# Technology Assessment

**Technology Assessment Program**

# Reviews of Selected Pharmacogenetic Tests for Non-Cancer and Cancer Conditions

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# Reviews of Selected Pharmacogenetic Tests for Non-Cancer and Cancer Conditions

## Technology Assessment Report

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# Abstract

**Objective:** We assessed four pharmacogenetic tests: 1) cytochrome P450, subfamily IIC, polypeptide 9 (*CYP2C9*), 2) vitamin K epoxide reductase subunit protein 1 (*VKORC1)*, 3) apolipoprotein E (*Apo E*), and 4) methylenetetrahydrofolate reductase (*MTHFR*) for their associations with patient's response to therapy with warfarin (*CYP2C9* and *VKORC1*), statins (*Apo E*), or antifolate chemotherapy (*MTHFR*).

**Data Sources:** Published studies were identified through an electronic search up to October 2007, and relevant bibliographies were reviewed. Focused searches for specific topics were conducted through April 2008 to identify published randomized controlled trials, systematic reviews, and ongoing clinical trials.

**Methods:** We included studies of any design that evaluated adults and abstracted data on all relevant clinical and laboratory outcomes. When sufficient data were available from studies making the same comparisons, the data were summarized in a meta-analysis. Additional subgroup, sensitivity, and meta-regression analyses were conducted as appropriate.

**Results:** The 99 included articles reported 103 studies: 29 tested the association of *CYP2C9* and the response to warfarin; 19 tested the association of *VKORC1* and the response to warfarin; 44 tested the association of *Apo E* and the response to statins; and 11 tested the association of *MTHFR* with the response to antifolate chemotherapy.

***CYP2C9* and *VKORC1* gene polymorphisms and response to warfarin therapy**

Of the 29 studies of *CYP2C9* gene polymorphisms, 26 evaluated their association with responses to maintenance does of warfarin. The remaining three studies were randomized controlled trials that evaluated response to therapy based on dosage-based algorithms among patients with pharmacogenetic test results. Carriers of the *CYP2C9* gene variant alleles **2* or **3*

had lower mean maintenance warfarin dose requirements than did non-carriers. Few studies investigated the relationship between genetic variations in *CYP2C9* or *VKORC1* and warfarin dose requirements in the induction phase. *CYP2C9* variants were associated with an increased rate of bleeding complications during the induction phase of warfarin therapy, but the studies did not report whether affected patients had normal or supratherapeutic INR ranges. As with the *CYP2C9* variants, carriers of the three common *VKORC1* variants (alleles T, G, and C) required lower mean maintenance doses of warfarin than did non-carriers. Studies of *CYP2C9* and *VKORC1* had significant between-study heterogeneity. Few studies evaluated the relationship between pharmacogenetic test results and patient- and disease-related factors or response to therapy. No study addressed how therapeutic choices affected the benefits, harms, or adverse effects of patients from subsequent therapeutic management after pharmacogenetic testing for *CYP2C9* and *VKORC1*.

***Apo E* genotype and statin treatment**

In studies of the *Apo E* genotype (e2 carriers, e3 homozygotes, and e4 carriers) and statin treatment, the pooled reduction in total and LDL cholesterol from baseline values was lower for all three genotypes but did not differ significantly among them. These studies also had significant between-study heterogeneity. Although few studies included certain subgroups, factors that may affect the associations between all three *Apo E* genotypes and response to statin therapy were ethnicity, sex, familial hyperlipidemia, the type of statin used, and possibly the presence of diabetes. No studies addressed the effects of therapeutic choice: there were no data on the benefits, harms, or adverse effects on patients from subsequent therapeutic management after pharmacogenetic testing for the three *Apo E* genotypes.

***MTHFR* gene polymorphisms and response to chemotherapy**

Limited data preclude making meaningful inferences about the relationship between common variants in *MTHFR* and chemotherapy of the folate metabolic pathway.

**Conclusions:** Certain *CYP2C9* and *VKORC1* variants are associated with lower warfarin maintenance doses, and *CYP2C9* variants are associated with increased bleeding rates among patients who use warfarin. Total and LDL cholesterol levels among patients on statin therapy were lower than baseline values among patients with the three *ApoE* genotypes. Response to chemotherapy based on the folate metabolic pathway in solid organ cancers was not associated with genetic variations in *MTHFR*. Overall, studies evaluating associations between the pharmacogenetic test results and the patient's response to therapy for non-cancer and cancer conditions showed considerable variation in study designs, study populations, medication dosages, and the type of medications. This variation warrants caution when interpreting our results. Data on the relationships among pharmacogenetic test results and patient- and disease-related factors and on the patient's response to therapy are limited. We found no data on the benefits, harms, or adverse effects from subsequent therapeutic management after pharmacogenetic testing. Detailed patient-level analyses are needed to adjust estimates for the effects of modifiers, such as age or tumor stage.

# Background

Genetic tests are frequently used to assess persons at risk for a heritable health condition, to confirm the diagnosis of a condition, and to guide treatment decisions. These tests may provide insights into the individual variability in drug responses. The information from pharmacogenetic testing, when applied in a clinical setting, could potentially identify non-responders and those likely to suffer adverse drug reactions, and thus could prevent the prescription of unsafe or ineffective drugs.

The results of diagnostic tests initiate a cascade of decisions on further testing, prevention, or treatment, which determine the course of illness and the cost of healthcare for patients. In 2005, $56 billion was spent on diagnostic services in the US.[1] Although diagnostics account for only 1.6% of total Medicare costs, they influence 60 percent to 70 percent of treatment decisions.[2]

The Coverage and Analysis Group at the Centers for Medicare and Medicaid Services (CMS) requested from The Technology Assessment Program (TAP) at the Agency for Healthcare Research and Quality (AHRQ) an assessment of pharmacogenetic tests for patients with non-cancer and cancer conditions relevant to the Medicare population. AHRQ assigned this report to the following Evidence-based Practice Center: the Tufts Medical Center Evidence-based Practice Center (Tufts EPC) (Contract No. 290-02-0022), who had previously produced two "horizon scans" on gene tests currently in clinical use and development. These reports identified 62 gene tests for cancer conditions and several hundred tests for non-cancer conditions. Five or six new gene tests become commercially available each month.

In this report, the Tufts EPC describes its systematic review of pharmacogenetic tests that evaluate cancer and non-cancer conditions common in the Medicare population. Tests that have recently been or are currently being reviewed by other groups at CMS or AHRQ (eg, Her-2/neu testing for solid tumors) were excluded. Also excluded were tests with such a large number of published studies that the literature could not be reviewed within the contracted period. Tests that qualified for a focused evaluation were: 1) testing for selected single nucleotide polymorphisms (SNPs) in the cytochrome P450, subfamily IIC, polypeptide 9 gene (*CYP2C9*) with respect to responses to warfarin therapy, 2) testing for selected SNPs in the Vitamin K epoxide reductase subunit protein 1 gene (*VKORC1)* with respect to responses to warfarin therapy, 3) testing for selected SNPs in the apolipoprotein E gene (*Apo E*) with respect to responses to statin therapy, and 4) testing for a SNP in the methylenetetrahydrofolate reductase gene (*MTHFR*) for responses to antifolate chemotherapy. Details on the examined SNPs are given in the corresponding sections.

Tufts EPC used the general methodological approach for evaluating gene expression profiling and gene tests: ACCE.[3] ACCE takes its name from its four components of evaluation—Analytic validity, Clinical validity, Clinical utility, and associated Ethical, legal, and social implications. The process includes collecting, evaluating, interpreting, and reporting data about DNA (and related) testing for disorders with a genetic component in a format that allows policy makers to have access to up-to-date and reliable information for decision making.

Because the CMS requested reviews on the clinical validity and utility of pharmacogenetic tests, the Tufts EPC also used the 6-level hierarchical model frequently used for evaluating diagnostic technologies as a base for this review.[4,5] Diagnostic tests are most commonly evaluated on 6 criteria: a) their technical feasibility and optimization; b) their

diagnostic accuracy; c) their diagnostic thinking impact; d) their impact on subsequent therapeutic choices; e) their impact on patient outcomes; and f) their societal impact. Pharmacogenetic tests are considered here as diagnostic and prognostic tests for predicting a patient's response to a specific drug. This report focuses on the association between pharmacogenetic test results and the therapeutic impact and patient outcomes of specific therapies.

## Key Questions Addressed in this Report

The approach, methodology, and criteria for the review were agreed on by consensus of the EPC, CMS, and AHRQ.

**SECTION 1: CYP2C9/VKORC1 - Warfarin**

Key Question #1: Among patients taking warfarin, is there any association between genetic variations (SNPs) in *CYP2C9* or *VKORC1* and:

- Clinical variables, such as the effective dose of warfarin?
- Biochemical variables, such as therapeutic INR levels?

Key Question #2: Among patients taking warfarin, is there any association between genetic variations (SNPs) in *CYP2C9* or *VKORC1* and adverse outcomes, such as serious bleeding events or thrombotic events?

Key Question #3: What demographic or clinical variables mediate the association between pharmacogenetic test results and biochemical or clinical outcomes among patients who use warfarin?

Key Question #4: How does the pharmacogenetic test result affect the decision to use warfarin; that is, how often has therapy changed in response to the test result?

Key Question #5: What benefits, harms, or adverse effects are experienced by patients on warfarin from change in treatment received after the pharmacogenetic test results are known?

5a. Does pharmacogenetic testing among patients on warfarin and with supratherapeutic INRs result in better maintenance of therapeutic INRs and fewer episodes of serious bleeding?

5b. Does pharmacogenetic testing among patients on warfarin and with subtherapeutic INRs result in better maintenance of therapeutic INRs and fewer serious thrombotic events, such as stroke or pulmonary embolus?

**SECTION 2: Apo E – statin therapy**

Key Question #1: Among patients taking statin therapy, is there any association between carrying the Apo E genotypes and:

- Clinical outcomes, such as cardiovascular events, mortality, or other composite clinical endpoint?
- Biochemical variables, such as total, LDL, or HDL cholesterol or triglyceride levels?

Key Question #2: What demographic or clinical variables mediate the association between pharmacogenetic test results and biochemical or clinical outcomes among patients taking statin therapy?

Key Question #3: How does the pharmacogenetic test result affects the decision to use statin therapy; that is, how often is therapy changed in response to the test result?

Key Question #4: What benefits, harms, or adverse effects are experienced by patients on statins from change in treatment received after pharmacogenetic testing?

**SECTION 3: MTHFR-Chemotherapy**

Key Question #1: Among patients undergoing chemotherapy, is there any association between genetic variations (SNPs) in *MTHFR* and complete response, partial response, or stable disease?

Key Question #2: What demographic or clinical variables mediate the association between pharmacogenetic test results and response to chemotherapy?

Key Question #3: How does the pharmacogenetic test result affect the decision to undergo chemotherapy; that is, how often is therapy changed in response to the test result?

Key Question #4: What benefits, harms, or adverse effects are experienced by patients on chemotherapy from change in treatment received after pharmacogenetic testing?

# Methods

This evaluation of pharmacogenetic tests for non-cancer and cancer conditions is based on a systematic review of the literature.

## Literature Search Strategy

We conducted a comprehensive search of the peer-reviewed scientific literature to identify relevant studies addressing the key questions. Medline was searched from its inception through September 2007. We used relevant terms for the pharmacogenetic tests and limited the search to studies on humans that were published in English (see **Appendix 1** for the complete search strategy). We did not search for unpublished data. Additional searches were conducted through April 2008 of Medline and clinicaltrials.gov to identify published randomized controlled trials, systematic reviews, and ongoing clinical trials of *CYP2C9* and *VKORC1* for warfarin therapy.

## Study Selection

We assessed titles and abstracts of citations identified from the literature searches using the eligibility criteria listed below. A low threshold was used to retrieve articles. The full text of articles that potentially met the inclusion criteria were retrieved and reviewed for inclusion. References cited in the retrieved studies and selected reviews were examined to identify additional published articles. Duplicate reports or multiple publications of the same article were identified by comparing authors and study recruitment centres. Studies were included only once, except when duplicate reports of the same study provided complementary information. This

report addresses four different pharmacogenetic tests. In addition to the general eligibility criteria, each topic also had specific eligibility criteria.

**Population**

We included only studies of adult humans who were: taking warfarin and underwent genetic testing for *CYP2C9* and *VKORC1* SNPs; taking any type of statin and underwent genetic testing for *Apo E* SNPs; receiving chemotherapy directed to the folate metabolic pathway and underwent genetic testing *MTHFR* SNPs. When data on racial descent were reported separately, we analyzed the results by racial descent strata.

**Exposure**

Relevant tests were those that evaluated specific genetic polymorphisms and that were used in conjunction with specific drug therapy. We included studies that evaluated allelic variants **2* and/or**3* for *CYP2C9*, various common allelic variants for *VKORC1,* alleles *e2, e3*, and *e4* for *Apo E*, and common allelic variants for *MTHFR* were included (see individual results sections for more details on how the genetic risk factors were selected).

**Comparisons**

In each study, we used a dominant genetic model to contrast carriers vs non-carriers of the variant alleles. (In the following sections, we refer to people who do not have the genetic variant of interest as "non-carriers".) We did not evaluate additive genetic models (i.e., allele-based analyses on an assumption of a constant odds ratio per copy of the variant allele) or other comparisons (e.g., comparison of extreme homozygotes). Details of genetic comparisons are available in individual results sections.

### Outcomes

We included all relevant clinical and laboratory outcomes. The outcomes evaluated are described in detail in each individual results section.

### Design

We accepted studies of any design that recruited at least 10 subjects per group.

## Data Extraction

For each included study, we extracted the following data: year of publication, country, clinical setting, study design, eligibility criteria, patient characteristics, details of the genotyping methodology, therapy used, co-medications, co-morbidities, definitions of outcomes, and baseline and final results for outcomes of interest (**Appendix 1** has the data extraction forms). We extracted data for time points as reported in individual studies. All complications (adverse events) were extracted exactly as reported. Data were extracted by single reviewer and verified by a second reviewer. Disagreements were resolved through consensus.

It is difficult to assign a single quality score for pharmacogenetic studies. No established method comprehensively describes all features pertinent to the validity of these studies. In addition, in any study, quality is assessed on information reported in the article and not necessarily on how the study was conducted. Thus, we systematically evaluated individual studies for the following characteristics without assigning a quality score: homogeneity of study groups, whether genotyping personnel was blinded to clinical results, genotyping methodology, replication or verification of genotyping with another protocol, reporting of loss to follow up, clear description of patient recruitment, assessment of deviations of the genotype frequencies from those predicted by the Hardy-Weinberg law (deviations from the Hardy-Weinberg

equilibrium [HWE]), assessment of gene-gene interactions, and control for possible clinical (age, sex, severity of disease) and other (smoking, diet) modifiers between genotypes. Details of these items in each study are reported in **Appendix 2**. In subgroup analyses we examined whether any observed heterogeneity could be explained by these factors.

## Data Synthesis and Analysis

### Meta-Analyses

Generally, when two or more studies made the same comparison, and when the data were sufficient, we performed meta-analyses to identify any association between pharmacogenetic testing results and patient outcomes with therapeutic management. The exception was the analysis of *VKORC1* variants and response to warfarin, where meta-analyses were performed when at least 4 studies were available (for practical purposes, since 22 different variants had been studied in the eligible reports).

For the main analyses, we used DerSimonian and Laird's random-effects model for all syntheses.[6] The random-effects model assigns a weight to each study based on both the individual study variance and the estimated between-study heterogeneity. Compared with the fixed-effect model, the random-effects model is more conservative in that it generally results in wider confidence intervals when between-study heterogeneity is present. We tested for heterogeneity using Cochran's Q and assessed its extent with $I^2$. $I^2$ expresses the proportion of between-study variability that is attributed to heterogeneity rather than chance.[7]

### Subgroup, Sensitivity Analyses, and Meta-regression

To address key question 2, we analyzed the data by demographic and disease subgroups. In addition, to exploring potential differences in results across studies and in the presence of

significant heterogeneity, further subgroup analyses were conducted based on study-design factors and duration of follow-up. More information is given in the individual result section. Heterogeneity of results across studies was explored by investigating the consistency of estimates between subgroups.

In addition, random effects meta-regression was performed when applicable. Weights were defined as the inverse variance of the odds ratio (OR) incorporating the residual heterogeneity variance. Meta-regression can reveal the effects of particular study characteristics on response to therapy.

# *CYP2C9* Gene Polymorphisms and Response to Warfarin Therapy

## Background

Warfarin is used to manage the risk of arterial and venous thromboembolism.[8] As a result of its narrow therapeutic index, managing warfarin therapy is challenging. An insufficient dose will fail to prevent thrombosis, and an excessive dose increases the risk of bleeding complications.[9,10]

Warfarin anticoagulation is used for very different indications. Typically, when treating patients with warfarin, physicians adjust doses to achieve desirable anticoagulation levels during a treatment induction period. Once the dosing scheme is stabilized patients are kept on a (largely) stable maintenance dose such that their international normalized ratio (INR) is within the desired therapeutic (target) range. Warfarin maintenance doses can vary greatly among patients (ranging from 0.5 mg/day to 60 mg/day).[11] For some patients, even seasoned specialists can face difficulties in finding a stable warfarin maintenance dose. Age, body weight, co-medications, co-morbid conditions, diet, alcohol consumption, and genetic factors affect patient response to warfarin therapy.

The cytochrome P450 complex is a group of hepatic microsomal enzymes involved in the oxidative metabolism of many drugs. Cytochrome P450, subfamily IIC, polypeptide 9 (*CYP2C9*), is the principal drug-metabolizing enzyme for warfarin. *CYP2C9* inactivates the S-enantiomer of warfarin by hydroxylation. Two variants of *CYP2C9* reduce the metabolism of warfarin (*CYP2C9*2* (430C>T) and *CYP2C9*3* (1075A>C)[12] or have been associated with a reduced dose of warfarin[13] and an increased risk for bleeding.[12,14]

Bleeding is a serious complication in patients who are on warfarin. Thromboembolism is also a serious complication among those under-dosed on warfarin therapy. Knowing whether a patient carries the aforementioned gene variants may help identify whether he or she is at an increased risk of bleeding or thromboembolism. At least in theory, warfarin doses could be tailored to take into account each patient's genetic risk factors. However, it remains unclear whether pharmacogenetic testing has a favorable impact on patient-relevant clinical outcomes.

**Data Synthesis and Analysis for Associations between *CYP2C9* Genotypes and Warfarin Therapy**

In the following sections, we will be referring to the *CYP2C9* alleles *CYP2C9 *2* and *CYP2C9 *3. CYP2C9 *2* (rs1799853) is also referred to as 430C>T. *CYP2C9 *3* (rs1057910) is also referred to as 1075A>C.

We performed meta-analyses of weighted mean differences in the average warfarin maintenance dose and the rate of bleeding complications across genotypes. We contrasted carriers versus non-carriers of the genetic risk factors, essentially assuming a dominant genetic model. More specifically, we contrasted carriers versus non-carries of *CYP2C9*2*, *CYP2C9*3*, and either *CYP2C9*2* or *CYP2C9*3*. When studies did not report standard deviations (SD) of the average maintenance dose of warfarin, we imputed means and standard deviations based on medians and ranges.[15]

Subgroup analyses by ethnic descent (European, Asian) were performed. In sensitivity analyses, we excluded studies in which the genotype proportions deviated significantly from the HWE-predicted proportions and those studies for which we imputed means and standard deviations on the basis of medians and ranges.[15]

## Results for Section 1: *CYP2C9/VKORC1* – Warfarin

The literature search identified 270 citations, from which 63 full-text articles were retrieved. Of these, 29 evaluated *CYP2C9 *2* allele and *CYP2C9*3* allele and were included in the systematic review,[16-44] including three recently published RCTs from the updated literature search.[41,43,44] Of 29 studies, 10 recruited patients in the induction phase of warfarin therapy.[21,25,31-33,40-44] Of these 10 studies, three continued follow-up until the maintenance phase. Another 12 of the 27 studies recruited patients in the maintenance phase, when patients were on stable warfarin dosing. The studies were published between 1995 and 2007 (**Table 1.1**). **Appendix 1** presents a flow chart of retrieved and excluded studies, along with the reasons for exclusion.

**Table 1.1. Characteristics of 29 studies evaluating associations between the *CYP2C9* genotypes and response to warfarin therapy**

| Characteristics | Description: number of studies (references) |
|---|---|
| Total | 29 studies[16-44] |
| Study design | Prospective cohort: 14[17,18,23,24,26,27,29,30,33-36,39,40]<br>Retrospective cohort: 8[19-22,25,28,31,32]<br>Retrospective and Prospective cohort: 2[37,42]<br>Case-control: 2[16,38]<br>Randomized controlled trial: 3[41,43,44] |
| Ethnicity | Caucasians: 12[16,20,25-27,31-34,38,41,42]<br>Asians: 5[17,29,30,36,39]<br>Mixed: 6[18,21,24,28,35,43]<br>Not documented: 6[19,22,23,37,40,44] |
| Setting | Anticoagulation clinic: 16[16,19-28,34,37-39,42]<br>Other: 11[17,18,29,30,32,33,35,40,41,43,44]<br>Not documented: 2[31,36] |
| Beginning of patient recruitment | Warfarin initiation phase: 10[21,25,31-33,40-44]<br>Warfarin maintenance phase: 12[16-19,23,24,26,30,34-36,38]<br>Not documented: 7[20,22,27-29,39,41] |
| Outcomes assessed | Mean maintenance dose: 25[16-40]<br>Rate of over-coagulation: 6[21,25,32,33,37,40,43,44]<br>Risk of bleeding: 9[21,25,27,31,33,38,40-44]<br>Thromboembolism: 2[38,40] |

***Key Question #1a: Among patients taking warfarin, is there any association between carrying the genotype and the effective dose of warfarin?***

Outcomes reported in the studies included warfarin dosage, rate of over-anticoagulation (defined as higher-than-target INR levels as reported in individual studies), rates of bleeding, and rates of thromboembolism or pulmonary embolism.

**Warfarin dose**

Of the 25 studies evaluating warfarin dosing and CYP2C9 genotypes,[16-40] five included only Asian populations.[17,29,30,36,39] Most (14) studies were prospective cohorts,[17,18,23,24,26,27,29,30,33-36,39,40] 10 were retrospective cohorts or case control studies,[16,19-22,25,28,31,32,38] and one used both research designs.[37] Most studies used polymerase chain reaction for genotyping, but only eight replicated or verified their methodology or reported quality controls for genotyping.[21,22,28,35,36,38-40] Only two studies reported blinding outcome assessors to genotype.[21,27]

Twenty of the studies reported that carriers of *CYP2C9 *2* allele and *CYP2C9*3* allele required significantly lower doses of warfarin than did non-carriers (**Table 1.2**).[16-21,23-25,28-35,37-39] The clinical significance of these differences is unclear.

**Table 1.2. Mean differences in maintenance warfarin dose between patients with and without *CYP2C9* variant genotypes**

| Genotype | N studies | N patients | Mean difference in Dose (95% CI), mg/day | Heterogeneity |
|---|---|---|---|---|
| *CYP2C9 *2* allele | 12 | 2530 | -0.8 (-1.05, -0.68) | NS |
| *CYP2C9 *3* allele | | | | |
| Overall | 16 | 3853 | -1.79 (-2.12, -1.45) | <0.05 |
| European descent | 13 | 3367 | -1.79 (-2.17, -1.40) | <0.05 |
| Asian descent | 3 | 486 | -1.76 (-2.34, -1.18) | NS |
| *CYP2C9 *2 or *3* allele | | | | |
| Overall | 18 | 3882 | -1.47 (-1.73, -1.21) | <0.05 |
| European descent | 15 | 3396 | -1.47 (-1.74, -1.20) | <0.05 |
| Asian descent | 3 | 486 | -1.55 (-2.96, -0.40) | <0.05 |

CI, confidence interval; NS, non significant at the 0.05 level

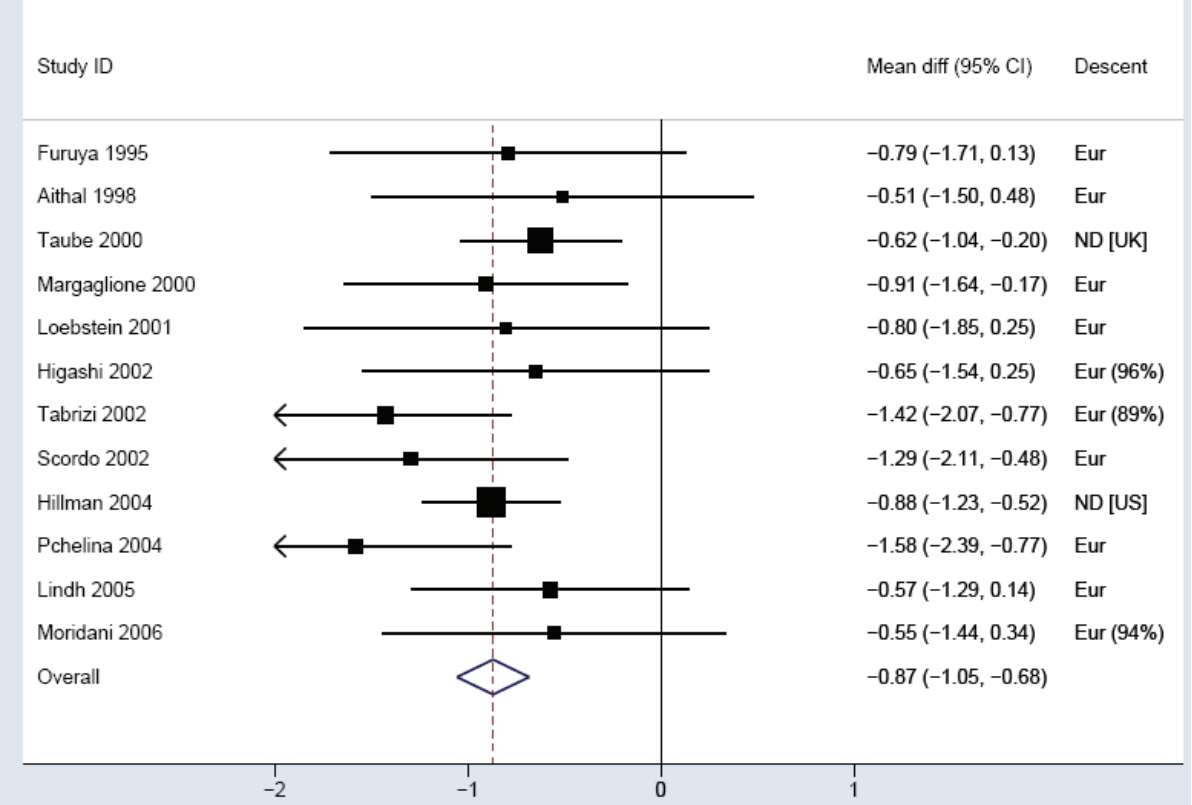
**Associations between carriers of *CYP2C9 *2* allele and *CYP2C9*3* allele and the maintenance dose of warfarin**

*Carriers of CYP2C9*2 allele*

Twelve studies (2,530 patients) reported data on the average difference in warfarin maintenance doses between carriers of *CYP2C9 *2* allele and all other patients (**Figure 1.1**).[16,19,21,22,25-28,32,34,35,37] Only four of the studies were prospective.[26,27,34,35] All 12 studies were conducted in patients predominantly of European or Caucasian descent. Three studies in Asian populations[29,30,36] did not identify any carriers of *CYP2C9*2* allele among 1,125 patients and are not included in this analyses.

Overall, carrying at least one copy of the *CYP2C9*2* allele was associated with a 0.87-mg/day (95% CI: 0.68 to 1.05) lower mean maintenance dose. The clinical significance of these differences is unclear. There was no between-study heterogeneity.

**Figure 1.1. Differences in the average daily maintenance dose of warfarin between carriers and non-carriers of the *CYP2C9*2* allele.**

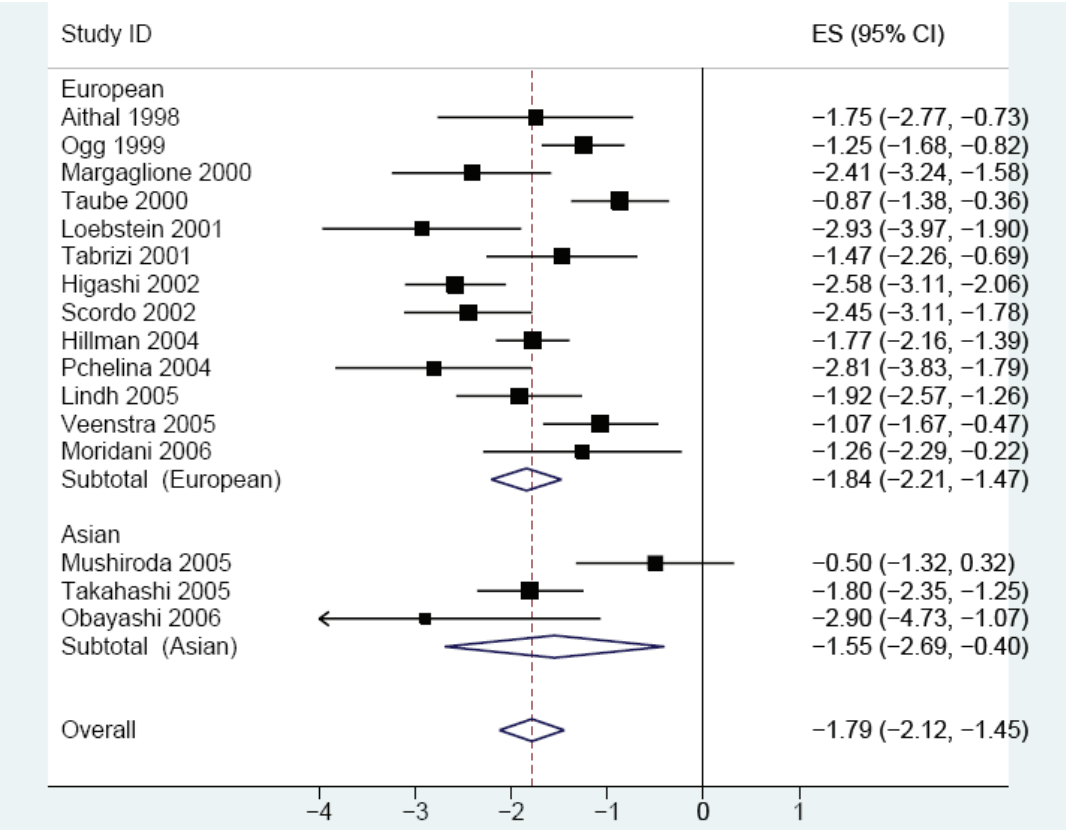



X label axis indicates difference in the mean maintenance dose of warfarin, in mg/day
95%CI, 95% confidence interval; Diff, Difference in the mean maintenance dose of warfarin, in mg, between *CYP2C9*2* allele carriers and non-carriers; Eur, Europe; ND, not documented

*Carriers of CYP2C9*3 allele*

Of the 16 studies (3,853 patients) reporting data on the average difference in doses between carriers and non-carriers of *CYP2C9 *3* allele,[16,21,22,25-32,34-37,39] 8 studies were prospective (**Figure 1.2**).[26,27,29,30,34-36,39] The presence of the allele was associated with a 1.79 mg (95% CI 1.45 to 2.12 mg) lower mean maintenance dose, but the between-study heterogeneity was significant. The mean difference in maintenance doses was not significantly different between people of European and Asian descent (1.84 mg vs. 1.55 mg, respectively). There was statistically significant between-study heterogeneity within both racial descent subgroups.

**Figure 1.2. Differences in the average daily maintenance dose of warfarin between carriers and non-carriers of the *CYP2C9*3* allele.**




X label axis indicates difference in the mean maintenance dose of warfarin, in mg/day
95%CI: 95% confidence interval; Diff: Difference in the mean maintenance dose of warfarin between *3 allele carriers and non-carriers;

*Carriers of either CYP2C9*2 or *3 allele*

Of 18 studies (3,882 patients) reporting data on the average difference in doses between carriers of either *CYP2C9*2* or **3* alleles (Figure 1.3),[16,18,21,22,25-30,32,34-40] nine studies were prospective.[18,26,27,29,30,34-36,39] Three studies of Asian patients (1,125 patients) found no carriers of *CYP2C9*2* alleles.

Overall, carrying at least one variant allele is associated with a 1.47-mg/day (95% CI 1.21 to 1.73 mg) lower mean maintenance dose, with significant between-study heterogeneity. The difference in mean maintenance doses was similar between people of European and Asian descent (1.47 mg vs. 1.55 mg, respectively), and again there was between-study heterogeneity.

