EPIDEMIOLOGICAL SCIENCE

Vitamin K antagonist anticoagulant usage is associated with increased incidence and progression of osteoarthritis

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ABSTRACT

Objectives Vitamin K is hypothesised to play a role in osteoarthritis (OA) pathogenesis through effects on vitamin K-dependent bone and cartilage proteins, and therefore may represent a modifiable risk factor. A genetic variant in a vitamin K-dependent protein that is an essential inhibitor for cartilage calcification, matrix Gla protein (MGP), was associated with an increased risk for OA. Vitamin K antagonist anticoagulants (VKAs), such as warfarin and acenocoumarol, act as anticoagulants through inhibition of vitamin K-dependent blood coagulation proteins. VKAs likely also affect the functioning of other vitamin K-dependent proteins such as MGP.

Methods We investigated the effect of acenocoumarol usage on progression and incidence of radiographic OA in 3494 participants of the Rotterdam Study cohort. We also examined the effect of MGP and VKORC1 single nucleotide variants on this association.

Results Acenocoumarol usage was associated with an increased risk of OA incidence and progression (OR=2.50, 95% CI=1.94–3.20), both for knee (OR=2.34, 95% CI=1.67–3.22) and hip OA (OR=2.74, 95% CI=2.08–3.61). Among acenocoumarol users, carriers of the high VKORC1(BB) expression haplotype together with the MGP OA risk allele (rs1800801-T) had an increased risk of OA incidence and progression (OR=4.18, 95% CI=2.69–6.50), while this relationship was not present in non-users of that group (OR=1.01, 95% CI=0.78–1.33).

Conclusions These findings support the importance of vitamin K and vitamin K-dependent proteins, as MGP, in the pathogenesis of OA. Additionally, these results may have direct implications for the clinical prevention of OA, supporting the consideration of direct oral anticoagulants in favour of VKAs.

INTRODUCTION

Osteoarthritis (OA) is a chronic disabling joint disease that also increases in prevalence with age. It is the most common form of arthritis, one of the fastest growing chronic diseases worldwide,1 and is the fourth leading cause of years lived with disability globally.2 To date, there are no known therapies that can alter its progression or prevent its occurrence. Apart from obesity and knee injury, very few other modifiable risk factors have been identified. Vitamin K has been hypothesised to play a role in OA pathogenesis through its effects on several vitamin K-dependent bone and cartilage proteins,3 and therefore may represent a modifiable risk factor. A number of observational studies reported an association between vitamin K status and prevalence and incidence of OA.4–6 There has been one modestly sized clinical trial studying the effect of vitamin K supplementation on OA progression. This ancillary study, originally designed to study vascular calcification, reported no overall beneficial effects of vitamin K supplementation. However, in individuals with insufficient vitamin K levels at baseline, a beneficial effect was observed.7 No studies to

What is already known about this subject?

► Osteoarthritis (OA) is the most common form of arthritis worldwide, affecting 320 million people, and is a leading cause of disability. To date, there are no disease-modifying therapies available, and treatment development has been hampered by existence of only few recognised modifiable risk factors.

► Vitamin K and vitamin K-dependent proteins, such as matrix Gla protein (MGP), have been implicated in OA by epidemiological studies, genetic studies and subsequent in functional genomics studies, indicating vitamin K as possible modifiable risk factor for OA.

What does this study add?

► This study shows that the use of vitamin K antagonist anticoagulants (VKAs) significantly increases the risk of progression of hip and knee OA, by inhibiting the vitamin K pathway.

► This study also demonstrates that known OA genetic risk variants in MGP and pharmacogenetic variants known to affect vitamin K metabolism increase the risk of OA progression when using VKAs.

How might this impact on clinical practice or future developments?

► The findings suggest the consideration of novel (or direct) oral anticoagulants in favour of VKAs, such as acenocoumarol and warfarin, in people with OA.
date have evaluated the relation between vitamin K antagonist anticoagulants (VKAs) and OA, which can be expected to result in low vitamin K functioning, which may lead to increased OA incidence or progression.4-6

Vitamin K is an essential cofactor in the post-translational γ-carboxylation of glutamic acid to form γ-carboxyglutamic acid (Gla) residues, which confer functionality to Gla proteins. VKAs deplete the active form of vitamin K by inhibiting the enzyme vitamin K epoxide reductase complex 1 (VKORC1). Genetic variants of VKORC1 account for approximately 25% of the variance in VKA dose.6 Matrix Gla protein (MGP) is a vitamin K-dependent Gla protein that is an essential inhibitor of cartilage and vascular mineralisation.7,8 Recently, genome-wide association study (GWAS) and functional studies identified MGP to be causally involved in OA.9,10

