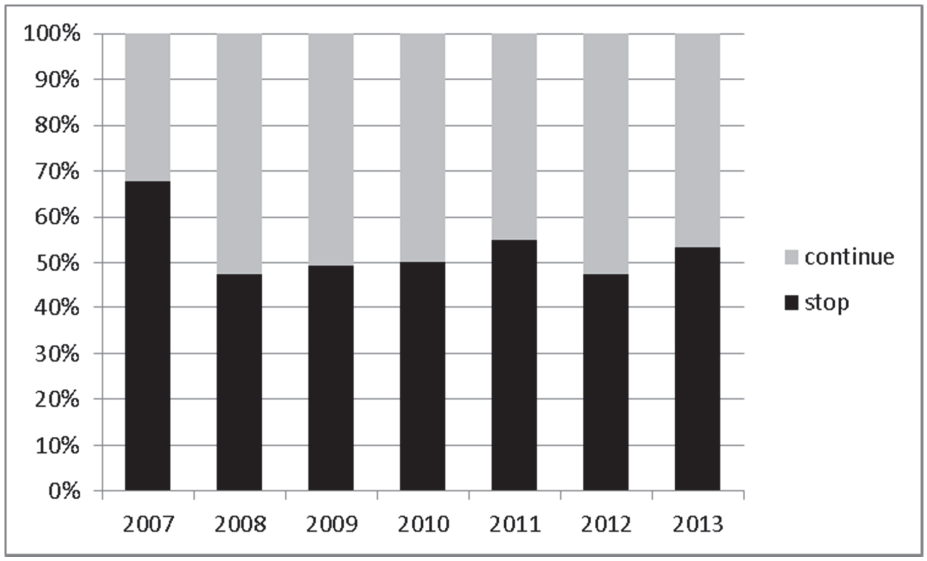

Figure 1. The yearly percentage of patients who continued ACEi therapy after a first angioedema.

the first angioedema, 28 developed a recurrent angioedema, cumulative incidence 12.7% (95%CI 8.9%-17.7%). Out of these 28 recurrent events, 9 occurred within the ACEi exposure time of the last prescription prior to the first angioedema. When it is assumed that these patients did not stop treatment after the first event, these 9 events were also ACEi-related recurrent angioedema and the cumulative incidence of recurrent angioedema in patients continuing ACEi would be 25.2% (95% CI 19.9%-31.4%); consequently in patients that discontinued ACEi use the cumulative incidence would be 9% (95% CI 5.8%-13.6%). The Kaplan-Meier curves for risk of second angioedema, during ACEi exposure (totally 54 patients) and after ACEi exposure (totally 23 patients) are presented in Figure 2 and are statistically significantly different (p-value<0.001).

DISCUSSION

This large population based study shows that in the UK almost half of ACEi-related angioedema cases, despite the guidelines [8], continue ACEi treatment which is associated with a high risk of recurrent angioedema. The probability to develop angioedema for patients who continue ACEis after a first event is between 137 to 158 times higher compared to new ACEi users and up to 2.8 times higher compared to patients who discontinued ACEi use. The main limitation of the study is that the diagnosis of angioedema was recorded by general practitioners and that we were not able to verify whether the angioedema was indeed caused by the use of an ACEi.

Considering the growing numbers of ACEi users, the high percentage of patients continuing an ACEi after a first angioedema event and the high risk of recurrent angioedema in these patients, physicians should be more aware of the serious risks of continuation of ACEi therapy after the occurrence of angioedema. This is particularly relevant because recently it has been shown that angioedema itself can potentially harm the heart and coronary arteries [9,10].

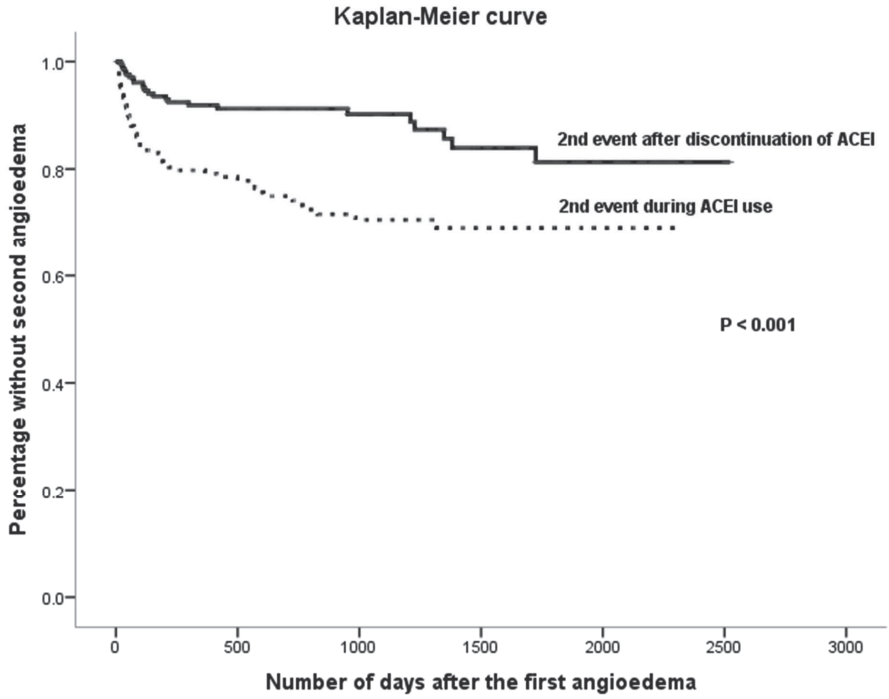

Figure 2. Comparing the rate of second angioedema during and after ACEi exposure.

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CONFLICTS OF INTEREST

The authors report no relationships that could be construed as a conflict of interest.

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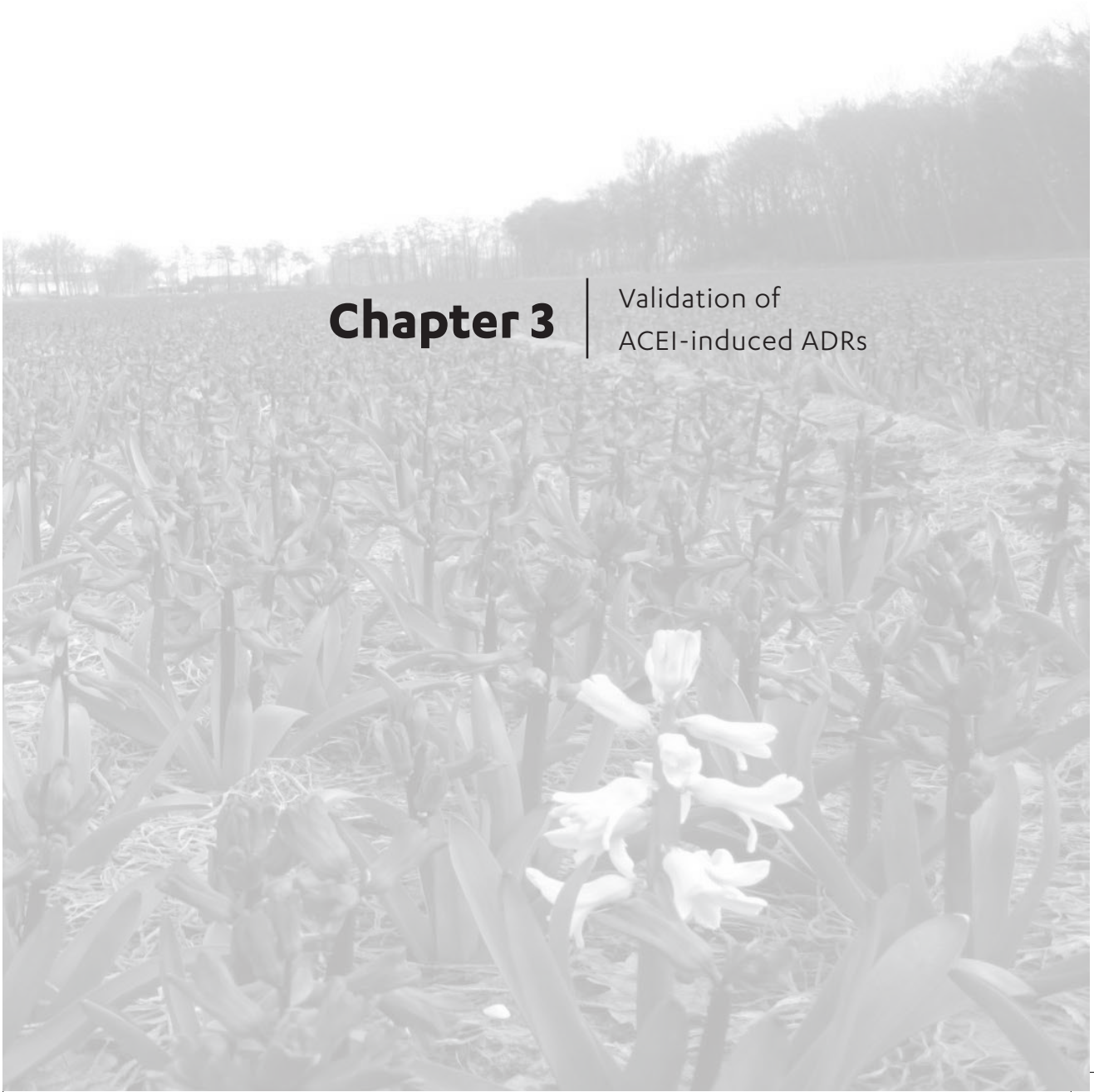

Chapter 3

Validation of
ACEI-induced ADRs

Chapter 3.1

Change in prescription pattern as a potential marker for adverse drug reactions of angiotensin converting enzyme inhibitors

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ABSTRACT

Background

Angiotensin converting enzyme inhibitors (ACEIs) are among the most frequently prescribed groups of medications. ACEI-induced adverse drug reactions (ADRs) are the main reason to discontinue or switch ACEI treatment. ADRs information is not available in prescription databases.

Objective

To identify a proxy for ACEI-induced ADRs in prescription databases.

Setting

The Rotterdam Study is an ongoing prospective cohort study that started in 1990 in the Netherlands and has included 14,926 subjects aged 45 years or older.

Methods

All ACEI starters from 2000 to 2011 were identified using prescription data within the Rotterdam Study. Participants were classified into 4 mutually exclusive groups: continuing, discontinuing, switching to angiotensin receptor blockers (ARBs), and switching to other antihypertensives. For categorization, the maximum time-interval between two prescription periods was set at 3 and 6 months. Subsequently, primary care physician files were searched and clinical events were classified as definite ADRs, probable ADRs, possible ADRs and definite non-ADRs. Finally the accuracy of different prescription patterns as indicators of ADRs was evaluated.

Main outcome measure

Positive predictive values (PPVs), negative predictive values (NPVs), sensitivity and specificity of the prescription patterns of the 4 groups were calculated.

Results

Totally, 1,132 ACEI starters were included. The PPV for a definite ADR was 56.1% for switchers to ARB, while the PPVs for switchers to other antihypertensives, and discontinuation were 39.5% and 19.5%, respectively. Including probable and possible ADRs, increased these values to 68.3% and 90.5%. A 6-month interval gave slightly higher PPVs compared to a 3-month interval (maximum 6.1% higher). The differences in NPVs between 3 and 6-months interval groups were approximately 1.0%.

Conclusions

Switching ACEIs to ARBs is the best marker for ACEI-induced ADRs in prescription databases.

Key words

Angiotensin Converting Enzyme Inhibitors, Adverse Drug Reaction, Electronic Healthcare Database, Positive Predictive Value, Pharmacoepidemiology, pharmacovigilance, ACEI, PPV, ADR.

INTRODUCTION

Angiotensin converting enzyme inhibitors (ACEIs) are commonly prescribed for a wide range of indications in both cardiovascular and renal disease, including hypertension, heart failure, myocardial infarction, renal failure and diabetic nephropathy [1]. They are first choice in cardiovascular protection in the group of renin angiotensin aldosterone system (RAAS) inhibitors [2]. It has been shown that ACEIs reduce the risk of all-cause mortality and cardiovascular mortality in both patients with hypertension or diabetes mellitus [3,4].

ACEIs are one of the most frequently prescribed groups of medications worldwide, in the US they were prescribed more than 150 million times per year since 2006 [5]. In the Netherlands there were around 9 million ACEI prescriptions in 2013 [6]. Furthermore, ramipril was the first antihypertensive medication in 2013 with more than 24 million prescriptions dispensed in community pharmacies in the United Kingdom [7]. Adverse drug reactions (ADRs) are one of the main reasons for discontinuation of ACEIs. 19% of ACEI starters discontinued therapy due to ADRs in a retrospective cohort study of outpatients who were prescribed an ACEI for the first time in a mixed ethnicity US population with 18 months follow-up [8].

Cough is among the most prevalent ADRs to ACEIs with a reported incidence ranging from 5% to 35%. Cough may occur months and even years after ACEI initiation [9,10]. More rarely, patients can develop potentially life-threatening angioedema that occurs in an estimated 0.1–0.7% of patients [11]. Population based studies showed that a large proportion of patients (44.2%) who discontinued ACEIs switched to an alternative antihypertensive drug within 90 days of discontinuation, indicating that they still need treatment [12]; however reason for discontinuation or switching was not clear in prescription datasets [12,13]. According to the medical guidelines, ACEIs have to be replaced by angiotensin receptor blockers (ARBs) when ADRs occur [9].

Electronic healthcare and prescription databases have been widely used in ACEIs epidemiologic studies and many of them have been linked to other data including genetic data or laboratory test data [14,15]. A major difficulty with conducting studies of ADRs is the fact that these are poorly registered in clinical practice, thus health care databases are generally incomplete sources in this respect [16,17]. Identifying proxies for ADRs based on prescription patterns in prescription databases can facilitate detection of ADRs for pharmacovigilance studies particularly when the dispensing data is linked to other data, like hospital admission data. Such a proxy will also create the opportunity for the large scale studies of biomarkers (such as genetic markers) that might predict the risk of developing ACEI-induced ADRs. ACEI-induced cough can lead to discontinuation of therapy and thereby to a higher risk of cardiovascular events. Angioedema on the other hand is a severe ADR, that might even be life threatening. Other effective antihypertensive drugs are available for patients at risk, and therefore predicting ACEI-induced ADRs is of clinical importance.

Aim of the study

The objective of this study was to test changes in prescription pattern as an appropriate proxy indicator for detecting the signal of potential ACEI-induced ADRs using data from the Rotterdam Study which contains both detailed drug dispensing data as well as primary care physician data.

Ethical approval

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 gave written informed consent

to participate in the study, and to obtain information by retrieval of medical records, use of blood and DNA for research purposes, and publication of results, separately.

METHODS

Data source

The Rotterdam Study is an ongoing prospective cohort study that started in 1990 in Ommoord, a suburb of Rotterdam, the Netherlands. This study has included 14,926 subjects aged 45 years or older. The overall participation was 72.0% (14,926 of 20,744 eligible invited people). The age distribution and social class of the participants is representative for the Dutch elderly society. The aims and details of the Rotterdam study have been described in detail previously [15,18]. In the Rotterdam Study, pharmacy dispensing data are available from January 1st, 1991. These records include details about drug names and contents, anatomical therapeutic chemical (ATC)-codes of medications, dosage forms, dispensing dates, number of units dispensed, and prescribed daily dose. Therefore, calculating the duration of drug therapy is possible by dividing the total number of tablets per prescription by the prescribed daily number, so the theoretical end date of prescriptions were calculated accordingly. Additionally the electronic primary care medical records were also available. The electronic medical records contained the notes and diagnoses of the treating primary care physician.

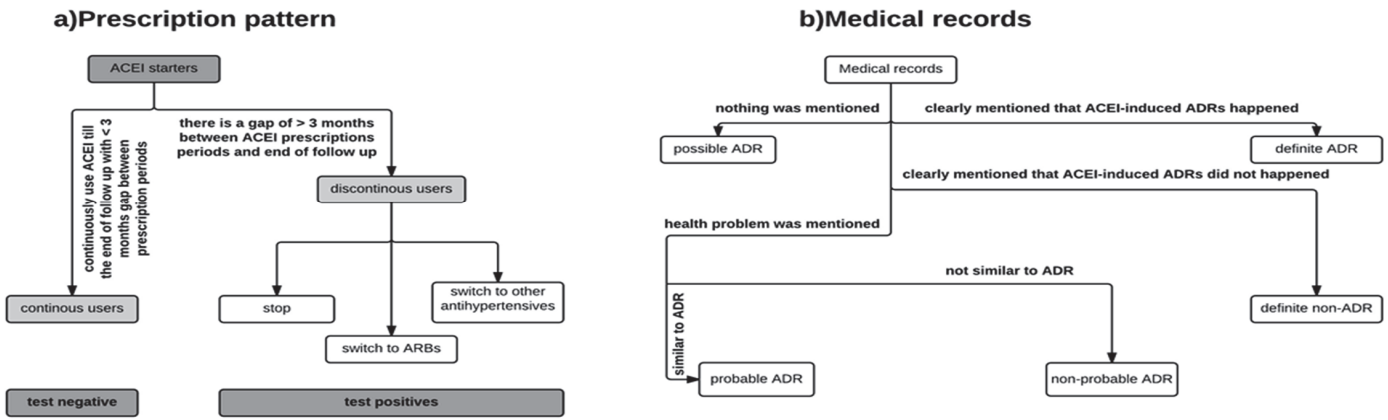
Study population

A cohort of patients who newly started ACEIs after January 1st, 2000 was identified retrospectively within the Rotterdam Study. The inclusion criteria were: having at least 6 months of valid medication history before starting the ACEI and not having any ACEI prescription within that period to ascertain that they are real ACEI starters. These patients were followed until the end of the study period which was January 1st 2011, or the date a patient died or moved outside of the catchment area (loss of follow-up), whichever came first. Patients whose medical records from general practitioners (GP) were not available were excluded from the study population.

Outcome measure

Outcomes were measured as below in both, prescription dispensing data and primary care medical records:

A. Prescription dispensing data were identified for all included patients, based on ATC codes including ACEIs (C09A, C09B), ARBs (C09C, C09D), beta blockers (C07), calcium channel blockers (C08), diuretics (C03) and/or antihypertensives (C02).Subsequently the cohort was divided with the following definitions: (Figure 1a)
    1. Continuation of ACEIs: patients who started ACEIs and continued until the end of the follow up. We allowed a maximum period of 3 months between a renewal of an ACEI dispensing date and the theoretical end date of the previous prescription because the maximum time duration for a prescription to be dispensed in the Netherlands is 3 months. This group was further subdivided into 3 categories depending on their situation when the follow up is ended (end of study, out of study, death). These categories were analysed separately and concomitantly. Furthermore "end of study" and "out of study" groups were analysed together (total minus death), to study whether



c)calculations of values

		Patients with ACEI-side effect as confirmed in physicians' reports		
		Positive	Negative	
ACEI pattern according to prescription data	Test Positive (stop or switch)	**True Positive** (TP)	**False Positive** (FP)	**Positive predictive value** = TP / (TP + FP)
	Test Negative (Continuation)	**False Negative** (FN)	**True Negative** (TN)	**Negative predictive value** = TN / (FN + TN)
		Sensitivity = TP / (TP + FN)	**Specificity** = TN / (FP + TN)	

ACEI: angiotensin converting enzyme inhibitors, ARB: angiotensin receptor blocker, ADR: adverse drug reaction

Figure 1. The outcome measurement in both prescription dispensing data and primary care medical records and a table for calculating of the values.

change in definitions would lead to differences in results. These patients were assumed not to have experienced ADR and they were considered as test negative group.

2. Discontinuation of ACEIs: patients who did not renew their ACEI prescription within maximum 3 months after the theoretical end date of the last ACEI prescription. Depending on their prescription data within 3 months after the end of ACEIs they were considered as stop (no new antihypertensive), switchers to either ARBs or another antihypertensive drug. These patients were assumed to have possibly experienced an ADR and were considered the test positive groups. The theoretical end date of last ACEI prescription would be the switch date or stop date.

B. In the primary care medical records, for the switching and discontinuation groups, two medical students manually searched 6 month before and 3 months after the switch or stop date to identify the reason for discontinuation or switching of ACEIs. This was done by looking for registered clinical events which might be related to ACEI use. Finally these reports were checked and confirmed by a pharmacist.

   Information from medical records was categorized into 4 groups:

   1. Definite ADR: ADR due to ACEI was clearly mentioned in the physician's records and/or the health problem resolved after discontinuation, thus, the reason for discontinuation was an ADR.
   2. Definite non-ADR: it was clearly mentioned that a physician decided to change or stop medication due to other reasons than an ACEI-induced ADR.
   3. Nothing mentioned: Medical records were available but there was no relevant clinical event mentioned within the required evaluation period. Occurrence of ADR is still possible in this group.
   4. Health problem mentioned: in this category, a clinical event was recorded but it was unclear whether it was due to the use of ACEIs. This category was divided into 2 subgroups according to the characteristics and nature of the mentioned clinical event (probable and non-probable ADR). (Figure 1b)

Data analyses

Positive predictive values (PPVs) which are the probability of correctly classifying a patient as having experienced an ACEI-induced ADR were calculated for the test positive groups separately, for these calculations we considered the proportion of test positive cohort (patients discontinued or switched ACEIs) that were identified as definite ADR cases, at least probable ADR cases (definite and probable ADR), and the at least possible ADR cases (definite, probable and possible ADR). Furthermore, PPVs were separately calculated as the proportion of definite ACEI-induced cough cases within patients that discontinued ACEI or switched to other antihypertensives, since this is the most frequently occurring ADR to ACEIs.

In order to calculate sensitivity and specificity, for each patient from the discontinuation or switch group (test positive), a patient from the continuation group (test negative) was selected and medical records were searched from the start date of an ACEI for the same duration of ACEI use that a test positive patient used ACEI; this approach was applied to harmonize the time course between test positive and test negative groups. Sensitivity and specificity were calculated considering definite ADRs only as probable and possible ADRs were not applicable within the continuation group because there was no switch or stop date by definition. Sensitivity in this study was calculated as the proportion of actual ADR cases which are correctly identified as ADR

cases and specificity was also calculated as the proportion of non-ADR cases which are correctly identified as non-ADR.

Negative predictive values (NPVs) which are the probability of correctly classifying a patient as not having experienced an ACEI-induced ADR were calculated in test negative group for the at least possible (only the definite ADR cases were deducted from the total number of patients that continued ACEI use), and for at least probable cases (both the definite ADR cases and the probable ADR cases were deducted from the total). Two sided 95% confidence intervals (CI) were calculated for PPVs, NPVs, sensitivity and specificity. (Figure 1c)

The sensitivity analyses were also performed with a 6 months interval instead of 3 months for defining the prescription patterns.