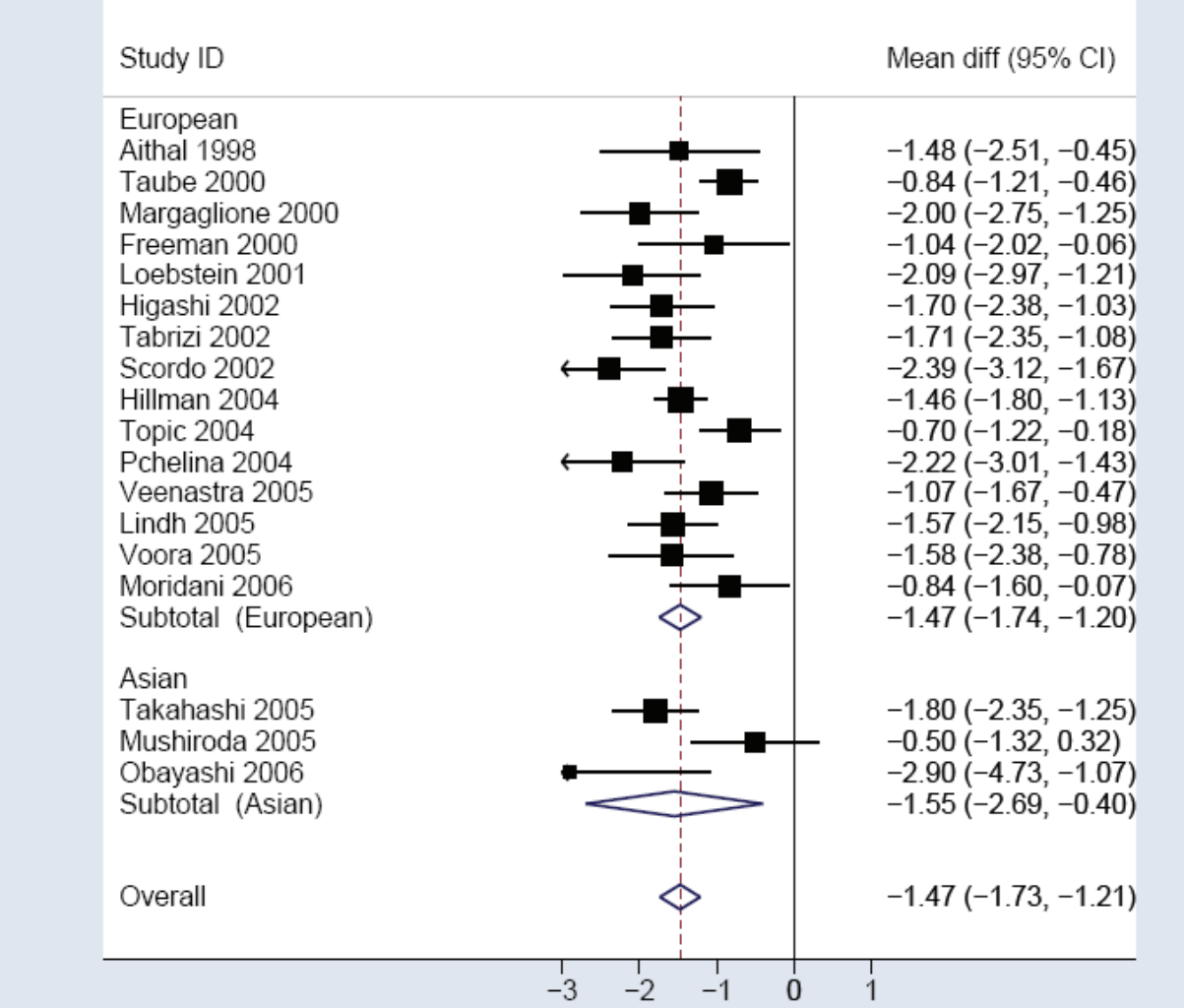
The results did not change when studies for which we imputed standard deviations were included or excluded from the analyses.

Two studies reported statistically significant odds ratios (OR) for lower warfarin dosing between variant genotype groups. Aithal reported that patients with one or more *CYP2C9* variants had a significant six-fold increase in the odds of having a low dose requirement (OR 6.21, 95% CI; 2.48 to 15.6). [16] Taube noted a significant five-fold increase in the odds (OR, 5.42; 95% CI 1.68 to 17.4) of having a lower warfarin mean maintenance dose (1.5 mg or less) in patients with the *CYP2C9 *2* allele.[37]

Two studies reported an association between *CYP2C9 *2* or **3* alleles and the time to achieve stable warfarin dosing.[21,27] Of these, Higashi reported that the variant genotype group required more time (hazards ratio, 0.65; 95% CI 0.45 to 0.94) to achieve stable dosing.[21]

No single subgroup explains the observed heterogeneity across study results for *CYP2C9 *3* allele and *CYP2C9 *2* or **3* allele (**Table 1.3**). Differences in the average maintenance warfarin dose in all analyses suggested that patients with *CYP2C9 *2* alleles consistently had lower maintenance dose requirements. There was no study heterogeneity.

**Figure 1.3. Differences in the average daily maintenance dose of warfarin between carriers and non-carriers of either *CYP2C9*2* or *3 alleles.**




X label axis indicates difference in the mean maintenance dose of warfarin, in mg/day; 95%CI: 95% confidence interval; Diff: Difference

**Table 1.3. Subgroup and Sensitivity Analyses on the Effects of the *CYP2C9* alleles **2* or **3* on Mean Warfarin Maintenance Dose**

| Subgroup | *CYP2C9 *2 carriers vs. non-carriers* | | | *CYP2C9 *3 carriers vs. non-carriers* | | | *CYP2C9 *2 or *3 carriers vs. non-carriers* | | |
|---|---|---|---|---|---|---|---|---|---|
| | N (patients) | Mean difference, mg/d (95% CI) | $P_{Het}$ ($I^2$ [%]) | N (patients) | Mean difference, mg/d (95% CI) | $P_{Het}$ ($I^2$ [%]) | N (patients) | Mean difference, mg/d (95% CI) | $P_{Het}$ ($I^2$ [%]) |
| All studies | 12 (2530) | -0.87 (-1.06, -0.68) | 0.52 (0) | 16 (3853) | -1.79 (-2.12, -1.45) | <0.01 (74) | 18 (3882) | -1.47 (-1.73, -1.21) | <0.01 (64) |
| Racial descent | | | | | | | | | |
| European | 12 (2530) | -0.87 (-1.06, -0.68) | 0.52 (0) | 13 (3367) | -1.84 (-2.21, -1.47) | <0.01 (75) | 15 (2757) | -1.47 (-1.74, -1.20) | <0.01 (63) |
| Asian | NA | NA | NA | 3 (486) | -1.55 (-2.69, -0.40) | 0.01 (79) | 3 (1125) | -1.55 (-2.70, -0.40) | 0.01 (78) |
| Only patients with stable dose | | | | | | | | | |
| Yes | 5 (678) | -1.07 (-1.45, -0.70) | 0.51 (0) | 6 (887) | -1.79 (-2.12, -1.45) | 0.15 (39) | 8 (1106) | -1.66 (-2.14, -1.18) | 0.01 (62) |
| No | 7 (1852) | -0.80 (-1.02, -0.59) | 0.50 (0) | 10 (2966) | -1.62 (-2.06, -1.19) | <0.01 (80) | 10 (2776) | -1.36 (-1.67, -1.05) | <0.01 (67) |
| Prospective study | | | | | | | | | |
| Yes | 4 (582) | -1.16 (-1.55, -0.78) | 0.65 (0) | 8 (1760) | -1.84 (-2.40, -1.29) | <0.01 (75) | 10 (1841) | -1.64 (-2.01, -1.27) | 0.02 (56) |
| No | 8 (1948) | -0.78 (-0.99, -0.56) | 0.59 (0) | 8 (2093) | -1.75 (-2.20, -1.29) | <0.01 (77) | 8 (2041) | -1.31 (-1.65, -0.96) | 0.01 (66) |
| Deviation from HWE | | | | | | | | | |
| Yes | NA | NA | NA | 1 (185) | -2.58 (-3.11, -2.06) | NA | 1 (185) | -1.70 (-2.34, -1.03) | NA |
| No | 12 (2530) | -0.87 (-1.06, -0.68) | 0.52 (0) | 15 (3668) | -1.71 (-2.04, -1.38) | <0.01 (70) | 17 (3697) | -1.46 (-1.74, -1.18) | <0.01 (65) |
| Blinding | | | | | | | | | |
| Genotypic personnel | 2 (365) | -0.80 (-1.37, -0.24) | 0.66 (0) | 2 (365) | -2.53 (-2.98, -2.09) | 0.74 (0) | 2 (365) | -1.84 (-2.34, -1.34) | 0.57 (0) |
| No or not reported | 10 (2165) | -0.88 (-1.10, -0.67) | 0.36 (9) | 14 (3488) | -1.66 (-2.00, -1.33) | <0.01 (70) | 16 (3517) | -1.43 (-1.71, -1.15) | <0.01 (66) |

CI: confidence interval; HWE: Hardy-Weinberg equilibrium; N: number; $P_{Het}$: p-value for heterogeneity

***Key Question #1b: Among patients taking warfarin, is there any association between carrying the variant genotype and therapeutic INR levels?***

**Rate of Over-Anticoagulation (INRs above Therapeutic Levels)**

Six studies **(Table 1.4)** evaluated the risk for over anticoagulation among carriers of *CYP2C9 *2* or *3* allele.[21,25,32,33,37,40] Two were prospective,[33,40] and four, retrospective.[21,25,32,37] With few exceptions, (e.g., patients with prosthetic valves), the targeted therapeutic INR was between 2 and 3 in the majority of patients. However, the threshold INR levels defining over-anticoagulation varied across studies from 3 to more than 8. One study (Taube 2000) that used a threshold INR greater than 8 found no association between carrying the *CYP2C9 *2* or *3* alleles and INR above the therapeutic threshold.[37] Differences in the definitions of over-anticoagulation and the differences in the time interval over which over anticoagulation events were examined precluded meta-analyses.

A positive association was reported between *CYP2C9 *2* and *3* alleles and INRs above 3 during the first 2 weeks of anticoagulation therapy.[25] The rate of over-anticoagulation among carriers and non-carriers did not differ during the third week of warfarin dose titration for patients with INRs greater than 3 or 4. The four remaining studies found significant associations between *CYP2C9 *2* and *3* alleles in patients starting on warfarin with INRs greater than 3 or 4, with relative effects ranging from 1.4 to 11.1 (reported as either risk ratios, or odds ratios, or hazard ratios) (**Table 1.4**).

Only one study provided a pharmacogenetic tool to assist titrating the warfarin maintenance dose depending on *CYP2C9* genotypes.[40] Despite the availability of the tool, among 48 orthopedic patients starting on anticoagulation with INRs greater than 4, the hazard ratio for

over-anticoagulation was 4.6, suggesting that over-anticoagulation was more common in patients who carried the genetic variants.

**Table 1.4. Characteristics of Six Studies Evaluating the Association between the Carrying the *CYP2C9 *2* or **3* alleles and Over-Anticoagulation**

| Study, year | Analyzed only patients initiating warfarin | Stable dose an inclusion criterion | Target INR | Definition of over anticoagulation, INR | $N_{All}$ | **2* or **3* Carriers | Crude genetic effect size | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Metric | **2* or **3* allele carriers |
| Taube, 2000[37] | No | No | 2.5 | >8 (over >2 mo) | 561 | 169 | OR | 1.52 (0.64, 3.58) |
| Lindh, 2005[25] | Yes | No | 2-3 | >3 (over 1st wk) | 219 | 84 | RR | **2.15 (1.59, 2.92)** |
| | | | | >3 (over 2nd wk) | 217 | 84 | RR | **2.05 (1.51, 2.79)** |
| | | | | >3 (over 3rd wk) | 210 | 84 | RR | 1.00 (0.68, 1.48) |
| | | | | >4 (over 1st wk) | 219 | 84 | RR | 1.48 (0.80, 2.72) |
| | | | | I>4 (over 2nd wk) | 217 | 84 | RR | **2.24 (1.64, 3.07)** |
| | | | | >4 (over 3rd wk) | 210 | 84 | RR | 1.27 (0.70, 2.29) |
| Peyvandi, 2004[33] | Yes | No | 2-3 | >3 (between days 4 and 24) | 125 | 50 | RR | **2.01 (1.28, 3.15)**[a] |
| Pchelina, 2004[32] | Yes | No | 2-3 (2.5-3.5 for some) | >3 or 3.5 as applicable (over 4 wk) | 62 | 16 | OR | **11.1 (2.9, 41.9)**[b] |
| Voora, 2005[40] | Yes | No | 2.5 | >4 (over 40d) | 48 | 16 | HR | **4.6 (1.4, 14.7)**[c] |
| Higashi, 2002[21] | Yes | No | 2-3 (2.5-3.5 for some) | >4 or 4.5 (over 2.2 y on average) | 185 | 58 | HR | **1.40 (1.03, 1.90)** |

$N_{All}$: all analyzed patients

[a] Assuming that all 4 double heterozygotes were over-anticoagulated. The RR would be 2.53 (95% CI, 1.54 to 4.14) if none of the 4 double heterozygotes were over anticoagulated.

[b] Odds ration would be 14.7 (95% CI, 3.8 to 57.2) if the single double-heterozygote was not over-anticoagulated

[c] Based on the maximum P value reported for this hazard ratio (0.01)

Three studies evaluated the time to reach the target INR.[21,32,42] Two studies reported no significant differences between the carriers and non-carriers of *CYP2C9 *2* or **3* alleles,[21,42] while the third reported that the time to reach therapeutic INR for non-carriers was significantly longer than it was for carriers of *CYP2C9 *2* or **3* alleles.[32]

None of the studies reported any statistically significant interactions between patient- or disease-related factors and the genetic variants for time to achieving the therapeutic INR range.

***Key Question #2: Among patients taking warfarin, is there any association between carrying the genotype and adverse outcomes, such as serious bleeding events or thrombotic events?***

**Risk of bleeding complications**

Nine studies examined the association between bleeding complications and carriers of *CYP2C9 *2, *3*.[21,25,27,31,33,38,40-42] Seven provided quantitative data suitable for meta-analysis.[21,25,27,31,33,38,40] The two studies that could not be included in the meta-analyses were an RCT that evaluated pharmacogenetic-based warfarin dosing versus standard approaches[41] and a retrospective study on warfarin-receiving patients.[42] The RCT by Hillman reported lower bleeding complication rates among 18 patients treated with pharmacogenetic-based dosing than among 20 patients treated with standard dosing (11% versus 25%).[41] One retrospective study reported that the risk of serious or life-threatening complications among carriers of *CYP2C9 *2, *3* was 2 to 3 times higher than it was among non-carriers (P=0.03).[42]

**Meta-analyses on the association between *CYP2C9*2 and *3* variants and bleeding events**

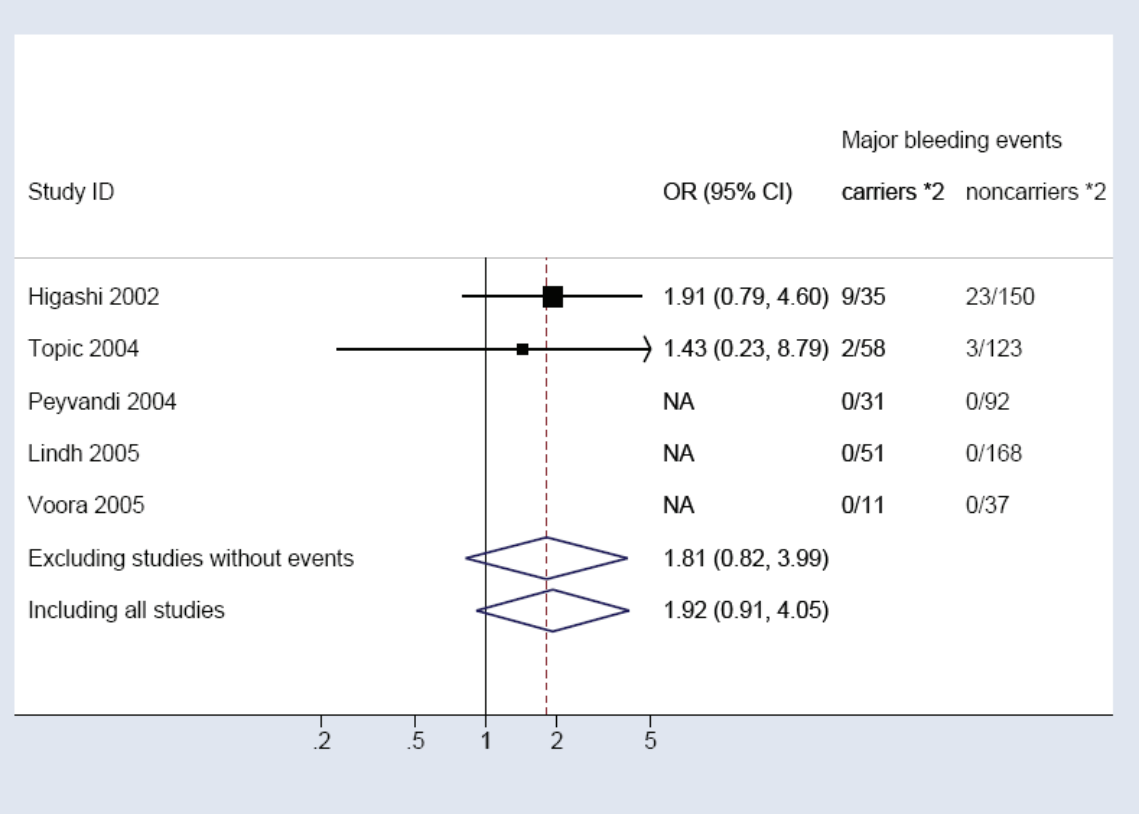
Bleeding events were defined differently in the seven studies included in the meta-analyses.[21,25,27,33,38,40] Two studies were prospective[27,33] and five were retrospective.[21,25,38,40] Only one study[21] used the Fihn criteria[45] to define serious and potentially life-threatening bleeding. Major bleeding was defined variously as bleeding warranting intervention or additional testing;[27] bleeding resulting in a life-threatening or disabling event, or rehospitalization or death;[25,40] macroscopic hematuria and melena[38]; and clinical symptoms or a drop in hematocrit.[33] The severity of bleeding was not defined in one study.[31]

*Carriers of the CYP2C9*2 allele*

The association between *CYP2C9 *2* allele and only major bleeding events was reported in five studies (756 patients) (**Figure 1.4**). Both studies reporting major bleeding events were retrospective.[21,38] The pooled odds ratio for increased bleeding events was 1.81 (95% CI, 0.82 to 3.99) favoring patients who do not carry *CYP2C9 *2* allele. The pooled estimate after excluding studies with no major bleeding events in any patients or including all studies in the analyses did not change inferences. There was little statistical evidence for heterogeneity in either analysis.

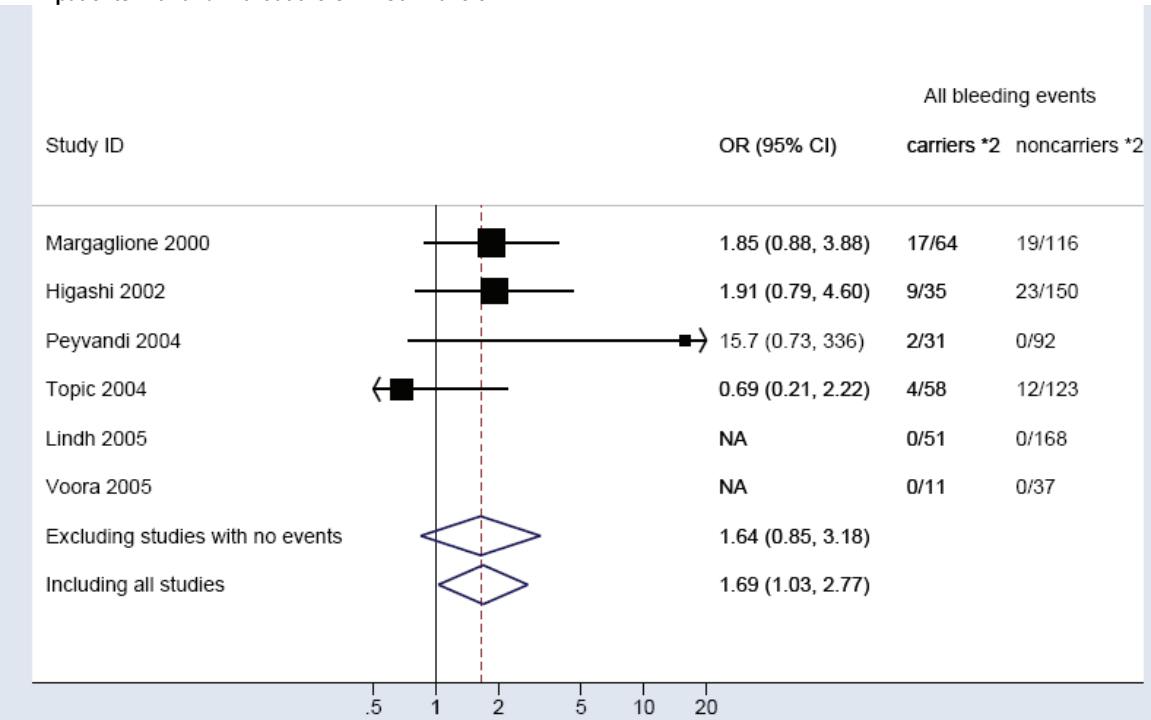
The association between *CYP2C9 *2* allele and bleeding of *any severity* was not significant (OR, 1.64; 95% CI, 0.85 to 3.18) (**Figure 1.5**). This analysis excluded the two studies with no bleeding events in any genotypic group. However, including all studies in the analyses resulted in a statistically significant odds ratio for increased bleeding events. There was no statistical heterogeneity in either analysis.

**Figure 1.4. Individual and pooled estimates for an association between warfarin treatment and major bleeding events in patients with and without the *CYP2C9 *2* allele**




X label axis indicates odds ratio (OR) and ratio greater than 1 indicates increased major bleeding events in the carriers of *CYP2C9 *2* allele.

**Figure 1.5. Individual and pooled estimates for an association between warfarin treatment and bleeding events of any severity in patients with and without the *CYP2C9 *2* allele.**



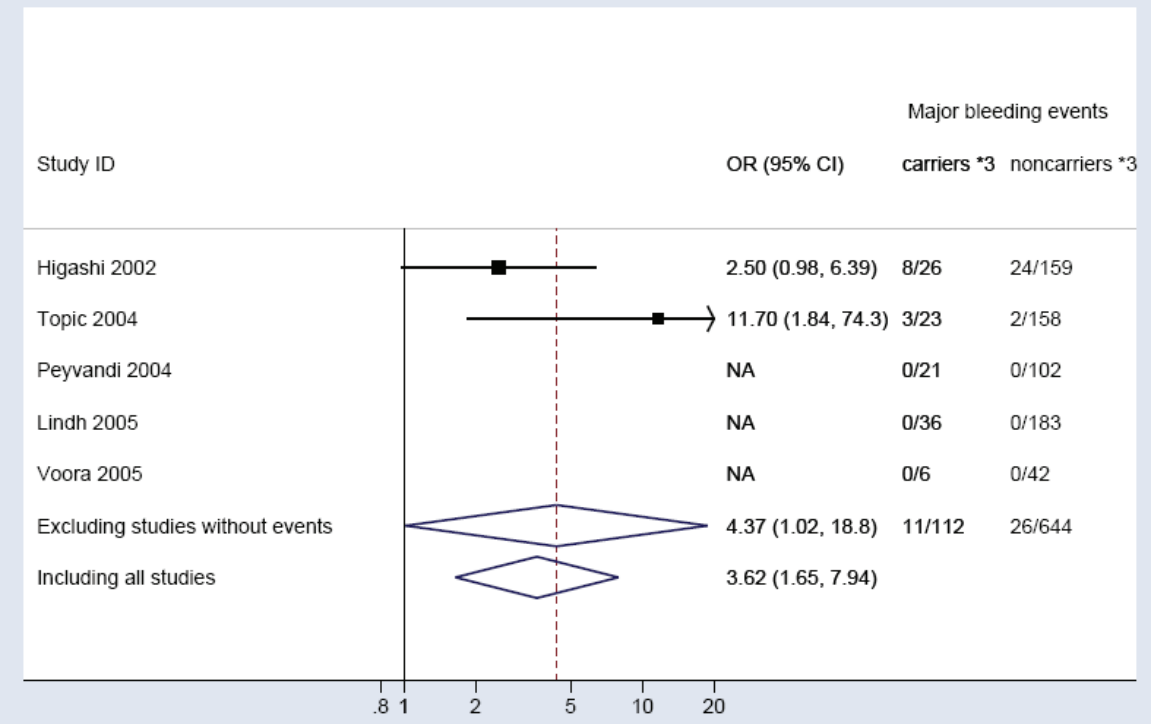

X label axis indicates odds ratio (OR) and ratio greater than 1 indicates increased bleeding events in the carriers of *CYP2C9 *2* allele.

*Carriers of the CYP2C9*3 allele*

The association between carriers of *CYP2C9 *3* allele and *major bleeding events* was assessed in five studies (756 patients; **Figure 1.6**). Both studies that reported major bleeding events were retrospective.[21,38] In three of the five studies, major bleeding rates ranged from 0 percent to 31 percent (8/26) among carriers of *CYP2C9 *3* allele and from 0 percent (3 studies) to 16 percent (24/159) among non-carriers. Excluding studies without major bleeding events in any patients, the pooled odds ratio for major bleeding was 4.37 (95% CI 1.02 to 18.8) favoring patients who do not carry *CYP2C9 *3* allele. Including all studies in the analyses resulted in a pooled odds ratio of 3.62 (1.65 to 7.94). Heterogeneity was not statistically significant in either analysis. With the exception of Higashi,[21] genotype distributions of patients taking warfarin were not statistically different from HWE-predicted proportions. When corrected for deviations from the HWE-predicted proportions, the pooled estimates did not change appreciably.[21]

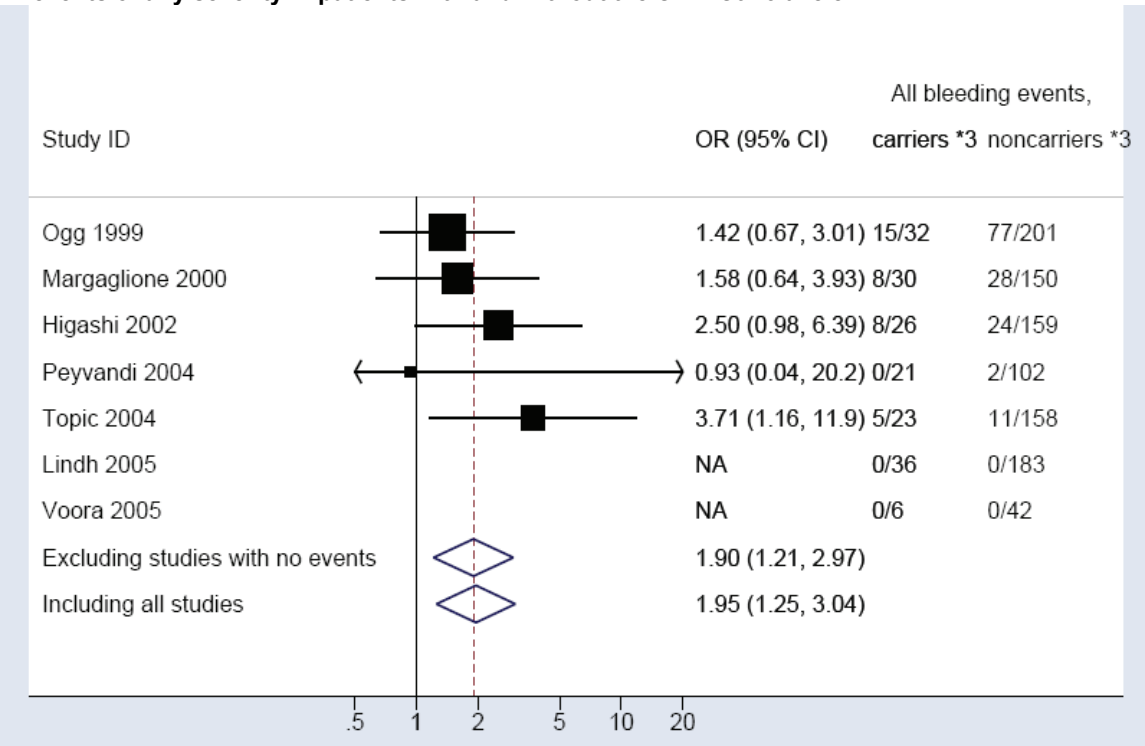
The association between carrying *CYP2C9 *3* allele and bleeding of *any severity* was significant (OR 1.90, 95% CI 1.21 to 2.97) in **Figure 1.7**. Including studies with no events in the analyses did not change the inferences, and there was no significant heterogeneity among studies.

**Figure 1.6. Individual and pooled estimates for an association between warfarin treatment and major bleeding events in patients with and without the *CYP2C9 *3* allele**




X label axis indicates odds ratio (OR) and ratio greater than 1 indicates increased major bleeding events in the carriers of *CYP2C9 *3* allele.

**Figure 1.7. Individual and pooled estimates for an association between warfarin treatment and bleeding events of any severity in patients with and without the *CYP2C9 *3* allele**



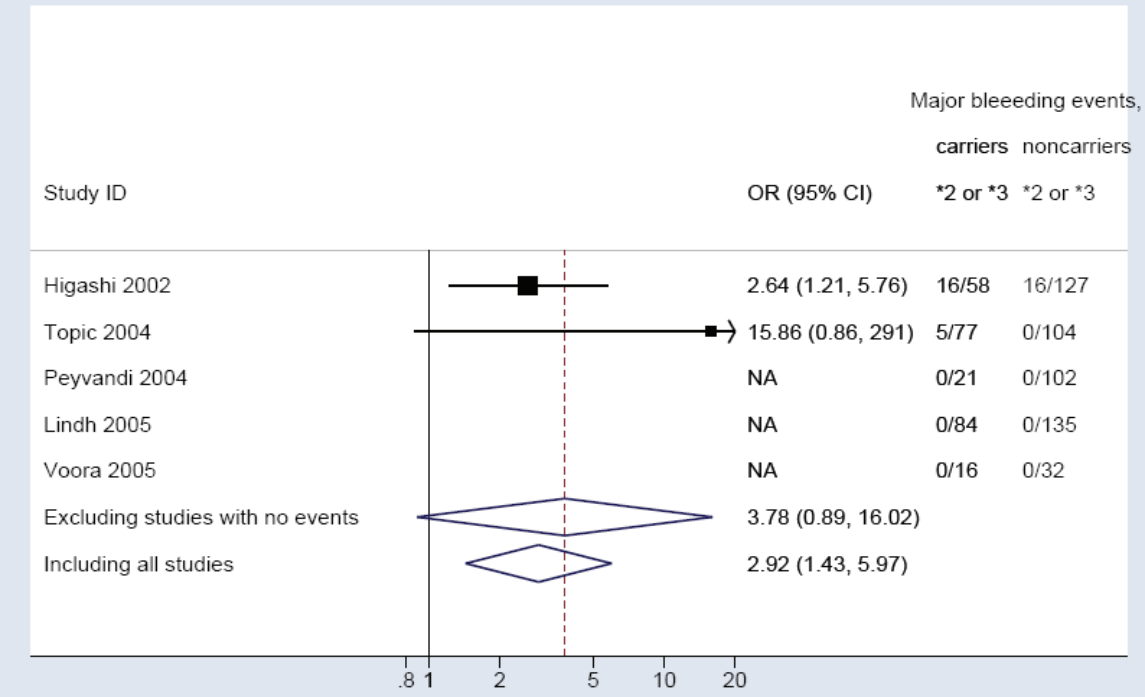

X label axis indicates odds ratio (OR) and ratio greater than 1 indicates increased bleeding events in the carriers of *CYP2C9 *3* allele.

*Carriers of either CYP2C9*2 or *3 alleles*

The association between carrying either *CYP2C9*2 or *3* alleles and *major bleeding events* was evaluated in five studies (756 patients). Two of the studies did not clearly distinguish events of at least "major" severity. In the remaining three studies, major bleeding rates ranged from 0 percent to 28 percent (16/58) among carriers of either alleles. Among people who did not carry *CYP2C9*2 or *3* alleles, rates were 0 percent in 4 studies and 13 percent (16/127) in the Higashi study.[21] Excluding studies with no major bleeding events in any patients, the pooled odds ratio was non-significant: 3.78 (95% CI 0.89 to 16.02), favoring patients who do not carry *CYP2C9*2 or *3* alleles (**Figure 1.8**). Including all studies in the analyses resulted in a significant pooled odds ratio of 2.92 (95% CI 1.43 to 5.97). Heterogeneity was statistically non-significant for both analyses. The association between carrying *CYP2C9* allele **2* or **3* and

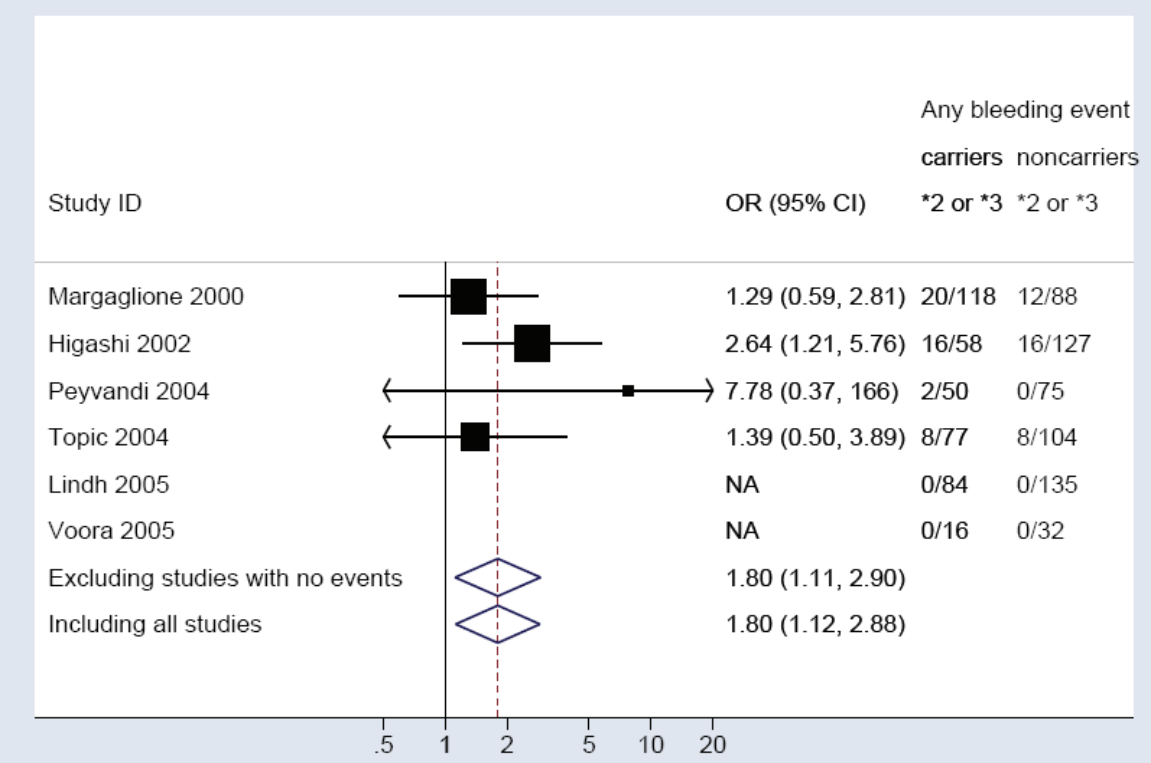
bleeding of *any severity* compared to non-carrier was significant, with an OR of 1.80 (95% CI 1.12 to 1.90) (**Figure 1.9**). Including all studies in the analyses did not change the inferences, and there was no statistical heterogeneity.