VKAs such as warfarin and acenocoumarol are primarily prescribed for the prevention of thromboembolic events in patients with atrial fibrillation (AF).11 With age-related increases in prevalence of AF, the projected number of individuals with AF needing anticoagulation is predicted to rise to 17.9 million by 2060 in the European Union.12 While a new class of anticoagulants are available, the direct oral anticoagulants (DOACs), VKAs are still widely prescribed, particularly to older adults.13 Whether long-term VKA use with resultant impairment of vitamin K-dependent proteins such as MGP increases risk of OA incidence or progression is not known. Given the high prevalence of VKA users in addition to the high prevalence of OA globally, clarifying this relationship would have substantial public health impact by identifying a potentially modifiable risk factor for OA.

We therefore examined the relation of VKA use to progression and incidence of hip and knee OA in two subcohorts of the large prospective population-based cohort of the Rotterdam study (RS). We additionally examined how the impact of VKA use varies by the presence of the MGP risk allele that influences MGP expression and single nucleotide variations (SNVs) affecting VKORC1 gene expression, which impact VKA dosage.

METHODS
Study population and clinical data
The Rotterdam Study (RS) is a large prospective population-based cohort study ongoing since 1990 to study determinants of chronic disabling diseases in the elderly.14 It consists of separate subcohorts (RS-I, RS-II, RS-III). All RS cohort participants live in the Ommoord district of the city of Rotterdam, the Netherlands. Residents of 55 years and older were first recruited in 1990. In 2000, a second cohort, RS-II, was started with individuals who had become 55 years of age or moved into the study district since the start of the study. Follow-up data were collected at follow-up visits every ~5–6 years. Details of the design and rationale of the RS have been published elsewhere.15 Participant measurements at baseline and follow-up were obtained during visits to the research centre for physical examinations, computerised pharmacy records and from home interviews. Our study included participants of RS-I and RS-II for whom radiographs of knee and hip joints at baseline and follow-up visit were present, obtained and scored (online supplemental figure S1). Additional information included sex, age at baseline visit, body mass index (BMI, kg/m²), physical activity (metabolic equivalent of task/ hours per week), smoking (never, former and current smoker), locomotor disability, education level (UNESCO education classification), diabetes mellitus, hypertension, femoral neck bone mineral density (FN-BMD), HDL/total cholesterol ratio and the Stanford Health Assessment Questionnaire (see online supplemental text for details).

The RS has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports. The approval has been renewed every 5 years (MEC 02.1015). All participants provided written informed consent for participation in the RS.

Incidence and progression of OA
Our study included participants of RS-I and RS-II for whom radiographs of knee and hip joints at baseline and follow-up visit were obtained and scored by trained medical professionals for OA severity using Kellgren and Lawrence Grade (KLG)17,18 (online supplemental figure S1). Individuals who had at baseline locomotor disability were excluded from our study population19 (online supplemental figure S1). We analysed OA incidence and progression together, as both definitions cannot be accurately defined based on radiographic examination alone, and by combining both into one definition reduces this bias.20 We evaluated any OA progression, defined as an increase of KLG between baseline and follow-up of ≥1 and/or joint replacement; if baseline KLG was 0, progression was defined as an increase of KLG ≥2 (incidence).21 Joints with a baseline KLG of 4 or baseline joint replacement were excluded from analysis (online supplemental figure S1). OA progression was defined in a joint-specific and side-specific manner (knee, hip; left and right). Joints with no progression of OA comprised the referent group. Joints with missing data were excluded (online supplemental figure S1), with the exception of joints with missing baseline data and a KLG of ≤1 at follow-up, which were included in the referent group (online supplemental figure S1).

Vitamin K antagonist anticoagulants
For each participant, we extracted the usage of VKAs (acenocoumarol) for the period between baseline visit (RS-I-1, RS-II-1) and follow-up visits (RS-I-3, RS-II-2), from computerised pharmacy data (online supplemental text). Aacenocoumarol is the main prescribed VKA in the Netherlands as warfarin is not registered for use as a drug. All participants taking VKAs attended an anticoagulation clinic, which is standard practice in the Netherlands.22 We excluded participants who were taking VKA (n=148) during the baseline visit to avoid prevalent user bias. We defined VKA usage as any acenocoumarol usage during the period between the baseline (RS-I, RS-II) and follow-up visit (RS-I-3, RS-II-2), regardless of duration or dosage. To examine the effects of increasing duration of use, we defined duration of use by tertiles: ≤180 days, between >180 days and ≤556 days of use, and >556 days of use.