RESULTS

General characteristics and prescription patterns

In total, 1,414 ACEI starters were found in the Rotterdam study within the study period; 282 patients (19.9%) did not have medical records available and finally 1,132 patients were included in this study (44.4% male, mean age 63.7 years). The mean and median follow up time for the included patients were 1,602 and 1,496 days respectively. Table 1 shows the baseline characteristics and the duration

Table 1. General characteristics of included patients in the study

3 months interval		Number (%of total)	Mean age (years) [SD]	Gender (%male)	Median ACEI treatment duration (days)	Mean ACEI treatment duration (days) [SD]
Continuation (N=503) (44.5%)	End of study	**267 (23.5%)**	**62 [6.7]**	**50.2%**	**1219**	**1340 [1035]**
	Out of study	**135 (12%)**	**62.9 [6.2]**	**49.6%**	**1350**	**1451 [1046]**
	Death	**101 (9%)**	**68.8 [7.3]**	**51.5%**	**508**	**756 [706]**
Stop		**308 (27%)**	**64.5 [7]**	**40.6%**	**207.5**	**477 [628]**
Switch to other antihypertensive than ARB		**134 (12%)**	**63.9 [6.5]**	**45.5%**	**116.5**	**419 [656]**
Switch to ARB		**187 (16.5%)**	**62.7 [5.9]**	**34.2%**	**115**	**296 [464]**
Total		**1132 (100%)**	**63.7 [6.9]**	**44.4%**	**343**	**785 [909]**
6 months interval		**Number (%of total)**	**Mean age (years) [SD]**	**Gender (%male)**	**Median ACEI treatment duration (days)**	**Mean ACEI treatment duration (days) [SD]**
Continuation (N=585) (51.5%)	End of study	**299 (26.5%)**	**62 [6.8]**	**50.5%**	**1207**	**1347 [1049]**
	Out of study	**167 (14.5%)**	**62.9 [6.1]**	**48.5%**	**1410**	**1533 [1047]**
	Death	**119 (10.5%)**	**69 [7.2]**	**51.3%**	**564**	**736 [673]**
Stop		**261 (23%)**	**64.3 [6.9]**	**40.2%**	**179**	**466 [643]**
Switch to other antihypertensive than ARB		**106 (9.5%)**	**64 [6.3]**	**40.6%**	**102.5**	**343 [532]**
Switch to ARB		**180 (16%)**	**62.8 [5.7]**	**34.4%**	**115**	**293 [473]**
Total		**1132 (100%)**	**63.7 [6.9]**	**44.4%**	**397**	**845 [948]**

ARB: Angiotensin receptor blocker, SD: standard deviation.

of ACEI use stratified by ACEI use categories. Data are shown both for the 3 and 6 months-time interval between the theoretical end of ACEI prescription and start of a new prescription. For both 3 and 6 months-time intervals, approximately half of the ACEI starters discontinued their medication (55.5% and 48.5%, respectively) and the average ACEI treatment duration for all patients was 2 months longer when a 6 months-time interval was applied instead of a 3 months interval. Switchers to ARBs had the shortest mean duration of ACEI use of almost 10 months of ACEI consumption for both the 3- and 6 months interval. When the time interval was changed from 3 months to 6 months in the prescription data in total 96 patients changed categories which is 8.5% of the study population and most of them (82 out of 96) were from the switching or discontinuation group to the continuation group.

Primary care medical records

Table 2 only shows the detailed categorization of the study population considering the 6 months interval because there were only minor differences between 3 and 6 months interval results. Within the group of definite ADRs, cough and dizziness were the two most prevalent ADRs (73.5% and 4.5% respectively). Angioedema occurred in 3.0% of the definite ADRs, and is shown separately as the most dangerous ADR. Details of definite ADRs and probable ADRs are presented in the annotation of tables 2.

Test positive groups

The highest PPVs were found for the switchers to ARBs in all categories (definite ADR 56.1% (95% CI 48.8%-63.1%), at least probable ADR 68.9% (95% CI 62.0%-75.1%) and at least possible ADR 90.9% (95% CI 85.9%-94.2%)). The PPV for definite ADR was 56.1% (95% CI 48.8%-63.1%) when the 6 months-time interval was taken into account which was slightly higher than 55.0% (95% CI 47.9%-62.0%) for the 3 months-time interval. Except for the category of at least possible, for all other categories these higher values for the 6 months interval were observed. Cough is the most prevalent ADR of ACEIs, so PPVs for the definite ACEI-induced cough cases were calculated separately. The highest value was 46.1% (95% CI 38.9%-53.4%) for the switchers to ARBs considering the 6 months-time interval, in all groups which were considered as test positive, the 6 months interval showed higher PPVs for ACEI-induced cough, (Table 3)

Sensitivity was 91.8% (95% CI 85.1%-95.9%) and specificity was 68.4% (95% CI 62.4%-73.8%) in switchers to ARBs when 6 months interval was applied and both were higher compared with the 3 months gap in the definition. (Table 3)

Test negative groups

The differences in NPVs for both at least probable and at least possible between 3 and 6 months interval group were very small (approximately 1 %) and inconsistent. Within the groups, the differences between the highest and lowest NPVs for subgroups of "end of study", "out of study", "death", "total minus death" and "total continuation" were also small with a maximum of 2.4%. (Table 4)

DISCUSSION

Based on PPV, NPV, sensitivity and specificity, this study showed that switching from an ACEI to an ARB allowing 6 months-time interval between last use of ACEI and start of ARB, is the best marker

Table 2. Number of ACEI starters in different categories both according to the prescription data and medical records

Total (N=1132)		Switchers to ARB (N=180) (16%)	Switchers to other antihypertensive than ARB (N=106) (9.5%)	Stoppers (N=261) (23%)	Continuation (N=585) (51.5%)		
					Out of study (N=167) (14.5%)	Death (N=119) (10.5%)	End of study (N=299) (26.5%)
Definite ADR **(N=222) (19.5%)**	Cough **(N=163)**	**83**	**25**	**35**	**4**	**3**	**13**
	Angioedema **(N=7)**	**3**	**1**	**1**	**0**	**1**	**1**
	Others[a] **(N=52)**	**15**	**16**	**15**	**3**	**1**	**2**
	Total	**101 (56%)**	**42 (39.5%)**	**51 (19.5%)**	**7**	**5**	**16**
					28 (5%)		
Definite Non-ADR **(N=48) (4.3%)**	No need **(N=11)**	**0**	**1**	**10**	**N/A**	**N/A**	**N/A**
	Not effective **(N=15)**	**4**	**4**	**7**	**N/A**	**N/A**	**N/A**
	Others[b] **(N=22)**	**6**	**5**	**11**	**N/A**	**N/A**	**N/A**
	Total	**10 (5.5%)**	**10 (9.5%)**	**28 (11%)**	**N/A**		
Nothing mentioned **(N=628) (55.5%)**		**40 (22.5%)**	**25 (23.5%)**	**132 (50.5%)**	**124**	**89**	**218**
					431 (73.5%)		
Health problem mentioned **(N=234) (20.7%)**	Probable ADR[c] **(N=197)**	**22**	**20**	**43**	**35**	**21**	**56**
	Non-probable ADR[d] **(N=37)**	**7**	**9**	**7**	**1**	**4**	**9**
	Total	**29 (16%)**	**29 (27.5%)**	**50 (19%)**	**36**	**25**	**65**
					126 (21.5%)		

The interval between a renewal of an ACEI prescription and the theoretical end date of the previous prescription was 6 months. ACEI: Angiotensin converting enzyme inhibitor, ARB: Angiotensin receptor blocker, N/A: Not applicable.

[a] Allergic reaction (N=1), Renal dysfunction (N=2), Runny nose (N=2), Sexual dysfunction (N=1), tiredness (N=6), Not mentioned (N=9), Chest pain (N=1) Decreased taste (N=1), Dizziness (N=9), Dizziness plus (N=1), Gastrointestinal (N=4), Headache (N=2), Hiccups (N=1), Hyperkalemia (N=2), Itching (N=3), Itching rash (N=5), Muscular cramps (N=1), Nausea (N=1).

[b] Angioedema history (N=1), Bad taste (N=1), Cerebrovascular event (N=1), do not like (N=1), Drug interaction (N=2), Disease interaction (N=3), Hypotension (N=2), Self-stop (N=3), Surgery (N=1), Not mentioned (N=5), Short change (N=2).

[c] Allergic reaction(N=1), Angioedema(N=2), Cough(N=48), Cough plus(N=11), Asthma(N=4), Bronchitis(N=26), Common cold (N=6), COPD(N=8), Dry cough(N=7), Flu(N=1), Infectious cough(N=28), Pneumonia(N=7), Cough with sputum(N=4), Dizziness(N=16), Dizziness plus (N=1), Dyspnea(N=5), Hypersensitivity (N=1), Itching(N=2), Itching rash(N=3), Itching throat (N=2), Rash (N=3), Tiredness plus (N=1), Tiredness(N=3),Sexual dysfunction(N=2), Shortness breath (N=5).

[d] Anxiety(N=1), Bad feeling(N=6), Body pain(N=1), Edema(N=1), Gastrointestinal (N=5), Hair loss(N=1), Hospitalization (N=1), Hand hypoxia(N=1),Increased blood urea(N=1), Muscular cramps (N=5), Muscular pain (N=1), Nausea(N=2), Not tolerated(N=4), Renal dysfunction (N=3), Runny nose(N=1), Swollen feet(N=1), Not mentioned (N=1) Pulmonary embolism(N=1).

Table 3. Positive predictive values for the total adverse drug reactions and cough only cases within the test positive groups (patients who discontinued or switched), sensitivity and specificity considering the definite adverse drug reactions

		Switchers to ARB	Switchers to other than ARB	Stoppers	Switchers total	Total discontinuation
PPV definite % (95% CI)	3M	**55.0 (47.9-62.0)**	**33.5 (26.1-41.9)**	**17.5 (13.6-22.1)**	**46.1 (40.7-51.5)**	**32.1 (28.5-35.8)**
	6M	**56.1 (48.8-63.1)**	**39.6 (30.8-49.1)**	**19.5 (15.1-24.7)**	**50.0 (44.2-55.7)**	**35.4 (31.5-39.5)**
PPV at least probable % (95% CI)	3M	**68.9 (62.0-75.1)**	**52.2 (43.8-60.5)**	**35.7 (30.5-41.2)**	**61.9 (56.5-67.1)**	**49.1 (45.2-53.0)**
	6M	**68.3 (61.2-74.6)**	**58.4 (48.9-67.4)**	**36.0 (30.4-42.0)**	**64.6 (58.9-70.0)**	**51.0 (46.8-55.1)**
PPV at least Possible % (95% CI)	3M	**90.9 (85.9-94.2)**	**83.5 (76.3-88.9)**	**87.6 (83.5-90.8)**	**87.8 (83.8-90.9)**	**87.7 (84.9-90.0)**
	6M	**90.5 (85.4-94.0)**	**82.0 (73.7-88.2)**	**86.5 (81.9-90.2)**	**87.4 (83.0-90.7)**	**87.0 (83.9-89.5)**
PPV definite Cough cases only % (95% CI)	3M	**45.4 (38.4-52.6)**	**19.4 (13.6-26.9)**	**11.6 (8.5-15.7)**	**34.5 (29.5-39.9)**	**23.3 (20.2-26.8)**
	6M	**46.1 (38.9-53.4)**	**23.5 (16.5-32.5)**	**13.4 (9.8-18.0)**	**37.7 (32.3-43.5)**	**26.1 (22.6-29.9)**
Sensitivity[a] definite cases % (95% CI)	3M	**91.1 (84.4-95.1)**	**95.7 (85.7-98.8)**	**80.6 (69.5-88.3)**	**92.5 (87.3-95.6)**	**N/A**
	6M	**91.8 (85.1-95.9)**	**93.3 (82.1-97.7)**	**80.9 (69.5-88.7)**	**92.2 (86.9-95.5)**	**N/A**
Specificity[a] definite cases % (95% CI)	3M	**67.8 (61.9-73.1)**	**59.7 (53.1-65.9)**	**53.7 (49.5-57.8)**	**64.1 (59.7-68.2)**	**N/A**
	6M	**68.4 (62.4-73.8)**	**61.6 (54.1-68.7)**	**54.2 (49.6-58.7)**	**65.7 (61.0-70.1)**	**N/A**

PPV: Positive predictive value, ARB: Angiotensin receptor blocker, CI: Confidence interval, N/A: Not applicable.
[a] To calculate the sensitivity and specificity, where it was possible, for each patient from the test positive groups (discontinuation or switch), a patient from the test negative group (continuation) was selected and medical records were searched for the same duration of ACEI use.
3M and 6M denote time intervals in months between a renewal of an ACEI prescription and the theoretical end date of the previous prescription.

in the prescription database of the Rotterdam Study for ACEI-induced ADRs. This finding offers the possibility to use prescription databases to identify patients who have experienced ACEI-induced ADRs even in the absence of clinical data or specific ADR registrations. This was also demonstrated for ACEI-induced cough specifically, because switchers from ACEIs to ARBs had the highest PPVs among all groups of ACEI prescription patterns for either definite, probable or possible ADRs and also for the definite ACEI-induced cough cases only. A 6 months interval gave slightly higher PPV compared with a 3 months interval, and both sensitivity and specificity were higher using a 6 months interval.

In all studies that compared discontinuation between different classes of antihypertensive drugs ARBs were used without switching or discontinuation for the longest period followed by

Table 4. Negative predictive values within the test negative group (patients who continued angiotensin converting enzyme inhibitor)

		End of study [a]	Out of study [b]	Death [c]	Total minus death	Total continuation
NPV at least possible % (95% CI)	3M	**95.5 (92.3-97.4)**	**95.5 (90.6-97.9)**	**95.0 (88.9-97.8)**	**95.5 (93.0-97.1)**	**95.4 (93.2-96.9)**
	6M	**94.6 (91.4-96.6)**	**95.8 (91.6-97.9)**	**95.8 (90.5-98.1)**	**95.0 (92.7-96.6)**	**95.2 (93.1-96.6)**
NPV at least probable % (95% CI)	3M	**77.9 (72.5-82.4)**	**75.5 (67.6-82.0)**	**78.2 (69.2-85.1)**	**77.1 (72.7-80.9)**	**77.3 (73.4-80.7)**
	6M	**75.9 (70.7-80.4)**	**74.8 (67.7-80.8)**	**78.1 (69.9-84.9)**	**75.5 (71.4-79.2)**	**76.0 (72.4-79.3)**

CI: Confidence interval, NPV: Negative predictive value.
[a] "end of study" group are patients who continued ACEIs till January 1st 2011
[b] "out of study" group are patients who continued ACEIs till they went out of the area
[c] "death" group are patients who continued ACEIs till date of death
3M and 6M denote time intervals in months between a renewal of an ACEI prescription and the theoretical end date of the previous prescription.

ACEI, while the time intervals for defining discontinuation or switch in prescription data were not consistent in all of them [12,19,20]. In this study, 3 and 6 months-time intervals were used to find the best interval in terms of indicating ADRs and accuracy to include real stoppers, switchers and continuers because previous studies have shown that time-interval influence the categorization in hypertensive therapy [21]. Out of the total 96 patients who changed categories when the interval changed from 3 months to 6 months, 82 changes (85.5%) were from groups of switching and discontinuation to the continuation group, which suggests that using the 6 month interval is probably better to prevent misclassification because patients who restart are not expected to have stopped due to an ADR previously. Morimoto et al investigated ACEI-induced ADRs and found that 32.4% of ACEI starters discontinued ACEI, of whom 19% discontinued use, due to ADRs after a maximum of 18 months follow up [8]. In our study, 48.5% of the ACEI starters discontinued their ACEI when the 6 months-time interval was used and ACEIs were on average used for about 28 months in the whole study population when considering a maximum interval of 6 months within the prescriptions.

Other examples where prescription data were validated as a marker for clinical events have been published. For instance, in the Rotterdam Study, using repeated nitrate prescription has been shown to be a suitable marker for angina pectoris in electronic healthcare databases [22] and also changes in prescription data were used previously as an indicator of ADR due to statins [23]. In a sample of 63 cases that switched, discontinued or reduced the dose of their statin therapy, 68% suffered from ADRs induced by statins and this proxy was used within prescription data for genetic association studies where large numbers of cases are needed [24]. This study tried to identify the reason for discontinuation and switching in general practitioners (GP) files to find the best marker in prescription data for ACEI-induced ADRs, and specifically cough. This study was conducted in the Rotterdam Study, which is a large cohort study within the Netherlands with a good generalizability to the Caucasian population of 45 years and older [25], so the results can be translated to other similar databases.

Pharmacies in the Netherlands are allowed to deliver medication for a maximum of 90 days; therefore the regular time interval for refilling a prescription is 3 months. Results of this study should be used with caution in countries with different intervals for prescription refill. Additionally the proxy cannot differentiate between the different ACEI-induced ADRs, however for cough as the most prevalent ADR, results showed a high predictive value for definite cases (46.1% (95% CI 38.9%–53.4%)).

Because usually ADRs are not well registered, the use of electronic healthcare databases can increase the number of cases of ADRs that can be found, and can decrease the amount of time and costs spent in searching for these cases in epidemiologic studies. Many prescription databases can be linked to other types of data, including but not limited to hospital data, genetic data, socio-demographic data and laboratories-test data [26].

Hospital data have been used previously to detect and report ADRs for pharmacovigilance studies [27]. If linkage to hospital data is possible, this can strengthen the validity for the detection of ACEI related ADRs, especially those ADRs that need hospital admission like angioedema. However, for ADRs that do not require hospitalization (like cough) the use of drug dispensing databases might be a good alternative for pharmacovigilance studies.

An important limitation of our study is that only the diagnoses of general practitioners (GP) records were considered. It was not possible to check specialist records or to interview patients. This might have led to misclassification because some clinical events might have been missed, misdiagnosed or not been registered in GP records [28]. The number of general practitioners visited by the patients in the Rotterdam Study is limited to the specific region and that is a single centre study, so the variation in physician's attitude to diagnose the ACEI-induced ADRs might be less comparing to multicentre studies, however it cannot be ruled out.

CONCLUSION

In conclusion, switching from ACEIs to ARBs is the best marker in prescription databases and might be useful to investigate genetic and environmental risk factors associated with the occurrence of ACEI-induced ADRs. Using such data might increase the efficiency of epidemiological studies of ADRs, especially of the ones which are not coded and found back in health care databases.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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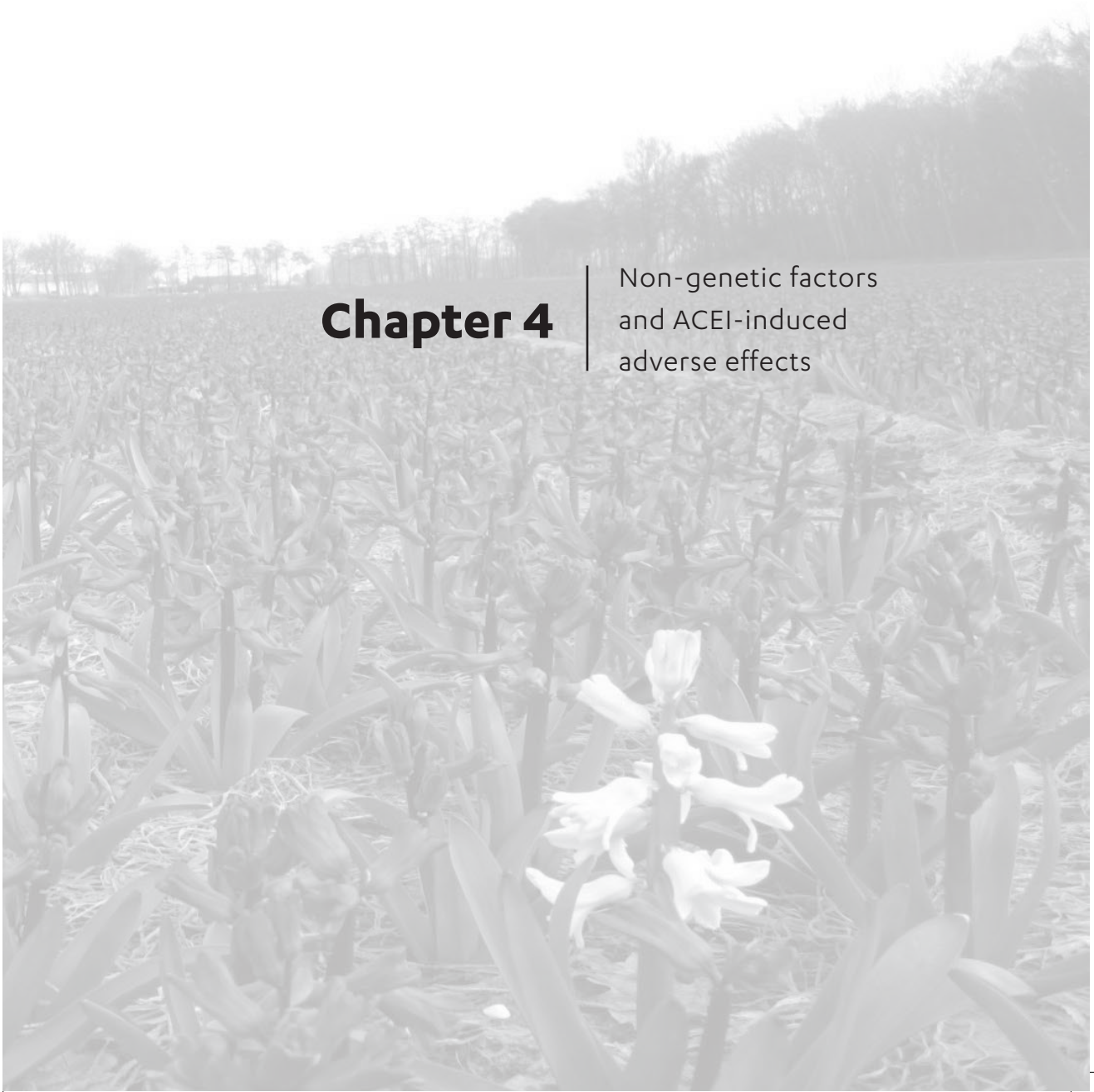

Chapter 4

Non-genetic factors and ACEI-induced adverse effects

Chapter 4.1

Determinants of angiotensin converting enzyme inhibitor (ACEI) intolerance and angioedema in the UK Clinical Practice Research Datalink

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ABSTRACT

Aim

In this study we aimed to describe the occurrence and determinants of ACE-inhibitor (ACEI) intolerance and angioedema (AE) among patients initiating ACEI therapy in a real-world primary care population.

Methods

Two nested case-control studies were conducted in a cohort of 276,977 patients aged ≥ 45 years initiating ACEIs from 2007 to 2014 in the UK Clinical Practice Research Datalink (CPRD). Cases of AE occurring for the first time during ACEI therapy (n=416) were matched with AE-free controls (n=4,335) on the duration of ACEI treatment. Switching to ARBs in the prescription records was used to identify ACEI intolerance cases (n=24,709) which were matched with continuous ACEI users (n=84,238) on the duration of ACEI therapy. Conditional logistic regression was used to assess the association of demographic factors, co-morbidities and co-medication with AE and ACEI intolerance.

Results

AE during ACEI therapy was associated with age over 65 years (OR 1.36, 95%CI: 1.07–1.73), history of allergy (OR 1.53, 95%CI: 1.19–1.96), use of calcium channel blockers (OR 1.57, 95% CI 1.23; 2.01), anti-histamines (OR 21.25, 95%CI 16.44; 27.46) and systemic corticosteroids (OR 4.52, 95% CI: 3.26, 6.27). ACEI intolerance was significantly associated with more co-morbidities and co-medication compared to AE, including allergy (OR 2.02, 95% CI 1.96; 2.09), use of anti-asthmatic drugs (OR 1.51, 95% CI 1.42; 1.61) and anti-histamines (OR 1.53, 95% CI 1.43; 1.63).

Conclusions

Among ACEI users developing AE or ACEI intolerance several co-morbidities and co-medication classes were significantly more prevalent compared to ACEI users not developing these adverse reactions.

Key words

ACE inhibitors, angiotensin II receptor blockers, angioedema, ACE-inhibitor intolerance, adverse drug reaction, case-control study

INTRODUCTION

Angiotensin-converting enzyme inhibitors (ACEIs) are among the most commonly prescribed antihypertensive agents to date used for hypertension, heart failure, diabetic nephropathy and secondary prevention following a myocardial infarction (MI). The estimated number of ACEIs prescriptions has substantially increased over the past years, with 35 to 40 million prescriptions worldwide in 2001 to approximately 160 million prescriptions issued in 2011 in the US alone [1,2]. In the UK, ramipril is the leading drug among medications for hypertension and heart failure, with almost 26 million prescriptions of ramipril having been dispensed in 2014, which shows an increase of 17 million prescriptions since 2004 [3].

