Precision medicine

steps towards improving treatment with vitamin K antagonists and ACE-inhibitors

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Precision medicine

steps towards improving treatment with vitamin K antagonists and ACE-inhibitors

Precisiegeneeskunde

op weg naar verbetering van behandeling met vitamine K antagonisten en ACE-remmers

(met een samenvatting in het Nederlands)

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CHAPTER 1

GENERAL INTRODUCTION

Precision medicine is “an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person”.[1] This approach considers a variety of patient-specific characteristics, such as age, sex, family history of disease, concomitant diseases and comedications.[2] Also, it can entail the analysis of DNA sequence variation that determines interindividual heterogeneity in drug metabolism and transport, a person’s response to a drug and the risk of drug toxicity.[3] Pharmacogenomics is an important element of precision medicine that applies genetic information to personalize drug therapy with the goal of maximizing its effectiveness and minimizing adverse drug reactions (ADRs).[2] Pharmacogenomics of cardiovascular (CV) drugs has been making great strides over the past few years, and a lot of pharmacogenetic data has accumulated for statins, *β*-blockers, novel oral anticoagulants, $P2Y_{12}$-antagonists and other drugs.[4] Among the earliest evidence of genetic factors contributing to CV drug response was the discovery of inherited deficiency in protein C responsible for skin necrosis induced by vitamin K antagonists (VKAs).[5] Improvement of treatment with VKAs in terms of safety and efficacy has been greatly anticipated, because of a narrow therapeutic index and a large interpatient dose variability of these commonly used oral anticoagulants. Genetic variants influencing the dose response to VKAs are well studied; variants in the *CYP2C9* and *VKORC1* genes (coding for the main metabolizing enzyme of VKAs and their pharmacological target) explain about one third of the dose variation in Caucasians.[6] Genotype-guided dose prediction algorithms, including these genetic variants along with clinical factors to inform VKA prescribing have been tested in randomized clinical trials (RCTs).[7-9] The European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) trial, alongside with the Clarification of Optimal Anticoagulation Through Genetics (COAG) trial, investigated the clinical utility of pharmacogenetic testing before the initiation of warfarin, acenocoumarol and phenprocoumon. These trials raised important questions about the way trials of pharmacogenetic tests should be conducted and interpreted, and also about the dosing algorithms used.[10,11] The warfarin genotype-guided algorithm did not seem to be effective in African American participants of COAG,[9] and new population-specific algorithms are being developed.[12] A lot of progress over the past decade also has been made in the field of ADRs associated with the use of CV drugs. For instance, the *SLOC1B1* rs4363657 polymorphism was identified as a risk factor for simvastatin-induced myotoxicity.[13]

SLOC1B1 encodes the organic anion transporting polypeptide 1 (OATP1B1), a membrane transporter that facilitates hepatic uptake of statins. To decrease the chance of statin-induced myotoxicity the Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends to prescribe a lower simvastatin dose or consider an alternative statin in carriers of *SLOC1B1* alleles associated with a low-function OATP1B1 that causes a reduced hepatic uptake of statins.[14] Another example of pharmacogenomic research of ADRs to CV drugs is the persistent dry cough associated with angiotensin-converting enzyme (ACE)-inhibitors. Most of the evidence supporting the association of genetic variants with this ADR (e.g., ACE gene deletion/insertion polymorphism) came from candidate-gene studies and was often conflicting.[15] It has also been suggested that genetic polymorphisms could contribute to the development of ACE-inhibitor-induced angioedema of the upper airways, however no particular genetic marker with a large effect size has been associated with angioedema so far.[16] The underlying mechanisms of cough and angioedema are not fully understood and are thought to involve the bradykinin pathway.[17,18] To gain more understanding of the mechanism of both ACE-inhibitor- and statin-induced ADRs and potentially identify patients at an increased risk of these ADRs the international Personalization of Treatment in Cardiovascular Disease through Next Generation Sequencing in Adverse Drug Reactions (PREDICTION-ADR) project will use a whole-exome sequencing approach.

Objectives of the thesis

The first objective of this thesis is to evaluate the effect of dose prediction algorithms for personalized VKA treatment on anticoagulation control and the (differences in) performance of these algorithms. The second objective is to study various determinants (comorbidities, co-medications and genetic factors) of angioedema related to ACE-inhibitor use, and to assess economic aspects of performing pharmacogenetic tests to predict the development of angioedema before the start of ACE-inhibitor therapy.

Outline of the thesis

This thesis consists of two parts (the first one dedicated to VKAs and the second one to ACE-inhibitors) that are preceded by a general introduction (**chapter 1**) and concluded by a general discussion (**chapter 4**).

The first part starts with a background paper (**chapter 2.1**), in which we describe different factors influencing the VKA dose variability, review the existing dose prediction algorithms for VKAs, describe the results of RCTs of genotype-guided VKA dosing and cost-effectiveness of this dosing strategy. In the next paper (**chapter 2.2**) we report the results of a secondary analysis of the EU-PACT acenocoumarol and phenprocoumon trial, in which we compared anticoagulation control between the trial arms after stratification by *VKORC1* and *CYP2C9* genotypes. In **chapter 2.3** the performance of genotype-guided and clinical dosing algorithms for acenocoumarol and phenprocoumon published in literature is compared with the EU-PACT algorithms.

The second part of this thesis contains four studies dedicated to adverse reactions to ACE-inhibitors, primarily focusing on ACE-inhibitor-induced angioedema. **Chapter 3.1** describes two case-control studies conducted in the UK Clinical Practice Research Datalink to investigate the association of comorbidities and comedications with ACE-inhibitor intolerance and angioedema during ACE-inhibitor therapy. In **chapter 3.2** we address the methods and results of patient enrollment into the PREDICTION-ADR project and describe the characteristics of patients with ACE-inhibitor-induced angioedema. **Chapter 3.3** is dedicated to the genome-wide association study of ACE-inhibitor-induced angioedema to identify genetic markers of this ADR using the novel Haplotype Reference Consortium imputation panel. In **chapter 3.4** we carry out a cost-effectiveness analysis to investigate the characteristics of a pharmacogenetic test for ACE-inhibitor-induced angioedema that are required to make it an economically attractive diagnostic option.

Lastly, we place our main findings into a wider context and describe possible implications for clinical practice and future research in the general discussion (**chapter 4)**.

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CHAPTER 2

PERSONALIZED TREATMENT WITH VITAMIN K ANTAGONISTS

CHAPTER 2.1

Genotype - guided coumarin dosing: where are we now and where do we need to go next?

EV Baranova, TI Verhoef, FW Asselbergs, A de Boer, AH Maitland-van der Zee.

Expert Opin Drug Metab Toxicol. 2015 Apr;11(4):509-22.

Abstract

Introduction: A large proportion of the coumarin dose variability is explained by environmental factors and by common genetic variants in the *VKORC1* and *CYP2C9* genes. Genotype-guided coumarin dosing has been proposed for a more accurate prediction of the coumarin dose in order to reduce the incidence of coumarin-related complications.

Areas covered: This review discusses the current state of coumarin pharmacogenetics, the evidence from recent randomized controlled trials and economic evaluations regarding the possible clinical implementation of genotype-guided coumarin dosing.

Expert opinion: When the *VKORC1* and *CYP2C9* genotypes are available before the start of coumarin therapy in individuals of European ancestry, a genotype-guided algorithm should be used for dose determination. Ethnicity-specific pharmacogenetic algorithms should be tested in other populations. At this moment, the evidence is not sufficient to support genotyping before coumarin therapy initiation. Based on results of recent randomized controlled trials, a clinical dosing algorithm could be considered in the initial phase of coumarin treatment. Current economic studies indicate that genotype-guided dosing could be cost-effective, but the clinical implementation of genotype-guided coumarin therapy will depend on the cost of pharmacogenetic tests and the availability of novel oral anticoagulants.

1. Introduction

Interindividual differences in drug response caused by multiple environmental, disease-related and genetic factors can lead to a reduction of efficacy or an increase in adverse reactions to a drug.[1] Identifying risk factors for the stratification of patients who are likely to have poor therapeutic responses may amend therapeutic choices and has the potential to minimize the number of adverse drug reactions.[2] Pharmacogenetics uses individual genetic information for the prediction of pharmacologic effect of a given drug. Among the most widely studied drugs in the field of pharmacogenetics are coumarin derivates, including warfarin, acenocoumarol and phenprocoumon. Worldwide warfarin is the most prescribed oral anticoagulant for the treatment of patients with venous thrombosis and prophylaxis of thromboembolic complications related to chronic atrial fibrillation and cardiac valves replacement surgery.[3] Acenocoumarol and phenprocoumon are more frequently used only in some European countries.[4] Due to the narrow therapeutic window of coumarins and the large inter- and intrapatient dose variability, treatment with these drugs is associated with an increased rate of bleedings.[5] Warfarin-related bleedings accounted for as much as one-third of hospitalizations for adverse drug events among older adults in the USA.[6] The individual warfarin dose may vary by a factor of 10 among patients, but in most countries the typical starting warfarin dose is fixed (5 mg) and titrations are performed based on the international normalized ratio (INR), which should remain within the 2.0-3.0 range for most indications.[1]

Among the important determinants of coumarin dose requirement are clinical factors, such as the intake of vitamin K, age, gender, concurrent medication, renal function and comorbidity.[7] However, it has been well-established that the dose of coumarin derivates is substantially influenced by the genotype.[8] In the past years, a more “personalized” approach to coumarin dosing, guided by an individual’s genetic information has been investigated in randomized controlled trials (RCTs) and more trials are still ongoing. Furthermore, the availability of novel oral anticoagulants (NOACs) had an impact on use of coumarins and will probably be an important factor for the clinical implementation of genotype-guided coumarin dosing strategies in the future.[1] In this review, the role of genetic factors influencing the coumarin response and the algorithms for calculating the coumarin dose based on genotype are addressed, along with the clinical evidence from recent RCTs examining the genetic-

guided coumarin dosing. The review mostly focuses on warfarin; however, the findings on pharmacogenetics of acenocoumarol and phenprocoumon are also evaluated. Moreover, economic considerations related to the realization of genotype-guided coumarin dosing in practice are discussed and suggestions for future research are given.

2. Pharmacogenetics of coumarins

2.1 Genetic variants predicting the coumarin dose

Coumarins act in the liver by inhibiting vitamin K epoxide reductase (VKOR), an enzyme converting inactive oxidized vitamin K back to its active reduced form, which is required as a cofactor for functional coagulation factors II, VII, IX, and X (**Figure 1**).[9] Coumarins exist as a racemic mixture of S- and R- enantiomers, with the S-enantiomer being several times more potent.[9] The two genes that influence warfarin response the most are vitamin K epoxide reductase subunit 1 gene (*VKORC1*), which encodes the warfarin target VKOR, and the liver enzyme cytochrome p450 2C9 gene (*CYP2C9*), metabolizing S-warfarin (**Figure 1**).[7,10,11] Cytochrome p450 2C9 also metabolizes acenocoumarol, but is less important for phenprocoumon, which is primarily metabolized by CYP3A4.[12,13] The influence of common *CYP2C9* polymorphisms on the warfarin dose was first described in the late 1990s, whereas the effect of *VKORC1* variants was reported in 2005.[14-16] Since that time, several investigators studied the contribution of common genetic polymorphisms to the variation in coumarin dose requirement and genome-wide association studies (GWAS) were performed, which confirmed the earlier findings (**Table 1**).[10,11,17-19] The results of the GWAS in acenocoumarol and phenprocoumon showed genetic associations for dose similar to the studies in warfarin (**Table 1**).[17,20] Scott *et al.* described rare missense mutations the *VKORC1* gene to be associated with warfarin resistance in Ashkenazi and Sephardi Jewish populations, where extremely high doses (> 20 mg/day) are needed for the therapeutic effect.[21] In the general population, functional single nucleotide polymorphisms (SNPs) in the *VKORC1* promoter (-1639 G > A, rs9923231) and intron 1 (1173 C > T, rs9934438) are responsible for ~ 25% of the warfarin dose variability.[22,23] These two SNPs are in almost complete linkage disequilibrium and similarly predict warfarin dose across all racial groups.[24] The -1639 G allele results in increased *VKORC1* promoter activity and mRNA levels, which leads to a higher

warfarin dose requirement by the G carriers in comparison to individuals with the A allele.[23,25] The homozygotes for the A allele have the highest sensitivity to warfarin and require lowest doses.[23] The GG genotype is most common in African Americans and a higher frequency of the AA genotype is observed in Asians, whereas ~50% of individuals of European ancestry have the AG genotype.[24] Most of the genetic variants in the *CYP2C9* gene lead to a reduced activity of the enzyme and an increased sensitivity to warfarin.[26] The most common variants in Europeans *2 (R144C, rs1799853) and *3 (I359L, rs1057910) polymorphisms are located in the exonic regions of *CYP2C9*, whereas the *6 variant (818delA, rs9332131), primarily present in the African-Americans, is a deletion with a reading frame shift.[26] The *CYP2C9* *2 variant is very rare in Chinese populations.[27] The common *CYP2C9* SNPs account for ~10% of the variation in warfarin dose requirement.[28] Altogether the variants in the *CYP2C9* and *VKORC9* explain ~35% of the warfarin dose variability, and when they are combined with clinical data, up to 50% of dose variability can be explained.[7,9] A few other genes with smaller effects than *CYP2C9* and *VKORC1* have been associated with warfarin dosing, including *CYP4F2* (V433M, rs2108622), *CYP2C18* (G4A, rs12777823), calumelin (*CALU*) and *GGCX* CAA16/17 repeat polymorphism).[18,29,30] The *CYP4F2* V433M is a nonsynonymous polymorphism causing decreased oxidation of vitamin K in the liver and thereby increasing warfarin dose requirement in homozygotes for the variant allele.[29] The association of warfarin dose with CYP4F2 rs2108622 is present in Europeans and Asians but not in African-Americans, because of a lower allele frequency in this population.[19,31] Cavallari *et al*. reported an association of an SNP in gamma-glutamyl carboxylase (*GGCX*) rs10654848 (CAA) 16 or 17 repeat with a higher warfarin dose in African-Americans.[30] The *GGCX* SNP explained 2% of the warfarin dose variability and is 10 times more frequent in African-Americans than in Caucasians (where the minor allele frequency is 0.27%).[30] The effect of another SNP, *CYP2C18* rs12777823, on the warfarin dose was also discovered in a population of African-American ancestry.[18] Carriers of the minor A allele had reduced clearance of S-warfarin and lower warfarin doses.[18] It is notable, that despite the same allele frequency across different populations, the effect of rs12777823 was only evident in African-Americans, so it is probably not the causal variant but is inherited together with a rare causal variant in African-Americans.[18]

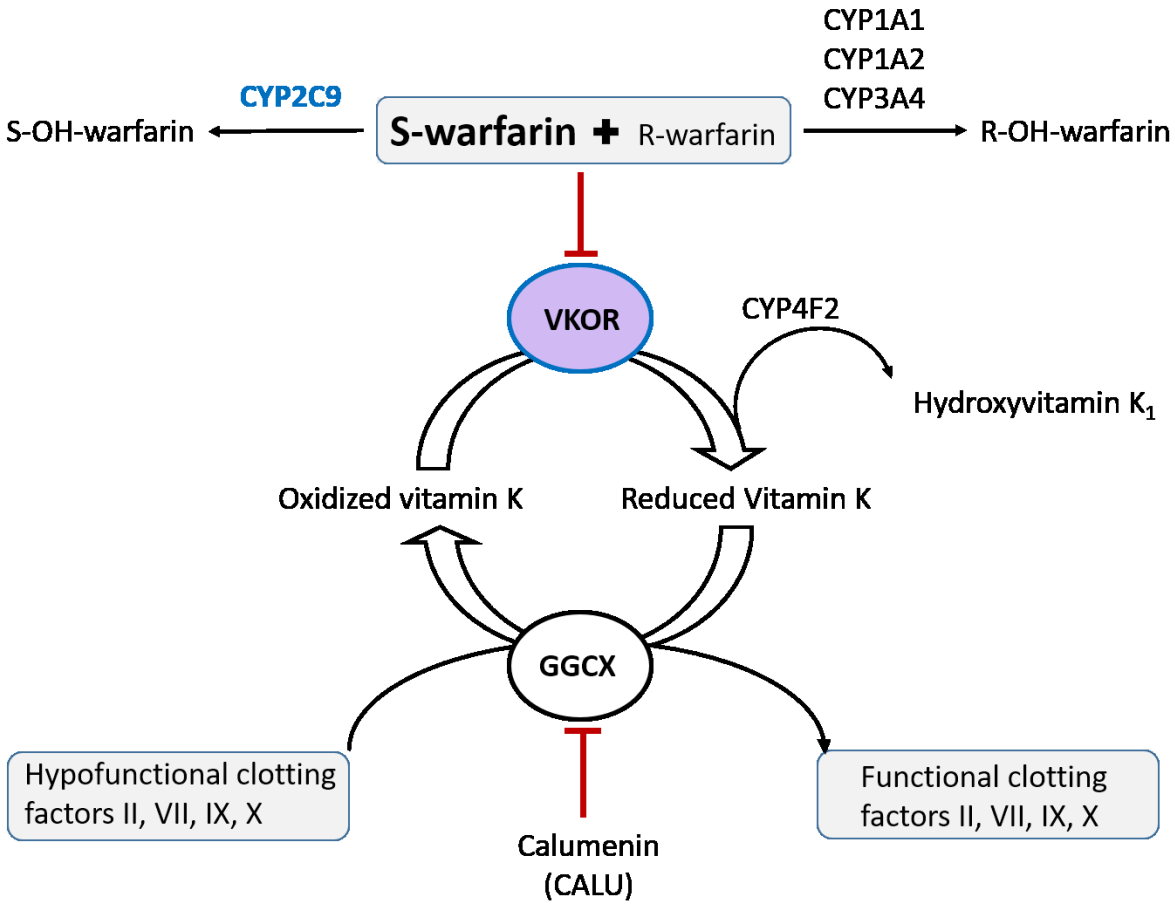

FIGURE 1. The role of enzymes involved in pharmacodynamics and pharmacokinetics of warfarin. Coumarin dose variation significantly depends on the SNPs in the genes encoding enzymes involved in the vitamin K cycle and the metabolism of warfarin. The most active S-enantiomer of warfarin is primarily metabolized by CYP2C9, whereas R-warfarin is metabolized by several other CYP isoforms[7]. GGCX: Gamma-glutamyl carboxylase; SNPs: Single nucleotide polymorphisms; VKOR: Vitamin K epoxide reductase.

The SNP rs339097 in calumelin (a chaperon protein capable of inhibiting GGCX) has been demonstrated to confer an 11 - 15% higher warfarin dose in African-Americans.[32] This minor allele frequency of rs339097 is ~1% in Europeans as opposed to 25% in African-Americans.[33]

2.2 Genetic associations of coumarin-related complications

The first 3-6 months of warfarin therapy are marked by an increased risk of excessive anticoagulation (INR above therapeutic range) and bleedings.[34,35] Genetic factors influencing warfarin dose contribute to the risk of over-anticoagulation. The *VKORC1* -1639 G > A has been associated with higher INR levels during the first month of treatment and with a longer time spent out of the therapeutic INR range, however not all studies found an association of the *VKORC1* SNP with bleeding risk.[10,34,36-38]

TABLE 1. Overview of genome-wide association studies of coumarin maintenance dose.

Author, year	Study sample (initial/replication)	Ancestry	Reported genes	Most significant SNP	P - value
Warfarin maintenance dose					
Cooper *et al.*[11], 2008	181 / 374	European	*VKORC1* *CYP2C9* *CACNA1C*	rs10871454 rs4917639 rs216013	6.2×10^{-13} 9.7×10^{-5} 8.6×10^{-7}
Takeuchi *et al.*[10], 2009	1053 / 588	European	*VKORC1* *CYP2C9* *CYP4F2*	rs9923231 rs1057910 rs1799853 rs2108622	3×10^{-181} 3×10^{-79} 1×10^{-31} 3×10^{-10}
Cha *et al.*[19], 2010	807 low dose, 701 high dose / 444	Japanese	*VKORC1* *CYP2C9* *CYP4F2*	rs9923231 rs10509680 rs2108622	9×10^{-31} 3×10^{-8} 4×10^{-7}
Perera *et al.*[18], 2013	533 / 432	African American	*CYP2C18* *CYP2C9* *CYP2C8* *CYP2C19*	rs12777823	5×10^{-12}
Acenocoumarol maintenance dose					
Teichert *et al.*[17], 2009	1451 / 287	European	*VKORC1* *CYP2C9* *CYP2C18* *CYP4F2*	rs10871454 rs4086116	2.0×10^{-123} 3.3×10^{-24}

Note: The rs10871454 SNP is in perfect linkage disequilibrium ($r^2 = 1.0$) with the *VKORC1* −1639 G>A rs9923231 SNP.[11]

Several studies showed that *CYP2C9* *2 and *3 polymorphisms were associated with over-anticoagulation and an increased major bleeding risk, particularly in the first week of warfarin therapy.[35,36] A meta-analysis found that the relative bleeding risk for *CYP2C9* *2 was 1.91 (95% CI: 1.16 - 3.17), for *CYP2C9* *3 1.77 (95% CI: 1.07- 2.91) and for either variant it was 2.26 (95% CI: 1.36 - 3.75).[39] A recent study in Indian population found that carriers of *VKORC1* AA and *CYP2C9* *3 homozygous genotypes were at significantly higher risk of over-anticoagulation (INR > 4).[40] The study by Tomek *et al*. reported a higher major bleeding risk in carriers of several variant alleles, both during therapy initiation and in a follow-up period of 26 months.[41] A comprehensive meta-analysis including 6272 patients from 22 studies concluded that *CYP2C9* *3 was a stronger risk factor for warfarin-related bleeding compared to *CYP2C9* *2 and found no significant associations of the *VKORC1* -1639 G > A variant with any hemorrhagic complications.[42] The association between the *CYP2C9* (*2 and *3) and *VKORC1* (GA and AA carriers) with over-anticoagulation (INR > 4) was confirmed in this meta-analysis.[42] The effect of *VKORC1* -1639 G > A on over-anticoagulation was shorter than that *CYP2C9* *3, which persisted during the entire

treatment period.[42] Increased risk of over-anticoagulation was found in *VKORC1* variant carriers up to 6 months after the start of therapy with acenocoumarol, but no such effect was observed for *CYP2C9* variants.[43] Interestingly, in the same study an increased risk of a subtherapeutic INR was described in *CYP2C9* wild-type individuals during the first month and in *VKORC1* wild-type individuals during 3 months after therapy initiation.[43] This suggests that *VKORC1* and *CYP2C9* wild-type patients might be underdosed when the standard fixed-dose approach is used.[43] In wild-type *VKORC1* and *CYP2C9* phenprocoumon users, the first month of therapy was characterized by an increased risk of underdosing and subtherapeutic INR measurements.[44] Additionally, the risk of overdosing was highest in phenprocoumon users with *VKORC1* or *CYP2C9* variant alleles.[44] However, beyond 1 month of treatment with phenprocoumon, there were no statistically significant differences in the risk of out-of-range INRs between different genotypes.[44] A detailed summary of studies on bleeding risk during coumarin therapy can be found in a recently published review.[45]

2.3 Genotype-guided algorithms for the prediction of coumarin dose

Coumarins have become a target for genotype-guided therapy, because only a small number of genetic variants explain such a substantial proportion in coumarin dose variability and the occurrence of hemorrhagic complications. To date, over forty pharmacogenetic algorithms have been developed for the calculation of warfarin maintenance dose in various populations.[45] The first algorithms only included *CYP2C9* variants and subsequently the information on the *VKORC1* and a few other genes, including *CYP4F2* and *APOE* genotypes, was being used.[46,47] Typically, a pharmacogenetic algorithm also includes demographic characteristics: age, body size, weight, smoking status and the use of amiodarone. Amiodarone intake is an important factor, because this drug inhibits CYP2C9, leading to increased plasma concentrations of warfarin and a higher risk of bleeding. Some pharmacogenetic algorithms include prosthetic valve replacement status, heart failure status, and the amount of vitamin K intake.[33] An algorithm developed by Gage *et al.* explained 57% of warfarin dose variation in Caucasians, but the predictive value of this algorithm was lower (31%) in African-Americans.[48] Another genotype-guided algorithm explained 59% of the dose variability in a Swedish population by the *VKORC1* and *CYP2C9* genotypes, age, race, sex and co-medications capable of increasing the INR.[37] Compared to Caucasians,

lower daily doses of warfarin are generally required for Chinese patients.[27] Studies in Chinese populations reported that combining the genetic information on *CYP2C9* *3 and *VKORC1* -1639 G > A to the clinical factors could explain 48 - 74% of the warfarin dose variation.[27] At the moment more than ten genetic-guided dosing algorithms have been developed and validated in the Chinese populations.[27] An international group of experts on pharmacogenomics of warfarin (Warfarin Pharmacogenetics Consortium, IWPC) developed a highly reliable warfarin dosing algorithm in a large diverse population from nine countries.[49] The IWPC algorithm predicted 47% of the warfarin dose variation among Caucasians by using the information on *CYP2C9* and *VKORC1* SNPs, age, height, weight, amiodarone use, race and number of CYP enzyme inducers.[49] Earlier studies indicated that pharmacogenetic algorithms in general predict warfarin dose more accurately than do other dosing methods.[50] The warfarin label updated by the FDA in 2010 contains a pharmacogenetic dosing table, which may be used for selection of an initial warfarin dose when the patient's *CYP2C9* and *VKORC1* genotype is available.[51] Finkelman *et al.* reported that a genotype-guided algorithm predicted more doses within 20% of the actual dose than a clinical dosing algorithm, the dosing table on the warfarin label and the 5 mg/day fixed-dose approach.[50] Genetic-guided strategy was particularly more accurate than other dosing approaches in patients requiring low (i.e., ≤ 3 mg/day) or high (i.e., ≥ 7 mg/day) warfarin doses.[49] A guideline for physicians on the interpretation and use of the *CYP2C9* and *VKORC1* genotype was developed by The Clinical Pharmacogenetics Implementation Consortium (CPIC).[52] The CPIC recommends considering the use of a pharmacogenetic algorithm for warfarin dosing, if genetic information is available.[52] The recommended warfarin dosing algorithm is available online at Warfarindosing.org (http://www.warfarindosing.org). Compared to warfarin dosing, somewhat less pharmacogenetic-guided algorithms for acenocoumarol and phenprocoumon maintenance dose were created.[53-56] An example is the acenocoumarol and phenprocoumon algorithm by Van Schie *et al.*, developed and validated in a Dutch population.[57,58]

3. Genotype-guided coumarin dosing in randomized controlled trials

Despite the promising results of earlier non-randomized studies on pharmacogenetic warfarin dosing, evidence from larger RCTs was required to assess the feasibility of

clinical implementation of the genotype-guided approach.[3,59] An overview of recent randomized clinical trials on genotype-guided coumarin dosing is presented in **Table 2**. At the end of 2013, the results of The Clarification of Optimal Anticoagulation through Genetics (COAG) and The European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) have been simultaneously published in the New England Journal of Medicine.[60-62] The COAG trial, conducted in the USA, was a multi-center, double-blinded RCT comparing genotype-guided warfarin dosing with a clinical dosing algorithm.[60] The EU-PACT trial was a single-blinded, multi-center RCT, which had a warfarin and an acenocoumarol-phenprocoumon part and was conducted Sweden, UK, the Netherlands and Greece. Both trials evaluated the effect of genotype-guided dosing strategy on percentage of time in therapeutic INR range (TTR).[61,62] The COAG trial utilized the dose initiation algorithm by Gage *et al.* and a dose revision algorithm by Lenzini *et al.* after 4-5 days.[63] The EU-PACT warfarin trial used the modified IWPC algorithm during therapy initiation in comparison to a standard warfarin loading dose (usual care) and the same dose revision algorithm.[61] The results of these trials turned out to bring slightly more confusion than clarity with respect to the clinical implementation of genetic-guided warfarin dosing. The COAG authors found no between-group differences in the mean TTR after 4 months of therapy.[60] Furthermore, TTR in African-American patients was decreased by 8%-points in the genotype-guided arm.[60] In contrast to COAG, results of the EU-PACT warfarin trial showed a 7%-point increase in the TTR in the genotype guided arm after 3 months of treatment.[61] The EU-PACT acenocoumarol-phenprocoumon trial found a 5% increase in TTR with genetic-guided dosing only during the first 4 weeks of coumarin treatment, but not 12 weeks after the initiation of therapy.[62] Such varying results could be explained by the choice of the control group, the differential influence of genetic variants on dosing in different ethnic groups, the regional variability in clinical practice and, possibly, by the differences in the used algorithms. Choosing the standard of care as a comparator arm in the EU-PACT warfarin trial over a clinical algorithm, including age, co-medications and other factors (as it was done in COAG) has been suggested to contribute to the detection of significant differences in TTR.[64] Furthermore, the ethnical differences between COAG and EU-PACT populations could also in part explain the discordant results.[3] Moreover, the overall number of individuals with variant alleles was greater in EU-PACT than in COAG, which might have had in impact on the findings. Finally, the

implemented genotype-guided algorithms were different as well. A more detailed comparison of the EU-PACT and COAG trial design can be found in recently published reviews.[3,59]

A few randomized clinical trials have already been performed in Asian populations. Huang *et al.* demonstrated that a pharmacogenetic algorithm allowed more accurate dosing and reduced the time to achieve a therapeutic stable warfarin dose in Chinese patients undergoing heart valve replacement therapy.[65] A randomized controlled trial by Wang *et al.* showed similar results favoring the genotype-guided warfarin dosing strategy over a fixed loading dose with adjustments according to INR.[66] At the moment at least three trials for genotype-guided warfarin dosing in the Chinese populations are recruiting participants. One of these studies (ClinicalTrials.gov Identifier NCT01855737) aims to assess the performance of a pharmacogenetic algorithm including *VKORC1*, *CYP2C9* and *CYP4F2* genotypes compared to the actual dose. Another trial will compare a genetic-guided algorithm and using a fixed warfarin dose (standard of practice) with respect to percent time out-of-range INRs, TTR, time to reach TTR, warfarin-related bleedings and thromboembolisms (ClinicalTrials.gov Identifier NCT01610141). A trial on genotype-guided warfarin therapy in Chinese elderly people will compare the IWPC dosing algorithm with the standard care using percentage of time in therapeutic INR range as primary outcome (ClinicalTrials.gov Identifier NCT02211326). Recently, meta-analyses of the largest RCTs have been published to provide more evidence on the effect of the genotype-guided warfarin dosing on thromboembolic and hemorrhagic complications of coumarins.[67-71] The meta-analysis performed by Stergiopoulos *et al*. included data from nine RCTs and a total of 2812 patients receiving warfarin, acenocoumarol or phenprocoumon.[67] The TTR, percentage of time with INR > 4 and the number of bleeding episodes were compared in the genotype-guided arm and the clinical-guided algorithm or the usual care comparator arms.[67] The authors found no statistically significant differences in any of these endpoints, although the TTR definitions and the clinical dosing approaches differed across the included studies.[67] Of note is that the meta-analysis by Franchini *et al*., which evaluated the same RCTs as the study by Stergiopoulos, concluded that serious bleeding events could be reduced by ~50% with the genotype-guided coumarin dosing as compared to the clinical dosing approach.[70] The reasons for such discrepancies might be that the latter study did not include the data from one

of the trials into the final analysis, and there were some differences in the study design between the two meta-analyses.[67,70] Another meta-analysis only included RCTs on genotype-guided dosing of warfarin, but not acenocoumarol and phenprocoumon, and pooled the data on TTR, number of bleedings and deaths across 1910 patients in seven trials.[68] In this study the analysis was split for the trials using a fixed coumarin dose or a clinical algorithm as a comparator to the genotype-guided strategy.[68] Compared to fixed-dose strategies (reflecting usual anticoagulation care), the genotype-guided warfarin dosing resulted in an increased TTR, but no significant reduction in the incidences of adverse events and death rates was observed.[68] According to this meta-analysis, the genotype-guided approach was not superior to a non-fixed initial dose that was calculated with clinical algorithms.[68] The meta-analysis by Goulding *et al.* found that genotype-guided warfarin dosing resulted in a statistically significant reduction of warfarin-related bleedings and thromboembolic events.[69] The differences in the results of these meta-analyses could probably in part be explained by the choice and number of included studies and by different approaches to the analysis of the data.[68,69] The meta-analysis by Tang *et al.* reported an improvement in TTR and a reduction in the number of bleeding events with pharmacogenetic-guided warfarin dosing, showing a significant TTR increase for Asians in a subgroup analysis.[71] Overall, the conflicting (at least to some extent) results of the meta-analyses suggest that even pooled, the data from existing trials might be insufficient to detect statistically significant differences in clinically relevant endpoints. Ongoing clinical trials powered to detect the effects of genotype-guided dosing on warfarin-related complications are currently underway.[72] In the Genetics Informatics Trial of Warfarin Therapy to Prevent Deep Vein Thrombosis, 1600 elderly patients undergoing elective hip or knee replacement surgery will be genotyped for *VKORC1* -1639 G > A, *CYP2C9* *2, *3 and additionally for the *CYP4F2* V433M variant.[72] The IWPC algorithm available on the website WarfarinDosing.org will be used for dosing during a minimum of the first 11 days of treatment for warfarin dose determination.[72] Another RCT in patients older than 65 years (the Warfarin Adverse Event Reduction for Adults Receiving Genetic Testing at Therapy Initiation [WARFARIN] trial) will also compare genetic-guided and clinically guided dosing (Clinicaltrials.gov identifier NCT01305148). The trial anticipates inclusion of 4300 patients and will utilize the

incidence of warfarin-related clinical events (major bleedings and thromboembolic events) as the primary endpoint.

4. Cost-effectiveness of genotype-guided coumarin therapy

The evidence of cost-effectiveness is essential for the clinical implementation of genetic-guided coumarin therapy. Since 2003 when the first economic analysis of warfarin pharmacogenetic testing was performed, a number of cost-effectiveness studies aimed to assess the genetic-guided versus clinical coumarin dosing.[73-79] Earlier studies have only evaluated the cost-effectiveness of *CYP2C9* genotyping; however, after 2005 the majority of the analyses included *VKORC1* genotyping. Furthermore, before 2010 data on clinical effectiveness of genotyping from RCTs was not available for the analyses and they relied mainly on assumptions.[79] Cost-effectiveness of genetic-guided warfarin therapy ranged from US$171,000 to 347,000 per quality-adjusted life-year (QALY) gained and the willingness to pay was estimated US$50,000 to 100,000 per QALY gained.[80] Meckley *et al.* estimated a 46% chance that genetic-guided dosing would be cost-effective at a threshold of US$50,000 per QALY gained.[78] Patrick *et al.* showed that a 5% increase in TTR after 3 months of therapy would be required to achieve the incremental cost-effectiveness ratio (ICER) of less than US$100,000 per QALY gained in the USA.[75] To bring the ICER under US$50,000 per QALY gained, a 9% increase in TTR with genetic-guided dosing would be needed.[75] A comprehensive report on the cost-effectiveness analyses performed before 2010 can be found in a previously published review.[81] A cost-effectiveness analysis of pharmacogenetic dosing of phenprocoumon was performed in 2013 by Verhoef *et al*..[82] This study concluded that the genetic-guided approach slightly increased QALYs in comparison to standard dosing (ICER = €2658 per QALY gained).[82] A more recent study performed in the EU-PACT acenocoumarol-phenprocoumon data evaluating the cost-effectiveness of genotype-guided versus clinical algorithm in the Netherlands showed that the genetic-guided dosing increased costs by €33 and QALYs by 0.001.[83] The ICERs for acenocoumarol and phenprocoumon were €28,349 and €24,427 per QALY gained, respectively.[83] The authors concluded that the cost per QALY would be below the willingness to pay threshold of €20,000 if genotyping costs were to decrease to approximately €30.[83]

TABLE 2. Overview of randomized clinical trials of genotype-guided coumarin dosing.