**Figure 1.8. Individual and pooled estimates for an association between warfarin treatment and major bleeding events in patients with either the *CYP2C9* *2 or *3 allele and patients with neither allele.**




X label axis indicates odds ratio (OR) and ratio greater than 1 indicates increased major bleeding events in the carriers of *CYP2C9 *2* or *3* allele.

**Figure 1.9. Individual and pooled estimates for an association between warfarin treatment and bleeding events of any severity in patients with either the *CYP2C9* *2 or *3 allele and patients with neither allele**




X label axis indicates odds ratio (OR) and ratio greater than 1 indicates increased bleeding events in the carriers of *CYP2C9* *2 or *3 allele.

### Association with the Risk of Thromboembolism

Two studies reported that patients with *CYP2C9*2 or *3* alleles had rates of thromboembolism or pulmonary embolism while on warfarin therapy that were similar to those of patients with no variants.[38,40] It was not clear whether these two studies actively monitored the occurrence of thromboembolic events, or whether these were passively reported.

### *Key Question #3: What demographic or clinical variables mediate the association between pharmacogenetic test results and biochemical or clinical outcomes among patients who use warfarin?*

Of 25 studies, 10 assessed genetic effects on the average warfarin maintenance dose and provided additional data on patient- and disease-related factors that may explain the inter-

individual variability of warfarin dosing.[20-27,35,36] Across all studies, patients' age at the start of warfarin therapy, along with genotype, significantly influenced the variability in warfarin dosing among patients. Patient age and the allele contributed 20 percent to 33.7 percent of the inter-individual variability.

Two studies examined the association between age and warfarin dose for the carriers and non-carriers of *CYP2C9 *2, *3*.[24,26] In a two-way ANOVA test evaluating the combined effect of age and *CYP2C9* genotypes, patients 66 years and older at least one copy of *CYP2C9 *3* required a lower mean (SD) daily dose than did younger patients with the *CYP2C9 *1/*1*, (2.2 (1.2) mg versus 7.9 (3.7) mg, respectively; $P < 0.001$).[26] Warfarin dose depends on age, and the relationship between age and dose was different for the carriers and non-carriers of *CYP2C9 *2, *3*.[24] In this study, simple linear regression analyses of the carriers and non-carriers of *CYP2C9 *2, *3* revealed an inverse relationship between maintenance warfarin dose and advancing age only among carriers of *CYP2C9 *2, *3*.

Other notable factors that influenced warfarin dosing for the carriers and non-carriers of *CYP2C9 *2, *3* included body weight in four studies,[20,22,35,36] sex in two studies,[22,24] co-medications that are metabolic inducers of warfarin in one study,[20] and diabetes in two studies.[21,41] All four studies consistently reported that patient's body weight had a significant linear relationship with warfarin dose in the variant groups. Two studies examined the association of sex and warfarin dosing in the carriers and non-carriers of *CYP2C9 *2, *3* reported discordant results on the interaction of sex.[22,24] Although Hillman, in a multivariate analysis, concluded that being male accounted for 5 percent of the variability in dosing,[22] Linder[24] found no statistically significant association between sex and warfarin dosing in the carriers and non-carriers of *CYP2C9*2, *3*.

One study reported a significant interaction between local bleeding and gene variants.[27] In multivariate analyses, in addition to the gene variants, female sex, the source of bleeding, and the duration of bleeding were associated with an increased risk of bleeding.

***Key Question #4: How does the pharmacogenetic test result affect the decision to use warfarin; that is, how often is therapy changed in response to the test result)?***

We identified three published RCTs on pharmacogenetic testing of *CYP2C9* and warfarin dosing,[41,43,44] two of which compared genotype-guided warfarin dosing with standard warfarin dosing beginning from the induction phase.[43,44] Hillman reported that the rate of bleeding complications among 18 patients treated with pharmacogenetic-based dosing was lower than among 20 patients treated with standard dosing (11% versus 25%).[41]

Caraco randomly assigned 191 patients to receive warfarin in the induction phase, either by six different *CYP2C9* genotype-adjusted algorithms (study group) or by a validated algorithm that did not include genetic information (control group).[44] Patients were followed for 2 years. Investigators were blinded to patient genotype until after the completion of the induction phase, at 8 weeks. The study did not report the accuracy of the *CYP2C9* genotype-adjusted algorithms.

Groups were similar at baseline, except that controls were significantly more likely to have a second indication for warfarin (deep venous thrombosis and pulmonary embolism) and to have the clinical characteristics of hyperlipidemia. The proportion of patients with the *CYP2C9*1/*1* was higher in the study group than in control group. The primary endpoints of the study were time to restore INR to a therapeutic range of greater than 2 and time to reach stable anticoagulation defined as two consecutive INR values within 1-week were within the therapeutic range, without any intervening dose alteration. The study group reached both a

therapeutic INR and stable anticoagulation significantly earlier by 2.7 days and 18.1 days, respectively than the control group. Higher proportions of study group patients were within a therapeutic INR range for longer periods, and study patients experienced fewer minor bleeding events than did controls. This study did not evaluate the influence of *VKORC1* in the variability of warfarin maintenance dose. Different follow-up times were reported for each group. The results were evaluated first for day 1-8 for both groups, then for days 9-22 for study group and days 9-40 (approximately) for control group. The interpretation of the percent time in therapeutic range is difficult.

Anderson compared 101 patients treated with pharmacogenetic-guided dosing to 99 patients treated with standard dosing in an RCT.[43] Pharmacogenetic-guided dosing was derived from a regression equation based on 3 genetic variants (*CYP2C9* *2 and *3, and *VKORC1* 1173C>T), age, sex, and weight. Patients were followed up for 3 months. At baseline, the study group included more number of older patients and a higher proportion of patients with hypertension, and the control group had a higher proportion of variant genotypes. The primary end point was the per-patient percentage of out-of range INR (<1.8 or >3.2) measurements. The groups did not differ on the primary outcome, but the study group required statistically significantly fewer dosing changes and fewer INR measurements than did the control group. Patients who carried both *CYP2C9* and *VKORC1* had an increased risk (26 of 57 patients; P=0.01) for an INR of 4 or higher, and the pharmacogenetic dosing group had more adverse events, defined as clinical events plus instances of INRs of 4 or higher, than did the standard dosing group. Finally, individuals with wild type genotype required significantly increased dose of Warfarin compared to the standard model. While that did not result in a significant difference in out of range INRs (primary endpoint), the authors note that both multiple variant allele carriers

and wild-type patients experienced a 10% reduction in out of range INRs with pharmacogenetic guidance. Of note, both Caraco and Anderson used different standard loading dose. These trials indicate the need for consensus with regard to the warfarin induction therapy and to develop a prediction model for a loading dose.

***Key Question #5: What benefits, harms, or adverse effects are experienced by patients on warfarin from treatment received after the pharmacogenetic test results are known?***

***5a. Does pharmacogenetic testing among patients on warfarin and with supratherapeutic INRs result in better maintenance of therapeutic INRs and fewer episodes of serious bleeding?***

***5b. Does pharmacogenetic testing among patients on warfarin and with subtherapeutic INRs result in better maintenance of therapeutic INRs and fewer serious thrombotic events, such as stroke or pulmonary embolus?***

We identified no studies that evaluated key question 5 or its subquestions.

**Published reviews and ongoing clinical trials**

The meta-analysis of 11 studies by Sanderson reported that patients with *CYP2C9 *2* or **3* required 1.4 mg/day lower mean maintenance warfarin doses and had twice the risk for bleeding complications than non-carriers. The authors concluded that the clinical utility and cost-effectiveness of genotyping should be assessed before recommending routine testing.[12]

A rapid ACCE review of *CYP2C9* and *VKORC1* published in February 2008, which included selected grey literature sources, determined that although the analytic validity was at least 98% for *CYP2C9* genotyping, the evidence for analytic validity was weak for both genotypes.[46] The review also reported strong evidence for the clinical validity of both genes in

predicting stable warfarin dose. However, the evidence was weak for the association between *CYP2C9* testing and severe bleeding, and no studies assessed the association between the presence of *VKORC1* and severe bleeding. Data documenting the clinical utility of genotyping before warfarin initiation were not yet available at the time of the review.

On the basis of this ACCE review, the American College of Medical Genetics issued a policy statement and guidelines for using pharmacogenetic testing of *CYP2C9* and *VKORC1* to change therapeutic goals and to reduce adverse events from warfarin therapy.[47] The statement concluded that the evidence is not yet sufficient to recommend for or against routine *CYP2C9* and *VKORC1* testing in warfarin-naïve patients. The statement also identified the need for additional prospective clinical trials to provide direct evidence for the utility of *CYP2C9* and *VKORC1* pharmacogenetic testing in the setting of initial warfarin dosing. Ongoing clinical trials are summarized in Table 1.5

**Summary**

Several studies have evaluated the use of *CYP2C9* gene polymorphisms in guiding warfarin maintenance dosing. Carriers of the variant *CYP2C9* genotypes **2* or **3* require lower mean maintenance doses than do non-carriers. Four of six studies found significant associations between INRs greater than 3 or greater than 4 and patients on maintenance warfarin with *CYP2C9* genotypes **2* or **3*. However, studies found no significant differences between the carriers and non-carriers of *CYP2C9 *2, *3* in the time to reach target INR. The rates of major bleeding were higher in the *CYP2C9* genotypes **2* or **3* than in the *CYP2C9* genotype **1/*1*, but the differences were not statistically significant.

No studies evaluated whether pharmacogenetic testing among patients who are on warfarin and who have supratherapeutic INRs will result in better maintenance of therapeutic INR, fewer episodes of serious bleeding, or fewer serious thrombotic events.

**Table 1.5 Ongoing Clinical Trials of Warfarin Dosing Based on Pharmacogenetic Testing of CYP2C9 and VKORC1**

| Official title (Clinical trials.gov identifier) | Study Design | Intervention Comparator | Primary Outcome | Country where Conducted; Current status |
|---|---|---|---|---|
| Prospective CYP2C9 And VKORC1 Genotyping for Total Hip or Knee Replacement Patients Receiving Warfarin (Coumadin) For Anticoagulation (NCT00634907) | Treatment, Randomized, Open Label, Active Control, Parallel Assignment, Safety/Efficacy Study | Pharmacogenetic-based warfarin dosing<br><br>Usual care of warfarin dosing | Reduction in the number of adverse events associated with warfarin anticoagulation after total hip and total knee replacement from the time of warfarin initiation to 3 months after completion of warfarin therapy | US Recruiting |
| Prospective Evaluation Comparing Initiation of Warfarin StrategiEs (PRECISE): Pharmacogenetic-Guided Versus Usual Care (NCT00634907) | Treatment, Randomized, Single Blind, Dose Comparison, Parallel Assignment, Safety/Efficacy Study Phase IV | Pharmacogenetic-based warfarin dosing<br><br>Usual care of warfarin dosing | Accuracy of the initial versus the stable warfarin dose, measured as mean absolute difference in initial versus stable dose | US Recruiting |
| A Controlled Clinical Pharmacogenetic Study of a CYP2C9 Plus VKORC1 Polymorphism-Based Individualized Dosing Algorithm for Warfarin to Increase Efficiency of Achieving Therapeutic Dosing (NCT00334464) | Treatment, Randomized, Double-Blind, Active Control, Parallel Assignment, Pharmacokinetics Study | Pharmacogenetic-based warfarin dosing<br><br>Usual care of warfarin dosing | A comparison of the per-patient percentage of out-of-range INRs (<2, >3) over the observation period of up to 3 months | US Recruiting |
| Warfarin Induction Regimen Based Upon CYP2C9, VKORC1 Factor VII Genotyping, PMR and INR Monitoring, as Compared to the Conventional Regimen: a Prospective Controlled Study (NCT00162435) | Treatment, Randomized, Single Blind, Active Control, Parallel Assignment, Pharmacokinetics/Dynamics Study | Pharmacogenetic-based warfarin induction<br><br>Conventional regimen | Warfarin clearance Maintenance dose of warfarin at steady state.<br>Time to reach INR >2, pharmacodynamic steady state.<br>Time spent at therapeutic INR <3 and >2; >3; <2<br>Incidence of minor and major bleeding episodes. | Israel Recruiting |
| Study of the Association of Warfarin | Treatment, Randomized, Open Label, Placebo | Not documented | Not documented | Taiwan Recruiting |

| Official title (Clinical trials.gov identifier) | Study Design | Intervention Comparator | Primary Outcome | Country where Conducted; Current status |
|---|---|---|---|---|
| Dosage and Plasma Enantiomer Concentration With the Gene Polymorphisms of CYP and VKOR (NCT00247702) | Control, Single Group Assignment | | | |
| Warfarin Dosing: Pharmacogenetic Algorithm Compared to Pharmacist's Dosing (NCT00511173) | Diagnostic, Randomized, Single-Blind, Active Control, Parallel Assignment, Safety/Efficacy Study | Pharmacogenetic-Algorithm-based dosing<br>Warfarin dosing by pharmacist | Not documented | US<br>Recruiting |
| Creating an Optimal Warfarin Nomogram (CROWN) Trial (NCT00401414) | Treatment, Non-Randomized, Open Label, Uncontrolled, Single Group Assignment, Safety/Efficacy Study | Genetic testing and nomogram modified warfarin induction and maintenance dosing | Frequency of maintaining the target INR using the Partners nomogram will be compared with a historical control group<br>Extent to which the Partners nomogram misses the target INR | US<br>Recruiting |
| Modeling Genotype and Other Factors to Enhance the Safety of Coumadin Prescribing (NCT00484640) | Treatment, Randomized, Double-Blind, Active Control, Parallel Assignment, Safety Study | Pharmacogenetic-based warfarin dosing<br><br>Usual care of warfarin dosing | Weighted time in therapeutic range<br>Absolute deviation from clinically optimal dose | US<br>Not yet open for patient participation |
| Prospective Study Comparing Between the Commonly-Used and Pharmacogenetically Guided Warfarin Administration Protocols (NCT00654823) | Cohort, Prospective | Novel pharmacogenetic model for predicting warfarin (Coumadin) dose response<br><br>None | ND | Israel<br>Not yet open for patient participation |

## Discussion

For the majority of the studies evaluating *CYP2C9* gene polymorphisms and warfarin therapy, the primary outcome was the effect on the warfarin maintenance dose between carriers and non-carriers of genetic variants. Overall, the evidence supports the conclusion that carriers of the variant alleles **2* or **3* have lower average maintenance requirements than do non-carriers.

Most of the data pertain to patients of European descent, although studies of Asian patients show similar genetic effects.

*CYP2C9* variants are also associated with increased rates of bleeding complications among patients in induction phase. This association was true for all bleeding complications, irrespective of severity, as well as for major bleeding complications that required assistance or intervention. The studies did not explicitly describe whether the association between genotype and adverse clinical outcomes occurred in patients with normal or supratherapeutic INR ranges. Because of marked differences in the definition of bleeding complications across studies, and the possibility of selective reporting for this outcome, the pooled effect sizes from the corresponding meta-analyses should be interpreted with caution. Additionally majority of the association data were from a variety of observational studies that included cross-sectional, retrospective, and prospective designs. While most of the studies reported associations of warfarin and *CYP2C9* with regard to bleeding events and overmedication, studies in general, failed to report the outcome of thrombotic events and under dosing. Thrombotic events could be considered only in prospective studies in which patients with variant genotypes are given a lower dose due to the patient's genotype.

The clinical utility of genetic testing for *CYP2C9* in everyday clinical practice is not straightforward. Currently, physicians use rules of thumb to select a starting dose for warfarin treatment and rely on phenotypic (INR) feedback to titrate maintenance doses. It is unclear whether dose-prediction algorithms using genetic information improve clinical outcomes (fewer bleeding complications and fewer thromboembolic events) over those of standard practice. Only a few clinical trials have addressed this question, essentially three RCTs, each of which has their flaws in the design, inclusion criteria and power to reach statistical conclusions. Additionally,

there are new genetic associations that are currently under investigation, such as the relationship between genetic variations in cytochrome 4F2 (*CYP4F2*) and response to warfarin.[48] Evaluating the interactions among *CYP2C9*, *VKORC1*, and *CYP4F2* variants (i.e., gene-gene interactions) as well as gene-environmental interactions can result in better risk predictive instruments for clinical use.

Although we used a different analytic methodology to evaluate pharmacogenetic testing of *CYP2C9* among warfarin-treated patients, our results are consistent with those from the ACCE review and the American College of Medical Genetics guideline on the pharmacogenetic testing of *CYP2C9* and *VKORC1* in changing therapeutic goals and reducing adverse events related to warfarin therapy. [46,47] Several ongoing trials may provide answers in the near future. Depending on these results, future overall conclusions might greatly change with regard to the clinical utility of the pharmacogenetic tests.

# *VKORC1* Gene Polymorphisms and Response to Warfarin Therapy

## Background

Warfarin inhibits Vitamin K epoxide reductase (*VKOR*), an enzyme complex that facilitates recycling reduced Vitamin K through its interaction with subunit protein 1 (*VKORC1*). Rare mutations in the *VKORC1* gene that lead to amino acid changes in the protein product have been found in familial cases of patients with both a deficiency of Vitamin-K-dependent clotting factors type 2 (online Mendelian inheritance in man, OMIM, #607473) and resistance to coumarin-type anticoagulant drugs (OMIM #122700).[49,50] These rare mutations are unlikely to explain the large variability in patient response to warfarin at the population level. However, the common non-coding variants of the *VKORC1* gene may explain part of the variability in warfarin sensitivity and dose requirements at the population level.[51-53] To date, several common variants of VKORC1 have been studied for their effects on response to warfarin.

Apart from *VKORC1* gene polymorphisms, common variants of the gene encoding the *CYP2C9* enzyme (belonging to the cytochrome P450 family) do influence warfarin maintenance dose requirements.[12,14] We performed a systematic review to evaluate the effects of common *VKORC1* variants on warfarin maintenance dose and measurements of anticoagulation status.

### Data Synthesis and Analysis for an Association between the *VKORC1* Gene Variant and Warfarin Therapy

We evaluated all common *VKORC1* variants that had at least 5% frequency among unrelated patients in at least one study. For all identified common *VKORC1* genetic variations and their relationship with eligible outcomes, we recorded whether the association was

statistically significant at the 0.05 level and the direction of the association. We evaluated both qualitative and quantitative data on potential associations. We performed meta-analyses whenever four or more studies had available data on the same outcome and the same polymorphism. Therefore, we performed meta-analyses only for the differences in the maintenance dose across *VKORC1* genotypes for 3 SNPs: rs9934438 (g.6484C>T or c.1173C>T), rs7294 (g.9041G>A or c.3730G>A), and rs8050894 (g.6853G>C or c.1542G>C). Two of these SNPs (namely rs9934438 and rs7294) are in strong linkage disequilibrium between them; in addition, the third SNP (rs7294) as well as at least 2 other SNPs (namely rs9923231 [g.3673G>A or c.-1639G>A] and rs2359612 [g.7566T>C or c.2255T>C]) are also in linkage disequilibrium between them and with the first two. This means that the alleles of these SNPs tend to be inherited together. For this reason all analyses across these SNPs are stochastically dependent (correlated).

We assumed dominant or recessive inheritance by combining the average maintenance doses for the proper genotypic groups in each study and also compared the results with those of homozygotes. We used an exact test to evaluate deviations from the HWE-predicted proportions in each study and per ethnic descent stratum when applicable.[54] We tested for deviations from HWE across studies and their similarity across studies using exact tests.[55]

Subgroup analyses by ethnic descent (European, Asian, and African American) were performed. In sensitivity analyses, we excluded studies in which the genotype proportions deviated significantly from the HWE-predicted proportions and studies for which means and standard deviations were approximated from medians or ranges.[15]

## Results

From 288 citations, 28 full-text articles were reviewed; 18 studies[20,29,30,36,39,51,52,56-66] reported data on the association of common *VKORC1* genetic variations (all were SNPs) and outcomes of interest.

### Description of studies

Nineteen studies evaluating 21 *VKORC1* SNPs contained information on 23 racial descent strata; European descent patients (14 strata), Asian descent patients (7 strata), and African American descent patients (2 strata) (**Figure 2.1**). All studies recruited warfarin-treated patients from anticoagulation clinics. The therapeutic INR target was between 2 and 3 in the majority of studies. About 50 percent of the studies enrolled only patients with stabilized maintenance doses, and the remaining studies included patients during the induction phase.

Nine studies were prospective (**Table 2.1**). Mean ages of warfarin-treated patients ranged from 43 to 72 years. More than one-half of the participants were male. Only one of 18 studies reported that the personnel who did the genotyping were blinded to the laboratory or clinical data of the patients, and only three studies clearly reported quality control of the reliability and reproducibility of genotyping.

**Table 2.1. Characteristics of Studies Testing the Association between the *VKORC1* Genotype and Response to Warfarin Therapy**

| Characteristics | Description: number of studies (references) |
|---|---|
| Total | 19 studies[20,29,30,36,39,43,51,52,56-66] |
| Study design | Prospective cohort: 9[29,30,36,39,57,59,61,62,66]<br>Retrospective cohort: 9[51,52,56,58,60,63-65,67]<br>RCT: 1[43] |
| Ethnicity | Caucasians: 8[51,52,57,59,60,64,65,67]<br>Asians: 5[29,30,39,58,66]<br>Mixed: 4[36,56,61,63]<br>Not documented: 2[43,62] |
| Setting | Anticoagulation clinic: 14[20,39,51,52,56,57,59-66]<br>Other: 4[29,30,43,58]<br>Not documented: 1[36] |
| Beginning of patient recruitment | Warfarin initiation phase: 5[43,51,58,61,68]<br>Warfarin maintenance phase: 9 |

| | Not documented: 5[29,39,52,59,60] |
|---|---|
| Commonly examined SNPs | rs9934438 (g.6484C>T): 13[29,30,36,51,52,57-61,66-68]<br>rs7294 (g.9041G>A): 11[36,39,51,52,58-60,63,66-68]<br>rs8050894 (g.6853G>C): 7[29,30,39,51,59,64,66] |

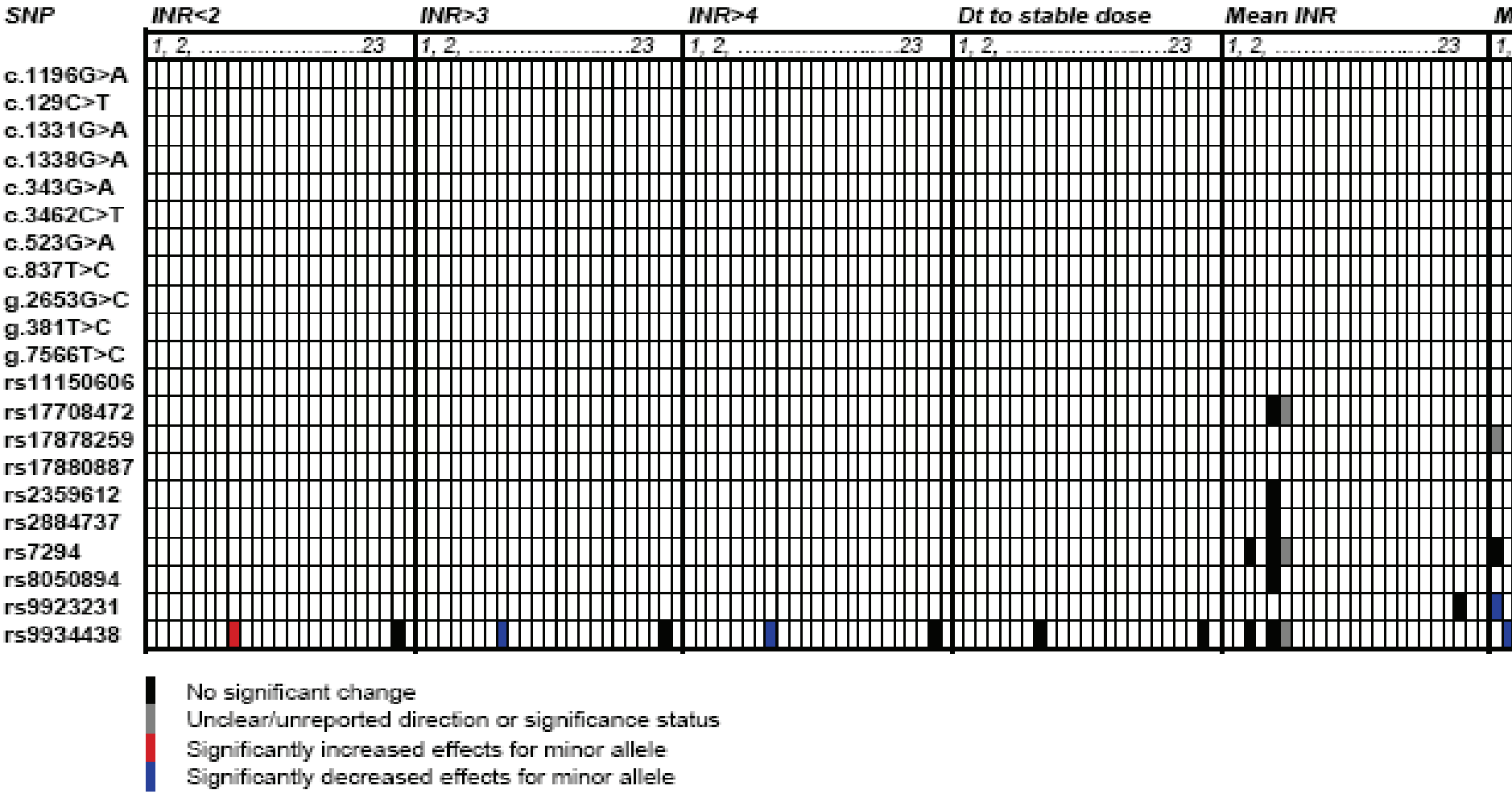
RCT: randomized controlled trial; SNP: single nucleotide polymorphism

**Overview of the reported associations**

Eighteen studies evaluating 21 *VKORC1* SNPs identified associations between SNPs and mean warfarin maintenance dose or other anticoagulation variables. No study reported associations between *VKORC1* SNPs and bleeding outcomes. The most commonly examined association was between three SNPs [rs9934438 (g.6484C>T), rs7294 (g.9041G>A), and rs8050894 (g.6853G>C)] and the mean maintenance warfarin dose (**Table 2 in Appendix 2**, **Figure 2.1**).

In the individual studies, most associations were described as statistically significant (red and blue cells in the figure). Studies agreed in the direction and statistical significance of associations between genetic effects for all SNPs and the outcome.

**Figure 2.1. Evidence map of associations between *VKORC1* single nucleotide polymorphisms with mean warfarin maintenance dose or anticoagulation outcomes by genetic descent strata. Numbers 1 through 23 under each outcome identify the specific genetic descent strata: see the key below the table.**



Dt: Time (to stable dose); INR: International normalized ratio; mean dose: mean maintenance dose. “Minor allele” is the less frequent of the two alleles in the population. We coined the genetic comparisons by considering as “variant allele” the allele with the lowest frequency (i.e., the minor allele) in European descent populations, to ensure correct representation of the direction of the genetic effects. The color code of each cell is based on what is reported by each study. Statistical significance status in the primary studies could be based on different analyses (e.g. haplotype-based analyses or adjusted analyses) than the ones we performed in this technology assessment.

**Key to the genetic descent strata and studies summarized in Figure 2.1**

| Order in figure | Author | Year | Descent | Reference |
|---|---|---|---|---|
| 1 | Aquilante | 2006 | European | 56 |
| 2 | Carlquist | 2006 | European | 57 |
| 3 | D'Andrea | 2005 | European | 52 |
| 4 | Herman | 2006 | European | 20 |
| 5 | Li | 2006 | European | 59 |
| 6 | Osman | 2006 | European | 60 |
| 7 | Rieder | 2005 | European | 51 |
| 8 | Schelleman [Eur] | 2007 | European | 61 |
| 9 | Sconce | 2005 | European | 62 |
| 10 | Takahashi [Eur] | 2006 | European | 36 |
| 11 | Tham [Indian] | 2006 | European | 63 |
| 12 | Vecsler | 2006 | European | 64 |
| 13 | Veenstra | 2005 | European | 39 |
| 14 | Wadelius | 2005 | European | 65 |
| 15 | Kimura | 2007 | Asian | 58 |
| 16 | Mushiroda | 2006 | Asian | 29 |
| 17 | Obayashi | 2006 | Asian | 30 |
| 18 | Takahashi [As] | 2006 | Asian | 36 |
| 19 | Tham [Chinese] | 2006 | Asian | 63 |
| 20 | Tham [Malay] | 2006 | Asian | 63 |
| 21 | Yuan | 2005 | Asian | 66 |
| 22 | Schelleman [Afr] | 2007 | African American | 61 |
| 23 | Takahashi [Afr] | 2006 | African American | 36 |

**Key to the nomenclature of the SNPs that are mentioned in the report above**

| rsNo | snp_c | snp_g |
|---|---|---|
| - | c.1196G>A | - |
| - | c.129C>T | - |
| - | c.1331G>A | - |
| - | c.1338G>A | - |
| - | c.343G>A | - |
| - | c.3462C>T | - |
| - | c.523G>A | - |
| - | c.837T>C | - |
| - | - | g.2653G>C |
| - | - | g.381T>C |
| - | - | g.7566T>C |
| rs11150606 | - | g.7040A>G |
| rs17708472 | c.689C>T | g.6009C>T |
| rs17878259 | - | g.4719T>C |
| rs17880887 | c.-4451G>T | g.861G>T |
| rs2359612 | c.2255T>C | g.7566T>C |
| rs2884737 | c.497T>G | g.5808A>C |
| rs7294 | c.3730G>A | g.9041G>A |
| rs8050894 | c.1542G>C | g.6853G>C |
| rs9923231 | c.-1639G>A | g.3673G>A |
| rs9934438 | c.1173C>T | g.6484C>T |

***Key Question #1a: Among patients taking warfarin, is there any association between carrying the genotype and clinical variables, such as the effective dose of warfarin?***

***VKORC1* rs9934438 (g.6484C>T or c.1173C>T)**

*Association between VKORC1 rs9934438 (g.6484C>T or c.1173C>T) and mean warfarin maintenance dose*

Thirteen studies examined the association between *VKORC1* rs9934438 (g.6484C>T or c.1173C>T) and mean warfarin maintenance dose in 17 ethnic-descent strata (10 in European descent, 5 in Asian descent, and 2 in African American descent) (**Table 2 in Appendix 2**). Data for meta-analyses were available in eight studies of 13 studies totaling 1,873 patients[20,29,30,36,57-59,61] (5 European-descent strata, 4 Asian-descent strata, and 1 African American stratum). Five studies reporting seven ethnic descent strata (not included in the meta-analysis) reported estimates similar to that of the meta-analysis (**Figure 2.1**).

In three of the 10 strata, the genotype distribution deviated significantly from the HWE-predicted proportions (European descent in Herman 2006[67] and Schelleman 2005[61] and Asian descent in Takahashi 2005[36]; exact P value <0.03 in all three cases). Standard deviations of warfarin maintenance doses were imputed in six of 10 ethnic descent strata described in four studies.[29,36,59,61]

Overall, among 1,748 patients and under a dominant genetic model, the mean maintenance dose among carriers of the T allele was lower than it was in CC homozygotes (**Figure 2.2**, panel A), without between-strata heterogeneity. Under a recessive model and 1,804 patients, TT homozygotes had lower maintenance doses than did those with CT or CC genotypes (**Figure 2.2**, panel B), although with substantial and significant heterogeneity. Among 1,123 patients with TT genotypes also had lower mean maintenance doses than did CC homozygotes

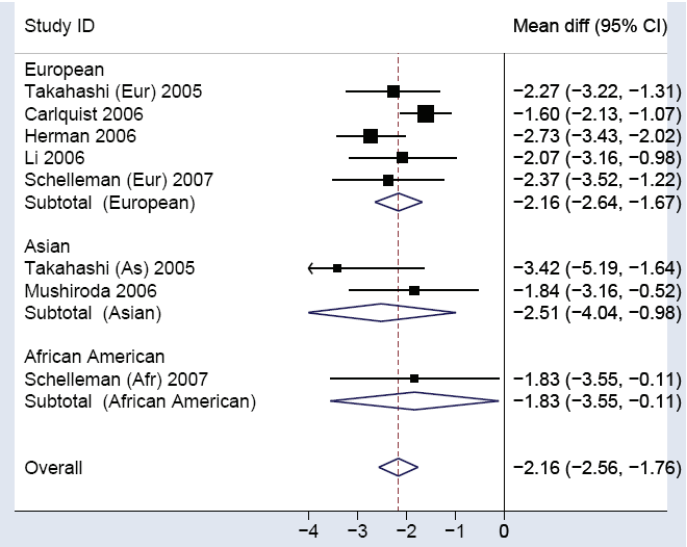
(**Figure 2.2**, panel C), without statistical heterogeneity across strata. These findings suggest an additive genetic effect for the T allele of rs9934438.

No single subgroup explains the observed between-strata heterogeneity across all three genotype comparisons (**Table 2.2)**. However, the mean differences in the average maintenance dose in all analyses consistently suggest that carriers of T allele have lower dose requirements.