ACEIs have been proven to reduce all-cause mortality in patients with hypertension as well as major cardiovascular (CV) events, all-cause and CV mortality in patients with diabetes mellitus (DM) [4,5]. Beneficial effect of ACEIs on the risk of subsequent CV events and mortality was also found in secondary prevention after MI [6]. However, according to observational studies 19–30% of patients initiating ACEIs discontinue treatment due to adverse effects [7,8]. One of the most common adverse effects of ACEIs is a persistent dry cough described in 9.9% to 35% of the patients in randomized clinical trials [9,10]. A far more uncommon, but potentially life-threatening adverse effect of ACEIs is angioedema (AE) of the head and neck region and visceral ACEI-induced AE. In randomized clinical trials the incidence of ACEI-induced AE was estimated to be 0.3–0.7 % [11]. In emergency care ACEI-induced AE of the larynx accounts for a third of all hospitalizations for AE [1,12,13].

Most cases of ACEI-induced cough occur early in the course of treatment, while ACEI-induced AE may develop either in the first weeks or several years after the start of treatment [1]. Although the exact mechanism of ACEI-induced AE and cough is not known, it was proposed to be similar for both adverse reactions and involves the reduction in catabolism of vasoactive substances (bradykinin and substance P) as a result of ACE inhibition [14]. Furthermore, ACEI-induced AE and cough share some similar clinical predictors [15]. For instance, ethnical origin is an important risk factor, with African American patients having an almost 3-fold higher risk for ACEI-induced AE and East Asians being at a higher risk for ACEI-induced cough [16,17].

Some of previous studies on predictors of ACEI-related adverse effects were limited by a relatively small sample size and an incomplete registering of adverse effects outside randomized clinical trials. A way allowing to bypass this limitation is to use a large patient database and to ascertain a drug prescribing pattern indicative of adverse reactions. Generally, ACEI intolerant patients are advised to avoid ACEIs and frequently switch to angiotensin-II receptor blockers (ARBs) [9,18]. A recent study found that approximately half of patients with ACEI-induced cough discontinued ACEIs and switched to ARBs [19]. The use of ARBs in patients with a history of ACEI-induced AE should be weighed against the therapeutic need for angiotensin inhibition in each patient, because a risk of recurrent AE while on ARBs remains [20,21]. An analysis of medical records identified a prescription pattern reflecting ACEI intolerance in a Dutch population [19]. Switching to ARBs within a 6 months interval from the end of an ACEI prescription in the Rotterdam study was an indicator for definitive ACEI-related adverse events with a positive predictive value (PPV) of 56.1% [19]. The PPV for the combined probable and definitive ACEI-related ADRs was 68.3% and combined for possible, probable and definitive ADRs it was 90.5 %.

Given the increasing utilisation of ACEIs, the purpose of this study was to describe the occurrence of AE and ACEI intolerance defined by a switch to ARBs among primary care patients newly treated with ACEIs. Secondly, we assessed the associated demographic factors, co-morbidities and co-medications to gain more insight into patient groups more likely to experience ACEI-related adverse reactions.

METHODS

Data source

Data for this study were obtained from the UK Clinical Practice Research Datalink (CPRD), an anonymized database containing approximately 12 million complete electronic medical records from over 600 participating general practices across the UK [22]. Primary care diagnoses, prescriptions, laboratory test results, referrals, patient demographics and lifestyle information are recorded in the CPRD using a hierarchical clinical coding system (Read codes) [23]. Hospital diagnoses are available for a sub-group of patients and are coded according to the International Classification of Diseases (ICD-10). Validity and a complete description of available CPRD data have been reported elsewhere [22,23]. The protocol for this study was reviewed and approved by the independent scientific advisory committee (ISAC) of CPRD (protocol number: 14_030R).

Study design and population

As a source population we identified all new users of ACEIs of 45 years or older registered between 01 January 2007 and 01 January 2014 in the CPRD. Date of the first ACEI prescription within this time period was considered as the cohort entry date. A new ACEI user was defined as a subject without ACEI prescription records in the CPRD prior to the cohort entry date. All included ACEI users had at least 12 months of valid prescription history available before the start of ACEI. To identify patients with AE and switching to ARBs during follow-up subjects were followed until the end of study, death or moving out of the practice area, whichever came first.

Within the cohort of new ACEI users we conducted two retrospective nested case-control studies to identify determinants of AE occurring during ACEI therapy and with switching to ARBs, as a proxy for an ACEI–induced adverse reaction. In the AE study, the index date was defined by the AE diagnosis date. For ACEI intolerance, the date of switching to ARBs was considered the index date.

The first ever registered AE episode among ACEI users was assumed to be an ACEI-related AE, if the AE diagnosis was entered into the CPRD any time during ACEI therapy or within a maximum of three months after expiration of the last ACEI prescription. Thus, AE cases were individuals in whom AE occurred for the first time during ACEI therapy. Individuals who had a diagnosis of AE at any time while not receiving ACEI therapy were excluded. Cases had no AE records both before the start and after the discontinuation of ACEI treatment. An individual also became a case if multiples episodes of AE have occurred while on ACEI therapy, but only the first AE during ACEI therapy was considered an event. For each case, up to 20 controls were selected from new ACEI users who didn't have a diagnosis of AE in CPRD records. Controls were ACEI users at the time of AE of the corresponding cases and were matched to cases on the duration of ACEI therapy. Cases were excluded if no matching controls were available in the cohort.

ACEI intolerant cases were defined by the switching from ACEIs to ARBs in the prescription records, allowing a six months-time interval between the theoretical last use of ACEIs and the start of ARBs, as described previously [19]. ACEI users continuously filling ACEI prescriptions, without discontinuing ACEI or switching to another antihypertensive drug at the index date of the relevant case were selected as controls. For each case of ACEI intolerance up to 4 controls were sampled. Controls were matched to cases on the duration of ACEI treatment at the index date.

Information on GP-prescribed medications was extracted using appropriate British National Formulary (BNF) medicine codes. The theoretical duration of an ACEI prescription was calculated as a ratio between the quantity of medication and the defined daily dose (DDD), estimated

according to the WHO. The duration of ACEI treatment was defined as time between the start of the first ACEI prescription until the end of the last ACEI prescription, allowing a gap of less than six months between two consecutive ACEI prescriptions. Discontinuation of ACEI therapy was defined as the absence of a new ACEI prescription record for at least six months after the theoretical end date of the last ACEI prescription.

Determinants

As possible determinants of AE during ACEI therapy and switching to ARBs we considered sex, age over 65 years at the index date, the use of co-medication and medical history of chronic co-morbidities. The use of co-medication, including anti-diabetic drugs, antihistamines, anti-asthmatic medications, non-steroidal anti-inflammatory drugs (NSAIDs), systemic corticosteroids, calcium channel blockers and statins, was assessed by any prescription record within a three-month time window before the index date, regardless to the duration of that prescription. Therefore drugs for which the theoretical end date of the previous prescription would be in this time window were not included into the analysis. We did not discriminate between various product names, but rather investigated the association between different classes of co-medication and AE or ACEI intolerance. Exposure to each co-medication class was included in the models as a dichotomous variable (yes vs. no use). The history of co-morbidities, including asthma, allergy, chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM) and rheumatoid arthritis (RA), was retrieved from medical records using Read codes any time before the index date. Drug prescriptions were not used to classify individuals on disease status when ascertaining co-morbidities. Additionally, the occurrence of any type of cough within three months before the AE date was included into analyses.

For descriptive purposes, demographic characteristics of the study population were determined on the cohort entry date. Information on lifestyle factors was not available for multiple subjects at the cohort entry date, therefore the most recent recording of body mass index (BMI) was retrieved within a time interval of 365 days around the cohort entry date. Smoking status and alcohol consumption were assessed at the most recent date ever before the baseline. Smoking status ever before the index date was used for the association analyses. The indication for ACEI therapy was obtained from medical records any time before the cohort entry date or any time within one year after this date.

Statistical analyses

The results are presented as means and standard deviations for continuous variables and as proportions for categorical variables. Differences in baseline characteristics between cases and controls were assessed with Student's t-test for continuous variables and using the chi-squared test for categorical variables. Kaplan-Meier curves were constructed to estimate time to AE and switching to ARBs. Odds ratios and 95% confidence intervals for the association of AE and switching to ARBs with age, sex, smoking, co-morbidities and co-medication were estimated by univariate logistic regression. The analyses with co-morbidities and co-medications were further adjusted for age and sex. Subsequently forward stepwise multivariable logistic regression was performed including all determinants significantly ($p < 0.05$) associated with the outcomes in univariate analyses. A two-sided P-value of less than 0.05 was considered statistically significant. Data analyses were performed IBM SPSS for Windows, version 23.0 (IBM SPSS Statistics for Windows Version 23.0. Armonk, NY: IBM Corp).

RESULTS

The cohort comprised 276,977 ACEI users aged 45 years or older initiating ACEI therapy between 2007 and 2014. Among these individuals we identified 416 cases of AE occurring for the first time during ACEI therapy and matched them with 4,335 controls. We determined that 24,709 individuals switched to ARBs in 6 months since the end of the last ACEI prescription. Switchers to ARBs were matched with 84,238 continuous ACEI users, after having excluded those who stopped ACEI therapy or switched to another antihypertensive drug.

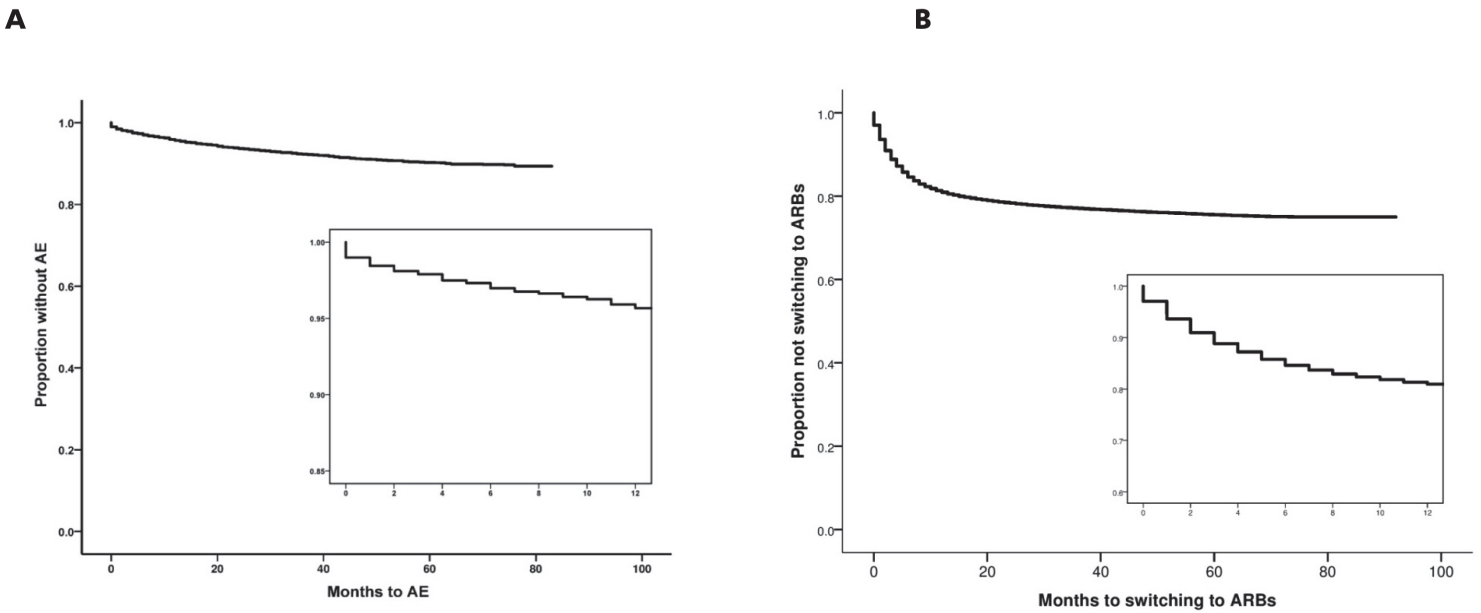
Clinical characteristics of the study populations are presented in Table 1. The proportion of women was statistically significantly higher among the switchers as compared to the continuous ACEI users (58.6% against 45.5%). The most frequent indication for ACEI therapy was hypertension, followed by the category of more than one indication, myocardial infarction (MI), renal disease and heart failure (HF). Figure 1 depicts the time to AE and switching to ARBs among ACEI users. The mean time to AE was 76.7 months, while the mean time to switching to ARBs was 71.6 months.

Table 1. Baseline characteristics of study populations (at cohort entry date).

	AE cases n=416	Controls n=4335	P	Switchers to ARBs n=24709	Continuous users of ACEIs n=84238	P
Gender, n (%)			0.472			<0.005
Female	204 (49.0)	2045 (47.2)		14482 (58.6)	38297 (45.5)	
Male	212 (51.0)	2290 (52.8)		10227(41.4)	45941 (54.5)	
Age (years), mean ± SD	67.8 ± 11.6	65.6 ± 11.8	<0.005	65.2 ± 11.8	65.7 ± 11.1	<0.005
BMI (kg/m2), mean ± SD	29.3 ± 5.8	29.4 ± 5.8	0.706	29.4 ± 5.9	29.5± 5.8	0.001
BMI unknown, n (%)	129 (31.0)	1249 (28.8)		23482 (27.9)	6963 (28.2)	
Alcohol consumption, n (%)			0.335			<0.001
No	86 (20.7)	771 (17.8)		4136 (16.7)	13857 (16.4)	
Yes	296 (71.2)	3210 (74.0)		18630 (75.4)	62760 (74.5)	
Unknown	34 (8.2)	354 (8.2)		1943 (7.9)	7621 (9.0)	
Smoking status, n (%)			0.156			<0.001
No	212 (51.0)	2412 (55.6)		15292 (61.9)	46584 (55.3)	
Yes	186 (44.7)	1729 (39.9)		8502 (34.4)	34208 (40.6)	
Unknown	18 (4.3)	194 (4.5)		915 (3.7)	3446 (4.1)	
Indications for ACEI therapy, n (%)			0.031			<0.005
Heart failure	6 (1.4)	50 (1.2)		319 (1.3)	1244 (1.5)	
Hypertension	246 (59.1)	2468 (56.9)		15160 (61.4)	50354 (59.8)	
Myocardial infarction	18 (4.3)	196 (4.5)		765 (3.1)	3060 (3.6)	
Renal disease	17 (4.1)	159 (3.7)		901 (3.6)	2843 (3.4)	
More than one of the above	96 (23.1)	866 (20.0)		4492 (18.2)	14736 (17.5)	
Unknown	33 (7.9)	596 (13.7)		3072 (12.4)	12001 (14.2)	

ACEI – angiotensin converting enzyme inhibitor. AE – angioedema. ARBs – angiotensin receptor blockers. BMI – body mass index. SD – standard deviation.
Smoking status and alcohol consumption were determined ever before the cohort entry date (start of ACEI therapy). BMI was retrieved within a time interval of 365 days around the cohort entry date.
Indications for ACEI therapy were assessed any time prior to the start of ACEI.
Age was assessed at the date of the first ACEI prescription.

	AE	Switch to ARBs
Mean time to event (months)	76.7	71.6
95% Confidence interval	(76.2; 77.3)	(71.4; 71.8)



A - Kaplan-Meier curves for time to the development of AE during ACEI therapy. The bottom right panel shows time to event within the first year of follow-up.

B - Kaplan-Meier curves for time to switching to ARBs. The bottom right panel shows time to event within the first year of follow-up.

Figure 1. Time to the development of ACEI intolerance and AE during ACEI therapy

Crude and adjusted ORs for the association of co-morbidities and co-medications with AE during ACEI therapy and ACEI intolerance are provided in Table 2 and Table 3. Overall, univariate analyses yielded similar associations for AE and switching to ARB. Age over 65 years at index date was statistically significantly associated with an increased risk of both AE and ACEI intolerance

Table 2. Determinants of angioedema during ACEI therapy.

	No. (%)		**Crude OR (95% CI)**	**P**	**Adjusted OR^ (95% CI)**	**P**
	Cases (n=416)	*Controls (n=4335)*				
Gender						
Male	212 (51.0)	2290 (52.8)	ref.	-	-	-
Female	204 (49.0)	2045 (47.2)	1.08 (0.88; 1.32)	0.470	-	-
Age > 65 years						
No	158 (38.0)	2086 (48.1)	ref.	**-**	**-**	**-**
Yes	258 (62.0)	2249 (51.9)	**1.51** (1.23; 1.86)	**<0.001**	**-**	**-**
Smoking						
No	215 (51.7)	2455 (56.6)	ref.	**-**	ref.	**-**
Yes	184 (44.2)	1735 (40.0)	1.21 (0.89; 1.49)	0.069	**1.27** (1.03; 1.56)	**0.027**
History of co-morbidities*						
Asthma						
No	333 (80.0)	3816 (88.0)	ref.	**-**	ref.	**-**
Yes	83 (20.0)	519 (12.0)	**1.83** (1.42; 2.37)	**<0.001**	**1.84** (1.42; 2.39)	**<0.001**
Allergy						
No	134 (32.2)	2129 (49.1)	ref.	**-**	ref.	**-**
Yes	282 (67.8)	2206 (50.9)	**2.03** (1.64; 2.52)	**<0.001**	**2.02** (1.62;2.50)	**<0.001**
COPD						
No	363 (87.3)	4051 (93.4)	ref.	**-**	ref.	**-**
Yes	53 (12.7)	284 (6.6)	**2.08** (1.52; 2.85)	**<0.001**	**1.96** (1.43;2.68)	**<0.001**
Diabetes mellitus						
No	360 (86.5)	3573 (82.4)	ref.	**-**	ref.	**-**
Yes	56 (13.5)	762 (17.6)	**0.73** (0.54; 0.98)	**0.034**	**0.73** (0.55; 0.98)	**0.037**
Rheumatoid arthritis						
No	397 (95.4)	4263 (98.3)	ref.	**-**	ref.	**-**
Yes	19 (4.6)	72 (1.7)	**2.83** (1.69; 4.75)	**<0.001**	**2.68** (1.59; 4.49)	**<0.001**

Table 2. Determinants of angioedema during ACEI therapy. *(Continued)*

	No. (%)		Crude OR (95% CI)	P	Adjusted OR^ (95% CI)	P
	Cases (n=416)	*Controls (n=4335)*				
Co-medications**						
Anti-diabetic drugs						
No	378 (90.9)	3773 (87.0)	ref.	-	ref.	-
Yes	38 (9.1)	562 (13.0)	**0.67** (0.48; 0.95)	**0.026**	**0.69** (0.49; 0.98)	**0.036**
Anti-histamines						
No	202 (48.6)	4163 (96.0)	ref.	-	ref.	-
Yes	214 (51.4)	172 (4.0)	**25.64** (20.06; 32.77)	**<0.001**	**26.62** (20.72; 34.20)	**<0.001**
Anti-asthmatic drugs						
No	326 (78.4)	3849 (88.8)	ref.	**-**	ref.	**-**
Yes	90 (21.6)	486 (11.2)	**2.19** (1.70; 2.81)	**<0.001**	**2.14** (1.66; 2.75)	**<0.001**
Calcium channel blockers						
No	257 (61.8)	3143 (72.5)	ref.		ref.	**-**
Yes	159 (38.2)	1192 (27.5)	**1.63** (1.32; 2.01)	**<0.001**	**1.59** (1.29; 1.96)	**<0.001**
NSAIDs						
No	375 (90.1)	3920 (90.4)	ref.	-	ref.	-
Yes	41 (9.9)	415 (9.6)	1.03 (0.74; 1.45)	0.852	1.07 (0.76; 1.51)	0.687
Systemic corticosteroids						
No	312 (75.0)	4142 (95.5)	ref.	-	ref.	-
Yes	104 (25.0)	193 (4.5)	**7.15** (5.49; 9.32)	**<0.001**	**6.93** (5.31; 9.05)	**<0.001**
Statins						
No	215 (51.7)	2104 (48.5)	ref.	-	ref.	-
Yes	201 (48.3)	2231 (51.5)	0.88 (0.72; 1.08)	0.220	0.85 (0.69; 1.04)	0.112
Any type of cough**						
No	383 (92.1)	4123 (95.1)	ref.	-	ref.	-
Yes	33 (7.9)	212 (4.9)	**1.68** (1.14; 2.45)	**0.008**	**1.63** (1.11; 2.39)	**0.012**

* History of co-morbidities was assessed any time before the AE date.
**Co-medication was assessed 3 months before AE date.
^ Sex and age adjusted ORs. Smoking was assessed ever before the index date. OR – odds ratio. CI – confidence interval. COPD - Chronic obstructive pulmonary disease. NSAIDs - Non-steroidal anti-inflammatory drugs.
The number of individuals with unknown smoking status 3 months before the index date was 17 (4.1%) of AE cases and 145 (3.3%) of AE controls.

Table 3. Determinants of ACEI intolerance defined by a switch to ARBs in prescription records.