Author, year	N	Main indication for coumarins	Blinding	Primary endpoint	Genotypes	Dosing strategy		Results
						Pharmacogenetic	**Clinical**	
Anderson *et al.,*[93] 2007	206	Preoperative orthopedic (60%)	Double-blinded	INR outside 1.8 - 3.2 range	*CYP2C9* *VKORC1*	Regression equation developed by the authors, based on observational data	10-mg warfarin nomogram by Kovacs *et al.*[98]	No differences in primary endpoint; doses were predicted more exact with genotype-guided algorithm
Caraco *et al.,*[94] 2008	191	DVT and PE (66%)	NR	INR 2.0-3.0	*CYP2C9*	Based on different algorithms using *CYP2C9* variants	Clinical algorithm by Ageno *et al*.[99]	Genotype-guided arm had higher TTR and less minor bleedings
Burmester *et al*.,[95] 2011	230	Atrial fibrillation (46%)	Single-blinded	INR 2.0-3.5	*CYP2C9* *VKORC1* *CYP4F2*	Marshfield Pharmacogenetic model[100]	Dosing according to the Marshfield Anticoagulation Service guidelines	No effect on TTR; more accurate dose prediction by pharmacogenetic model
Borgman *et al*.,[96] 2012	26	DVT (46%)	Single-blinded	INR 1.8-3.2	*CYP2C9* *VKORC1*	PerMIT dose calculation software	Standard clinical care by thrombosis service and warfarin nomogram by Kovacs *et al.*[98]	PerMIT led to increase in TTR and a decrease in the frequency of warfarin dose adjustments per INR
Huang *et al*.,[65] 2009	121	Heart valve replacement	Single-blinded	mean time to reach a stable warfarin maintenance dose	*CYP2C9* *VKORC1*	Pharmacogenetic algorithm developed by the authors	Usual AC: warfarin starting dose 2.5 mg/day with adjustments based on INR	HR for the time to reach stable dose was 1.9 for AC vs. genotype-guided dosing
Jonas *et al*.,[97] 2013	109	Atrial fibrillation (34%), DVT (30%)	Double-blinded	INR 2.0-3.0 or 2.5-3.5	*CYP2C9* *VKORC1*	Washington University School of Medicine pharmacogenetic algorithm	Same algorithm but including only clinical factors	genotype-guided dosing did not improve TTR or decrease the number of anticoagulation visits
Kimmel *et al*.,[60] 2013	1015	DVT or PE (58%)	Double-blinded	INR 2.0-3.0	*CYP2C9* *VKORC1*	Algorithm by Gage *et al.* and a pharmacogenetic dose revision algorithm by Lenzini *et al.*[63]	Clinical dosing algorithm	No difference between arms
Pirmohamed *et al*.,[61]	455	Atrial fibrillation (73%)	Single-blinded	INR 2.0-3.0	*CYP2C9* *VKORC1*	Modified IWPC algorithm	Patients aged ≤75 y: warfarin 10 mg	Genotype-guided dosing superior to clinical care

2013							on day 1 -3, patients aged >75 y: warfarin 5 mg on days 1-3 with; dosing on days 4-5 according to local clinical practice	
Verhoef *et al.,*[62] 2013	548	Atrial fibrillation (83%)	Single-blinded	INR 2.0-3.0	*CYP2C9 VKORC1*	Pharmacogenetic algorithm by van Schie *et al.*[58]	Clinical dosing algorithm	No difference between arms
Wang *et al.,*[66] 2012	101	Heart valve replacement	Single-blinded	mean time to reach a stable warfarin maintenance dose	*CYP2C9 VKORC1*	Pharmacogenetic algorithm developed by Huang *et al.*[65]	Usual AC: warfarin starting dose 2.5 mg/day with adjustments based on INR	Mean time to reach a stable dose was shorter in the genetic-guided group

DVT - deep venous thrombosis. INR – international normalized ratio. PE - pulmonary embolism. NR - not reported. HR – hazard ratio. AC –anticoagulation care.

To make genotyping cost-effective in patients older than 70 years, the costs of the pharmacogenetic test would have to be even lower.[83] The cost-effectiveness of the genetic-guided coumarin dosing can be determined by several factors, including the population where it is tested and the indication, the age of the patients and the cost of the pharmacogenetic test as well as how often it will be used.[79] Currently, one of the most important factors influencing the cost-effectiveness of genotype-guided warfarin therapy is the availability of NOACs, that is, the direct thrombin inhibitors and activated factor X inhibitors (dabigatran, apixaban, and rivaroxaban). Compelling data from RCTs shows that these novel agents can be a good alternative to warfarin for stroke prevention in patients with atrial fibrillation.[84,85] Unlike coumarins, NOACs do not require frequent INR monitoring, but they do have certain limitations, including high costs, the lack of a specific antidote and the anticipated decrease in therapy adherence.[1] The latest cost-effectiveness analyses provide a comparison between the genotype-guided warfarin dosing and treatment with NOACs (**Table 3**). The study by Pink *et al.* used a clinical trial simulation approach to compare genotype-guided dosing with clinical dosing and then performed a discrete-event simulation for comparison of genotype-guided dosing with NOACs.[86] Genotype-guided dosing in this study was more cost-effective than clinical dosing with an ICER of £13,226 (€16,792).[86] However, apixaban would be the most cost-effective option as compared to clinical and genotype-guide dosing algorithms, with an ICER of £20,671 (€26,245).[86] Previously it has been shown that the cost-effectiveness of NOACs depends on the INR control in the warfarin comparator group.[84] Supporting this evidence was a study, comparing the genotype-guided and clinical algorithms with dabigatran, which concluded that dabigatran had an ICER of US$13,810 (€11,173) per QALY gained, but would only be cost effective if TTR is < 64%.[87]

An interesting approach to assess the cost-effectiveness of genotype-guided warfarin dosing implied simulation of a situation where the decision which anticoagulant to choose would be made based on a warfarin pharmacogenetic test.[88] According to this approach, genotyping would separate *VKORC1* and *CYP2C9* wild-type patients from those with variant alleles and susceptible to over-anticoagulation.[88] The patients with the *VKORC1* GA and *CYP2C9* *1*1 genotype would be prescribed genetic-guided warfarin, whereas the other patients would receive NOAC treatment. In this stratified approach, pharmacogenetic dosing was very cost-effective with an ICER of US$2843

per QALY (which is well below than the willingness to pay threshold of US$50,000).[88] The use of genetic-guided dosing was also more cost-effective (ICER = US$12,080 per QALY) than the usual anticoagulation care.[88] Prospective, randomized trials are underway to provide clinical utility data for cost-effectiveness analyses. In particular, the Clinical and Economic Implications of Genetic Testing for Warfarin Management study (ClinicalTrials.gov Identifier NCT00964353) aims to assess the clinical effectiveness and cost-effectiveness of genotype-guided warfarin algorithms.

5. Conclusion

Coumarin dose requirements are largely determined by the common genetic variants in the *VKORC1* and *CYP2C9* genes. Over the past decade, in attempt to reduce the number of coumarin-related complications pharmacogenetic dosing algorithms were developed to provide more accurate coumarin doses than clinical algorithms and the usual "one-fits-all" strategy. A few observational and randomized clinical trials suggested the benefit of genetic-guided dosing, whereas some others failed to detect any improvements of the anticoagulation status with this approach.

Recent large RCTs of genotype-guided coumarin therapy produced varying results with respect to TTR and were not designed to evaluate clinically relevant endpoints, such as bleedings and thromboembolic events. This will be assessed in ongoing trials. Furthermore, the genotype-guided coumarin dosing must be cost-effective to be able to compete with newly developed anticoagulants. Earlier studies were not sufficient to determine whether or not pharmacogenetic coumarin dosing was cost-effective. Currently, with more clinical effectiveness data available from RCTs, more reliable cost-effectiveness studies can be performed. The data so far indicates that genotype-guided therapy could be more cost-effective than clinical dosing, but this depends on the cost of genetic tests. With suboptimal INR control during coumarin therapy, the cost-effectiveness of NOACs increases.

6. Expert opinion

The environmental and genetic factors defining the coumarin dose required to achieve and maintain therapeutic anticoagulation have been extensively studied. However, the knowledge about these factors is often omitted in clinical practice.

TABLE 3. Overview of published studies on the cost-effectiveness of genotype-guided warfarin dosing.

Author, year	Comparators	Population	Outcomes	Time horizon	Events included	Perspective	Conclusions
Pink J *et al.*,[86] 2014	clinical algorithm for warfarin rivaroxaban dabigatran apixaban	average profile of the AF population in UK	QALYs gained	Lifetime	Stroke, systemic embolism, TIA, major bleed (including intracranial hemorrhage), myocardial infarction	UK National Health Service (NHS)	Apixaban and genotype-guided warfarin are cost-effective against clinical dosing algorithm. Apixaban had the highest gain in QALYs.
You JH *et al.*,[87] 2012	usual AC with warfarin dabigatran	newly diagnosed AF patients ≥ 65 years old with a high risk for stroke	total direct medical cost and QALYs gained	maximum period of 25 years	dyspepsia, major bleeding, ischemic stroke, myocardial infarction, death	healthcare payers	Genotype-guided warfarin would be most cost-effective when TTR is > 77% and the utility value of warfarin was the same or higher than that of dabigatran.
You JH *et al.*,[88] 2014	usual AC with warfarin patients with *VKORC1* GA and *CYP2C9* *1*1 were assigned to a NOAC and patients with polymorphisms in *VKORC1* and *CYP2C9* received genetic-guided warfarin	newly diagnosed AF patients ≥ 65 years old	total direct medical cost and QALYs gained	maximum period of 25 years	major bleeding, ischemic stroke, myocardial infarction, death	healthcare payers	Compared to usual AC with TTR of 60%, assigning patients by the genotype to either NOACs or pharmacogenetic warfarin was highly cost effective.

AF – atrial fibrillation. NOACs – novel oral anticoagulants. AC-anticoagulation care.

The recent RCTs conducted in the USA and Europe, COAG and EU-PACT, aimed to provide more evidence on the clinical utility of pharmacogenetic dosing for coumarin anticoagulants. The differences in trial design and used algorithms and the absence of a third trial arm, which would compare the standard anticoagulation care and the clinical dosing algorithm, complicate the interpretation of the findings. The results of COAG and of some of the recent meta-analyses do not directly support using a pharmacogenetic dosing algorithm before the start of anticoagulation therapy with warfarin.[3] Nevertheless, if the genetic information is already available before the start of treatment, the utilization of the *VKORC1* and *CYP2C9* genotypes for the initial warfarin dose determination should be considered in individuals of European ancestry. When the *CYP2C9* and *VKORC1* genotypes are not known, a clinical algorithm could be considered preferable for the coumarin dose determination.[3,8] The question still remains, whether the implementation of clinical algorithms could take place without the evidence from randomized trials. Furthermore, in the absence of additional data showing that genetic-guided strategy not only improves TTR, but also reduces the number of coumarin-related bleedings and thromboembolic events, there is yet no consensus in current clinical management guidelines to advice for or against *VKORC1* and *CYP2C9* genotyping before the start of coumarin therapy.[3] The recent meta-analyses of RCTs on genetic-guided coumarin dosing produced somewhat different results with respect to the coumarin-related complications, which indicate that even pooling the data from several trials might be insufficient to assess the clinically relevant endpoints. The availability and the cost of a reliable pharmacogenetic test are also important factors that could influence the implementation of genetic-guided coumarin dosing in clinical practice. It has been suggested that a new point-of-care test for *VKORC1* and *CYP2C9* variants would cost US$50, but the price would be lower if the test is used more often.[89] In the long term, it is possible that pharmacogenetic testing will be a part of standard care, and in the meantime several clinics in the USA are using pharmacogenetic data in real-life setting.[90] Data collected through this practice would assist the comparison of outcomes between genotype-guided and clinical dosing.[8] The results of COAG emphasized the importance of developing and utilizing coumarin dosing algorithms specific for certain ethnic groups. In the African-American patients it is especially true because of the different genetic variants that are important for determining the coumarin dose in this ethnical group (e.g., *CYP2C9* *5, *6, *8, and

*11). A reliable genetic-guided algorithm for African-Americans, which would include the ethnic-specific *CYP2C9* variants responsible for a lower warfarin dose requirement, is under development.[91] In Asian populations, trials are also currently underway to address the clinical utility of pharmacogenetic coumarin dosing in this ethnic population. Cost-effectiveness analyses are essential for the decisions surrounding the clinical implementation of coumarin pharmacogenetic testing, especially after the development of direct antithrombin and anti-Xa inhibitors. It is possible that from the economic perspective pharmacogenetic coumarin dosing for certain indications might be preferable to the use of novel oral anticoagulants.[92] Currently, more cost-effectiveness analyses comparing these two therapeutic options are required.

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Declaration of interest

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CHAPTER 2.2

The efficacy of dosing algorithms for acenocoumarol and phenprocoumon across *VKORC1* and *CYP2C9* genotypes.

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Abstract

Background: The multicenter, single-blind, randomized EU-PACT trial compared safety and efficacy of genotype-guided and non-genetic dosing algorithms for acenocoumarol and phenprocoumon in patients with atrial fibrillation or deep venous thrombosis. The trial showed no differences in the primary outcome between the two dosing strategies.

Objectives: To explore possible reasons for the lack of differences between trial arms this secondary analysis of EU-PACT data evaluated the performance of both dosing algorithms across *VKORC1-CYP2C9* genetic sub-groups.

Patients/Methods: Anticoagulation control measured by international normalized ratio (INR) below (INR<2), within (INR 2-3) and above (INR>3) the therapeutic range was compared across *VKORC1-CYP2C9* sub-groups. Due to a low number of patients per sub-group, trials for acenocoumarol and phenprocoumon were combined for analysis.

Results: Four weeks after therapy initiation genotype-guided dosing increased the mean percentage of time in therapeutic INR range (PTIR) in the *VKORC1* GG-*CYP2C9* *1*1 sub-group as compared to the non-genetic dosing (%-point difference 14.68, 95% CI [5.38; 23.98], p=0.002). For the *VKORC1* AA-*CYP2C9* *1*1 sub-group, there was a higher risk of under-anticoagulation with the genotype-guided algorithm (19.9 %-points; 95% CI, 11.6 to 28.2, p<0.001). Twelve weeks after therapy initiation no statistically significant differences in anticoagulation control between trial arms were noted across the *VKORC1-CYP2C9* genetic sub-groups.

Conclusions: EU-PACT genetic-guided dose initiation algorithms for acenocoumarol and phenprocoumon could have predicted the dose extra cautiously in the *VKORC1* AA-*CYP2C9* *1*1 sub-group. Adjustment of the genotype-guided algorithm could lead to a higher benefit of genotyping.

Introduction

Coumarin anticoagulants, acenocoumarol and phenprocoumon, are commonly used in many countries for the prevention of thromboembolic complications of atrial fibrillation (AF) and for the treatment of deep venous thrombosis (DVT). Owing to a narrow therapeutic window and a large inter- and intrapersonal variability in coumarin dose requirements, the dose-finding process during therapy initiation remains a challenge, leading to an increased number of bleeding episodes and hospitalizations.[1] Among many factors that influence coumarin dose variability, including patients' anthropomorphic characteristics, (non) compliance, diet, co-morbidities and co-medications, genetic variants in the vitamin K epoxide reductase (*VKORC1*) and hepatic drug-metabolizing enzyme cytochrome P450 2C9 (*CYP2C9)* genes are responsible for a large proportion of variation in dose required.[1] Taken together, *VKORC1* -1639 G>A (rs9923231), *CYP2C9* *2 (rs1799853) and *3 (rs1057910) polymorphisms explain up to 30%-40% of dose variability and have been associated with anticoagulation effects of coumarins in populations of European descent.[1-3] The utility of pharmacogenetic-guided (PG) coumarin prescribing during therapy initiation has recently been investigated in prospective randomized trials.[4-7] Two warfarin trials (the European Pharmacogenetics of Anticoagulant Therapy, EU-PACT warfarin arm, and the Clarification of Optimal Anticoagulation through Genetics, COAG) produced divergent results.[5,6] The EU-PACT warfarin trial showed a 7% improvement in the mean percentage of time in therapeutic INR range (PTIR; INR 2.0-3.0) with PG dosing as compared to the UK standard clinical practice.[5] Conducted in the US COAG trial, in contrast, demonstrated no difference in PTIR between PG dosing algorithms, including genetic and clinical information, and non-PG dosing algorithms, including only clinical information.[6] Similarly, the combined EU-PACT acenocoumarol and phenprocoumon arm showed no statistically significant difference in PTIR over 12 weeks between the PG and the non-PG control arm.[4] To explore the potential reasons for these findings, we performed sub-analyses of EU-PACT acenocoumarol and phenprocoumon data stratified by the *VKORC1* and *CYP2C9* genotypes. We aimed to investigate whether the effect of PG and non-PG dosing on the anticoagulation control in certain genetic sub-groups differed from the overall effect in the whole trial population and whether any differences across sub-groups were present 4 and 12 weeks after the start of treatment.

Materials and Methods

Trial design and participants

We used the combined data of two multicenter, single-blind, randomized controlled trials, comparing a PG with a non-PG dosing algorithm for the initiation of acenocoumarol or phenprocoumon treatment in patients with AF and in patients with VTE (www.clinicaltrials.gov NCT01119274 and NCT01119261).[4] A detailed description of EU-PACT trial design, procedures and results can be found elsewhere.[4,8] In brief, patients ≥ 18 years old diagnosed with AF or VTE, initiating acenocoumarol or phenprocoumon therapy for at least 12 weeks, having a target INR in the low intensity range and being able to attend scheduled visits were recruited and randomized to either of the dosing groups.[4] During the first 5-7 days of the trial doses of acenocoumarol and phenprocoumon were determined by a PG algorithm in the intervention group and by a non-PG algorithm in the control group. The EU-PACT loading and maintenance dosing algorithms were developed and validated by van Schie *et al*. and are described in detail elsewhere.[9,10] Non-PG algorithms predicted dose based on age, height, weight, gender and amiodarone use, while PG algorithms also used *VKORC1* -1639 G>A, *CYP2C9* *2 and *3 genotypes. After initial 5-7 days, dose adjustments were performed using INR values in accordance with the local clinical practice of participating trial centers. Coumarin doses, INR and the occurrence of possible adverse events were being monitored during the 12-week follow-up. Patients taking acenocoumarol were recruited at the department of Cardiology and the department of Internal medicine of the Democritus University of Thrace in Alexandroupolis, Greece and at the Cardiology department of the Onassis Cardiac Surgery Center in Athens, Greece. Phenprocoumon and acenocoumarol patients were recruited at four anticoagulant clinics in The Netherlands from November 2010 to March 2013. The trial protocol was approved by the Leiden Medical Ethics Committee in the Netherlands and by the Scientific Council and Ethics Committee of the Academic General Hospital of Alexandroupolis and the institutional review board of the Onassis Cardiac Surgery Center in Athens, Greece. All patients provided written informed consent upon inclusion into the trial.

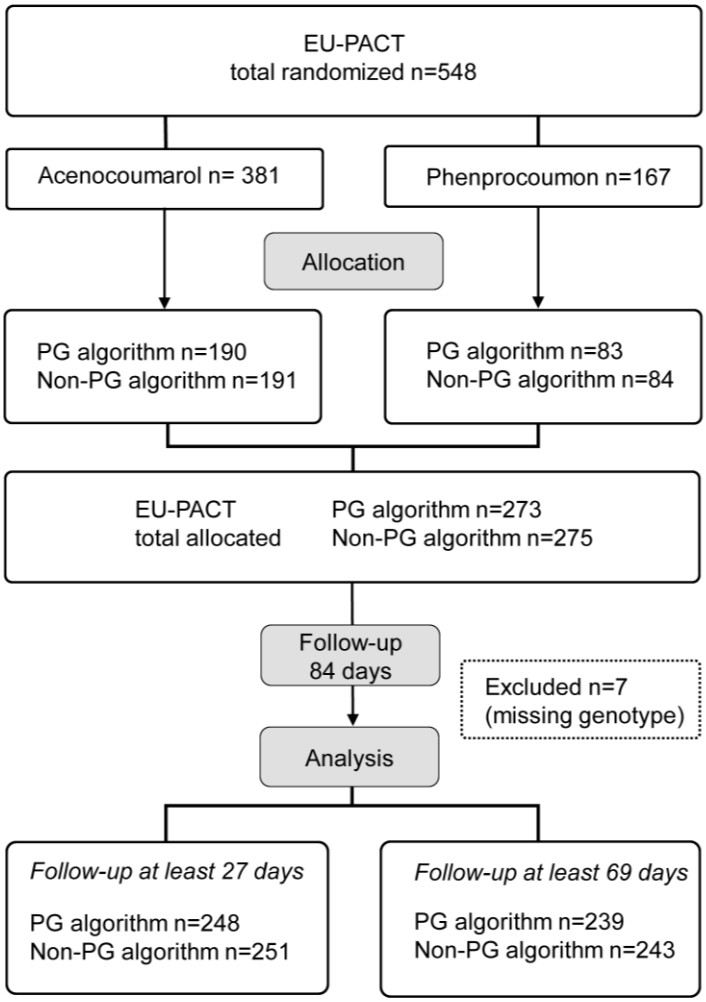

FIGURE 1. Flowchart of patients included into the analyses.
PG, pharmacogenetic-guided. Non-PG, non-pharmacogenetic-guided (using clinical information only).

Outcome measures

The primary outcome of the EU-PACT trial was PTIR during 12 weeks following the start of therapy with acenocoumarol or phenprocoumon, calculated by the linear interpolation method by Rosendaal *et al*.[11] The secondary outcomes included, among others, the percentage of time with an INR > 4 and INR < 2, the time it took to reach a therapeutic INR, the time it took to achieve a stable dose, and the percentage of patients with a stable dose within 12 weeks[4]. In the present analysis, trial participants were stratified by *VKORC1* and *CYP2C9* genotypes and differences in the INR response 4 and 12 weeks after therapy initiation were assessed across the sub-groups. The time intervals were chosen based on the follow-up duration and considering earlier reports indicating importance of the PG dosing during first few

weeks of therapy. Due to a low rate of thromboembolic events, minor and major bleedings in the trial, these outcomes were not evaluated.

Statistical analysis

In EU-PACT, a sample size of 200 patients per group was required to detect a 7% improvement in PTIR over 12 weeks. The sample size calculation was based on a standard deviation of 23%, a two-sided significance level of 5% and an 80% power. As a consequence of low enrollment, both acenocoumarol and phenprocoumon trials were concluded before reaching the enrollment goal and were combined for analysis. Patients with a follow-up of at least 27-days were included into the analyses 4 weeks after therapy initiation. For analyses 12 weeks after therapy initiation data of patients with a minimum of 69 days of follow-up were used.

The joined effect of *VKORC1* and *CYP2C9* variants on anticoagulation control was investigated by creating six sub-groups, in which each of the three *VKORC1* genotypes was combined with either the wild type, or the variant *CYP2C9* alleles, as previously described.[2,9,12-14] Due to a low number of patients with *CYP2C9* variant alleles, homozygous and heterozygous *CYP2C9* *2 and *3 carriers were placed into the same sub-group, as follows:

VKORC1 GG – *CYP2C9* *1/*1 (wt - wt);
VKORC1 GG – *CYP2C9* *1/*2, *2/*2, *1/*3, *2/*3, and *3/*3 (wt - any variant);
VKORC1 GA – *CYP2C9* *1/*1 (GA - wt);
VKORC1 GA – *CYP2C9* *1/*2, *2/*2, *1/*3, *2/*3 and *3/*3 (GA - any variant);
VKORC1 AA – *CYP2C9* *1/*1 (AA - wt);
VKORC1 AA – *CYP2C9* *1/*2, *2/*2, *1/*3, *2/*3 and *3/*3 (AA - any variant).

Between group differences in baseline characteristics were assessed using a two-sample t-test and a Chi-squared test where appropriate. Ninety-five percent confidence intervals (CI) were constructed for the differences in mean PTIR, mean time above and below the therapeutic INR range. Means and 95% CI's were also calculated for the INR measurements in week 1 of the trial. One-way analysis of variance (ANOVA) was performed to assess differences in the INR response between trial arms across genetic sub-groups. A two-sided p-value of less than 0.05 was considered nominally statistically significant. After the Bonferroni correction for multiple testing, a p-value threshold of less than 0.001 was considered statistically significant. All analyses were

carried out using SPSS Statistics for Windows, version 23.0 (Armonk, NY: IBM Corp., USA).

Results

Data from 548 trial participants were available, of whom 7 patients of the non-PG arm were excluded due to missing genotypes, which left 273 patients in the PG group and 268 patients in the control group (**Figure 1**). Baseline clinical characteristics of the trial population are shown in **Table 1.**

TABLE 1. Baseline clinical characteristics of the trial population.

	Acenocoumarol		**Phenprocoumon**		**Combined**	
	PG n=190	**Non-PG** n=191	**PG** n=83	**Non-PG** n=84	**PG** n=273	**Non-PG** n=275
Age, years	68 ± 14	68 ±13	67 ±11	67 ±11	68 ±13	68 ±13
Male sex, n (%)	121 (64)	107 (56)	51 (61)	47 (56)	172 (63)	154 (56)
Caucasian, n (%)*	184 (97)	189 (99)	79 (95)	81 (96)	263 (96)	270 (98)
Atrial fibrillation, n (%)	158 (83)	158 (83)	68 (82)	70 (83)	226 (83)	228 (84)
Height, cm	172 ± 11	171 ± 11	174 ± 9	173 ± 10	172 ± 10	171 ± 11
Weight, kg	84 ±15	82 ±18	87 ± 17	83 ±16	85 ±16	82 ± 17
CYP2C9, n (%)						
*1*1	111 (58)	107 (57)	55 (66)	57 (70)	166 (61)	164 (60)
*1*2	39 (21)	33 (18)	14 (17)	14 (17)	53 (19)	47 (17)
*1*3	29 (15)	32 (17)	11 (13)	7 (9)	40 (15)	39 (14)
*2*2	4 (2)	11 (6)	2 (2)	2 (3)	6 (2)	13 (5)
*2*3	5 (3)	4 (2)	1 (1)	1 (1)	6 (2)	5 (2)
*3*3	2 (1)	0 (0)	0 (0)	0 (0)	2 (1)	0 (0)
Missing, n (%)	0 (0)	4 (2)	0 (0)	3 (4)	0 (0)	7 (3)
P-value of HWE for *CYP2C9*	0.37	0.002	0.66	0.77	0.89	0.002
VKORC1, n (%)						
GG	70 (37)	55 (29)	24 (29)	33 (41)	94 (34)	88 (32)
GA	84 (44)	93 (50)	40 (48)	33 (41)	124 (45)	126 (46)
AA	36 (19)	39 (20)	19 (23)	15 (19)	55 (20)	54 (20)
Missing, n (%)	0 (0)	4 (2)	0 (0)	3 (4)	0 (0)	7 (3)
P-value of HWE for *VKORC1*	0.23	0.97	0.77	0.20	0.23	0.47
Amiodarone use, n (%)	22 (12)	23 (12)	0 (0)	0 (0)	22 (8)	23 (8)

The ± values are means with standard deviations. HWE – Hardy-Weinberg equilibrium. PG – pharmacogenetic-guided. Non-PG – non-pharmacogenetic-guided (using clinical information only). * - Ethnicity was self-reported.

Demographic characteristics were comparable between the intervention and the control groups. The most frequent indication for coumarin therapy was AF. Results of analyses across *VKORC1-CYP2C9* sub-groups over the first 4 and 12 weeks of treatment are presented in **Table 2** and **Table 3**. Four weeks after therapy initiation in the PG dosed *VKORC1* GG-*CYP2C9* *1*1 sub-group a nominally statistically significant 14.7%-point increase in PTIR was observed (54.9 ± 23.9 % versus 40.2 ± 27.0 %; p = 0.002, **Table 2**). In the *VKORC1* AA-*CYP2C9* *1*1 sub-group there was a higher risk of under-anticoagulation when dosed with the PG strategy than with the non-PG strategy (29.1 ± 23.3 % versus 9.3 ± 6.1 %; p < 0.001, **Table 2**). Twelve weeks after therapy initiation the difference in PTIR between trial arms was no longer statistically significant in the *VKORC1* GG-*CYP2C9* *1*1 sub-group (62.9 ± 21.4% versus 55.7 ± 23.5 %; p=0.087; **Table 3**). For the PG-dosed *VKORC1* AA-*CYP2C9* *1*1 sub-group percentage of time below therapeutic range remained increased, but it was not statistically significant after the correction for multiple testing (21.9 ± 22.3 % versus 8.9 ± 13.0 %; p=0.006; **Table 3**). No statistically significant differences were observed for the INR above therapeutic range. Sensitivity analyses were also performed per coumarin separately and in the per-protocol dataset and the results for sub-groups were similar (data not shown).

Discussion

This study addressed the issue of robustness of the EU-PACT dose prediction algorithms for acenocoumarol and phenprocoumon and explored possible explanations for the lack of benefit of the PG-guided over non-PG dosing on the anticoagulation control in this trial.The analysis of the EU-PACT data across the, while the *VKORC1* AA-*CYP2C9* *1*1 sub-group was dosed extra cautiously and had an increased mean time below the therapeutic INR range. Using a PG strategy could allow to start with higher doses in *VKORC1* GG-*CYP2C9* *1*1 carriers and thereby reduce under-anticoagulation and the risk of thrombosis.

More time below the therapeutic INR range in the *VKORC1* GG-*CYP2C9* *1*1 sub-group dosed with the EU-PACT clinical algorithm is in accordance with previous reports of an increased risk of subtherapeutic INRs in these patients when an insufficiently high dose was prescribed using a standardized dosing algorithm.[12,13]

TABLE 2. Anticoagulation control 4 weeks after therapy initiation across *VKORC1-CYP2C9* sub-groups.

	INR 2-3, %				INR < 2, %				INR > 3, %			
***VKORC1-CYP2C9* genotype**	**PG algorithm**	**Non-PG algorithm**	**Difference in %-points (95% CI)**	**P**	**PG algorithm**	**Non-PG algorithm**	**Difference in %-points (95% CI)**	**P**	**PG algorithm**	**Non-PG algorithm**	**Difference in %-points (95% CI)**	**P**
GG - *1*1	**n=60**	**n=58**			**n=60**	**n=58**			**n=60**	**n=58**		
	54.9±23.9	40.2±27.0	14.7 (5.4; 23.9)	**0.002**†	30.6±22.8	50.9±30.9	-20.3 (-30.2; -0.4)	**<0.001**‡	14.5±22.9	8.9±17.1	5.6 (-1.8; 13.0)	0.136
GG - var[a]	**n=28**	**n=28**			**n=28**	**n=28**			**n=28**	**n=28**		
	49.7±30.6	40.9±26.9	8.8 (-6.7; 24.2)	0.259	27.6±29.7	38.4±31.6	-10.9 (-27.3; 5.6)	0.191	22.7±25.6	20.6±24.6	2.08 (-11.4; 15.6)	0.758
GA -*1*1	**n=62**	**n=60**			**n=62**	**n=60**			**n=62**	**n=60**		
	56.2±25.3	53.2±27.8	3.0 (-6.5; 12.5)	0.532	28.1±26.4	25.0±22.1	3.1 (-5.7; 11.8)	0.485	15.7±21.5	21.8±27.6	-6.10 (-14.9; 2.7)	0.174
GA - var[a]	**n=50**	**n=56**			**n=50**	**n=56**			**n=50**	**n=56**		
	53.2±22.4	50.8±25.7	2.37 (-6.9; 11.7)	0.617	26.9±23.3	21.5±20.6	5.5 (-2.9; 13.9)	0.198	19.9±21.8	27.8±26.4	-7.90 (-12.3; 1.5)	0.098
AA - *1*1	**n=29**	**n=34**			**n=29**	**n=34**			**n=29**	**n=34**		
	41.9±24.6	49.9±27.5	-8.07 (-21.3;5.2)	0.229	29.1±23.3	9.3±6.1	19.9 (11.6; 28.2)	**<0.001**§	28.9±26.2	40.8±27.4	-11.82 (25.4; 1.8)	0.087
AA - var[a]	**n=19**	**n=15**			**n=19**	**n=15**			**n=19**	**n=15**		
	54.9±24.4	45.5±27.8	9.44 (-8.8;27.7)	0.300	26.2±19.3	15.2±14.6	11.1 (-1.1; 23.3)	0.075	18.9±23.2	39.4±31.9	-20.50 (-39.7; -1.3)	0.037

Frequencies of *VKORC1* and *CYP2C9* genotypes per sub-group in the PG arm: **GG -*1*1**: *VKORC1* GG n=60, *CYP2C9**1*1 n=60; **GG-variant**: *VKORC1* GG n=28, *CYP2C9* *1*2 n=15, *1*3 n= 10, *2*2 n=1, *2*3 n=2, *3*3 n=0; **GA-*1*1:** *VKORC1* GA n=62, *CYP2C9**1*1 n=62; **GA-var:** *VKORC1* GA n=50, *CYP2C9* *1*2 n=26, *1*3 n=19, *2*2 n=4, *2*3 n=1, *3*3 n=0; **AA-*1*1:** *VKORC1* AA n=29, *CYP2C9* *1*1 n=29; **AA-var:** *VKORC1* AA n=19, *CYP2C9* *1*2 n=8, *1*3 n=8, *2*2 n=0, *2*3 n=3, *3*3 n=0.Frequencies of *VKORC1* and *CYP2C9* genotypes per sub-group in the non-PG arm: **GG -*1*1**: *VKORC1* GG n=58, *CYP2C9**1*1 n=58; **GG-variant**: *VKORC1* GG n=28, *CYP2C9* *1*2 n= 16, *1*3 n=10, *2*2 n=1, *2*3 n=1, *3*3 n=0; GA-*1*1: VKORC1 GA n=60, CYP2C9*1*1 n=60; GA-var: VKORC1 GA n=56, CYP2C9 *1*2 n=22, *1*3 n=21, *2*2 n=9, *2*3 n=4, *3*3 n=0; AA-*1*1: VKORC1 AA n=34, CYP2C9 *1*1 n=34; AA-var: VKORC1 AA n=15, CYP2C9 *1*2 n=8, *1*3 n=5, *2*2 n=2, *2*3 n=0, *3*3 n=0.The ± values are means with standard deviations. PG, pharmacogenetic-guided. Non-PG, non-pharmacogenetic-guided (using clinical information only). The carriers with the following *CYP2C9* genotypes were combined into *CYP2C9* "variant" category: *1*2, *1*3, *2*2, *2*3, *3*3. After the Bonferroni correction for multiple testing the threshold for statistical significance is $P < 0.001$. †$P=2.2 \times 10^{-3}$. ‡$P=8.4 \times 10^{-5}$. §$P=1.1 \times 10^{-5}$.

TABLE 3. Anticoagulation control 12 weeks after therapy initiation across combined *VKORC1-CYP2C9* sub-groups.

VKORC1-CYP2C9 genotype	INR 2-3, % PG algorithm	Non-PG algorithm	Difference in %-points (95% CI)	P	INR < 2, % PG algorithm	Non-PG algorithm	Difference in %-points (95% CI)	P	INR > 3, % PG algorithm	Non-PG algorithm	Difference in %-points (95% CI)	P
GG -*1*1	**n=58**	**n=57**			**n=58**	**n=57**			**n=58**	**n=57**		
	62.9±21.4	55.7±23.5	7.2 (-1.1;15.5)	0.087	24.9±23.5	32.5±23.1	-7.6 (-16.2; 1.0)	0.083	12.2±16.2	11.8±17.3	0.4 (-5.8; 6.6)	0.902
GG - var	**n=28**	**n=28**			**n=28**	**n=28**			**n=28**	**n=28**		
	57.1±25.9	54.1±22.5	3.0 (-9.9;16.0)	0.644	22.9±23.4	28.3±24.0	-5.4 (-18.1; 7.3)	0.395	19.9±21.8	17.6±21.5	2.4 (-9.2; 14.0)	0.678
GA -*1*1	**n=60**	**n=57**			**n=60**	**n=57**			**n=60**	**n=57**		
	62.5±22.7	67.7±19.4	-5.5 (-12.9;2.5)	0.186	22.7±21.9	16.6±14.8	6.1 (-0.8; 12.9)	0.081	14.8±22.2	15.7±17.6	-0.9 (-8.3; 6.5)	0.811
GA - var	**n=46**	**n=53**			**n=46**	**n=53**			**n=46**	**n=53**		
	62.5±25.4	59.4±24.9	3.2 (-6.9;13.2)	0.532	19.4±20.5	15.7±16.5	3.6 (-3.7; 11.0)	0.330	18.1±23.8	24.9±24.9	-6.8 (-16.6; 2.9)	0.169
AA -*1*1	**n=28**	**n=34**			**n=28**	**n=34**			**n=28**	**n=34**		
	59.7±21.9	62.1±24.8	-2.4 (-14.4;9.7)	0.694	21.9±22.3	8.9±13.0	12.9 (3.9; 22.1)	***0.006*†**	18.4±17.3	29.0±24.4	-10.6 (-21.6; 0.4)	0.057
AA - var	**n=19**	**n=14**			**n=19**	**n=14**			**n=19**	**n=14**		
	61.3±25.9	54.8±25.5	6.5 (-11.9;24.9)	0.478	18.6±19.1	15.2±15.4	3.4 (-9.3; 16.1)	0.585	20.1±22.9	29.9±26.8	-9.9 (-27.7; 7.8)	0.261

Frequencies of *VKORC1* and *CYP2C9* genotypes per sub-group in the PG arm: **GG -*1*1**: *VKORC1* GG n=58, *CYP2C9**1*1 n=58; **GG-variant**: *VKORC1* GG n=28, *CYP2C9* *1*2 n=15, *1*3 n=10, *2*2 n=1, *2*3 n=2, *3*3 n=0; **GA-*1*1:** *VKORC1* GA n=60, *CYP2C9**1*1 n=60; **GA-var:** *VKORC1* GA n=46, *CYP2C9* *1*2 n=24, *1*3 n=18, *2*2 n=4, *2*3 n=0, *3*3 n=0; **AA-*1*1:** *VKORC1* AA n=28, *CYP2C9* *1*1 n=28; **AA-var:** *VKORC1* AA n=19, *CYP2C9* *1*2 n=8, *1*3 n=8, *2*2 n=3, *2*3 n=0, *3*3 n=0. Frequencies of *VKORC1* and *CYP2C9* genotypes per sub-group in the non-PG arm: **GG -*1*1**: *VKORC1* GG n=57, *CYP2C9**1*1 n=57; **GG-variant**: *VKORC1* GG n=28, *CYP2C9* *1*2 n= 16, *1*3 n=10, *2*2 n=1, *2*3 n= 1, *3*3 n=0; **GA-*1*1:** *VKORC1* GA n=57, *CYP2C9**1*1 n=57; **GA-var:** *VKORC1* GA n=53, *CYP2C9* *1*2 n=20, *1*3 n=20, *2*2 n=9, *2*3 n=4, *3*3 n=0; **AA-*1*1:** *VKORC1* AA n=34, *CYP2C9* *1*1 n=34; **AA-var:** *VKORC1* AA n=14, *CYP2C9* *1*2 n=8 ,*1*3 n=5, *2*2 n=1, *2*3 n=0, *3*3 n=0.