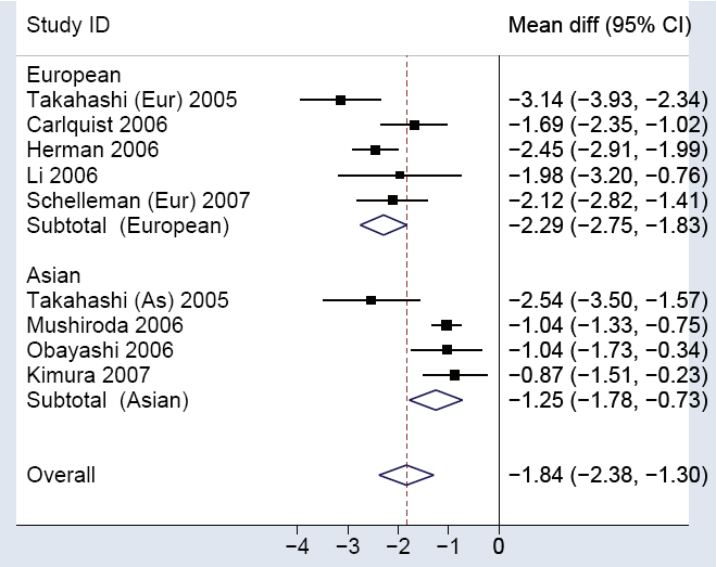
**Figure 2.2. Individual and pooled estimates for an association between *VKORC1* rs9934438 and mean warfarin maintenance dose**

A) Dominant model for rs9934438 (CT+TT vs. CC), B) Recessive model for rs9934438 (TT vs. CT+CC), and c) Extreme homozygotes for rs9934438 (TT vs. CC)

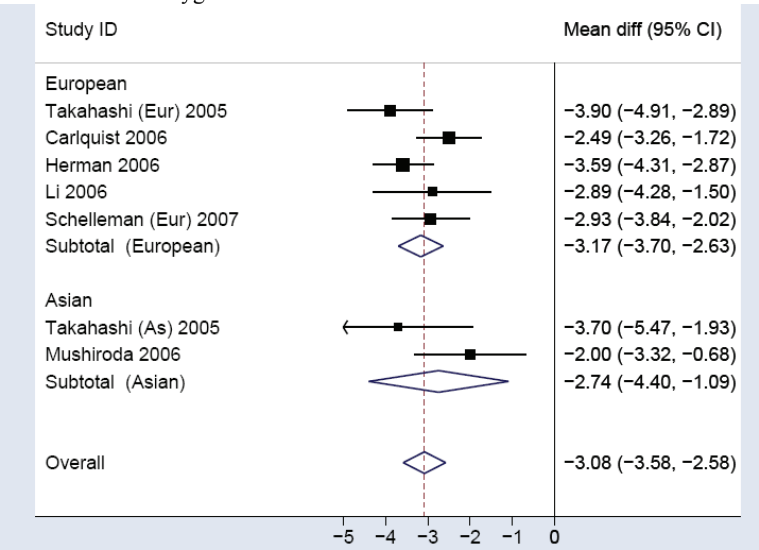
A. Dominant Model




B. Recessive Model




C. Extreme homozygotes




X-axis indicates Mean diff: mean difference in the average daily maintenance dose of warfarin (in mg).

Obayashi and Kimura had 1 and 0 CC homozygotes and therefore are not included in panels A and C. The African American stratum of Shelleman had 1 TT homozygote and therefore is not included in panels B and C.

Overall, when the pooled effect sizes are considered, results are consistent with an additive genetic model.

**Table 2.2. Subgroup Comparisons on the Genetic Effects of rs9934438 on Mean Warfarin Maintenance Dose**

| Study or summary | Dominant model (CT +TT vs. CC)* | | | Recessive model (TT vs. CT+CC)* | | | Extreme homozygotes (TT vs. CC)* | | |
|---|---|---|---|---|---|---|---|---|---|
| | N (patients) | Mean difference, mg/d (95% CI) | $P_{Het}$ ($I^2$ [%]) | N (patients) | Mean difference, mg/d (95% CI) | $P_{Het}$ ($I^2$ [%]) | N (patients) | Mean difference, mg/d (95% CI) | $P_{Het}$ ($I^2$ [%]) |
| All studies | 8 (1748) | -2.16 (-2.56, -1.76) | 0.24 (24) | 9 (1804) | - 1.84 (-2.38, -1.30) | <0.01 (86) | 7 (1123) | -3.08 (-3.58, -2.58) | 0.13 (40) |
| Racial descent | | | | | | | | | |
| European | 5 (696) | -2.16 (-2.64, -1.67) | 0.15 (41) | 5 (696) | -2.29 (-2.75, -1.83) | 0.08 (53) | 5 (373) | -3.17 (-3.70, -2.64) | 0.15 (41) |
| Asian | 2 (890) | -2.51 (-2.64, 1.67) | 0.16 (49) | 4 (1108) | -1.25 (-1.78, 0.73) | 0.03 (67) | 2 (750) | -2.74 (-4.40, -1.09) | 0.13 (56) |
| African American | 1 (162) | -1.83 (-3.55, 1.08) | NA | 0 (0) | NA | NA | 0 (0) | NA | NA |
| Only patients with stable dose | | | | | | | | | |
| Yes | 4 (510) | -2.32 (-3.05, -1.59) | 0.03 (66) | 5 (635) | -2.15 (-2.84, -1.47) | <0.01 (80) | 4 (301) | -3.34 (-4.04, -2.64) | 0.09 (53) |
| No | 4 (1238) | -2.08 (-2.71, -1.44) | 0.93 (0) | 4 (1169) | -1.40 (-1.98, -0.81) | 0.02 (71) | 3 (822) | -2.69 (-3.35, -2.03) | 0.50 (0) |
| Prospective study | | | | | | | | | |
| Yes | 7 (1583) | -1.93 (-2.30, -1.57) | 0.50 (0) | 7 (1546) | -1.88 (-2.50, -1.26) | <0.01 (83) | 6 (1023) | -2.94 (-3.49, -2.39) | 0.19 (33) |
| No | 1 (165) | -2.73 (-3.43, -2.02) | NA | 2 (258) | -1.68 (-3.22, -0.13) | <0.01 (94) | 1 (100) | -3.59 (-4.31, -2.87) | NA |
| Deviation from HWE | | | | | | | | | |
| Yes | 3 (382) | -2.71 (-3.30, -2.14) | 0.62 (0) | 3 (382) | -1.57 (-2.18, -0.95) | 0.69 (0) | 3 (227) | -3.37 (-3.91, -2.83) | 0.50 (0) |
| No | 5 (1366) | -1.81 (-2.20, 1.41) | 0.79 (0) | 6 (1422) | -2.38 (-2.73, -2.02) | <0.01 (82) | 4 (896) | -2.85 (-3.65, -2.04) | 0.09 (55) |
| Blinding | | | | | | | | | |
| Genotypic personnel | 2 (317) | -2.20 (-3.16, -1.25) | 0.61 (0) | 1 (155) | -2.12 (-2.82, -1.41) | NA | 1 (73) | -2.93 (-3.84, -2.02) | NA |
| No or not reported | 6 (1431) | -2.19 (-2.70, -1.68) | 0.12 (43) | 8 (1649) | -1.81 (-2.40, -1.21) | <0.01 (87) | 6 (1050) | -3.10 (-3.71, -2.50) | 0.08 (49) |

CI: confidence interval; d, day; HWE: Hardy-Weinberg equilibrium; N: number of strata in the meta-analysis; $P_{Het}$: p-value for heterogeneity

* Obayashi and Kimura had 1 and 0 CC homozygotes and therefore are not included in the meta-analyses for the dominant model and for the extreme homozygotes. The African American stratum of Shelleman had 1 TT homozygote and therefore is not included in the meta-analyses for the recessive model and the extreme homozygotes.

***Key Question #1b: Among patients taking warfarin, is there any association between carrying a genotype and biochemical variables, such as therapeutic INR levels?***

*Association between VKORC1 rs9934438 (g.6484C>T) and over anticoagulation*

Only one study reported an association between rs9934438 and INR values above 3 or 4 among patients of European and African American descent.[61] During the induction phase, the odds for over-anticoagulation (INR >3) were increased among patients of European descent who carry the T allele when compared to CC homozygotes. Statistical analyses were adjusted for dose, having a variant *CYP2C9* allele, having a copy of *APOE e4*, gender, BMI, comedications and other factors. The odds ratio for TT vs. CC was 3.02 (95% CI, 1.20 to 7.57), and that for CT vs. CC was 2.49 (95% CI, 1.46 to 4.25). In adjusted analyses, carriers of at least one copy of the T allele had an OR of 3.10 (95% CI, 1.73, 5.55) for overcoagulation (INR>3). This result was significant in the unadjusted analyses as well. The corresponding adjusted odds ratio for an INR>4 was 11.4 (95% CI, 1.3 to 102.0). This association was not statistically significant in unadjusted analyses. Finally, among patients of African American descent, these associations were not statistically significant.

In any racial subgroup, after dose stabilization, there was no association between over-anticoagulation (defined as either an INR greater than 3 or than 4) and the carriers of rs9934438 polymorphism (P >0.10).

*Association between VKORC1 rs9934438 (g.6484C>T or c.1173C>T) and time to maintenance dose*

Only one study reported an association between rs9934438 and the time to attain dose stability among patients of European and African American descent,[61] but there was no association under a dominant genetic model.

*Effects of environmental factors on the associations*

None of the eligible studies reported any interaction between the rs9934438 polymorphism and any environmental factor.

***Key Question #1a: Among patients taking warfarin, is there any association between carrying the genotype and clinical variables, such as the effective dose of warfarin?***

***VKORC1* rs7294 (g.9041G>A or c.3730G>A)**

*Association between VKORC1 rs7294 (g.9041G>A or c.3730G>A) and average warfarin maintenance dose*

Eleven studies examined the association between VKORC1 rs7294 (g.9041G>A or c.3730G>A) and mean warfarin maintenance dose for 15 ethnic descent strata (10 European, 4 Asian, and 1 of African American) (**Table 2 in Appendix 2**). Data for meta-analyses were available in four studies with 498 patients[52,58,59,67] (3 European strata and 1 Asian strata). None of the four studies deviated significantly from HWE-predicted proportions (exact P > 0.14 for all four studies). Numerical data were not reported in the remaining seven studies.

Among seven studies that did not provide quantitative data, three reported significant associations between rs7294 and mean warfarin maintenance dose (**Figure 2.1)**. The effects of rs7294 in these three studies were in the same direction as those identified in the meta-analyses.[39,51,65] One additional study had no significant association between rs7294 and mean warfarin maintenance dose.[56] In the remaining two studies (describing 6 ethnic descent strata), results on this association were not reported.[36,63]

Overall, in 498 analyzed patients under a dominant genetic model, the mean maintenance dose among carriers of the A allele was higher than it was in GG homozygotes (**Figure 2.3**,

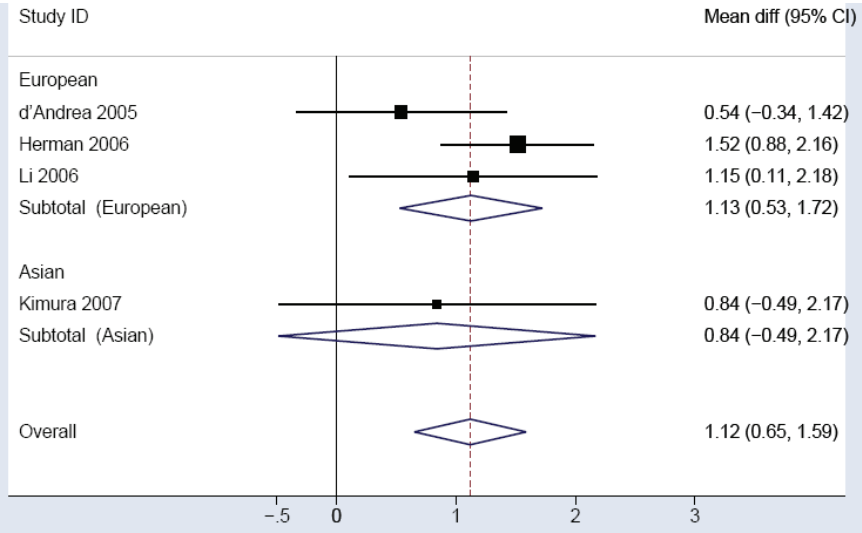
panel A). Under a recessive model, AA homozygotes had a higher average maintenance doses (405 patients) than did those with the AG or GG genotypes (**Figure 2.3**, panel B). Compared with GG homozygotes, patients with AA genotypes had higher mean maintenance doses (231 analyzed patients) (**Figure 2.3**, panel C). No statistical heterogeneity was noted across strata in these analyses. As a result of the small number of studies, no subgroup or sensitivity analyses were performed.

*Association between VKORC1 rs7294 (g.9041G>A or c.3730G>A) and other outcomes*

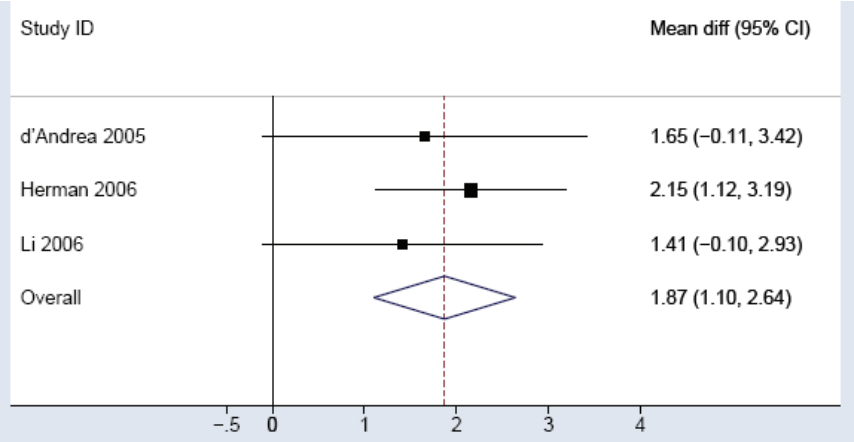
None of the studies evaluated the association between the rs7294 polymorphism and over-anticoagulation outcomes or time to achieve stable maintenance dose, and none evaluated interactions between the polymorphism and environmental factors.

**Figure 2.3. Individual and pooled estimates for an association between *VKORC1* rs7294 and mean warfarin maintenance dose**
A) Dominant model for rs7294 (AG+AA vs. GG), B) Recessive model for rs7294 (AA vs. AG+GG), and c) Extreme homozygotes for rs7294 (AA vs. GG)

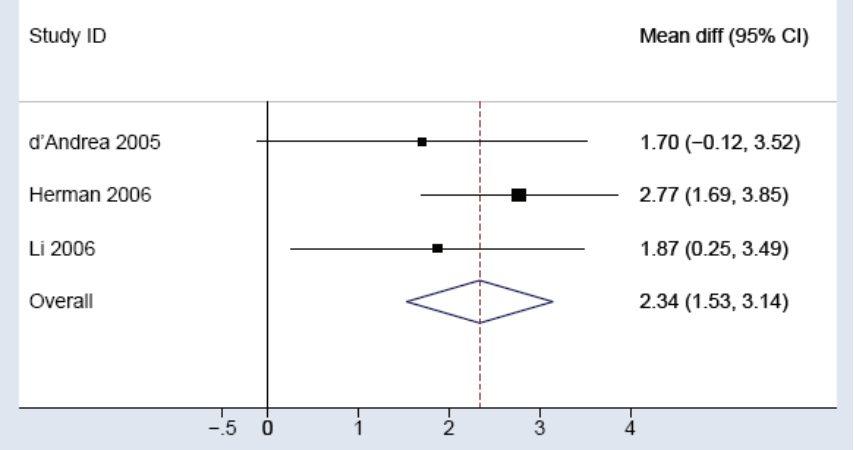
A. Dominant Model




B. Recessive Model




C. Extreme Homozygotes




X-axis indicates Mean diff: mean difference in the average daily maintenance dose of warfarin (in mg).
Kimura had 0 GG homozygotes and therefore is not included in panels B and C.

***Key Question #1: Among patients taking warfarin, is there any association between carrying the genotype and clinical variables, such as the effective dose of warfarin?***

***VKORC1* rs8050894 (g.6853C>G or c.1542G>C)**

*Association between* VKORC1 *rs8050894 (g.6853C>G or c.1542G>C) and mean warfarin maintenance dose*

The seven studies that examined the association between *VKORC1* rs8050894 (g.6853C>G or c.1542G>C) and mean warfarin maintenance dose[29,30,39,51,59,63,64] included nine ethnic descent strata (5 European and 4 Asian) (**Table 2 in Appendix 2**). Data needed for meta-analyses were available from 4 studies[29,30,59,64] (1,139 patients; 2 European strata and 2 Asian strata). None of the four studies deviated significantly from the HWE-predicted proportions (exact P > 0.14 for all four studies).

No numerical data were available for meta-analysis, but qualitative data were available for 3 studies.[39,51,63] In two of these three studies, significant associations with mean warfarin maintenance dose were reported in the same direction as the meta-analysis **(Figure 2.1**). [39,51] One study by Tham (3 ethnic descent strata) used haplotype-based analyses and did not mention results specifically on rs8050894.

Overall, under a dominant genetic model, the mean maintenance dose among carriers of the C allele was lower than it was among GG homozygotes (1,014 analyzed patients; (**Figure 2.4**, panel A). There was no between-study heterogeneity. Assuming a recessive model, CC homozygotes had an average lower maintenance dose than did patients with the CG or GG genotype (1,139 patients**; Figure 2.4**, panel B). There was little evidence for between-study heterogeneity in this analysis. When compared with GG homozygotes, patients with CC genotypes had an average lower mean maintenance dose (801 patients; **Figure 2.4**, panel C);

again, there was no statistical heterogeneity across studies. Because only 4 studies were quantitatively analyzed, no subgroup or sensitivity analyses were performed.

*Association between* VKORC1 *rs8050894 (g.6853C>G or c.1542G>C) and other outcomes*

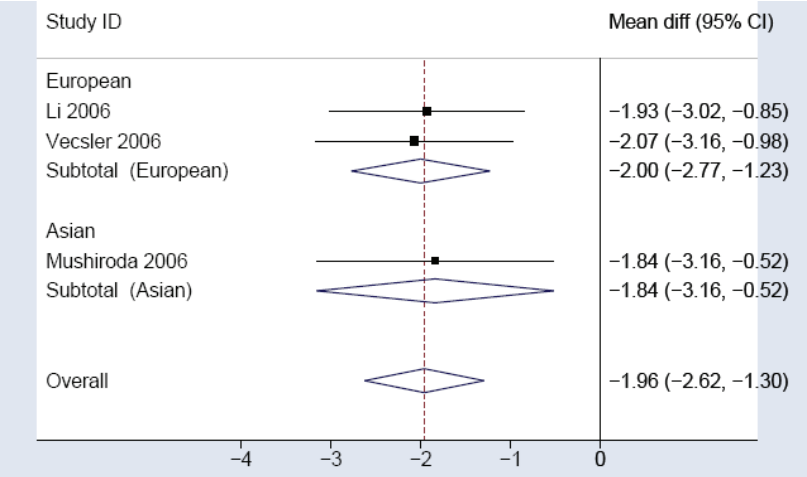
None of the studies evaluated the association between the rs8050894 polymorphism and over-anticoagulation outcomes or time to stable maintenance dose, and none evaluated interactions between the rs8050894 polymorphism and any environmental factors.

**Summary**

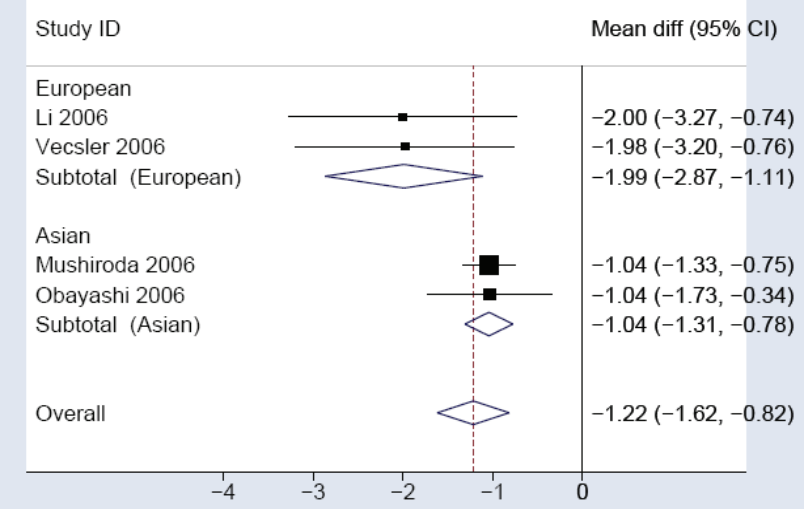
Carriers of the three common *VKORC1* variants T, G, and C alleles were associated with lower average maintenance warfarin doses than were non-carriers. Data were not adequate to address any other key questions.

**Figure 2.4. Individual and pooled estimates for an association between *VKORC1* rs8050894 and mean warfarin maintenance dose**
A) Dominant model for rs8050894 (CG+CC vs. GG), B) Recessive model for rs8050894 (CC vs. CG+GG), and c) extreme homozygotes for rs8050894 (GG vs. CC)

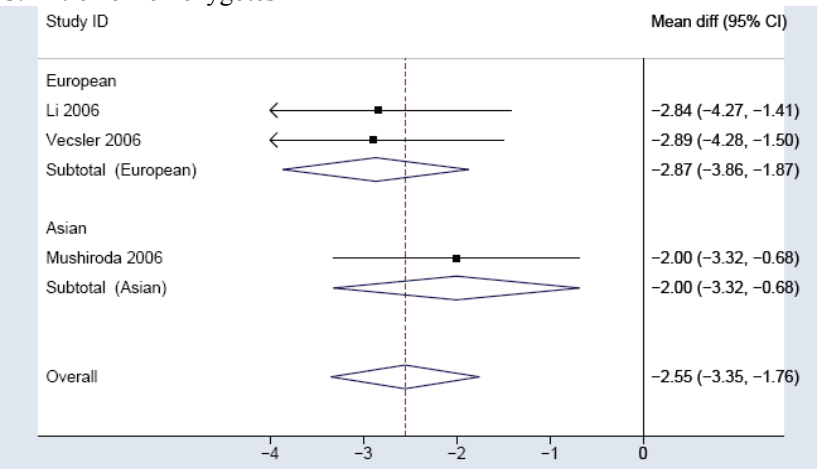
A. Dominant Model




B. Recessive Model




C. Extreme Homozygotes




X-axis indicates Mean diff: mean difference in the average daily maintenance dose of warfarin (in mg). Obayashi had 0 GG homozygotes and therefore is not included in panels A and C.

**Discussion**

We found no published information on the association of common *VKORC1* variants and clinical outcomes. Meta-analyses of the published literature provide substantial information that three common *VKORC1* variants [rs9934438 (g.6484C>T or c.1173C>T), rs7294 (g.9041G>A or c.3730G>A), and rs8050894 (g.6853G>C or c.1542G>C)] affect average warfarin maintenance dose. Carriers of the T, G and C alleles, respectively, have, on average, lower maintenance doses than non-carriers. However, the clinical utility of the routine use of *VKORC1* genotyping in anticoagulation clinics is uncertain.

Studies included in the meta-analyses were mostly of patients of European descent, and only limited information was available on other ethnic descent groups (e.g., Asians and African American). We did not identify any evidence for differences in genetic effects on warfarin dose across racial subgroups. This is in agreement with large scale empirical explorations of common genetic variations for common diseases: the effects of genetic factors were consistent across populations of different racial descent, although the frequencies of the genetic risk factors in the populations were very different.[69]

Moreover, the meta-analyses included only studies that reported sufficient numerical data for a quantitative synthesis. Many studies had data on the same associations, but could not be included in the analyses, because they did not report necessary data. However, the direction of genetic effects in the studies not included in the meta-analyses is the same as in the included studies. We performed meta-analyses only for the differences in the maintenance dose across *VKORC1* genotypes for 3 SNPs: rs9934438 (g.6484C>T or c.1173C>T), rs7294 (g.9041G>A or c.3730G>A), and rs8050894 (g.6853G>C or c.1542G>C). Two of these SNPs (namely rs9934438 and rs7294) are in strong linkage disequilibrium between them; in addition, the third

SNP (rs7294) as well as at least 2 other SNPs (namely rs9923231 [g.3673G>A or c.-1639G>A] and rs2359612 [g.7566T>C or c.2255T>C]) are also in linkage disequilibrium between them and with the first two. This means that the alleles of these SNPs tend to be inherited together. For this reason all analyses across these SNPs are stochastically dependent (correlated).

In the absence of clinical studies that directly compare using vs not using pharmacogenetic testing with respect to patient-relevant clinical outcomes, the role of pharmacogenetic testing in everyday clinical practice remains unclear. (Meta-) analyses of individual patient data are likely needed to analyze and describe possible interactions between genetic factors, and genetic and environmental factors and their exact effects on warfarin maintenance doses. However, ultimately, the value of pharmacogenetic testing is going to be determined by its ability to affect patient relevant clinical outcomes.

# Addendum

During the preparation of this report, domain experts identified 10 articles of observational designs that were published after the last search date. In this section we include four publications on nonoverlapping populations that are relevant to the key questions.[70-73] Three studies evaluated the effects of variants of both *CYP2C9* and *VKORC1* on response to warfarin therapy.[70-72] One study evaluated only *VKORC1* variants.[73] One study tested associations in populations of European descent,[71] and three included populations of European and African American descent.[70,72,73] All four studies were prospective.[70-73] Schwarz found no association between *CYP2C9* alleles and warfarin response (time to first INR within therapeutic range) in 297 patients starting on warfarin therapy.[70] Less than 5% of patients had the *CYP2C9 *2/*2, *2/*3, *3/*3* genotypes. Schwartz found associations between A/A haplotype of *VKORC1* (A haplotype includes variants of rs9923231, rs2884737, rs9934438, rs8050894, and rs2359612) and decreased time to the first INR within the therapeutic range as well as time to the first INR greater than 4. Limdi and Wadelius found no association between *VKORC1* alleles rs9934438 (in Limdi), and rs9923231 (in Wadelius) and risk of bleeding. [71,72] In each of these studies, homozygotes for the aforementioned *VKORC1* variant alleles had increased odds for overanticoagulation (early INR values greater than 4) and increased risk for major bleeding among patients of European descent. In patients of African-American descent *CYP2C9* or *VKORC1* variants were not statistically significantly associated with response to warfarin therapy.[72] Finally, Wang found an association between increased warfarin dose requirement and lower frequency of *VKORC1* rs9923231 allele.[73]

# *Apo E* gene polymorphisms and response to statin therapy

## Background

The *Apo E* gene is located on human chromosome 19. The primary product of the *Apo E* gene is a 317-amino acid protein that gives rise to the 299-amino acid mature protein by cleavage of an 18-amino acid signal peptide.[74] The primary role of *Apo E* in plasma lipid metabolism is to mediate the interaction of chylomicron remnants and intermediate-density lipoprotein particles with lipoprotein receptors, including the low-density lipoprotein (LDL) receptor and the chylomicron remnant or *Apo E* receptor.

Three major Apo E isoforms are coded by three alleles at the *Apo E* gene, designated as e2, e3, and e4 (dbSNP accession numbers rs7412 and rs429358). In the general population, e2 carriers are consistently associated with lower levels of total cholesterol (TC), LDL cholesterol, and elevated levels of triglyceride (TG) than are e3 carriers. Conversely, e4 carriers are associated with higher levels of total and LDL cholesterol. In general, the lower TC levels in e2 carriers are associated with reduced coronary and peripheral artery atherosclerosis, and the higher cholesterol levels in e4 carriers are associated with a higher prevalence of cardiovascular disease (CVD).[75,76]

Statins are competitive inhibitors of 3-hydroxy-3- methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in the cholesterol biosynthesis pathway.[77] Inhibition of cholesterol biosynthesis reduces secretion of Apo B-containing lipoproteins from the liver and up-regulates LDL receptor activity, both of which contribute to LDL-C lowering in the plasma.[78]

The inter-individual variation in lipid response to statin therapy is considerable, in terms of reduced LDL levels and clinical outcomes. This variability has been attributed to individual

environmental or genetic factors.[79,80] The most studied genetic variation is the *Apo E* polymorphism and its association with reductions in LDL levels with statin therapy.[80-82]

One meta-analysis of five studies[83] investigated differences in the lipid response to statins with genetic variation. In that study, LDL-C was lowered more in e2 carriers (37%, $P < 0.05$) than in e3 homozygotes and e4 carriers (35%, 33%, respectively).

**Data Synthesis and Analysis for the association of *Apo E* gene and statin therapy**

Studies provide data on lipid level (mean [SD]) in e2 carriers (e2e2+e2e3), e3 homozygotes (e3e3), and e4 carriers (e3e4+e4e4). Among the studies we evaluated, e2e4 data were not reported. Studies compared these three groups (e2 carriers, e3 homozygotes, and e4 carriers).

We calculated the change in lipid levels as percent improvement (or decline) from baseline for each genotype within a study with the formula: [(final value − baseline value)/baseline value] x 100%]. We performed meta-analyses on the percent change in lipid levels ($\Delta\mu$, (95% CI)) for e2 carriers, e3 homozygotes, and e4 carriers using the inverse of the variance of $\Delta\mu$ as a weighting factor.

Overall, for each outcome, the genotypes were compared (e2 carriers, vs. e3 homozygotes and e4 carriers vs. e3 homozygotes) using a general linear model under a random-effects assumption and the inverse of the variance of $\Delta\mu$ as a weighting factor. Because the data in individual studies were based on carrier contrasts but were not homogeneous with respect to allelic contrasts, these differences were evaluated quantitatively with point estimates of summary $\Delta\mu$. Thus, results for carrier contrasts should be interpreted with caution. In subgroups, the genotypes were compared with z scores because the number of studies was limited. Significance levels for the comparisons (Pc-value) were adjusted using Bonferroni's correction. Differences in

subgroups of genotypes were also evaluated quantitatively on the basis of the point estimates of summary $\Delta\mu$ to investigate possible trends.

For key question 2, concerning the effect of the APO E genotype on statin response in particular subpopulations, subgroup and sensitivity analyses for potential effect modifiers were performed for the following three categories:

1. Patient-related factors, including analyses for sex and ethnicity
2. Disease-related factors, including analyses for studies with and without diabetic patients and studies with and without familial hyperlipidemia.
3. Study design-related factors, including analyses for the duration of follow up (short ≤12 weeks versus long >12 weeks), and type and dosage of statin.

**Results for Section 2: *Apo E* and Response to Statin Therapy**

*Eligible studies*

Of 262 studies, 44 met the inclusion criteria for a systematic review of *Apo E* genotype association with statin therapy.[53,83-125] The studies were published between 1999 and 2007. Of the 44 published articles, 21 [53,83,85,91,95,100,101,107,112-114,116,118,122,124,125] provided quantitative data for genotypes and outcomes and were included in our meta-analyses. **Appendix 1** presents a flow chart of retrieved and excluded studies, with the reasons for exclusion.

The study and population characteristics are summarized in **Table 3.1** and detailed in **Appendix 2**.

**Table 3.1. Characteristics of studies testing for associations between the ApoE genotype and response to statin therapy**

| Characteristics | Description: number of studies (references) |
|---|---|
| Study design | Cohort studies: 35 [53,83-86,88-92,95-97,99-107,109-116,118-122,124] of which 14 studies were from randomized control trial-arm study[83-85,88,90,96,97,99,104,107,112,113,119,121]<br>Cross-sectional: 2 [108,123]<br>Retrospective studies: 5 [87,93,98,117,125] |
| Ethnicity/Descent | Caucasians: 32 [53,86-92,94-99,101-115,119,121,125]<br>Asians: 5 [100,117,122,124,126]<br>Jewish: 1 [102]<br>Mixed: 3 [85,120,123]<br>Not documented: 2 [83,84] |
| Data on Sex | Men: 9 [87,91,92,99,106,107,110,113,114]<br>Women: 5 [87,91,110,113,114] |
| Outcomes assessed: | *CV events*<br>• Mortality: 2 [97,105]<br>• Clinical events: 3 [85,97,105]<br>• Intracranial hemorrhage: 1 [123]<br>*CAD response*<br>• Angiographical change of CAD: [85,107]<br>*Lipid response*<br>• Total cholesterol (TC): 30 [53,83-89,92,93,95,96,98-101,103,107-109,112-114,116-119,122,124,125]<br>• Low density lipoprotein (LDL): 36 [53,83-93,95,96,98-103,107,108,110-114,116-119,121,122,124,125]<br>• High density lipoprotein (HDL): 26 [53,83-85,87,88,90-92,95,96,99-101,107-110,112-114,116,118,119,122,125]<br>• Triglyceride (TG): 28 [53,83-87,91-93,95,96,99-101,107-110,112-116,118,119,122,124,125]<br>• Apolipoprotein (ApoA1): 7 [53,84,100,101,108,116,122]<br>• Apolipoprotein (Apo B): 10[53,84,86,89,100,103,108,116,122,124]<br>• Apolipoprotein E (*Apo E*): 4 [53,86,100,122]<br>• Apolipoprotein (ApoA2): 1 [116]<br>• Apolipoprotein (ApoCII): 2 [84,122]<br>• C-reactive protein (CRP): 1 [94]<br>• Cholesteryl ester transfer protein (CETP) activity: 1 [101]<br>• Plasma phospholipid transfer protein (PLTP) activity: 1[104]<br>• Non-HDLC: 1 [120]<br>• Pseudocholinesterase (Pche) activity: 1 [115]<br>• Lipoprotein a (LP(a)): 1 [84]<br>*Adverse reactions response*<br>Discontinuation of statins: 1 [106] |
| Type of Hyperlipidemia | Familial: 12[53,86,87,89-91,98,102,103,110,111,121]<br>Non-Familial: 33[53,83-85,88,92-97,99-101,104,106-109,111-120,122-125] |
| Presence of Diabetes Mellitus: | Diabetic cohort: 4 [104,115,118,122]<br>Diabetic patients excluded from study: 15 [83,85,87,91,95,100,101,107,110,112-114,116,124,125] |
| Type of statin | Pravastatin: 8 [83,86,92,107,114,115,117,122]<br>Simavastatin: 10 [89-91,95,97,98,109,118,121,124]<br>Atorvastatin: 6 [53,87,96,99,104,113]<br>Lovastatin: 8 [84,87,101,103,110,112,116,118]<br>Fluvastatin: 3 [85,92,102]<br>Not documented: 7 [93,94,106,108,120,123,125] |
| Duration of follow-up | Short (≤12 weeks): 23 [85-87,89-93,98,100,101,103,109-112,115,116,118-122,124]<br>Long (>12 weeks): 16 [53,83-85,88,95-97,99,102,104,107,108,113,114,117] |

## Results

***Key Question #1: Among patients taking statin therapy, is there any association between carrying the Apo E genotype and clinical outcomes, such as cardiovascular events, mortality, or other composite clinical endpoint?***

**Coronary artery disease response**

Two studies evaluated the association of *Apo E* polymorphisms on angiographic progression or regression of CAD among patients treated with statins.[85,107] Both studies reported no significant treatment–genotype interaction between *Apo E* genotype and statin therapy with respect to progression or regression of CAD. However, a small angiographic benefit in e2 carriers was noted in both studies.