	No. (%)		Crude OR (95% CI)	P	Adjusted OR^ (95% CI)	P
	Cases (n=24709)	*Controls (n=84238)*				
Gender						
Male	10227 (41.4)	45941 (54.5)	ref.	-	-	-
Female	14482 (58.6)	38297 (45.5)	**1.70** (1.65; 1.75)	**<0.001**	**-**	**-**
Age >65 years						
No	11839 (47.9)	43331 (51.4)	ref.	-	-	-
Yes	12870 (52.1)	40907 (48.6)	**1.15** (1.12; 1.18)	**<0.001**	**-**	**-**
Smoking						
No	15360 (62.2)	46959 (55.7)	ref.	**-**	ref.	**-**
Yes	8577 (34.7)	34289 (40.7)	**0.77** (0.74; 0.79)	**<0.001**	**0.83** (0.81; 0.86)	**<0.001**
History of co-morbidities*						
Asthma						
No	21150 (85.6)	73986 (87.8)	ref.	-	ref.	-
Yes	3559 (14.4)	10252 (12.2)	**1.21** (1.17; 1.27)	**<0.001**	**1.17** (1.12; 1.22)	**<0.001**
Allergy						
No	7840 (31.7)	42265 (50.2)	ref.	-	ref.	-
Yes	16869 (68.3)	41973 (49.8)	**2.17** (2.10; 2.23)	**<0.001**	**2.06** (1.99; 2.12)	**<0.001**
COPD						
No	23417 (94.8)	79412 (94.3)	ref.	-	ref.	-
Yes	1292 (5.2)	4826 (5.7)	**0.91** (0.85; 0.97)	**0.003**	**0.90** (0.84; 0.96)	**<0.001**
Diabetes mellitus						
No	21210 (85.8)	69361 (82.3)	ref.	-	ref.	-
Yes	3499 (14.2)	14877 (17.7)	**0.77** (0.74; 0.80)	**<0.001**	**0.80** (0.77; 0.83)	**<0.001**
Rheumatoid arthritis						
No	24263 (98.2)	82766 (98.3)	ref.	-	ref.	-
Yes	446 (1.8)	1472 (1.7)	1.03 (0.93; 1.15)	0.545	0.93 (0.83; 1.03)	0.171
Co-medications**						
Anti-diabetic drugs						
No	22317 (90.3)	73938 (87.8)	ref.	-	ref.	-
Yes	2392 (9.7)	10300 (12.2)	**0.77** (0.73; 0.81)	**<0.001**	**0.81** (0.77; 0.85)	**<0.001**

Table 3. Determinants of ACEI intolerance defined by a switch to ARBs in prescription records. *(Continued)*

	No. (%)		Crude OR (95% CI)	P	Adjusted OR^ (95% CI)	P
	Cases (n=24709)	*Controls (n=84238)*				
Anti-histamines						
No	23119 (93.6)	81213 (96.4)	ref.	-	ref.	-
Yes	1590 (6.4)	3025 (3.6)	**1.85** (1.73; 1.97)	**<0.001**	**1.77** (1.66; 1.88)	**<0.001**
Anti-asthmatic drugs						
No	21266 (86.1)	75412 (89.5)	ref.	-	ref.	-
Yes	3443 (13.9)	8826 (10.5)	**1.38** (1.33; 1.44)	**<0.001**	**1.34** (1.28; 1.39)	**<0.001**
Calcium channel blockers						
No	17660 (71.5)	63210 (76.2)	ref.	**-**	ref.	**-**
Yes	7049 (28.5)	21028 (23.8)	**1.20** (1.16; 1.24)	**<0.001**	**1.21** (1.17; 1.25)	**<0.001**
NSAIDs						
No	22416 (90.7)	77169 (91.6)	ref.	-	ref.	-
Yes	2293 (9.3)	7069 (8.4)	**1.12** (1.06; 1.17)	**<0.001**	**1.10** (1.05; 1.16)	**<0.001**
Systemic corticosteroids						
No	23514 (95.2)	81144 (96.3)	ref.	-	ref.	-
Yes	1195 (4.8)	3094 (3.7)	**1.33** (1.24; 1.43)	**<0.001**	**1.25** (1.17; 1.34)	**<0.001**
Statins						
No	14064 (56.9)	44426 (52.7)	ref.	-	ref.	-
Yes	10645 (43.1)	39812 (47.3)	**0.84** (0.82; 0.87)	**<0.001**	**0.89** (0.86; 0.92)	**<0.001**

* History of co-morbidities was assessed any time before the switch to ARBs date.
**Co-medication was assessed 3 months before the switch to ARBs date.
^ Sex and age adjusted ORs. Smoking was assessed ever before the index date. OR – odds ratio. CI – confidence interval. COPD – Chronic obstructive pulmonary disease. NSAIDs - Non-steroidal anti-inflammatory drugs.
The number of individuals with unknown smoking status 3 months before the index date was 772 (3.1%) of switchers to ARBs and 2990 (3.5%) of continuous ACEI users.

in univariate models (OR 1.51, 95% CI: 1.23–1.86; OR 1.15, 95% CI: 1.12–1.18). Female sex was not associated with AE (OR 1.08, 95% CI: 0.88–1.32), but was associated with an increased risk of ACEI-intolerance (OR 1.70, 95% CI: 1.65–1.75). History of asthma and allergy were associated with both AE and ACEI intolerance (OR 1.83, 95% CI: 1.42–2.37; OR 1.21, 95% CI: 1.17–1.27 for asthma and OR 2.03, 95% CI: 1.64–2.52; OR 2.17, 95% CI: 2.10–2.23 for allergy, respectively). History of COPD appeared to increase the risk of AE (OR 2.08, 95% CI: 1.52–2.85), but not of ACEI intolerance (OR 0.91, 95% CI: 0.85–0.97). Patients with DM had a lower risk of AE and ACEI intolerance (OR 0.73, 95% CI: 0.54–0.98 and OR 0.77, 95% CI: 0.74–0.80, respectively). The proportion of patients with RA was higher in AE cases than in the controls (OR 2.83, 95% CI: 1.69–4.75).

The strongest associations for AE in univariate models were found with antihistamines and systemic corticosteroids within three months before the index date (OR 25.64, 95% CI: 20.06–32.77; OR 7.15; 95% CI: 5.49–9.32). These associations were also significant but less strong in ACEI intolerance (OR1.85, 95% CI: 1.73–1.97; OR 1.33, 95% CI: 1.24–1.43). Furthermore, when these determinants were examined at the cohort entry date instead of index date, association with AE remained statistically significant with OR 4.48 (95% CI: 3.41–5.88) for antihistamines and OR 2.90 (95% CI: 2.16–3.91) for systemic corticosteroids (Supplementary Table 1, 2). Anti-diabetic drugs contributed to a lower risk of both AE and ACEI intolerance in univariate analyses (Table 2, 3). Recent NSAID use was associated with a higher risk of ACEI intolerance (OR 1.12, 95% CI: 1.06–1.17). We observed similar effect size for statin use and ACEI intolerance (OR 0.84, 95% CI: 0.82–0.87) and AE during ACEI therapy (OR 0.88, 95% CI: 0.72–1.08), but the association was not statistically significant for AE.

To evaluate whether having cough during ACEI therapy could be predictive of developing ACEI-related AE in our dataset, we assessed the association of any type of cough with AE during ACEI therapy. We chose any type of cough, because it was not possible to discriminate adverse drug reactions, such as ACEI-induced cough, in the CPRD. Indeed, any type of cough was associated with AE during ACEI therapy (OR 1.68, 95% CI: 1.14–2.45).

In the forward stepwise multivariable analysis switching to ARB was associated with more factors as compared to AE. Age over 65 years (OR 1.36, 95% CI: 1.07–1.73), history of allergy (OR 1.53; 95% CI: 1.19–1.96), the use of antihistamines (OR 21.25; 95% CI: 16.44–27.46), systemic corticosteroids (OR 4.52; 95% CI: 3.26- 6.27) and calcium channel blockers (OR 1.57; 95% CI: 1.23–2.01) were associated with AE during ACEI therapy (Table 4). Age over 65 years (OR 1.06; 95% CI: 1.03–1.09), history of allergy (OR 2.02; 95% CI: 1.96–2.09), the use of antihistamines (OR 1.53; 95% CI: 1.43–1.63) and calcium channel blockers (OR 1.19; 95% CI: 1.15–1.23) were also associated with ACEI intolerance in multivariable model (Table 5). Other determinants associated with ACEI intolerance were female gender (OR 1.49; 95% CI 1.44;- 1.53), smoking (OR 0.83; 95% CI: 0.81–0.86), asthma (OR 0.88; 95% CI: 0.83–0.93), COPD (OR 0.69; 95% CI: 0.64–0.75), DM (OR 0.80; 95% CI: 0.77–0.84) and the use of anti-asthmatic drugs (OR 1.51; 95% CI: 1.42–1.61), NSAIDs (OR 1.07; 95% CI: 1.02–1.13) and statins (OR 0.92; 95% CI: 0.89–0.95).

Table 4. Determinants of angioedema during ACEI therapy in the multivariable model.

Determinants	OR	95%CI	P-value
Age >65 years	1.36	1.07;1.73	0.013
History of co-morbidities			
Allergy	1.53	1.19; 1.96	<0.001
Co-medications			
Antihistamines	21.25	16.44; 27.46	<0.001
Systemic corticosteroids	4.52	3.26; 6.27	<0.001
Calcium channel blockers	1.57	1.23; 2.01	<0.001

Co-morbidities were assessed any time before the AE date. Co-medication was assessed 3 months before the AE date. Smoking was assessed ever before the index date. OR – odds ratio. CI – confidence interval.

Table 5. Determinants of ACEI intolerance (defined by switching to ARBs) in the multivariable model.

Determinants	OR	95%CI	P-value
Female sex	1.49	1.44; 1.53	<0.001
Age >65 years	1.06	1.03; 1.09	<0.001
Smoking	0.83	0.81; 0.86	<0.001
History of co-morbidities			
Allergy	2.02	1.96; 2.09	<0.001
Asthma	0.88	0.83; 0.93	<0.001
COPD	0.69	0.64; 0.75	<0.001
Diabetes mellitus	0.80	0.77; 0.84	<0.001
Co-medications			
Anti-asthmatic drugs	1.51	1.42; 1.61	<0.001
Anti-histamines	1.53	1.43; 1.63	<0.001
NSAIDs	1.07	1.02; 1.13	0.008
Statins	0.92	0.89; 0.95	<0.001
Calcium channel blockers	1.19	1.15; 1.23	<0.001

Co-morbidities were assessed any time before the AE date. Co-medication was assessed 3 months before the AE date. Smoking was assessed ever before the index date. OR – odds ratio. CI – confidence interval. COPD - Chronic obstructive pulmonary disease. NSAIDs - Non-steroidal anti-inflammatory drugs.

DISCUSSION

We conducted two exploratory case-control studies in an extensive real-world primary care database to evaluate the association between history of co-morbidities and co-medication use with ACEI intolerance (defined by switching to ARB) and AE during ACEI therapy. The main finding of both studies is that several co-morbidities and prescriptions for different co-medication classes within 3 months before the event were significantly more prevalent in ACEI starters developing AE and ACEI intolerance, as compared to ACEI users who did not develop these adverse reactions. Moreover, although some of the associations were similar for both outcomes, we observed a larger number of associations with switching to ARB than with AE. The knowledge gained through these studies might be helpful for further research by using history of co-morbidities and recent co-medication as potential risk factors for ACEI-related adverse reactions. Several risk factors for AE which we report here have been described earlier, including among others age, female gender, smoking, allergies and some of the drug exposures [7,15,16,24–30]. We replicated the association of AE with older age, but contrary to prior observations, did not replicate an increased risk for ACEI-related AE in females and in smokers [7,15,24]. Furthermore, our finding for a positive association of AE with allergies is also in accordance with previous observations, which showed that seasonal allergies, history of drug rash, sensitization to certain food components and pollen season were all associated with a higher count of AE episodes [25,27].

It was evident from univariate analyses that history of asthma, COPD and RA were more frequent and DM less frequent among ACEI users who developed AE during ACEI therapy. However, in multivariable analyses none of these co-morbidities remained associated with AE during ACEI therapy. To the authors' knowledge, no reports on associations with asthma and COPD and ACEI-

related AE have been published. A study Byrd *et al*. found no association between RA and ACEI-related AE [26]. An explanation for a possible higher number of ACEI-related AE in RA could be an observation that patients with autoimmune disorders (such as systemic lupus erythematosus) might have an acquired antibody-mediated c1 inhibitor (c1-INH) deficiency contributing to the development of AE [31]. Under this scenario it is probable that starting an ACEI in RA could more likely trigger AE. Regarding DM it was suggested AE is less likely to develop in diabetic patients due to a higher activity of DPP-IV which is seen in hyperglycaemia [32]. A number of studies also reported that simultaneous use of DPP-IV inhibitors and ACEIs increases the risk of AE [33,34].

Our results showed that asthma and allergies occurred more frequently in switchers to ARB, while DM and COPD were less common in these patients. A study by Wyskida *et al*. found that asthma and COPD were associated with ACEI-related cough with age-adjusted OR of 1.60 and 1.70, respectively [35]. Based on validation of the database marker for ACEI intolerance used in our analyses, at least half of the cases of ACEI intolerance in the present study might be considered as having ACEI-related cough [19]. However, it is important to acknowledge the possibility of other undesirable side-effects of ACEIs or different reasons leading to a switch to ARBs. Therefore, only an indirect comparison with the results of previous studies on ACEI-induced cough is possible. While we confirmed the association with asthma, the association with COPD was in the opposite direction compared to the abovementioned study [35]. A potential explanation for this could be that ACEI users with COPD who already experience cough as a symptom of COPD, might be less likely to attribute cough to ACEIs and therefore less likely to be switched to ARBs. Similarly, we could not replicate a recently reported finding that statin use was independently associated with a higher risk for ACEI-induced cough [17].

We observed that prescriptions for antihistamines, systemic corticosteroids and calcium channel blockers within 3 months prior to the index date were most prevalent in the AE study population. Although the association with anti-histamines and systemic corticosteroids could be attributed to prescription of these drugs for the treatment of AE (reverse causation), it persisted irrespective of the moment when drug exposure was assessed, i.e. recent to the event date and at baseline and after adjusting for sex and age. Similar results for corticosteroids and other immunosuppressants have been reported in previous studies and are thought to be due to a reduced activity of the DPP-IV enzyme during immunosuppressant use [15,26,28]. In the setting of DPP-IV suppression its normal function to degrade substance P and bradykinin is compromised, causing the accumulation of these substances ultimately leading to AE [36].

The strengths of this study include using a real-world primary care patient population, a large number of events, complete data on the medication prescriptions and co-morbidities and the use of a validated marker for ACEI intolerance in prescription databases. We acknowledge a number of limitations to our study. Firstly, we could not assess the causal relationship in this observational exploratory study, because the actual reason behind AE cannot be retrieved directly from the CPRD. The Read coding system does not allow for the differentiation of hereditary AE, drug-induced AE or AE secondary to acquired C1 esterase deficiency. We believe that the contribution of ACEI to AE is likely, because we considered only AE occurring during ACEI therapy. However in cases of AE occurring after years of ACEI treatment we cannot completely exclude another trigger of AE. Furthermore, there is a possibility that diagnostic codes for allergy were entered into the CPRD together with diagnostic codes for AE to indicate the AE event, which could affect the associations described in this study. Another reason for possible errors in the ascertainment of AE, possibly compromising the relation with ACEI use, is the difference in time of the actual AE episode and the time it was entered into the CPRD. Secondly, the CPRD provides information on drug

prescriptions, but not drug dispensing, and it is not possible to verify the actual intake of a drug. Thirdly, we could not assess the influence of co-morbidities and co-medication on ACEI-induced cough, since the information on this adverse reaction was not available. Using a prescription pattern for the identification of ACEI-induced cough could have resulted in misclassification of the outcome of ACEI intolerance. Particularly, an increased number of switchers to ARBs among patients with COPD, asthma and users of systemic corticosteroids (a marker of an exacerbation) might indicate a preventive measure for patients more prone to cough, rather than the presence of ACEI-related adverse effect itself.

In conclusion, this study showed that several co-morbidities and recently prescribed co-medication were significantly more prevalent in ACEI starters developing AE and ACEI intolerance as opposed to ACEI users who did not develop these adverse reactions. Attention to the history of co-morbidities and co-medication when ACEI treatment is required might assist in identification of patients potentially at a higher risk for ACEI-related adverse drug reactions.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work.

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SUPPLEMENT

Supplementary Table 1. Association of angioedema with history of co-morbidities measured any time before ACEI start and co-medications use 3 months around ACEI start date.

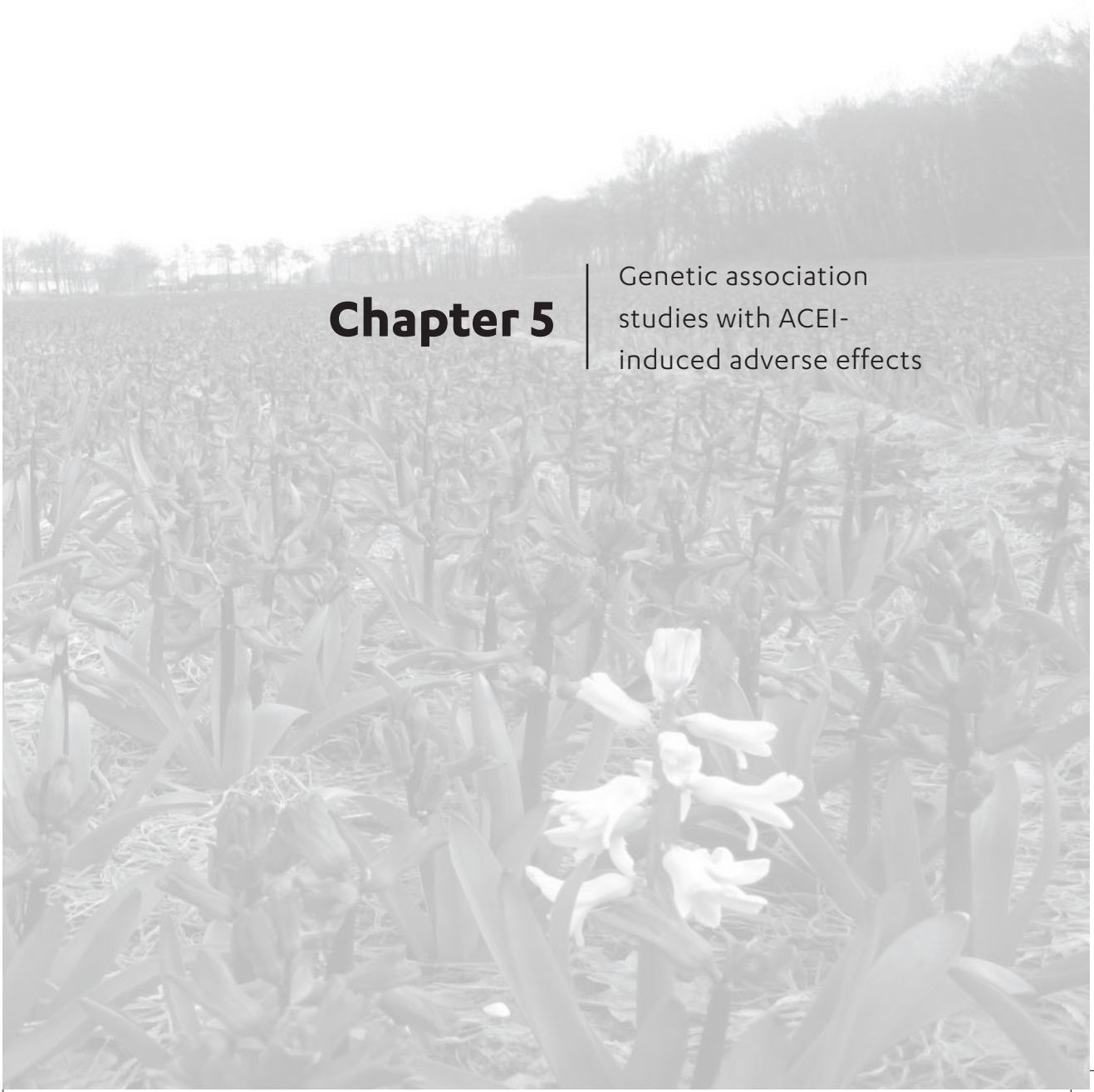
Variables	Crude OR (95% CI)	P	Adjusted OR^ (95% CI)	P
History of co-morbidities				
Asthma	1.88 (1.45-2.43)	<0.001	1.88 (1.45; 2.45)	<0.001
Allergy	1.71 (1.39-2.09)	<0.001	1.70 (1.38; 2.08)	<0.001
COPD	2.05 (1.46-2.87)	<0.001	1.90 (1.35; 2.68)	<0.001
Diabetes mellitus	0.65 (0.47-0.91)	0.011	0.65 (0.47; 0.91)	0.011
Rheumatoid arthritis	2.88 (1.69-4.89)	<0.001	2.69 (1.58; 4.58)	<0.001
Co-medications				
Anti-asthmatic drugs	1.95 (1.52-2.50)	<0.001	1.92 (1.50; 2.47)	<0.001
Anti-histamines	4.48 (3.41-5.88)	<0.001	4.49 (3.41; 5.91)	<0.001
Anti-diabetic drugs	0.62 (0.43-0.90)	0.012	0.63 (0.44; 0.92)	0.016
Calcium channel blockers	1.52 (1.24-1.87)	<0.001	1.45 (1.18; 1.78)	<0.001
Systemic corticosteroids	2.90 (2.16-3.91)	<0.001	2.79 (2.07; 3.76)	<0.001
NSAIDs	1.16 (0.88-1.52)	0.283	1.18 (0.90; 1.55)	0.228
Statins	0.86 (0.82-1.05)	0.130	0.82 (0.67; 1.01)	0.061

OR – odds ratio. ^ Sex and age adjusted ORs. CI – confidence interval. COPD - Chronic obstructive pulmonary disease. NSAIDs - Non-steroidal anti-inflammatory drugs.

Supplementary Table 2. Association of switching to ARBs with history of co-morbidities measured any time before ACEI start and co-medications use 3 months around ACEI start date.

Variables	Crude OR (95% CI)	P	Adjusted OR^ (95% CI)	P
History of co-morbidities				
Asthma	1.19 (1.15-1.25)	<0.001	1.15 (1.10; 1.20)	<0.001
Allergy	1.28 (1.24-1.31)	<0.001	1.20 (1.17; 1.24)	<0.001
COPD	0.89 (0.84-0.96)	0.001	0.88 (0.82; 0.94)	<0.001
Diabetes mellitus	0.76 (0.73-0.79)	<0.001	0.79 (0.76; 0.83)	<0.001
Rheumatoid arthritis	1.03 (0.92-1.15)	0.595	0.93 (0.83; 1.03)	0.163
Co-medications				
Anti-asthmatic drugs	1.21 (1.16-1.26)	<0.001	1.17 1.12; 1.22	<0.001
Anti-histamines	1.51 (1.43-1.59)	<0.001	1.43 (1.35; 1.51)	<0.001
Anti-diabetic drugs	0.74 (0.70-0.77)	<0.001	0.77 (0.73; 0.81)	<0.001
Calcium channel blockers	1.18 (1.16-1.24)	<0.001	1.16 (1.13; 1.20)	<0.001
Systemic corticosteroids	1.25 (1.18-1.32)	<0.001	1.17 (1.10; 1.24)	<0.001
NSAIDs	1.09 (1.04-1.13)	<0.001	1.08 (1.03; 1.12)	<0.001
Statins	0.79 (0.77- 0.81)	<0.001	0.83 (0.80; 0.85)	<0.001

OR – odds ratio. ^ Sex and age adjusted ORs. CI – confidence interval. COPD - Chronic obstructive pulmonary disease. NSAIDs - Non-steroidal anti-inflammatory drugs.



Chapter 5

Genetic association studies with ACEI-induced adverse effects

Chapter 5.1

Pharmacogenetics of ACE inhibitor-induced angioedema and cough: a systematic review and meta-analysis

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ABSTRACT

Aim

Angioedema and cough are the two most important adverse effects of ACE inhibitors (ACEIs). Evidence exists that ACEI-related angioedema/cough is partly genetically determined and several genes have been identified to play a role in the development of ACEI-related adverse effects.

Materials & methods

This study was performed in order to evaluate the evidence of these genetic associations and ACEIs' adverse effects. After removing duplicates and critical appraisal, 19 studies were considered to be eligible to review; 14 articles about cough and five articles about angioedema. A separate meta-analysis was performed for the most studied ACE insertion/deletion polymorphism (rs4646994) and its association with cough.

Results & conclusion

One gene region (*XPNPEP2*) was associated with ACEI-induced angioedema in three studies. In our meta-analysis we did not find a significant association between the ACE insertion/deletion polymorphism and ACEI cough.

INTRODUCTION

Angiotensin converting enzyme inhibitors (ACEIs) are commonly used in the management of hypertension, heart failure, myocardial infarction, renal failure, and diabetic nephropathy. Compared to placebo, ACEIs decreased the mortality of cardiovascular disease up to 18% during three years of follow up [1,2].

In 2010, ACEIs were the fifth on the list of most frequently used medications, with 168 million prescriptions in the United States [101].

In a cohort study 19% of patients discontinued ACEIs because of adverse drug reactions (ADRs) of which the most common is persistent, dry cough [3]. Cough occurs in 5–20% of patients treated with ACE inhibitors and is more frequent in women. ACEI-induced cough is supposed to be caused by Bradykinin and substance P accumulation due to ACE inhibition [4]. Angioedema is the most severe adverse effect of ACEIs with an increasing incidence of 0.1–0.2% to 1% during the last decade [2]. The first case of angioedema associated with ACEIs was described more than 30 years ago [5]. Angioedema is defined as quickly developing inflammation in dermis, subcutaneous tissue, mucosa and sub mucosal tissues. The mechanism is not fully understood but it is proposed that Bradykinin has an important role in angioedema as a potent vasodilating agent that increases permeability of vessels, therefore fluid will go out of the vessels and that might lead to inflammation6. Life-threatening edema of the upper airway, which is present in 25–39% of cases of ACEI-induced angioedema, can be developed in a very short time so within few minutes the upper airway can be completely blocked. Therefore, patients need intravenous medication rapidly, and in some cases intubation is indicated. If intubation is not possible anymore because of the swelling, acute tracheotomy can be necessary. Eventually some of these angioedema's can be resistant to treatment and even lethal [7,8].