Genetic data and haplotype analysis
Methods for DNA isolation, genotyping, quality control and data processing have been described elsewhere.11 Data from 11 SNVs were extracted from the genotyped and imputed genetic dataset: the MGP SNV previously found associated with OA11 and 10 SNVs needed for the VKORC1 H-haplotypes as described on the PharmGKB database23 (online supplemental table S1). Haplotypes were inferred from all available genotypes (N=8448), using imputed genotype dosage data (HRC panel v.1.1.120) and the R-package haplo.stats.24 Haplotypes were grouped based on VKA maintenance dose/VKORC1 expression association: (A) low-dose VKA requirement/low VKORC1 expression and (B) high-dose VKA requirement/high VKORC1 expression.26 Study
participants were further stratified into: low expression/dose (AA), intermediate expression/dose (AB) and high expression/dose (BB) groups (table 1 and online supplemental table S1).

**Statistical analysis**

We evaluated the relation of VKA to the risk of overall progression of OA of either the knee or hip using logistic regression with generalised estimating equations (GEE) to account for correlations between joints within an individual. We repeated the analysis stratified by VKORC1 and MGP genotype/haplotype. The following covariates were included in all analyses: sex, age, BMI, physical activity, smoking, education level, diabetes mellitus, hypertension, FN-BMD, HDL/total cholesterol ratio, baseline OA severity, time between follow-up visits, joint modelled and RS cohort (online supplemental text).

**RESULTS**

**Relation of acenocoumarol use to OA progression**

A total 3494 of participants of two large prospective older-age population-based cohorts, the Rotterdam Study (RS), were included in this study, with RS-I contributing 2601 individuals, while RSII contributed 894 participants. See table 1 for the general characteristics of the study population. At baseline, there were 363 individuals with OA (KLG ≥2), 75 with hip OA and 288 with knee OA. We identified 239 new users of acenocoumarol (VKA) (RS-I n=207, RS-II n=33) in our study population in our follow-up period.

When we examined the incidence/progression of OA in acenocoumarol users and non-users, there was a >2-fold higher risk for overall OA incidence/progression (ie, OA of the knee or hip) in acenocoumarol users compared with non-users (OR=2.50, 95% CI=1.94–3.20; table 2, online supplemental table S2). This association was also observed in each subcohort (RS-I and RS-II) separately (online supplemental table S3) and for OA incidence and OA progression separately (online supplemental table S4). Overall OA incidence/progression risk estimates remained similar for longer duration of acenocoumarol use (tertiles): ≤180 days (OR=2.82, 95% CI=1.90–4.20), >180 days and ≤556 days (OR=2.94, 95% CI=2.00–4.32), with only a reduction of risk with long-term use, >556 days of acenocoumarol use (OR=1.74, 95% CI=1.10–2.76) (table 2).

Increased risk of overall OA incidence/progression in acenocoumarol users was also observed in the knee and hip joints separately (table 2) (knee OA (OR=2.34, 95% CI=1.69–3.22) and hip OA (OR=2.74, 95% CI=1.82–4.11)), as well as in each subcohort separately (online supplemental table S3). Interestingly, longer duration of acenocoumarol use does seem to have slight different effects on overall knee OA incidence/progression than on hip OA. Knee OA risk seems to increase for longer duration of acenocoumarol use, whereas risk for hip OA seems to decrease for longer durations of acenocoumarol use. These differences may represent true biological differences between the joints. However, this is more likely the effect of low statistical power (tertiles analysis), the statistical power difference between the joints as there were more cases of overall knee OA incidence/progression (n=385 joints) in our study cohorts than for hip OA (n=216). In addition, the absence of a stronger effect in long-term acenocoumarol users could be explained by depletion of susceptible bias. This bias suggests that the cohort is depleted of all its susceptible subjects who had the event, OA incidence/progression, early on. Seemingly decreasing the risk with longer acenocoumarol (exposure) use. Thus, the remaining acenocoumarol users will include fewer OA incidence/progression predisposed subjects over the longer follow-up, causing the risk to decrease over time.

**MGP, VKORC1 genetics and acenocoumarol affect OA progression**

Maintenance dosages of VKAs are dependent on genetic variants (SNVs) affecting VKORC1 expression activity. This altered VKORC1 expression can also affect MGP γ-carboxylation, as VKORC1 is needed for γ-carboxylation by vitamin K, a process that is needed to activate MGP. We therefore examined the extent to which acenocoumarol users with a genetic predisposition for decreased MGP and/or altered VKORC1 expression had an altered risk for overall incidence/progression of OA. The study population was stratified in MGP risk allele carriers (T/*) and non-carriers (A/A) (table 1).