**Mortality**

Two studies evaluated the association of *Apo E* polymorphisms on mortality among patients treated with statins.[97,105] In a substudy of the Scandinavian Simvastatin Survival Study (the 4S study),[97] during the follow-up of myocardial infarction (MI) survivors, treatment with simvastatin reduced the number of deaths in 187 e4 carriers from 15.7% to 6.0%, and decreased mortality risk by 0.33 (95% CI 0.16 to 0.69). In 301 non-e4 carriers, simvastatin also decreased mortality from 9.0% to 5.1%, and decreased mortality risk to 0.66 (95% CI, 0.35 to 1.24). In the same study, 166 placebo-treated e4 carriers had almost twice the mortality risk as that of 312 non-carriers (15.7% and 9%; RR 1.8, 95% CI: 1.1 to 3.1). The Kaplan-Meier curves for all-cause mortality for both simvastatin treated e4 carriers and non-carriers were similar, suggesting that simvastatin negated the increased risk associated with e4 carrier status. However, in the

Rotterdam study for all the *Apo E* genotypes, the relative risk of all-cause mortality in patients with and without statin treatment was similar.[105]

**Composite clinical events**

Three studies examined the effect of *Apo E* polymorphisms on the composite endpoint of "clinical events" in patients treated with statins.[85,97,105] The definition of "clinical events" varied among studies. Gerdes defined clinical events as "coronary death, nonfatal definite or probable MI, silent MI, or resuscitated cardiac arrest;"[97] Ballantyne described them as "percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, definite or probable MI, unstable angina, death from any cause;" [85] and Maitland van der Zee defined it as MI, stroke, or death. [105] The genotypes did not differ in the rate of composite clinical events in any of the three studies.

**Intracerebral Hemorrhage**

In one study, among 78 patients treated with statins, e3e3 homozygotes had a lower risk for intracerebral hemorrhage (OR 0.08, 95% CI 0.02 to 0.39) than did the 40 e2 and e4 carriers (OR 0.69, 95% 0.29 to 1.78).[123]

***Key Question #1: Among patients taking statin therapy, is there any association between carrying the Apo E genotype and biochemical variables, such as total, LDL, or HDL cholesterol or triglyceride levels?***

**Total cholesterol**

All 30 studies evaluating the association of *Apo E* genotype with total cholesterol (TC) levels in patients treated with statins [53,83-89,92,93,95,96,98-101,103,106,107,109,112-114,116-119,122,124,125] reported that statins significantly lowered TC levels in e2 carriers, e3e3 homozygotes, and e4

carriers. However, 21 of these studies found no significant differences between the groups in the amount of reduction among genotypic groups .[53,83,84,86,89,91,92,95,96,98,99,101,104,106,112-114,116,117,122,124,125] Four studies reported that e2 carriers had significantly greater reductions in TC levels from baseline than did other genotypes, [100,107,109,113] three studies reported that e4 carriers had significantly smaller reductions of TC levels, [85,92,111] and only one study reported that e4 carriers had greater reductions in TC than e2 carriers.[118] Eighteen studies that provided quantitative data were included in the meta-analysis.[53,83,85,91,95,100,101,107,112-114,116,118,122,124,125]

The meta-analysis included 2,848 patients from studies with different designs, thus comprising a non-homogeneous study group. The meta-analysis for e2-carriers, e3 homozygotes, and e4-carriers showed that between-study heterogeneity was significant ($P_Q < 0.10$, $I^2 \geq 75\%$). In the main analysis, the pooled change in TC from baseline was significant ($\Delta\mu$ = -27.7% (-32.5% to -22.8%), $\Delta\mu$ = -25.3% (-28.0% to -22.6%) and $\Delta\mu$ = -25.1% (-29.3% to -21.0%), respectively (**Table 3.2; Figure 3.1-3.3)**.

Overall, there was no statistical difference between the genotypes ($P \geq 0.05$), although e2 carriers had a better response, followed by e3 homozygotes and e4 carriers. In addition, the confidence intervals of the three genotypes overlapped, indicating a lack of significant differences.

***Key Question #2: What demographic or clinical variables mediate the association between pharmacogenomic test results and biochemical or clinical outcomes among patients taking statin therapy?***

Baseline TC levels had a significant effect ($P < 0.01$) on the relative change in TC after statin therapy. Other possible sources of heterogeneity were explored through subgroup analyses.

Subgroup analysis was also used to evaluate patient- and disease-related factors that might affect the response to *Apo E*. Subgroups analyzed were the type of statin, dosage, ethnicity (racial descent), sex, familial hyperlipidemia, and duration of follow-up (**Table 3.2; Figure 3.1-3.3)**. Only familial hyperlipidemia with e4 carriers differed significantly from the other groups, having the weakest response to statin therapy.

**Type of statin**

In comparisons between genotypes, simvastatin, lovastatin, and atorvastatin were associated with a much greater lowering of TC levels than was pravastatin. In four studies that evaluated lovastatin, e2 and e4 carriers had a greater mean reduction in TC than did e3e3 homozygotes.[87,101,112,116] For all the other type of statins, e2 carriers had a better response, followed by e3e3 homozygotes. On the basis of the dose of statin, five studies on pravastatin had extractable data for a meta-analysis.[83,100,107,114,122]

Pravastatin studies were classified as high-dose (more than 20 mg) or low-dose (20 mg or less) for analysis. With pravastatin therapy, e4 carriers had the smallest pooled reduction in TC. These results were consistent with the main analyses.

**Ethnicity**

The subgroup analysis based on the ethnicity (racial descent) showed that the reduction in TC with statin therapy among Caucasians was consistent with the main analyses.[53,85,87,91,95,101,107,112-114,116,125] However, in studies conducted among Asians, e4 carriers were associated with a greater response, followed by the e2 carriers and e3 homozygotes (**Table 3.2**).[118,122,124,125] In addition, in Asians, e2 carriers and e3e3 homozygotes had poorer responses than did Caucasians, indicating that ethnicity might mediate the association between *Apo E* and response to statin therapy.

**Sex**

With statin therapy, the male e2 carriers in three studies[77,127,128] and the female e2 carriers in three other studies[87,113,114] had a greater reduction in TC than did the opposite-sex groups. However in men, the e4 carriers had the lowest response to statin therapy, suggesting a possible sex-specific interaction between *Apo E* genotypes and TC response to statin therapy.

**Familial hyperlipidemia**

Three studies included in the meta-analysis were conducted in heterozygous familial hyperlipidemia patients,[53,87,91] and 15 studies enrolled non-familial hyperlipidemia patients. [83,85,95,100,101,107,112-114,116,118,122,124,125] In the familial hyperlipidemia studies, the e2 allele was associated with the greatest response among the genetic subgroups. The e3e3 homozygotes had greater reductions in TC than did the e4 carriers. The mean change in TC differed moderately between genotype groups in patients with non-familial hyperlipidemia, as e2 and e4 carriers showed a slightly better response than did e3 homozygotes.

**Duration of follow-up**

The e2 carriers had a greater mean decrease in TC in studies with both short (less than 12 weeks) [85,87,91,99-101,113,116,118,122,124] and long follow-ups.[53,83,95,107,114] The decrease was larger in the short follow-up studies.

The Egger test and the Begg-Mazumdar test for changes in TC among e2 carries indicated that there was no differential magnitude of effect in large versus small studies (P=0.43 and P=0.11, respectively).

**Table 3.2. Results of the meta-analysis and subgroup analyses for the association between the *Apo E* Genotype and mean change in total cholesterol levels in patients treated with statins**

| Variable | Number of studies | Mean change from baseline in TC (%) for e2 carriers ***(95% CI)*** | Mean change from baseline in TC (%) for e3 homozygotes (95% CI) | Mean change from baseline in TC (%) for e4 carriers (95% CI) |
|---|---|---|---|---|
| Overall | 18 | -27.7 (-32.5, -22.8) | -25.3 (-28.0, -22.6) | -25.1 (-29.3, -21.0) |
| Pravastatin | 5 | -23.1 (-27.6, -18.6) | -19.3 (-22.3, -16.2) | -17.3 (-19.1, -15.6) (Pc<0.01) |
| Pravastatin high dose | 2 | -22.2 (-25.2, -19.1) | -20.3 (-23.6, -17.0) | -17.9 (-18.4, -17.4) *(Pc=0.02)* |
| Pravastatin low dose | 3 | -23.2 (-33.6, -12.9) | -18.6 (-23.8, -13.5) | -17.0 (-20.3, -13.6) |
| Simvastatin | 3 | -32.0 (-37.1, -26.9) | -30.3 (-34.2, -26.5) | -31.3 (-34.3, -28.3) |
| Lovastatin | 4 | -32.3 (-42.6, -22.0) | -30.3 (-34.7, -25.93) | -30.0 (-34.0, -26.0) |
| Atorvastatin | 3 | -32.8 (-40.6, -25.0) | -27.7 (-28.8, -26.6) | -26.2 (-27.5, -24.9) |
| Caucasians | 13 | -28.4 (-29.1, -27.6) | -26.6 (-29.8, -23.5) | -25.4 (-29.6, -21.3) |
| Asians | 4 | -25.5 (-32.3, -18.8) | -22.8 (-29.1, -16.6) | -25.8 (-35.2, -16.4) |
| Men | 3 | -31.9 (-43.9, -19.8) | -26.6 (-36.0, -17.3) | -22.1 (-29.4, -14.8) |
| Women | 3 | -28.2 (-42.9, -13.5) | -26.5 (-36.1, -16.7) | -26.1 (-36.6, -15.5) |
| Familial hyperlipidemia | 3 | -39.3 (-43.4, -35.3) | -32.8 (-37.2, -28.3) | -29.6 (-32.1, -27.1)[1] (Pc<0.01) |
| Non-Familial hyperlipidemia | 15 | -24.9 (-27.2, -22.5) | -23.8 (-25.9, -21.7) | -24.3 (-28.0, -20.7) |
| Short follow-up | 12 | -28.3 (-34.4, -22.2) | -26.8 (-30.1, -23.5) | -26.9 (-30.7, -23.0) |
| Long follow-up | 5 | -24.9 (-35.8, -14.0) | -22.3 (-26.0, -18.7) | -20.8 (-25.2, -16.5) |

TC = total cholesterol

[1]PQ > 0.14, $I^2$ < 55%; Significant and large heterogeneity ($P_Q$ < 0.01, $I^2$ ≥ 75%) is presented in all settings

**Figure 3.1. Individual and pooled estimates of percent reductions in total cholesterol from baseline for e2-carriers treated with statins.**

(Data are plotted in ascending order of baseline TC levels. The analysis used a random-effects model.)

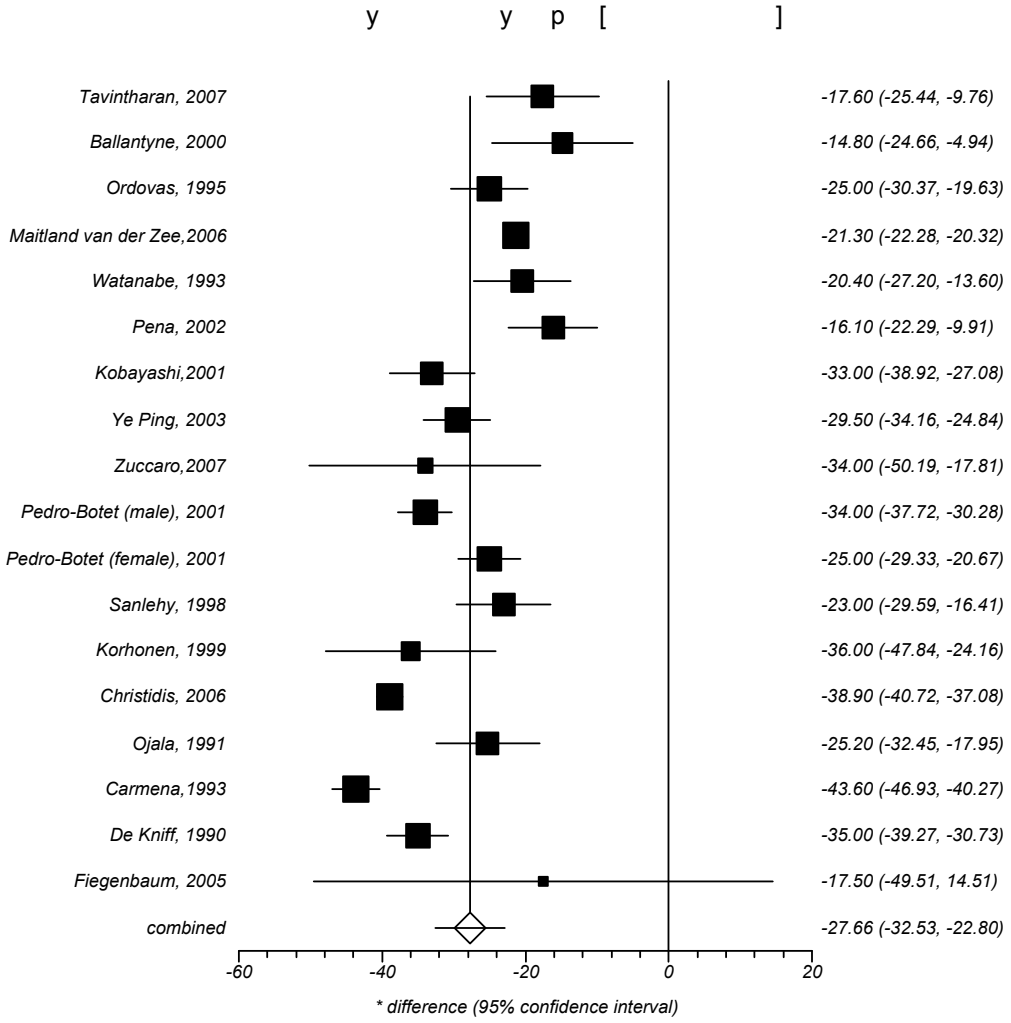


**Figure 3.2. Individual and pooled estimates of percent reductions in total cholesterol from baseline in e3 homozygote treated with statins.**

(Data are plotted in ascending order of baseline TC levels. The analysis used a random-effects model.)

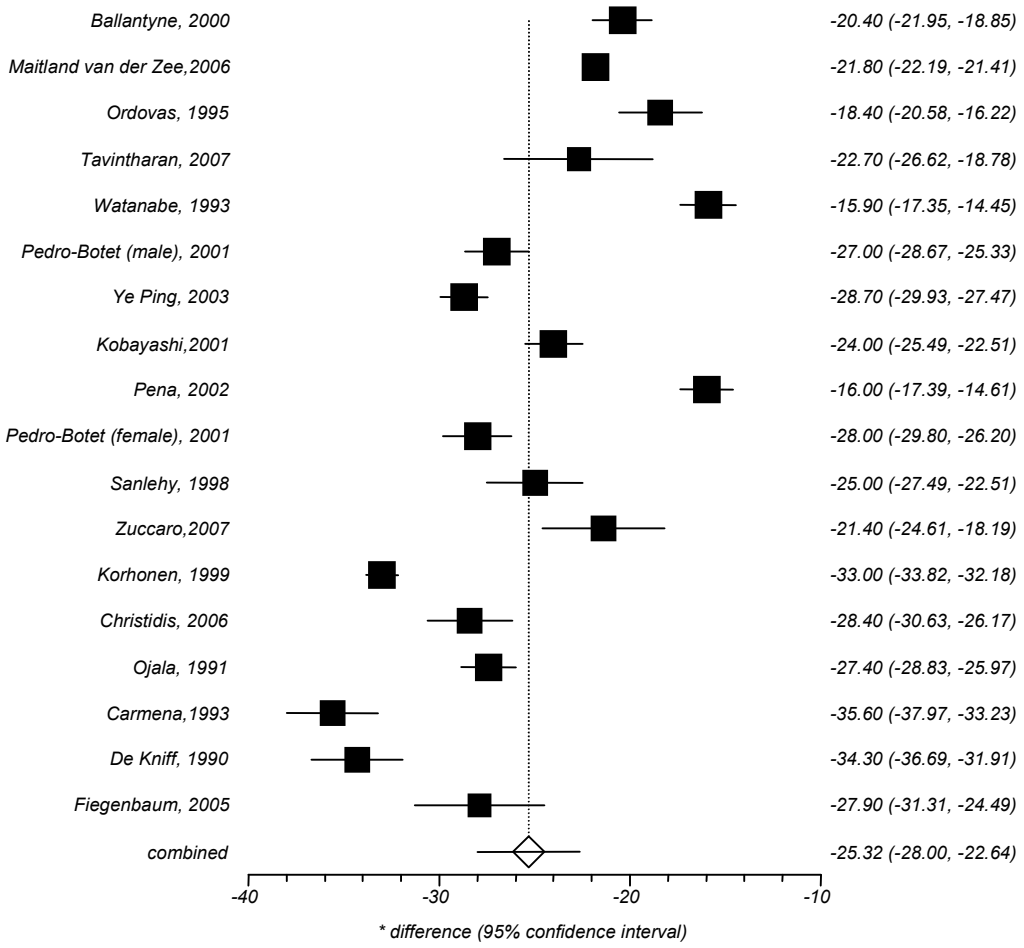
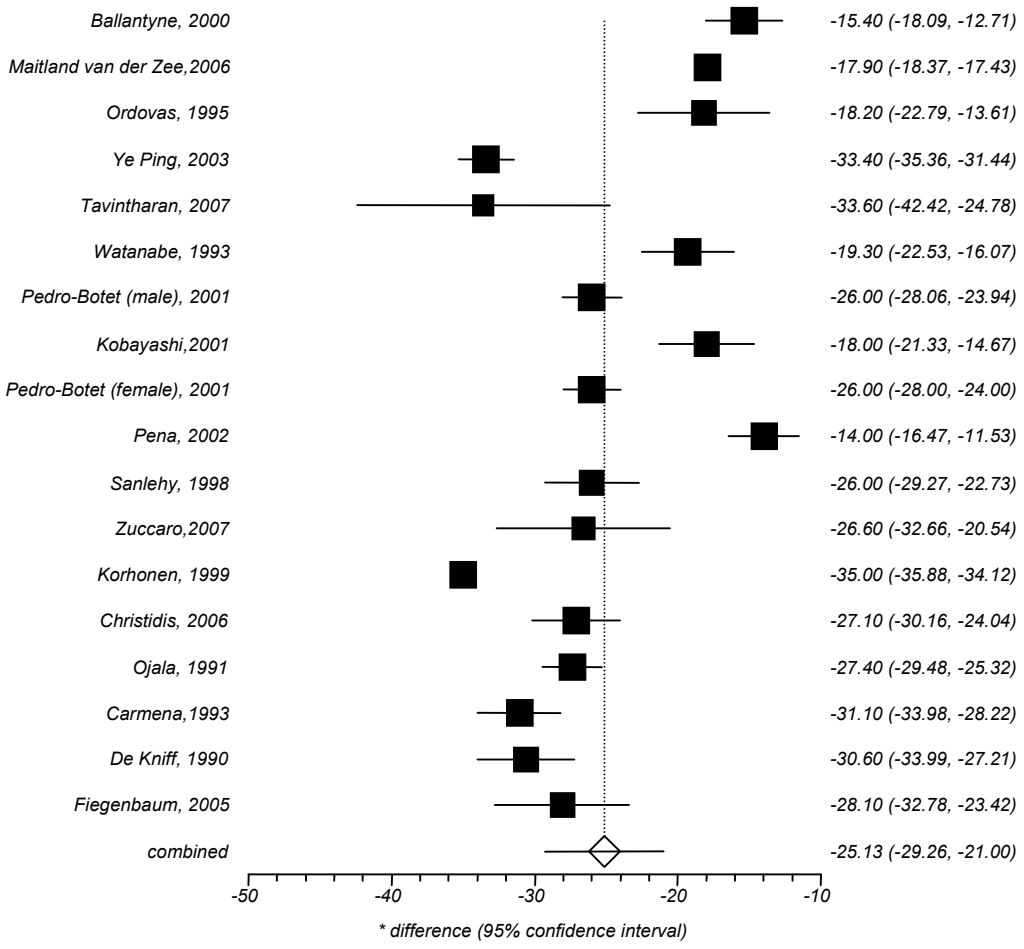


**Figure 3.3. Individual and pooled estimates of percent reductions in total cholesterol from baseline in e4 carriers treated with statins.**

**(Data are plotted in ascending order of baseline TC levels. The analysis used a random-effects model.)**



***Key Question #1: Among patients taking statin therapy, is there any association between carrying the Apo E genotype and biochemical variables, such as total, LDL, or HDL cholesterol or triglyceride levels?***

## Low-density-lipoprotein cholesterol

Thirty-six studies investigated the LDL-cholesterol-lowering effect of statins in association with the *Apo E* genotype.[53,83-93,95,96,98-103,107,108,110-114,116-119,121,122,124,125] All studies reported that the e2-carriers, e3e3 homozygotes, and e4-carriers had significant reductions in LDL levels compared to their baseline values. Of the 36 studies, 18 reported no significant differences in the amount of reduction of LDL levels among genotypic groups.[53,83,84,86,89-92,95,96,98,110,112,114,116,122,124,125] Seven studies reported that e2 carriers had greater reductions in LDL levels than did other genotypes,[90,99,100,107,111,113,121] five studies reported that e4 carriers had smaller reductions in LDL levels,[85,87,93,108,117] and two studies reported that e4 carriers responded better than e2 carriers.[102,118]

Twenty studies were included in the meta-analysis.[53,83,85,87,95,100,101,107,112-114,116,118,122,124,125] The meta-analysis for e2-carriers, e3e3 homozygotes, and e4-carriers found large heterogeneity among studies ($P < 0.10$, $I^2 \geq 50\%$), and the pooled mean (95% CI) reduction in LDL was significantly lower for all genotypic groups; $\Delta\mu = -35.9\%$ (-39.9% to -31.9%), $\Delta\mu = -33.6$ (-36.5% to -30.8%) and $\Delta\mu = -31.8\%$ (-35.7% to -27.9%), respectively (**Table 3.3** and **Figures 3.4-Figure 3.6**). Significant and large heterogeneity ($P_Q < 0.10$, $I^2 \geq 75\%$) was present in all or most of the settings.

Overall, there was no statistical difference between the genotypes ($Pc \geq 0.05$), although e2 carriers had greater reductions, followed by the e3 homozygotes and the e4 carriers.

**Table 3.3. Results of the meta-analysis and subgroup analyses for associations between the *Apo E* genotype and low-density lipoprotein levels in patients treated with statins**

| Variable | Number of studies | Mean change in LDL (%) from baseline for e2 carriers (95% CI) | Mean change in LDL (%) from baseline for e3 homozygotes (95% CI) | Mean change in LDL (%) from baseline for e4 carriers (95% CI) |
|---|---|---|---|---|
| **Overall** | **20** | **-35.9 (-39.9, -31.9)** | **-33.6 (-36.5, -30.8)** | **-31.8 (-35.7, -27.9)** |
| **Pravastatin** | **5** | **-32.5 (-40.9, -24.1)** | **-27.5 (-32.0, -23.1)** | **-24.7 (-28.6, -20.7)** |
| **Pravastatin high dose** | **2** | **-32.7 (-34.6, -30.8)** | **-29.1 (-30.7, -27.4)** ***(Pc<0.02)*** | **-26.4 (-27.3, -25.5)** ***(Pc<0.01)*** |
| **Pravastatin low dose** | **3** | **-30.9 (-50.2, -11.5)** | **-26.9 (-35.7, -18.2)** | **-23.8 (-30.8, -16.9)** |
| **Simvastatin** | **3** | **-39.7 (-43.6, -35.9)** | **-38.5 (-39.9, -37.1)** | **-34.7 (-37.8, -31.6)** |
| **Lovastatin** | **6** | **-36.7 (-43.0, -30.4)** | **-36.7 (-41.2, -32.3)** | **-34.8 (-41.4, -28.3)** |
| **Atorvastatin** | **3** | **-41.9 (-48.1, -35.7)** | **-37.0 (-39.3, -34.7)** | **-34.1 (-35.7, -32.5)**[1] ***(Pc=0.04)*** |
| **Caucasians** | **15** | **-36.3 (-40.9, -31.8)** | **-34.4 (-37.3, -31.6)** | **-31.9 (-35.3, -28.6)** |
| **Asians** | **4** | **-35.2 (-45.8, -24.6)** | **-32.1 (-39.8, -24.4)** | **-31.9 (-37.6, -26.2)** |
| **Men** | **4** | **-41.1 (-44.3, -38.5)** | **-33.8 (-41.7, -25.9)** | **-30.3 (-37.0, -23.6)** |
| **Women** | **4** | **-38.1 (-47.2, -28.8)** | **-36.7 (-47.2, -26.3)** | **-32.1 (-41.9, -22.2)** |
| **Familial hyperlipidemia** | **5** | **-39.9 (-46.9, -32.9)** | **-36.3 (-40.0, -32.7)** | **-32.8 (-36.3, -29.4)** |
| **Non- Familial hyperlipidemia** | **15** | **-34.2 (-38.1, -30.4)** | **-32.7 (-35.4, -29.9)** | **-31.7 (-35.9, -27.6)** |
| **Short follow-up** | **14** | **-36.3 (-40.8, -31.8)** | **-35.1 (-38.3, -32.0)** | **-33.6 (-37.2, -30.0)** |
| **Long follow-up** | **5** | **-32.8 (-42.9, -22.7)** | **-30.3 (-35.5, -25.1)** | **-28.3 (-34.1, -22.5)** |
| **Diabetes** | **2** | **-27.8 (-34.6, -21.0)**[2] | **-27.2 (-35.5, -18.9)** | **-37.7 (-57.1, -18.2)** |
| **No diabetes** | **18** | -37.0 (-41.0, -33.0) | **-34.3 (-36.9, -31.7)** | **-31.3 (-34.3, -28.4)** |

[1] $P_Q = 0.97$, $I^2 = 0$; [2] $P_Q = 0.98$

**Figure 3.4. Individual and pooled estimates of percent reduction in low-density lipoprotein from baseline in e2-carriers treated with statins.**

**(Data are plotted in ascending order of baseline LDL levels. The analysis used a random-effects model.)**

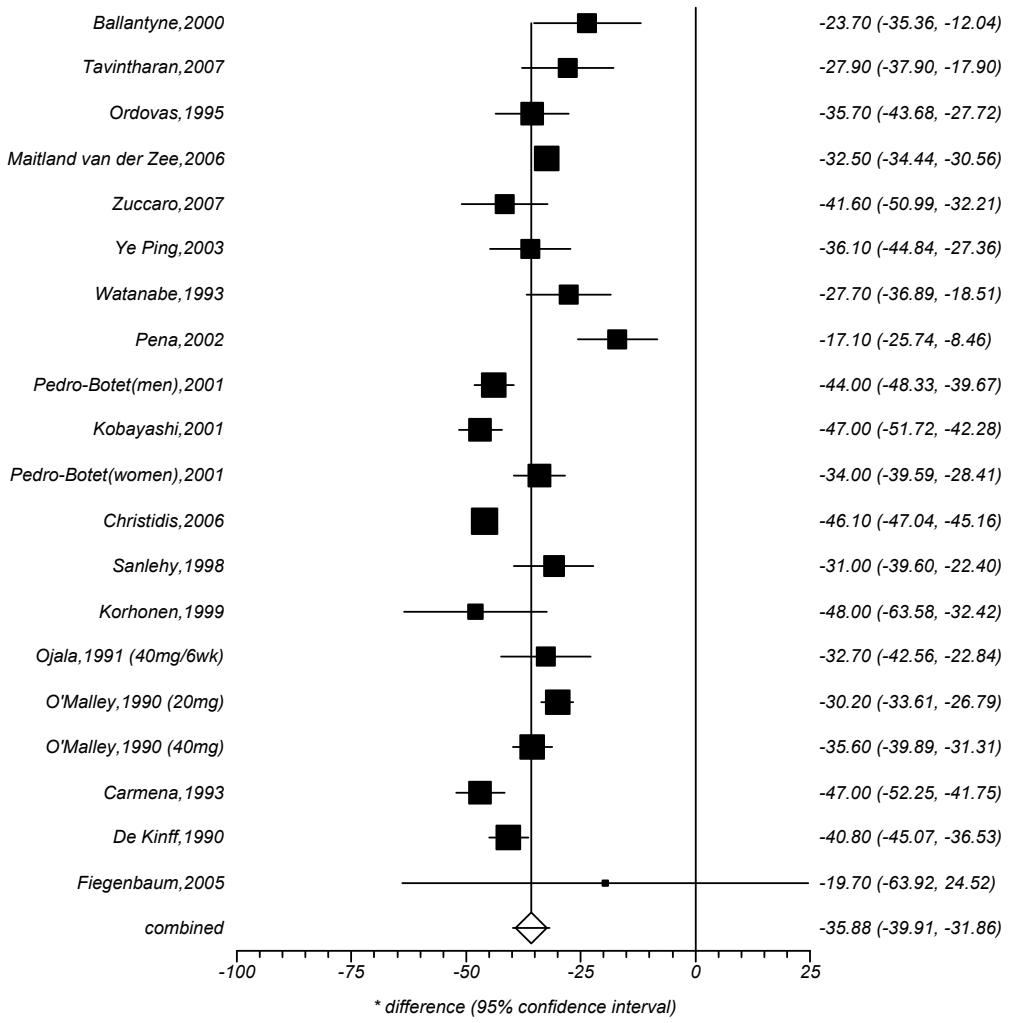


**Figure 3.5. Individual and pooled estimates of percent reduction in low-density lipoprotein from baseline in e3 homozygotes treated with statins.**

**(Data are plotted in ascending order of baseline LDL levels. The analysis used a random-effects model.)**

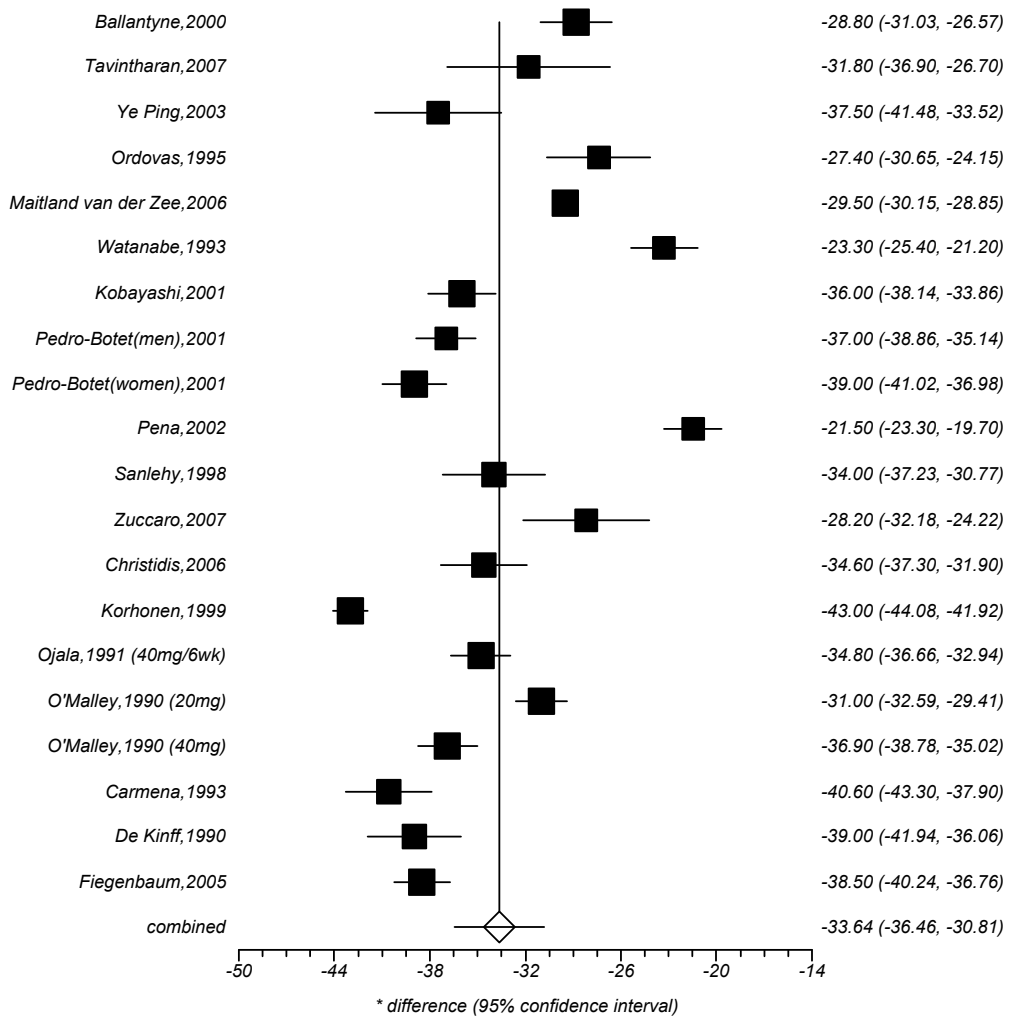
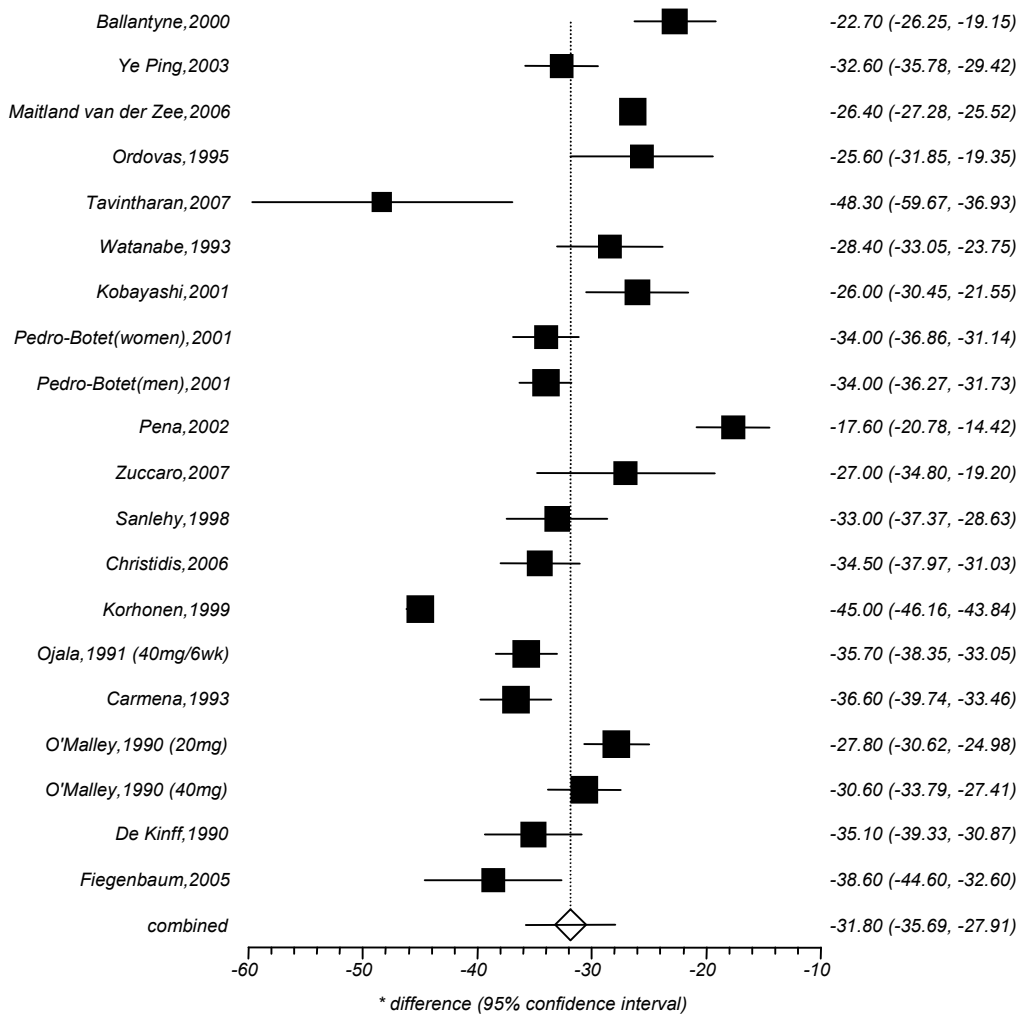


**Figure 3.6. Individual and pooled estimates of percent reduction in low-density lipoprotein from baseline in e4 carriers treated with statins.**

**(Data are plotted in ascending order of baseline LDL levels. The analysis used a random-effects model.)**



***Key Question #2: What demographic or clinical variables mediate the association between pharmacogenomic test results and biochemical or clinical outcomes among patients taking statin therapy?***

Patient- and disease-related factors were analyzed for their effects on response by genotype (**Table 3.3**). The results were significant only for atorvastatin and high doses (>20 mg) of pravastatin.