There is variation in the risk of developing ACEI-induced cough and angioedema for individual patients and the underlying mechanism of this difference is not clear, but there are clues that indicate a role for genetic factors, for instance, angioedema patients often have affected relatives and the frequency of these two side effects varies among different racial groups [9–11]. The incidence of drug withdrawal because of persistent dry cough is higher in Chinese patients (23%) treated with ACE inhibitors compared with Caucasians (5–11%) [12,13].

The ACEI-induced angioedema rates are significantly higher in blacks than in whites [14,15] and the risk in women is higher than in men; however, diabetic patients have a lower risk than non-diabetics [16,17].

Moreover several studies found genetic variations related to the development of ACEI side effects confirming the hypothesis that genetics may play a role. However, results of the different candidate gene studies are inconsistent and most of the studies are small in size.

The aim of this study was to review available pharmacogenetic studies on angioedema and cough induced by ACEI use and to aggregate results of individual studies to see which genes are in an association with ACEI induced cough and angioedema for future implementation in clinic for personalized medicine.

MATERIALS & METHODS

Search strategy

The MOOSE guidelines for the meta-analysis of observational studies in epidemiology were used [18]. The search was conducted in PubMed, Scopus, Embase, Web of Knowledge and Cochrane through title, abstract and keywords of articles from the earliest available dates till 12th April 2012 without any limitation. Search terms and search strategy are listed in Appendix 1.

Study selection

After removing duplicates, title and abstracts of the articles were screened manually in order to exclude irrelevant studies. Studies were included in the current review if they evaluated the association between genetic polymorphism and ACEI-induced cough or angioedema in ACEIs consumers. Case reports, review articles and abstracts of conferences were excluded from the review. All references quoted in the included studies or review studies were also evaluated to identify additional published articles not included in the original search or not indexed in the database. Full texts of all included studies were retrieved via the library of Utrecht University or via contact with the authors. The following information was extracted from the included studies: first author, year of publication, ethnicity, gender, study design, number of cases and controls, gene polymorphism, and study findings.

Meta-analysis

Number of cases and controls for different ACE I/D genotypes were extracted separately; authors were contacted through e-mail for articles when the study details could not be extracted or when articles were not in English language. Studies were excluded if the author did not respond to e-mail.

The significance of the association for the allele contrast model (I versus D) was calculated for all studies separately and was indicated as odds ratios (ORs) with 95% confidence intervals (CI). The random effects (RE) model was used with inverse variance weighting to calculate pooled ORs based on the individual ORs. Statistical heterogeneity between studies was tested via calculation of I^2.

Review Manager (RevMan). Version 5.1. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2011 and R software (Windows, version 2.15.1) were utilized for the meta-analysis and calculation of the heterogeneity tests and for creating funnel plots.

A chi-square (χ^2) test was used to test Hardy-Weinberg equilibrium (HWE) in the control population. Level of significance was considered to be 0.05 for HWE.

RESULTS

Eligible studies

After removing duplicates, 106 articles were found with the described search strategy. Nineteen articles were included in the review according to the inclusion and exclusion criteria's. Figure 1 shows a flowchart of retrieved articles and excluded articles with illustrating reasons. Five studies that investigated the relationship between ACEI-induced angioedema and gene polymorphisms were descriptively reviewed. Fourteen studies that investigated the relationship between ACEI-induced cough and single nucleotide polymorphisms (SNPs) were reviewed. Among them twelve studies were appropriate for a meta-analysis as they all studied the association of the ACE Insertion/Deletion polymorphism with ACEI-induced cough.

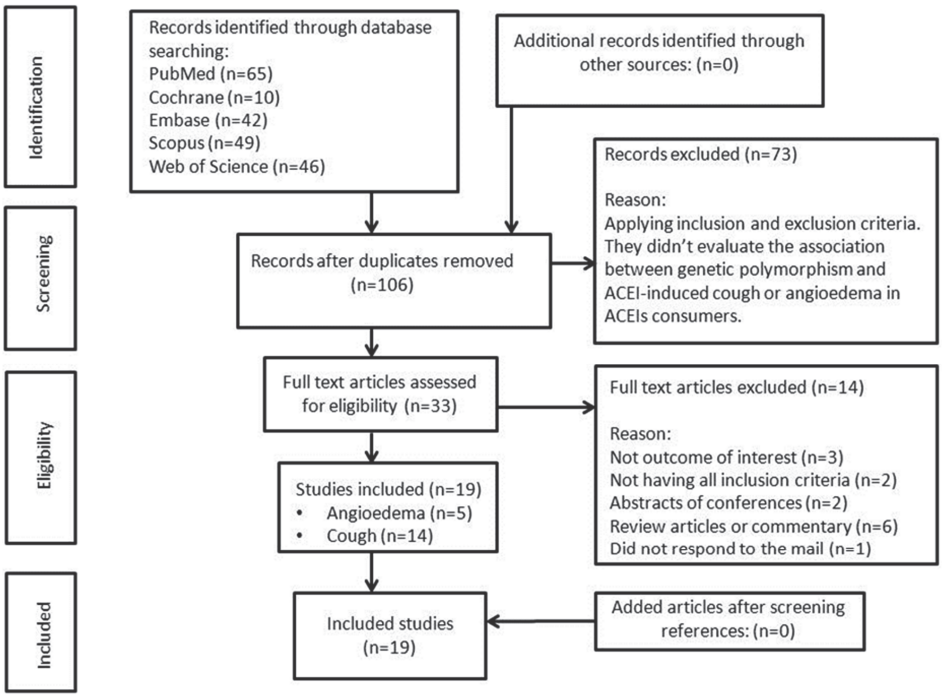


Figure 1. Literature search results.

Characteristics and reported results of the selected studies for angioedema

All five articles that explored the relationship between ACEI-induced angioedema and genetic polymorphisms were case-control studies. Two of the studies were performed in Caucasians, one had a mixed population of African-Americans and Caucasians, and two other studies did not mention the ethnicity of the population (see Table 1). The first article was published in 2005 [19] and the last one in 2011[20]. Four gene polymorphisms and one haplotype were investigated for their relationship with ACEI related angioedema. XPNPEP2 C-2399A (rs3788853) was positively associated with ACEI-angioedema in all three studies which it was investigated in. The total population of these three studies consisted of 223 cases and 584 controls. However there are some degrees of overlap between the population of the first study and the last study and the populations of both studies are smaller compared with the study from the US. All three studies reported the association of C-2399A polymorphism (rs3788853) with ACEI-induced angioedema [19–21]. It was suggested that this angioedema is related to the lower plasma Amino Peptidase P (APP) activity. Therefore 25 SNPs within the XPNPEP2 gene were identified to test whether genetic factors contribute to the variation in plasma APP activity and the risk of angioedema. Three of them (C-2399A; rs3788853, G-1612T; rs2050011, G-393A; rs2235444) were significantly associated with APP activity in the study population consisted of 34 cases and 127 controls, matched according to the gender and country [20]. Extended data analysis showed that a common ATG haplotype of these three SNPs was strongly correlated with reduced plasma APP activity. This functional

Table 1. Characteristics and reported results of studies that investigated the relationship between Gene polymorphism and Angioedema induced by ACEIs.

Country	Ethnicity	Age (years)	Gender (F %)	Population & treatment duration	Genotyping method	Gene Polymorphism	Results
USA, Canada, Belgium [19]	Caucasian	NR	65	20 cases (<8 years) 60 unrelated controls	Radiolabeled PCR	XPNPEP2 C-2399A (rs3788853)	Significant association between the C-2399A and AE-ACEI. (P = 0.0364)
Turkey [23]	Caucasian	58 ± 9	28	32 cases (1 - 36 months) 46 controls (NR)	PCR	ACE I/D (rs4646994)	No effect of ACE gene polymorphism on ACEI or ATRB induced angioedema
USA [21]	Black & Caucasian	57 ± 14	52	169 cases (median 5 months) 397 controls (median 42 months)	Allele specific PCR	XPNPEP2 C-2399A (rs3788853)	XPNPEP2 -2399 A/associated with increased risk of angioedema in men OR = 2.17 (95%CI:1.09–4.32) (P =0.03)
Germany [22]	NR	62 ± 2	47	65 cases (37±5 months) 65 controls (49±3 months)	Allele specific PCR	ACE I/D (rs4646994)	ACE I/D: No significant differences in allele frequencies (P = 0.095)
						BDKRB2 2/3	BDKRB2 2/3: No significant differences in allele frequencies (P = 0.26)
						BDKRB2 c.C181T	BDKRB2 c.C181T: No significant differences in allele frequencies (P = 0.1)
USA, Canada, Belgium [20]	NR	NR	NR	34 cases (NR) 127 controls (NR)	Assay by design SNP detection system	XPNPEP2 C-2399A (rs3788853)	a functional haplotype in the XPNPEP2 5' regulatory region is associated with reduced plasma APP activity and increased risk for AE-ACEI OR = 4.87 (95%CI:1.78–13.35) (P = 0.002). In contrast, the odds ratio for subjects possessing the C-2399A allele compared with those homozygous for the C allele was 3.30 (95%CI:1.35,8.10) (P = 0.009)
						XPNPEP2 ATG haplotype	a common ATG haplotype in the XPNPEP2 5' regulatory region, caused a greater increased risk for AE-ACEI than the c-2399C>A polymorphism alone (4.9-fold compared with 3.0-fold increased risk, respectively)

Abbreviations: NR = Not Reported; AE-ACEI = Angioedema Induced by ACEI; ARB = Angiotensin Receptor Blocker; P = P-Value; OR = Odds Ratio; CI = confidence interval, PCR=Polymerase Chain Reaction, SNP=Single Nucleotide Polymorphism.

ATG haplotype in the XPNPEP2, 5' regulatory region was also more informative than the C-2399A polymorphism alone when comparing the increased risk of ACEI-angioedema [20].

The ACE I/D polymorphism did not show any statistical significant association with ACEI related angioedema in two studies. However, one of them did find a trend for II genotype of this polymorphism with ACEI mediated angioedema [22, 23]. Two gene polymorphisms within the Bradykinin receptor B2 gene were also not associated with ACEI related angioedema.

Characteristics and reported results of the selected studies for cough

Fourteen studies evaluated the relationship between different gene polymorphisms and ACEI-Induced cough, six studies considered only ACE I/D polymorphism, six studies considered ACE I/D in addition to other gene polymorphisms separately and two studies did not consider ACE I/D polymorphism at all.

According to above statements, a descriptive review was performed for gene polymorphisms other than ACE I/D (eight studies were included in this part) and a meta-analysis was done for twelve published studies that investigated the ACE I/D polymorphism and its association with ACEI-Induced cough.

A: ACEI-cough and gene polymorphisms other than ACE I/D

The study characteristics and reported results of the eight studies are summarized in Table 2.

The Bradykinin B2 receptor gene (BDKRB2) was the second most researched gene after ACE gene with seven studies on different polymorphisms. The BDKRB2–58T/C polymorphism (rs1799722) was explored in five studies. Four of them were in populations of East-Asian origin; among these four East-Asian studies, three were previously combined in a meta-analysis and, in combination, showed a significant association with ACEI related cough [24]. But this association was not observed in the fourth study in Chinese Non-Insulin Dependent Diabetes Mellitus (NIDDM) patients [25]. One Spanish study in Caucasians also did not show relationship with the BDKRB2–58T/C. However, a relationship was found with another SNP in the BDKRB2 gene in this Spanish study [26]. In another Spanish study four different SNPs in the BDKRB2 gene were found to be associated with ACEI-cough in Caucasians [27]. SNPs in the Bradykinin B1 receptor gene were not correlated with cough induced by ACEIs [26, 27].

A Korean cohort study reported an association between ACEI related cough and a Neurokinin 2 receptor gene polymorphism in East-Asians but this has not been replicated in other studies yet [28]. Two large Spanish studies found significant associations for SNPs within the following genes; ABO (Glycosyltransferases), MME (Membrane Metallo Endopeptidase), PTGER3 (Prostaglandin E receptor 3) and ACE (angiotensin converting enzyme). But none of these have been replicated in other populations.

The abbreviations, products and functions of all mentioned genes in this review article are listed in Appendix 2.

Table 2. Characteristics and reported results of studies that investigated the relationship between Gene polymorphism and Cough induced by ACEIs.

Country & Study type	Ethnicity	Age (years)	sex (F %)	Population & treatment duration	Genotyping method	Gene Polymorphism	Results
Japan Case-control [29]	East-Asian	51±8	57	70 cases (2 weeks) 120 controls (more than 1 year)	PCR, restriction isotyping, Single-strand conformation polymorphism	AGTR1-1166 A/C	There was an association between cough and T allele and TT genotype especially in females for BDKRB2 gene (P<0.001) but no association was seen with the other 3 polymorphisms in this study
						AGTR2 C/A	
						BDKRB2-58 T/C	
						BDKRB2 I/D	
China Prospective cohort [25]	East-Asian	47 - 72	56	(189 NIDDM patients for 8 weeks treatment) 93 cough + 96 cough –	PCR, single-strand conformation polymorphism	BDKRB2-58 T/C	There was no association between BDKRB2-58T/C and cough in NIDDM patients
Japan Case-control [30]	East-Asian	51±7	63	30 cases (2 weeks) 30 controls (NR)	PCR, single-strand conformation polymorphism	BDKRB2-58 T/C	There was an association between cough and T allele and TT genotype (p=0.001), especially in females
International Nested case-control [31]	Caucasian	57±10	58	99 cases (6 weeks) 70 controls(NR)	PCR-RLFP technique, radiolabeled PCR	BDKRB2 +/-	There were no associations
						MCC BstXI	
South Korea Case-control [32]	East-Asian	58±11	38	50 cases 60 controls (more than 3 months)	PCR, sequencing	BDKRB2-58 T/C	There were no significant associations
						BDKRB2-59 C/A	
South Korea Prospective cohort [28]	East-Asian	58±13	40	22 cough + 69 cough – (6 months)	PCR, single-base extension	NK2R	The study indicates that the Gly231Glu polymorphism was associated with a lower prevalence of ACE inhibitor-related cough (P=0.029)
Spain Retrospective cohort [27]	Caucasian	69±1	50	102 cough + 179 cough –	Mass ARRAY genotyping system	ACE	59 SNPs were analysed in 6 gene regions. The study showed associations between cough and polymorphisms in the BDKRB2 (rs945032,P=0.018) (rs8016905,P=0.003) (rs4900312,P=0.038) (rs8013400,P=0.042), ABO (rs495828,P=0.001), and ACE (rs4459610,P=0.005) (rs4267385,P=0.004)
						BDKRB2	
						XPNPEP2	
						ABO	
						AGTR1	
						BDKRB1	

Table 2. Characteristics and reported results of studies that investigated the relationship between Gene polymorphism and Cough induced by ACEIs. *(Continued)*

Country & Study type	Ethnicity	Age (years)	sex (F %)	Population & treatment duration	Genotyping method	Gene Polymorphism	Results
Spain Case-control [26]	Caucasian	Mean 57	50	144 cases(NR) 105 controls (more than 1 year)	PCR, single-base extension	ACE	39 polymorphisms in 19 genes were studied. Associations were shown between cough and the BDKRB2 (rs8012552) (P=0.012), MME (rs2016848) (P=0.002), PTGER3 (rs11209716) (P=0.002) and ACE (rs4344) (P=0.027 Male), (P=0.031 Female) genes.
						ACE2	
						AGTR1	
						BDKRB1	
						BDKRB2	
						CPN1	
						CPN2	
						MME	
						NOS1	
						NOS2A	
						PTGER1	
						PTGER2	
						PTGER3	
						PTGER4	
						PTGES	
						PTGIR	
						PTGIS	
						XPNPEP1	
						XPNPEP2	

Abbreviations: NR = Not Reported, PCR=Polymerase Chain Reaction, RLFP= Restriction Fragment Length Polymorphism.

B: Meta-analysis of ACE I/D gene polymorphism

In total, a population of 2623 hypertensive patients (in twelve studies) with ACEI consumption was investigated, including 1136 cases (patients with cough due to ACEIs) and 1487 controls (patients without cough due to ACEIs). Table 3 shows the population characteristics of the twelve studies that were included in meta-analysis. Seven studies concerned East-Asian (three Chinese, three Japanese and one Korean) and five studies concerned Caucasian patients (two Spanish, one French, one English, one international). Meta-analysis was performed for all the studies combined, and we also performed the meta-analysis stratified for ethnicity. Population size in these studies ranged from 96 to 716 patients. As hypertension is a disease of older ages, not surprisingly the mean age of all studies was higher than fifty years.

Table 3. Population characteristics and study type of the included studies in the meta-analysis.

Country & study type	Ethnicity	Population & treatment duration	Genotyping methods	Age (years)	Gender (%F)
Japan [33] Retrospective Cohort	East-Asian	102 NA	PCR	47 - 83	49
France [34] Case-control	Caucasian	146 At least 6 months	NA	53 ± 10	NA
England [35] Case-control	Caucasian	252 NA	NA	NA	NA
International [31] Nested Case-control	Caucasian	169 6 weeks	PCR	57 ± 10	58
China [25] Prospective Cohort	East-Asian	189 8 weeks	PCR	47 - 72	56
Japan [36] Nested Case-control	East-Asian	96 NA	PCR	60 ± 10	42
Japan [29] Retrospective Case-control	East-Asian	190 At least 2 weeks	PCR	51 ± 8	57
China [37] Nested Case-control	East-Asian	716 NA	NA	NA	NA
South Korea [32] Case-control	East-Asian	110 At least 3 months	PCR	58 ± 11	38
Spain [26] Case-control	Caucasian	249 At least one year	PCR	Mean 57	50
Spain [27] Retrospective Cohort	Caucasian	281 but 4 missed so 277 in total At least 8 moths	Conventional PCR	69 ± 1	50
China [38] Cohort	East-Asian	127 8 weeks	NA	NA	NA

Abbreviations: NA = Not Available, %F=Female percentage, PCR=Polymerase Chain Reaction.

Table 4 shows the distribution of different genotypes and allele frequencies in each study populations in meta-analysis. P-value for Hardy-Weinberg Equilibrium (HWE) was calculated for cases and controls in each study as they are shown in Table 4 and this p-value was significant in the control group of one study, which means that the control population of just one study is not in the HWE. Deviation from HWE in the case groups can be due to the small sizes of the studies, but can also reflect an effect of the genetic variant for developing cough.

Table 4. The different Genotypes distribution of ACE I/D polymorphism for Cases and Controls.

		Cases						Controls					
		Distribution of I/D genotype			Allele frequency		HWE	Distribution of I/D genotype			Allele frequency		HWE
Reference	Year	II	ID	DD	I	D	*P*	II	ID	DD	I	D	*P*
Furuya K	1994	19	12	0	81	19	0.18	25	35	11	60	40	0.83
Chadwick IG	1994	6	12	13	39	61	0.31	58	97	66	48	52	0.07
Kreft-Jais C	1994	12	33	30	38	62	0.57	15	29	27	42	58	0.18
Zee RYL	1998	23	41	35	44	56	0.11	12	37	21	44	56	0.53
Lee YJ	2001	46	44	3	73	27	0.05	33	48	15	59.4	40.6	0.72
Okumura H	2001	20	16	6	67	33	0.35	19	31	4	64	36	0.07
Mukae S	2002	8	45	17	44	56	0.01	28	73	19	54	46	0.01
Lu J	2003	148	163	40	65	35	0.63	160	165	40	66	34	0.79
Ye RJ	2004	27	14	7	71	29	0.04	16	35	28	42	58	0.41
Woo SW	2009	26	16	8	68	32	0.06	24	31	5	66	34	0.25
Grilo A	2011	15	80	49	38	62	0.03	14	55	36	40	60	0.33
Mas S	2011	14	47	41	37	63	0.93	32	80	63	41	59	0.46

Abbreviation: HWE=Hardy-Weinberg Equilibrium, P=P-value, I= Insertion, D=deletion

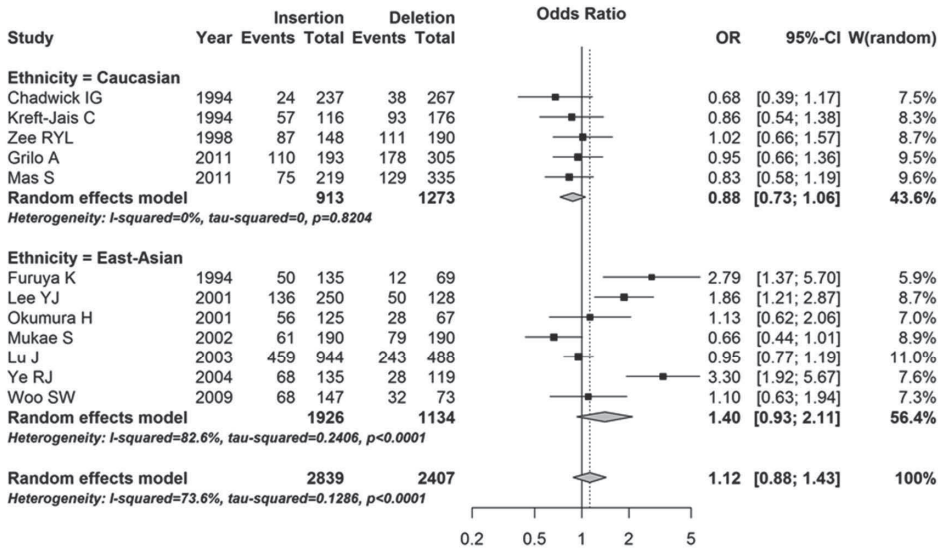
The pooled OR for the combination of all studies was 1.12 (95%CI 0.88–1.43), but the meta-analysis of all the studies showed high heterogeneity (I^2=73.6%) as expected. Therefore, subgroup analyses were performed. No heterogeneity was observed within Caucasians while the heterogeneity increased between East-Asian (Figure 2). Three Chinese studies and three Japanese studies existed in the East-Asian subgroup. Both the Japanese and the Chinese studies showed heterogeneity when analyzed separately; however, the Chinese population showed more heterogeneity than the Japanese (I^2 =92% vs. I^2 =83%).

A small protective effect of I allele can be seen in Caucasians (OR=0.88, 95% CI 0.73–1.06); however, for East-Asians this allele increases the risk of cough (OR 1.40, 95% CI 0.93–2.11) but with high heterogeneity between results from East-Asian populations. On the other hand the allele frequency for the ACE I/D polymorphism is not the same in Caucasians and East-Asians, it seems that the frequency of D allele is relatively higher in Caucasians comparing to East-Asians [39].

In the funnel plot in Figure 3 all studies in the meta-analysis have been included. This funnel plot is not adjusted to correct for publication bias because otherwise unverifiable assumptions should have been made [40]. Clear publication bias was not seen for the allele contrast of I versus D. However two East-Asian studies were published with a large OR and small sample size [33, 38]. This might account for some degree of publication bias.

DISCUSSION

ACEI-angioedema was not significantly associated with the ACE I/D polymorphism and larger studies might provide more definitive conclusions to clarify the impact of this polymorphism on ACEI-angioedema. However, three studies found a significant association with SNPs in the XPNPEP2 gene. The common ATG haplotype within this gene seems to be most informative



Abbreviation: OR = Odds Ratio, CI = Confidence Interval, W = weight.

Figure 2. Random effect pooled ORs for the allele contrast (I vs. D) model. Subgroup analyses were also carried out for Caucasians and East-Asian separately.

(4.9-fold increased risk for haplotype compared to a 3-fold increased risk for polymorphism alone). This haplotype might be a suitable predictor for ACEI induced angioedema if these study results will be replicated in other populations [20].

ACEI- cough was not significantly associated with the ACE I/D polymorphism in our meta-analyses of the allele contrast model within the Caucasian population, the East-Asian population and the total population. However, heterogeneity was present in the total population and in the East-Asian population but not in the Caucasian population. For both East-Asians and Caucasians larger studies showed smaller associations, and all studies showing large effects are relatively small. Therefore, it seems that the large odds ratios in the smaller studies are due to chance. This would suggest that there is no real effect of the insertion/deletion, which is also supported by the meta-analysis.