The ± values are means with standard deviations. PG, pharmacogenetic-guided. Non-PG, non-pharmacogenetic-guided (using clinical information only). The carriers with the following *CYP2C9* genotypes were combined into *CYP2C9* "variant" category: *1*2, *1*3, *2*2, *2*3, *3*3. After the Bonferroni correction for multiple testing the threshold for statistical significance is $P < 0.001$. †$P = 5.8 \times 10^{-3}$.

We suggest that one of the many reasons for the controversy between existing trial results could be the possible limitations of a particular dosing algorithm used. The prediction of coumarin dose variability is not entirely similar between existing algorithms. It depends on the characteristics of the derivation cohort and on the variables included into the algorithm and could perform differently in another population.[3,15] The EU-PACT algorithms were developed in large populations of acenocoumarol and phenprocoumon users, but the derivation cohort did not contain a sufficient number of rare *CYP2C9* variant allele carriers to account for dose variability in these patients.[9,10] A recent randomized controlled trial of genotype-guided dosing of acenocoumarol used a PG dosing algorithm developed in a Spanish population which included the *VKORC1*, *CYP2C9* and *CYP4F2* genotype.[15] In this trial the PG approach was superior to the standard care in terms of the number of patients with stable dose and the mean percentage of therapeutic INRs after 90 days of follow-up.[7] While there are certainly differences in study design between this trial and EU-PACT, it is possible that including extra genetic variants might affect the precision of dose prediction and impact the outcome.

One of the study limitations is a small sample size and combining the acenocoumarol and phenprocoumon data, not accounting for pharmacological differences between the two drugs and between their dosing algorithms. However, the results of our sensitivity analyses by drug did not show substantial differences. The *CYP2C9* *2 and *3 genotypes were combined in *VKORC1-CYP2C9* sub-groups, but the low frequencies of *CYP2C9* *2/*2, *3/*3 and *2/*3 genotypes in our data probably had minor effects on the results.

In conclusion, the use of PG algorithms for therapy initiation with acenocoumarol and phenprocoumon could be advantageous in certain patient sub-groups, particularly during the first month of coumarin therapy. Adjustment and refinement of the EU-PACT PG algorithms could increase the benefit of genotyping for *VKORC1* and *CYP2C9* variant allele carriers. This study also highlights the need to consider potential limitations of dose prediction algorithms when interpreting the results of clinical trials of coumarin PG dosing.

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CHAPTER 2.3

Comparative evaluation of dose prediction algorithms for acenocoumarol and phenprocoumon.

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Abstract

Background: The European pharmacogenetics of anticoagulant therapy (EU-PACT) randomized controlled trial prospectively compared the effect of pharmacogenetic (PG) versus clinical dose prediction algorithms for acenocoumarol and phenprocoumon on the percentage of time within therapeutic International Normalized Ratio (INR) range.

Objectives: To assess the performance of previously published dose prediction algorithms for acenocoumarol and phenprocoumon compared to algorithms used in EU-PACT trial.

Methods: Five (two clinical and three PG) algorithms for acenocoumarol and two PG algorithms for phenprocoumon were selected through a literature search. The predictive ability and accuracy of the algorithms was evaluated in 157 acenocoumarol and 97 phenprocoumon EU-PACT trial participants who reached stable dose.

Results: The percentage of stable dose variability explained by the PG algorithms (adjusted R^2) varied from 53.9 to 61.1% and from 53.7 - 56.9% for acenocoumarol and phenprocoumon, respectively. The R^2 of acenocoumarol clinical algorithms was 18.5 - 28.9%. The (percentage) mean absolute error (MAE) ranged between 0.47 - 0.49 mg/day (19.4 - 21.0%) for acenocoumarol PG algorithms; for acenocoumarol clinical algorithms it was 0.57 and 0.64 mg/day (27.9%). The lowest MAE was observed with the EU-PACT PG (0.39 mg/day; 16.2%) and clinical (0.49 mg/day; 20.5%) algorithms. For phenprocoumon PG algorithms MAE ranged between 0.35-0.39 mg/day (15.4-17.4%).

Conclusions: The algorithms had comparable predictive abilities. EU-PACT acenocoumarol algorithms had a highest accuracy, and the accuracy of phenprocoumon PG algorithms was nearly similar.

Introduction

Vitamin K antagonists (VKAs), warfarin, acenocoumarol and phenprocoumon, are commonly used oral anticoagulants in patients with atrial fibrillation (AF) and venous thromboembolism (VTE). Optimal VKA dosing is often hindered by a narrow therapeutic index of these drugs, potential interactions with other medications and a considerable interpatient variability in dose required for therapeutic anticoagulation. The maintenance dose of VKAs is influenced by multiple factors, including age, sex, weight, dietary vitamin K intake, comorbidities, comedications and genetic polymorphisms.[1,2] In Caucasians single nucleotide polymorphisms (SNPs) in the vitamin K epoxide reductase complex 1 gene (*VKORC1*) and the hepatic metabolizing enzyme cytochrome P450 gene (*CYP2C9*) explain some of the variation in dose requirements for acenocoumarol, phenprocoumon and warfarin.[3-7] Other SNPs in genes of the VKA pharmacokinetic pathways, such as calumenin (*CALU*), cytochrome P450 family 4 subfamily F member 2 (*CYP4F2*), *γ*-glutamyl carboxylase (*GGCX*) and apolipoprotein E (*APOE*), have been associated with warfarin and acenocoumarol dose variability in African American and Asians.[8-12]

For over a decade dose prediction algorithms for VKAs based on genetic and clinical information have been developed using multiple linear regression analysis and mainly in individuals of European ancestry.[13] Development of additional ethnicity-specific dosing algorithms has been suggested to account for the varying SNP frequencies across ethnicities and to include more SNPs explaining dose variability in non-Caucasians.[14,15] Indeed, recently two new genotype-guided algorithms for acenocoumarol have been developed in Indian patients, a warfarin algorithm was published for Puerto Rican patients and a large number of studies also evaluated VKA dosing algorithms in Asians.[16-22] Some of the published algorithms were not evaluated and validated in external data, therefore their application outside of the derivation cohorts could give a better idea of their generalizability. In order to assess the performance of dosing algorithms for acenocoumarol and phenprocoumon from published studies in patients of European ancestry we evaluated their predictive ability and accuracy in the population of the European pharmacogenetics of anticoagulant therapy (EU-PACT) trial.

Methods

Study population

Design and results of the EU-PACT trial were described elsewhere.[23] In brief, EU-PACT was a multicenter, single-blind, randomized controlled trial comparing genotype-guided dosing algorithms (including *VKORC1* and *CYP2C9* polymorphisms) for the initiation of treatment with acenocoumarol and phenprocoumon with dosing algorithms based only on non-genetic factors (control group) (www.clinicaltrials.gov identifiers: NCT01119274, NCT01119261).[23] Patients ≥ 18 years old with AF or VTE and a target International Normalized Ratio (INR) in the low intensity range (between 2 and 3) were randomly assigned to either of the dosing strategies and followed for twelve weeks.[23] Genotyping for *CYP2C9* *1, *3 (rs1057910) and *2 (rs1799853) and *VKORC1* -1639 G>A (rs9923231) was performed using a point-of-care assay with HyBeacon® probes (LGC Ltd, Middlesex, UK).[24] The trial protocol was approved by the Leiden Medical Ethics Committee in the Netherlands and by the Scientific Council and Ethics Committee of the Academic General Hospital of Alexandroupolis and the institutional review board of the Onassis Cardiac Surgery Center in Athens, Greece. All patients provided written informed consent upon inclusion into the trial.

One-hundred-sixty-seven patients taking phenprocoumon were recruited in the Netherlands and 381 patients taking acenocoumarol were enrolled in Greece and the Netherlands (**Figure 1**). One-hundred-fifty-nine phenprocoumon patients and 325 acenocoumarol patients had a follow-up of at least 10 weeks; of these 151 acenocoumarol patients and 97 phenprocoumon patients reached a stable dose within 12 weeks in both randomization groups and were included in the present analysis.

Selection of dosing algorithms

We searched studies describing genotype-guided and clinical dosing algorithms for acenocoumarol and phenprocoumon in individuals of European ancestry published between January 01, 2007 and February 01, 2017 on PubMed and Embase. **Supplementary Table 1** provides an overview of publications considered for inclusion in the present study. Only algorithms based on linear regression analysis and containing a complete dosing equation were eligible for the analysis.

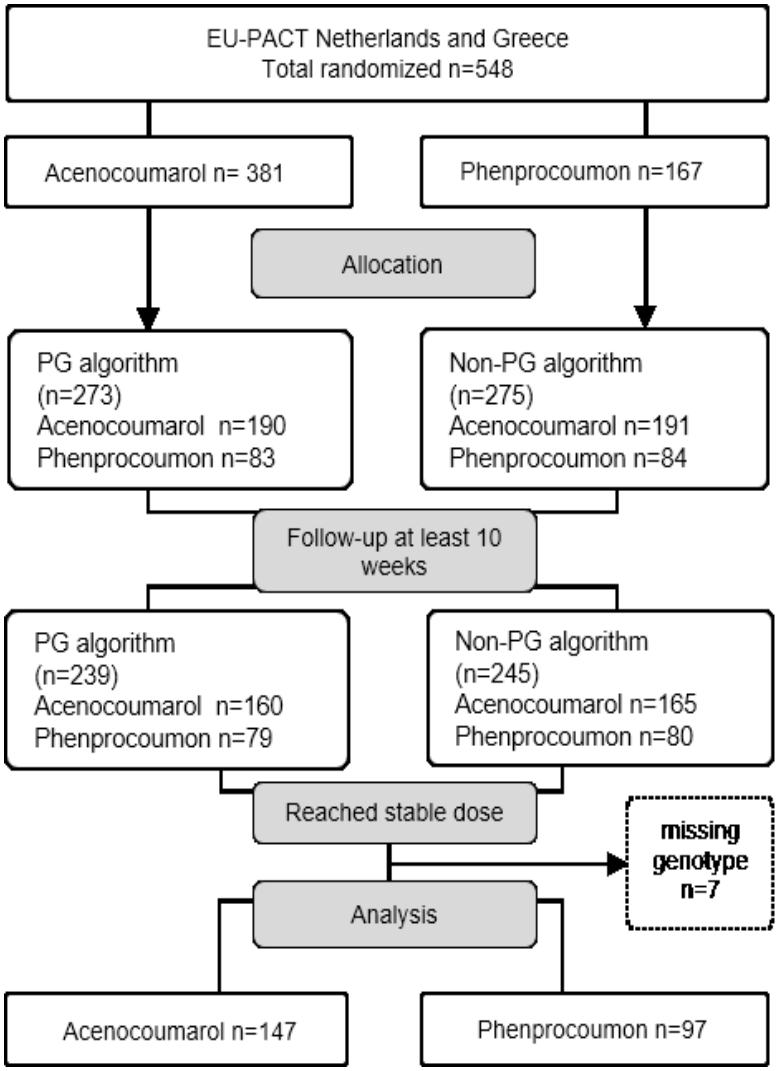

FIGURE 1. Flowchart of EU-PACT participants included in the analysis.
PG, pharmacogenetic-guided. Non-PG, non-pharmacogenetic-guided (using clinical information only).

The algorithms consisted of different combinations of demographic, clinical, pharmacogenetic, dose response and comedication variables, and the inclusion of algorithms was determined by the availability of these variables in the EU-PACT dataset. Genotype-guided algorithms including SNPs other than *CYP2C9* (rs1057910, rs1799853) and *VKORC1* (rs9923231) were not included, because genotyping for these variants was not performed in EU-PACT.[25-28] In total, three genotype-guided and two clinical algorithms for acenocoumarol, and two genotype-guided algorithms for phenprocoumon were used in the analysis. The dosing equation by Borobia *et al*. for acenocoumarol was excluded, because the intercept for the model was not provided in the article.[26] The clinical algorithm for phenprocoumon by Caduff-Good *et al*. was excluded, because of the absence of

information about serum albumin and operations conducted within a week of the start of VKA therapy in the EU-PACT dataset.[29] The acenocoumarol algorithm by Ragia *et al*. was excluded, because it was developed in same population (Greek patients of EU-PACT).[30]

Genotype-guided EU-PACT dosing algorithms and algorithms selected from the literature were applied in all acenocoumarol and phenprocoumon patients who reached a stable dose (also patients initially randomized to the control group). The clinical EU-PACT algorithm for acenocoumarol was applied and compared with the published clinical algorithms in a subset of acenocoumarol patients who reached a stable dose. A stable dose of acenocoumarol and phenprocoumon in EU-PACT was defined as the dose a patient used, when the INR was within the target range for a period of at least 3 weeks, with at least three consecutive INR measurements within the target range and a less than 10% change in dose.[23]

Statistical analysis

Continuous data are presented as means with standard deviations, and non-normally distributed data are presented as medians (25^{th}, 75^{th} percentiles). Mean daily doses of acenocoumarol and phenprocoumon predicted by the published algorithms were compared with doses predicted by EU-PACT algorithms using the paired-samples Student's t-test. Scatter plots of observed stable daily doses against predicted daily doses were constructed, and a linear regression line fitted. The accuracy of dosing algorithms was assessed by the mean absolute error (MAE, defined as the mean of the absolute difference between predicted and observed stable VKA doses) and the mean percentage absolute error (MAE, %). Wilcoxon signed-ranks test was used to compare MAE between the algorithms. Multiple linear regression with stable acenocoumarol or phenprocoumon doses as the outcomes was used to estimate the intercept and the R^2 statistic of the algorithms to estimate their ability to explain the variability of stable dose in our dataset. Each model was fitted using variables included in the algorithms, coded as described in the original studies. The actual stable dose achieved in EU-PACT was transformed according to the scale of each algorithm (square root, logarithm to the base 10 or natural logarithm). A two-sided p-value of 0.05 was considered statistically significant. Statistical analyses were carried out using SPSS Statistics for Windows, version 24.0 (IBM Corp., NY, USA).

Results

The demographics and clinical information of the EU-PACT patients who reached a stable dose are presented in **Table 1**. Maintenance doses of acenocoumarol and phenprocoumon predicted by the algorithms were calculated for these patients using coefficients in **Tables 2** and **3**. All genotype-guided acenocoumarol algorithms included age, and the EU-PACT genotype-guided algorithm was the only one to include sex, height and amiodarone use (**Table 2**).[31-34]

TABLE 1. Baseline characteristics of EU-PACT participants who reached a stable dose.

Characteristic	Acenocoumarol N=151	Phenprocoumon N=97
Age, years*	67.4 ± 11.4	66.9 ± 10.2
Male sex, n (%)	93 (61.6)	52 (53.6)
Caucasian, n (%)†	149 (98.7)	93 (95.9)
Atrial fibrillation, n (%)	128 (84.8)	80 (82.5)
Venous thromboembolism, n (%)	23 (15.2)	17 (17.5)
Height, cm	173.1 ± 10.4	173.6 ± 9.3
Weight, kg	84.8 ± 16.5	83.3 ± 15.2
CYP2C9, n (%)		
*1*1	93 (61.1)	71 (73.2)
*1*2	27 (17.9)	14 (14.4)
*1*3	20 (13.2)	8 (8.2)
*2*2	7 (4.6)	3 (3.1)
*2*3	4 (2.6)	1 (1.0)
*3*3	0 (0)	0 (0)
VKORC1 rs9923231, n (%)		
GG	53 (35.1)	32 (33.0)
GA	73 (48.3)	47 (48.5)
AA	25 (16.6)	18 (18.6)
Amiodarone, if yes, n (%)	10 (6.6)	0 (0)
Treatment arm, PG/non-PG, n (%)	70 (46.4) / 81 (53.6)	49 (50.5) / 48 (49.5)

*Plus–minus values are means ±SD. † Ethnicity was self-reported. *CYP2C9*, cytochrome P450 2C9; *VKORC1*, vitamin K epoxide reductase complex subunit 1.
Additional variables used in the models: for acenocoumarol, simvastatin use n=39 (26.5%), creatinine clearance < 40 ml/min n=7 (4.8%), body mass index (BMI), kg/m^2 (mean ± SD) 28.1 ± 4.6. There was no rifampicin, carbamazepine, phenytoin in the acenocoumarol dataset. For phenprocoumon, β-blockers (any of the following: atenolol, carvedilol, metoprolol, propranolol) n=68 (41.0%).

The algorithms by Markatos *et al*., Wolkanin-Bartnik *et al*. and Pop *et al*. explained respectively 55%, 45.5 - 49.0% (if vitamin K intake was included into the model) and 41% of the stable acenocoumarol dose in the derivation cohorts.[32-34]

TABLE 2. Acenocoumarol dose prediction algorithms.

	Genotype-guided				Clinical		
First author, year	**van Schie, 2011 EU-PACT**	**Markatos, 2008**	**Pop, 2013**	**Wolkanin-Bartnik, 2013**	**van Schie, 2011 EU-PACT**	**Jiménez-Varo, 2014**	**Tong, 2016**
Outcome	Sqrt weekly dose (mg/week)	Log_{10} daily dose (mg/day)	Log_{10} weekly dose (mg/week)	Ln daily dose (mg/day)	Sqrt weekly dose (mg/week)	Weekly dose (mg/week)	Ln weekly dose (mg/week)
Intercept	4.117	1.083	1.402	1.831	2.635	15.470	2.951
VKORC1 rs9923231							
GG	0[a]	-0.188	0[a]	0[a]	-	-	-
GA	-0.572	-0.376	-0.135	-0.339	-	-	-
AA	-1.267	-0.564	-0.285	-0.651	-	-	-
CYP2C9							
*1/*1	0[a]	-0.073	0[a]	0[a]	-	-	-
*1/*2	-0.093	-0.146	-	-	-	-	-
*1/*3	-0.519	-0.219	-	-	-	-	-
*2/*2	-0.435	-0.292	-	-	-	-	-
*2/*3	-0.466	-0.365	-	-	-	-	-
*3/*3	-1.375	-	-	-	-	-	-
CYP2C9 *2[b]	-	-	-0.094	-	-	-	-
CYP2C9 *3[b]	-	-	-0.099	-	-	-	-
CYP2C9 non-*1*1[c]	-	-	-	-0.145	-	-	-
Age, years	-0.027	-0.004	-0.005	-0.013	-0.027	-0.145	-0.011

Sex, if female	0.271		-		-		-	0.386		-		-
Height, cm	0.009		-		-		-	0.013		-		-
Weight, kg	0.010		-		-	0.004		0.013		-		0.004
BMI, kg/m2		-	-	0.009		-		-	0.276			-
Amiodarone, if yes	-0.377		-		-		-	-0.167		-		-0.290
Simvastatin, if yes		-	-		-		-		-	2.506		-
CYP2C9 inducers, if yes[d]		-	-		-		-		-		-	0.045
Creatinine clearance < 40 ml/min		-	-		-	-0.197			-		-	-
INR target range		-	-		-		-		-		-	0.086[e]

Numbers are standardized regression coefficients. [a]The value of this parameter is zero because it is the reference group. [b]In the model of Radu Pop *et al.*, CYP2C9 was divided in two groups: CYP2C9*2 and CYP2C9*3; CYP2C9 *2*3 was included in both groups. [c] In the model of Wolkanin-Bartnik *et al.*, CYP2C9 was divided into *1*1 and non-*1*1. [d] CYP2C9 inducers: phenytoin, carbamazepine and rifampin (absent in the dataset). [e] This coefficient should only be added to the equation, if the INR target range is 2.5-3.5. Sqrt, square root; Log_{10}, logarithm to the base 10; Ln, natural logarithm; BMI, body mass index.

Clinical algorithms by Jiménez-Varo *et al*. and Tong *et al.* explained 14% and 21.1% of the stable dose variability in the original studies.[27] The EU-PACT genotype-guided algorithm for phenprocoumon included the same variables as the acenocoumarol algorithm (**Table 2**).[31] The phenprocoumon dosing algorithm by Geisen *et al*. explained 48.6% of the interindividual phenprocoumon dose variability in the original cohort.[35] Botton *et al*. developed a dosing algorithm including age, sex, use of *β*-blockers and genotype, that explained 46.1% of phenprocoumon dose variability in their study population (**Table 3**).[36]

TABLE 3. Phenprocoumon dose prediction algorithms.

First author, year	**van Schie, 2011 EU-PACT**	**Geisen, 2011**	**Botton, 2014**
Outcome	Sqrt weekly dose (mg/week)	Sqrt daily dose (mg/day)	Log_{10} weekly dose (mg/week)
Intercept	2.874	0.460	1.779
VKORC1 rs9923231			
GG	0[a]	0.238	0[a]
GA	-0.601	0[a]	-0.103
AA	-1.394	-0.271	-0.265
CYP2C9			
*1/*1	0[a]	-	-
*1/*2	-0.259	-	-
*1/*3	-0.342	-	-
*2/*2	-0.447	-	-
*2/*3	-0.684	-	-0.062
*3/*3	-0.681	-	-
Age, years	-0.015	-0.004	-0.008
Sex, if female	0.026	-	-0.056
Height, cm	0.011	0.007	-
Weight, kg	0.008	-	-
Amiodarone, if yes	-0.345	-	-
β-blockers, if yes[b]	-	-	-0.050

Numbers are standardized regression coefficients.
[a] The value of this parameter is zero because it is the reference group.
[b] β-blockers: atenolol, carvedilol, metoprolol, propranolol.
Sqrt, square root; Log_{10}, logarithm to the base 10; BMI, body mass index.

TABLE 4. Performance of acenocoumarol and phenprocoumon dose prediction algorithms.

First author	Predicted dose, mg/day	P	MAE, mg/day	P	MAE, %	P	Intercept	R^2, %	R^2 adj, %
ACENOCOUMAROL									
Genotype-guided									
van Schie (EU-PACT)	2.39 ± 0.76	-	0.39 (0.16; 0.66)	-	16.12 (6.90; 27.95)	-	1.298	64.9	62.1
Markatos	2.39 ± 0.89	0.918	0.47 (0.20; 0.71)	<0.001	19.37 (9.92; 32.60)	<0.001	0.936	57.1	55.0
Pop	2.22 ± 0.75	<0.001	0.48 (0.21; 0.76)	<0.001	19.66 (9.94; 32.80)	0.002	1.706	58.5	53.9
Wolkanin-Bartnik	2.75 ± 0.94	<0.001	0.49 (0.20; 0.83)	<0.001	21.04 (8.80; 45.25)	<0.001	1.533	57.8	56.0
Clinical									
van Schie (EUPACT)	2.50 ± 0.56	-	0.49 (0.17; 0.99)	-	20.48 (6.85; 44.45)	-	-2.433	31.1	28.8
Jiménez-Varo	2.02 ± 0.33	<0.001	0.57 (0.31; 1.08)	0.002	27.92 (16.23; 42.64)	<0.001	-29.734	31.3	28.9
Tong	1.82 ± 0.32	<0.001	0.64 (0.32; 1.06)	0.004	27.89 (15.61; 40.88)	<0.001	3.074	20.1	18.5
PHENPROCOUMON									
Genotype-guided									
van Schie (EU-PACT)	2.08 ± 0.59	-	0.35 (0.12; 0.62)	-	15.35 (5.91; 24.37)	-	5.128	61.4	56.9
Geisen	2.10 ± 0.57	0.408	0.35 (0.11; 0.64)	0.419	12.66 (6.65; 23.86)	0.466	3.678	56.7	54.8
Botton	2.08 ± 0.58	0.854	0.39 (0.13; 73.50)	0.035	17.64 (7.03; 29.63)	0.054	2.134	56.6	53.7

*Plus–minus values are means ±SD. Numbers in brackets are the 25th and the 75th percentils (P_{25}; P_{75}).P-values for predicted doses are from a paired Student's t-test. P-values for MAE are from Wilcoxon signed-ranked test.R^2, coefficient of determination. R^2 adj, adjusted coefficient of determination. MAE, mean absolute error (mean of the absolute difference between predicted and observed stable coumarin doses). MAE=ABS(predicted dose-observed stable dose). MAE(%)=(ABS(predicted dose-observed stable dose))/observed stable dose*100.

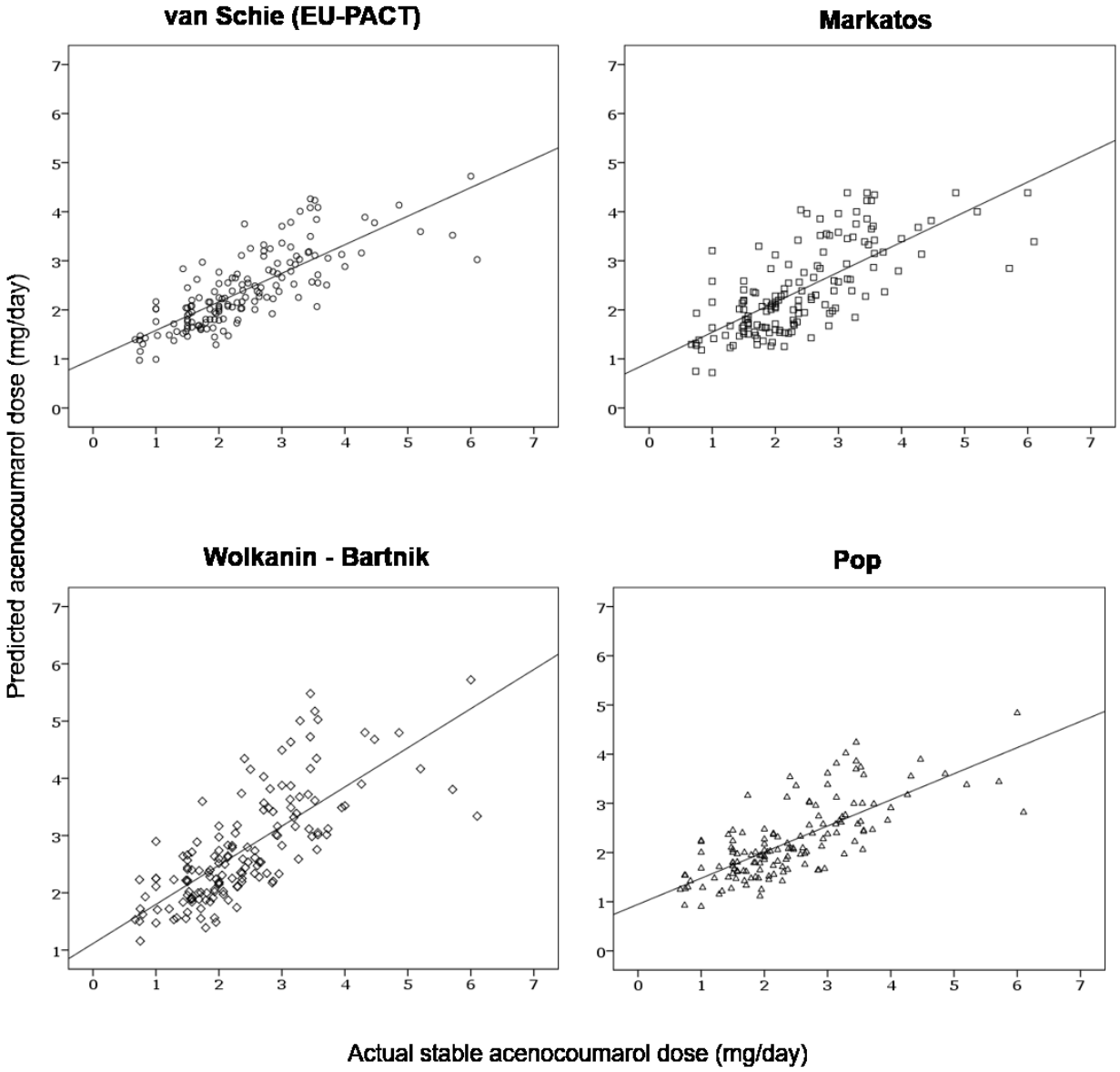

FIGURE 2. Scatter plots of actual stable acenocoumarol dose and dose predicted by genotype-guided models.

The mean dose predicted by each of the algorithms, mean absolute error, intercept and R^2 statistic obtained from multiple linear regression are shown in **Table 4**. For acenocoumarol, the mean predicted daily dose was not statistically significantly different between the EU-PACT (2.39 ± 0.76 mg/day) and Markatos *et al*. (2.39 ± 0.89 mg/day) genotype-guided algorithms (p=0.92). The algorithm by Pop *et al*. predicted a lower daily dose (2.22 ± 0.75 mg/day), while the Wolkanin-Bartnik model predicted a higher daily dose of acenocoumarol (2.75 ± 0.94 mg/day; p<0.001; **Table 4**; **Figure 2**). The mean acenocoumarol daily dose predicted by the clinical EU-PACT algorithm was the highest among the three studied clinical algorithms (2.50 ± 0.56 mg/day; **Table 4**;

Fig. 3). The highest MAE for acenocoumarol was observed for the algorithms by Wolkanin-Bartnik *et al*. and Tong *et al*. (0.49 mg/day and 0.64 mg/day, respectively). For phenprocoumon, there were no statistically significant differences in terms of predicted doses and MAE between the analyzed algorithms (**Table 4**; **Fig.4**).

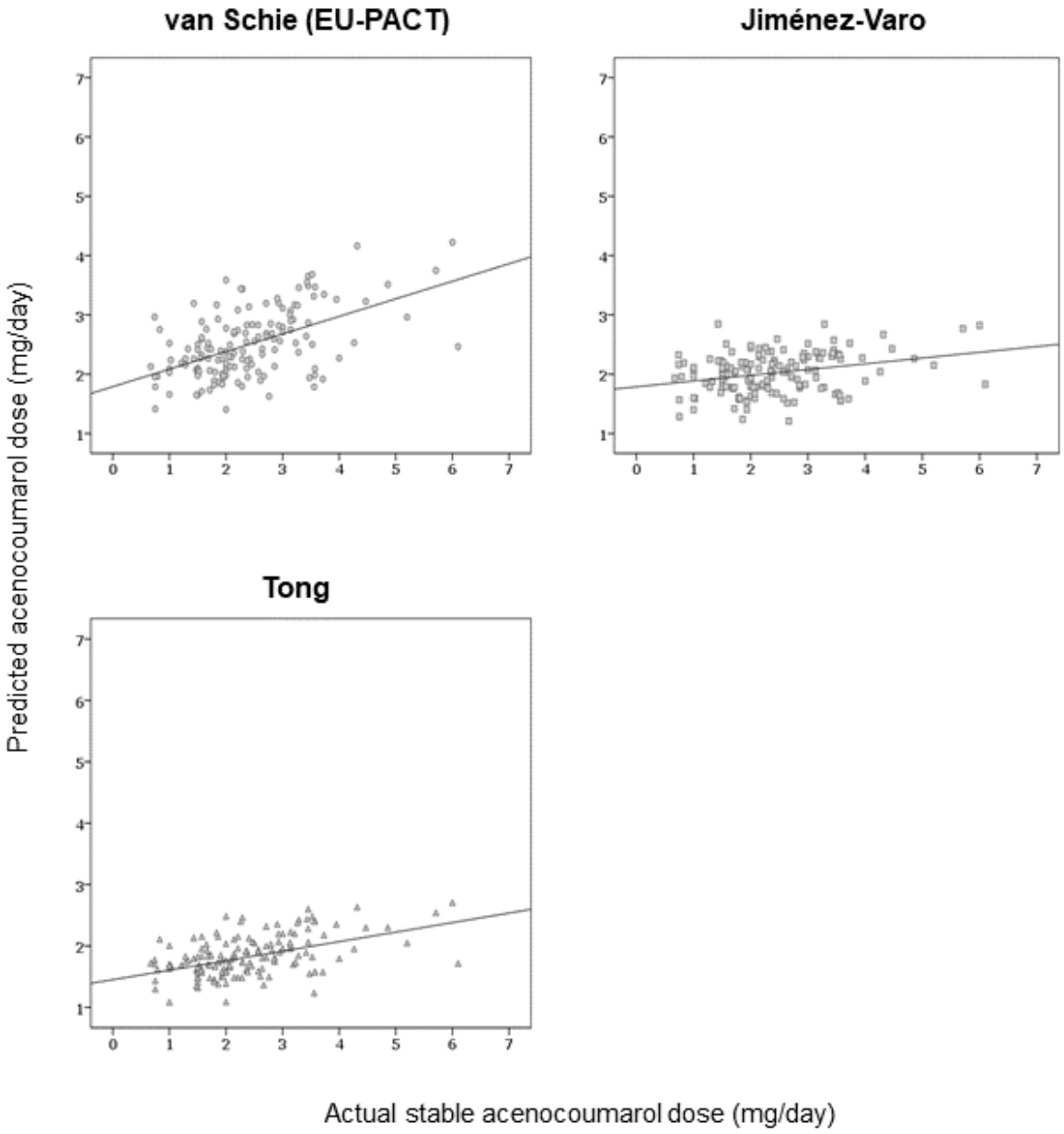

FIGURE 3. Scatter plots of actual stable acenocoumarol dose and dose predicted by clinical models.

The EU-PACT genotype-guided algorithm explained 62.1% of the acenocoumarol dose variability in our dataset, while the clinical algorithm explained 28.8%. The EU-PACT algorithm for phenprocoumon explained 56.9% of the interindividual dose

variability. All other genotype-guided models for acenocoumarol and phenprocoumon explained over 50% of the dose variability. Among them, the Wolkanin-Bartnik algorithm for acenocoumarol had the highest R^2 (56.0%), and the model by Geisen *et al*. explained 54.8% of the phenprocoumon dose variability.