Studies of Caucasians[53,85,87,91,95,101,107,110,112-114,116,125] had results similar to those of the overall analysis, whereas in studies of Asians[100,118,122,124] pooled changes in LDL from baseline did not differ greatly among the genotypes. Subgroup analysis by sex[87,91,113,114] and familial hyperlipidemia[53,87,91,110] had results similar to those of the overall analysis. However, in familial hyperlipidemia studies,[53,87,91,110] the mean reduction in LDL was greater than it was in the non-familial hyperlipidemia studies[83,85,95,101,107,112-114,116,118,122,124,125]. In studies with both short [85,87,91,100,101,110,112,113,116,118,124] and long follow-ups,[53,83,95,107,114] the e2 carriers showed greater decreases in LDL. Diabetes could be a potential source of heterogeneity. Contrary to the main analysis, e4 carriers with diabetes,[118,122] had a greater response to statin therapy than did patients with other genotypes and those without diabetes.[53,83,85,87,91,95,100,101,107,110,112-114,116,124,125]

***Key Question #1: Among patients taking statin therapy, is there any association between carrying the Apo E genotype and biochemical variables, such as total, LDL, or HDL cholesterol or triglyceride levels?***

**High-Density Lipoprotein Cholesterol**

Twenty-six studies investigated the association between the *Apo E* genotype with the effect of statins in increasing HDL cholesterol levels.[53,83-85,87,88,91,92,95,96,99-101,107-110,112-114,116,118,119,122,125] Twenty-two studies reported that statin therapy was not associated with significant changes in HDL among *Apo E* genotypes.[53,83,84,86,90-92,95,96,99-101,108,110,112-114,116,118,122] Three studies reported that e2 carriers responded better than other genotypes,[85,107,125] and only one study reported that e4 carriers responded better than did e2 carriers.[87]

Twenty-one studies were included in the meta-analysis.[53,83,85,87,91,95,100,101,107,108,110,112-114,118,125] The meta-analysis for e2-carriers, e3e3 homozygotes, and e4-carriers showed great heterogeneity among studies ($P < 0.10$, $I^2 \geq 50\%$), and the pooled mean increase in HDL (95% CI) was significant: $\Delta\mu = +9.44\%$ (3.91% to 15.0%), $\Delta\mu = +7.45\%$ (5.59% to 9.49%) and $\Delta\mu = +7.61\%$ (4.47% to 10.7%), respectively (**Table 3.4 and Figures 3.7 - 3.9**).

Although statin therapy increased HDL levels most in e2 carriers, followed by the e4 carriers and then by e3 homozygotes, the overall differences were small, and the three genotypes did not differ significantly ($Pc \geq 0.05$).

**Table 3.4. Results of the meta-analysis and subgroup analyses for associations between the *Apo E* genotype and high-density lipoprotein levels in patients treated with statins**

| Variable | **Number of studies** | Mean change in percentage HDL for e2 carriers (95% CI) | Mean change in percentage HDL for e3e3 homozygotes (95% CI) | Mean change in percentage HDL for e4 carriers (95% CI) |
|---|---|---|---|---|
| All | 21 | 9.44 (3.91, 15.0) | 7.45 (5.59, 9.49) | 7.61 (4.47, 10.7) |
| Pravastatin | 5 | 6.20 (-5.30, 17.7) ns | 6.36 (2.14, 10.6) | 6.34 (-0.16, 12.8) ns |
| Pravastatin high dose | 2 | 10.3 (-4.28, 25.0) ns | 9.6 (9.31, 9.09) | 8.73 (-1.49, 19.0) ns |
| Pravastatin low dose | 3 | 2.46 (-4.40, 9.36) ns | 4.00 (1.31, 6.61) | 4.60 (-1.18, 10.4) ns |
| Simvastatin | 2 | 9.34 (3.28, 15.4) | 5.45 (1.94, 8.96) | 6.48 (2.04, 10.9) |
| Lovastatin | 8 | 11.27 (6.74, 15.8) | 11.1 (9.05, 13.1) | 8.74 (6.28, 11.2) |
| Atorvastatin | 3 | -2.32 (-5.00, 0.36) ns | 3.92 (-1.12, 8.96) ns | 9.10 (6.87, 11.3)[1] (Pc<0.01) |
| Caucasians | 17 | 11.1 (6.92, 15.3) | 8.10 (6.79, 9.41) | 7.92 (5.41, 10.4) |
| Asians | 3 | 3.58 (2.46, 4.70)[2] | 3.88 (-0.06, 7.83) | 7.96 (4.37, 11.6) (Pc<0.01) |
| Men | 5 | 11.5 (5.28, 17.6)[3] | 6.73 (4.07, 9.38) | 10.1 (4.01, 16.3) |
| Women | 5 | 10.8 (6.62, 15.0)[4] | 12.2 (3.94, 20.5) | 8.33 (3.07, 13.6) |
| Familial hyperlipidemia | 7 | 7.66 (3.25, 12.1) | 9.77 (6.78, 12.8) | 10.3 (5.93, 14.6) |
| Non- Familial hyperlipidemia | 14 | 10.4 (3.85, 16.9) | 6.26 (3.67, 8.84) | 5.23 (0.95, 9.51) |
| Short follow-up | 15 | 8.21 (5.67, 10.7) | 8.29 (6.05, 10.6) | 7.42 (5.40, 9.44) |
| Long follow-up | 5 | 6.86 (-7.21, 20.9) ns | 5.06 (0.30, 9.82) | 6.40 (-0.86, 13.7) ns |
| Diabetes | 2 | -1.89 (-3.96, 0.18)[5] ns (Pc<0.01) | 2.00 (0.86, 3.15)[6] | 8.57 (4.46, 12.7)[7] |
| No diabetes | 19 | 10.4 (6.44, 14.30) | 8.12 (6.87, 9.36) | 7.63 (5.23, 10.0) |

[1]$P_Q = 0.70$, $I^2 = 0$; [2]$P_Q = 0.43$, $I^2 = 0$; [3]$P_Q = 0.30$, $I^2 = 18\%$; [4]$P_Q = 0.56$, $I^2 = 0\%$; [5]$P_Q = 0.72$; [6]$P_Q = 0.98$; [7]$P_Q = 0.62$; ns, non-significant

**Figure 3.7. Individual and pooled estimates of percent changes in high-density lipoprotein from baseline in e2-carriers with treated with statins.**

(Data are plotted in ascending order of baseline HDL levels. The analysis used a random-effects model)

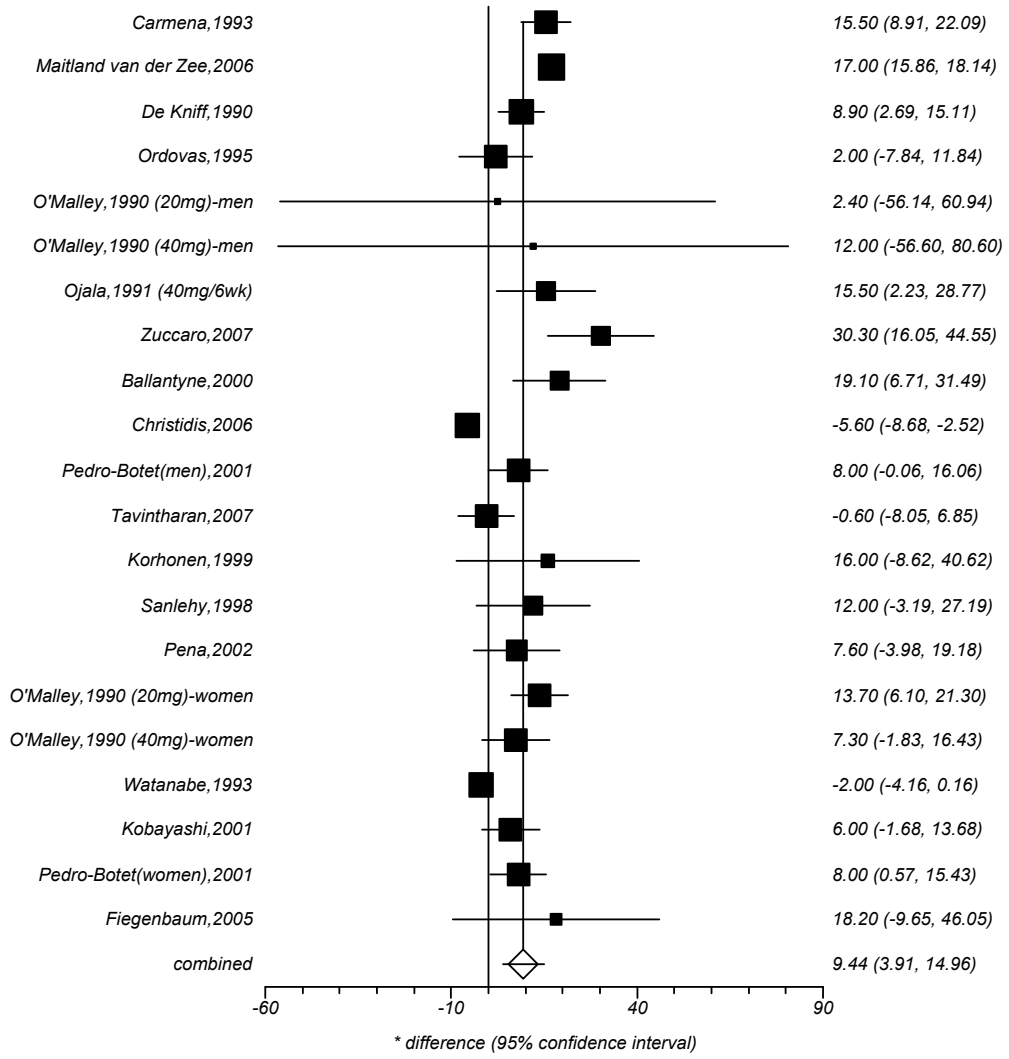


**Figure 3.8. Individual and pooled estimates of percent reduction in high-density lipoprotein from baseline in e3 homozygotes with statins.**
(Data are plotted in ascending order of baseline HDL levels. The analysis used a random-effects model)

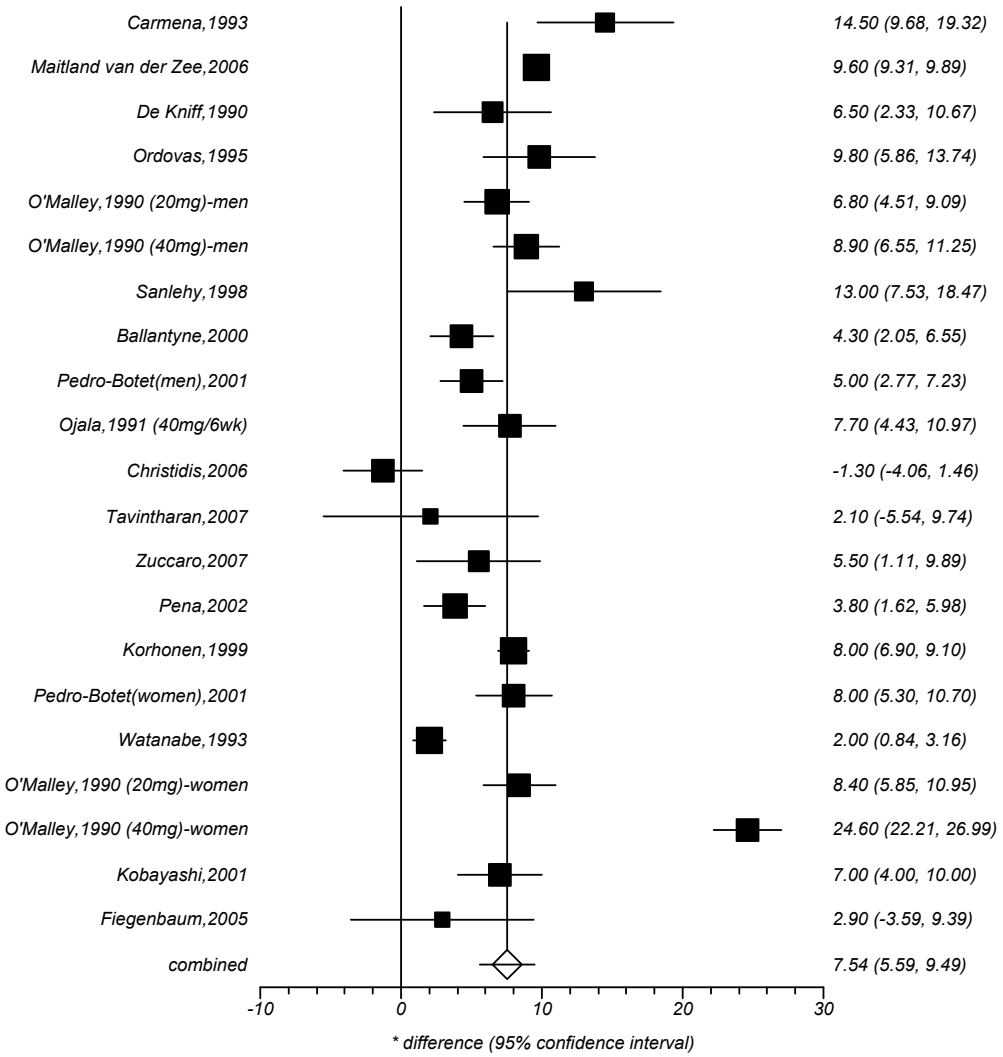
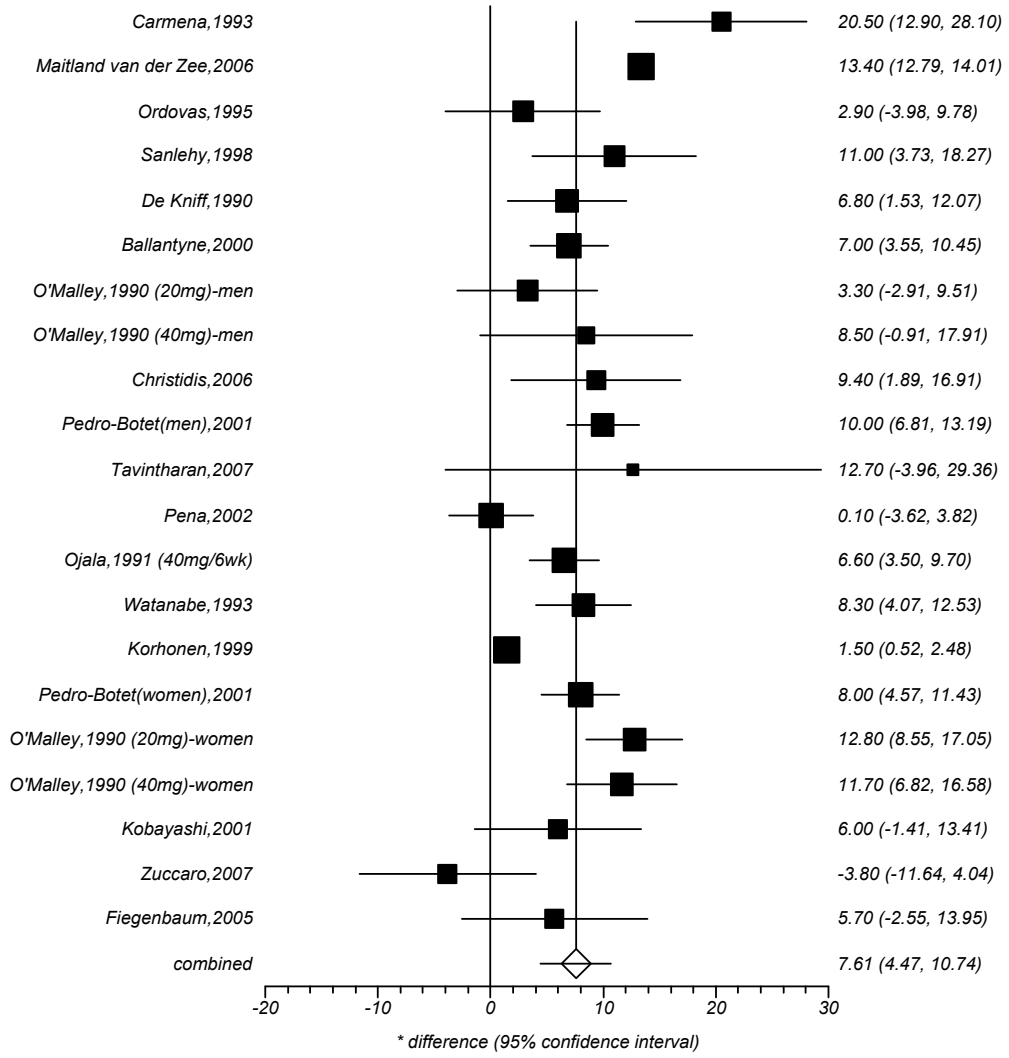


**Figure 3.9. Individual and pooled estimates of the reduction in high-density lipoprotein among e4 carriers treated with statins.**

(Data are plotted in ascending order of baseline HDL levels. The analysis used a random-effects model)



***Key Question #2: What demographic or clinical variables mediate the association between pharmacogenomic test results and biochemical or clinical outcomes among patients taking statin therapy?***

For comparisons among genotypic groups, the response to statin therapy for HDL levels was significantly associated with atorvastatin, Asian descent, and diabetes (**Table 3.4**). Analyses by type of statin were contradictory. The studies of lovastatin had results similar to the main analysis. In studies of simvastatin, e2 carriers responded better, followed by e4 carriers. Increases in HDL were significant for simvastatin and lovastatin. For atorvastatin, only e4 carriers had a significant increase in HDL. For both low-and high-doses of pravastatin, only e3 homozygotes had a significant increase.

In Caucasians, the response to statin therapy was consistent with the main analysis. In Asians, the presence of the e4 allele was associated with a significantly greater response. In men, e2 and e4 carriers had a better response, whereas women who were e3 homozygotes had the greatest increase. In studies of patients without familial hyperlipidemia and diabetes, results were similar to those of the main analysis. In patients with familial hyperlipidemia, e4 carriers had the highest response, followed by the e3 homozygotes. In patients with diabetes, HDL levels increased significantly ($Pc < 0.01$) in e4 carriers but decreased in e2 carriers. In studies with long follow-ups, the results were significant only for the e3 homozygotes.

***Key Question #1: Among patients taking statin therapy, is there any association between carrying the Apo E genotype and biochemical variables, such as total, LDL, or HDL cholesterol or triglyceride levels?***

**Triglycerides**

Twenty-eight studies investigated the association between the *Apo E* genotype and decreases in TG levels with statin therapy.[53,83-87,91-93,95,96,99-101,107,108,110,112-116,118,119,122,124,125] In 24 of the 28 studies, treatment with statins was not associated with significant changes in TG levels in any setting.[53,83-87,91,92,95,96,99-101,107,108,110,112-116,119,122,124] Three studies reported that e4 carriers had significantly lower TG levels with therapy than did those with other genotypes; [93,113,118] Two other studies reported that e2 carriers had a better response to statins. [109,125] Quantitative data from 20 studies were included in the meta-analysis.[83,85,87,91,95,100,101,107,110,113-115,118,122,124,125]

The meta-analysis for e2 carriers, e3e3 homozygotes, and e4 carriers showed substantial heterogeneity between studies ($P < 0.10$, $I^2 \geq 50\%$) as well as a significant percentage reduction in the pooled mean TG levels: $\Delta\mu = -17.0\%$ (-22.5% to -11.5%), $\Delta\mu = -12.3\%$ (-16.4% to -8.22%), and $\Delta\mu = -13.6\%$ (-16.2% to -10.9%), respectively (**Table 3.5; Figures 3.10-3.12**). Overall, the genotypes did not differ significantly ($Pc \geq 0.05$).

**Table 3.5. Results of the meta-analysis and subgroup analyses for associations between the *Apo E* genotype and triglyceride levels in patients treated with statins.**

| Variable | Number of studies | Mean change in TG (%) from baseline for e2 carriers (95% CI) | Mean change in TG (%) from baseline for e3e3 homozygotes (95% CI) | Mean change in TG (%) from baseline for e4 carriers (95% CI) |
|---|---|---|---|---|
| All | 20 | -15.6 (-19.5, -11.7) | -12.1 (-15.4, -8.77) | -13.3 (-15.9, -10.7) |
| Pravastatin | 6 | -14.0 (-21.6, -6.42) | -6.48 (-9.53, -3.43) | -9.2 (-15.2, -3.05) |
| Pravastatin high dose | 3 | -13.4 (-26.9, 0.18) ns | -9.89 (-19.0, -0.78) | -11.1 (-24.3, 2.13) ns |
| Pravastatin low dose | 3 | -15.6 (-26.2, -5.04) | -5.67 (-8.59, -2.75) (Pc = 0.04) | -7.54 (-14.6, -0.42) |
| Simvastatin | 3 | -25.8 (-34.5, -17.1) | -13.5 (-22.6, -4.37) | -11.9 (-25.3, 1.44) ns |
| Lovastatin | 5 | -15.1 (-22.9, -7.30) | -17.4 (-21.0, -13.9) | -17.5 (-21.2, -13.2) |
| Atorvastatin | 3 | -22.9 (-29.1, -16.7) | -15.3 (-18.4, -12.2) | -21.6 (-25.7, -17.5) |
| Caucasians | 15 | -17.5 (-22.1, -12.9) | -12.8 (-17.8, -7.81) | -15.7 (-20.5, -10.8) |
| Asians | 4 | -11.6 (-25.9, 2.72) ns | -10.4 (-16.3, -4.16) | -6.84 (-12.6, -1.04) |
| Men | **3** | -26.7 (-36.2, -17.1) | -11.5 (-16.6, -6.48) (Pc < 0.01) | -12.0 (-27.1, 3.11) ns |
| Women | 3 | -26.5 (-34.5, -18.4) pQ = 0.13 | -13.7 (-26.1, -1.27) | -18.6 (-31.1, -6.07) |
| Familial hyperlipidemia | 6 | -16.4 (-22.4, -10.4) | -17.9 (-21.2, 14.6) | -19.6 (-24.0, -15.3) |
| Non- Familial hyperlipidemia | 15 | -14.3 (-18.9, -9.69) | -10.5 (-14.1, -6.79) | -10.5 (-13.2, -7.92) |
| Short follow-up | 14 | -15.1 (-20.2, -10.0) | -14.2 (-17.9, -10.6) | -15.3 (-19.7, -10.9) |
| Long follow-up | 5 | -6.79 (-7.53, -6.05) | -6.79 (-11.4, -2.17) | -9.27 (-17.2, -1.32) |
| Diabetes | 3 | -12.6 (-26.5, 1.35) ns | -11.2 (-16.9, -5.56) | -13.1 (-20.9, -5.37) |
| No diabetes | 17 | -16.9 (-21.1, -12.6) | -12.5 (-17.2, -7.75) | -13.8 (-16.7, -11.0) |

**Figure 3.10. Individual and pooled estimates of the reduction in triglycerides among e2-carriers treated with statins.**

(Data are plotted in ascending order of baseline triglyceride levels. The analysis used a random-effects model)

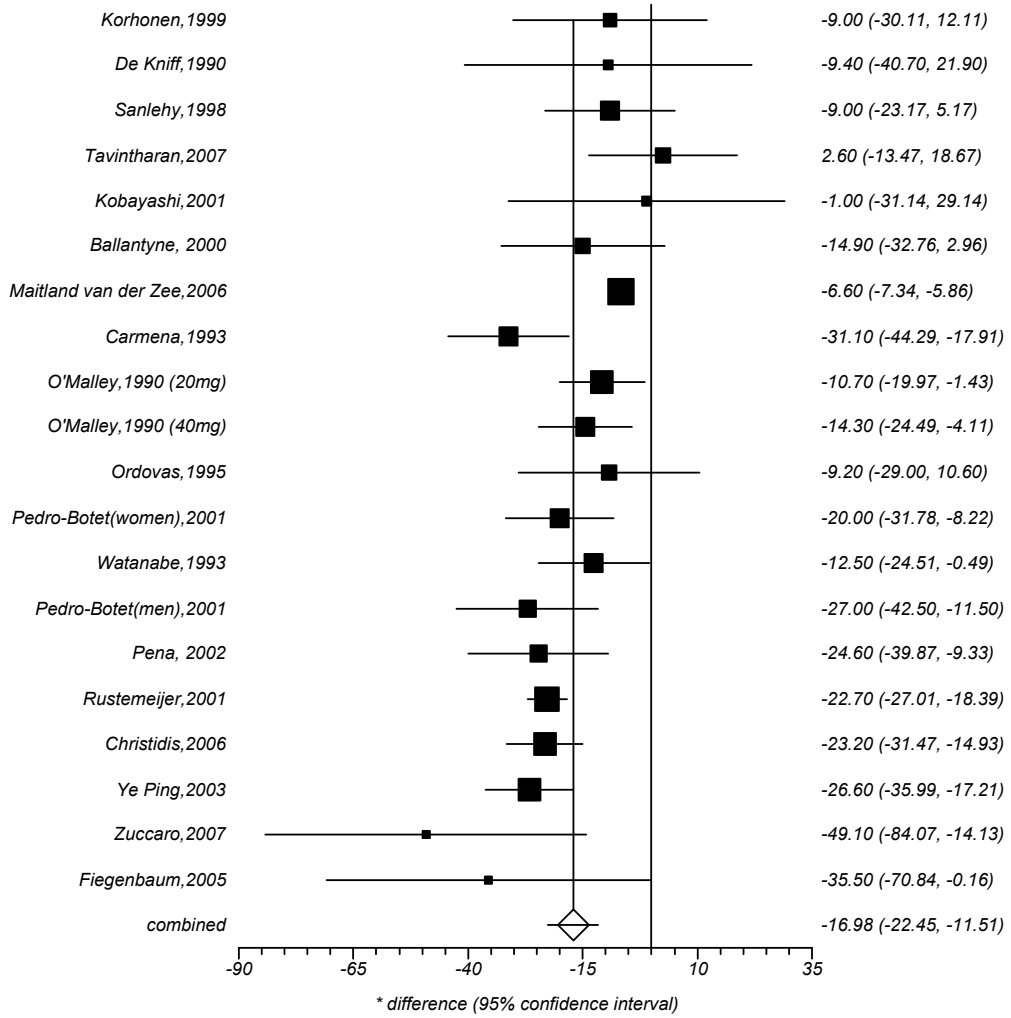


**Figure 3.11. Individual and pooled estimates of the reduction in triglycerides among e3 homozygotes treated with statins.**

(Data are plotted in ascending order of baseline triglyceride levels. The analysis used a random-effects model)

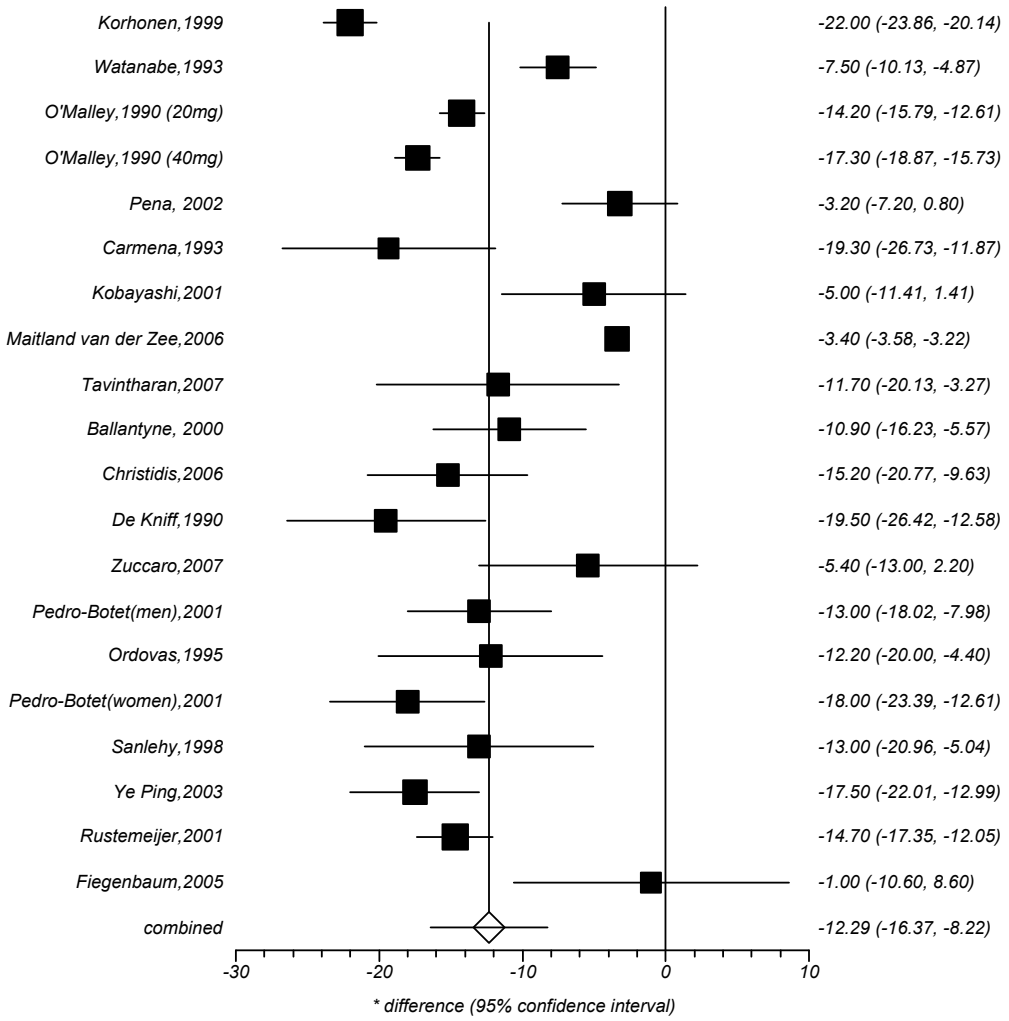
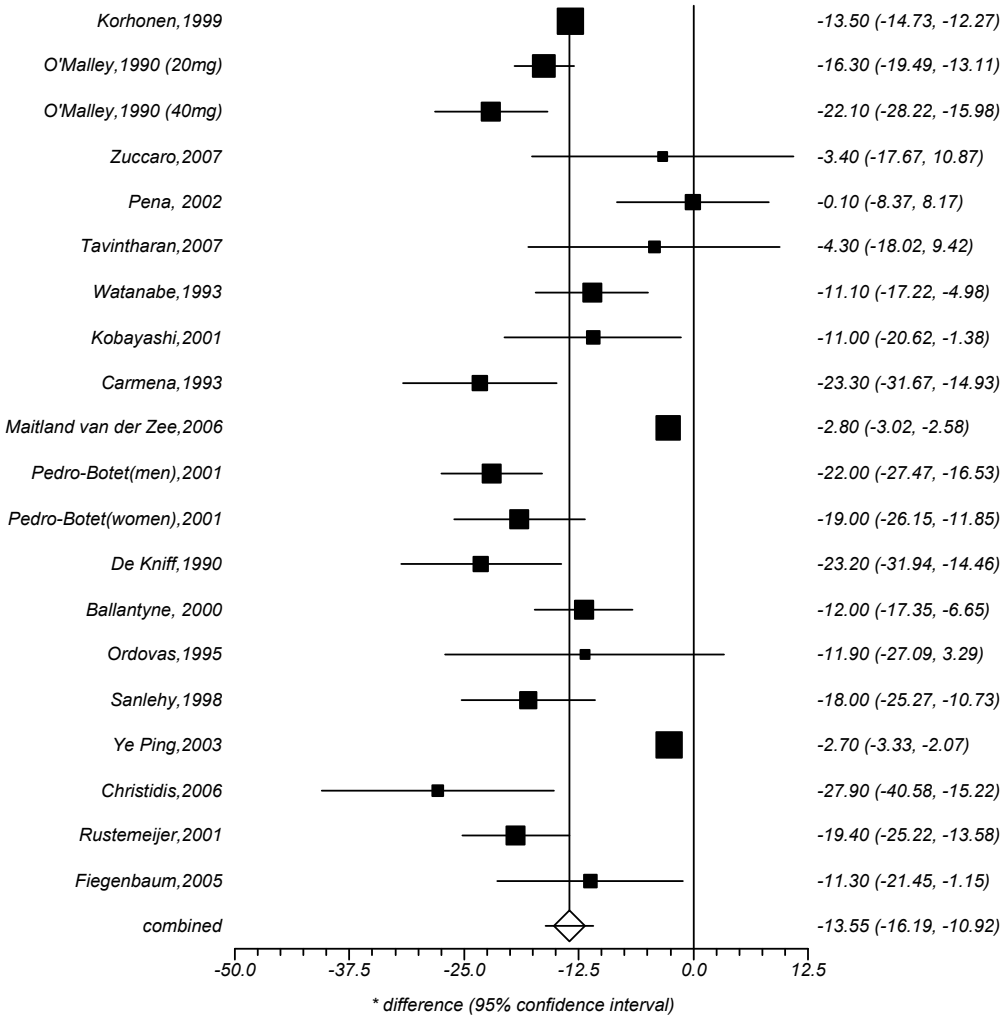


**Figure 3.12. Individual and pooled estimates of the reduction in triglycerides among e4 carriers treated with statins.**
(Data are plotted in ascending order of baseline triglyceride levels. The analysis used a random-effects model)



***Key Question #2: What demographic or clinical variables mediate the association between pharmacogenomic test results and biochemical or clinical outcomes among patients taking statin therapy?***

Pooled differences in TG levels by genotype were significant only for men. Results for the type of statin were contradictory. The results for atorvastatin were similar to those of the main analysis. In studies with pravastatin and simvastatin, e2 carriers responded better, followed by e4 carriers. In studies with lovastatin, e4 carriers had a greater mean decrease, followed by e3e3 homozygotes. Caucasians, women, and patients without diabetes had results similar to those of the main analysis. In Asians, the e2 carriers and the e3 homozygotes had a greater response than did e4 carriers. In patients with familial hyperlipidemia, the e4 allele was associated with a better response, followed by the e3 homozygotes and e2 carriers.