Biologically, the pathogenesis of ACEI related angioedema and cough can be due to failure in many pathways. By the B2 receptor stimulus, Bradykinin increases vascular permeability. Also, substance P is released from nerve terminals through Bradykinin stimulation and then substance P itself stimulates the NK1 and maybe NK2 receptors and increases vascular permeability again through this stimulation. Blocking either B2 or NK1 receptors can be preventive of ACEI related angioedema in animal models [41, 42]. It has also been demonstrated that NK2 receptor stimulation plays a role in the development of airway hyperresponsiveness and that NK1 receptor stimulation influences microvascular leakage hypersensitivity in animals [43]. Thus, difference in enzymes responsible for the Bradykinin inactivation including ACE, APP, Neutral Endopeptidase P (NEP), or enzymes that inactivate substance P such as ACE, Dipeptidyl Peptidase IV (DPP IV), NEP, or variation in the receptors of Bradykinin (B1 and B2) and substance P (NK1 and NK2) may contribute to angioedema and cough; for instance elevated levels of the sensory neuropeptide

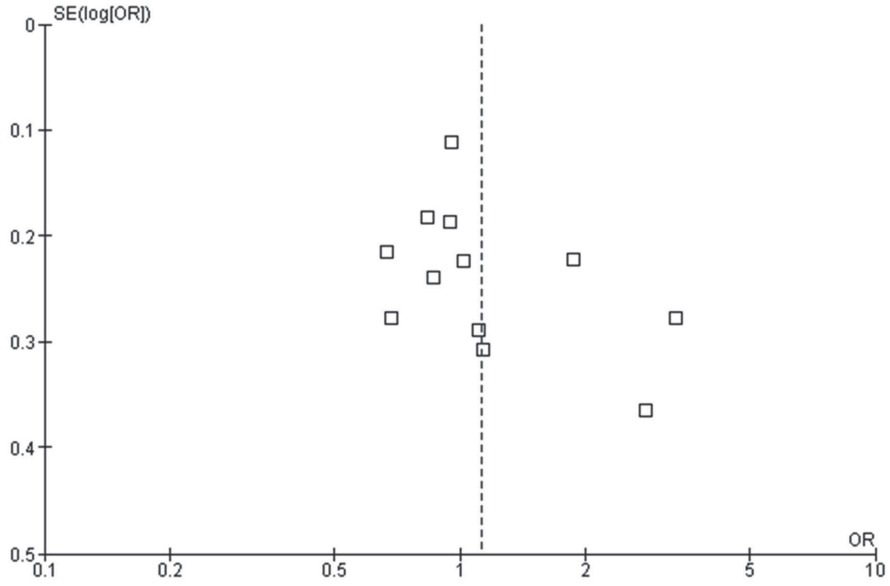


Figure 3. Funnel plot of SE against OR

substance P are related to decreased DPP IV activity and reduced activity of DPP IV was reported in some ACEI-angioedema cases [44, 45]. Additionally, low plasma amino peptidase P activity was significantly associated with ACEI-induced angioedema [46] and in 2005 Duan et al showed that a variant in XPNPEP2 (candidate gene encoding membrane-bound APP) was associated with angioedema induced by ACEIs [19]. Also patients with ACEI-related cough have been found to have higher levels of substance P. This is supposed to be the result of suppressing the catabolism of substance P trough ACE inhibition [47]. In another experiment, one genotype in the BDKRB2 gene was described to influence Bradykinin-related vasodilation during ACE inhibition [48]. There were more studies available about the relationship between ACEI-cough and Bradykinin B2 receptor and one meta-analysis confirmed that BDKRB2–58 T/C polymorphism showed a significant association with ACEI-cough within East Asians [24]. ACEIs inhibit Bradykinin degradation; some articles have shown a higher level of Bradykinin and its active metabolite, des-Arg 9 -Bradykinin during ACEI treatment or in the angioedema attacks [49, 50]. A linkage between manifestations of ACEI induced angioedema and the ACE I/D polymorphism would be probable because previous studies showed the effect of this polymorphism on the Bradykinin metabolism [51]. In 1990 Rigat et al showed that the serum level of ACE is associated with an Insertion/Deletion (I/D) polymorphism in the ACE gene [52]. The ACE I/D polymorphism was found to be correlated with serum ACE activity as well [53]. Following that, in 1994 Furuya et al found a relationship between ACE I/D polymorphism in the ACE gene and susceptibility to ACEI-induced cough [33]. One study showed that the sensitivity to cough response is significantly associated with ACE I/D polymorphism [54]. Takahashi et al performed a randomized, placebo-controlled, double-blind, cross-over study, using different concentrations of capsaicin solutions as a cough trigger. They showed that the cough threshold can be modified by ACE I/D polymorphism after using Cilazapril [55]. Another

study did not find association between ACE I/D polymorphism and susceptibility to develop chronic cough regardless the ACEI consuming [56].

One meta-analysis of seven studies reported association between ACE I/D polymorphism and cough in East-Asians but not in Caucasians [24]. We included all of its studies except one study in Chinese language. Another recent meta-analysis indicated that the ACE I/D polymorphism might be a genetic, age-dependent risk factor for ACEI-cough [57], this study included data from the China National Knowledge Infrastructure (CNK1) database which was not available to us. However, we included two new Spanish studies. Heterogeneity was observed in both analyses and was not present in Caucasians. This heterogeneity can be due to large association that is reported by small studies as mentioned previously. Besides that, East-Asian studies were more diverse than Caucasians studies.

A limitation of this review is the variation between designs of included studies. For example some of them were cohort and others were case-control studies. Furthermore the sample size of all studies was relatively small.

Because of the huge amount of data in this paper we only performed the allele contrast analysis and no other models were investigated.

In conclusion, one polymorphism was associated with ACEI-angioedema in three studies and two polymorphisms were associated with ACEI-cough in more than two studies. In our meta-analysis we have not found a significant association between the ACE I/D polymorphism and ACEI-induced cough although it is possible that the I allele has a small protective effect in Caucasians.

Future perspective

According to new developments in pharmacogenetics of ACEIs in the future it might be possible to predict the efficacy of ACEIs in individual patients [58]. New initiatives in pharmacogenetic research are needed to determine the efficacy of ACEIs in each patient and to live up the promise of personalized medicine. If physicians are able to predict which patients will experience ADRs due to ACEI use, they can make an informed decision to prescribe an ACEI for a patient or provide an alternative to increase compliance and subsequently decrease adverse clinical events. However, at this moment it is not clear which genetic variants should be determined to predict efficacy and safety in daily clinical practice.

Therefore we recommend that large scale targeted sequencing approach studies using genes involved in the Bradykinin and RAAS (Renin Angiotensin Aldosterone System) pathway should be performed in the future. Next to that large scale genome wide association studies (GWAS) should be carried out to identify other genes/SNPs that might be associated with ACEI side effects and that will be never discovered using a candidate gene approach. If SNPs are identified cost-effectiveness studies should be designed to evaluate the cost-effectiveness of implementing the genotype-guided prescription of ACEIs in clinical practice.

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APPENDIXES

Appendix 1: search terms and search strategy

"cough" OR "coughs"	**OR**	"angioedema" OR "angioedemas" OR "Quincke's Edema" OR "Edema, Quincke's" OR "Quincke Edema" OR "Quinckes Edema" OR "Angioneurotic Edema" OR "Angioneurotic Edemas" OR "Edema, Angioneurotic" OR "Edemas, Angioneurotic"
AND		
"ACEI" OR "Angiotensin Converting Enzyme Inhibitors" OR "Antagonists, Angiotensin-Converting Enzyme" OR "Antagonists, Angiotensin Converting Enzyme" OR "Angiotensin I-Converting Enzyme Inhibitors" OR "Angiotensin I Converting Enzyme Inhibitors" OR "Inhibitors, Angiotensin-Converting Enzyme" OR "Enzyme Inhibitors, Angiotensin-Converting" OR "Inhibitors, Angiotensin Converting Enzyme" OR "ACE Inhibitors" OR "Kininase II Antagonists" OR "Inhibitors, Kininase II" OR "Kininase II Inhibitors" OR "Inhibitors, ACE" OR "Angiotensin-Converting Enzyme Antagonists" OR "Angiotensin Converting Enzyme Antagonists" OR "Enzyme Antagonists, Angiotensin-Converting" OR "Antagonists, Kininase II"		
AND		
"Pharmacogenetics" OR "pharmacogenomics"	**OR**	"Genetic Polymorphisms" Or "Polymorphism (Genetics)" Or "Polymorphisms, Genetic" Or "Genetic Polymorphism"

Appendix 2: abbreviation, name, product and function of all genes that were reviewed in this article.

Gene name	Product	Function
ACE ACE2	Angiotensin converting enzyme Angiotensin converting enzyme2	Catalyzes the conversion of decapeptide angiotensin I to octapeptide angiotensin II. an exopeptidase that catalyses the conversion of angiotensin I to the nonapeptide angiotensin or the conversion of angiotensin II to angiotensin
BDKRB1 BDKRB2	B1 receptor of bradykinin B2 receptor of bradykinin	After bounding to bradykinin can increase the cytosolic calcium, resulting in chronic and acute inflammatory responses.
XPNPEP1 XPNPEP2	X-prolyl aminopeptidase Membrane bound APP (aminopeptidase P)	A proline-specific metalloaminopeptidase that specifically catalyzes the removal of any unsubstituted N-terminal amino acid that is adjacent to a penultimate proline residue. A hydrolase specific for N-terminal imido bonds, which are common to several collagen degradation products, neuropeptides, vasoactive peptides, and cytokines.
MME	Membrane metalloendopeptidase	a zinc-dependent metalloprotease enzyme that degrades a number of small secreted peptides
PTGER1 PTGER2 PTGER3 PTGER4	Prostaglandin E receptor 1 Prostaglandin E receptor 2 Prostaglandin E receptor 3 Prostaglandin E receptor 4	A G-protein that depends on the tissue can have several biological functions.
NK2R	Neurokinin 2 receptor	Involve in inflammatory and pain response

Appendix 2: abbreviation, name, product and function of all genes that were reviewed in this article. *(Continued)*

Gene name	Product	Function
ABO	Glycosyltransferases	Determine the ABO blood group of an individual by modifying the oligosaccharides on cell surface glycoproteins. Variations in the sequence of the protein between individuals determine the type of modification and the blood group.
AGTR1 AGTR2	Angiotensin II receptor type 1 Angiotensin II receptor type 2	Responsible for the signal transduction of the vasoconstricting stimulus of the main effector hormone, angiotensin II.
MCC	Mast cell chymase	Show broad peptidolytic activity and are involved in a variety of functions, Chymases are also known to convert angiotensin I to angiotensin II.
CPN1 CPN2	Carboxypeptidase N	A plasma metallo-protease that cleaves basic amino acids from the C terminal of peptides and proteins.
NOS1 NOS2A	Nitric oxide synthase 1 (neuronal) Nitric oxide synthase 2A	Family of enzymes that catalyze the production of nitric oxide (NO) from L-arginine. NO is an important cellular signaling molecule.
PTGES	Prostaglandin E synthase	An enzyme involved in eicosanoid and glutathione metabolism, a member of MAPEG family (Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism). It generates prostaglandin E (PGE) from prostaglandin H2.
PTGIR	prostaglandin I2(Prostacyclin) receptor	Prostacyclin, the major product of cyclooxygenase in macrovascular endothelium, elicits a potent vasodilation and inhibition of platelet aggregation through binding to this receptor.
PTGIS	prostaglandin I2 (prostacyclin) synthase	This enzyme belongs to the family of cytochrome P450 isomerases and catalyzes the conversion of prostaglandin H2 to prostacyclin

Chapter 5.2

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

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ABSTRACT

Background

Angiotensin converting enzyme inhibitors (ACE-inhibitors) are frequently used to treat hypertension and heart failure. Cough and angioedema are the two main adverse drug reactions (ADRs) associated with ACE-inhibitor use that occur in up to 20% of the patients and are the main reason of therapy discontinuation.

Objectives

To identify single nucleotide polymorphisms (SNPs) associated with switching of an ACE-inhibitor to an angiotensin receptor blocker (ARB) as a marker of ADRs.

Methods

A cohort of patients starting ACE-inhibitors was identified within the Rotterdam Study in the Netherlands and the GoDARTS study in Scotland. Cases were defined as intolerant subjects who switched from an ACE-inhibitor to an ARB during follow up, while controls were subjects who used ACE-inhibitors continuously for at least two years and did not switch. A GWAS using an additive model was run in these sets and results were meta-analysed using METAL and GWAMA.

Results

In total, 5161 starters of ACE-inhibitors were included in the study of which 972 subjects were identified as cases. Eight SNPs within four genes reached the GWAS significance level ($P< 5\times10^{-8}$) in the meta-analysis (*RBFOX3*, *GABRG2*, *SH2B1* and *MBOAT1*). The strongest associated SNP was located in an intron of *RBFOX3*, which contains a RNA binding protein (rs2061538: MAF=0.16, OR=1.52 [95%CI: 1.32–1.76], $p=6.2\times10^{-9}$).

Conclusion

These results indicate that genetic variation in *RBFOX3, GABRG2, SH2B1* and *MBOAT1* may increase the risk of ACE-inhibitors induced ADRs.

Key words

ACE inhibitors, ACE-inhibitor intolerance, adverse drug reaction, cough, angioedema, Genome Wide Association Study.

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

The mechanism of ACE-inhibitor induced cough and angioedema is not completely understood. ACE-inhibitors inhibit Angiotensin I Converting Enzyme (ACE) that cleaves several target proteins including angiotensin I and pro-inflammatory kinins. The blood pressure modification takes place through angiotensin I [8]. Accumulation of these inflammatory kinins is hypothesized to be the main reason of ACE-inhibitor induced angioedema 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 angioedema have also been conducted with the same approach; 3 of them found a statistically significant association between ACE-inhibitor induced angioedema 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 angioedema [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 angioedema cases and 489 controls that also used ACE-inhibitors, found no genome-wide association, which might be due to the small sample size [21]. For ACE-inhibitor induced cough, the only GWAS with 1,595 cases and 5,485 controls identified genome-wide significant associations in *KCNIP4* gene at chromosome 4 (rs145489027, p=1.0x10−8) which was replicated in 2 independent populations [22].

Based on the probable similar mechanism of ACE-inhibitor induced ADRs (cough and angioedema), 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 performed in 2 separate European populations:

A. 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 sub-cohorts (RS1, RS2, and RS3), started in 1990 in Ommoord a suburb of Rotterdam that has included 14,926 subjects aged 45 years or older (72.0 % of 20,744 eligible invited people). 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 gave informed consent to participate in the study and to obtain information from treating physicians and pharmacies, separately.

B. The Genetics of Diabetes Audit and Research in Tayside Scotland (Go-DARTS study) which is a genetic sub-study of The Diabetes Audit and Research Tayside, Scotland (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, allowing 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 7 596 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 was approved by the East of Scotland Research and Ethics Committee, in compliance with the declaration of Helsinki.

5.2

Phenotype

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

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.

For defining continuation, discontinuation or switching, a maximum of 6 months gap between 2 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 subjects were genotyped with Illumina 500(+duo) and Illumina 610 quad and 11,496 subjects passed genotyping quality control. Exclusion criteria were a call rate <98%, Hardy-Weinberg p-value <1 × 10^-6, minor allele frequency <0.01%, excess autosomal heterozygosity >0.336, sex mismatch and outlying identity-by-state clustering estimates. Data was 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, subjects were genotyped on the Affymetrix 6.0 (Affymetrix, Santa Clara, CA, USA) or Illumina HumanOmniExpress (Illumina, San Diego, CA, USA) platforms. Both platforms were imputed using IMPUTE2 and the 1000 Genomes reference panel. SNPs deviating from Hardy–Weinberg equation (P<1 × 10^−6) or with an Info Score <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 gender. PLINK v1.07 was used for the Dutch cohort [27] and SNPTEST-v2.5-beta was used for the Scottish cohort [28]. The fixed effect meta-analyses were done at both sites using the inverse variance weighting, in the Netherlands using METAL and Scotland using GWAMA [29,30]. The final SNP list in the Netherlands analysis was filtered based on the index of heterogeneity (I^2<60) and the number of cohorts that covered a SNP (more than two cohorts) [31]. 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 [32]. All other analyses were performed using SAS v9.3 (SAS Institute, Cary, NC, USA). R packages were used to plot the graphs. Metafor R package used for forest plot [33] and qqman package for Manhattan and QQ plot [34].

RESULTS

A total of 710 cases of 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 Go-DARTS population were genotyped using the Illumina chip (GD1) and the rest (2305 patients) were genotyped using the Affymetrix chip (GD2). Within Rotterdam study, RS1, RS2 and RS3 had 630, 170 and 52 patients respectively). In both cohorts the mean age of included patients was not statistically significantly different between cases and controls. The proportion of females was significantly higher within cases compared to controls in both cohorts (Table 1).

Table 1. General characteristics of the included ACE-inhibitors starters

		GoDARTS			Rotterdam study		
		Case (n=710)	Control (n=3599)	P-value	Case (n=262)	Control (N=590)	P-value
gender	Male	51.4%	59.8%	<0.001	33.59%	53.2%	<0.001
	Female	48.6%	40.2%		66.41%	46.8%	
Mean age years [SD]		62.77 [9.98]	62.45 [10.84]	0.4631	64.47 [6.79]	65.15 [7.69]	0.2177

SD: standard deviation.

In the meta-analysis of both cohorts using multivariable regression analyses adjusting for gender and age, 8 SNPs located on chromosome 5 (one SNP), 6 (one SNP), 16 (one SNP) and 17 (five SNPs) reached genome-wide significance level (P-value less than $5x10^{-08}$) (Figure 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 insertion/deletion polymorphism on chromosome 17 position 77112502). The most significantly associated SNP (rs2061538) was located within the gene *RBFOX3* (RNA Binding Protein, Fox-1 Homolog (C. Elegans) 3). There were several other strongly associated SNPs in high linkage disequilibrium (LD) with this SNP in that region (Figure 3A). The second most statistically significant SNP (rs77370934) was located within the gene *GABRG2* (Gamma-Aminobutyric Acid Receptor Subunit Gamma-2), however, there were no other SNPs with a high level of LD in that locus (Figure 3B).

There were also genome wide statistically significant SNPs within the *MBOAT1* gene (Membrane Bound O-Acyltransferase Domain Containing 1) and *SH2B1* gene (SH2B Adaptor Protein 1).

Figure 4 presents the odds ratio and the 95% confidence interval (95% CI) 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 meta-analyses results from both sites using the GWAMA and METAL, particularly for the most significantly associated SNPs.

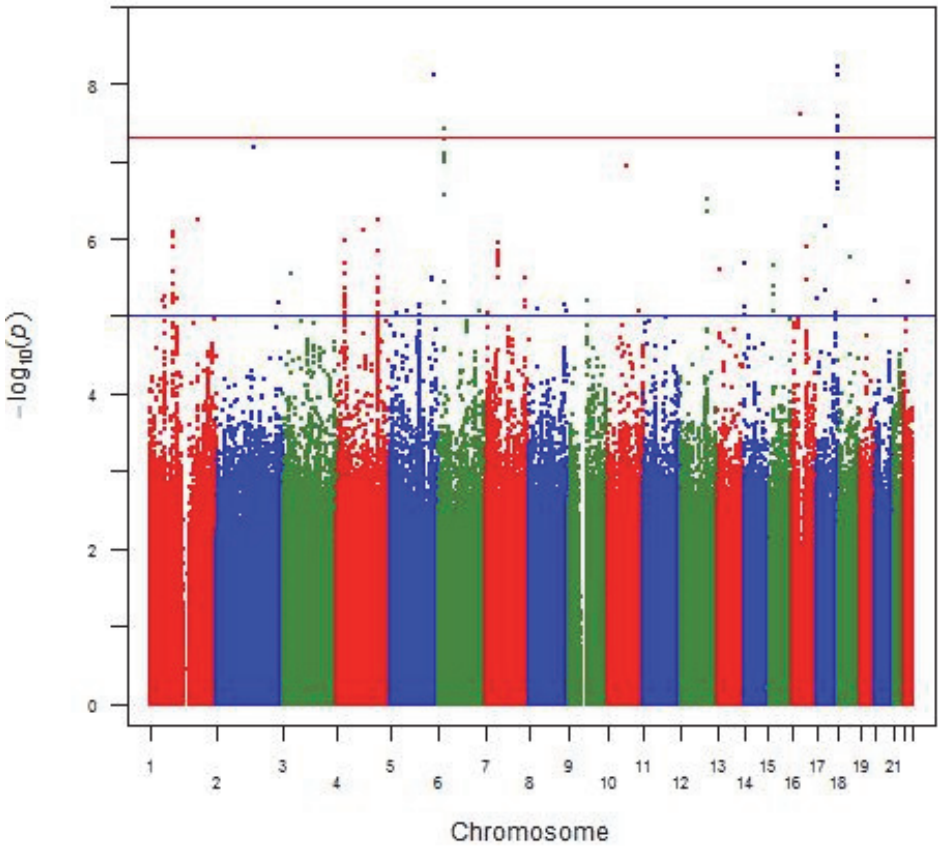


Figure 1. Manhattan plot of genotyped SNPs associated with ACE-inhibitor intolerance using an additive model adjusted for age and gender. The red line indicates the genome-wide significance threshold of alpha=$5x10^{-8}$.