Acenocoumarol users among both MGP genotype groups had a significantly higher risk of overall OA incidence/progression than non-users (table 3). Using the VKORC1-Haplotype, the study population could be also stratified into low (AA), intermediate (AB) and high (BB) VKORC1 expression/VKA dose groups (table 1 and online supplemental table S1). Similar to the MGP genotypes, we observed no effect of the VKORC1 genotypes on the risk for overall OA incidence/progression (table 3), although
Table 2. Association between acenocoumarol use and risk of OA incidence and progression in RSI and RSII

<table>
<thead>
<tr>
<th>Joints Incidence/Progression</th>
<th>Incidence/Progression N (%)</th>
<th>OR adj.† 95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-users</td>
<td>128 (9.8%)</td>
<td>1</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Duration of acenocoumarol usage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤180 days</td>
<td>25 (19.3%)</td>
<td>2.14</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>&gt;180 days and ≤556 days</td>
<td>29 (15.5%)</td>
<td>1.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;556 days</td>
<td>22 (7.6%)</td>
<td>1.10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

†Unadjusted (raw) ORs are reported in online supplemental table S2. BMI, body mass index; HDL, high-density lipoprotein; RS, Rotterdam Study.

DISCUSSION

We demonstrated that use of the acenocoumarol was associated with a higher risk of overall OA incidence/progression in non-users. We observed that the increased OA risk in acenocoumarol users varied based on genetic variants affecting the vitamin K cycle with VKA use and MGP risk allele status. Acenocoumarol users with the MGP risk allele and VKORC1 BB-haplotype had a fourfold higher risk for overall OA incidence/progression.

The VKORC1 BB-haplotype is associated with a higher expression of VKORC1, associated with greater vitamin K activity; however, individuals who are VKORC1 BB-haplotype carriers also require and receive higher dosages of VKA for the desired anticoagulation effect.\(^\text{26}\) Intuitively, the anticoagulation, amount also require and receive higher dosages of VKA; however, DOACs are more costly and difficult to reverse.\(^\text{36–38}\) With ageing of the population, the number of people with OA and antiphospholipid antibody syndrome, mechanical heart valves.

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Table 3  Acenocoumarol use interacts with MGP OA risk variants and VKORC1 haplotype groups, leading to increased risk of overall incidence/progression of osteoarthritis

<table>
<thead>
<tr>
<th>Acenocoumarol use</th>
<th>Joints*</th>
<th>Incidence/Progression</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N (%)</td>
<td>Adj.</td>
<td>Adj.</td>
<td>Adj.</td>
</tr>
<tr>
<td>MGP rs1800801 alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-users MGP non-risk allele carriers (A/A)</td>
<td>4332</td>
<td>205 (4.7%)</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Non-users MGP risk allele carriers (T/*)</td>
<td>7211</td>
<td>274 (3.8%)</td>
<td>0.83</td>
<td>0.69 to 1.00</td>
<td>0.06</td>
</tr>
<tr>
<td>Users MGP non-risk allele carriers (A/A)</td>
<td>286</td>
<td>26 (9.1%)</td>
<td>2.11</td>
<td>1.37 to 3.24</td>
<td>2.01</td>
</tr>
<tr>
<td>Users MGP risk allele carriers (T/*)</td>
<td>402</td>
<td>58 (11.8%)</td>
<td>2.92</td>
<td>2.09 to 3.84</td>
<td>2.97</td>
</tr>
<tr>
<td>VKORC1 haplotype groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-users low VKORC1 group (AA)</td>
<td>1735</td>
<td>75 (4.3%)</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Non-users intermediate VKORC1 group (AA)</td>
<td>5525</td>
<td>203 (3.7%)</td>
<td>0.84</td>
<td>0.64 to 1.11</td>
<td>0.84</td>
</tr>
<tr>
<td>Users high VKORC1 group (BB)</td>
<td>4448</td>
<td>201 (4.5%)</td>
<td>1.05</td>
<td>0.80 to 1.37</td>
<td>1.04</td>
</tr>
<tr>
<td>Users low VKORC1 group (AA)</td>
<td>152</td>
<td>16 (10.1%)</td>
<td>2.6</td>
<td>1.48 to 4.59</td>
<td>2.31</td>
</tr>
<tr>
<td>Users intermediate VKORC1 group (AB)</td>
<td>317</td>
<td>24 (7.6%)</td>
<td>1.81</td>
<td>1.13 to 2.92</td>
<td>1.53</td>
</tr>
<tr>
<td>Users high VKORC1 group (BB)</td>
<td>305</td>
<td>42 (13.8%)</td>
<td>3.53</td>
<td>2.37 to 5.27</td>
<td>3.35</td>
</tr>
</tbody>
</table>