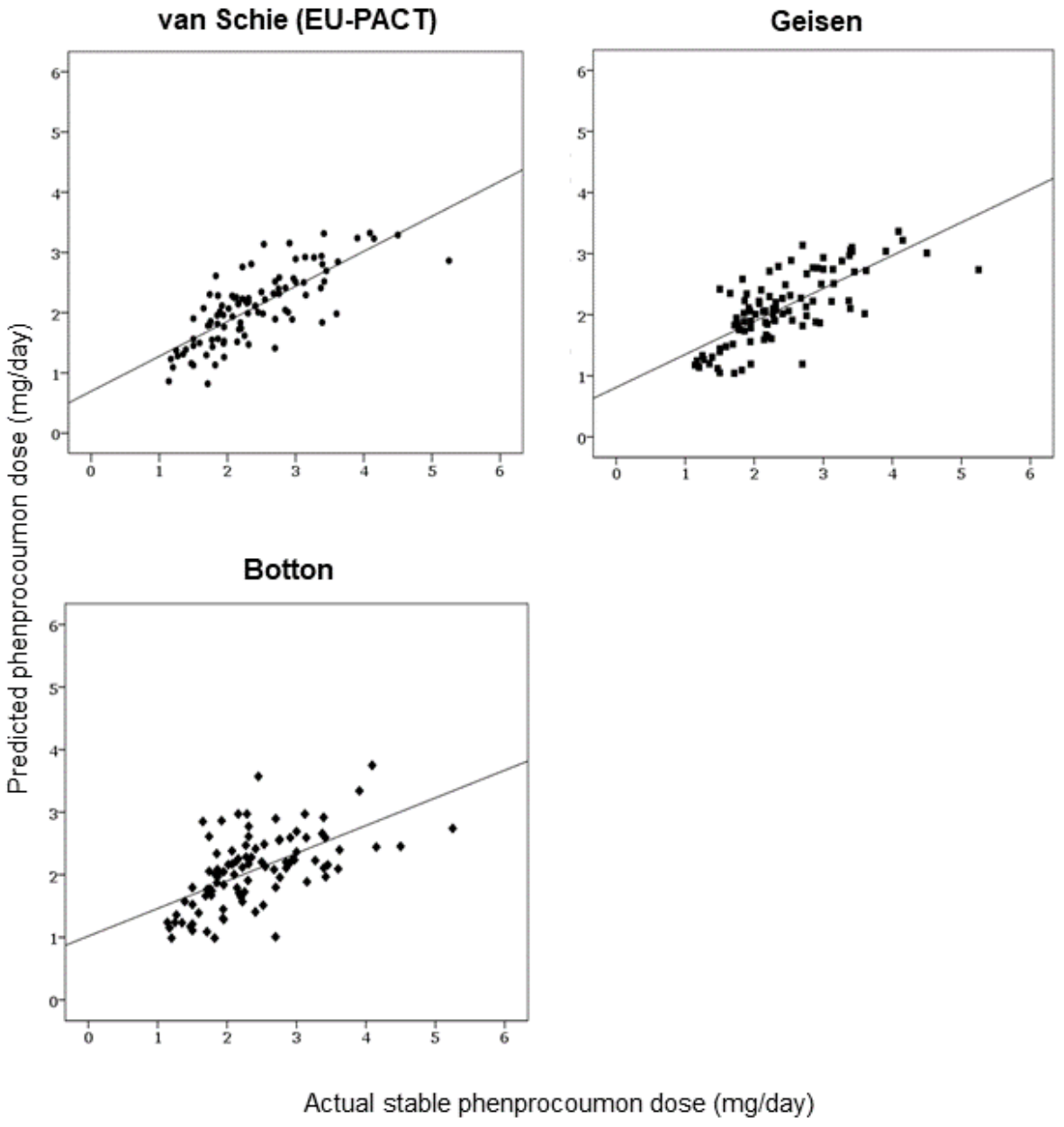

FIGURE 4. Scatter plots of actual stable phenprocoumon dose and dose predicted by genotype-guided algorithms.

The model by Pop *et al*. had the lowest R^2 (53.9%) of the genotype-guided models for acenocoumarol. The least amount of acenocoumarol dose variability in our dataset was explained by the clinical algorithm by Tong *et al*.

Discussion

In this study, we compared the predictive performance of seven acenocoumarol and three phenprocoumon dosing algorithms by re-fitting each linear regression model in the study population of the EU-PACT trial. Overall the percentage of dose variability explained by the investigated algorithms was not lower in comparison to their respective derivation cohorts, and for EU-PACT algorithms it was even slightly increased. Also, the genotype-guided EU-PACT algorithms for acenocoumarol and phenprocoumon had a markedly high adjusted R^2 coefficient in this dataset in comparison to the other studied models. This observation could be explained by the fact that the observed stable dose was achieved after the initiation of VKA therapy using EU-PACT algorithms during the trial. Other possible explanations for the differences between EU-PACT and the other algorithms include the diversity of demographic characteristics, different *VKORC1* and *CYP2C9* genotype frequencies across the derivation cohorts, and different covariates used in the models. Also, medical management of patients in a clinical trial setting could differ from observational data used to develop the algorithms.

In accordance with our findings, a study in 134 Spanish patients with AF and VTE showed that the EU-PACT genotype-guided algorithm for acenocoumarol had an R^2 of 53.1%, very close to the algorithm developed in this population (56.6%).[27] The percentage of dose variability explained by the EU-PACT algorithm in the study by Wolkanin-Bartnik *et al*.[34] was 61%, as compared to 64% explained by the algorithm derived from the population of this study.

We found that the mean predicted daily doses of phenprocoumon were not statistically significantly different between the algorithms, while more discrepancy was found in predicted acenocoumarol doses. EU-PACT acenocoumarol algorithms had the lowest MAE (0.39 mg/day for the genotype-guided and 0.49 mg/day for the clinical algorithm), and among all phenprocoumon models MAE was similar (0.35-0.39 mg/day). MAE shows how close the predicted doses are to the actual doses and is important for assessing the best predictive ability of an algorithm. However, the clinically relevant value of MAE for acenocoumarol and phenprocoumon is not strictly defined. For warfarin, for example, a change in 1 mg/day from a baseline of 5 mg is sufficient to change INR by 0.5.[37] Judging by MAE and mean daily dose, the investigated clinical

and genotype-guided models for acenocoumarol were less accurate than the EU-PACT algorithms.

In contrast to our findings, multiple studies found that dosing algorithms for VKAs often do not perform similarly to the derivation cohorts, if applied to external datasets or in ethnically mixed populations.[38-40] A recent meta-analysis found that 22 published warfarin algorithms under-predicted warfarin dose in patients requiring ≥7 mg/day.[39] The inability to replicate findings from the derivation cohorts led to an opinion that using linear regression models may not be the best approach to develop dosing algorithms for VKAs.[38,41] When stable doses are used as the outcome of the linear regression, the coefficient values and significance can be different in other data, because of the differences in the definition of stable dose.[38] Excluding patients with a non-stable dose could also lead to overlooking important sources of dose variability.[38]

A comparison of the EU-PACT genotype-guided algorithm for acenocoumarol with a new model generated using the data of Greek participants of the EU-PACT trial showed that the EU-PACT algorithm overestimated acenocoumarol dose.[30] The genotype-guided algorithm developed by Markatos *et al.*[32] in a Greek population overestimated the acenocoumarol dose only in normal responders to VKA (carriers of *VKORC1* GG-*CYP2C9*1*1*, or *VKORC1* GG-*CYP2C9 *1*2* or *VKORC1* GA-*CYP2C9 *1*1* genotypes) .[30] It has been therefore suggested that differences between the Dutch and the Greek populations, such as demographic characteristics and dietary habits could be the underlying factors determining the algorithm performance.[30]

One of limitations of this study is the inability to compare all existing pharmacogenetic algorithms for acenocoumarol (that include the *CYP4F2* and *APOE* genotypes) and clinical algorithms for phenprocoumon, because of the absence of some of the required variables. Secondly, the trial data might not be representative of standard clinical care; EU-PACT dosing algorithms for acenocoumarol and phenprocoumon were used for the initiation of VKA therapy in our patient population. However, the effect of this intervention can be expected to be diminished after four weeks of VKA treatment.[42,43] Moreover, after the first 5-7 days of the EU-PACT trial dosing was performed based on the INR values and according to standard clinical care.

In conclusion, genotype-guided dose prediction algorithms for acenocoumarol and phenprocoumon developed in Caucasian populations, and including *VKORC1* and/or *CYP2C9* genotypes, explained over 50% of dose variability in our dataset, similarly to

their derivation cohorts. Clinical dosing algorithms for acenocoumarol explained less than a third of acenocoumarol dose variability. EU-PACT acenocoumarol algorithms had a higher accuracy in comparison to other models, while the accuracy of phenprocoumon algorithms was nearly similar. Although dosing algorithms for VKAs can be optimized for any country or population, it would be preferable if a single algorithm for each VKA that includes the most universally important variables could be used across populations. Certain differences in the algorithm performance cannot be avoided, due to intrinsic characteristics of the derivation cohorts and the presence of difficult to measure variables, such as dietary, lifestyle factors and patient compliance. We suggest, that developing similar algorithms for VKAs in different populations is unnecessary, unless it concerns algorithms for specific ethnic groups in which predictors of VKA dose are known to be determined by ethnicity.

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Supplement

Supplementary Table 1. Genotype-guided and clinical dose prediction algorithms for acenocoumarol and phenprocoumon.

First author, year, [ref]	Country	N*	Genes**	Clinical variables	R^2, %	MAE (mg/day)
Acenocoumarol						
Markatos, 2008 [32]	Greece	98	*CYP2C9, VKORC1*	Age, gender	55	-
van Schie, 2011§ [31]	Netherlands	471	*CYP2C9, VKORC1*	Age, height, weight, gender, amiodarone	53 (PG) 24 (clin)	0.52
Borobia, 2012 [26]	Spain	117	*CYP2C9, VKORC1, CYP4F2, APOE*	Age, BMI	61	0.52
Cerezo-Manchado, 2013 [25]	Spain	973	*CYP2C9, VKORC1, CYP4F2*	Age, BSA, gender	50	-
Wolkanin-Bartnik, 2013 [34]	Poland	226	*CYP2C9, VKORC1*	Age, weight, creatinine clearance, vitamin K intake	49	0.63
Pop, 2013 [33]	Romania	200	*CYP2C9, VKORC1*	Age, BMI	41	0.82
Jiménez-Varo, 2014§ [27]	Spain	134	*CYP2C9, VKORC1, CYP4F2, APOE*	Age, BMI, simvastatin, amiodarone	56 (PG) 14 (clin)	0.35
Tong, 2016§ [28]	Spain	554	*CYP2C9, VKORC1, CYP4F2*	Age, weight, CYP2C9 inducers, target INR range	52 (PG) 15 (clin)	0.53
Ragia, 2017 [30]	Greece	140	*CYP2C9, VKORC1*			
Phenprocoumon						
Geisen, 2011 [44]	Germany	75	*VKORC1*	Age, weight	49	-
van Schie, 2011 [31]	Netherlands	624	*CYP2C9, VKORC1*	Age, height, weight, gender, amiodarone	56 17	0.45

Caduff Good, 2007† [29]	Switzerland	300	-	Weight, Albumin, Age, Age>60 years, Alcohol > 20 g/day, Operation within 1 week	-	-
Caduff Good, 2013§ [45]	Switzerland	301	*VKORC1*	Age, weight, INR before start, recent operation	57	-
					19	-
Botton, 2014‡ [36]	Brazil	198	*CYP2C9, VKORC1*	Age, sex, beta-blockers	48	-

BMI, body mass index. MAE, mean absolute error in the discovery cohort. R^2, R squared of the linear regression model (in the derivation cohort). PG, genotype-guided; clin, clinical algorithm. *Number of participants in the derivation cohort. **For genotype-guided algorithms.
§Studies describing both clinical and genotype-guided algorithms. †Studies describing clinical algorithms only. ‡Developed an algorithm for European and mixed Southern Brazilian populations.

CHAPTER 3

STUDIES OF ADVERSE REACTIONS TO ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

CHAPTER 3.1

Determinants of angiotensin-converting enzyme inhibitor intolerance and angioedema in the UK Clinical Practice Research Datalink.

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Abstract

Aim: The aim of the present study was to describe the occurrence and determinants of angiotensin-converting enzyme 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 = 4335) on the duration of ACEI treatment. Documented switches to angiotensin-II receptor blockers in the prescription records were used to identify ACEI-intolerance cases (n = 24,709), and these were matched with continuous ACEI users (n = 84,238) on the duration of ACEI therapy. Conditional logistic regression was used to assess the associations of demographic factors, comorbidities and comedication with AE and ACEI intolerance.

Results: AE during ACEI therapy was associated with age over 65 years [odds ratio (OR) 1.36, 95% confidence interval (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), use of antihistamines (OR 21.25, 95% CI 16.44, 27.46) and use of systemic corticosteroids (OR 4.52, 95% CI 3.26, 6.27). ACEI intolerance was significantly associated with more comorbidities and comedication compared with AE, including allergy (OR 2.02, 95% CI 1.96, 2.09), use of antiasthmatic drugs (OR 1.51, 95% CI 1.42, 1.61) and use of antihistamines (OR 1.53, 95% CI 1.43, 1.63).

Conclusions: Among ACEI users developing AE or ACEI intolerance, several comorbidities and comedication classes were significantly more prevalent compared with ACEI users not developing these adverse reactions.

What is already known about this subject

Angioedema (AE) and dry cough related to angiotensin-converting enzymes (ACEIs) often result in the discontinuation of ACEI treatment. Knowledge of potential risk factors could be helpful in identifying patients more likely to develop these adverse reactions and assist in prescribing decisions. Previous studies identified a number of risk factors, including, for instance, female gender, ethnicity and older age.

What this study adds

We investigated whether history of chronic disease and comedication use were associated with ACEI intolerance (defined by switching to angiotensin II receptor blockers (ARBs) in prescription records) and AE during ACEI therapy in two explorative case - control studies.

We found that age over 65 years, history of allergy and prescriptions for calcium channel blockers, antihistamines and systemic corticosteroids were more prevalent in ACEI users developing AE than in AE-free ACEI users.

The number of observed associations with comorbidities and comedications was higher in patients developing ACEI intolerance than in those developing AE. A history of allergy, and the use of antihistamines, antiasthmatic drugs and calcium channel blockers were more frequent in switchers to ARBs compared with continuous ACEI users. A history of diabetes or chronic obstructive pulmonary disease, and use of statins were less prevalent among switchers to ARBs.

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 ACEI prescriptions has substantially increased over the past few years, from 35–40 million worldwide in 2001 to approximately 160 million in 2011 in the USA alone.[1,2] In the UK, ramipril is the leading drug among medications for hypertension and heart failure, with almost 26 million prescriptions 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, and all-cause and CV mortality in patients with diabetes mellitus (DM).[4,5] A 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 owing to adverse effects.[7,8] One of the most common adverse effects of ACEIs is a persistent dry cough, described in 9.9–35% 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 the viscera. 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 mechanisms of ACEI-induced AE and cough are not known, they have been proposed to be similar, and to involve a reduction in the 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 threefold higher risk for ACEI-induced AE and East Asians being at a higher risk for ACEI-induced cough.[16,17] Some of the 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. One way of enabling this limitation to be bypassed is to use a large patient database and to ascertain a drug prescribing pattern

indicative of adverse drug reactions (ADRs). 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 decision to use 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-month interval from the end of an ACEI prescription in the latter 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 adverse effects was 68.3%, and combined for possible, probable and definitive adverse effects it was 90.5%. Given the increasing utilization of ACEIs, the purpose of the present study was, firstly, 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, comorbidities and comedications to gain more insight into patient groups more likely to experience ACEI-related adverse reactions.

Methods

Data source

Data for the present 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 subgroup 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 the present 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 of age or older, registered between 1 January 2007 and 1 January 2014 in the CPRD. The 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 use. To identify patients with AE and switching to ARBs during follow-up, subjects were followed until the end of the 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 the occurrence of AE during ACEI therapy and on 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 as 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 at any time during ACEI therapy or within a maximum of 3 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, either before the start or after discontinuation of ACEI treatment. An individual also became a case if multiple episodes of AE had occurred while on ACEI therapy, but only the first AE during ACEI therapy was considered as an event. For each case, up to 20 controls were selected from new ACEI users who did not 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 6-month 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 ACEIs 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 four controls were sampled. Controls were matched to cases on the duration of ACEI treatment at the index date. Information on

general practitioner-prescribed medications was extracted using appropriate British National Formulary medicine codes. The theoretical duration of an ACEI prescription was calculated as a ratio between the quantity of medication and the defined daily dose, estimated according to the World Health Organization. The duration of ACEI treatment was defined as the time between the start of the first ACEI prescription until the end of the last ACEI prescription, allowing a gap of less than 6 months between two consecutive ACEI prescriptions. Discontinuation of ACEI therapy was defined as the absence of a new ACEI prescription record for at least 6 months after the theoretical end date of the last ACEI prescription.

Determinants

As possible determinants of AE during ACEI therapy and a switch to ARBs, we considered gender, age over 65 years at the index date, the use of comedication and medical history of chronic comorbidities. The use of comedication, including antidiabetic drugs, antihistamines, antiasthmatic medications, nonsteroidal anti-inflammatory drugs (NSAIDs), systemic corticosteroids, calcium channel blockers and statins, was assessed by any prescription record within a 3-month time window before the index date, regardless of the duration of that prescription. Therefore, drugs for which the theoretical end date of the previous prescription would occur in this time window were not included in the analysis. We did not discriminate between various product names, but rather investigated the association between different classes of comedication and AE or ACEI intolerance. Exposure to each comedication class was included in the models as a dichotomous variable (use vs. no use). The history of comorbidities, including asthma, allergy, chronic obstructive pulmonary disease (COPD), 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 comorbidities. Additionally, the occurrence of any type of cough within 3 months before the AE date was included in the analyses. For descriptive purposes, the 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 on either side of the cohort entry date. When

assessing baseline characteristics of study populations, we selected records of alcohol consumption and smoking closest to the index date from those entered into the CPRD at any stage before the index date. In association analyses, we only considered the presence or the absence of information regarding smoking status in the CPRD at any stage before the index date. The indication for ACEI therapy was obtained from medical records any time before the cohort entry date or at any time within 1 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 using Student's t-test for continuous variables and using the chi-squared test for categorical variables. Kaplan–Meier curves were constructed to estimate the time to AE and switching to ARBs. Odds ratios and 95% confidence intervals for the association of AE and switching to ARBs with age, gender, smoking, comorbidities and comedication were estimated by univariate logistic regression. The analyses with comorbidities and comedications were further adjusted for age and gender. Subsequently, forward stepwise multivariable logistic regression was performed, including all determinants significantly ($P < 0.05$) associated with the outcomes in univariate analyses. Additionally, a stratified analysis was performed to compare the occurrence of AE and ACEI intolerance, depending on the type of ACEI. 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. IBM Corporation, Armonk, NY, USA).

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 4335 controls. We determined that 24,709 individuals switched to ARBs within 6 months of the end of the last ACEI prescription. Switchers to ARBs were matched with 84,238 continuous ACEI users, after excluding those who stopped ACEI therapy or switched to another antihypertensive drug.

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

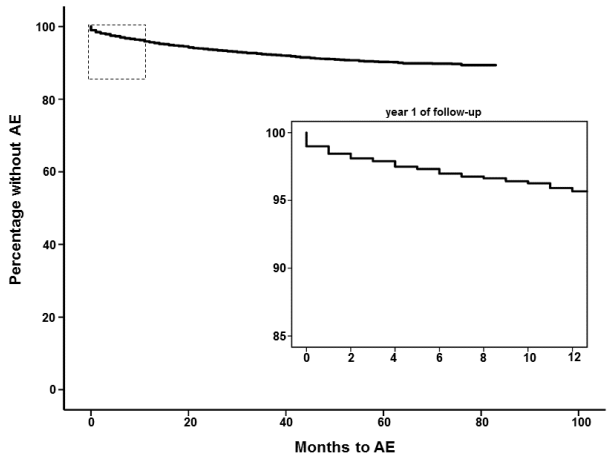
	AE cases n=416	Controls n=4335	P	Switchers to ARBs n=24709	Continuous users of ACEIs n=84238	P
Gender, n (%)			0.472			<0.005
Female	204 (49.0)	2045 (47.2)		14482 (58.6)	38297 (45.5)	
Male	212 (51.0)	2290 (52.8)		10227(41.4)	45941 (54.5)	
Age (years), mean ± SD	67.8 ± 11.6	65.6 ± 11.8	<0.005	65.2 ± 11.8	65.7 ± 11.1	<0.005
BMI (kg/m^2), 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)	
Type of ACEI used, n (%)			0.992			<0.001
Captopril, n (%)	0 (0)	1 (0)		5 (0)	26 (0)	
Cilazapril, n (%)	0 (0)	0 (0)		0 (0)	5 (0)	
Enalapril, n (%)	9 (2.2)	89 (2.2)		433 (1.8)	1794 (2.1)	
Fosinopril, n (%)	0 (0)	1 (0)		2 (0)	7 (0)	
Imidapril, n (%	0 (0)	1 (0)		0 (0)	0 (0)	
Lisinopril, n (%)	118 (28.0)	1213 (28.4)		6079 (24.6)	19110 (22.7)	
Perindopril, n (%)	56 (13.5)	526 (12.1)		1890 (7.6)	6807 (8.1)	
Quinapril, n (%)	0 (0)	1 (0)		4 (0)	26 (0)	
Ramipril, n (%)	233 (57.7)	2503 (56.0)		16280 (65.9)	56377 (66.9)	
Trandolapril, n (%)	0 (0)	0 (0)		16 (0.1)	86 (0.1)	
Fosinopril, n (%)	0 (0)	1 (0)		2 (0)	7 (0)	
Imidapril, n (%	0 (0)	1 (0)		0 (0)	0 (0)	

ACEI, angiotensin-converting enzyme inhibitor; AE, angioedema; ARBs, angiotensin II receptor blockers; BMI, body mass index; SD, standard deviation. Smoking status and alcohol consumption were determined at any stage 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 at any time prior to the start of ACEI. Age was assessed at the date of the first ACEI prescription.

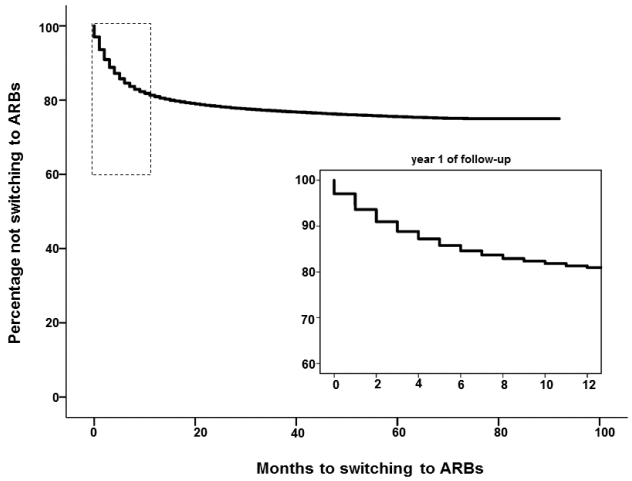
Clinical characteristics of the study populations are presented in **Table 1**. The proportion of women was statistically significantly higher among the switchers compared with the continuous ACEI users (58.6% vs. 45.5%). Hypertension as the only indication for ACEI therapy was used most frequently (approximately 60% in cases and controls of each study). About 20% of study participants in the AE study and 18% of participants in the ACEI intolerance study had more than one indication. Myocardial infarction, renal disease and heart failure as isolated indications were used much less frequently. The most frequent ACEI prescribed was ramipril, followed by lisinopril, perindopril and enalapril. There were no major differences in the frequency of use of these ACEI's between cases and controls (**Table 1**). **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.

There were no statistically significant differences in the occurrence of AE and switching to ARBs in the analyses stratified by type of ACEI (see **Tables S3** and **S4**). Crude and adjusted ORs for the association of comorbidities and comedications 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 in univariate models [odds ratio (OR) 1.51, 95% confidence interval (CI) 1.23, 1.86; OR 1.15, 95% CI 1.12, 1.18]. Female gender 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 3 months before the index date (OR 25.64, 95% CI 20.06, 32.77, and OR 7.15, 95% CI 5.49, 9.32, respectively).

A



B



B

FIGURE 1. Time to the development of ACEI intolerance and angioedema during ACEI therapy.

(A) Kaplan-Meier curves for time to the development of AE during ACEI therapy. Kaplan-Meier curves were constructed for cases only. The top left rectangle indicates the area of Kaplan-Meier curve depicted in the bottom right. The bottom right panel shows time to event during the first year of follow-up.
(B) Kaplan -Meier curves for time to switching to ARBs. Kaplan-Meier curves were constructed for cases only. The top left rectangle indicates the area of Kaplan-Meier curve depicted in the bottom right. The bottom right panel shows time to event within the first year of follow-up. AE, angioedema; ARB, angiotensin II receptor blocker.

These associations were also significant but less strong in ACEI intolerance (OR 1.85, 95% CI 1.73, 1.97, and OR 1.33, 95% CI 1.24, 1.43, respectively). Furthermore, when these determinants were examined at the cohort entry date instead of the index date, the association with AE remained statistically significant, with an OR of 4.48 (95% CI 3.41, 5.88) for antihistamines and an OR of 2.90 (95% CI 2.16, 3.91) for systemic corticosteroids (**Tables S1**, and **S2**). Antidiabetic drugs contributed to a lower risk of both AE and ACEI intolerance in univariate analyses (**Tables S2** and **S3**).

TABLE 2. Determinants of angioedema during ACEI therapy.

	No. (%)		OR crude (95% CI)	P	OR adj.^ (95% CI)	P
	Cases	Controls				
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**
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**

Cases n=416; controls n=4335. Smoking was assessed at any stage before the index date. The number of individuals with unknown smoking status 3 months before the index date was 17 (4.1%) out of AE cases and 145 (3.3%) out of AE controls. [a]History of comorbidities was assessed at any time before the AE date. [b]Comedication was assessed 3 months before the AE date. [c]Gender and age-adjusted ORs. CI, confidence interval; COPD, chronic obstructive pulmonary disease; NSAIDs, nonsteroidal anti-inflammatory drugs; OR, odds ratio.

Recent NSAID use was associated with a higher risk of ACEI intolerance (OR 1.12, 95% CI 1.06, 1.17). We observed a 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 specify adverse effects, 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).

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

	No. (%)		OR crude (95% CI)	P	OR adj.^ (95% CI)	P
	Cases	Controls				
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**
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**

Total cases n=24709; controls n=84238. Smoking was assessed at any stage before the index date. The number of individuals with unknown smoking status 3 months before the index date was 772 (3.1%) of the switchers to ARBs and 2990 (3.5%) of continuous ACEI users. [a] History of comorbidities was assessed at any time before the switch to ARBs. [b] Comedication was assessed 3 months before the switch to ARBs. [c] Gender and age-adjusted ORs. CI, confidence interval; COPD, chronic obstructive pulmonary disease; NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio.

Compared to AE, switching to ARBs was associated with more risk factors in the forward stepwise multivariable analysis. 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), and 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**).

TABLE 4. Determinants of angioedema during ACEI therapy in the multivariable model.			
Determinants	**OR**	**95%CI**	**P**
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

Chronic comorbidities were assessed at any stage within a period of time from the start of available CPRD records until the date of AE entered into the CPRD. Comedication was assessed within 3 months before the date of AE entered into the CPRD. Smoking was defined as presence or absence of information regarding smoking status in the CPRD at any stage before the index date (date of AE). CI, confidence interval; OR, odds ratio.

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), and 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 a 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 antiasthmatic 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 5. Determinants of ACEI intolerance (defined by switching to ARBs) in the multivariable model.			
Determinants	**OR**	**95%CI**	**P**
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

Chronic comorbidities were assessed at any stage within a period of time from the start of available CPRD records until the date of switching to ARBs. Comedication was assessed within 3 months before the date of switching to ARBs. Smoking was defined as presence or absence of information regarding smoking status in the CPRD at any stage before the index date (date of switching to ARBs). CI, confidence interval; COPD, chronic obstructive pulmonary disease; NSAID, Nonsteroidal anti-inflammatory drug; OR, odds ratio.

Discussion

We conducted two exploratory case-control studies in a cohort of patients in an extensive real-world primary care database to evaluate the association between a history of comorbidities and comedication use, and ACEI intolerance (defined by switching to ARBs) and AE during ACEI therapy. The main finding of both studies was that several comorbidities and prescriptions for different comedication classes within 3 months before the event were significantly more prevalent in ACEI starters developing AE and ACEI intolerance, compared with 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 ARBs than with AE. The knowledge gained through these studies might be helpful for further research by using the history of comorbidities and recent comedication as potential risk factors for ACEI-related adverse reactions. Several of the risk factors for AE which we report here have

been described earlier, including, among others, age, female gender, smoking, allergies and some 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 of a positive association of AE with allergies is also in accordance with previous observations, which showed that seasonal allergies, a history of drug rash, sensitization to certain food components and pollen season were all associated with a higher number of AE episodes.[25,26] It was evident from univariate analyses that a 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 comorbidities remained associated with AE during ACEI therapy. To the authors' knowledge, no reports on associations between asthma or COPD, and ACEI-related AE have been published. A study by Byrd *et al.* found no association between RA and ACEI-related AE.[26] An explanation for a possible higher number of episodes of ACEI-related AE in RA could be the observation by Habibagahi *et al.* 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] In this scenario, it is probable that starting an ACEI in RA could more likely trigger AE. It has been suggested that AE is less likely to develop in diabetic

patients owing to the higher dipeptidyl peptidase-4 (DPP-IV) activity that 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 ARBs, 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 ORs 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 had ACEI-related cough.[19] However, it is important to acknowledge the possibility that there were 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 to that found in the above-mentioned 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 antihistamines 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 gender and age. Similar results for corticosteroids and other immunosuppressants have been reported in previous studies and are thought to be due to reduced DPP-IV activity 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] Furthermore, there are data suggesting that ACEIs may affect local microvascular perfusion in the skin by a bradykinin-dependent mechanism.[37] In animal models and in humans, captopril was shown to increase skin microvascular blood flow owing to an increase in endogenous tissue bradykinin and the subsequent release of prostaglandins and nitric oxide.[37]

The strengths of the present study included using a real-world primary care patient population, a large number of events, complete data on the medication prescriptions and comorbidities, and the use of a validated marker for ACEI intolerance in prescription databases. We acknowledge that there were 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 ACEI treatment is likely to contribute to AE because we considered only AE occurring during ACEI therapy. However, in cases of AE occurring after several years of ACEI treatment, we cannot completely exclude another trigger for 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 have affected the associations described in the present study. Another reason for possible errors in the ascertainment of AE, possibly compromising the relationship with ACEI use, is the difference between the time of the actual AE episode and the time that 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 comorbidities and comedication on ACEI-induced cough as 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. In particular, 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 the ACEI-related adverse effect itself.

In conclusion, the present study showed that several comorbidities and recently prescribed comedication 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 comorbidities and comedication when ACEI treatment is required might assist in identifying patients potentially at a higher risk for ACEI-related adverse effects.

Competing interests

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: A.H.M. had support from grants from the European Union FP7 Collaborative grant EU-PACT, during the conduct of the study and an unrestricted research grant from GSK, outside the submitted work; P.S. had grants from Top-Institute Pharma, grants from EU Innovative Medicines Initiative (IMI) , grants from Respiratory Effectiveness Group, outside the submitted work; S.H.M. and E.V.B. had support from European Union FP7 Grant no. 602 108 during the conduct of the study. F.W.A. and A.D.B. had no support from any organization for the submitted work. This research was conducted as a part of the Personalisation of treatment In Cardiovascular disease through next generation sequencing in Adverse Drug Reactions (PREDICTION-ADR) consortium. The PREDICTION-ADR project is supported by the European Union FP7 Grant no. 602108. The authors would like to thank the members of PREDICTION-ADR consortium, particularly Colin NA Palmer (University of Dundee, Dundee, UK), Ana Alfirevic (University of Liverpool, Liverpool, UK) Mia Wadelius (Uppsala University, Uppsala, Sweden), Alun McCarthy (Pharmacogenomic Innovative Solutions Ltd, UK) and Anu Aaspollu (Asper Biotech Ltd, Tartu, Estonia) for their support and contribution to this work. F.W.A. is supported by the UCL Hospitals NIHR Biomedical Research Centre and by a Dekker scholarship (Junior Staff Member 2014 T001) from the Dutch Heart Foundation.

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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

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

SUPPLEMENTARY TABLE 3. Odds ratios for angioedema by type of ACEI.

Type of ACEI	OR	95% CI	P-value
Ramipril	Ref.	-	-
Captopril	0	-	1
Enalapril	1.09	(0.54; 2.18)	0.82
Fosinopril	0	-	1
Imidapril	0	-	1
Lisinopril	1.05	(0.83; 1.32)	0.71
Perindopril	1.14	(0.84; 1.55)	0.39
Quinapril	0.93	-	1

SUPPLEMENTARY TABLE 4. Odds ratios for ACEI intolerance by type of ACEI.

Type of ACEI	OR	95% CI	P-value
Trandolapril	Ref.	-	-
Captopril	1.03	(0.35; 3.09)	0.95
Cilazapril	0	-	0.99
Enalapril	1.30	(0.75; 2.24)	0.35
Fosinopril	1.54	(0.29; 8.07)	0.61
Lisinopril	1.71	(1.00; 2.92)	0.05
Perindopril	1.49	(0.87; 2.55)	0.14
Quinapril	0.83	(0.25; 2.69)	0.75
Ramipril	1.55	(0.91; 2.65)	0.11

CHAPTER 3.2

PREDICTION-ADR project: characterization of 392 patients with angioedema related to angiotensin-converting enzyme-inhibitors and angiotensin II receptor blockers.

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Manuscript in preparation.

Abstract

Background: The Personalization of treatment in cardiovascular disease through next generation sequencing in adverse drug reactions (PREDICTION-ADR) project was an international multicenter case-control study, set up to investigate the genetic etiology of angioedema related to agents acting on renin-angiotensin-aldosterone system (RAAS) and statin-induced myopathy.

Methods: Here we characterize 392 angioedema cases recruited in PREDICTION-ADR: 345 related to angiotensin-converting enzyme inhibitors (ACEIs) and 47 related to angiotensin II receptor 1 blockers (ARBs). Cases were recruited using standardized phenotypic criteria; 641 ACEI-treated controls were defined in existing databases. Association with co-morbidities was assessed using conditional logistic regression in a subset of patients with ACEI-induced angioedema, matched with controls in a 1:1 ratio for ethnicity, gender and age.

Results: Among all cases 54% were males, and the mean age at the time of angioedema was 64.1 and 65.5 years for the ARB and ACEI groups, respectively. Angioedema was most commonly localized in the tongue or lips. A history of non-drug-related allergies was found in 44.9% of the ACEI patients and 40.4% of the ARB patients. The median time to onset of angioedema was 912.5 days for ACEIs and 1095.0 days for ARBs. In logistic regression analysis of 286 matched pairs of cases and controls, the OR was 1.03 (95% CI: 0.58; 1.82) for asthma and 0.56 (95% CI: 0.39-0.81) for diabetes.

Conclusions: ACEIs and ARBs should be recognized as potential causes of angioedema, even after years of treatment. Diabetes was associated with a lower risk of ACEI-induced angioedema, while asthma was not statistically significantly associated.

Introduction

Angioedema due to agents acting on the renin-angiotensin-aldosterone system is an acute and uncommon adverse drug reaction (ADR). It typically presents as a well-demarcated, non-pitting, skin-colored swelling of the head and neck region or the mucous membranes of the upper aerodigestive tract or the airways, and is not associated with urticaria.[1] Angioedema may be life-threatening, when it involves the upper airways. Occasional cases of visceral angioedema have also been described.[2,3] Angiotensin-converting enzyme inhibitor (ACEI)-induced angioedema can occur unexpectedly days or years after the initiation of treatment, and can recur several months after cessation of treatment in up to 50% of the patients.[4-6] A retrospective study in 134,945 patients prescribed an ACEI showed that 0.7% of patients developed ACEI - induced angioedema within 5 years of an ACEI prescription.[7] In an analysis of 1,845,138 patients taking ACEI, the cumulative incidence of angioedema was 1.79 (95% confidence interval (CI): 1.73-1.85) per 1,000 persons during the first year of treatment. The cumulative incidence rate of angioedema due to angiotensin II receptor 1 blockers (ARBs) was 0.62 (95% CI: 0.55–0.69) per 1,000 person-years.[8]

The exact pathophysiological mechanism of angioedema induced by ACEI or ARB treatment is not fully elucidated. In addition to inhibiting ACE, ACEIs inhibit the degradation of bradykinin to inactive metabolites, which can lead to an accumulation of bradykinin. Higher levels of bradykinin act on the B2 bradykinin receptor inducing vasodilation and increasing vascular permeability.[9,10] Furthermore, other vasoactive molecules, such as substance P, have been implicated in the reaction.[11] The enzymes aminopeptidase P (APP) and dipeptidyl peptidase-IV (DPP-IV) take over the degradation of substance P and bradykinin, when ACE is inhibited. Impaired function of APP and DPP-IV could therefore contribute to angioedema.[9] ARB-induced angioedema is assumed to be caused by an increase in bradykinin levels through indirect inhibition of ACE and the metallo-endopeptidase enzyme (capable of inactivating bradykinin and substance P), since ARBs have no direct effect on ACE or bradykinin breakdown.[10,12]

It is believed that individual constitutional factors predispose to the development of ACEI-induced angioedema. Studies of clinical risk factors for ACEI-induced angioedema demonstrated a 4-fold increase in the incidence of angioedema among patients with a history of drug rash and an almost 2-fold higher incidence in patients

with seasonal allergies.[1] Lower rates of angioedema were found in patients with diabetes, while higher rates were reported in African-Americans.[13] Genetic variants in enzymes and receptors involved in the bradykinin pathway have been implicated in some studies,[14,15] but to-date, no single genetic variant with a large effect size has been associated with ACEI- or ARB-induced angioedema.