Table 2. Most significantly associated SNPs

SNP	Chr	Position	MA	MAF	OR	95% CI	P-value	Gene
rs2061538	17	77112562	G	0.16	1.52	1.3-1.7	$6.2x10^{-09}$	*RBFOX3*
rs77370934	5	161604254	G	0.03	3.16	2.1-4.6	$7.7x10^{-09}$	*GABRG2*
rs56209714	17	77113268	G	0.14	1.54	1.3-1.7	$7.9x10^{-09}$	*RBFOX3*
rs192613545	16	28863901	T	0.07	2.33	1.7-3.1	$2.5x10^{-08}$	*SH2B1*
chr17:77112502:I	17	77112502	C	0.14	1.62	1.3-1.9	$2.7x10^{-08}$	
rs62063838	17	77114028	C	0.17	1.47	1.2-1.6	$3.7x10^{-08}$	*RBFOX3*
rs10946364	6	20177222	T	0.39	1.34	1.2-1.4	$3.8x10^{-08}$	*MBOAT1*
rs56044629	17	77109653	G	0.14	1.51	1.3-1.7	$4.2x10^{-08}$	*RBFOX3*

SNP: single nucleotide polymorphism, Chr: chromosome, MA: minor allele, MAF: minor allele frequency, OR: odds ratio, CI: confidence interval,

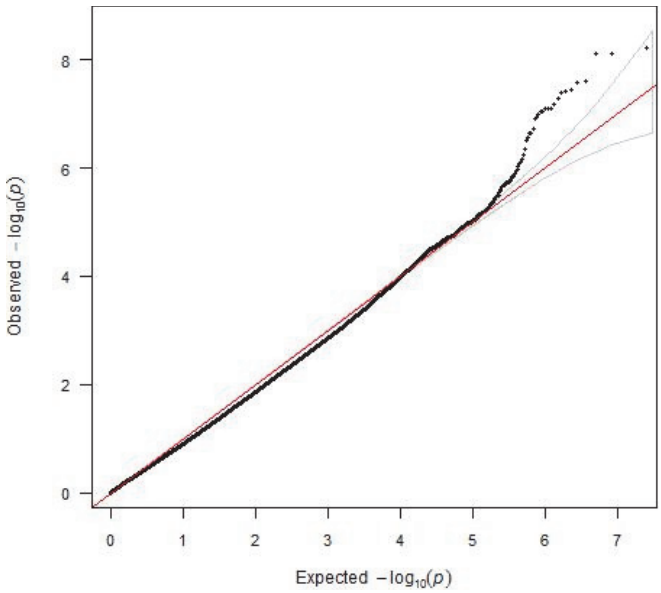


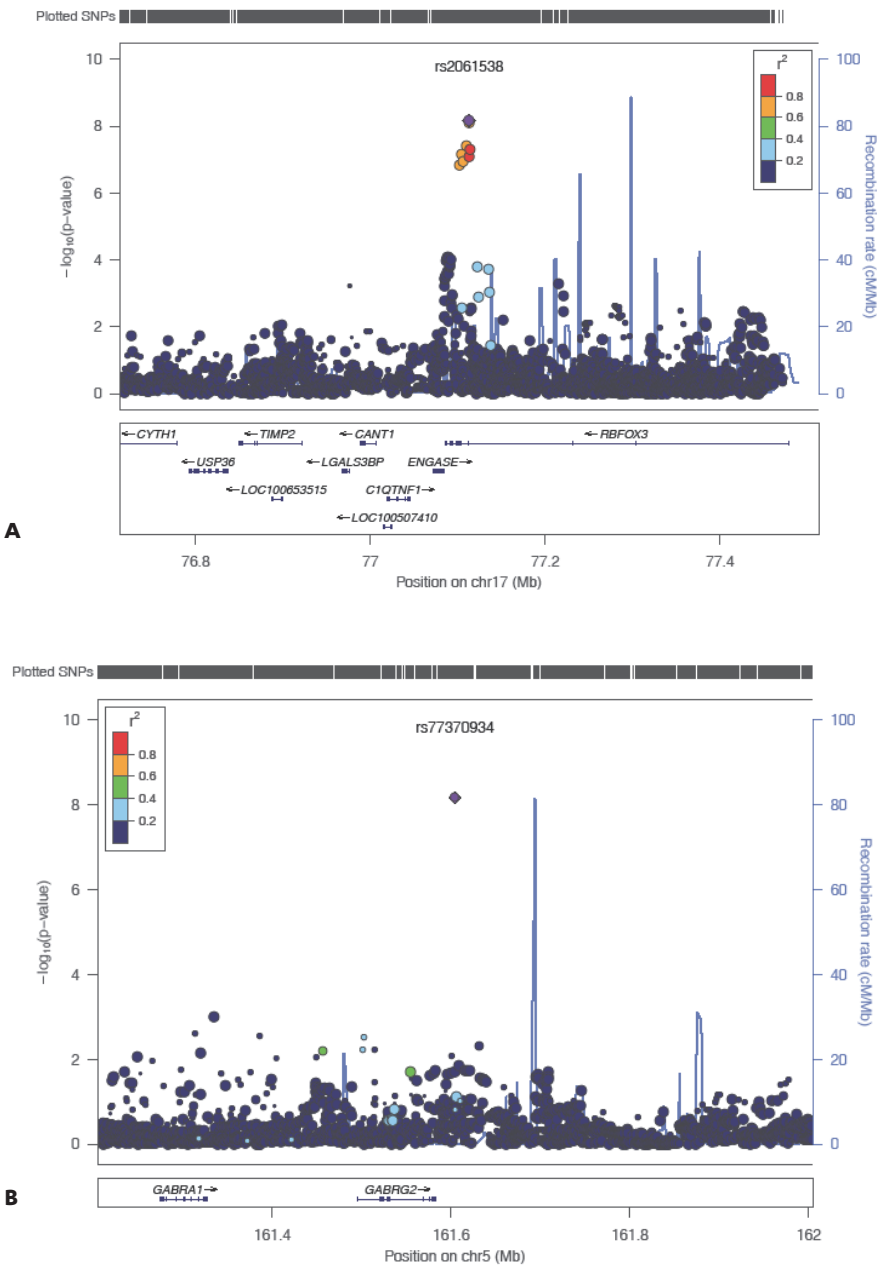
Figure 2. A QQ plot for SNP associations from a meta-analysis of GWAS of ACE-inhibitor intolerance using an additive model adjusted for age and gender. (Lambda=0.88)

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 (GoDARTS) 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 which may play a role in the adverse drug reactions 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 diversity of gene products in higher eukaryotes [35,36].

The other most strongly associated SNP was located on chromosome 5 within the gene *GABRG2* which encodes a gamma-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: alpha, beta, gamma, delta and rho [37]. 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 [38]. Dicpinigaitis *et al* showed that Baclofen (as a GABA receptor agonist) can supress cough induced by ACE-inhibitors [39]. They also proved in a prospective clinical trial that baclofen can inhibit capsaicin-induced cough [40].



A. The *RBFOX3* (chromosome 17 centred around SNP rs2061538 (shown in purple). Linkage disequilibrium (based on r^2 values) with respect to rs2061538 are based on the CEU reference population.
B. The *GABRG2* (chromosome 5 centred around SNP rs77370934 (shown in purple). Linkage disequilibrium (based on r^2 values) with respect to rs77370934 are based on the CEU reference population.

Figure 3. LocusZoom plot of most strongly associated SNPs from the meta-analysis located in A) the region of most significantly associated genes

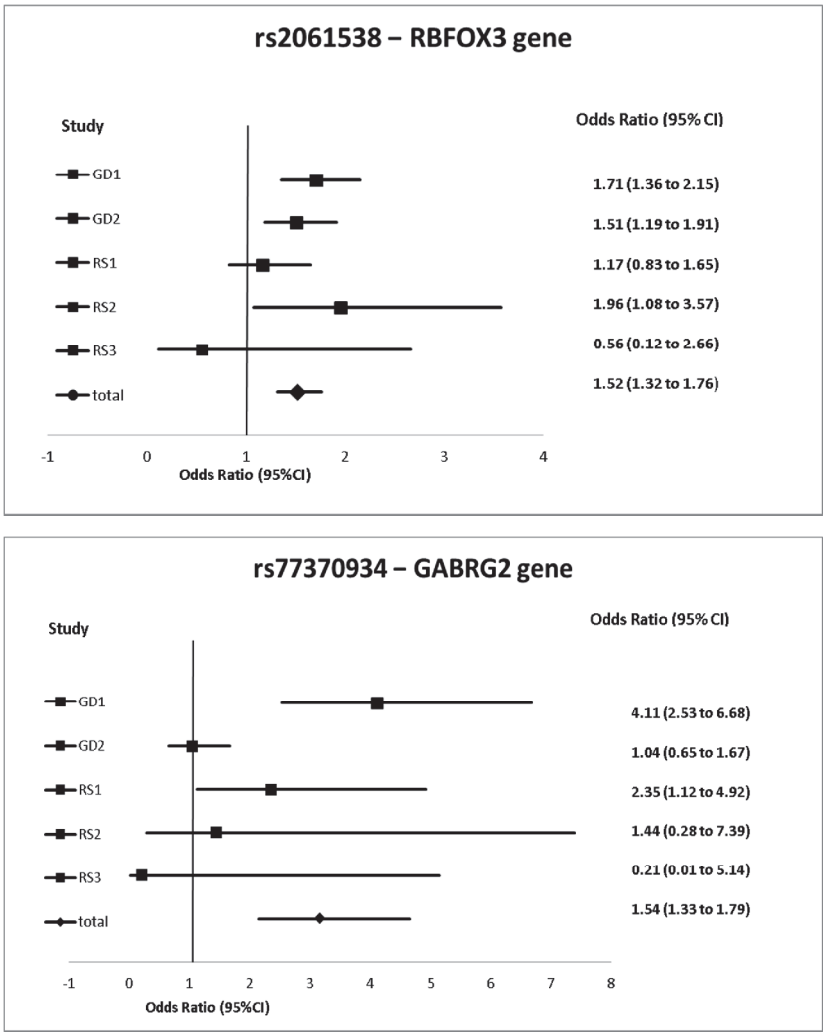


Figure 4. The forest plot from the meta-analyses of most strongly associated SNPs

SH2B1 (sarcoma (Src) homology 2 (SH2) B adaptor protein 1) is a member of a family of scaffold proteins implicated in signalling downstream of a variety of receptor tyrosine kinases and cytokine receptors [41]. Variations in this gene have been reported to be associated with obesity [42]; however its role in the abnormal glucose homeostasis has not been proved [43]. The significant association of this gene with the intolerance of ACE-inhibitors needs to be further investigated because there was no previous report of this gene contributing in cough or angioedema.

MBOAT1 (membrane bound O-acyltransferase domain containing 1) belongs to the superfamily of *MBOAT* that transfer organic compounds, usually fatty acids onto hydroxyl groups of membrane-embedded targets [44]. This trans-membrane protein has been reported to be involved in developmental processes [45].

The main hypothesized mechanism of ACE-inhibitor induced ADRs (mainly cough and angioedema) is 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 variation in inflammatory pathways, however findings of those candidate gene studies were replicated inconsistently and the meta-analyses of loci that had enough studies, did not find the significant effect for the insertion/deletion polymorphism within ACE gene [15]. Hypothesis free GWA studies may lead to finding 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 (*KCNIP4*) which is predominantly expressed in nervous systems [22]. However the only available GWAS on the ACE-inhibitor induced angioedema with 175 ACE-inhibitor induced angioedema cases and 489 controls could not find any significant association on a genome wide level which could be due to the relatively small sample size and lack of the 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 are playing 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 clear yet and needs to be further investigated, 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 to develop ACE-inhibitor induced ADRs including angioedema, which is a life threatening event. We recently showed that approximately 50% of ACE-inhibitor users continue ACE-inhibitors after the first episode of angioedema [46]; Identification of those patients at high risk could help physicians guide their treatment choice. ACE-inhibitor induced cough is not as life threatening as angioedema but it can be misdiagnosed and mistreated which significantly decreases the compliance of patients and might finally result in unsuccessful drug therapy [47,48]. Therefore in the context of precision medicine, the ultimate application of these findings within the clinic would be the prediction of susceptible patients and treating them with an alternative medication with comparable effect such as ARBs [49].

An important limitation of this study is defining phenotype based on the electronic medical records which can 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 cannot detect associations for rare SNPs (MAF< 0.01%). The study results are restricted to the European ancestor populations.

Due to 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 in addition to the epigenetic and molecular studies are needed to explore the functional roles of variations within genes reported in this study specifically the *GABRG2* gene for which several clinical studies also showed its role in susceptibility to cough [38–40]. The standard clinical criteria have been described for ACE-inhibitor induced angioedema [50] and to make it possible to combine results it would be good if new genetic association studies would use this standard phenotype in the future.

In 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.

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CONFLICTS OF INTEREST

Authors declare no potential conflict of interest.

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Chapter 6 | General discussion

Scope of the thesis

The introduction of angiotensin converting enzyme inhibitors (ACEIs) in the 1980s was an enormous step forward in the treatment of high blood pressure. Currently, ACEIs are being prescribed for several indications within cardiovascular and renal diseases. Although the majority of patients can be treated satisfactorily, there is a considerable proportion of patients that suffers from adverse drug reactions (ADRs) due to ACEIs, mainly cough (more than 10%) and in a small group of patients angioedema (around 0.2%)[1]. The number of ACEIs prescriptions is globally increasing from 35 to 40 million prescriptions worldwide in 2001 to more than 160 million prescriptions in 2011 in the US alone [2,3]. This leads to a substantial absolute number of ADRs worldwide. Therefore, investigation of factors that can predispose patients to ADRs is important in order to optimise the benefit-risk ratio of ACEIs therapy. In this thesis the focus was predominantly on ACEIs induced ADRs and genetic or non-genetic factors that are associated with the risk on these ADRs. Studies in this thesis were conducted as part of a European project called PREDICTION-ADR (Personalisation of tREatment In Cardiovascular disease through next generation sequencing in Adverse Drug Reactions). PREDICTION-ADR aims to discover genetic factors predisposing patients to ADRs from drugs used in prevention of cardiovascular disease (CVD) (statins and ACEIs). Our studies aimed to identify ADRs in large databases that can be used for several ADRs studies including replication of novel genetic associations and discovery of new genetic loci through genome wide association studies (GWAS).

This general discussion will elaborate on the results described in this thesis. First, the main findings will be placed in a broader perspective, second, databases in which our studies were conducted will be discussed and finally, ideas for future research and the potential clinical use of our results will be presented.

Main findings of this thesis

In **Chapter 2.1** we investigated patterns of ACEI use in patients with different indications and we showed that for all indications the five years persistence rate was less than 70%. The lowest persistence rate was found in patients who started ACEIs for the indication renal failure. Although this high non-persistence rate can be due to different factors (like non adherence or non-response to treatment), we expect that at least part of it is caused by ADRs [4]. We showed that patients with renal disease are more likely to stop their ACEI therapy, it is important that physicians are aware of this. If risk factors of discontinuation are known this might help to prevent the large number of unsuccessful ACEI therapies.

In **chapter 2.2** we investigated the most severe ADR of ACEIs, which is life-threatening angioedema. The cumulative incidence of angioedema in ACEIs users is approximately 0.2% and it mostly occurs at the larynx or tongue [5]. This chapter showed that despite all clinical recommendations [6,7], ACEIs are being continued after the first angioedema event in almost 50% of cases which leads to around 3 times higher risk of recurrent angioedema in for patients who continued comparing to those who stopped. This is particularly important with regard to angioedema although it has been shown previously that ACEI-induced cough is also misdiagnosed and that resulted in mistreatment of ACEI-induced cough in clinical practice [8]. Observational studies from real life clinical data showed that these ADRs are frequently misdiagnosed; therefore it is clinically relevant to identify patients at higher risk of developing ADRs before initiation of ACEI therapy. Many of those high risk patients can be treated with alternative medicines like angiotensin receptor blockers (ARBs), and if that is not possible they should be closely monitored for ADRs when ACEI prescription is inevitable.

In **chapter 3** we defined and compared different patterns of ACEI use, as a proxy of ACEI-induced ADRs We concluded that switching from ACEIs to ARBs was the best marker for an ADR in a prescription database based on the positive predictive value (PPV), negative predictive value, sensitivity and specificity. We hypothesized that this marker is a useful tool to detect the ACEI-induced ADRs in other prescription databases as well. This approach of using prescription patterns as a marker has been used before. For example, in the Rotterdam Study, using repeated nitrate prescription has been shown to be a suitable marker for angina pectoris in electronic healthcare databases [9]. For the detection of statin-induced ADRs the same approach was utilized successfully to investigate prescription data as a proxy of ADRs [10,11]. The main strength of this approach is to make it possible to investigate ADRs within prescription databases where ADRs are usually not well recorded and thereby it can create numerous opportunities (e.g, running GWAS using these phenotype, and investigating the occurrence of ADRs associated with sociodemographic variables), however misclassifications remain possible and results of those studies should be interpreted with caution.

Chapter 4 is a combination of two nested case control studies within a cohort of ACEI starters in CPRD. In this chapter we studied non-genetic factors associated with ACEI-induced angioedema and also ACEI-intolerance (defined as switching from ACEI to ARB as defined in **chapter 3**); among others we showed that patients with a history of allergic-related disease or use of anti-allergic medications were more susceptible to ACEI-intolerance and also to angioedema. It was also shown previously that the use of immunosuppressant medicines is associated with ACE-induced angioedema [12–14]. Therefore the involvement of immune system is probable. On the other hand diabetic patients were less susceptible. We could not investigate the causality of those factors in our database; however most of our results were in line with the previous risk factors reported for ACEI-induced ADRs [12,13,15]. The high number of factors that were associated with both angioedema and intolerance (mainly cough), suggests that there might be similar molecular pathways involved in both ADRs. For example, biological evidence supports our finding that angioedema is less likely to develop in diabetic patients due to a higher activity of dipeptidyl peptidase IV (DPP-IV) which is seen in hyperglycaemia and supposed to degrade substance P and bradykinin [16,17]. The kinin metabolic pathway is considered to play an important role in the pathogenesis of both ACEI-induced cough and angioedema. During ACE inhibition, aminopeptidase-P (APP) acts as the major metabolising enzyme of bradykinin and its active metabolite (des-Arg9-bradykinin). Therefore, a hereditary or acquired deficiency in this enzyme may result in the accumulation of these vasoactive peptides in the body and may underlie the pathogenesis of ACEI-induced ADRs [18]. Our population based studies are supporting the hypothesis that similar pathways are involved in development of both angioedema and cough induced by ACEIs.

In **chapter 5** genetic factors were studied in relation to ACEI-induced ADRs. In **chapter 5.1** all available evidence was reviewed and a meta-analysis was performed for the association between the ACE insertion/deletion (I/D) polymorphism and ACEI-induced cough. Our meta-analyses showed no significant association. Other polymorphisms were also reviewed for ACEI-induced cough but there was no polymorphism for which results were consistent. Only variation within the *XPNPEP2* gene was consistently associated with ACEI-induced angioedema in three studies. This gene encodes the membrane-bound form of APP [19–21]. Most of the candidate gene approach studies have been proven difficult to replicate and their results should therefore be interpreted with caution [22]. At the time of the review no GWAS was available on either ACEI-induced cough or angioedema. However after that, one GWAS on ACEI-induced angioedema did not show a genome wide significant locus association [23]. Another recent GWAS was published on ACEI-

induced cough from the eMERGE network (The Electronic Medical Records and Genomics) [24]. They identified 1595 cases of ACEI-induced cough and 5485 controls using electronic health care records (EHR). This study found a novel locus within the *KCNIP4* gene statistically significant associated on the genome wide level with ACEI-induced cough and these results were replicated in GoDARTS study (Genetics of Diabetes Audit and Research in Tayside Scotland) using our validated definition for phenotyping [25]. In **chapter 5.2** we ran a GWAS on both the Rotterdam study and GoDARTS using our validated definition for the ACEI-intolerance. The meta-analyses of both studies showed that eight single nucleotide polymorphisms (SNPs) within four genes reached the GWAS significance level (*RBFOX3*, *GABRG2*, *SH2B1* and *MBOAT1*). These genes -if replicated in other populations- are suitable targets for further molecular and functional investigations related to ACEI-induced cough.

Within the PREDICTION-ADR project 250 cases of ACEI-induced angioedema have been recruited with the standardized phenotype [26]. Currently the DNA samples from those cases and corresponding controls are sequenced at 3 centres simultaneously using an innovative next generation sequencing strategy to find predisposing genetic changes to ACEI-induced angioedema.

Genotyping can be performed through a variety of methods, depending on the variants of interest and resources available. For studying many variants at once, especially common variants, genotyping chips or arrays are an efficient and accurate option. These do, however, require prior knowledge of the variants you want to analyse. Sequencing is a method used to determine the exact sequence of a certain part of DNA. You can sequence a short piece, the whole genome, or parts of the genome such as the exomes (parts of the genome that contain genes). Thus, sequencing can be used to genotype someone for known variants, however it can also identify variants that are rare and may be unique for that person. This is the most important advantage of sequencing compared to genotyping particularly when you study rare phenotypes like angioedema. However, even though there are many new developments in sequencing technology and prices are rapidly coming down analysing sequence data is still a challenge and sequencing is still far more expensive than using GWAS chips. PREDICTION-ADR applies whole exome sequencing to identify genetic variants associated with ACEI-induced angioedema.

Data sources and challenges

In this thesis we used the data from three different databases, the Clinical Practice Research Datalink (CPRD), the Rotterdam study and the GoDARTS study. Each database has its own characteristics, strengths and limitations which made them suitable for special types of studies. Below we will further elaborate on the reasons that we used these different databases, and the challenges that we faced working with them.

CPRD formerly known as General Practice Research Database (GPRD) is a governmental, not-for-profit research service, jointly funded by the National Institute for Health Research (NIHR) and the Medicines and Healthcare products Regulatory Agency (MHRA), a part of the Department of Health which has been providing anonymised primary care records for public health research since 1987. CPRD contains the computerized information entered by almost 700 primary care practices in the UK and currently covers clinical records of almost 12 million patients [27,28]. CPRD data was used in **chapter 2** and **chapter 4** where we investigated the use of ACEIs and the ADRs in real life clinical data. The CPRD suited for this purpose because of its huge patient numbers and because it is a well representative sample of the UK population. Specifically, in **chapter 2.2** and **chapter 4**

this is important because a rare clinical event (angioedema) was investigated. However, the unique structure and coding of the CPRD present challenges for analyses and for comparisons with other databases. For instance, it is difficult to construct complete code sets in CPRD because of varying terminologies for the same medical event. Therefore, for determining indications we compiled code lists together with expert clinical cardiologist; however misclassifications cannot be ruled out. The other challenge particularly for **chapter 2.1** and **chapter 4** was the timing of registration for lifestyle data and retrieving the indications for ACEI users relative to the start of treatment which made it necessary to consider different time windows. Furthermore for lifestyle data, we used the closest registered data to the event of interest. For body mass index (BMI) we only used data that was within 1 year of the event. For exposure to cigarettes or alcohol we considered any time before the event of interest. To check the importance of timing of the measurement several sensitivity analyses were performed for example considering the closest registered measurement ever. However, this did not influence the results. Although having the longitudinal primary care prescription data is one of the strengths of the CPRD, missing values can make it difficult to define prescription patterns and therefore we imputed missing values to be able to define the defined daily dose (DDD) of medication within the prescription data[29,30]. Having prescription data could be a limitation comparing to drug dispensing data when you want to be sure if patients actually filled the prescriptions.

In general, CPRD is a great option for pharmaco-epidemiologic and drug utilisation studies, and for studying rare clinical events; however for studying ADRs, where precise diagnosis and a clear time relation between drug use and the potential ADR are needed, it has several limitations.

The Rotterdam study is a much smaller prospective cohort ongoing since 1990 among, initially, 7,983 persons living in the well-defined Ommoord district in the city of Rotterdam in The Netherlands (78 % of 10,215 invitees participated). After 3 rounds of recruitment, by the end of 2008, the Rotterdam Study comprised 14,926 subjects aged 45 years or over [31,32]. The participants were all examined at baseline. They were interviewed at home (2 hours) and then had an extensive set of examinations (a total of 5 hours) in a specially built research facility in the centre of their district. The physical examinations were repeated every 3–4 years; longitudinal drug dispensing data from linked pharmacies are also available for included patients [33]. There were 3 main reasons that we used the Rotterdam study in **chapter 3** and **chapter 5.2**. First of all ACEIs are predominantly prescribed for older people, secondly the extensive primary care clinical data that was available for the patients in the Rotterdam study and thirdly the availability of the GWAS data in this cohort. Because of the relatively small number in the GWAS and in order to increase the power, we meta-analysed the results of the Rotterdam study with the GoDARTS study. This is a genetic sub-study of The Diabetes Audit and Research Tayside, Scotland (DARTS). This data source is based on linking clinical records by a patient-specific identifier, allowing the creation and maintenance of regional health informatics systems. The DARTS project electronically followed all residents in Tayside, since January 1996 (n=391,274 including 7,596 individuals with diabetes) through linking clinical datasets with a high degree of reliability and accuracy [34]. A GWAS is also available for the GoDARTS population. The main drawback for both GoDARTS and Rotterdam study is their limitation in recording clinical information. Therefore we could only identify possible ADRs by using the proxy that we validated in **chapter 3**.

To conclude our discussion regarding the data sources, we believe that there will be an emerging need for databases to record ADRs more precisely and more specifically. In this regard, primary care physicians together with pharmacists play a key role because most of the time, they are the first person dealing with patients and probably recording signs and symptoms of relevant

ADRs. This need will be more relevant when we see that even in ADRs databases a large number of ADRs was under reported [35]. Inman and his team investigated reasons for under reported ADRs in 1970s and identified seven major reasons for why suspected ADRs were not reported. These reasons, later referred to as Inman's seven deadly sins like lack of confidence in diagnosis of ADRs, or guilt of having administered the treatment which may have harmed a patient, etc [36]. However, later only one of those seven deadly sins appeared to be confirmed which was that the heavy workload deterred doctors from reporting suspected ADRs [37]. This leads to a discussion that other healthcare team member should be also responsible for reporting ADRs mainly nurses and pharmacists, although their clinical knowledge and information about patients history is limited [38]. Abovementioned challenges make it difficult for pharmacogenetic researchers to study ADRs. Therefore innovative solutions are needed for example investigating genetic markers in a small but well defined phenotype of patients who developed ADRs and subsequently confirm the results in large available databases using the proxy markers. This is the ongoing approach within PREDICTION-ADR.