Overall progression of osteoarthritis (OA) in RSI and RSII within the follow-up time associated with acenocoumarol use effect MGP rs1800801 OA risk variant and VKORC1 expression/VKA dosage haplotypes. Model used is a GEE (generalised estimated equation) multivariate logistic regression model including acenocoumarol use adjusted (Adj.) for age, sex, BMI, smoking, time between baseline and follow-up, baseline OA severity in Kellgren-Lawrence score, joint modelled, femoral neck BMD, HDL/total cholesterol ratio, physical activity, education level, hypertension, diabetes mellitus and Rotterdam Study cohort. VKORC1 haplotype groups are based on the H haplotypes, see online supplemental table S1. For MGP risk variants and carriers, see Table 1. Progression: number of joints showing overall OA progression; T/*: MGP osteoarthritis risk variant carrier (T/A) or (T/T).

The strengths of our study include the robust underlying biological hypothesis, large sample size and high-quality prospectively requiring anticoagulation medication will continue to rise. Given our findings of increased risk of OA incidence/progression with VKA use and the lack of effective treatment options for OA, it may be reasonable to consider DOACs over VKAs for medical indications in which N=DOACs can be used. This may be particularly the case for those who are carriers of the MGP risk allele and the VKORC1 BB-haplotype, which is an estimated 21% of individuals of European ancestry.
collected data. However, some limitations of our study should be acknowledged. First, while we found similar results in RS-I and RS-II, analyses in other independent and even larger cohorts are warranted, as in our large sample size, the numbers of acenocoumarol users is still relatively low. Specifically in RSII, which has a much smaller sample size and number of acenocoumarol users compared with RS-I. Also replication in non-Central European ancestry-based cohorts is warranted. Second, the association we noted in this study may be due to a shared disease pathology between OA and VKA indications.\textsuperscript{39, 40} We addressed this issue by adjusting for multiple cardiovascular disease risk factors in our analysis (hypertension, HDL/total cholesterol ratio, diabetes mellitus, BMI, physical activity, smoking and age). However, we cannot rule out possible confounding by indication. This potential bias needs to be addressed more directly. This could be done by examining direct (new) oral anticoagulants (DOAC)/NOAC users as a comparator group, as these oral anticoagulants do not inhibit the vitamin K cycle. Unfortunately, our study population contains too few DOAC/NOAC users for such an analysis (n=9). Third, as we only have radiographs available of participants whom were able, healthy and survived long enough to come to our research centre at baseline and follow-up visits, our study may contain health and survivor bias. However, this could also possibly indicate that our found effect may even be larger.\textsuperscript{20} Last, as with all observational studies, we cannot rule out residual confounding.

In summary, we found an increased risk of overall OA incidence/progression in users of the VKA acenocoumarol, which was further increased in VKORC1 BB-haplotype and MGP risk allele carriers. These findings are consistent with the known biology of MGP and vitamin K, and are in keeping with prior studies of vitamin K in OA. Taken together, these studies, including the current one, highlight the importance of vitamin K and vitamin K-dependent Gla proteins such as MGP in the pathogenesis of OA. Importantly, our results may also indicate a role for other vitamin K-dependent proteins which occur and function in cartilage and bone tissues, such as osteocalcin and Gla-rich protein.\textsuperscript{3} Given that there are as yet no treatment options for preventing OA onset or progression, vitamin K may represent a modifiable risk factor. These data provide strong rationale for a properly powered randomised clinical trial of vitamin K in an appropriate patient population, such as those with insufficient vitamin K levels and/or MGP risk allele carriers. Additionally, these data lend support to the consideration of DOACs in favour of VKAs when appropriate for a supported indication, and highlight the future possibility of genetic screening to identify individuals at high risk of OA incidence/progression.

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Contributors CGB designed the study, performed the analyses, made the figures and tables, and wrote the manuscript. IS contributed to study design. NLM performed analysis. TN and IM contributed to study design. AGU and MAI provided access to the Rotterdam study dataset. SB-Z contributed to study design. BS provided pharmacological data, contributed to study design and analysis. JBVM designed the study and supervised this work. All authors critically assessed the manuscript.

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