There is currently no diagnostic biomarker for ACEI- or ARB-induced angioedema, and the diagnosis is based on clinical features. The conventional treatment of angioedema consists of discontinuation of the culprit drug, airway management and administration of antihistamines, corticosteroids, and epinephrine. However, in ACEI-induced angioedema, traditional anti-allergic therapies are generally ineffective, since ACEI-induced angioedema is not a histamine-mediated reaction.[16] A selective bradykinin B2 receptor antagonist, icatibant, approved for the treatment of hereditary angioedema, was reported to significantly decrease the time to complete resolution of ACEI-induced angioedema in Caucasian patients, as compared to treatment with intravenous prednisolone plus clemastine.[17] However, other randomized placebo-controlled trials including African-American patients, did not support the efficacy of icatibant against placebo, when administered in addition to standard-of-care therapy.[18,19] In the study of Straka *et al.*, time to resolution of symptoms was similar between icatibant and placebo, and it did not differ between Caucasian and African-American patients.[18] A recent placebo-controlled trial by Sinert *et al.*, found no statistically significant improvement in time to meeting discharge criteria between icatibant and placebo.[19]

An international multicenter project, Personalisation of tREatment In Cardiovascular disease through next generation sequencing in Adverse Drug Reactions (PREDICTION-ADR), was set up to investigate the genetic etiology of rare ADRs to cardiovascular drugs, focusing on drugs acting on the renin-angiotensin-aldosterone system and on statins.[20] One of aims was to discover genetic variants, predisposing to ACEI-induced angioedema and statin-induced myopathy, using a next generation sequencing strategy. The objective of the present study was to describe the demographic characteristics of patients with ACEI- and ARB-induced angioedema and ACEI-treated controls included in PREDICTION-ADR; to evaluate the clinical presentation, types of causative drugs and the duration of treatment before onset of angioedema; to summarize the recruitment process in the participating centers (Sweden, England, Scotland, Denmark and the Netherlands). Additionally, we

assessed the association of co-morbidities available in our dataset (asthma and diabetes mellitus) with ACEI-induced angioedema.

Methods

Inclusion and exclusion criteria for angioedema cases

The consensus process of defining inclusion and exclusion criteria for angioedema cases by the collaborating centers of PREDICTION-ADR has been described previously.[9] Eligible patients were older than 18 years, able to give informed consent and fulfilling the phenotype standardization criteria for ACEI- or ARB-induced angioedema (**Table 1**).[9]

TABLE 1. Phenotype standardization criteria for angioedema cases.[9]

Inclusion criteria	Exclusion criteria
Symptoms in the head and neck region judged to be angioedema by a physician	Symptoms coinciding with urticaria
The initial event should occur during treatment with an ACEI or ARB	Another likely cause, such as severe facial trauma or infection
	Association with CI -INH or complement deficiency (if these data are available)
	Mutation in the CI inhibitor (*SERPINGI*) or factor XII (*F12*) gene (if these data are available)

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor type 1 blocker; C1-INH, complement C1 inhibitor; *SERPINGI,* serpin family G member 1 gene; *F12*, coagulation factor XII gene.

The first angioedema episode should have occurred during ACEI or ARB treatment and should have been judged by the treating physician as caused by either of these drugs; after the event, ACEI or ARB should have been discontinued. Exclusion criteria included hereditary and acquired angioedema due to complement CI-esterase inhibitor deficiency, presence of any other likely trigger of angioedema and urticaria. Cases with itching were recruited, but excluded from the present analysis. Compliance with inclusion and exclusion criteria in all recruited cases was verified by an allergist. Information about comorbidities (including asthma and diabetes) was obtained through questionnaires and during interviews with research nurses.

Recruitment of angioedema cases

Recruitment for PREDICTION-ADR took place in Sweden, England, Scotland, Denmark and the Netherlands. Swedish cases were recruited by SWEDEGENE, a national biobank for ADRs, which is a collaborative project between Uppsala

University, the Swedish Medical Products Agency (MPA) and Karolinska Institutet.[21] All cases of angioedema due to ACEIs or ARBs, which were reported to the Swedish national ADR-registry at the MPA between January 1, 1990 and March 31, 2016 were retrieved. Also, a small number of the patients were referred from collaborating clinicians.

In the Netherlands, patient recruitment took place at the Medical Centre of Vrije Universiteit (VU) Amsterdam, Academic Medical Centre (AMC) Amsterdam, University Medical Centre (UMC) Groningen, Westfriesgasthuis in Hoorn, Noordwest Hospital Group, UMC Utrecht and UMC Maastricht from December 02, 2013 until February 28, 2017. Patients were identified by direct referral from physicians of the participating hospitals and by screening of electronical medical records of patients with angioedema. All hospital admissions for angioedema for the past 10 years prior to the moment of screening were reviewed to find patients fulfilling the inclusion criteria for PREDICTION-ADR. In Denmark, patients with ACEI- or ARB-induced angioedema were referred by physicians at Roskilde Hospital, University Hospital of Copenhagen (Rigshospitalet), Gentofte Hospital, University Hospital of Zealand, Slagelse Hospital, Bispebjerg Hospital, University Hospital of Odense and by a collaborating general practitioner from May 01, 2015 until September 31, 2016. In the UK, patients were identified from December 01, 2014 until October 30, 2015 by direct referral from two consultant immunologists at Royal Liverpool Hospital, Southampton General Hospital and a consultant immunologist at Ninewells Hospital and Medical School in Dundee. Furthermore, patients were identified by a review of archived clinical letters at Ninewells Hospital.

Patients were approached either by telephone or received an invitation letter. Participating patients signed an informed consent form, and were provided with a patient information leaflet and a questionnaire surveying demographics, type of causative drug, symptoms of angioedema, other possible triggers and family history of angioedema, co-medication taken around the time of angioedema and a few items of medical history. The questionnaire was either completed during a telephone interview, by the patients themselves at home or during an appointment with a research nurse. When needed, participants' medical records were used to complete the questionnaire. For DNA analyses, patients provided a blood sample or collected saliva into a 2-ml Oragene® OG-500 collection kit (DNA Genotek, Canada).

Definition of control subjects

The same control subjects were used in both arms of PREDICTION-ADR: the ACEI-induced angioedema arm and the statin-induced myopathy arm (not described in the present study). Swedish controls for PREDICTION-ADR were unrelated individuals from the Swedish Twin registry, treated with ACEI (one twin from a pair was included).[22] The Twin registry is linked to the National Prescription Register, which contains dispensed drugs,[23] and the National Patient Register, which contains all diagnosis from in-patient care and outpatient visits except those in primary care.[24] Additionally, a minor part of control subjects was enrolled by collaborating physicians, and they answered a questionnaire in the same way as the cases.

Dutch controls were selected from the database of the Utrecht Cardiovascular Pharmacogenomics (UCP) studies.[25] Drug dispensing data for the UCP participants and hospital discharge diagnoses were obtained through the Dutch population-based Pharmaco-Morbidity Record Linkage System (PHARMO) database.[25] British controls were recruited from the Pharmacogenetics of Statin-Induced Muscle Toxicity (STAGE) study, which recruited eligible subjects through Clinical Practice Research Datalink (CPRD), and the Pharmacogenetics of Acute Coronary Syndrome (PhACS) study.[26] Scottish controls were selected from the Genetics of Diabetes Audit and Research Tayside Study (GoDARTS).[27] No Danish controls were used.

Eligible controls for PREDICTION-ADR fulfilled the following criteria: Caucasian ethnicity, had prescriptions for ACEIs and statins for at least 1 year, no former history of angioedema, larynx edema or myopathy (associated with statin use) and DNA samples available. The identification of former angioedema and myopathy cases in the databases was performed according to the codes of International Classification of Diseases (ICD-9 and ICD-10). If information on asthma and diabetes mellitus was not available, a proxy definition in the prescription databases was used; it required a minimum of 2 consecutive prescriptions for antiasthmatic and antidiabetic drugs, identified with Anatomical Therapeutic Chemical Classification System (ATC) and British National Formulary (BNF) codes.[28]

Ethics

Study procedures and data anonymization were in accordance with the standards of regional ethics committees in Sweden (Uppsala Dnr 2008/213 and Dnr 2010/231;

Stockholm Dnr 2007-644-31 and 2011/463-32). The study protocol was approved by the Central Medical Ethical Committees of the Medical Centre VU Amsterdam and UMC Utrecht and by the local Medical Ethical Committees of each participating hospital in the Netherlands. The UCP studies received approval from the Medical Ethics Committee of the UMC Utrecht. Approval was obtained by the Danish Data Protection Agency (Journal number 2008-58-0035) and Ethics Committee (ID S-20140165). The Liverpool part of the study was approved by the North-West Research Ethics Committee - Liverpool Central. The local ethic approvals were obtained from Liverpool Central Royal Liverpool Hospital and Southampton General Hospital. The Pharmacogenetics of Statin Induced Muscle Toxicity (STAGE) study (IRAS ID: 7086) was approved by the Sefton Research Ethics Committee, and the Pharmacogenetics of Acute Coronary Syndrome (PhACS) study (IRAS ID: 31492) by the Liverpool (Adult) Research Ethics Committee. Ethical approval from the East of Scotland Ethics Approval Board and Caldicott Approval were obtained for the Scottish part of the study. Research was carried out in accordance with the Declaration of Helsinki. All patients gave written informed consent.

Statistical analysis

Descriptive statistics were used to summarize the demographic characteristics of cases and controls including type and dose of the culprit drug, localization of angioedema, co-medications, comorbidities and duration of ACEI and ARB use. The results are presented as means with standard deviations for continuous normally distributed variables, as medians with interquartile ranges in case of a skewed distribution and as percentages for categorical variables. Student's t-test and Mann-Whitney U-test were used for comparison of continuous data, where appropriate. Chi-squared test and Fisher's exact test were used to compare categorical data. For the case-control analysis, we used a subgroup of Caucasians with ACEI-induced angioedema and ACEI-treated controls, for whom data on age, gender, ethnicity and the diagnoses diabetes and asthma were available. Within this subset cases and controls were matched for age at inclusion, gender and ethnicity in a 1:1 ratio. Odds ratio's and 95% confidence intervals for the association between ACEI-induced angioedema and history of diabetes and asthma were obtained with conditional logistic regression. A two-sided P-value of less than 0.05 was considered statistically

significant. Data analyses were performed with IBM SPSS for Windows, version 23.0 (IBM SPSS Statistics for Windows Version 23.0. Armonk, NY: IBM Corp).

Results

Recruitment and characteristics of angioedema cases

The process of patient recruitment is depicted in **Figure 1**.

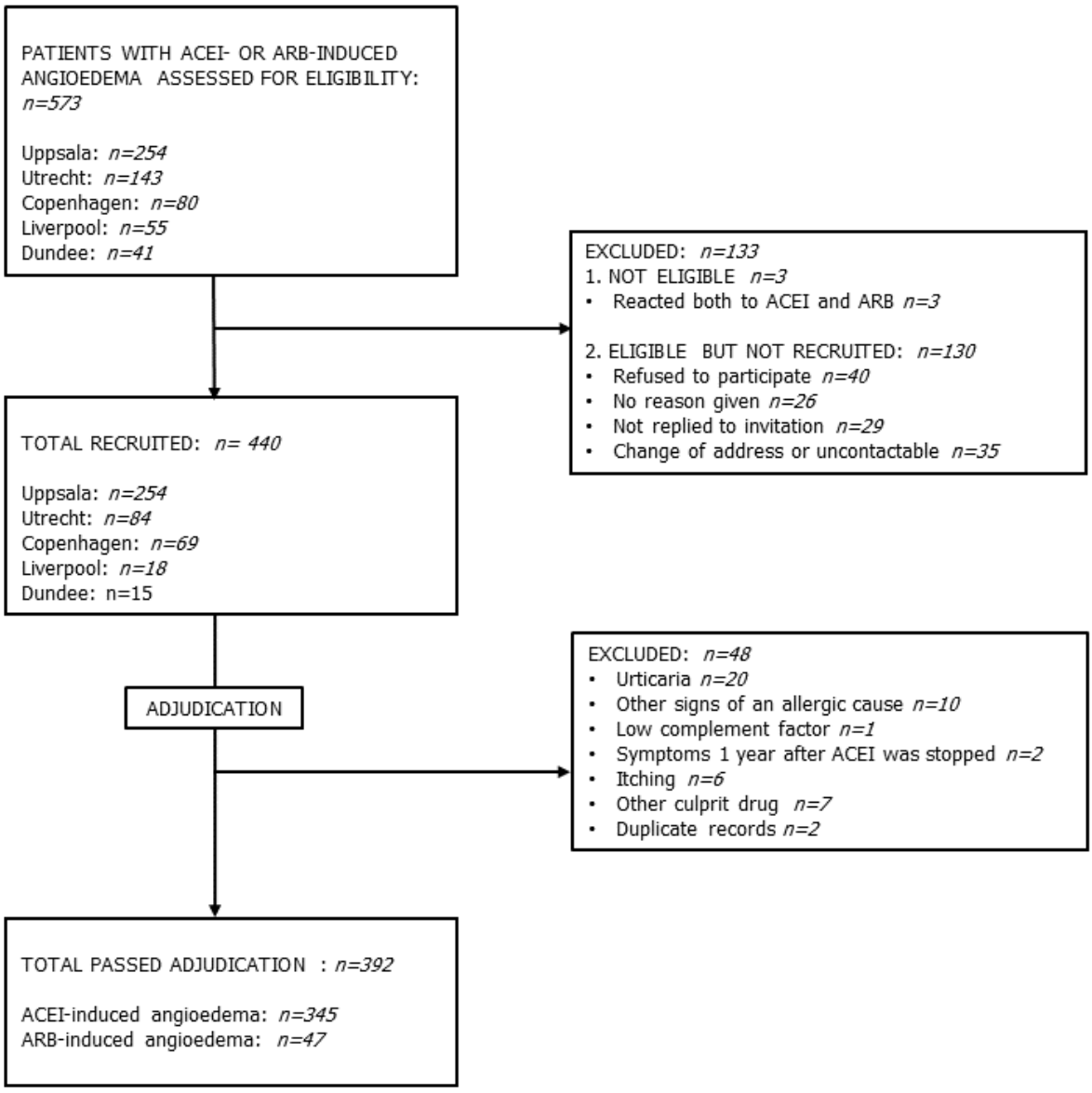


FIGURE 1. Overview of the recruitment of angioedema cases in the PREDICTION-ADR project.
ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor type 1 blocker.

A total of 573 potentially eligible cases of ACEI- or ARB-induced angioedema were identified by the PREDICTION-ADR centers. Of these, 133 (23.2%) were not recruited for one of the following reasons: reaction both to ACEIs and ARBs, refusal to participate, no response to invitation, change of address (not being able to reach the patient) or unknown reasons. After the review of 440 included cases by one allergist, another 46 cases were excluded, most of them (20; 43.5%) due to urticaria coinciding with the angioedema attack. In total, 345 cases of ACEI-induced angioedema and 47 cases of ARB-induced angioedema were included in the study.

TABLE 2. Characteristics of all angioedema cases and ACEI-treated controls included in the PREDICTION-ADR project.

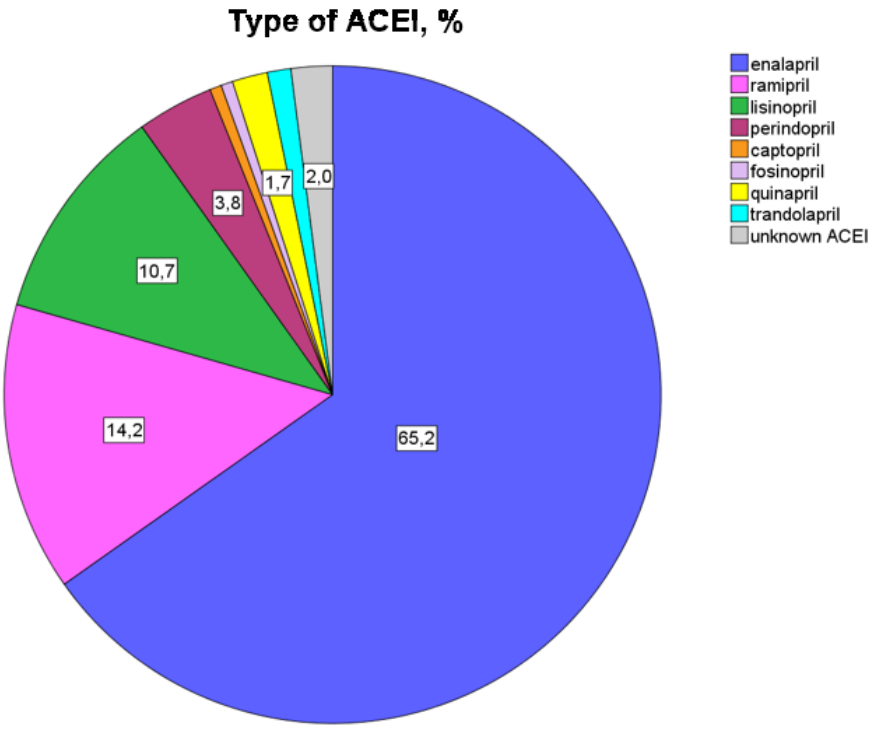
Characteristics	ACEI-induced angioedema (n=345)	ARB-induced angioedema (n=47)	ACEI-treated controls (n=641)
Age at the event, years	65.5 ± 10.7	64.1 ± 9.2	-
Age at inclusion, years	69.4 ± 10.8	70.4 ± 8.9	69.7 ± 10.1
Sex, n (%)			
Male	189 (54.8)	24 (51)	393 (61.3)
Female	156 (45.2)	23 (49)	248 (38.7)
Ethnicity, n (%)*			
Caucasian	333 (96.5)	45 (95.7)	641 (100)
African	4 (1.2)	0 (0)	-
Asian	2 (0.6)	1 (2.1)	-
Other	5 (1.4)	0 (0)	-
Height, m	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
Weight, kg	82.4 ± 17.5	81.5 ± 20.6	92.2 ± 28.1
BMI, kg/m^2	27.9 ± 5.3	27.3 ± 4.8	30.4 ± 7.5
Co-morbidities, n (%)**			
Allergic rhinitis	52 (15.1)	6 (12.8)	-
Eczema	27 (7.8)	3 (6.4)	-
Allergy to antibiotics	51 (14.8)	6 (12.8)	-
Allergy to drugs, other than antibiotics	38 (11.0)	8 (17.0)	-
History of any allergy	155 (44.9)	19 (40.4)	-
Food allergy	38 (11.0)	7 (14.9)	-
Pollen allergy	31 (9.0)	4 (8.5)	-
Dust allergy	8 (2.3)	0 (0)	-
Asthma	33 (9.6)	7 (14.9)	56 (8.7)
Diabetes mellitus	76 (22.0)	11 (23.4)	203 (31.7)
Co-medications, n (%)**			
Platelet-aggregation inhibitors[#]	137 (39.7)	19 (40.4)	-
Beta-blockers	112 (32.5)	13 (27.7)	-
Calcium antagonists	110 (31.9)	12 (25.5)	-
Diuretics	135 (39.1)	18 (38.3)	-
Statins	160 (46.4)	21 (44.7)	-

*Ethnicity was self-reported. **Some patients had more than one of the co-morbidities/co-medications, the total sum of individual values might not be 100%. [#] Includes aspirin, dipyridamole, carbasalate calcium, clopidogrel, ticagrelor. Diabetes mellitus: both type 1 and type 2 diabetes. Missing data (n): for ACEI - history of any allergy (2), asthma and/or diabetes (5), ethnicity (1), BMI (8), age at inclusion (1), age at event/date of event (4); for ARB: history of any allergy (3), asthma and/or diabetes (1), ethnicity (1), BMI (3), age (0); for controls - height, weight and BMI (576, 89.9%). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor type 1 blocker, BMI, body mass index.

Among patients with ACEI-induced angioedema, the majority were Caucasian (96.5%), 189 (54.8%) were male, and the mean age at the time of angioedema was 65.5 years (**Table 2**). There were 24 males and 23 females with ARB-induced angioedema, the mean age at event was 64.1 years, and most patients were Caucasians (95.7%). It was notable that 44.9% of the patients with ACEI-induced angioedema and 40.4% of patients with ARB-induced angioedema had a history of non-drug related allergies (**Table 2**). Allergic reactions to antibiotics were reported in 14.8% and 12.8% of patients with ACEI- and ARB-induced angioedema, respectively. Allergic reactions to other drugs were noted in 11% of patients with ACEI-induced angioedema and 17% of the patients with ARB-induced angioedema. Asthma was present in 9.6% of ACEI-induced angioedema patients and in 14.9% of patients with angioedema related to ARBs. The proportions of patients with diabetes were similar (22.0% and 23.4%). None of the characteristics were statistically significantly different between cases of ACEI- and ARB-induced angioedema (**Table 2**). Angioedema affected multiple anatomical sites of the head and neck region in most of the patients (61.2% for ARBs, and 66 % for ACEIs) (**Supplementary Figure 1**). There were only three patients with peripheral angioedema. Swelling of the tongue was the most prevalent of all locations in patients with angioedema due to ACEIs (56.5%), and 13% of the patients had angioedema with laryngeal involvement. In patients with angioedema related to ARBs, the most common locations of the swelling were lips (44.7%), tongue (23.4%) and pharynx (19.1%). Laryngeal location was found in 8.5% of the ARB-induced angioedema cases.

Enalapril and ramipril were most frequently identified as causative drugs among ACEIs (65.2% and 14.2%, respectively), while losartan (44.7%) and candesartan (31.9%) were the most common among implicated ARBs (**Figure 2**).

The duration of ACEI or ARB treatment was categorized from descriptions in patients' questionnaires and medical records (**Table 3**). The median duration of ACEI treatment before the onset of angioedema was 912.5 days (interquartile range (IQR) of 1935.6) and the median duration of ARB treatment was 1095.0 days (IQR 1352.9) (**Table 3**, **Supplementary Figure 2**).



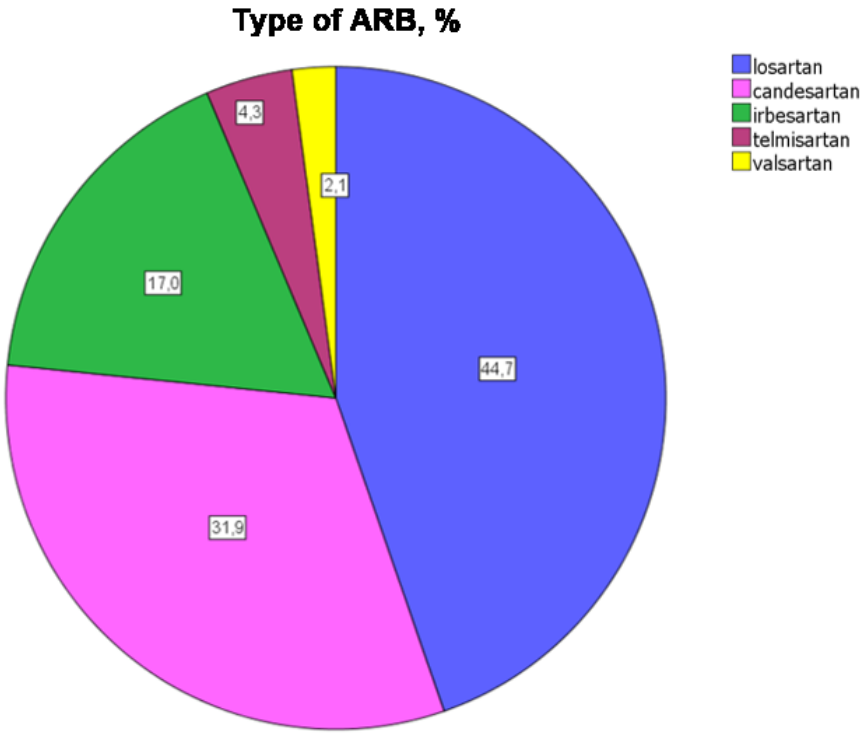


FIGURE 2. Reported causative drugs in the PREDICTION-ADR angioedema cases.
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor type 1 blocker. *Captopril and perindopril – 0,6%, Trandolapril – 1,2%.

TABLE 3. Duration of causative drug use before the onset of angioedema.		
	ACEI-induced angioedema (n=284)*	**ARB-induced angioedema (n=39)****
Duration (days), median (IQR)**	912.5 (1935.6)	1095.0 (1352.9)
≤ 2 first weeks, n (%)	30 (8.7)	3 (6.4)
≤ first 3 months, n (%)	43 (12.5)	3 (6.4)
Hours, n (%)	4 (1.2)	0 (0)
Days (0-6 days), n (%)	19 (5.5)	3 (6.4)
Weeks (≥ 7 - 28 days), n (%)	11 (3.2)	0 (0)
Months (≥ 29 - 364 days), n (%)	51 (14.8)	6 (12.8)
Years (≥ 365 days), n (%)	199 (57.7)	30 (63.8)
Unknown (categories), n (%)	61 (17.8)	8 (17.0)

*Missing data for categories of duration: ACEI-angioedema n=61, ARB-angioedema n=8. **Missing data for duration in days: ACEI-angioedema n=94 (37,5%) – total available 251; ARB-angioedema n=12 (25,5%) – total available n=35. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor type 1 blocker.

A small proportion of patients developed angioedema within the first 2 weeks (8.7% for ACEI and 6.4% for ARB) or within the first 3 months of treatment (12.5% and 6.4% for ACEI and ARB, respectively; **Table 3**). The majority of patients experienced the attack at least 365 days after the start of treatment (57.7% and 63.8% for ACEIs and ARBs, respectively). In one patient angioedema occurred 30 years after symptom-free use of an ACEI. The longest duration of ARB use before the onset of angioedema was 19 years (**Supplementary Figure 2**).

Characteristics of control subjects and case-control analysis

The demographic characteristics available for ACEI-treated controls are shown in **Table 2**. All controls were Caucasians, 61.3% of them were male, and the average age at inclusion was 69.7 years. For the case-control analysis**,** 286 Caucasian cases of ACEI-induced angioedema with available data on age, asthma and diabetes were matched for age, gender and ethnicity in a 1:1 ratio with 286 of the 641 PREDICTION-ADR controls. The results of conditional logistic regression are presented in **Table 4**.

TABLE 4. Association of angioedema with history of chronic comorbidities.		
	OR (95% CI)	**P**
Asthma	1.03 (0.58; 1.82)	0.921
Diabetes	0.56 (0.39; 0.81)	**0.002**

CI – confidence interval, OR – unadjusted odds ratio.

There were 167 males and 119 female cases and an equal number of male and female controls; the mean age was 70.6 years (range: 48-93 years). The likelihood of developing ACEI-induced angioedema was almost half in patients with diabetes as compared to no diabetes, OR 0.56 (95% CI: 0.39; 0.81, p=0.002). Asthma was not significantly associated with ACEI-induced angioedema, OR 1.03 (95% CI: 0.58; 1.82),

Discussion

PREDICTION-ADR included many patients with ACEI- and ARB-induced angioedema that fulfilled standardized phenotypic criteria. In the present study, we evaluated demographic characteristics, clinical presentation, type of causative drug, and the duration of treatment in recruited patients. The demographic characteristics of cases did not differ significantly between patients with ACEI- and ARB-induced angioedema, and their age and gender distributions were largely similar to other studies.[4,29-31] The average age at the time of the event was 65.5 and 64.1 years for ACEI and ARB, respectively. There was a slightly higher proportion of males than females in both groups (54.8% and 51.0%). However, this should be compared with the proportion of males prescribed ACEIs and ARBs, which at least in Sweden was higher among males than females, 53% in 2006-2016.[32] In other epidemiological studies, mostly using data of African-American patients with ACEI-induced angioedema, a higher percentage of females was observed (62.5% and 75.0%).[18,33] Frequencies of comorbidities among patients with angioedema in our study did not vary significantly from those described in the literature. Asthma was reported by different authors in 8.6% - 15.9% of patients with ACEI-induced angioedema, food allergy in 6.0% - 8.0%, allergic rhinitis in 3.4% - 9.0%, diabetes mellitus in 18.8% - 24.0%.[29,33,34]

In accordance with previous studies, we found that angioedema was localized in the head and neck region in the majority of cases.[29] The most frequently involved anatomical site in patients with ACEI-induced angioedema was the tongue (56.5%), as also reported for Caucasian patients in the study by Bas *et al.*[17] Different studies describe angioedema of the tongue in 39.7% - 60% of patients with ACEI-induced angioedema.[18,29,31,33,35] Of note, the studies by Straka *et al*. and Chan *et al*. found that the most frequent location of swelling in African-Americans was the lips (50.0% - 60.2%).[18,33] Angioedema of the larynx occurred in 13,5% of patients in our study, and in the literature the numbers vary from 13.0% to 29.5%.[4,29,31,33] Laryngeal and

pharyngeal angioedema can be potentially life-threatening, and even a mono-symptomatic angioedema of the tongue can be lethal, if the obstruction of the upper airways occurs.[13]

Compared to other studies, in which lisinopril was the most frequent causative drug (88.6% - 92.0%), in our data, enalapril was the causative agent in the majority of patients with ACEI-induced angioedema (65.2%).[18,33,34] As in the study by Faisant *et al*., we found candesartan to be a common causative drug in patients with ARB-induced angioedema, while valsartan was infrequent in our data (2.1% vs. 29.2%).[29] However, these observations are likely to be explained by the differences in prescribing patterns of ACEIs and ARBs, as well as the time when these drugs appeared on the market and when their patents' expired. Since angioedema is a drug class effect, no differences between individual drugs are expected.

In earlier reports, most of the ACEI-induced angioedema episodes (47% - 72%) occurred within the first days to weeks of treatment, but recent studies have consistently shown that the time to onset of angioedema is often much longer.[7,18,29,36] In the present study, symptoms of ACEI- and ARB-induced angioedema occurred within the first two weeks in 8.7% and 6.4% of patients, respectively. A retrospective cohort study of 888 patients with ACEI-induced angioedema found that 6.8% developed angioedema within the first two weeks of therapy.[7] A study by Faisant *et al*. reported that 30% of patients with ACEI-induced angioedema experienced the first attack within 3 months after starting treatment, but in our study, this was only 12,5%.[29] The median duration of ACEI use before the onset of angioedema was 912.5 days (2.5 years), which is similar to other published data.[18,29] In a study by Chan *et al*., 50.7% of patients presented with the first angioedema attack after taking ACEI for at least 1 year[33]; in our population, this proportion was 57.7% of patients. Our study confirmed that angioedema due to ACEIs was less frequent in the presence of diabetes mellitus.[13] However, we did not find increased odds of angioedema in patients with asthma, as previously reported.[37] Asthma was less frequent in patients with angioedema secondary to ACEI in the studies by Rasmussen *et al*. and Banerij *et al*.[34,38] We were not able to investigate the association of angioedema with other atopic traits, because of the absence of these data in the controls. It should be mentioned that about 40% of angioedema cases in our study had a history of non-drug related allergies. In our previous study using CPRD data, the history of allergy was statistically significantly

associated with ACEI-induced angioedema (OR 1.53 (95% CI, 1.19-1.96).[37] Byrd *et al.* found an OR for angioedema of 2.40 (95% CI, 1.42–4.07) among patients with a history of seasonal allergies.[39] Straka *et al.* reported the presentation of angioedema to be more frequent in months with increased pollen counts.[40] The history of rash and seasonal allergies was identified as independent risk factors for angioedema related to enalapril in the analysis of the Omapatrilat Cardiovascular Treatment vs. Enalapril (OCTAVE) trial.[41] These observations indicate that an atopic predisposition to certain aeroallergens could trigger angioedema, possibly justifying the occurrence of this ADR after prolonged treatment with ACEI.[40] The susceptibility to angioedema in certain months of the year could be explained by an increased production of bradykinin and substance P, mediated by allergic inflammation in patients with seasonal allergies and defects in bradykinin degradation pathways.[40] Other reported risk factors of angioedema, such as African descent (hazard ratio (HR): 3.5; 95% CI: 1.3–8.9) and history of smoking (HR: 2.7; 95% CI: 1.1–7.0) were not assessed in this study, because of a small number of participants of African descent, and no data on smoking among the controls.[42-44]

This study has some limitations, which need to be addressed. The recurrence of angioedema after cessation of treatment and other variables, such as duration of hospitalization or treatment of angioedema were not recorded. We also cannot exclude recall bias, due to the use of questionnaires. We may have a possible bias for later angioedema presentations, because only patients who were referred to hospitals were recruited. In case of an early presentation of angioedema, the causative link with ACEIs might be more obvious, leading to discontinuation of the ACEIs by the prescribing primary care physician. If data on asthma and diabetes were not recorded for the controls, we used proxy definitions, based on prescription data, which could have caused misclassification. Our findings concerning the associations with comorbidities need to be interpreted with caution, because we were not able to adjust for potential confounders, including some of the previously reported potential risk factors for angioedema, such as smoking and allergies, due to missing data in the controls. Due to logistic reasons, the source populations of the controls were different; this could have introduced selection bias into our case-control analysis. Moreover, not all available cases were included into the analysis, because of the limited number of available controls with exact matching for age and sex. The absence of information on comedications and comorbidities for the controls did not permit further analysis.

Conclusion

Most patients presenting with ACEI- or ARB-induced angioedema were in their 60s and frequently presented with angioedema, involving multiple anatomical sites of the head and neck region. Angioedema often occurred later during ACEI and ARB treatment. The time course of angioedema can make it clinically challenging to recognize a causative link with treatment, and this study should remind physicians to be aware of the possibility of late-onset angioedema. History of allergies was common among recruited angioedema patients. History of diabetes mellitus was associated with a decreased risk of angioedema, and no association was found with a history of asthma.

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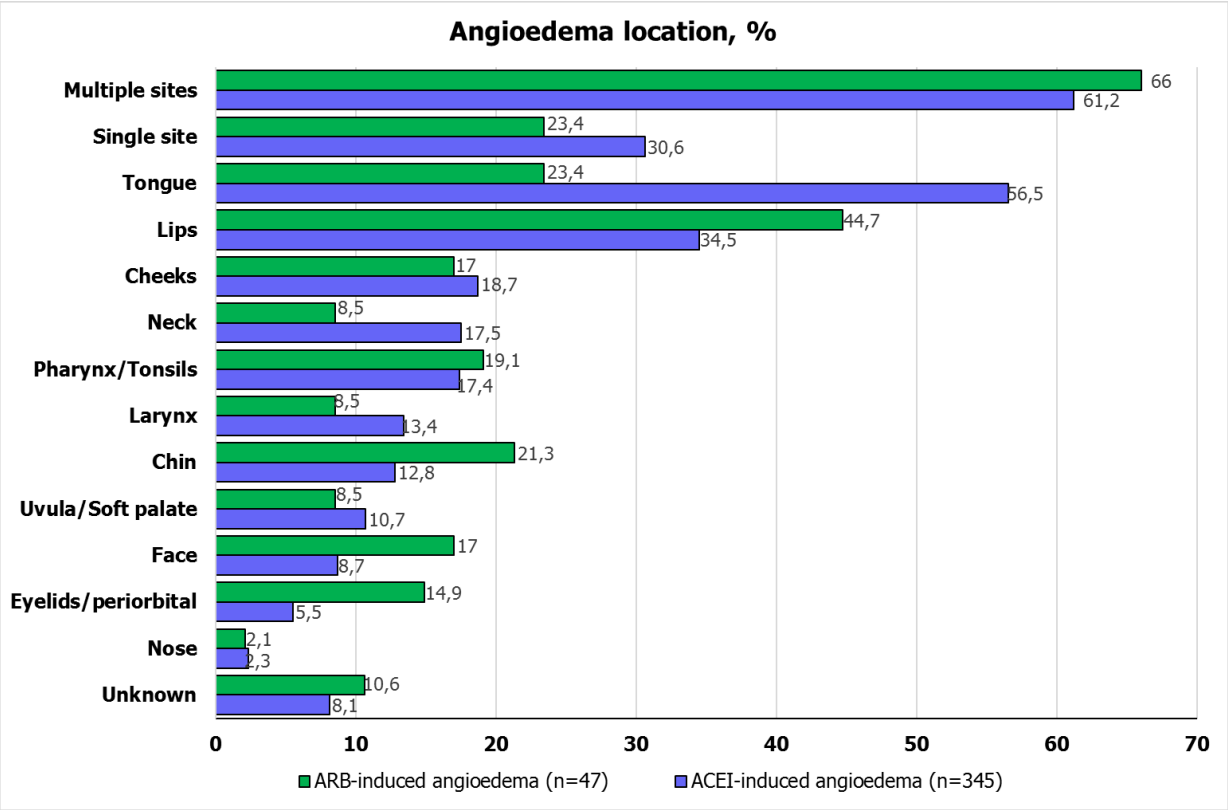
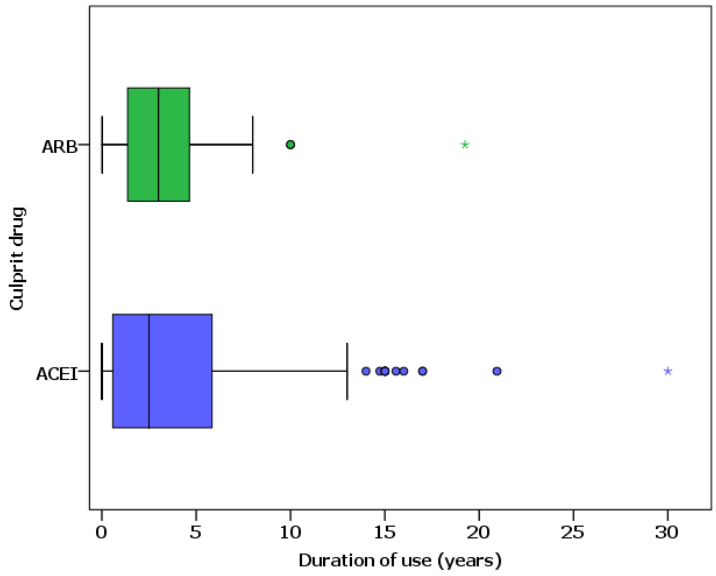
Supplement



SUPPLEMENTARY FIGURE 1. Location of angioedema by type of causative drug.