Future perspective and clinical application

Pharmacogenetics, pharmacogenomics, stratified medicine, personalised medicine or precision medicine; these are the words that have been used sometimes interchangeably during the last decades. Although they are not exactly the same, all of them can be placed under the umbrella of a key concept "the right medication for the right patient" [39]. The response to a medicine (efficacy and safety) on an individual level is determined by two main components: patient (genetic) and environmental factors, therefore if we understand these factors; it would be possible to predict the response of an individual patient to a drug. Although it seems easy in theory, it is not at all in practice. The non-genetic factors (mainly demographic and medical history) are much easier to be studied and implemented in practice, compared to the genetic component because they do not need an extra genotyping test in clinical practice or genotyping costs for research. Addition of biological samples (blood, DNA, urine, saliva, faeces etc.) to EHRs, and the use of new –omics methods and analysing this data with modern bioinformatics, epidemiological and statistical methods to assess the relationship between exposures and drug responses, will extend the knowledge that can be used to personalize therapy.

The use of pharmacogenetic tests in clinical practice is still limited mainly because of non-replicated results in pharmacogenetic studies in the last decade in addition to the small effect size of truly associated genetic variations [40]. However the convergence of genetics and informatics, along with other technologies such as epigenetics, proteomics, metabolomics and microbiomes is rapidly expanding the scope of precision medicine [41,42]. Among these new technologies, genetics and next generation DNA sequencing methods are having the greatest effect particularly considering the dramatically decreasing trend in their price [43]. The results described in this thesis are still far from ready for implementation in clinical practice. The genes found associated with the risk of switching from ACEIs to ARBs should be further investigated, and might be leads for markers that can be used to target ACEI therapy to the right patients in the future. However for the non-genetic component it was clear from our population based studies that there is a need to alert physicians to be more cautious while prescribing ACEIs for patients with a history of inflammatory diseases to prevent angioedema. Furthermore patients that already experienced angioedema during ACEI use should not continue their therapy. Finally, because of their high risk of discontinuation it might be important to better monitor patients with renal dysfunction.

Combining genetic and environmental data to create a statistical model for prediction of ADRs to ACEIs would be feasible in the near future. Therefore it would not be unlikely in the next decade that physicians and pharmacists have an application installed on their computer which records personal genetic data and relevant individual characteristics combined with the presence of actual environmental risk factors; these application will then predict the probability of intended and unintended effects of ACE-inhibitors. For this scenario to become true it is also important that cost-effectiveness analyses show benefits of implementation in clinical practice [44].

In conclusion

In this thesis, we report a variety of studies considering ACEIs utilisation, genetic and non-genetic factors associated with ADR risk. Some of our results should be considered as drug utilisation studies, some studies are hypotheses testing and some are hypotheses generating. Within the descriptive part of the thesis, we observed a need to alert physicians for a proper prescribing of ACEIs, considering ADRs. The hypothesis testing part of the thesis was subdivided in 2 parts: a non-genetic part and a genetic part, the non-genetic part showed that several variables were associated with both angioedema and intolerance of ACEIs, furthermore, occurrence of cough was associated with the risk of developing angioedema in our study, regardless of the reason of cough. These results suggest that the probable similar pathways for ACEI-induced ADRs need to be investigated in depth in the future because there is some evidence from molecular studies also supporting this hypothesis [18]. Within the hypothesis testing genetic part of the thesis we showed that the results of candidate gene approach studies on ACEI-induced ADRs are often difficult to replicate and we also showed that the ACE I/D polymorphism is not associated with ACEI-induced cough. In the hypothesis generating part of the thesis we introduced novel loci associated with ACEI-intolerance on a whole genome level. These loci should be investigated more in detail in the future.

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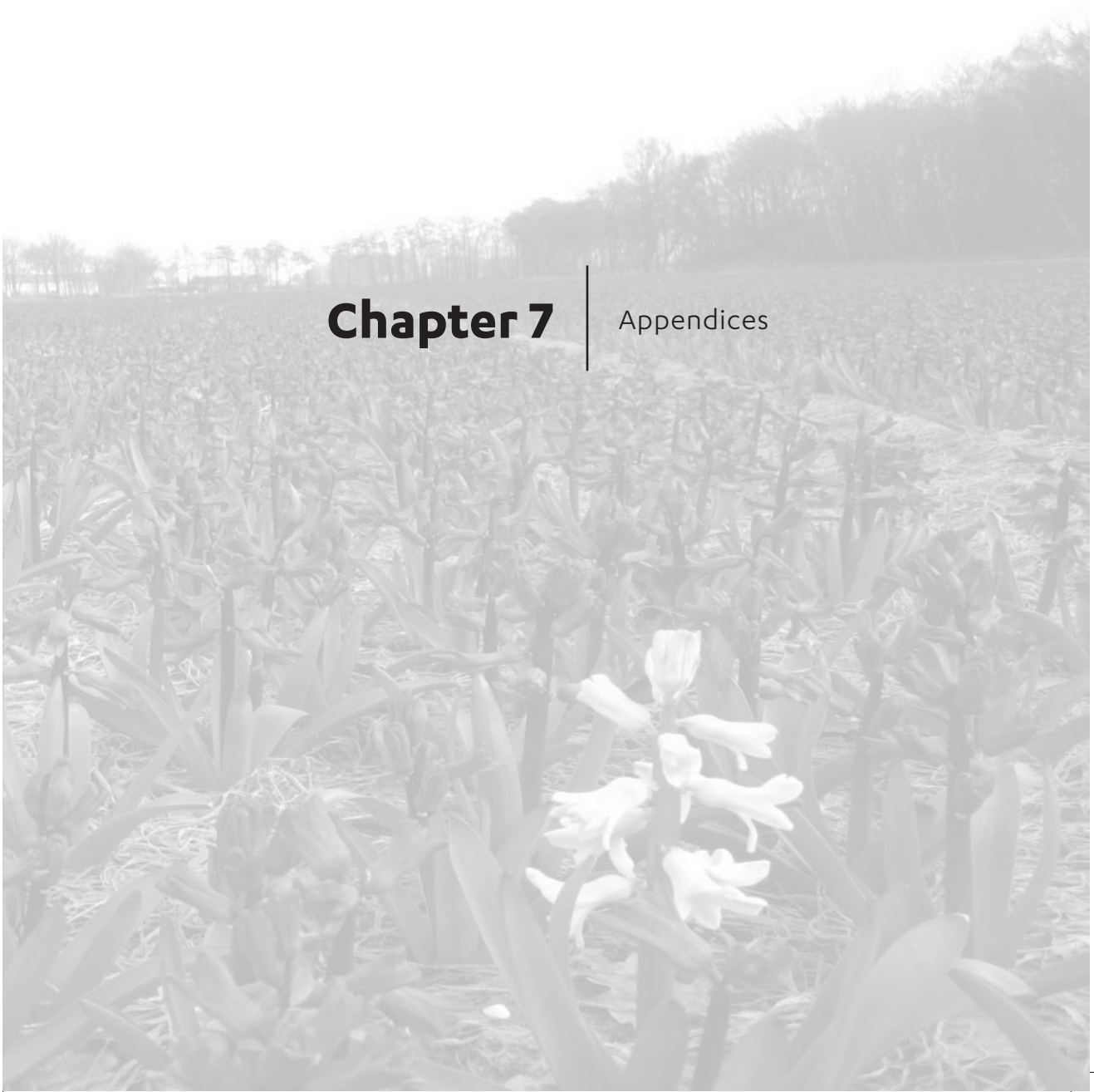

Chapter 7 | Appendices

Appendix 1 | English summary

Worldwide, millions of patients with cardiovascular and/or renal diseases are treated with angiotensin converting enzyme inhibitors (ACEIs) according to the international treatment guidelines based on the results of large clinical trials. Although this class of medications is generally well tolerated, adverse drug reactions (ADRs) may prevent their use in some individuals. The most common side effect is a persistent, non-productive cough that may require cessation of ACEI use. ACEIs can also cause angioedema, which is rare but extremely dangerous and can even be life-threatening because it occurs mainly at mouth, lips and upper airways. This ADR can be fatal within a few hours. Both of the above mentioned ACEI-induced ADRs can start within days to months after initiating therapy. **Chapter 1** of this thesis is the general introduction. The different indications of ACEIs are described in addition to their main ADRs and the putative modes of action; furthermore we have briefly described the context of personalized therapy.

Chapter 2 is dedicated to drug utilisation studies of ACEIs in large populations; both studies within this chapter were conducted in the clinical practice research datalink (CPRD) that is one of the world's largest electronic health record (HER) databases. The initial number of ACEI starters used for these studies was more than 270,000. In **chapter 2.1** we investigated the pattern of ACEI use for different indications. The patterns were defined as continuous users and non-continuous users, which were subdivided into stoppers, re-starters and switchers to other alternative medications. We showed that although ACEIs are often prescribed for lifetime treatment, a large percentage -more than 20%- of starters were not persistent to their ACEIs for all indications. This non-persistence rate was not the same for the different indications. Patients who started ACEIs for myocardial infarction and hypertension were more persistent with their therapy and used ACEIs for a longer period compared to patients who started ACEIs for renal diseases and heart failure. In **chapter 2.2** we tried to identify patients who developed angioedema for the first time during ACEI therapy and subsequently investigated the pattern of ACEI use following the angioedema. In this chapter we showed that almost half of those patients continued their ACEI therapy despite the occurrence of angioedema and this was associated with a considerable higher risk (around 3 times)on developing a second angioedema event comparing to those who stopped, which might be even more severe.

Because most of the times ADRs are not very well recorded in healthcare databases, in **chapter 3** we defined a proxy marker for ACEI-induced ADRs within a prescription database. We conducted a study in the Rotterdam study where both physician diagnoses and prescription dispensing data were available. In this chapter we found that switching from ACEIs to angiotensin receptor blockers (ARBs) is the best marker for ACEI-induced ADRs particularly ACEI-induced cough with a positive predictive value of around 70% for at least probable ADRs. In the rest of this thesis we applied this validated definition to investigate more in depth the ADRs of ACEIs.

In **chapter 4** the non-genetic factors that might be associated with ACEI-induced ADRs were studied, mainly focusing on the chronic co-morbidities and co-medications of ACEI users. We performed 2 separate nested case-control studies in this chapter, one with angioedema cases and one with the validated definition of ACEI-intolerance. We showed that patients with a disease with an allergic component like asthma, history of allergy or rheumatoid arthritis were at a higher risk of ACEI-induced ADRs. Several factors were associated with both outcomes (asthma and history of allergy for instance). This could indicate that the same pathway is responsible for developing ACEI related cough and angioedema, however there were some differences as well for example rheumatoid arthritis was associated with angioedema but not with switching outcome. In context of personalized therapy these factors -if replicated in other populations- can be incorporated in computerized modelling together with genetic markers to predict the risk for the individual patient

to develop ADRs when using ACEIs. **Chapter 5** is dedicated to studies searching for genetic factors that contribute to the risk of developing ACEI-induced ADRs. In **chapter 5.1** we systematically reviewed all pharmacogenetic studies regarding ACEI-induced cough and angioedema. All these studies used a candidate gene approach. We found that many of the reported associations were not replicated, and that the majority of the studies suffered from issues with statistical power. We meta-analysed the studies that considered the association between the ACE insertion/deletion polymorphism and ACEI-induced cough, however we did not find a statistically significant association between this polymorphism and ACEI-induced cough. In **chapter 5.2** we performed 2 hypothesis free genome wide association studies (GWAS) on the intolerance of ACEIs using the validated definition within a Scottish and a Dutch population (both with European ancestors). We furthermore meta-analysed those results and reported 4 new loci associated with ACE-intolerance on a genome wide significance level (*RBFOX3, GABRG2, SH2B1* and *MBOAT1*). These results generate the hypothesis that those genes are involved in the molecular mechanism of ACEI-ADRs, although more epigenetic and biological studies are needed to reveal the details.

In **Chapter 6** we have discussed methodological challenges and opportunities. We also discussed the possibilities to incorporate the findings of genetic and non-genetic association studies in the clinical setting. Finally, we described the future perspectives of precision medicine.

Appendix 2 | Samenvatting

Wereldwijd worden miljoenen patiënten met cardiovasculaire of renale ziekten behandeld met ACE (angiotensine converting enzyme)-remmers. Deze behandeling is gebaseerd op de resultaten van grote klinische trials en beschreven in internationale behandelingsrichtlijnen. Over het algemeen worden ACE-remmers goed verdragen door patiënten, maar bijwerkingen kunnen optreden. De meest voorkomende bijwerking is een droge, niet-productieve hoest. Deze hoest kan er toe leiden dat het gebruik van de ACE-remmer gestaakt moet worden. ACE-remmers kunnen ook angio-oedeem veroorzaken. Dit is een zeldzame, maar extreem gevaarlijke bijwerking die zelfs levensbedreigend kan zijn, omdat deze vooral voorkomt in de mond, lippen en bovenste luchtwegen. Deze bijwerking kan in een paar uur fataal zijn. Hoe snel deze bijwerkingen optreden kan sterk variëren. Dit kan al een paar dagen na de start met het middel gebeuren, maar ook pas als de patiënt het middel al maanden gebruikt.

Hoofdstuk 1 van die proefschrift is de introductie. In de introductie worden de verschillende indicaties van ACE-remmers beschreven, de belangrijkste bijwerkingen en de waarschijnlijke biologische mechanismes. Daarnaast beschrijven we kort de context van therapie op maat voor de individuele patiënt (Personalized therapy).

In **hoofdstuk 2** beschrijven we twee geneesmiddelgebruik studies met ACE-remmers in grote populaties. Beide studies in dit hoofdstuk werden uitgevoerd in de "Clinical Practice Research Datalink" (CPRD) studie. Dit is een van de grootste elektronische gezondheidsdatabases in de wereld. Het geneesmiddel gebruik van meer dan 270.000 starters met ACE-remmers is beschreven in dit hoofdstuk. In **hoofdstuk 2.1** onderzochten we het patroon van ACE-remmer gebruik bij verschillende indicaties. Het patroon was gedefinieerd als continu gebruik en niet-continu gebruik, deze laatste groep was onderverdeeld in stoppen, herstarten, en switchen naar andere therapie. We lieten zien dat ondanks het feit dat ACE-remmers meestal worden voorgeschreven voor levenslange therapie meer dan 20% van de starters toch niet door bleven gaan met gebruiken van ACE-remmers. Dit percentage was niet voor alle indicaties gelijk. Patiënten die ACE-remmers startten omdat ze hypertensie hadden of na een myocardinfarct hadden een grotere kans om door te gaan met de therapie en gebruikten ACE-remmers voor een langere tijd vergeleken met patiënten die ACE-remmers gebruikten voor nierziekten of hartfalen.

In **hoofdstuk 2.2** hebben we patiënten geïdentificeerd die voor de eerste keer angio-oedeem kregen tijdens het gebruik van ACE-remmers. Vervolgens hebben we gekeken hoe patiënten werden behandeld na dit event. In dit hoofdstuk laten we zien dat bijna de helft van de patiënten doorging met het gebruiken van ACE-remmers ondanks het optreden van het angio-oedeem en dat deze patiënten een ongeveer drie keer zo hoog risico hadden op het optreden van een (mogelijk ernstiger) tweede angio-oedeem vergeleken met de patiënten die wel waren gestopt.

Omdat in de meeste databases het optreden van bijwerkingen niet goed is vastgelegd zochten we in **hoofdstuk 3** naar een marker voor ACE-remmer geïnduceerde bijwerkingen in een apotheekgegevens database. We voerden deze studie uit in de Rotterdam studie waar zowel klinische diagnoses als apotheekgegevens beschikbaar zijn. In dit hoofdstuk vonden we dat switchen van een ACE-remmer naar een angiotensine receptor blokker (ARB) de beste marker was voor ACE-remmer geïnduceerde droge hoest met een positieve predictieve waarde van 70% (voor de cases die waren gedefinieerd als waarschijnlijk of zeker). In de rest van dit proefschrift hebben we deze definitie gebruikt om bijwerkingen van ACE-remmers te kunnen bestuderen in apotheekgegevens.

In **hoofdstuk 4** bestudeerden we niet-genetische factoren die geassocieerd waren met ACE-remmer geïnduceerde bijwerkingen. We richtten ons voornamelijk op chronische co-morbiditeit en comedicatie van de patiënten We voerden 2 geneste case-controle studies uit in dit hoofdstuk,

de eerste studie met angio-oedeem cases en de tweede studie met de switchers van ACE-remmers naar ARBs als cases. We lieten zien dat patiënten met een ziekte met een allergische component, zoals astma, allergie of reumatoïde artritis een hoger risico hadden op het optreden van ACE-remmer geïnduceerde bijwerkingen. Verschillende factoren (zoals bijvoorbeeld astma en allergie) waren geassocieerd met beide uitkomsten. Dit zou een aanwijzing kunnen zijn dat hetzelfde biologische mechanisme ten grondslag ligt aan beide bijwerkingen. Er waren echter ook verschillen. Reumatoïde artritis was alleen geassocieerd met angio-oedeem en niet met de switch van ACE-remmer naar ARB. In de context van therapie op maat zouden deze factoren –als ze in andere studies gerepliceerd worden- kunnen worden gebruikt in een model samen met genetische factoren om het risico van de individuele patiënt op het optreden van ACE-remmer geïnduceerde bijwerkingen te kunnen voorspellen.

In **hoofdstuk 5.1** hebben we alle farmacogenetische studies over ACE-remmer geïnduceerd hoesten en ACE-remmer geïnduceerd angio-oedeem systematisch gereviewed. Alle deze studies hadden een kandidaat-gen aanpak gebruikt. We vonden dat het grootste deel van de gevonden associaties niet was gerepliceerd en dat veel studies een powerprobleem hadden. We hebben een meta-analyse uitgevoerd van de studies die de associatie tussen het ACE insertie/deletie polymorfisme en ACE-remmer geïnduceerde hoest bestudeerden en we vonden geen statistisch significante associatie. In **hoofdstuk 5.2** voerden we twee genoom wijde associatie studies (GWAS) uit op het eindpunt switchen van ACE-remmer naar ARB. Deze studies werden uitgevoerd in een Schotse en een Nederlandse populatie (beide met Europese voorouders). We meta-analyseerden de resultaten van deze twee studies en vonden 4 nieuwe loci die waren geassocieerd met ACE-intolerantie op een genoomwijd significantie level (*RBFOX3*, *GABRG2*, *SH2B1* en *MBOAT1*). Deze resultaten leiden tot de hypothese dat deze genen een rol spelen in het moleculaire mechanisme van het optreden van bijwerkingen bij het gebruik van ACE-remmers. Meer (epi-) genetische en biologische studies zijn nodig om dit te bevestigen en de details te bestuderen.

In **hoofdstuk 6** hebben we methodologische uitdagingen en mogelijkheden besproken. We hebben ook gediscussieerd over de mogelijkheden om de resultaten uit de genetische en de niet-genetische studies in de klinische setting te implementeren. Tenslotte beschreven we de toekomst van precisie geneeskunde.

Chapter 7.3 | Acknowledgements

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Chapter 7.5 | List of publications

Publications related to this thesis

Pharmacogenetics of ACE inhibitor-induced Angioedema and Cough: a systematic review and meta-analysis.
S.H. Mahmoudpour, M. Leusink, L. van der Putten, I. Terreehorst, F.W. Asselbergs, A. de Boer, A.H. Maitland-van der Zee.
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Change in prescription pattern as a potential marker for adverse drug reactions of angiotensin converting enzyme inhibitors.
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International Journal of Clinical Pharmacy. 2015 Dec; 37(6): 1095–103.

Continuation of angiotensin converting enzyme inhibitor therapy, in spite of occurrence of angioedema.
S.H. Mahmoudpour, F.W. Asselbergs, I. Terreehorst, P.C. Souverein, A. de Boer, A.H. Maitland-van der Zee.
International Journal of Cardiology. 2015 Dec 15; 201: 644–5.

Patterns of Angiotensin Converting Enzyme Inhibitors prescribing for different indications: a population based study.
S.H. Mahmoudpour, F.W. Asselbergs, P.C. Souverein, A. de Boer, A.H. Maitland-van der Zee.
Submitted.

Determinants of Angiotensin Converting Enzyme-inhibitor (ACEI) intolerance and angioedema in the UK Clinical Practice Research Datalink.
S.H. Mahmoudpour, E.V. Baranova, F.W. Asselbergs, P.C. Souverein, A. de Boer, A.H. Maitland-van der Zee.
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7.5

Meta-analysis of the genome wide association studies (GWAS) on the intolerance of Angiotensin converting enzyme inhibitors (ACEIs).
S.H. Mahmoudpour, A. Veluchamy, M.K. Siddiqui, F.W. Asselbergs, P.C. Souverein, C.E. de Keyser, A. Hofman, C.C. Lang, A.S. F. Doney, B.H. Stricker, A. de Boer, A.H. Maitland-van der Zee, C.N.A. Palmer.
Submitted.

Publications unrelated to this thesis

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S. Fazeli Farsani, P. Eshaghi Jebeli, **S.H. Mahmoudpour.**
Pejvak publication 2006 (Persian)

A Genome-Wide Association Study Identifies Variants in KCNIP4 Associated with ACE Inhibitor Induced Cough.
J. Mosley, C. Shaffer, S. Van Driest, P. Weeke, Q. Wells, J. Karnes, D. Velez Edwards **S.H. Mahmoudpour** *et al*.
Pharmacogenomics Journal. 2015 Jul 14. [Epub ahead of print]

Chapter 7.6 | About the author

Seyed Hamidreza Mahmoudpour was born on the 6th of November 1982 in Esfahan, Iran. After completing His secondary school at "National Organization for Exceptional Talents (NODET)", he passed university entrance examination for Medical Sciences successfully with the national rank of 632 out of more than 450,000 participants. He started studying Pharmacy at Shahid Beheshti University of Medical Sciences and Health Services (SBUM) in Tehran, However after 5 semesters he went back to his hometown, Esfahan due to family reasons. He graduated as Doctor of Pharmacy (PharmD) in 2009 From Esfahan University of Medical Sciences.

During his pharmacy education both in Tehran and Esfahan, he was an active member of "Students' Scientific Research Center (SSRC)". He received the "Best poster presentation award" in 2004 in his first time attending an international conference for the outstanding presentation.

After his graduation from the pharmacy school, he worked about 3 years for educational pharmacies of university in addition to community pharmacies in Esfahan. In 2012 he moved to the Netherlands to continue his education which was started with a one year internship at the Division of Pharmacoepidemiology and Clinical Pharmacology of Utrecht University under the supervision of Prof. dr. A. de Boer, Prof. dr. F.W. Asselbergs, and Dr. A.H. Maitland-van der Zee. The output of that successful internship resulted in a PhD position on a European founded project called PREDICTION-ADR. The results obtained in this period are described in this thesis. During his PhD study he received several prizes, among them the ISPOR best poster presentation in Milan, Italy (2015) and the selected best student abstract for oral presentation at the ISPE mid-year meeting in Baltimore, the USA (2016). In addition to the scientific life he was an active member and representative of PhD students in the Utrech Institute for Pharmaceutical Sciences (UIPS) PhD council for about 2 years when he organized several events and initiated the regular monthly indoor soccer events.