***Key Question #1: Among patients taking statin therapy, is there any association between carrying the Apo E genotype and other lipid or biochemical variables?***

**Apolipoprotein A1**

Seven studies investigated the effect of statins on Apo A1 levels in association with the *Apo E* genotype.[53,84,100,101,108,116,122] Treatment with statins was not associated with significant changes in Apo A1 levels in any setting. No study found significant associations between the *Apo E* genotype and changes in Apo A1 levels following treatment with statins.

Five studies were included in the meta-analysis.[53,100,101,116,122] The meta-analysis showed substantial heterogeneity between studies ($P < 0.10$, $I^2 \geq 50\%$). The pooled increase in Apo A1 from baseline (95% CI) was significant for e2 carriers and e3e3 homozygotes, with $\Delta\mu = +2.76\%$ (0.81%

to 4.71%) and Δ μ = +2.41% (1.97% to 2.86%), respectively (**Table 3.6**). Overall, the genotypes did not differ significantly (Pc ≥ 0.05).

***Key Question #2: What demographic or clinical variables mediate the association between pharmacogenomic test results and biochemical or clinical outcomes among patients taking statin therapy?***

Changes in Apo A1 levels among Caucasian and Asian e2 carriers treated with pravastatin did not differ significantly, contradicting with the main analysis. Among patients treated with lovastatin, e3e3 homozygotes differed significantly from both e2 and e4 carriers (**Table 3.6**).

**Table 3.6. Results of the meta-analysis and subgroup analyses for associations between the *Apo E* genotype and Apo A1 levels in patients treated with statins**

| Variable | Number of studies | Mean change in Apo A1 (%) from baseline for e2 carriers (95% CI) | **Mean change in Apo A1 (%) from baseline for e3 homozygotes** (95% CI) | Mean change in percentage Apo A1 for e4 carriers (95% CI) |
|---|---|---|---|---|
| All | 5 | 2.76 (0.81, 4.71) | 2.41 (1.97, 2.86) | 1.99 (-0.94, 4.92)[1] ns |
| PRAVA low dose | 2 | 4.45 (-1.54, 10.4) ns | 2.17 (1.64, 2.70) | 2.22 (0.46, 3.99) |
| Lovastatin | 2 | 7.61 (0.01, 15.2) | 3.00 (2.22, 3.78) (Pc < 0.01) | 0.22 (-4.07, 4.50) ns (Pc < 0.01) |
| Caucasians | 3 | 2.66 (-0.63, 5.96) ns | 2.87 (2.13, 3.60) | 2.41 (-2.89, 7.70) ns |
| E. Asians | 2 | 4.45 (-1.54, 10.4) ns | 2.17 (1.64, 2.70) | 2.22 (0.46, 3.99) |

[1] $P_Q < 0.01$, $I^2 = 85\%$; ns = non-significant

## Apolipoprotein B

Ten studies investigated the effect of statins in lowering Apo B levels among patients with the *Apo E* genotype.[53,84,86,89,100,103,108,116,122,124] Six studies found no significant effect,[53,84,86,89,103,116] and one study reported that e4 carriers responded less well.[108] Another found that e2 carriers

responded less well,[122] whereas two studies found that e2 carriers responded better than did other groups.[100,124] Five studies were included in the meta-analysis.[53,94,100,116,122]

The meta-analysis of e2-carriers, e3e3 homozygotes, and e4-carriers on the effect of statin therapy on Apo B levels showed substantial heterogeneity between studies ($P < 0.10$, $I^2 \geq 50\%$). The mean reduction in Apo B (95% CI) was significant for each genotype: $\Delta\mu = -28.9\%$ (-39.9% to -17.8%), $\Delta\mu = -25.5\%$ (-31.2%, to-19.9%) and $\Delta\mu = -24.8\%$ (-31.3% to -18.3%), respectively **(Table 3.7)**. Patients with the e2 allele had the greatest reductions in Apo B levels, followed by those with the e3e3 and the e4 alleles (**Table 3.7**). The results of subgroup analyses (**Table 3.7**) were consistent with those of the main analysis.

**Table 3.7. Results of the meta-analysis and subgroup analyses for associations between the *Apo E* genotype and Apo B levels in patients treated with statins**

| Variable | Number of studies | Mean change in Apo B (%) from baseline for e2 carriers (95% CI) | **Mean change in Apo B (%) from baseline for e3e3 homozygotes** (95% CI) | Mean change in Apo B (%) from baseline for e4 carriers (95% CI) |
|---|---|---|---|---|
| All | 5 | -28.9 (-39.9, -17.8) (Pc<0.05) | -25.5 (-31.2, -19.9) | -24.8 (-31.3, -18.3) (Pc<0.05) |
| Pravastatin low dose | 2 | -24.6 (-54.6, 5.43) ns | -21.7 (-32.1, -11.3) | -16.7 (-18.3, -15.2) |
| Caucasians | 2 | -31.4 (-45.4, -17.3) | -28.0 (-33.3, -22.6) | -27.9 (-31.0, -24.9)[1] |
| Asians | **3** | -27.18 (-46.7, -7.64) | -24.0 (-32.7, -15.4) | -22.5 (-33.8, -11.2) |

[1] $P_Q$=0.98, $I^2$=0; ns=non-significant

## Serum Apolipoprotein E levels

Four studies investigated the ability of statins to lower serum Apo E in patients in association with the *Apo E* genotype.[53,86,100,122] Three studies were included in the meta-analysis.[53,100,122] Studies reported no significant association between the *Apo E* genotype and an Apo-E lowering effect of statins.

The meta-analysis for e2-carriers, e3e3 homozygotes, and e4-carriers showed that there was substantial heterogeneity between studies ($P < 0.10$, $I^2 \geq 50\%$) and that the mean pooled change in Apo E from baseline (95% CI) was significant for e3e3 homozygotes and e4 carriers: $\Delta\mu$ = -34.4% (-68.7% to -0.12%) and $\Delta\mu$ = -35.2% (-57.9% to -12.4%), respectively **(Table 3.8)**. Subgroup analyses indicated that Caucasian ethnicity might contribute to the heterogeneity of results.

**Table 3.8. Results of the meta-analysis and subgroup analyses for associations between the *Apo E* genotype and Apolipoprotein E levels in patients treated with statins**

| Variable | Number of studies | **Mean change in serum Apo E (%) from baseline for e2 carriers** | Mean change in serum Apo E (%) from baseline for e3 homozygotes | Mean change in serum Apo E (%) from baseline for e4 carriers |
|---|---|---|---|---|
| All | 3 | -45.0 (-95.9, 5.92) ns | -34.4 (-68.7, -0.12) | -35.2 (-57.9, -12.4) |
| Pravastatin low dose | 2 | -22.8 (-32.3, -13.8)[1] | -19.1 (-26.1, -12.0)[2] | -21.3 (-26.0, -16.7)[3] |
| Caucasians | 1 | -88.8 (-92.9, -83.1) | -68.6 (-74.5, 62.7) | -64.6 (-75.6, -53.6) *(Pc < 0.01)* |
| Asians | 2 | -22.8 (-32.3, -13.8)[1] | -19.1 (-26.1, -12.0)[2] | -21.3 (-26.0, -16.7)[3] |

[1] $P_Q = 0.91$; [2] $P_Q = 0.16$; [3] $P_Q = 0.73$; ns = not significant

## Miscellaneous biochemical variables

A few studies evaluated other lipid and biochemical variables: Apolipoprotein CII (Apo CII), C-reactive protein (CRP), cholesteryl ester transfer protein (CETP), plasma phospholipid transfer protein (PLTP), pseudocholinesterase (Pche), lipoprotein (a) (Lp (a)), and non-HDL cholesterol.

Statin therapy did not significantly change the levels of Apo CII, and this result was independent of the *Apo E* genotype.[101,117] One cross-sectional study of statin-treated individuals participating in the AGES-Reykjavik Study reported that one or two e4 allele carriers had significantly lower CRP levels than did non-carriers and that this effect was dose-dependent on the number of alleles.[94] E4 carriers had higher TC and lower TG levels irrespective of being treated with statins or not, suggesting that the e4 allele lowers CRP levels independently and perhaps by a different mechanism.

Lovastatin significantly reduced CETP activity independently of the *Apo E* genotype.[101] Atorvastatin decreased PLTP independently of the *Apo E*-genotype in one study.[104] Pravastatin

did not significantly influence Pche activity (an enzyme involved in TG metabolism) in any of the three *Apo E* genotype groups.[115] One study found that *Apo E* genotype did not affect the response to statin treatment for Lp (a).[84] Another found that statins significantly decreased non-HDL levels and that this effect was not influenced by the *Apo E* genotype. [120]

**Adverse reactions response - Discontinuation of statins**

Maitland-van der Zee reported that adherence was lower and the risk of discontinuation was 2.3 times higher among e4 homozygotic patients,[106] perhaps because of the lower therapeutic effectiveness of statins in these individuals.

***Key Question #3: How does the pharmacogenomic test result affect the decision to use statin therapy; that is, how often is therapy changed in response to the test result?***

***Key Question #4: What benefits, harms, or adverse effects are experienced by patients on statins from treatment received after pharmacogenomic testing***

No studies addressed these two key questions.

**Summary**

In the studies of the *Apo E* genotype and statin treatment, the pooled change in total and LDL cholesterol from baseline was lower for all genotypic groups (e2 carriers, e3e3 homozygotes and e4 carriers) but without significant difference among groups. However, this analysis also found significant between-study heterogeneity. Although only a few studies were available for certain subgroups, they indicate that some factors may affect the investigated associations of *Apo E* with statin therapy: ethnicity, sex, familial hyperlipidemia, the type of

statin used, and possibly the presence of diabetes. No studies evaluated the last two key questions on the impact of therapeutic choice and the benefits, harms, or adverse effects for patients from subsequent therapeutic management after pharmacogenetic testing for *Apo E* genotype.

**Discussion**

For most of the studies investigating the *Apo E* genotype and statin treatment, the primary clinical outcomes included the reduction of TC and LDL. The meta-analysis for e2-carriers, e3e3 homozygotes, and e4-carriers showed large and significant heterogeneity among studies. Overall, the pooled change in TC and LDL from baseline was significant for all three genotypic groups. However, the significant heterogeneity of our results warrants a cautious interpretation. Among the factors that may impact the investigated association are ethnicity, familial hyperlipidemia, the type of statin used, sex, and possibly the presence of diabetes. However, the limited number of studies for certain subgroups (i.e. diabetes) is another limitation of our analysis.

For the pooled change in TC and LDL levels, comparisons across genotypes showed that e2 carriers had a better response to statin therapy, with a pooled estimate better than that of the e3 homozygotes and e4 carriers. In a large replication study with a sample size similar to the number of patients included in our meta-analysis, and with a more homogeneous population and intervention, with statin therapy, e2 carriers lowered their LDL by 3.5% (P = 0.001) over other groups.[119] The direction and magnitude of this effect are the same as our findings and further support the association between the *Apo E* genotype and the lipid-lowering response to statin therapy. Although very small, this effect may be large enough to affect health on a population basis [88]. Intervention studies with statins in the general population have shown that cardiovascular event rates can be reduced by 1%, for every 1% reduction in LDL.[81]

# *MTHFR* gene polymorphism and response to chemotherapy

## Background

The methylenetetrahydrofolate reductase (*MTHFR*) gene is located on chromosome 1p36. Gene polymorphisms can reduce *MTHFR* enzyme activity. Two common single nucleotide polymorphisms (SNPs) that affect the activity of the *MTHFR* enzyme are the C-to-T nucleotide transition at position 677 (dbSNP accession number: rs1801133) (which results in an alanine-to-valine substitution) and the A-to-C nucleotide transversion at position 1298 (dbSNP accession number: rs1801131) (leading to an amino acid exchange from glutamine to alanine).[129] Reduced enzyme activity has been reported in 677TT and 1298CC homozygotes, as well as in combined carriers and to a lesser extent, in heterozygous individuals.[129-135]

The C677T polymorphism is the most frequent *MTHFR* polymorphism. Homozygotes (677TT) have 30% of the normal *MTHFR* enzyme activity, whereas heterozygotes (677CT) have 60% of the normal activity.[132] The C677T mutation increases the thermolability of *MTHFR*, which leads to lower levels of 5-methyltetrahydrofolate, an accumulation of 5,10-methylenetetrahydrofolate, and increases in plasma homocysteine levels in individuals with marginal folate status. The resulting changes in cellular composition of one-carbon folate derivatives may also impair remethylation of homocysteine to methionine and lead to DNA hypomethylation.

The *MTHFR A*1298C gene polymorphism also decreases enzymatic activity, but to a lesser extent than does the *MTHFR* C677T gene polymorphism. The *in vivo* functional relevance of the *A1298C* variant is less well defined. *A1298C* affects *in vitro* enzyme function to a lesser degree, and individuals carrying the variant often have normal homocysteine and plasma folate concentrations.[132]

These MTHFR SNPs often appear in the literature with alternative designations, such as Glu429Ala or E429A for A1298C and Ala222Val or A222V for C677T [129-135].

***MTHFR* and chemotherapy**

Folate is vital in DNA synthesis, and polymorphisms in the *MTHFR* gene may affect the response of cancer cells to chemotherapy. Two commonly used chemotherapeutic agents, 5-fluorouracil (5FU) and methotrexate (MTX), competitively interact with folate metabolism. In addition, *MTHFR* polymorphisms can lead to the accumulation of 5,10-methylenetetrahydrofolate that can affect the chemotherapeutic response of cancer cells.[133]

Alterations in the intracellular pool of folates caused by *MTHFR* polymorphisms can increase toxicity in patients receiving antifolate therapy. According to the common toxicity criteria of the National Cancer Institute, five of six patients who experienced grade-4 toxicity in their first cycle of adjuvant chemotherapy with cyclophosphamide, methotrexate and 5-FU for early breast cancer had the variant 677TT *MTHFR* genotype. In these patients, the antifolate, methotrexate, potentiated 5-FU toxicity as a result of thymidilate synthase inhibition by a covalently bound ternary complex and probably contributed to severe acute toxicity.[136-138]

We investigated *MTHFR* gene polymorphisms in patients with solid or hematologic cancers and determined their effect on the response to chemotherapy.

**Data synthesis and analyses of *MTHFR* gene polymorphism with chemotherapy**

For each study, the odds ratio (OR) and corresponding 95% confidence interval (CI) of responders versus non-responders to chemotherapy for the allele of each gene polymorphism were extracted or calculated from each article. Studies for the same type of cancers were combined in meta-analysis.

## Results

Of 136 abstracts screened for eligibility (**Appendix 1),** 11 studies that investigated the association between any of the *MTHFR* C677T or *MTHFR* A1298C gene polymorphisms and response to chemotherapy of the folate metabolic pathway were included (**Table 4.1**).[129-134,136-140]

**Table 4.1. Characteristics of the 11 Studies Evaluating the Association between the *MTHFR* Genotype and Response to Chemotherapy**

| Characteristics | Description: number of studies (references) |
|---|---|
| Total | 11 studies[129-134,136-140] |
| Ethnicity | Caucasians: 8[129,131-134,136,139,140]<br>Asians: 2[129,137]<br>Mixed: 1[138] |
| Countries | North America: 2[131,138]<br>Europe: 7[129,132-134,136,139,140]<br>Asia: 2[130,137] |
| Types of cancers | Upper gastrointestinal cancers: 4[129,133,138,140]<br>Lower gastrointestinal cancers: 6[131,132,134,136,137,139]<br>Cervical cancer: 1[130] |
| Commonly examined polymorphisms | C677T: 10[129-134,136,137,139,140]<br>A1298C: 4[132,134,136,139] |

The studies were published between 2003 and 2006 (**Table 4 in Appendix 2**). Eleven studies involved the *MTHFR* C677T gene polymorphism[129-134,136,137,139,140] and six involved the *MTHFR* A1298C polymorphism [130,132,134,136,138,139] . The final outcome of chemotherapy was classified as response or no response, using different criteria (e.g., shrinkage of tumor, histological evaluation, recurrence). Studies varied in their definitions of complete response (disappearance of the disease) or partial response (at least 50% reduction in tumor load of the lesions), stable disease (≤25% progression or <50% shrinkage) or cancer progression (size enlargement >25% or appearance of new lesions). The definitions of outcomes for individual studies are shown in Table 4 in Appendix 2.

Overall, the number of responders and nonresponders, respectively, was 561 and 667 for *MTHFR* C677T and 266 and 254 for *MTHFR* A1298C. The most common genotypes in both

responders and nonresponders were genotypes *MTHFR* 677 CT and *MTHFR* 1298 AA, and the most common alleles were C and A, respectively.

***Key Question #1: Among patients undergoing chemotherapy, is there any association between carrying the genotype and complete response, partial response, or stable disease?***

*MTHFR* C677T

No study showed significant associations among the alleles (T vs. C), the recessive model (TT vs. TC+CC), and the dominant model (TT+TC vs. CC) (**Figures 4.1-4.3**).[129-134,136,137,139,140]

*Colorectal cancer*

Six studies investigated the association between the response to chemotherapy and comparison between alleles (MTHFR 677T vs. C). The studies showed significant heterogeneity (P = 0.05, $I^2$ = 54%) among them[131,132,134,136,137,139] (**Table 4.2**). The pooled odds ratio, under a random-effects model, was not significant (OR: 0.97; 95% CI, 0.65 to 1.46). The recessive and dominant models for allele T showed non-significant associations (OR: 1.48; 95% CI, 0.67 to 3.25) and (OR: 0.72; 95% CI, 0.42 to 1.23), respectively.

A subgroup analysis of Caucasians (from five studies)[131,132,134,136,139] found no significant association for the allele contrast (OR: 1.05; 95% CI, 0.66 to 1.67). The recessive and dominant models showed similar results. Genotype data were not reported by age group, tumor type, or tumor stage.

The Egger test and the Begg-Mazumdar test for the comparison between alleles (MTHFR 677T vs. C) indicated that there is no differential magnitude of effect in large versus small studies (P = 0.14 and P = 0.17, respectively).

*Gastric cancer*

Two studies in Caucasians[129,140] found no significant association between the response to chemotherapy and comparison between alleles (MTHFR 677T vs. C) (OR: 0.78; 95% CI, 0.47 to 1.31). The recessive and dominant models for allele T also produced non-significant associations (OR: 0.61; 95% CI, 0.18 to 2.04) and (OR: 0.77; 95% CI, 0.38 to 1.56), respectively (**Table 4.3**). No study reported genotype data by age group, tumor type, or tumor stage.

*Esophageal cancer*

Two studies[130,135] found no significant association between the response to chemotherapy and the dominant models for allele T (OR:1.09, 95% CI, 0.64 to1.84) (**Table 4.4**).

*Cervical cancer*

One study[130] found no significant associations between response to chemotherapy and MTHFR C677T genotype in cervical cancer.

***MTHFR* A1298C**

No study showed a significant association for the allele contrast (C vs. A), the recessive model (CC vs. CA+AA), or the dominant model (CC+CA vs. AA) (**Figures 4.4-4.6**).

*Colorectal cancer*

Four studies of Caucasians investigated the association between the response to chemotherapy and comparison between alleles (1298C vs. A).[132,134,136,139] Heterogeneity among the four studies was significant (P = 0.09, $I^2$ = 54%).[132,134,136,139] The pooled odds ratio, under a random-effects model, was not significant (OR: 0.94; 95% CI, 0.58 to 1.52). The recessive and dominant models for allele C also produced non-significant associations (OR: 0.95; 95% CI, 0.26 to 3.50) and (OR: 0.94; 95% CI, 0.54 to 1.65), respectively (**Table 4.5**). Genotype data were not reported by age group, tumor type, or tumor stage.

***Key Question #2: What demographic or clinical variables mediate the association between pharmacogenomic test results and response to chemotherapy?***

***Key Question #3: How does the pharmacogenomic test result affect the decision to undergo chemotherapy; that is, how often is therapy changed in response to the test result)?***

***Key Question #4: What benefits, harms, or adverse effects are experienced by patients on chemotherapy from treatment received after pharmacogenomic testing?***

None of the identified studies investigated demographic or clinical variables that might mediate the association between pharmacogenomic test results and response to chemotherapy (key question 2). In addition, none of the studies investigated whether *MTHFR* gene testing influenced therapeutic choice (key question 3) or the benefits and harms or adverse effects for patients from their subsequent therapeutic management after gene testing (key question 4).

**Summary**

Few studies evaluated the relationship between *MTHFR* and the response to chemotherapy of folate metabolic pathway. We identified no studies that evaluated key questions 2, 3, or 4.

**Table 4.2. Results of the meta-analyses for the association between the *MTHFR C677T* genotype and the response to chemotherapy in patients with colorectal cancer**

| Contrast for C677T | Population | No. Studies | Fixed effects OR (95% CI) | Random effects OR (95% Ci) | $I^2$ (%) | P Q-test |
|---|---|---|---|---|---|---|
| T vs C | All | 6 | 0.92(0.71-1.20) | 0.97 (0.65-1.46) | 54 | 0.05 |
| | Caucasians | 5 | 0.96 (0.73-1.27) | 1.05 (0.66-1.67) | 60 | 0.04 |
| Recessive model | All | 6 | 1.43 (0.87-2.33) | 1.48 (0.68-3.25) | 50 | 0.07 |
| | Caucasians | 5 | 1.56 (0.94-2.61) | 1.75 (0.74-4.13) | 54 | 0.07 |
| Dominant model | All | 6 | 0.68 (0.47-1.00) | 0.72 (0.42-1.23) | 46 | 0.10 |
| | Caucasians | 5 | 0.71 (0.48-1.05) | 0.77 (0.41-1.41) | 55 | 0.06 |

**Table 4.3. Results of the meta-analyses for the association between the *MTHFR C677T* genotype and the response to chemotherapy in patients with gastric cancer**

| Comparisons for C677T | Population | No. Studies | Fixed-effects OR (95% CI) | Random-effects OR (95% CI) | $I^2$ (%) | P Q-test |
|---|---|---|---|---|---|---|
| T vs C | Caucasians | 2 | 0.81 (0.57-1.14) | 0.78 (0.47-1.31) | na | 0.16 |
| Recessive model | Caucasians | 2 | 0.70 (0.37-1.31) | 0.61 (0.18-2.04) | na | 0.20 |
| Dominant model | Caucasians | 2 | 0.78 (0.45-1.35) | 0.77 (0.38-1.56) | na | 0.20 |

na = non-applicable

**Table 4.4. Results of the meta-analyses for the association between the *MTHFR C677T* genotype and the response to chemotherapy in patients with esophageal cancer**

| Comparisons for C677T | Population | No. Studies | Fixed-effects OR (95% CI) | Random-effects OR (95% CI) | $I^2$ (%) | P Q-test |
|---|---|---|---|---|---|---|
| Dominant model | All | 2 | 0.78 (0.45-1.35) | 1.09 (0.64-1.84) | na | 0.86 |

na = non-applicable

**Table 4.5. Results of the meta-analyses for the association between the *MTHFR A1298C* genotype and the response to chemotherapy in patients with colorectal cancer**

| Comparisons for A1298C | Population | No. studies | Fixed-effects OR (95% Ci) | Random-effects OR (95% CI) | $I^2$ (%) | P Q test |
|---|---|---|---|---|---|---|
| C vs A | Caucasians | 4 | 0.96 (0.70-1.32) | 0.94 (0.58-1.52) | 54 | 0.09 |
| Recessive model | Caucasians | 4 | 0.89 (0.45-1.78) | 0.95 (0.26-3.50) | 57 | 0.07 |
| Dominant model | Caucasians | 4 | 0.98 (0.65-1.46) | 0.94 (0.54-1.65) | 45 | 0.14 |

**Figure 4.1. Individual odds ratios for the comparison between alleles *(MTHFR 677T* vs *C*) and response to chemotherapy, by type of cancer**

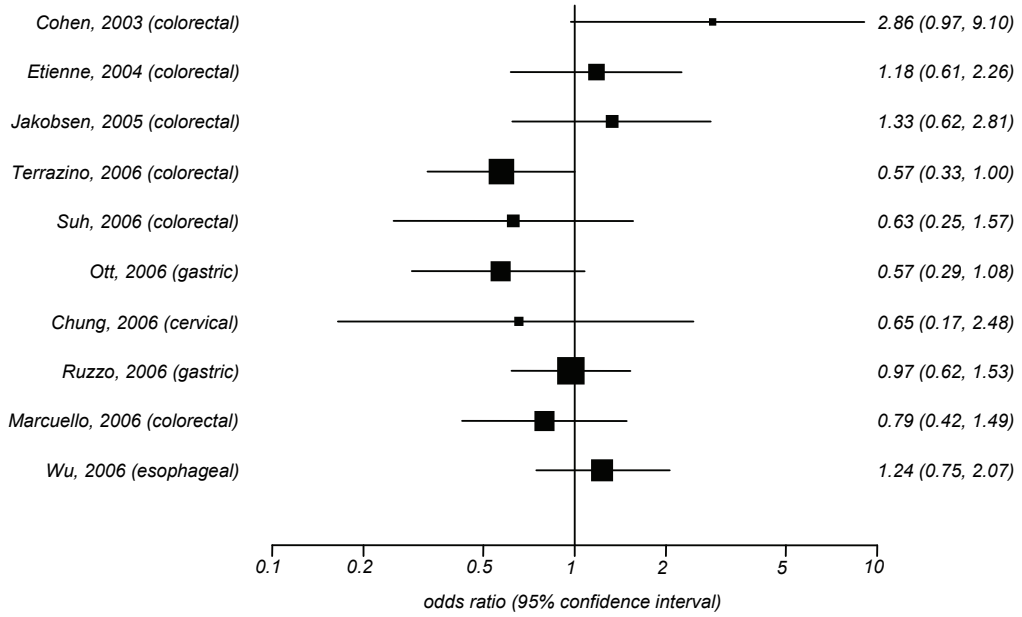



**Figure 4.2. Individual odds ratios for the recessive model of *MTHFR 677T* and response to chemotherapy, by type of cancer**

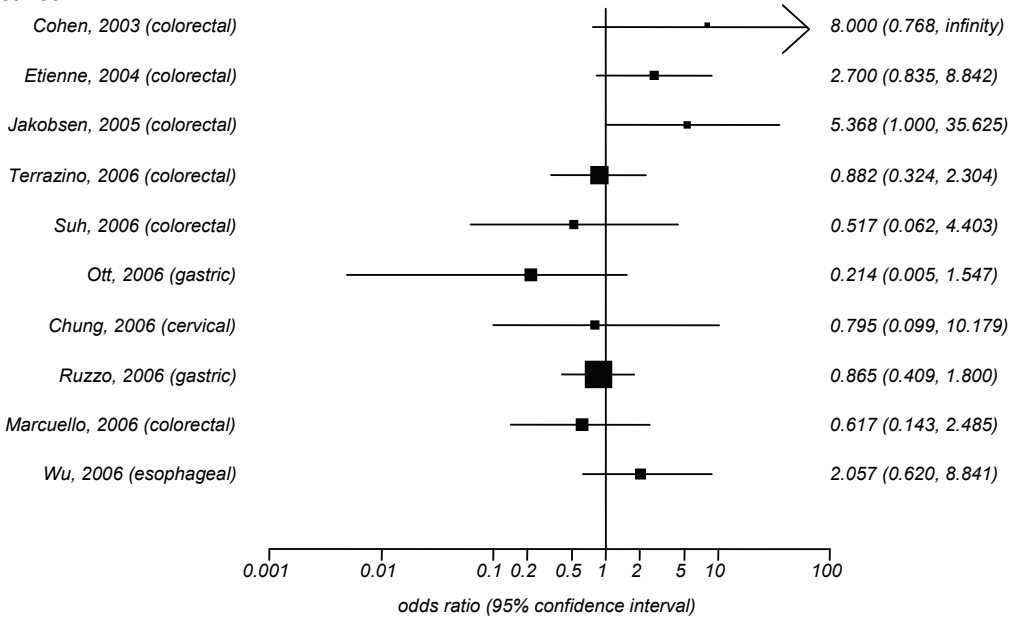


**Figure 4.3. Individual odds ratios for the dominant model of *MTHFR 677T* and response to chemotherapy, by type of cancer**

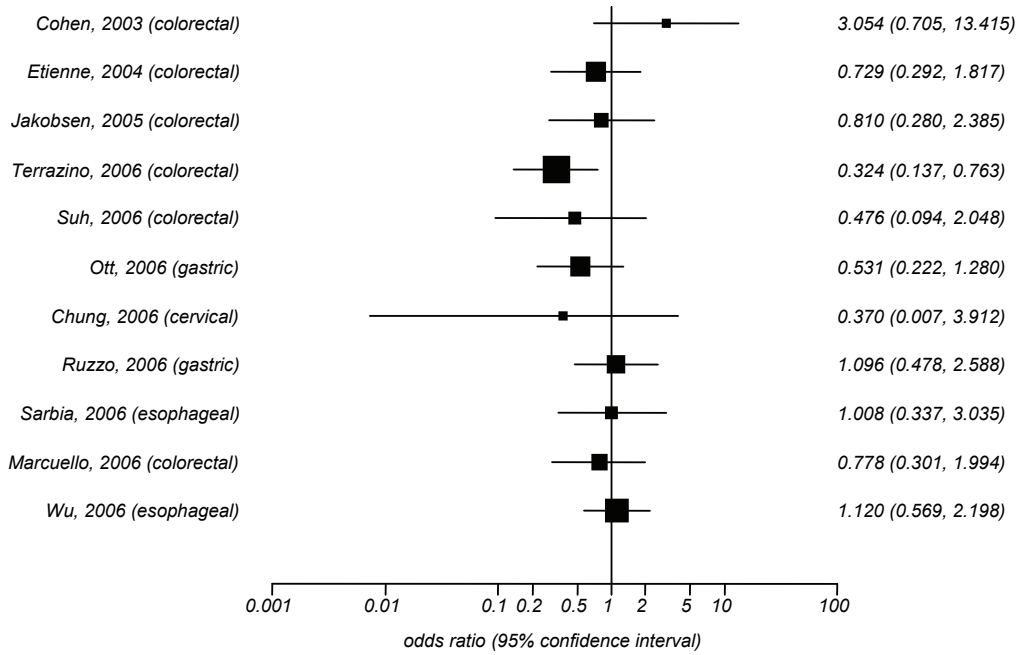



**Figure 4.4. Individual odds ratios for the comparison between alleles (*MTHFR 1298C vs. A*) and response to chemotherapy, by type of cancer**