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor type 1 blocker.The percentages reflect frequencies of different locations in the dataset; the total sum of individual values might not be 100%. Multiple sites: >2 anatomical sites in the head and neck region.



SUPPLEMENTARY FIGURE 2. Time to onset of angioedema after the start of ACEI or ARB use.

Blue box shows IQR of duration for ACEI; green box shows IQR of duration for ARB, whiskers depict the minimum and the maximum; blue and green circles and stars represent the outliers. The median duration of ACEI use before the angioedema attack is 2,5 years. The median duration of ARB use before the angioedema attack is 3 years. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor type 1 blocker.

CHAPTER 3.3

Genome-wide association study of angiotensin-converting-enzyme (ACE) inhibitor-induced angioedema imputed to the Haplotype Reference Consortium panel.

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Manuscript in preparation.

Abstract

Objectives: To identify single nucleotide polymorphisms (SNPs) associated with angiotensin-converting enzyme inhibitor (ACEI)-induced angioedema.

Participants and methods: Data of 174 angioedema cases and 489 controls of African and European descent, collected at Vanderbilt University and Marshfield Clinic were used for the analysis. Cases were patients who developed angioedema of the head and neck region while taking an ACEI, but had never had angioedema while not taking an ACEI. Controls were individuals who were treated with an ACEI for at least 6 months, and had never developed angioedema. Imputation of genetic variants was performed to the Haplotype Reference Consortium (HRC) reference panel, and a genome-wide association study (GWAS) approach was applied.

Results: No SNPs were associated at the genome-wide significance level. Considering the bradykinin-mediated mechanism of ACEI-induced angioedema, the biologically most plausible hit was rs55940712 near the bradykinin receptor 2 (*BDKRB2*) gene (odds ratio (OR), 0.41; 95% confidence interval (CI): 0.28-0.59, $P=2.06\times10^{-6}$). Two intronic SNPs, *EFCAB4B/CRACR2A* rs12425092 and *PSAT1* rs2998724, were associated with an increased risk of angioedema (OR, 2.15; 95% CI: 1.61-2.88; $P=2.88\times10^{-7}$ and OR 1.98; 95% CI: 1.49-2.62; $P=2.51\times10^{-6}$, respectively).

Conclusions: Variants in genes of bradykinin pathway (*BDKRB2*), and genes involved in pathways of innate immunity (*EFCAB4B/CRACR2A*) and serine biosynthesis (*PSAT1*) may be associated with ACEI-induced angioedema. Additional replication and functional follow-up is needed to confirm these findings.

Introduction

Angioedema induced by angiotensin-converting enzyme-inhibitors (ACEIs) is a rare and potentially life-threatening type B adverse drug reaction (ADR) with an incidence of 0.7-1% among ACEI users.[1,2] Studies in patients admitted to emergency departments (ED) show that ACEIs are among the most common causes of angioedema, with 30.0-56.6% of all ED angioedema cases being attributed to these drugs.[3-5] Epidemiological evidence suggests that individuals of African descent, females, older patients and smokers have an increased risk of ACEI-induced angioedema,[2,3,6] and there are also indications for a potential role of genetic factors. However, currently there is no accepted method to identify patients at risk of developing this ADR before the start of ACEI treatment.

Angioedema related to ACEIs is hypothesized to be mediated by vasoactive molecules, including bradykinin and substance P, which can accumulate under ACE inhibition and cause an increased vascular permeability and tissue edema.[7,8] Candidate-gene analyses found associations of angioedema with single nucleotide polymorphisms (SNPs) in genes encoding receptors and enzymes regulating the activity or breakdown of these substances. The bradykinin receptor 2 gene (*BDKRB2)* rs5810761 and the membrane metallo-endopeptidase (neprilysin) gene (*MME)* rs989692 were associated with angioedema in patients of African descent.[9,10] A study by Duan *et al.* in eight large pedigrees found that the C-2399A variant of the X-linked X-prolyl aminopeptidase 2 gene (*XPNPEP2,* rs3788853) encoding the bradykinin-inactivating aminopeptidase P, was associated with an increased incidence of angioedema.[11] A study by Woodard-Grice *et al.* showed that *XPNPEP2* rs3788853 was associated with ACEI-induced angioedema in both Caucasian and African-American males, but not females.[12] The only genome-wide association study on ACEI-induced angioedema published to-date was conducted in samples collected at Vanderbilt University and as part of the Marshfield Clinic Personalized Medicine Research Project.[10] The study analyzed 579,344 SNPs and found no associations at the genome-wide significance level (P-value < 5×10^{-8}). A replication candidate-gene analysis with 57 moderately associated SNPs (P-value < 10^{-4}) from the GWAS was performed in the Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial (ONTARGET).[10,13] It showed that a SNP in the protein kinase C θ gene

(*PRKCQ*, rs500766) was associated with a reduced risk of ACEI-induced angioedema in Caucasians, while a SNP in the ETS variant 6 gene (*ETV6,* rs2724635) was associated with an increased risk in both the original GWAS and ONTARGET participants of African descent.[10]

While the GWAS approach allows testing of multiple SNPs, it often lacks the coverage of rare genetic variants with potentially large effect sizes.[14] Using reference panels of haplotypes, genotyped SNPs can be used to impute a large number of variants that are not directly assayed.[15] Most commonly GWAS are imputed to the Phase 2 version of the HapMap Project (HapMap) or the Phase 1 version 3 of the 1000 Genomes Project (1000G), the latter covering low-frequency variants (minor allele frequency, MAF > 1%), and rare variants (MAF < 1%) that were previously not covered.[16] For instance, a GWAS of circulating fibrinogen comparing these two panels found that 1000G imputation enabled identification of 20% more associated loci compared to HapMap.[16] Recently, a Haplotype Reference Consortium (HRC) reference panel combining 64,976 human haplotypes at 39,235,157 SNPs was constructed using whole-genome sequence data from 20 studies of predominantly European ancestry.[17] This large panel has been applied in 20,032 Scottish individuals from the Generation Scotland: Scottish Family Health Study.[18] The results of that study confirmed known associations with some well-studied cardiometabolic and anthropometric traits and also showed novel associations.[18]

We hypothesized that by using a denser imputation reference panel, we could identify new association signals for ACEI-induced angioedema in the Vanderbilt/Marshfield samples. In this study we investigated the use of HRC imputation in a GWAS on ACEI-induced angioedema.

Methods

Participants

Blood samples of patients with ACEI-induced angioedema were collected for a case-control study at Vanderbilt University and as part of the Marshfield Clinic Personalized Medicine Research Project.[10] Patients were recruited at Vanderbilt at the time they presented with angioedema, by a direct referral from the treating physicians or by searching electronic medical records.[10] In Marshfield, patients were identified through

electronic medical records, and the medical history was reviewed by a trained study coordinator. A detailed case report form was used to confirm the medical history in both centers.[10] The Institutional Review Boards of Vanderbilt University and Marshfield Clinic approved the studies, and a written informed consent was obtained from all participants.

Definition of cases and controls

Cases were defined as having ACEI-induced angioedema if they had swelling of the lips, pharynx, or face while taking an ACEI, but had never had angioedema while not taking an ACEI.[10] Controls were individuals who had been exposed to an ACEI for at least 6 months, but had never developed angioedema.[10] Hereditary angioedema was excluded by the measurement of C1 esterase activity, when deemed necessary by the treating physician.

Genotyping and quality control

All the cases and controls were genotyped using a 610Quadv1.B Illumina Bead Chip platform. The poor-quality SNPs and samples were excluded based on the quality control (QC) criteria: SNP call rate <0.95, MAF<0.0001, Hardy-Weinberg equilibrium (HWE) $P<10^{-6}$, sample call rate <0.95, gender discrepancy, and sample duplicates (identity-by-descent, IBD>0.8). After QC, totally 174 cases, and 489 controls and 563,722 SNPs were used for the imputation. Using the Sanger imputation service,[19] samples of European American and African American ancestry were phased using SHAPEIT2[20] and imputation was carried out using the positional Burrows-Wheeler transform algorithm[21] and the HRC 1.1 release panel[17].

Statistical analysis

Genome-wide association analysis was performed on the imputed data. Low-quality imputed variants with an imputation quality score (INFO score) lower than 0.4 and SNPs with MAF below 5% were excluded. To detect population stratification, principal components were estimated by Smartpca 8000, EIGENSOFT package.[22] The λ inflation factor was calculated for genomic control analysis using an R script. Logistic regression analysis assuming an additive genetic model was carried out to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of genetic

markers with ACEI-induced angioedema using PLINK v. 1.9.[23] The model was adjusted for age, sex and ten principal components. The genome-wide statistical significance threshold was set at $P < 5\times10^{-8}$. SNPs were annotated to genes using Haploreg database[24] and UCSC genome browser.[25] Manhattan plots and Q-Q plots were generated using the R qqman package.[26] Regional plots for the most strongly associated SNPs were generated using the LocusZoom tool.[27] We used Genotype-Tissue Expression (GTEx) database[28] to explore the information about the *cis*-expression quantitative trait loci (eQTLs, variants within 1 Mb (megabase) on either side of a gene's transcriptional start site) for the top variants.

Results

The characteristics of 174 cases and 489 controls are shown in **Table 1**. Both in Vanderbilt and Marshfield the cases and controls were matched with respect to ethnicity and smoking status, as described previously.[10] Cases were more likely to have seasonal allergies than controls, and there were no other statistically significantly differences in age, sex and prevalence of diabetes mellitus. In multivariable logistic regression adjusting for sex, age and ten principal components to control for population stratification no SNPs reached genome-wide significance.

TABLE 1. Characteristics of the study participants.

Characteristic, n (%)	Cases (n=174)	Controls (n=489)
Sex		
male	78 (44.8)	229 (46.8)
female	96 (55.2)	260 (53.2)
Age (mean ± SD)	58.4 ± 14.1	61.7 ± 13.1
Ancestry		
African	66 (37.9)	157 (32.1)
European	108 (62.1)	330 (67.5)
missing	0 (0)	2 (0.4)
Current smoker	37 (21.3)	91 (18.6)
Diabetes mellitus	57 (32.8)	182 (37.2)
Seasonal allergy*	85 (48.9)	154 (31.5)

Missing information (cases/controls): smoking (28/110), diabetes (1/7), seasonal allergy (47/152). *For seasonal allergy P-value = 0.001 between cases ad controls. SD, standard deviation.

The Manhattan and Q-Q plots of the GWA analysis are provided in **Figure 1a** and **1b**. The top suggestive signals were located on chromosomes 9, 12 and 14 near the phosphoserine aminotransferase 1 gene (*PSAT1*), EF-hand calcium-binding domain-containing protein 4b/calcium release activated channel regulator 2A gene

(*EFCAB4B/CRACR2A*) and bradykinin receptor B2 gene (*BDKRB2*), respectively (**Table 2**). The strongest association was in the imputed SNP rs12425092 in the *EFCAB4B/CRACR2A* gene (MAF=21.14%; OR, 2.15; 95% CI: 1.61-2.88; $P=2.88\times10^{-7}$). This SNP is a proxy of rs758530 ($r^2 = 1.00$), one of the top two signals near *EFCAB4B/CRACR2A* in patients of European ancestry of the initial GWAS (**Supplementary Table 1**). We also replicated the association with the other top SNP, *EFCAB4B/CRACR2A* rs1015762 (MAF=25.48%; OR, 1.96; 95% CI: 1.49-2.59; $P=1.72\times10^{-6}$) and found associations with its proxy rs55940430 ($r^2 = 0.95$). There were other strongly associated SNPs in high linkage disequilibrium ($r^2>0.80$) with the imputed rs12425092 in that region (**Figure 2a**). Intronic SNP rs2998724 near the *PSAT1* gene (MAF=42.07%) was associated with a higher risk of ACEI-induced angioedema (OR, 1.98; 95% CI: 1.49-2.62; $P = 2.51\times10^{-6}$). The SNPs rs55940712 (MAF=21.00%) and rs34393530 (MAF=26.94%) in the *BDKRB2* gene appeared to have a protective effect with OR's of 0.41 (95% CI: 0.28-0.59; $P=2.06\times10^{-6}$) and 0.45 (95% CI: 0.32-0.63; $P=2.66\times10^{-6}$). Several other SNPs in high linkage disequilibrium ($r^2 > 0.80$) with rs55940712 were moderately associated ($P < 10^{-5}$) in that region (**Figure 2b**). According to the GTEx Portal, *BDKRB2* rs1889374 (in perfect LD with rs55940712) and two proxies of *BDKRB2* rs34393530 (rs7492727 and rs4905449, $r^2 = 0.87$) function as eQTLs expressed in the lungs. We replicated the association of rs500766 in the protein kinase C θ gene (*PRKCQ*) with a reduced risk of ACEI-induced angioedema, as reported in the initial GWAS for patients of European descent (OR, 0.56; 95% CI: 0.42-0.75, $P=8.27\times10^{-5}$; **Supplementary Table 1**).

Discussion

In this GWAS on ACEI-induced angioedema using imputation to the HRC reference panel, we did not find associations at the genome-wide significance level. However, using this denser imputation panel we identified novel suggestive signals in the bradykinin receptor B2 gene (*BDKRB2* rs55940712 and rs34393530), conferring a lower risk of angioedema. Two SNPs (*EFCAB4B/CRACR2A* rs12425092 and *PSAT1* rs2998724) were statistically significantly associated with an increased risk of ACEI-induced

TABLE 2. Six most significant signals in the GWAS on the ACEI-induced angioedema (cases n=174; controls n=489).

SNP	CHR	BP	Gene	MA	MAF, %	OR	95% CI	P-value
rs12425092	12	3759214	*EFCAB4B/CRACR2A*	A	21.14	2.15	1.61 - 2.88	2.88×10^{-7}
rs55940430	12	3776802	*EFCAB4B/CRACR2A*	G	25.63	1.99	1.51 - 2.62	1.20×10^{-6}
rs1015762	12	3772977	*EFCAB4B/CRACR2A*	C	25.48	1.96	1.49 - 2.59	1.72×10^{-6}
rs55940712	14	96605573	*BDKRB2*	A	21.00	0.41	0.28 - 0.59	2.06×10^{-6}
rs2998724	9	81007452	*PSAT1*	G	42.07	1.98	1.49 - 2.62	2.51×10^{-6}
rs34393530	14	96624517	*BDKRB2*	G	26.94	0.45	0.32 - 0.63	2.66×10^{-6}

CHR, chromosome. BP, base pair. MAF, minor allele frequency. P, P-value based on logistic regression analysis. SNP - single nucleotide polymorphism. OR, odds ratio. HWE, Hardy-Weinberg equilibrium. CI, confidence interval. *EFCAB4B/CRACR2A,* EF-hand CAlcium-Binding domain-containing protein 4b/Calcium Release Activated Channel Regulator 2A. *BDKRB2,* Bradykinin Receptor B2. *PSAT1,* Phosphoserine Aminotransferase 1. Base pair position is based on NCBI build 37.

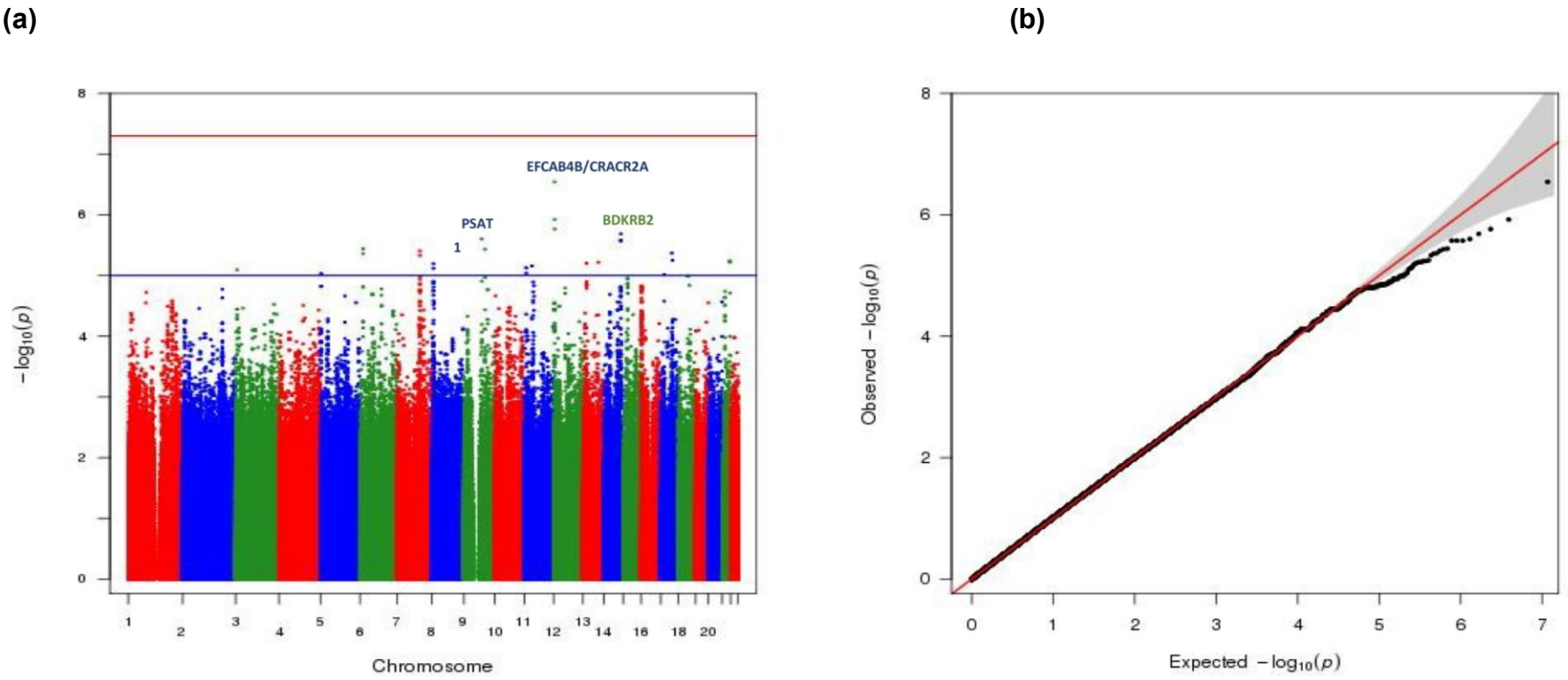

FIGURE 1. Manhattan and Q-Q plots of the GWAS analysis.

(a) Each point represents 1 of the 5,826,226 SNPs with MAF > 0.05, colored according to chromosome. The *x* axis represents genomic location, and the *y* axis represents the *P*-value for the SNPs' associations calculated using an additive genetic model with adjustment for age, sex and 10 PC's. The upper red line indicates the genome-wide significance threshold of $p = 5 \times 10^{-8}$. The lower blue line shows $p = 1 \times 10^{-5}$.
(b) Quantile-quantile plot of genome-wide association study. Observed *P*-values (*y*-axis) for ACEI-induced angioedema are plotted for 5,826,226 SNPs against expected *P*-values (*x*-axis) under the null distribution for no association.

INFO quality score>0.4; HWE $<10^{-6}$; MAF cut-off = 0.05. Lambda = 1.02.

EFCAB4B/CRACR2A - EF-hand CAlcium-Binding domain-containing protein 4b/Calcium Release Activated Channel Regulator 2A gene. *BDKRB2* - Bradykinin Receptor B2 gene. *PSAT1* - Phosphoserine Aminotransferase 1 gene.

The association with *BDKRB2* variants (rs55940712 and rs34393530) seems to be the most biologically plausible, considering the hypothesized bradykinin-mediated mechanism of ACEI-induced angioedema. Bradykinin exerts its vasodilatory action primarily through direct effects at the B2 receptor and through the B2-receptor-dependent release of substance P.[29] The contribution of genetic variation in *BDKRB2* has been previously investigated in candidate-gene analyses. A study in South African patients suggested a significant difference in the *BDKRB2* -9 (rs5810761) allele frequencies between patients with ACEI-induced angioedema and ACE inhibitor-induced cough and controls.[30] A study by Bas *et al.* found no association between angioedema and the *BDKRB2* exon 1 polymorphism (*2/3 polymorphism*) and c.C181T variant in patients of European ancestry.[31] Similarly, a candidate-gene analysis in ONTARGET found no associations with *BDKRB2* rs1799722 variant.[10] Notably, the top associated *BDKRB2* SNPs in our study were in high LD with markers functioning as eQTLs in the lungs. This could be helpful for the interpretation of a potential function of this locus in angioedema, should this association be confirmed in further studies.

The possible role of the *EFCAB4B/CRACR2A* and *PSAT1* genes in ACEI-induced angioedema is not clear. The *EFCAB4B/CRACR2A* belongs to the family of Rab GTPases and plays a key role in intracellular vesicle trafficking via store-operated Ca^{+2} entry in T-cells[32,33] and may be related to pathways of innate immunity. Variants in *EFCAB4B/CRACR2A* were found to be associated with lobular inflammation in non-alcoholic fatty liver disease, male infertility and cytomegalovirus antibody response.[34-36] The CRACR2A protein also seems to have a long variant which is present in the endothelial cells and lacks impact on CRAC channels, but its precise function is not well-characterized.[37] The pathways linked to the function of *PSAT1* include serine biosynthesis and viral mRNA translation.[38,39] Decreased expression of *PSAT1* may be linked to schizophrenia[40] and its mutations are associated with rare inherited disorders (phosphoserine aminotransferase deficiency and Neu-Laxova Syndrome).[41,42] Future studies will be required to validate the association with angioedema and assess the functionality of the *EFCAB4B/CRACR2A* and *PSAT1* variants. One of the limitations of this study is a small sample size and a lack of statistical power to achieve genome-wide significance, but this is a common issue in studies of rare ADRs. Replication studies or a meta-analysis in other cohorts of patients with ACEI-induced angioedema are warranted, preferably using data imputed to HRC.

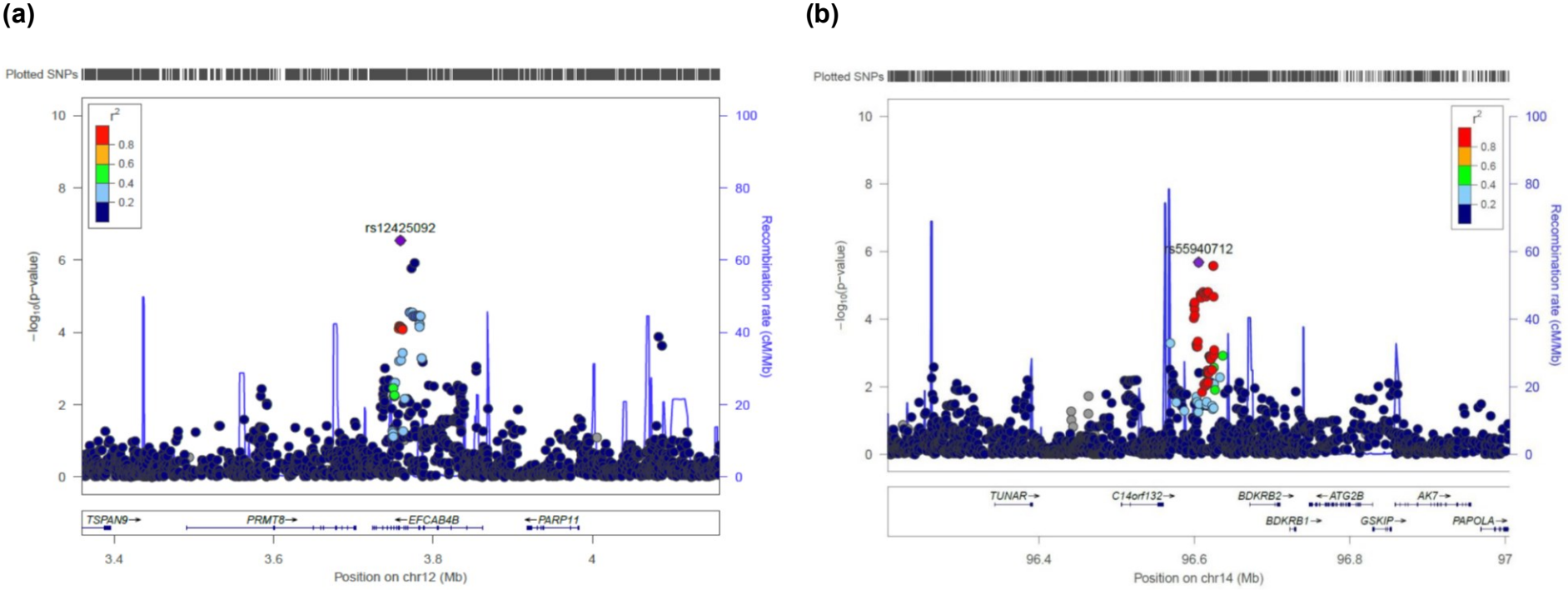

FIGURE 2. Genomic regional plots centered on (*a*) chromosome 12 and (*b*) chromosome 14 region, including the most strongly associated SNPs near *EFCAB4B/CRACR2A* and *BDKRB2* genes.

Plots **(a)** and **(b)** were generated using the LocusZoom tool for the lead SNP in genomic region 400 kb in either side of the significant signal. Blue spikes represent the estimated recombination rates. Colored circles and scale represent the pairwise correlation (r^2) between the top SNPs and other SNPs in the loci. Grey color indicates that LD information was not available in the reference population. The top variant for each region is indicated by a purple-colored solid diamond (indicated with rs number). The boxes below plots illustrate gene annotations in this region. The genome position is based on NCBI build 37.

SNP, single-nucleotide polymorphism. *EFCAB4B/CRACR2A,* EF-hand CAlcium-Binding domain-containing protein 4b/Calcium Release Activated Channel Regulator 2A gene. *BDKRB2,* Bradykinin Receptor B2 gene. LD, linkage disequilibrium.

Imputation allowed us to test a large set of genetic markers and identify novel suggestive SNPs, while the replicated signals had a lower p-value in comparison to the results of initial GWAS, as it has been observed in other studies imputed to HRC.[18] It has been shown that imputations with HRC improve the statistical power to identify both common and low-frequency variants, and weaker signals detected in genotyped GWAS could become stronger in imputed SNPs that potentially could be linked to the causal variant.[43]

In conclusion, we used a GWAS approach and an imputation to one of the largest reference panels to identify novel variants associated with ACEI-induced angioedema. We found that variants in genes of bradykinin pathway, and genes involved in pathways of innate immunity and serine biosynthesis may be associated with ACEI-induced angioedema. These findings should be viewed as hypothesis-generating; a replication and functional studies of the described variants are required to provide more insight into their role in the development of angioedema.

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Supplement

SUPPLEMENTARY TABLE 1. Replicated SNPs from the initial Vanderbilt/Marshfield GWAS

SNP	CHR	Gene	MA	OR, additive genetic model (95% CI)	P-value Pare *et al.*[10]	P-value this study
rs758530	12	*EFCAB4B/CRACR2A*	C	2.34 (1.67-3.30)	1.00×10^{-6}	$^{**}2.88\times10^{-7}$
rs1015762	12	*EFCAB4B/CRACR2A*	G	2.09 (1.51-2.90)	1.03×10^{-5}	1.72×10^{-6}
rs500766	10	*PRKCQ*	T	0.42 (0.28-0.63)	2.97×10^{-5}	8.27×10^{-5}

OR, odds ratio in the initial GWAS (Pare *et al.*, 2013).[10] CI, confidence interval. *EFCAB4B/CRACR2A*, EF-hand calcium-binding domain-containing protein 4b/calcium release activated channel regulator 2A gene. *PRKCQ*, protein kinase C θ gene. **P-value for *EFCAB4B/CRACR2A* rs12425092 (in perfect LD with rs758530).

CHAPTER 3.4

Early health technology assessment in pharmacogenomics: a case example in cardiovascular drugs.

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Abstract

Aims: To assess the required characteristics (cost, sensitivity and specificity) of a pharmacogenomic test for being a cost-effective prevention of ACEI-induced angioedema. Furthermore, we assessed the influence of only testing high risk populations.

Materials & Methods: A decision tree was used.

Results: With a willingness-to-pay (WTP) threshold of €20,000 and €80,000 per QALY, a 100% sensitive and specific test may have a maximum cost of €1.30 and €1.95, respectively. When only genotyping high-risk populations, the maximum test price would be €5.03 and €7.55, respectively.

Conclusions: This theoretical pharmacogenomic test is only cost-effective at high specificity, high sensitivity and a low price. Only testing high-risk populations yields more realistic maximum test prices for cost-effectiveness of the intervention.

Summary points

Angiotensin-converting enzyme inhibitors (ACEI) are commonly used cardiovascular drugs. They can cause the severe and possibly lethal adverse drug reaction angioedema in a very small part (0.2%) of the patients. A pharmacogenomic test could be used to identify patients at risk for this severe adverse drug reaction and advise them to use another drug.

With a willingness-to-pay threshold of €20,000 and €80,000 per quality adjusted life year, a 100% sensitive and specific test may have a maximum cost of €1.30 and €1.95, respectively. When only genotyping high-risk populations, the maximum test price would be €5.03 and €7.55, respectively.

Testing all patients starting an ACEI for developing angioedema is unlikely to be cost-effective as the test should have a high diagnostic accuracy combined with a low price. Selectively testing only populations that have an increased risk of developing ACEI-induced angioedema improves test characteristics needed.

While separate testing for this variation for all ACEI starters or subgroups is not cost-effective, implementing whole exome or genome sequencing in routine clinical practice will result in economically attractive benefits of finding genetic variations like the one discussed here.

Introduction

The use of pharmacogenomics is becoming more common in daily clinical practice. In many cases it improves patient outcomes by predicting the response to drugs or adverse events, allowing health care providers to adjust treatment accordingly.[1] Recent literature shows variation in the performance of pharmacogenomics: it varies from a large effect with a large increase in efficiency to a large increase of costs per patient without much benefit.[2] Technology in pharmacogenomics is advancing and the number of known single nucleotide polymorphisms (SNPs) impacting pharmacological treatment is rapidly increasing. Since both the advancement of technology, as well as an ageing population cause an increased pressure on health care budgets, cost-effectiveness of innovations is on the health care policy agenda of many countries. To determine the coverage of innovations from public funds, several countries use a threshold which indicates the maximum costs to be paid for the gain of one extra quality adjusted life year (QALY) by the new intervention.[3] For the UK for example, the threshold is indicated at £30,000 per QALY. For the Netherlands, the discussion on the threshold is ongoing. The current thresholds range from €20,000 to €80,000 per QALY gained, based on disease burden.[4] The price of testing as well as the effect of genetic variation on treatment response or adverse events is often unknown. From a health system perspective, it is therefore important to assess, at an early stage, the impact of a test in daily practice. When price and the sensitivity (true positive rate) and specificity (true negative rate) of the test are still unknown, the estimation of cost-effectiveness is done in a turn-around analysis: by investigating the required specifications that would make the test a cost-effective diagnostic. The threshold for costs per QALY is used as the basis of this evaluation. By evaluating an intervention at an early stage, its value becomes clear early and this can inform either further research, an implementation trajectory or an exit strategy. In this study, we take a case example of an early health technology assessment (HTA) of the prediction of angioedema caused by the use of angiotensin-converting enzyme inhibitors (ACEIs). ACEIs are amongst the most frequently prescribed drugs and serve as an important treatment modality for several, highly prevalent cardiovascular indications.[5-7] They are generally well-tolerated. Nonproductive, persistent cough is the most common adverse drug reaction (ADR) and occurs in approximately 9% of ACEI users.[8] Besides this mild

and well-known ADR, ACEI can cause rare ACEI-induced angioedema, a serious and frightening sudden swelling of the upper airways that can be fatal.[6,7,9-11]

ACEI-induced angioedema is characterized by a transient, localized swelling of the deep reticular dermis, subcutaneous or submucosal tissues of the head and neck region and occasionally the viscera.[12] It frequently affects the face, lips, tongue and upper airways and is usually accompanied by symptoms such as a lump in the throat, hoarse voice and difficulties in swallowing and breathing.[12] Typically, ACEI-induced angioedema develops over 4-6 hours and resolves within 1-2 days.[12,13] Rare lethal cases with severe airway obstruction have also been reported.[6,7] The factors predisposing to ACEI-induced angioedema are not fully elucidated. Among clinical risk factors of ACEI-induced angioedema are female sex, age over 65 years, African-American ethnicity, local trauma, smoking, history of drug rash, type 2 diabetes, seasonal allergies and ACEI-induced cough. The mechanism of ACEI-induced angioedema is thought to involve the accumulation of bradykinin, due to a dysregulation of its inactivation by ACE and alternative enzymes.[14] Genetic variants identified in the membrane metallo-endopeptidase gene (*MME*) and the X-prolyl aminopeptidase 2 gene (*XPNPEP2*), belonging to the bradykinin degradation pathway, could contribute to the development of AE in some of the patients.[14] However, the effect of genetic variation on the susceptibility to angioedema caused by ACEI is yet to be fully uncovered.

The identification of patients at risk of ACEI-induced angioedema using a pharmacogenomic (PG) test prior to treatment initiation could prevent harm caused by this ADR and reduce healthcare expenses. Hence, the goal of this study was to assess required test characteristics (cost, sensitivity & specificity) in order for the test to be a cost-effective measure for preventing ACEI-induced angioedema. In addition, we investigated the benefits of only testing specific populations that are known to have an increased risk of developing this serious ADR.

Methods

We used a decision tree model to compare genotyping versus no genotyping prior to starting an ACEI. The model reflects the patient pathway and is depicted in **Figure 1**. In constructing the model, we conformed to the ISPOR Modeling Good Research Practices.[15] As angioedema risk is the greatest immediately after starting an ACEI, and

because of scarce data on angioedema risk in long-term ACEI use, a decision tree was the preferred model to simulate patient pathways.

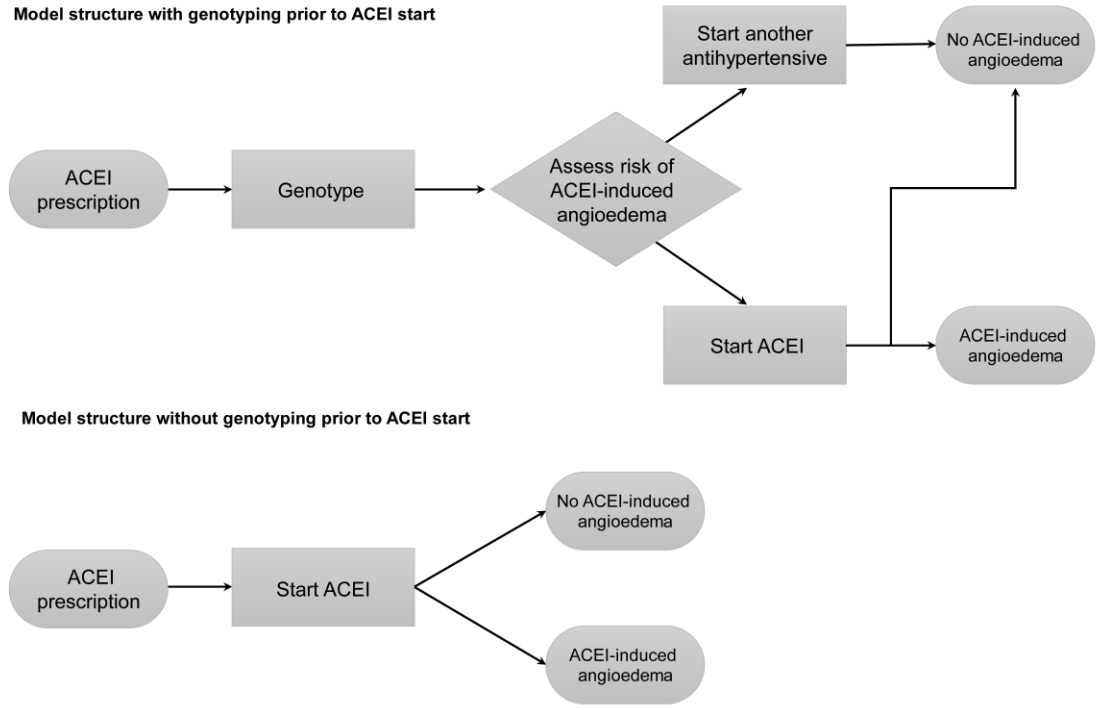

FIGURE 1. Model structures used for the analyses.

Angioedema incidence

ACEI-induced angioedema incidence rates (per 1000 person-years) have been reported to be in the 1.97 – 4.38 range in observational studies by Miller *et al*. and Toh *et al*.[16,17] The OCTAVE randomized controlled trial (omapatrilat versus enalapril) by Kostis *et al*. reported 0.68% of patients developing angioedema during 24 weeks of follow-up.[18] Cumulative incidence of 1.79 (1.73-1.85) per 1,000 persons reported by Toh *et al*. was used for the model input, based on 3,301 events in 1,845,138 exposed persons. 326 (9.88%) of these events were classified as 'serious', indicating the need for inpatient care.[17]

Characteristics of ACEI treatment

After the initial ACEI prescription, patients stop and/or switch to another drug class in up to 44% of cases.[19,20] However, it is unlikely that switching and discontinuation

patterns are influenced by being genotyped for angioedema prior to starting an ACEI. We therefore assumed that all patients stay on the ACEI for one year, unless they develop angioedema or receive a positive diagnosis by a genomic assay. In these cases, according to guidelines, they are switched to another antihypertensive. The price of 'other antihypertensive' is the weighed per person average of the cost per user*number of users of ATC-classes C03 (diuretics), C08 (calcium antagonists) and C07 (beta-blockers), yielding an average cost per user per year of €23.77. This is higher than the annual per user cost of ACEI at €13.62.[21,22] The difference between these two treatments (€10.15) was used as a model input. **Supplementary table 1** presents the data used for calculating treatment costs.

Subgroups

Subgroups of patients with an increased risk of developing ACEI-induced angioedema have been identified by Miller *et al*.[16] Individuals of African ancestry are at a highest risk for developing ACEI-induced angioedema, as shown in **Table 1**.

TABLE 1. Subgroups with increased risk of developing ACEI-induced angioedema.

Risk Factor	OR/HR [Reference]
African ancestry	RR: 3.88 [16]
Age 65-74	RR: 1.42 [16]
Female Gender	RR: 1.45 [16]

HR, hazard ratio. OR, odds ratio.

Estimation of QALYs

Mortality due to ACEI-induced angioedema is extremely rare but, per case, can result in a large loss of QALYs. Evidence on mortality is scarce and is mainly available in the form of case reports. To estimate the mortality risk, studies that recorded intensive care unit (ICU) admittance or direct mortality due to angioedema, were selected. The selected studies are shown in **Supplementary Table 2**. We assumed that all lethal cases would be admitted to the ICU. Then, lethal cases were divided by the total number of patients with angioedema admitted to the ICU to yield a mortality probability of 0.66% per ICU admittance. The average ACEI starter was 62 years old.[23] QALYs lost by premature mortality were calculated using life expectancy data from the Statistics Netherlands and data on quality of life (QoL) per age group, yielding 17.20 QALYs.[24,25]

Utilities

By making assumptions regarding answers to the validated EQ5D questionnaire and using the Dutch value set to calculate utility scores, specific health state utilities were generated.[26]

Costs – resource use

Banerji *et al*. assessed the percentage of ACEI-induced angioedema among all patients with angioedema presenting to the emergency department and described their healthcare requirements.[27] We combined these results with the data presented by Toh *et al*. to calculate the fraction of ICU stays of per total inpatient stays.[17] ICU stays were further specified using data from Soo Hoo *et al*.[28] They investigated ACEI-induced angioedema requiring ICU admission, yielding data on hospitalization duration.[28] Drug utilization for the treatment of angioedema was not assessed, as these costs are included in reference prices for hospital admittance.

Costs - prices

Costs for inpatient stays, GP and ED visits, and ambulance use were based on reference prices published by the Dutch Manual for Costing in Economic Evaluations.[29] Drug utilization and costs were retrieved from The Drug Information System and The Pharmacy Purchase Price database of the Dutch National Health Care Institute.[21,22] All costs are in Euros and, if applicable, indexed to 2016. Because of the one-year time horizon, discounting of future costs and effects was not necessary.

Analysis

The main outcome was the incremental cost-effectiveness ratio (ICER), which is a ratio indicating the extra costs per QALY gained. In the OCTAVE-randomized controlled trial, significantly more patients experienced angioedema in the first month of treatment (3.6/1,000 vs 0.4/1,000 after 24 weeks of follow-up).[18,30] Observational studies by Toh *et al*. and Miller *et al*. reported that respectively 66% and 55% of events occurred in the first 90 days after ACEI initiation.[16,17] Based on these findings, we assumed a one-year timeframe for the development of angioedema and all related healthcare utilization. Model parameters are shown in **Table 2**. Model parameter sensitivity was

assessed by the probabilistic and deterministic sensitivity analyses. In a deterministic sensitivity analysis, robustness of the model was tested for variations between the extremes of a plausible range of all parameters. In a probabilistic sensitivity analysis, uncertainty of the analysis was examined by first constructing distributions for all parameters in the model. Secondly, the model picked a random value for all parameters from these distributions and the results were recalculated. This was repeated 5,000 times and the results were depicted in a scatterplot. We did not vary the cost components, as these were based on reference prices.

TABLE 2. Model parameters and probability distributions.

Parameter	Value	Distribution	EQ5D input
Prob. of visiting ED*	0.4256	fixed	
Prob. of observational stay at ED*	0.0773	beta	
Prob. of patient stay (regular ward) *	0.0515	beta	
Prob. of ICU stay*	0.0472	beta	
Prob. of ambulance*	0.1141	beta	
Prob. of visiting GP*	0.574	beta	
Incidence rate of angioedema (per 1,000)	1.79	beta	
Prob. of mortality*	0.0004	beta	
Cost of visiting ED (€)	170.59	fixed	
Cost of observational stay at ED (€)	283.56	fixed	
Cost of inpatient stay (regular ward) (€)	737.14	fixed	
Cost of ICU stay (€)	8,434.26	fixed	
Cost of requiring ambulance (€)	331.00	fixed	
Cost of visiting GP (€)	28.00	fixed	
Additional cost on other antihypertensive (€)	10.15	fixed	
Utility during ED visit	0.569	fixed	33333
Utility during observational stay at ED	0.569	fixed	33333
Utility during inpatient stay (regular ward)	0.569	fixed	33333
Utility during ICU stay	0.115	fixed	55533
Utility during GP visit	0.638	fixed	22222
Quality of Life lost by fatal angioedema	17.78	fixed	

*Probability is per angioedema event. ED = emergency department, ICU = intensive care unit, GP = general practitioner.

Results

Base-case

The influence of sensitivity, specificity and test price on the ICER is shown in **Figures 2.1** and **2.2.** Data points represent a test price, at which ICER exactly matches the willingness-to-pay (WTP)-threshold (a red point reflects a negative test price, and a green point reflects a positive test price). With WTP-thresholds of €20,000 and €80,000 per QALY, a 100% sensitive and 100% specific test had a maximum cost of €1.30 and €1.95, respectively. A free and 100% sensitive test must be at least 87% and 81% specific to be cost-effective at the aforementioned WTP-thresholds. The ICER of a free and 100% specific test is, only in this scenario, not influenced by sensitivity, as it is

free, and therefore does not generate false positives. At 90% specificity, a free test should be at least 79% and 52% sensitive for €20,000 and €80,000 WTP-thresholds.

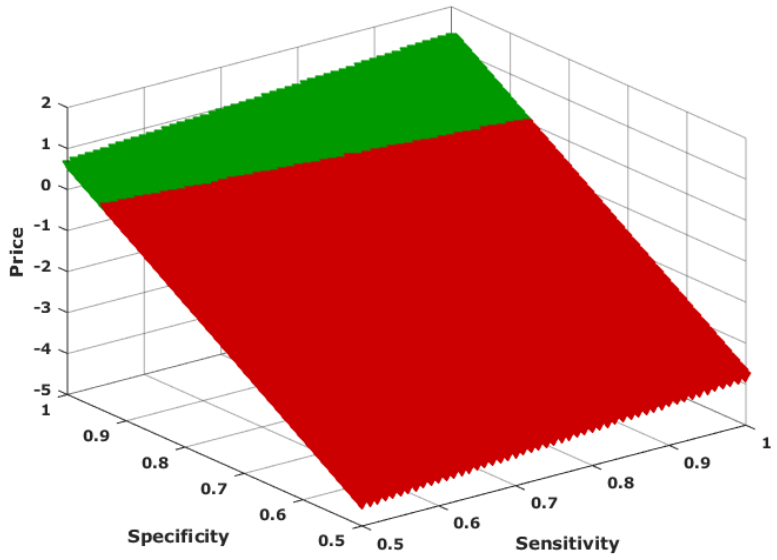

FIGURE 2.1. Base-case results. Maximum test price to meet a willingness-to-pay threshold of €20,000.

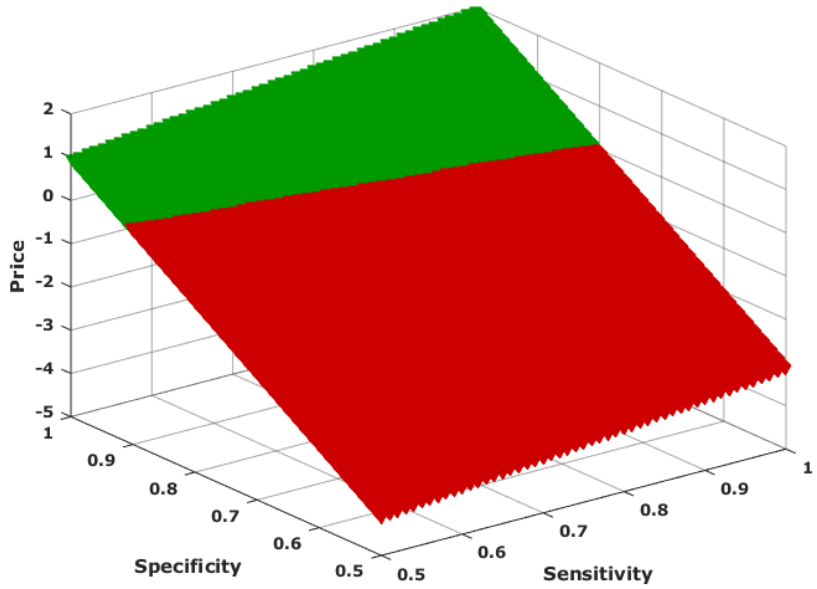

FIGURE 2.2. Base-case results. Maximum test price to meet a willingness-to-pay threshold of €80,000.

A change in specificity had a 3.5-fold higher impact on the ICER than a change in sensitivity. This is due to the additional cost of switching to another, more expensive, antihypertensive treatment in case of false positives. False negatives did not cause

additional costs; they only lowered the maximum, but ever positive price (indicated by a green dot) at 100% specificity and lowest (50%) sensitivity.

Subgroups

Limiting genotyping to individuals at a higher risk for ACEI-induced angioedema had a profound influence on the test requirements.

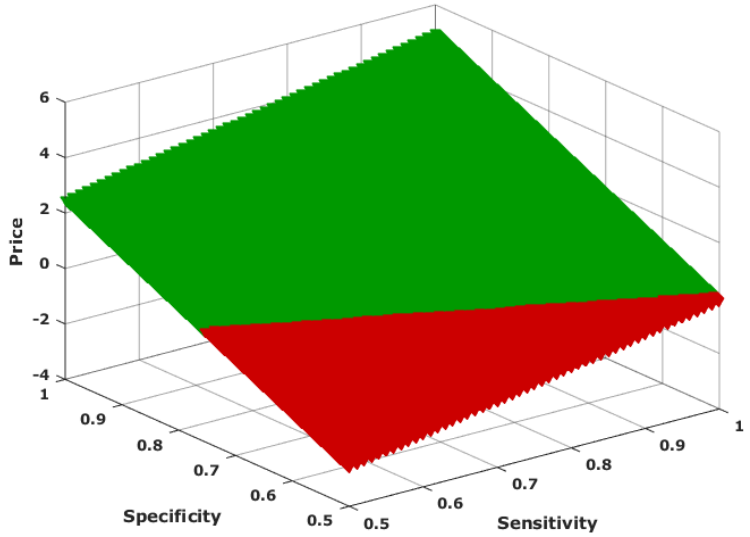

FIGURE 3.1. Subgroup results. Maximum test price when only testing people of African ancestry (HR=3.88) to meet a willingness-to-pay threshold of €20,000.

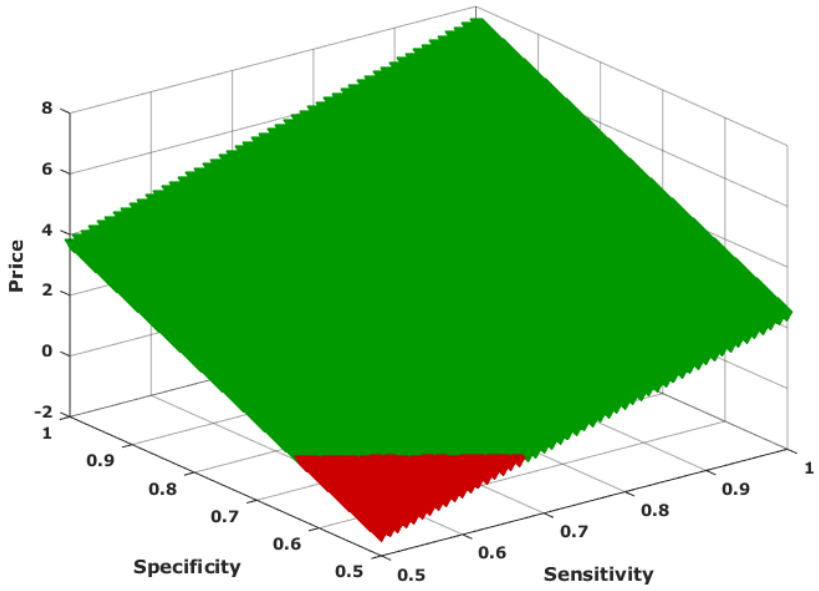

FIGURE 3.2. Subgroup results. Maximum test price when only testing people of African ancestry (HR=3.88) to meet a willingness-to-pay threshold of €80,000.

Figures 3.1 and **3.2** display the relation between test parameters and a maximum price to meet WTP-thresholds of €20,000 and €80,000, respectively. In this scenario, a perfect test met the WTP-thresholds at €5.03 and €7.55. The bandwidth for a positive test price had increased dramatically, as well as the spread between the two WTP-thresholds. The requirement of a high specificity was no longer present: for a 100% sensitive test costing €3.00, the minimum specificity was 81% and 56% for the aforementioned WTP-thresholds. Also, the influence of specificity versus sensitivity decreased from ±3.5:1 to 1:1.

Probabilistic sensitivity analysis

Probabilistic sensitivity analysis (PSA) was based on a 90% sensitive and 90% specific test costing €0.50. Results, shown in Figure 4, indicated a 100% probability for both QALY gain and higher costs. Furthermore, there was a 10.6% and 55.3% probability of meeting €20,000 and €80,000 WTP-thresholds, respectively. The base-case ICER at specified parameters was €56,896. The PSA results were higher and lower than this base case in 57% and 43% of cases.

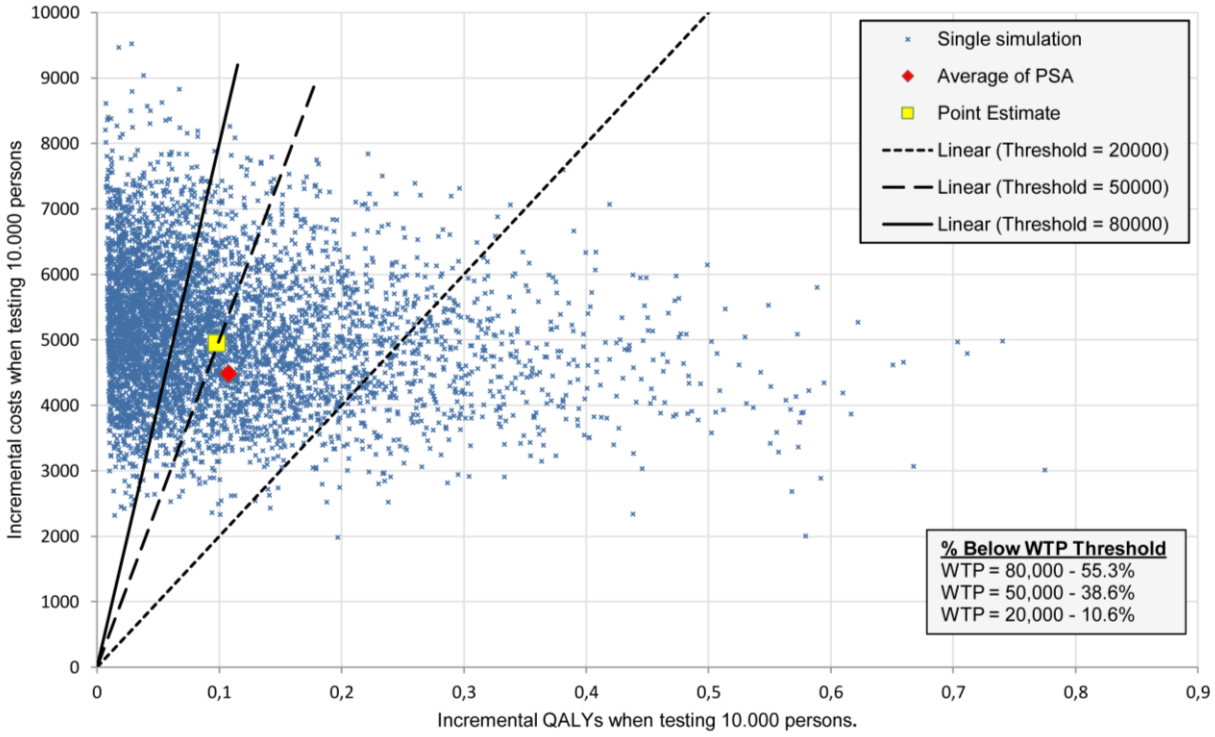

FIGURE 4. Probabilistic sensitivity analysis.
Parameters used: 5000 simulations, fixed test price = €0.50, fixed test sensitivity = 90%, fixed test specificity = 90%.

Deterministic sensitivity analysis

Deterministic sensitivity analysis (DSA), shown in Figure 5, was also performed with a 90% sensitive and 90% specific test costing €0.50.

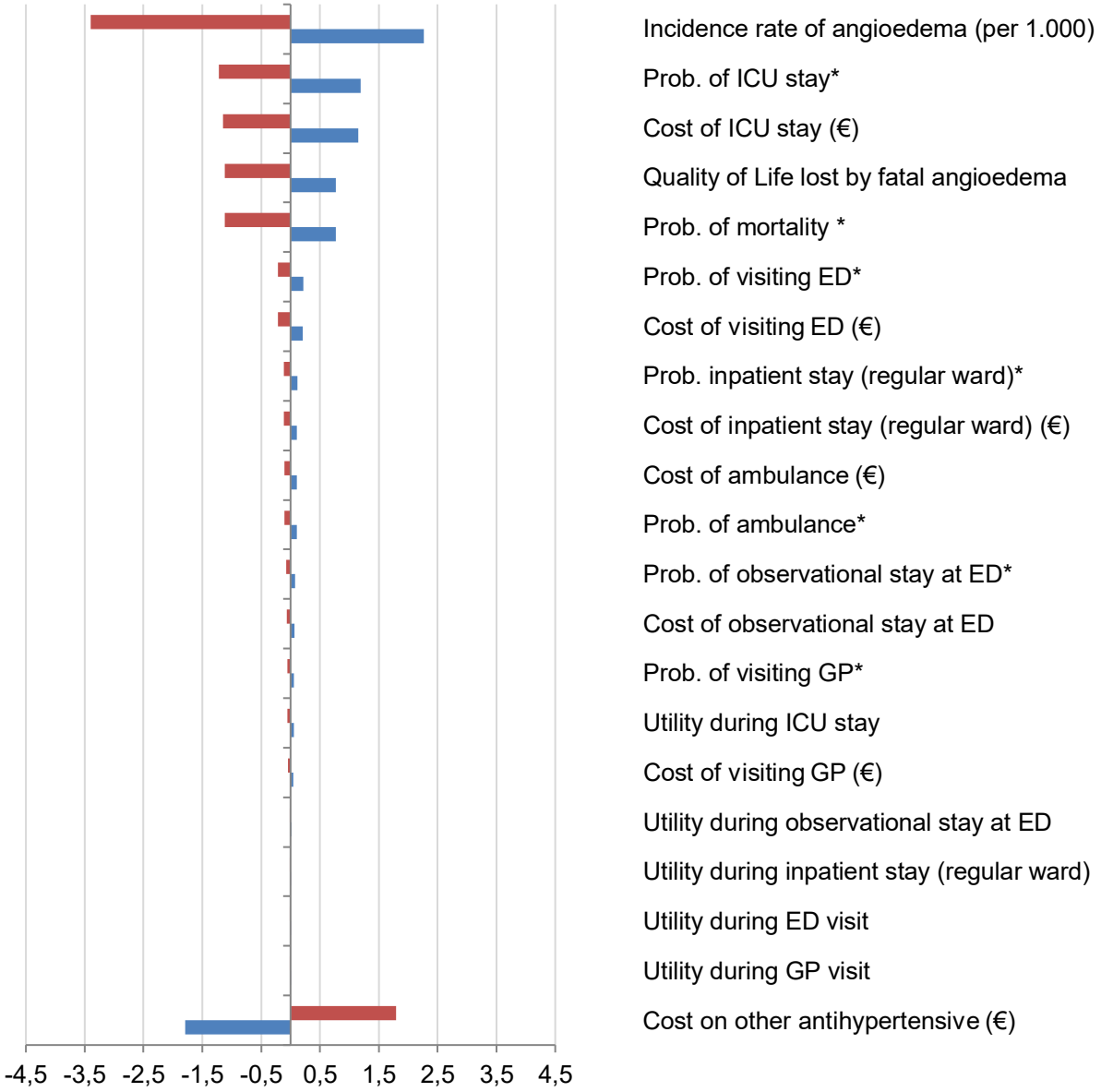

FIGURE 5. Deterministic sensitivity analysis.
X-axis indicates the magnitude of the difference in ICER compared with a parameter. Red indicates a negative parameter change, blue a positive change. Parameters used: fixed test price = €0.50, fixed test sensitivity = 90%, fixed test specificity = 90%. The x-axis indicates the factor of the response versus a change in a parameter.
* Prob, probability is per angioedema event.
ED, Emergency department; GP, General practitioner; ICU, Intensive care unit.

The incidence rate of angioedema resulted in the largest effect, followed by the additional cost of “other antihypertensive”. ICU admission and mortality had a substantial effect on the ICER. Other parameters had a small or negligible influence.

Discussion

We evaluated the specifications of a pharmacogenomic (PG) test for preventing ACEI-induced angioedema in terms of the required specificity, sensitivity and price for achieving cost-effectiveness. Our findings indicate that testing all ACEI starters is unlikely to be cost-effective, as >90% specificity, >93% sensitivity and a low (<€1.00) price would be required. Our results highlight that a focused approach of testing high-risk populations can be a fruitful endeavor for increasing cost-effectiveness. This statement is further supported by the DSA, demonstrating a major influence of angioedema incidence on ICER.

Miller *et al*. reported a relative risk of 3.88 and 1.45 for people of African-American ancestry and for women, respectively. In our model, this had a profound positive impact on parameter requirements. Further clarification of risk factors, for example women of African-American origin, could prove to lower diagnostic accuracy and test price to more favorable ranges, that could warrant actual development of a PG test for this specific indication.

Nevertheless, individual tests for rare ADRs may not be very efficient. Plumpton *et al*. have shown that single testing is not always cost-effective, even when a proper biomarker or SNP is present.[1] Their results indicate that mainly Human Leukocyte Antigen (HLA) polymorphisms are cost-effective single targets. These HLA polymorphisms predispose to hypersensitivity reactions, sometimes leading to very severe ADRs, such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), induced by carbamazepine, abacavir and allopurinol. Not only are these ADRs more severe than angioedema, with mortality ranging from 10% to 40% for TEN, their incidence rates (of up to 5%) are much higher than the incidence rates of ACEI-induced angioedema.[1,31]

There could be a solution to biomarkers that do have value, but are too costly to implement separately: to combine many of these tests into a single package or perform them together with a test that will be performed in routine daily practice. This way, the

fixed costs of sampling, transport to a lab and reporting of the results would be spread, and incremental costs per test could decrease dramatically. We can extend the idea of combining tests to whole-exome or whole-genome sequencing. Currently, these sequencing techniques are considered to be too costly for the implementation in routine clinical practice, but prices have been falling dramatically.[32] When efforts are focused towards making sequencing a routine part of daily clinical practice, all future genomic markers will deliver additional benefit to patients, regardless of the rarity of the predictor. Sadly, the full potential value that innovations may deliver in the future cannot be captured in traditional cost-effectiveness analysis.

The two most important limitations of our study need to be addressed. Firstly, the DSA indicates a strong influence of the additional cost of antihypertensive treatment. This is the cost of a false positive case. In Dutch practice, switching to another antihypertensive is more expensive than ACEI treatment. This price difference is likely to be country-specific. In other jurisdictions, where ACEI treatment is more expensive than other antihypertensive treatment, the genotyping strategy would result in drug-cost savings in the event of a (false) positive diagnosis.

Secondly, model parameters were based on multiple studies with different study designs possibly leading to biased estimates. Especially our assessment of mortality risk was based on suboptimal evidence that required some assumptions. However, the DSA indicates a relatively low influence of mortality risk on model outcomes. Utility scores were assessed by estimating the answers to the EQ5D questionnaire which is clearly sub-optimal. The DSA indicates that these parameters have a negligible effect on the results. Furthermore, cost-parameters could be underestimated. Only ICU-related costs seem, as shown by the DSA, to have some impact on the ICER.

Conclusion

Our study indicates that testing all patients starting an ACEI for developing angioedema is unlikely to be cost-effective, as the test should have a high diagnostic accuracy combined with a sub-€2.00 cost. Selectively testing only populations that have an increased risk of developing ACEI-induced angioedema improves test characteristics needed and price for an ICER below €20,000 and €80,000. While separate testing for this variation for all ACEI starters or subgroups is not cost-effective, implementing whole-exome or whole-genome sequencing in routine clinical practice

could result in economically attractive benefits of finding genetic variations like the one discussed here.

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Supplement

SUPPLEMENTARY TABLE 1. Cost of switching to another antihypertensive drug.

Other antihypertensive drugs	ATC	No. users	Cost/user (2014)	Total costs
Diuretics	C03	1,143,000	22.48	25,694,640
Calcium antagonists	C08	831,430	39.09	32,500,599
Beta blockers	C07	1,642,000	16.91	27,766,220
	sum:	**3,616,430**	sum:	**85,961,459**
Average cost per user per year:	**23.77**			
ACEI (cost/user)	13.62	Difference:	10.15	

SUPPLEMENTARY TABLE 2. Studies included in the mortality assessment.

Study [ref.]	No. Angioedema	No. ICU	No. mortality
OCTAVE [18]	86	0	0
ALLHAT [33]	38	1 (assumed)	1
Grant [34]	228	0	0
Soo Hoo [28]	50	50	0
Banerji [27]	220	24	0
Kyrmizakis [35]	31	1	0
Chan [13]	88	75	0

CHAPTER 4

GENERAL DISCUSSION

1. Introduction

Vitamin K antagonists (VKAs) and angiotensin-converting enzyme (ACE)-inhibitors are widely prescribed drugs that can be safely used in the majority of patients, but can also cause adverse drug reactions (ADRs).

VKAs have been the mainstay of oral anticoagulation therapy for many years and proved their efficacy for the management of atrial fibrillation (AF) and venous thromboembolism (VTE) in randomized controlled trials (RCTs).[1,2] The VKA dose necessary for optimal anticoagulation varies between patients and is affected by, among others, single nucleotide polymorphisms (SNPs) in the vitamin K oxidoreductase complex 1 (*VKORC1*) and cytochrome P450 (*CYP2C9*) genes. Suboptimal VKA dosing can lead to excessive or insufficient anticoagulation, increasing the risks for bleedings and thromboembolic complications.

ACE-inhibitors are indicated for hypertension, heart failure and chronic renal disease. Approximately 5%-20% of patients treated with ACE-inhibitors develop a persistent dry cough,[3,4] and between 0.2% and 0.7% develop bradykinin-mediated ACE-inhibitor-induced angioedema of the upper airways that can be life-threatening.[3,5,6]

The efficacy of anticoagulation therapy with VKAs could be optimized and ADRs associated with ACE-inhibitors avoided by studying the environmental and genetic factors that explain heterogeneity of drug response. In this thesis, we evaluated algorithms for individualized dosing of VKAs and carried out epidemiological, genetic and cost-effectiveness studies of angioedema induced by ACE-inhibitors. This chapter presents our main findings and their relevance from a broader perspective. We discuss the strengths and limitations of the methodology and address several considerations for clinical practice and future research.

2. Main findings and relevance

2.1 Revisiting approaches to improve VKA dosing

The first part of this thesis is dedicated to personalized VKA dosing that aims at improvement of anticoagulation control. The use of a genotype-guided dose prediction algorithm, containing information about the *VKORC1* and *CYP2C9* polymorphisms could allow to prescribe a personalized dose to patients commencing VKA therapy, thereby reducing unwanted complications of VKA treatment. In 2013, the added value

of genotyping before the initiation of VKA therapy for AF and VTE was investigated in RCTs. These clinical trials compared genotype-guided dose prediction algorithms with algorithms containing only clinical variables (EU-PACT acenocoumarol/phenprocoumon arm and COAG).[7,8] Also, genotype-guided dosing was compared with standard clinical care in the EU-PACT warfarin arm.[9] Results of clinical trials did not provide unanimous support for the clinical implementation of genotype-guided VKA dosing, based on the measurement of percentage of time in therapeutic INR range for different dosing strategies (**chapter 2.1**). When compared to standard clinical care, there was an improvement of anticoagulation control with genotype-guided warfarin dosing,[9] but no differences for the primary outcome were observed between the genotype-guided and clinical algorithms for warfarin, acenocoumarol and phenprocoumon.[7,8] When we assessed the parameters of EU-PACT and COAG algorithms, we hypothesized that algorithm performance in clinical trials and some of the disparity in trial results could be explained by a difference in distributions of *VKORC1* and *CYP2C9* genotypes in the trial population as opposed to patient cohorts used to derive the algorithms. We investigated, whether there were differences in over- or underanticoagulation between patients with different genotypes after dose titration by genotype-guided and clinical EU-PACT algorithms in the first 5-7 days of VKA therapy. We found that four weeks after therapy initiation genotype-guided strategy in the acenocoumarol/phenprocoumon arm of EU-PACT resulted in a higher percentage of time below therapeutic INR range in the *VKORC1* AA - *CYP2C9**1*1 carriers (**chapter 2.2**). Our findings from this sub-group analysis underscore the importance of using a representative and diverse derivation cohort for algorithm construction and suggest considering the robustness of algorithms when interpreting results of clinical trials.

The performance of a dosing algorithm can vary if parameters included in linear regression in the derivation cohort are less important for the dose variability in other patient populations. Since there is currently no universally used dosing algorithm for VKAs, novel algorithms are being developed for patients of European descent in different countries and for different ethnic populations. We used the EU-PACT acenocoumarol/phenprocoumon trial dataset to examine the performance of several dose prediction algorithms from published studies in patients of European ancestry (**chapter 2.3**). As expected, genotype-guided algorithms for acenocoumarol containing

VKORC1 and *CYP2C9* genotypes explained a higher percentage of dose variability (53.9-62.1%) than algorithms using clinical information only (18.5-28.9%). Genotype-guided algorithms for phenprocoumon also could explain over 50% of dose variability. The most accurate prediction of mean daily acenocoumarol maintenance dose was achieved with the EU-PACT algorithm, while no differences were seen between EU-PACT phenprocoumon algorithm and two other evaluated genotype-guided algorithms. Although there are differences in genetic variants influencing the VKA dose requirements between different ethnic groups, and yet unknown genes can contribute to the dose variability, the predictive ability of existing algorithms in European populations seems similar. From a clinical usefulness perspective, an ideal VKA dosing algorithm should include all variables of importance (for instance, ethnicity, anthropometric measures, vitamin K intake and interacting drugs) and still be applicable across populations.

2.1 Studies of ACE-inhibitor-induced ADRs

In the second part of this thesis, we described the recruitment of patients with ACE-inhibitor-induced angioedema in the PREDICTION-ADR project, addressed the role of chronic comorbidities, co-medication and common genetic variants in the development of ACE-inhibitor-induced angioedema and investigated economic issues surrounding the implementation of a potential pharmacogenetic test for this ADR. We performed two explorative nested case-control studies in a cohort of patients starting ACE-inhibitor therapy from a large anonymized UK primary care database containing electronic medical records and prescription data (Clinical Practice Research Datalink, CPRD). In these studies, we investigated the association between chronic comorbidities and co-medication with angioedema occurring for the first time during ACE-inhibitor therapy, and with ACE-inhibitor intolerance that was defined as a switch in prescriptions from ACE-inhibitors to angiotensin II receptor blockers (ARBs; **chapter 3.1**). Switching from ACE-inhibitors to ARBs has been shown to be the best marker of ACE-inhibitor-related ADRs in prescription databases and primarily indicates the presence of cough associated with ACE-inhibitor use.[10] In CPRD data we found that a history of allergy and use of anti-allergic medication were more prevalent in patients with angioedema and switching to ARBs. In accordance with previous studies, we also found that patients with diabetes may be less susceptible to angioedema.[11,12] Other

associations in our study were in line with results of previous analyses of risk factors of ACE-inhibitor-induced angioedema.[13] Our analyses could not establish causality and residual confounding cannot be ruled out, however the overlap between comorbidities and co-medication associated with both angioedema and ACE-inhibitor intolerance (essentially ACE-inhibitor-induced cough) could indicate a common underlying pathway for these ADRs. The bradykinin pathway was implicated in both angioedema[14] and cough[15] related to ACE-inhibitor therapy, and there is evidence of genetic susceptibility to these ADRs.[16-19] To investigate the genetic etiology of ACE-inhibitor-induced angioedema, DNA samples and clinical data of patients with angioedema have been collected for a multicenter case-control study as part of the European international Personalization of treatment in cardiovascular disease through next generation sequencing in adverse drug reactions (PREDICTION-ADR) project. We reviewed the recruitment process in PREDICTION-ADR centers, summarized the characteristics of 345 cases of ACE-inhibitor-induced angioedema and 47 cases of angioedema related to ARBs, and evaluated the association of angioedema with comorbidities (**chapter 3.2**). This study assessed the clinical presentation and culprit drugs in the largest dataset of patients with ACEI-induced angioedema available to-date. It showed that angioedema due to ACE-inhibitors in most of the patients occurred after years of ACE-inhibitor treatment.

While PREDICTION-ADR centers are currently analyzing the exome-sequencing data of patients with ACEI-induced angioedema, we conducted a genome-wide association study (GWAS) in 174 patients with angioedema and 489 ACE-inhibitor-treated controls in collaboration with Vanderbilt University (**chapter 3.3**). To provide improved coverage of SNPs and increase statistical power to detect an association at the genome-wide significant level ($P < 5\times10^{-8}$) we performed imputation the novel Haplotype Reference Consortium (HRC) reference panel.[20] One of the top associated signals was rs55940712 near the bradykinin receptor 2 (*BDKRB2*) gene that conferred a decreased risk of angioedema. Variants strongly associated with an increased risk of angioedema were SNPs near genes involved in innate immunity (*EFCAB4B*) and serine biosynthesis (*PSAT1*). None of the SNPs reached the genome-wide significance threshold, and a replication in a larger dataset preferably imputed to the HRC panel is required. Using the HRC imputation panel allowed us to analyze over 5 million SNPs as compared to approximately 500,000 genotyped SNPs reported in the first GWAS

conducted in the Vanderbilt samples.[21] This highlights the advantage of imputation for the identification of potentially causative variants that could be otherwise missed when only using genotyped SNPs.[22,23] Evidence from the first GWAS in Vanderbilt[21] and our own findings did not support a role of a single genetic variant with a large effect size in the development of ACE-inhibitor-induced angioedema (**chapter 3.3**), as shown, for instance, for the human leukocyte antigen HLA-DQB1 locus in clozapine-induced agranulocytosis.[24] On the other hand, whole exome-sequencing approach used in PREDICTION-ADR is advantageous to using a genotyping chip containing a predefined set of SNPs, because it could reveal rare variants unique for the patients' ACE-inhibitor-induced angioedema. If a pharmacogenetic marker for angioedema was discovered, genotyping patients before the start of ACE-inhibitor therapy would be likely to increase healthcare costs. We investigated the economic aspects of genotyping for a hypothetical pharmacogenetic marker of angioedema by evaluating the required sensitivity, specificity and price of this test that would make it a cost-effective intervention (**chapter 3.4**). We found that genotyping all patients starting ACE-inhibitor therapy is unlikely to be cost-effective, because it would require a test with a near-perfect diagnostic accuracy that would cost under €2. This hypothetical test could become cost-effective when restricting its application to patients at a higher risk of angioedema based on ethnical or demographic characteristics, or if it would be a part of a panel of pharmacogenetic tests.

3. Strengths and limitations

3.1 VKAs

For the studies described in the first part of this thesis we used data of the randomized controlled EU-PACT trial. One of the strengths of EU-PACT is that it included all available VKAs (warfarin in the UK and Sweden, acenocoumarol in the Netherlands and Greece, and phenprocoumon in the Netherlands). Secondly, the risk of bias, such as selection and information bias, and confounding is generally lower in RCTs than in observational studies. While a carefully selected population of a RCT could be not representative enough of standard clinical practice, the inclusion criteria of the EU-PACT trial were broad to ensure resemblance to a clinical setting in which genotype-guided VKA dosing for patients with AF or VTE could be applied.

By performing a secondary analysis of the EU-PACT data, we were limited by statistical power issues owing to the small size of the sub-groups. Sub-group analyses in general have been criticized for the methodological issues, power issues and interpretation.[25] Another limitation of these data is that the EU-PACT acenocoumarol and phenprocoumon algorithms were developed using retrospective observational data from the pre-EU-PACT study. Pre-EU-PACT included all patients requiring VKA therapy in the low intensity range (INR 2.0-3.5), while the EU-PACT trial had stricter inclusion criteria (among others, patients should have not been treated with VKA before and the duration of treatment should be at least 12 weeks).

3.2 ACE-inhibitors

3.2.1 Data source

The study of determinants of ACE-inhibitor-related ADRs in the second part of the thesis was carried out with the CPRD database, a large real-life longitudinal database of UK primary care often used for drug utilization and epidemiological studies. CPRD contains detailed anonymized electronical medical records, laboratory data, prescriptions and information about lifestyle factors in a representative sample of the UK population.[26] Compared to RCTs, the risk of selection, information and confounding bias is higher in observational data. CPRD does not contain drug-dispensing data, and therefore, an uncertainty remains about patients filling prescriptions, and some degree of misclassification of the exposure cannot be completely ruled out. There are no standard definitions for diagnoses in CPRD, so Read code lists were needed to identify exposures and outcomes. Furthermore, when defining ADRs, such as ACE-inhibitor-related angioedema in CPRD, it is not possible to be certain of the actual type of angioedema (drug-related ADR, hereditary angioedema or allergy). Also, variations between general practitioners in coding of diagnoses in electronic medical records and the timing of diagnose registration in CPRD could lead to misclassification.[27] Additionally, defining the moment to measure the lifestyle variables (body mass index, smoking, alcohol use) can be challenging in CPRD, since this information is not always available around the time of the event and could be measured more often in specific patient subgroups.[27,28] Missing values in the CPRD can make it difficult to define prescription patterns, therefore we used imputed values for the defined daily doses in the prescription data.

3.2.2 PREDICTION-ADR

One of the important advantages of the PREDICTION-ADR study design is that it utilized standardized phenotypic criteria for ACE-inhibitor induced angioedema,[29] that were applied during patient recruitment by all centers. The collaboration between international centers also enabled enrolment of a large number of patients, which is important for a rare ADR, such as angioedema. In contrast, the selection of control subjects for angioedema cases became a critical issue, because of the multicenter character of the project and due to the occurrence of angioedema years after treatment with ACE-inhibitors. To circumvent this in our CPRD study we matched cases and controls on the duration of ACE-inhibitor therapy. However, for PREDICTION-ADR we chose an arbitrary duration of at least 1 year without angioedema for control subjects. While we do not expect genetic analyses in PREDICTION-ADR to be affected by these decisions, they were a limitation in our analyses of comorbidities in this dataset (**chapter 3.2**).

Another advantage of PREDICTION-ADR is applying whole-exome sequencing (WES) instead of a GWAS approach to identify genetic markers of angioedema. Although over the past years GWA studies identified thousands of genetic loci associated with various phenotypic traits and diseases, genotyping microarrays cover only the known variation in the genome and not rare variants. It is challenging to interpret GWAS results, because most disease-associated loci have no clear functional roles in disease etiology, and only explain a small portion of disease heritability.[30] WES, on the other hand, allows to study all variants in coding regions in an individual, therefore giving more opportunities to identify causal variants.[31] The cost of sequencing technologies has been decreasing over the past years, but GWAS is often preferred due to its lower cost. Next to high costs of WES, the complexity of data analysis and summarizing is a disadvantage of this strategy.

With PREDICTION-ADR sequencing efforts still ongoing we did not have an actual pharmacogenetic marker of ACEI-induced angioedema for our cost-effectiveness analysis. We were limited by the uncertainty about the genotype frequency in various ethnic populations, that could have an impact on cost-effectiveness of a pharmacogenetic test. Also, the absence of data regarding the effectiveness of a pharmacogenetic test for angioedema contributed to the uncertainty of the analyses. Furthermore, our study assumed the Dutch healthcare setting and willingness-to-pay

thresholds, but the estimated characteristics of a pharmacogenetic test for ACE-inhibitor-induced angioedema could be different for other countries.

4. Implications

4.1 Implementation in clinical practice

Although the impact of the studies presented in this thesis on clinical implementation is limited it is important to address the use of pharmacogenomics in the clinic. A widespread adoption of pharmacogenetic testing has not yet taken place to-date, with an exception of a few examples, including testing for thiopurine methyltransferase (TPMT) polymorphisms prior to mercaptopurine prescription and HLA-B*57:01 genotyping prior to abacavir prescription.[32] These two tests illustrate application of pharmacogenomics in the areas of drug metabolism (TPMT) and ADRs (hypersensitivity reactions to abacavir).

Information about clinically actionable genetic biomarkers affecting effectiveness and safety of a drug ideally should be available at the point-of-care, through electronic medical records (EMR), and could be incorporated into a large panel of genes (instead of gene-drug pairs) to increase cost-effectiveness.[33] The design of workflow is challenging and requires decisions about the timing of genotyping (prior to start of therapy or for diagnosis of an ADR),[34] which professional should order the test and for which patient. Pharmacist and physician awareness and education about pharmacogenomics, integrating computerized tools into EMR to deliver pharmacogenetic alerts, and clear reimbursement policies for pharmacogenetic tests are some of the barriers for clinical implementation.[35] Additionally, more evidence of clinical utility of a panel of biomarkers is required.[35] Generally, RCTs are considered the best evidence for clinical utility. Thus, given mixed results of RCTs with algorithms for VKAs containing *VKORC1* and *CYP2C9* genotypes, the idea of integrating genotype into VKA dosing decisions has been questioned. However, the results of EU-PACT warfarin and EU-PACT acenocoumarol/phenprocoumon in the first 4 weeks of treatment were positive. Newly emerged data from the randomized clinical Genetic Informatics Trial (GIFT) of Warfarin to Prevent Deep Vein Thrombosis in patients initiating warfarin for elective hip or knee arthroplasty showed that genotype-guided warfarin dosing, compared with clinically-guided dosing, reduced the combined risk of major bleeding, INR of 4 or greater, VTE, or death.[36] More data on clinical

implementation of pharmacogenomics of warfarin and 42 other drugs in different ethnic groups is underway through collaboration of multiple centers in the US and Europe.[35] Finally, clinical implementation will probably depend on geographical differences in drug use and clinical practice in different countries. For example, the updated Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines 2017 recommend using published pharmacogenetic algorithms for warfarin dose determination if *VKORC1* and *CYP2C9* *2 and *3 genotypes are available.[37] The Dutch Pharmacogenetics Working Group (DPWG) additionally gives therapeutic recommendations for acenocoumarol and phenprocoumon, but only provides a decrease in the loading dose.[38] In the Netherlands, anticoagulation during VKA therapy is strictly monitored by anticoagulation clinics; this influences the need to use pharmacogenetic information during VKA therapy.

4.2 Future

There is still a place for warfarin and other VKAs in oral anticoagulation therapy, and this might be even more the case if genotyping to predict drug response will become routine in the future.[32] The role of (genotype-guided) VKA therapy will be also determined by the use of novel non-VKA oral anticoagulants (NOACs). In RCTs, NOACs are at least as effective in preventing stroke in AF as VKAs, with less life-threatening bleedings.[39,40] Drug utilization studies showed that dispensing of NOACs is becoming more prevalent than VKAs.[41,42] The actual number of patients starting VKA therapy according data from Dutch pharmacies has been declining since the introduction of NOACs in 2011, while the share of NOACs in oral anticoagulants has grown to 57% of prescriptions among new patients (van den Heuvel *et al.*, submitted for publication). However, VKAs remain the anticoagulants of choice in patients undergoing mechanic cardiac valve replacement surgery.[43] Furthermore, a recent RCT meta-analysis with a cost-effectiveness analysis found no strong evidence that NOACs should replace warfarin or low molecular weight heparin in primary prevention, treatment or secondary prevention of VTE.[44] At present the costs of NOACs is still high[32] and some challenges with NOAC use remain (antidotes not easily available and some patients have contraindications for the use of NOACs).[45]

Another factor to be taken into account in future personalized VKA therapy are population-specific variation in gene regulation and allele frequencies.[46,47] African

American, Asian and Hispanic populations have greater variability in VKA dose requirements and are at greater risk for experiencing adverse events compared with Europeans.[47,48] The SNPs affecting dose requirements also differ between ethnicities, and new genetic variants are being investigated. The COAG trial found no improvement in time in therapeutic INR range with genotype-guided algorithm in African Americans (27% of the trial participants).[8] New ethnicity-specific VKA dosing algorithms are being developed, but it is unclear whether an improved performance of an algorithm accounting for ethnic stratification will be clinically relevant.[49] It is doubtful that an accurate algorithm could be created for each nationality or individual ethnic group.[47] In future, novel computational methods that utilize knowledge of drug pathways and quantitative trait loci, could be used to effectively predict individualized VKA doses for different ethnicities.[50]

Predicting ADRs to ACE-inhibitors is not feasible with the current knowledge. However, recent GWAS of ACE-inhibitor-induced cough found interesting signals suitable for further replication and possibly functional research.[51,52] Future studies of ACE-inhibitor-induced angioedema and other rare ADRs should focus on reducing the heterogeneity of clinical phenotype definition across studies to facilitate replication and meta-analysis of the results. Recruitment into pharmacogenetic studies of severe ADRs is often difficult because of low occurrence rates. This can lead to statistically underpowered studies and warrants international collaboration efforts for identification of patients and data collection.

Advances in next-generation sequencing technologies allow to investigate the role of rare genetic variants in ADR predisposition.[53] However, to gain more insight into the mechanisms of drug response and ADRs not only analyses of DNA sequence variations, but also more comprehensive approaches of systems medicine (such as network and pathway analysis) are required.[54] Epigenetic modifications, metabolomics and proteomics data will be integrated to understand the global relationship among genotype, environment, and phenotype.[55] In the future we will not be relying on single biomarkers, but on multiple biomarker panels, constructed using ‘omics’ data.

5. Conclusion

The studies described in this thesis focused on personalized VKA dosing and ADRs to ACE-inhibitors. Our studies addressed the robustness of VKA dosing algorithms, and

indicated that they may not be equally effective among all *VKORC1* and *CYP2C9* genotypes. The percentage of dose variation explained by linear regression-based algorithms in Europeans is comparable, however the accuracy of dose prediction differs, because it depends on algorithm variables determined by the inherent characteristics of the derivation cohort. Our studies in ACE-inhibitors showed, among others, that individuals with a history of immunologic disorders may be more prone to angioedema and ACE-inhibitor intolerance. Analysis of patient data from PREDICTION-ADR reminded of the need for clinicians' awareness of angioedema even after years of ACE-inhibitor treatment. Genetic susceptibility to angioedema will be further studied in WES data, while our GWAS analysis indicated a possible involvement of variants in bradykinin and immune pathways. For any pharmacogenetic marker, it is important to assess cost-effectiveness. Using a marker for ACE-inhibitor-induced angioedema could be cost-effective, as genotyping becomes less costly and with an expectation that genomic data of the majority of patients will be routinely available in the clinic.

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APPENDICES

APPENDIX I

Scientific summary

Scientific summary

Vitamin K antagonists (VKAs) and angiotensin-converting enzyme (ACE)-inhibitors are commonly used cardiovascular drugs worldwide. In general, VKAs and ACE-inhibitors are well-tolerated by the majority of patinets, however adverse reactions do occur in some individuals. The initiation of VKA treatment is associated with a challenging dose-titration process due to a narrow therapeutic window and a large variability of the required dose among the patients. This can lead to over- or under-anticoagulation resulting in bleedings and thromboembolic complications. ACE-inhibitors can cause a rare and potentially life-threatening angioedema of the upper airways. This serious adverse drug reaction can manifest within a few days after the start of ACE-inhibitor therapy, however it can also occur many months later. The precision medicine approach can serve to improve the effectiveness of anticoagulation therapy with VKAs and the safety of ACE-inhibitor use by prescribing individualized VKA doses and being able to predict the development of angioedema.

Chapter 1 provides a general introduction and describes the aims of this thesis. We aimed to evaluate the effect on anticoagulation control and performance of genotype-guided and clinical dose prediction algorithms for VKAs. We also aimed to study various determinants (comorbidities, co-medication and genetic factors) of angioedema related to ACE-inhibitors, and to assess the cost-effectiveness of a pharmacogenetic test predicting angioedema before the start of ACE-inhibitor therapy.

Chapter 2 focusses on personalized treatment with VKAs and dosing algorithms for acenocoumarol and phenprocoumon. **Chapter 2.1** provides more background information on this subject, reviewing the evidence from recent RCTs of genotype-guided VKA dosing and discussing possible economic consequences of clinical implementation of VKA pharmacogenetics. The management of anticoagulant therapy with VKAs requires strict monitoring of the International Normalized Ratio (INR) in order to adjust the initial standard loading dose that is prescribed in the first few days of treatment. INR is frequently measured until a stable dose is reached and INR remains in therapeutic range. Since a stable VKA dose needed for therapeutic anticoagulation markedly varies between patients, dose prediction algorithms have been developed to prescribe a more personalized dose and improve the dose-finding process. This approach could reduce the chance of over- and under-anticoagulation, hence the number of bleedings and thromboembolic complications during VKA therapy would

decline. Dosing algorithms contain either only clinical factors affecting the VKA dose variability (such as demographics and co-medication) or a combination of clinical and genetic information. Genotype-guided dosing algorithms containing vitamin K epoxide reductase subunit 1 (*VKORC1*) and cytochrome p450 2C9 (*CYP2C9*) polymorphisms were compared with clinical algorithms (in the COAG and EU-PACT acenocoumarol / phenprocoumon trials) and with routine clinical care (in the EU-PACT warfarin trial) in their ability to improve percentage of time in therapeutic INR range (PTIR). The results of EU-PACT warfarin and the first four weeks of EU-PACT acenocoumarol / phenprocoumon trial showed benefits of the genotype-guided dosing, however in African American participants of COAG this strategy was less effective and resulted in a decreased PTIR. Given the differences in the importance of genetic variants between ethnicities, ethnicity-specific pharmacogenetic algorithms should be tested in other populations. Ongoing trials are expected to provide evidence on clinically bleedings and thromboembolic complications while genotype-guided dosing is applied. Cost-effectiveness studies indicated that genotype-guided dosing could be cost-effective, but its clinical implementation would depend on the cost of pharmacogenetic tests and the availability of novel oral anticoagulants.

The performance of dosing algorithm can vary across genotypes. In **chapter 2.2** we described a secondary analysis of the EU-PACT acenocoumarol / phenprocoumon data assessing the effect of algorithms on anticoagulation control after stratification by *VKORC1* and *CYP2C9* genotypes. Four weeks after therapy initiation genotype-guided dosing increased the mean PTIR in the *VKORC1* GG-*CYP2C9* *1*1 sub-group. For the *VKORC1* AA - *CYP2C9* *1*1 sub-group, there was a higher risk of under-anticoagulation with the genotype-guided algorithm. Twelve weeks after therapy initiation no statistically significant differences in anticoagulation control between trial arms (genotype-guided vs. clinical algorithm) were noted across the *VKORC1*-*CYP2C9* genetic sub-groups. Refinement of the EU-PACT PG algorithms could increase the benefit of genotyping for *VKORC1* and *CYP2C9* variant allele carriers.

We compared the performance of EU-PACT algorithms with seven previously published algorithms for acenocoumarol and phenprocoumon in **chapter 2.3.** The explained percentage of variability in stable dose was 53.9 - 61.1% and 53.7 - 56.9% for acenocoumarol and phenprocoumon genotype-guided algorithms. The R^2 of acenocoumarol clinical algorithms was 18.5 - 28.9%. As measured by the (percentage)

mean absolute error (MAE), EU-PACT acenocoumarol algorithms had a highest dose predicting accuracy, and the accuracy of phenprocoumon genotype-guided algorithms was nearly similar. We suggest that while ethnicity-specific algorithms may be needed in African Americans and Asians, the development of additional algorithms for acenocoumarol and phenprocoumon in European-ancestry populations seems unnecessary.

In **chapter 3** of this thesis we investigate ADRs to ACE-inhibitors, focusing on angioedema of the upper airways. **Chapter 3.1** describes the associations of concomitant chronic diseases and concomitant medication with ACE-inhibitor induced ADRs. We conducted two nested case-control studies in a UK primary care database Clinical Practice Research Datalink (CPRD), using angioedema during ACE-inhibitor therapy and switching from ACE-inhibitors to angiotensin receptor II blockers (ARBs) as outcomes. Switching to ARBs was previously shown to be a marker of ACE-inhibitor-induced ADRs in prescription databases (and mostly reflects the presence of ACE-inhibitor-induced dry cough). We found that a history of allergies and diseases with an allergic component (such as asthma) were more frequent among patients with ACE-inhibitor-induced ADRs than controls. Various comorbidities (for example, asthma and allergies) and co-medication classes (including calcium channel blockers, antihistamines and systemic corticosteroids) were associated with both outcomes, however there were some differences (for example, rheumatoid arthritis). In the context of precision medicine, knowledge of these associations – if replicated in other studies – could be potentially utilized in prediction models alongside genetic information to assess the risk of ACE-inhibitor-induced ADRs in individual patients, and to adjust therapy accordingly.

In **Chapter 3.2** we describe the characteristics of 392 angioedema cases recruited in the PREDICTION-ADR project: 345 related to ACE-inhibitors and 47 related to ARBs. We described the enrollment process and summarized our data on clinical presentation, culprit drugs and time of occurrence of angioedema. Using data of PREDICTION-ADR ACE-inhibitor-treated controls that were defined in databases of prescription and genetic data, we also assessed the association of ACE-inhibitor-induced angioedema with comorbidities. We found that diabetes was associated with a lower risk of angioedema, while asthma was not statistically significantly associated.

Studying the genetic etiology of ACE-inhibitor-induced angioedema could help to better understand its mechanism and provide genetic markers to identify patients who have an increased risk of this ADR and may need an alternative drug. To investigate what single nucleotide polymorphisms (SNPs) could be involved in the development of ACE-inhibitor-induced angioedema we applied a genome-wide association study approach in **chapter 3.3**.

To increase the coverage of genetic variants we used imputation to the Haplotype Reference Consortium (HRC) reference panel, containing over five million SNPs. Our analyses in 174 angioedema cases and 489 controls of African and European descent found no SNPs associated at the genome-wide significance level ($P < 5\times10^{-8}$). Considering the bradykinin-mediated mechanism of ACEI-induced angioedema, the biologically most plausible highly-associated hit was rs55940712 near the bradykinin receptor 2 (*BDKRB2*) gene. It was associated with a decreased risk of angioedema. Two intronic SNPs, *EFCAB4B/CRACR2A* rs12425092 and *PSAT1* rs2998724, were associated with an increased risk of angioedema. The denser HRC imputation panel enabled us to detect an association signal near the *BDKRB2* gene that was not found in the previous GWAS in this angioedema dataset.

Although a genetic marker for ACE-inhibitor-induced angioedema has not been discovered so far, it is important to evaluate the feasibility of a pharmacogenetic test for this ADR from the economic perspective. In **chapter 3.4** we investigated the cost, sensitivity and specificity of a hypothetical pharmacogenetic test for angioedema that would be required to make it cost-effective. If genotyping is performed in all patients starting an ACE-inhibitor to predict the development of angioedema, a 100%-sensitive and specific pharmacogenetic test may have a maximum cost of €1.30 and €1.95 with willingness-to-pay (WTP) thresholds of €20,000 and €80,000 per quality-adjusted life year (QALY), respectively. When only genotyping high-risk populations (defined from literature: African American ethnicity, female gender, age between 64-74 years) the maximum test price would be €5.03 and €7.55. Therefore, a pharmacogenetic test for angioedema in all ACE-inhibitor starters is unlikely to be cost-effective, unless it is a part of a larger panel of tests that could become increasingly available in the future after a wider implementation of whole-exome and whole-genome sequencing in the clinical practice.

In **chapter 4** we elaborate further on the studies described in this thesis, discuss their strengths and limitations and provide some implications for the clinical practice and directions for future research. Pharmacogenetic dosing algorithms for acenocoumarol and phenprocoumon in Europeans appear to have similar performance in our data, however it will be important in the future to develop algorithms in different ethnicities and assess their clinical relevance. The studies of ACE-inhibitor-induced angioedema showed that patients with a history of diseases with an allergic component experience this ADR more often. In a large proportion of patients angioedema developed months after the start of ACE-inhibitor therapy. The results of GWAS suggest an involvement of SNPs in genes of the bradykinin and immune pathways. A cost-effective pharmacogenetic test for predicting ACE-inhibitor-induced angioedema could be possible in the future, as genotyping becomes less costly and genetic information becomes routinely available in the daily clinical practice.

APPENDIX II

SAMENVATTING

Samenvatting

Vitamine K-antagonisten (VKA) en angiotensine-converterend enzym (ACE)-remmers zijn veel gebruikte cardiovasculaire geneesmiddelen. Over het algemeen worden VKA en ACE-remmers goed verdragen door het grootste deel van de patiënten, maar bijwerkingen kunnen optreden. De antistollingsbehandeling met VKA gaat gepaard met aanpassingen van de dosering op basis van een antistollingstest. Deze middelen hebben een smalle therapeutische breedte en er is een grote variabiliteit van de benodigde dosering tussen patiënten. Dit kan leiden tot een overmatige of onvoldoende antistollingseffect hetgeen weer kan leiden tot bloedingen of trombotische complicaties. Van de ACE-remmers is het bekend dat deze middelen een zeldzame en potentieel levensbedreigend angio-oedeem van de bovenste luchtwegen kunnen veroorzaken. Deze ernstige bijwerking kan een paar dagen na de start van een ACE-remmer optreden, echter dit kan ook als een patiënt het middel al maanden gebruikt. Precisiegeneeskunde kan de effectiviteit van de antistollingsbehandeling met VKA en de veiligheid van ACE-remmer gebruik verbeteren door het bepalen van een individuele VKA-dosering en de kans op angio-oedeem te kunnen voorspellen.

In **hoofdstuk 1** geven wij een algemene introductie op het onderwerp en beschrijven wij de doelen van dit proefschrift. We hebben ons gericht op het evalueren van het antistollingseffect bij gebruik van doseringsalgoritmes voor VKA en het voorspellend vermogen van klinische en genetische doseringsalgoritmen. Daarnaast was het doel om zowel genetische als andere factoren (comorbiditeit en comedicatie) te onderzoeken, die van invloed zijn op het optreden van ACE-remmer-geïnduceerd angio-oedeem, en om de kosteneffectiviteit van een farmacogenetische test voor het voorspellen van angio-oedeem voor het begin van de ACE-remmer behandeling te bestuderen.

Hoofdstuk 2 is gericht op de gepersonaliseerde behandeling met VKA en de doseeralgoritmen voor acenocoumarol en fenprocoumon. In **hoofdstuk 2.1** geven we meer achtergrondinformatie over dit onderwerp. We hebben de wetenschappelijke literatuur bestudeerd voor wat betreft recente gerandomiseerde, gecontroleerde klinische studies waarin de toegevoegde waarde van genotypering voor VKA-dosering werd onderzocht, en hebben de mogelijke economische consequenties van de implementatie van de farmacogenetica voor VKA-behandeling besproken. Het antistollingseffect tijdens de VKA-behandeling wordt regelmatig gecontroleerd door het

meten van de INR (International Normalized Ratio). Na een standaard oplaaddosering en tijdens de verdere antistollingsbehandeling wordt de dosering op basis van de INR zonodig aangepast. Bij de start van de behandeling is het de bedoeling zo snel mogelijk een stabiele dosering te bereiken met een INR in het therapeutische gebied. Er zijn doseeralgoritmen voor VKA ontwikkeld om bij individuele patiënten zo snel mogelijk een stabiele antistolling te bereiken. Deze benadering kan helpen om het aantal bloedingen en trombotische complicaties terug te dringen. De ontwikkelde doseeralgoritmen bevatten alleen klinische factoren die van invloed op de VKA-dosering zijn (met name leeftijd, geslacht, lengte en het gebruik van comedicatie), of zowel klinische als genetische informatie (*VKORC1* en *CYP2C9* genen). Gerandomiseerde, gecontroleerde klinische trials hebben de effectiviteit van doseringsalgoritmes op basis van klinische en genetische gegevens vergeleken met algoritmes op basis van alleen klinische gegevens (COAG en EU-PACT acenocoumarol/fenprocoumon) en met de standaard oplaaddosering (EU-PACT warfarine). Het primaire eindpunt van deze studies was het percentage tijd dat de INR zich binnen het therapeutisch gebied (INR 2.0-3.0) bevond gedurende de eerste 12 weken van de behandeling. De resultaten van de EU-PACT warfarine studie en de eerste vier weken van EU-PACT acenocoumarol/fenprocoumon waren gunstig voor de genetische algoritmen, echter bij Afro-Amerikaanse patiënten van in de COAG studie was het genetische algoritme niet effectief en was de tijd binnen het INR therapeutisch gebied zelfs verminderd.

Omdat de genetische variatie die van invloed is op de VKA-dosering per etniciteit kan verschillen, is er meer onderzoek nodig naar het effect van genetische algoritmen bij verschillende etniciteiten. Klinische studies die nog gaande zijn zullen meer kennis opleveren over bloedingen en trombotische complicaties tijdens het gebruik van genetische algoritmen. Economische studies lieten zien dat het gebruik van genetische algoritmen kosteneffectief kan zijn, echter de klinische implementatie van de algoritmen zal mede worden bepaald door de prijs van de farmacogenetische testen en de beschikbaarheid van de nieuwe orale antistollingsmiddelen (NOACs) waarbij een vaste dosering kan worden gebruikt zonder monitoring van het antistollingseffect. De INR-respons bij gebruik van doseeralgoritmen kan verschillen afhankelijk van het genotype. In **hoofdstuk 2.2** beschrijven wij een secundaire analyse van de gegevens van de EU-PACT acenocoumarol/fenprocoumon trial, waarbij het effect van de

klinische en genetische algoritmen op de INR-respons wordt vergeleken in de verschillende *VKORC1* en *CYP2C9* genotype subgroepen. Vier weken na het begin van de behandeling bleek het genetische algoritme de gemiddelde tijd dat de INR binnen het therapeutisch gebied bleef te verhogen in de *VKORC1* GG-*CYP2C9* *1*1 subgroep. Echter was het algoritme ook geassocieerd met een verhoogd risico op een INR-waarde, die onder het therapeutisch gebied lag in de *VKORC1* AA - *CYP2C9* *1*1 subgroep. Twaalf weken na het begin van de behandeling waren er geen significante verschillen in het effect op de INR-respons tussen klinische en genetische algoritmen in alle *VKORC1-CYP2C9* subgroepen. Het verbeteren van de genetische EU-PACT algoritmen (met meer voorspellende factoren, zoals de genotypen) zou het klinische belang van het genotyperen voor de *VKORC1* en *CYP2C9* kunnen verhogen.

In **hoofdstuk 2.3** hebben wij het voorspellend vermogen van de EU-PACT algoritmen voor acenocoumarol en fenprocoumon met zeven eerder gepubliceerde algoritmen vergeleken. De genetische algoritmen voor acenocoumarol en fenprocoumon hebben respectievelijk 53.9 - 61.1% en 53.7 - 56.9% van de variatie in de stabiele dosering kunnen verklaren. De R^2 ("coefficient of determination") van de klinische algoritmen voor acenocoumarol was 18.5 - 28.9%. De EU-PACT algoritmen waren het meest accuraat bij het voorspellen van de stabiele dosering van acenocoumarol (gemeten door "Mean Absolute Error"). Tussen de getoetste algoritmen bleek er vrijwel geen verschil in de nauwkeurigheid van de voorspelling van de stabiele dosering van fenprocoumon tussen de getoetste algoritmen. Hoewel de ontwikkeling van algoritmen specifiek voor Afro-Amerikaanse en Aziatische patiënten nodig is, lijkt het ontwikkelen van meer algoritmen voor acenocoumarol en fenprocoumon bij Europeanen niet noodzakelijk.

In **hoofdstuk 3** van dit proefschrift hebben wij de bijwerkingen van ACE-remmers bestudeerd, met name het ACE-remmer-geïnduceerd angio-oedeem van de bovenste luchtwegen. **Hoofdstuk 3.1** beschrijft de associaties van twee uitkomsten (angio-oedeem tijdens ACE-remmer gebruik en het switchen van een ACE-remmer naar angiotensine II-receptorantagonisten (ARB) als marker voor een ACE-remmer-geïnduceerde bijwerking) met chronische comorbiditeit en het gebruik van comedicatie. Eerdere studies vonden dat het switchen naar ARB als marker van ACE-remmer-geïnduceerde bijwerkingen (met name kriebelhoest) in elektronische databases kon worden gebruikt. Wij voerden twee geneste case-controle studies uit

gebruikmakend van de UK Clinical Practice Research Datalink (CPRD). We lieten zien dat patiënten met allergieën of een ziekte met een allergische component (zoals astma) een hoger risico hadden op het optreden van ACE-remmer-geïnduceerde bijwerkingen. Verschillende comorbiditeit (zoals bijvoorbeeld astma en allergie) en comedicatie waren geassocieerd met beide uitkomsten, maar er waren ook verschillen (bijvoorbeeld reumatoïde artritis). In de context van precisiegeneeskunde zouden deze associaties – als ze worden gerepliceerd in andere studies – kunnen worden gebruikt in een model samen met genetische factoren om het risico van de individuele patiënt op het optreden van de ACE-remmer-geïnduceerde bijwerkingen te kunnen voorspellen en de behandeling tijdig aan te passen.

In **hoofdstuk 3.2** beschreven wij de klinische karakteristieken van 392 patiënten met angio-oedeem (345 cases gerelateerd aan ACE-remmer gebruik, 47 cases gerelateerd aan ARB gebruik) ingesloten in het PREDICTION-ADR project. We hebben het inclusieproces samengevat en hebben de klinische presentatie en de tijd tot het optreden van angio-oedeem beschreven. Daarnaast hebben we de associatie tussen ACE-remmer-geïnduceerd angio-oedeem en comorbiditeit bestudeerd, gebruikmakend van de gegevens van de controle patiënten behandeld met ACE-remmers gedefinieerd voor PREDICTION-ADR in verschillende databases. Diabetes bleek dat diabetes het risico op angio-oedeem verlaagde, en er was geen statistisch significante associatie met astma.

Het bestuderen van de genetische etiologie van ACE-remmer-geïnduceerd angio-oedeem kan bijdragen zowel aan het beter begrijpen van het mechanisme achter deze bijwerking, als aan het ontdekken van biomarkers om patiënten met een verhoogd risico op angio-oedeem te kunnen identificeren en een alternatief middel voor te schrijven. In **hoofdstuk 3.3** voerden wij een genoomwijde associatie studie (GWAS) uit op het eindpunt ACE-remmer-geïnduceerd angio-oedeem om te onderzoeken welke single nucleotide polymorfismen (SNPs) mogelijk een rol spelen bij deze bijwerking. Om het aantal getoetste genetische varianten te vergroten hebben wij de data geïmputeerd naar de nieuwe Haplotype Reference Consortium (HRC) panel. Onze analyse van 174 cases met angio-oedeem en 489 controles van Afrikaanse en Europeaanse afkomst liet geen statistisch significante associaties op het genoom-wijde niveau ($P < 5\times10^{-8}$) zien. Omdat angio-oedeem bij ACE-remmer gebruik door bradykinine wordt gemedieerd, is een SNP in het bradykinine receptor 2 gen (*BDKRB2*

rs55940712) de meest biologisch relevante tophit; deze SNP was geassocieerd met een verlaagd risico op angio-oedeem. Twee SNPs, *EFCAB4B/ CRACR2A* rs12425092 en *PSAT1* rs2998724, waren geassocieerd met een verhoogd risico op angio-oedeem. Het gebruik van de HRC panel met een bredere SNP dekking heeft ons een associatie met het *BDKRB2* gen laten opsporen, die in de eerdere GWAS in dezelfde dataset niet gevonden was.

Ook al zijn er momenteel nog geen genetische biomarkers van ACE-remmer-geïnduceerd angio-oedeem bekend, het is belangrijk om de uitvoerbaarheid van een potentiele farmacogenetische test voor het voorspellen van deze bijwerking vanuit het standpunt van de kosteneffectiviteit te onderzoeken. In **hoofdstuk 3.4** hebben wij bestudeerd welke prijs, sensitiviteit en specificiteit nodig zijn om deze farmacogenetische test een kost-effectieve diagnostische optie voor angio-oedeem te maken. Als alle patiënten worden gegenotypeerd in het begin van de behandeling met een ACE-remmer om het optreden van angio-oedeem te voorspellen, mag een 100%-sensitief en -specifiek test respectievelijk maximaal €1.30 en €1.95 kosten bij een willingness-to-pay (WTP) drempel van €20,000 en €80,000 per voor kwaliteit van leven gecorrigeerd levensjaar (QALY). Als alleen de hoog-risico patiënten (zoals beschreven in de literatuur: Afro-Amerikaanse etniciteit, vrouwelijke seks, leeftijd tussen 64 en 74 jaar) worden gegenotypeerd, dan wordt de maximale prijs per test €5.03 en €7.55. Een farmacogenetische test om angio-oedeem te voorspellen bij de start van de behandeling met ACE-remmers lijkt dus niet kosteneffectief te zijn, tenzij deze test in de toekomst een onderdeel van een groter panel van testen wordt gebruikt, als de Whole Exome Sequencing en de Whole Genome Sequencing vaker in de dagelijkse klinische praktijk worden toegepast.

In **hoofdstuk 4** bespreken wij de resultaten van de studies in dit proefschrift. Daarbij beschreven wij ook de plus- en minpunten van de studies, de implicaties voor de klinische praktijk en enkele aanbevelingen voor toekomstig onderzoek. Doseeralgoritmen voor acenocoumarol en fenprocoumon in Europeanen hadden een vergelijkbaar voorspellend vermogen in onze data, echter in de toekomst is het belangrijk om algoritmen voor andere etniciteiten te ontwerpen en de klinische relevantie hiervan te evalueren. Uit de studies naar ACE-remmer-geïnduceerd angio-oedeem in dit proefschrift blijkt dat patiënten met ziekten die een allergische component hebben (zoals bijvoorbeeld astma) vaker bijwerkingen van ACE-remmers

ervaren. Bij een groot deel van de patiënten is angio-oedeem opgetreden maanden na start van de behandeling. De resultaten van GWAS suggereren een mogelijke rol van genetische varianten in de bradykinine en immuunrespons pathways. Een kosteneffectieve farmacogenetische test voor angio-oedeem zou in de toekomst mogelijk zijn, als het genotyperen goedkoper wordt en de genetische informatie in de klinische praktijk standaard beschikbaar wordt.

APPENDIX III

Dankwoord

Dankwoord

Wat is het een fijn gevoel om mijn proefschrift na vier jaar hard werken af te hebben. Maar helemaal alleen heb ik het natuurlijk niet gedaan. Er zijn veel mensen die ik graag wil bedanken voor alle hulp bij de totstandkoming van mijn proefschrift.

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APPENDIX IV

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APPENDIX V

List of publications

List of publications

Publications related to this thesis

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