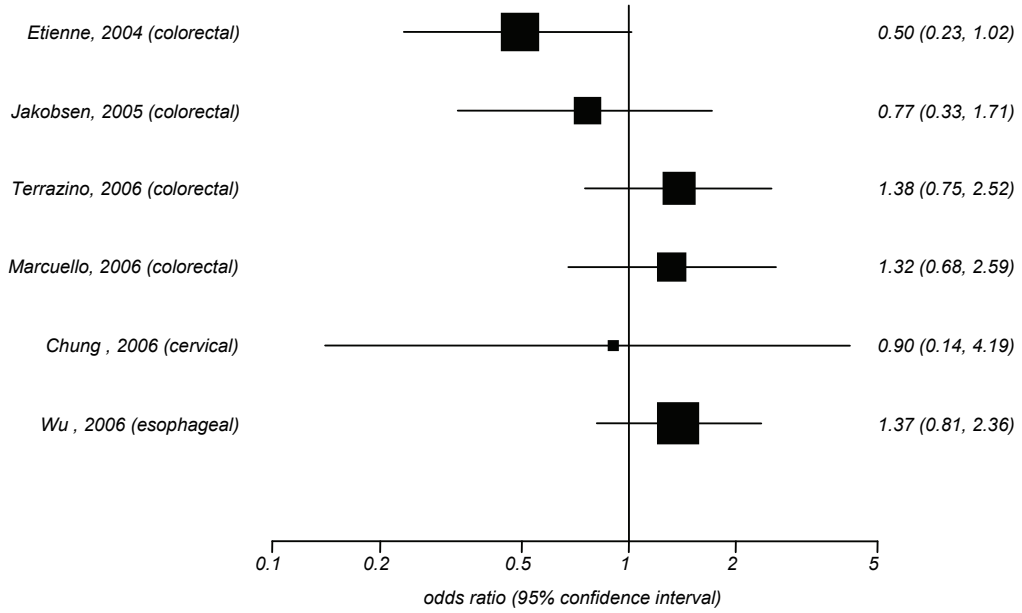


**Figure 4.5. Individual odds ratios for the recessive model of MTHFR 1298C and response to chemotherapy, by type of cancer**

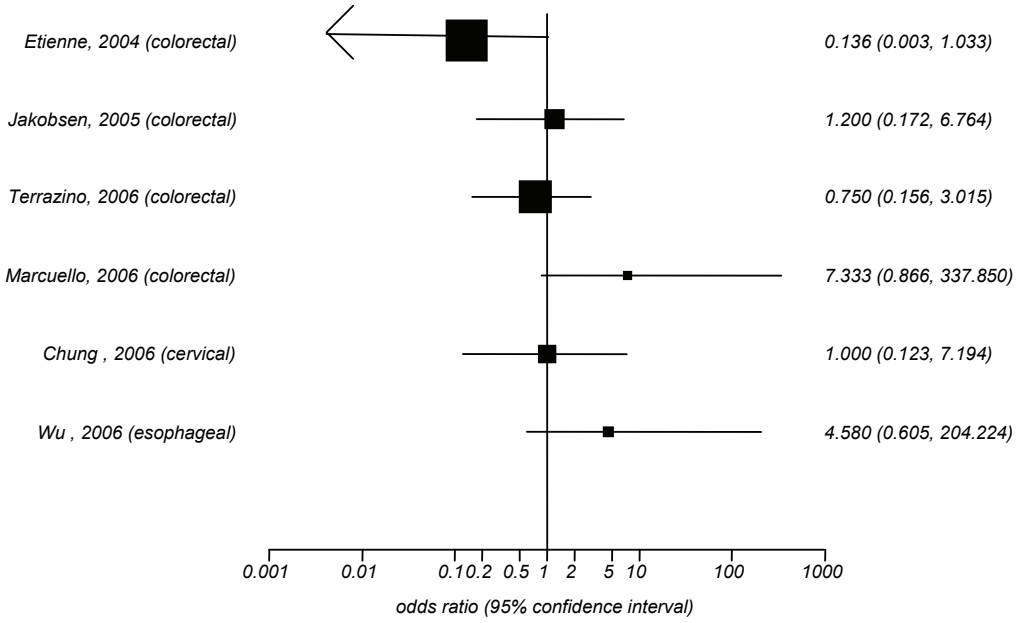
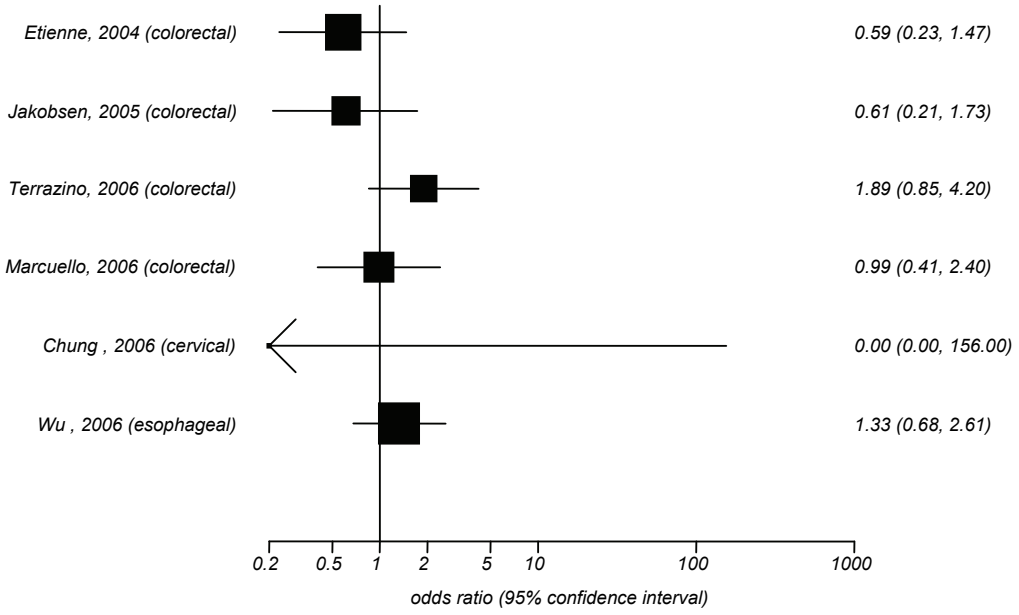



**Figure 4.6. Individual odds ratios for the dominant model of MTHFR 1298C and response to chemotherapy, by type of cancer**



**Discussion**

The evidence indicates that *MTHFR* gene polymorphisms do not predict response to chemotherapy. The results of the present report and meta-analysis were based on a relatively small number of studies and participants and, therefore, should be interpreted cautiously. The relationship between response to chemotherapy and the multiple gene polymorphisms involved in homocysteine-folate metabolism remains unresolved. Ethnicity had no effect on the association between *MTHFR* gene polymorphisms and response to chemotherapy. We do not know the relationship between effect modifiers such as age and tumor type or stage, and *MTHFR* gene polymorphisms and the response to chemotherapy because they were not clearly reported in the individual studies. The scarcity of data on *MTHFR* gene testing and therapeutic choice precluded any inference on this association. Data on the benefits and harms or adverse effects for patients from their subsequent therapeutic management after gene testing are also needed.

# Conclusion

In this report, we examined a large and varied literature on four pharmacogenetic tests for treatment of three non-cancer conditions and one cancer condition. Our systematic review found that the majority of studies evaluated the associations of pharmacogenetic test results with intermediate, not clinical, outcomes, such as the effectiveness of drug dose, and adverse clinical outcomes, such as bleeding events. Only a few studies evaluated the effects of patient- and disease-related characteristics on the association between test results and intermediate or clinical outcomes. Across all four topics, no studies investigated the influence of gene testing on the impact of therapeutic choices and on the benefits and harms or adverse effects for patients from their subsequent therapeutic management after pharmacogenetic testing.

**Limitations and Future Research Recommendations**

Genetic meta-analyses have been criticized for lack of consistency in selecting genotypes.[141-143] In practice, it is not easy to define *a priori* the proper, biologically meaningful genotype comparisons, because the actual inheritance models are unknown.[144] Early work in the candidate gene era suggests that associations based on highly selected genotype comparisons are almost 10 times more likely to be refuted than other associations.[145]

Detailed patient-level analyses of outcomes such as warfarin dosing are needed to derive and validate an algorithm that incorporates genetic information and to define the risk for bleeding complications and thromboembolic events among people who carry genetic variants. As described in several examples in the literature, and encouraged by the Network of Networks initiative,[146-148] meta-analysis of individual patient data is a particularly suitable framework for such efforts. A consortium of investigators has already been assembled to derive an optimal

dose-prediction algorithm, and further efforts to quantify genetic associations with clinical outcomes should be encouraged.

Another major limitation of our analyses is that we included studies with significant diversity in terms of clinical diagnosis, co-morbidities, polypharmacy, and outcome definitions. Future analyses with more studies of homogeneous groups, with strict inclusion criteria and definitions of phenotypes and responses to therapy, may alter the current findings. Moreover, if researchers can make their data on individual patients readily available, adjusted estimates for the effects of modifiers (such as age or tumor stage) can also be analyzed.

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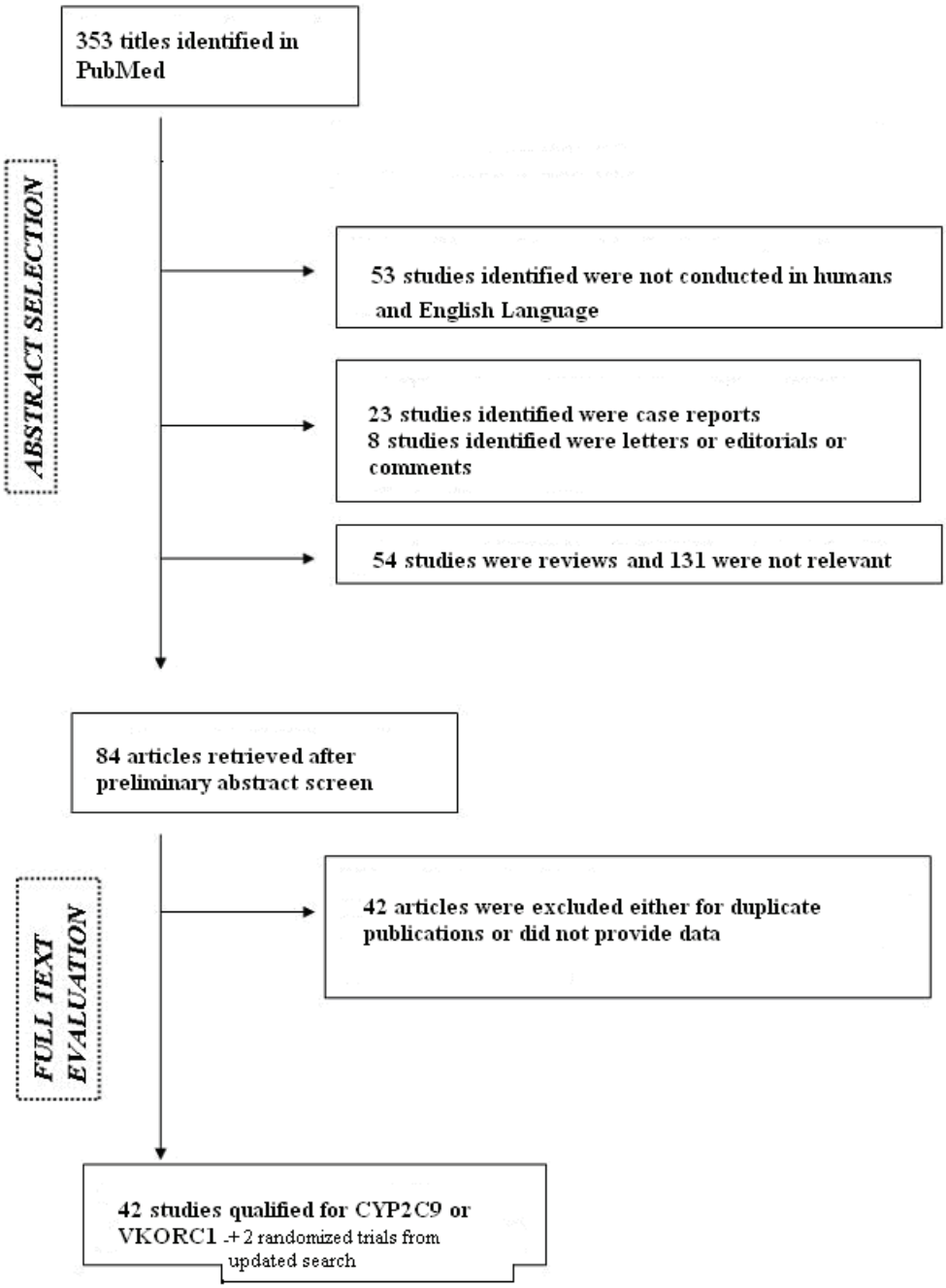
# Appendix 1 Search strategies, flow chart of included studies, and template of extraction forms

## CYP2C9 or VKORC1 and warfarin

(CYP2C9.mp. OR VKORC1.mp.OR *Mixed Function Oxygenases/ge OR *Mixed Function Oxygenases/me) AND (Warfarin.mp. or exp Warfarin/)

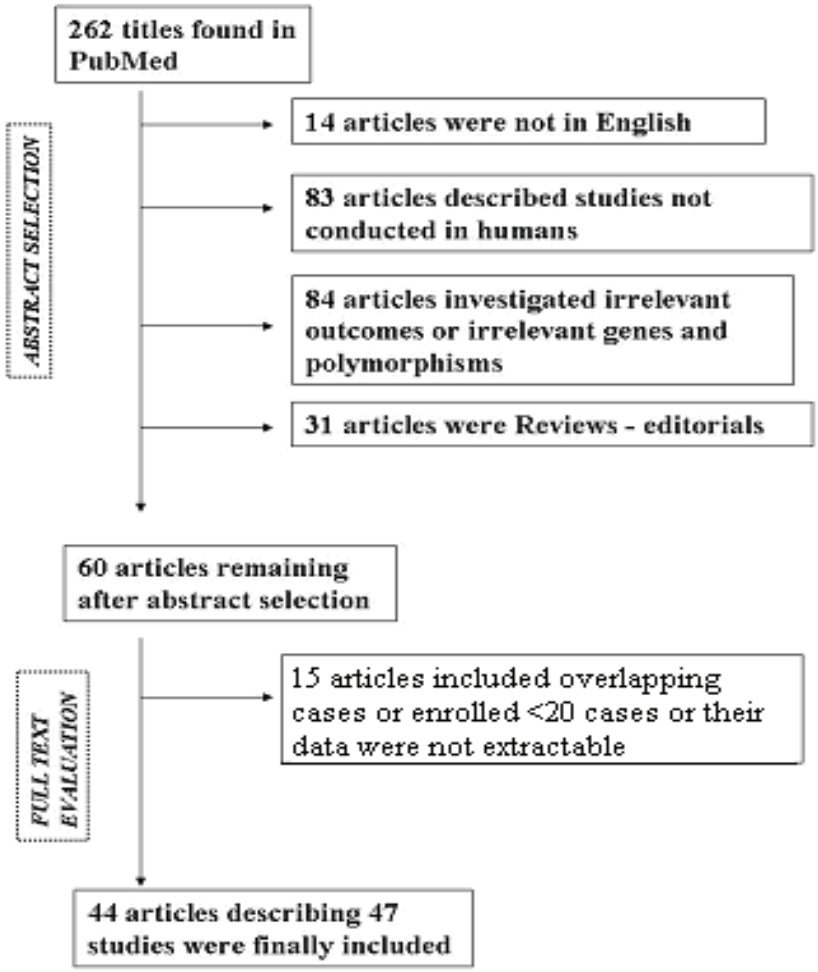
**Figure 1**. Flow diagram of eligible studies of CYP2C9, VKORC1 and warfarin



## Apo E genotype and response to statin treatment

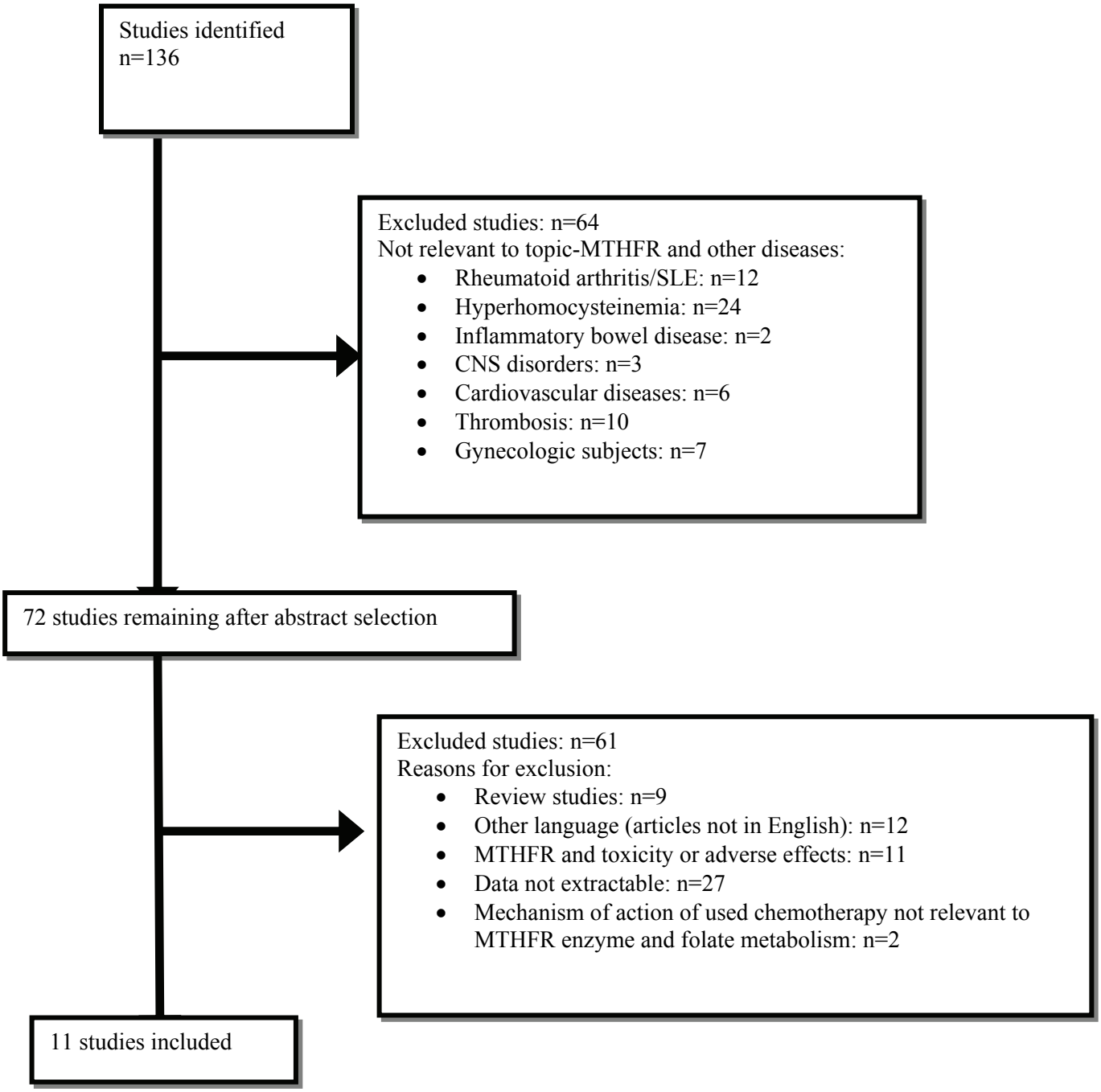
Search terms included (Apolipoprotein-E OR APOE OR APOE2 OR APOE3 OR APOE4) AND (statin OR hydroxymethylglutaryl-CoA reductase inhibitors OR lovastatin OR atorvastatin OR simvastatin OR pravastatin OR rosuvastatin OR fluvastatin).

**Figure 2**. Flow diagram of eligible studies of ApoE genotypes and statin therapy



**MTHFR and Chemotherapy**
Search terms included (methylenetetrahydrofolate reductase OR MTHFR) AND (gene OR polymorphism) AND chemotherapy.

**Figure 3.** Flow diagram of eligible studies of MTHFR genotype and response to chemotherapy.



Appendix 1. Table 1. CYP2C9/VKORC1 with warfarin therapy

**Study**

- First author:
- Year
- Country:
- Journal
- Study Design
- Duration of follow up

**Outcomes assessed (yes/no)**

- Time to achieve stable dose
- Overcoagulation / proportion high INR
- Time to first over the ceiling INR
- Serious or life threatening bleeding event
- Intensity of followup
- Change in management

**Cohort characteristics**

- Number of patients:
- Male/Female
- Male %
- Racial descent:
- Age (y):
- Inclusion criteria defefined
- Criteria of Diagnosis
- Type of Diseases
- Disease Duration
- Duration of follow-up
- Exclusion criteria defined

**Intervention Used**

- Type of warfarin
- Dosage of warfarin
- Duration of treatment
- Concomittant therapy
- Number of patients excluded

**Patient characteristics in the cohort (percentage)**

- Cohort medications
- Co medications

**Study Results**

- Genotype distribution
- Sex-specific
- Gene-gene interactions
- Gene-environment interactions
- VKORC1 methodology
- CYP2C9 methodology
- Was the genotype methodology replicated with
- another protocol

- Blindness of genotyping:
- Is data by gender provided?
- Power calculations provided?
- Cohort overlapping with previous studies
- How were cases recruited?
- Is HWE assessed?
- Predictor analyses controlled for other risk factors

**Appendix1. Table 2. Extracted data from each study of ApoE with statin therapy**

**Study**

- First author
- Year
- Country
- Journal
- Study design
- Duration of follow up

**Outcomes assesed (yes/no)**

- LR
  - TC
  - LDL
  - HDL
  - TGs
  - APoA1
  - ApoB
  - ApoE
  - ApoA2
  - ApoCII
  - CRP
  - CETP activity
  - PLTP activity
  - Non-HDLC
  - Pche activity
  - LP(a)
- CVER
  - Mortality
  - Clinical events*
  - Intracerebral hemorrhage (ICH)
- ADR
  - angiographical change of CAD
- ARR
  - Discontinuation of statins

**Cohort characteristics**

- Number of patients
- Male/Female
- Racial descent
- Age
- Inclusion criteria defined
- Criteria of Diagnosis
- Type of Disease
- Disease duration
- Duration of Follow-up
- Age at onset
- Exclusion criteria defined

**Patient characteristics in the cohort (percentage)**

- Hyperlipidemics
  - severe or mild hyperipidemia
- Primary or secondary hyperlipidemia
- Familial Hyperlipidemia
- Diabetics
- Hypertensives
- CAD

- Alcoholics (never/ever)
- Smoking (never/ever)

**Intervention used**

- Hypolipidemic diet:
- Type of statin:
- Dosage of statin:
- Duration of treatment:
- Concomittant therapy:
- Number of patients excluded because of SE:

**Study Results**

- Genotype distribution
- LR: Means of individual percentage change in lipid/lipoprotein/biochemical parameter following treatment with statins per genotype
- CADR: percentage of patients with angiographical change (progression/regression) of CAD lesions following treatment with statins per genotype
- CVER: percentage of new cardiovascular events following treatment with statins per genotype
- Mortality estimates following treatment with statins per genotype
- ARR: Percentage of patients dicsontinuating statin treatment per genotype
- Percentage of statin related side-effects per genotype
- Gender interaction
- Gene-gene interactions
- Genetic contrasts used
- Baseline differences in lipid profile according to genotype
- Gene-dose effect assesed

* Definition of Clinical events by individual studies.

**Appendix 1.Table 3. Extracted data from each study for MTHFR polymorphisms and response to chemotherapy**

**Study**

- First author
- Year
- Country
- Journal
- Study design

**Cohort characteristics**

- Number of patients
- Male/Female
- Racial descent
- Age
- Inclusion criteria defined
- Criteria of Diagnosis
- Disease duration
- Survival time
- Exclusion criteria defined

**Type of cancer or malignancy**

**MTHFR gene polymorphism studied**

**Tumor grade and stage**

**Clinical Outcomes investigated**

**Intervention used**

- Type of chemotherapy
- Dosage scheme:

**Definition of responders and non-responders**

**Patient comorbitidy**

**Study Results**

- Genotype distribution
- Gender interaction
- Age interactions
- Genetic contrasts used

# Appendix 2

## Evidence tables of included studies

**Appendix 2 Table 1. Study and population characteristics of studies reporting gene variants and warfarin stable dose among patients**

| Author, yr | Country Setting | Race | N subjects (N excluded) | Age yr | Male% | Study design | Target INR reported in the study | Stable dose as an inclusion criteria | Genotypes | Genotype methodology | Methodology replicated? Or quality control described? | Blinding | HWE assessed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Obayashi, 2006 [45] | Japan, CVD unit | Asian 100% | 125 (0) | 62.5 (SD 13.1) | 51% | P | ND [Mean INR 2.12 ± 0.44] | Yes Fixed warfarin dose for at least 2 mo | CYP2C9 *3 VKORC1 | PCR AvaII and KpnI | ND | ND | No |
| Mushiroda, 2006 [46] | Japan, Hospital | Asian 100% | 790 (38) | 68 (19-92) | 67% | P | ND | ND | CYP2C9 *3 VKORC1 | PCR | ND | ND | No |
| Moridani, 2006 [47] | Canada and US Anticoagulation clinic | Caucasian 94%; other 6% | 189 (0) | 62-71 | 56% | R | ND | ND | CYP2C9 *2, *3 | PCR AvaII and NsiI | Yes | ND | Yes |
| Veenastra, 2005 [48] | Hong Kong – China, Anticoagulation clinic | Asian 100% | 69 (28) | 58 (SD 10) | 46% | P | 1.8-3.2 | ND | CYP2C9 *3 | PCR | Yes | ND | ND |
| Herman, 2005 [49] | Slovenia Anticoagulation clinic | Caucasian 100% | 188 (0) | 71.9 (SD 9.0) | 50% | R | ND | ND | CYP2C9 *2 or *3 | ND | ND | ND | ND |
| Tabrizi, 2002 [50] | US, Hospital Inpatient and outpatient | Caucasian 78% Afr American 22% | 153 (0) | 58.7 (SEM1.2) | 56% | P | 1.8 – 3.5 | Yes <10% dose fluctuation over 4 wk | CYP2C9 *2, *3 | TDI-FP PCR | Yes | ND | ND |
| Scordo, 2002 [51] | Italy, Anticoagulation outpatient | Caucasian 100% | 93 (0) | 68 (SD 11) | 61% | P | 2.0 – 3.0 | Yes ≤15% variation over 3 visits | CYP2C9 *2, *3 | PCR AvaII and NsiI | ND | ND | Yes |
| Loebstein, | Israel, | Caucasian, | 174 (0) | 63 (SD | 56% | P | ND | Yes | CYP2C9 | PCR | ND | ND | ND |

| Author, yr | Country Setting | Race | N subjects (N excluded) | Age yr | Male% | Study design | Target INR reported in the study | Stable dose as an inclusion criteria | Genotypes | Genotype methodology | Methodology replicated? Or quality control described? | Blinding | HWE assessed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2001 [52] | Anticoagulation outpatient | 100% | | 15) | | | | ± 20% INR values for 4 clinic visits | *2, *3 | AvaII, NsiI and KpnI | | | |
| Freeman, 2000 [53] | US Inpatient | Caucasian 81% Afr American 19% | 38 (0) | 55.62 (SD 14.95) | 58% | P | 2.0-3.0 | Yes <10% variation in the last 4 wk | CYP2C9 *2 or *3 | PCR AvaII, NsiI and KpnI | ND | ND | Yes |
| Furuya, 1995 [54] | UK Anticoagulation clinic | ND | 100 (6) | ND | ND | R | 2.0-4.0 | Yes Stable over 3 consecutive visits | CYP2C9 *2 | PCR AvaII | ND | ND | ND |
| Chern, 2006 [55] | Taiwan Cardiac surgery clinic | 100% Chinese | 37(202) | 51.6 (SD 11.5) | 54% | P | 2.0-3.0 | Yes Stable over 3 months | CYP2C9 *3 | PCR HpaI | ND | ND | ND |
| Kamali, 2004 [56] | UK Anticoagulation clinic | ND | 121 | 72 (24-90) | 55% | P | 2.0-3.0 | Yes Stable over 3 clinic visits | CYP2C9 *3 | PCR AvaII, NsiI and KpnI | ND | ND | ND |
| Linder, 2002 [57] | US Anticoagulation clinic | Caucasian 98% Afr American 2% | 56 | 69(12.5) | 55% | P | 2.0-3.0 | Yes Stable over 3 months | CYP2C9 *2, *3 | Same as Aithal | ND | ND | Yes |
| Takahashi, 2006 [58] (only data for Japanese CYP2C9 is included; Caucasian data part of Scorda study) | Japan ND | Asian 100% | 172 | 61 (11) | 59% | P | 1.5-2.5 | Yes Stable over 1 month | CYP2C9 *3 | PCR | ND | ND | Yes |

| Author, yr | Country Setting | Race | N subjects (N excluded) | Age yr | Male% | Study design | Target INR reported in the study | Stable dose as an inclusion criteria | Genotypes | Genotype methodology | Methodology replicated? Or quality control described? | Blinding | HWE assessed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hillman, 2004 [59] | US Anticoagulation clinic | ND | 453 (17) | 70.6 | 56.7% | R | ND | ND | CYP2C9 *2, *3 430C>T 1075A>C | PCR | Yes | No | Yes |
| Higashi, 2002 [60] | US Anticoagulation clinic | Caucasian 96.2% Other 3.8% | 185 (36) | 59.9 | 63.8% | R | 2.0-3.0 | NA; recruited at induction | CYP2C9 *2, *3 | PCR | Yes | Yes | Yes |
| Lindh, 2005 [61] | Sweden Anticoagulation clinic | Caucasian 100% | 219(3) | 61 | 58% | R | 2.0-3.0 | ND | CYP2C9 *2, *3 430C>T 1075A>C | PCR | No | No | Yes |
| Margaglione, 2000 [62] | Italy Anticoagulation clinic | Caucasian 100% | 199 (19) | 41 (15-84) | 55% | P | NA | ND | CYP2C9 *2 *3 | PCR (blood leukocytes) | ND | Yes | No |
| Aithal, 1999 [63] | UK Anticoagulant clinic | Caucasian 100% | 36 cases | Cases: 73 (55-88) | Cases: 47% | Cc | 2.0-3.0 | Yes for at least 3 consecutive visits | CYP2C9 *2, *3 | PCR *Ava*II and *Nsi*I | ND | ND | Yes |
| | | | 52 controls | Controls: 70.5 (33-94) | Controls: 50% | | | | | | | | |
| Peyvandi, 2003 [64] | Italy Hospital based | Caucasian 100% | 125 (0) | 50 | 69% | P | 2.0-3.0 | NA (initiation phase study) | CYP2C9 *2, *3 | PCR (same as Taube) | ND | ND | ND |
| Pchelina, 2005 [65] | Russia Outpatient and inpatient | Caucasian 100% | 62 (236) | 47.6 | ND | R | 2.5-3.5 | NA (initiation phase study) | CYP2C9 *2, *3 | PCR *Kpn*I, *Ava*II, *Mph* 1103 | ND | ND | Yes |
| Hillman, 2005 [66] | US Inpatient and outpatient | Caucasian 100% | 38 (79) | 70.5 (standard dosing) 68.8 (model | 44% (standard dosing) 45% (model | RCT | ND | NA; initiation phase | CYP2C9 *2, *3 | PCR | ND | ND | ND |

| Author, yr | Country Setting | Race | N subjects (N excluded) | Age yr | Male% | Study design | Target INR reported in the study | Stable dose as an inclusion criteria | Genotypes | Genotype methodology | Methodology replicated? Or quality control described? | Blinding | HWE assessed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | based dosing) | based dosing) | | | | | | | | |
| Ogg, 1999 [67] | UK Part of RCT | Caucasian 100% | 233 (nd) | ND | 100% | R | 1.5 | NA (initiation phase study) | CYP2C9 *3 | ND | ND | ND | ND |
| Topic, 2004 [68] | Croatia Anticoagulant clinic | Caucasian 100% | 181 (nd) | 60 | 44 % | Cc | ND | Yes ( > 1 month) | CYP2C9 *2, *3 | PCR *Ava*II, *Nsi*I and *Kpn*I | Yes | ND | Yes |
| Voora, 2005 [69] | USA Orthopedic patients | ND | 48 (8) | 61 | 58% | P | 2.5 | NA (initiation phase study) | CYP2C9 *2, *3 | PCR *Ava*II, and *Kpn*I | Yes | ND | Yes |
| Veenstra, 2005 [70] | US Anticoagulant clinic | Caucasian 100% | 196 (4) | 60 | 63.8% | R; P | 2.0-3.0 | NA (initiation phase study) | CYP2C9 *2, *3 | PCR | Yes | ND | Yes |
| Taube 2000 [71] | UK Anticoagulant clinic | ND | 561 (122) | ND | ND | R; P | <2.5 | ND | CYP2C9 *2, *3 | PCR *Ava*II, *Nsi*I | ND | ND | Yes |
| Anderson 2007[98] | USA ND | Caucasian 95% | 200 (6) | 63 | 50% | RCT | 1.8-3.2 | NA | CYP2C9 *2, *3 VKORC1 | PCR | Yes | ND | ND |
| Caraco 2008[99] | Israel ND | ND | 191 (283) | 59 | 49% | RCT | 1.8-3.4 | NA | CYP2C9 *2, *3 | PCR | Yes | Yes | Yes |

**Table 2. Characteristics of the identified studies on VKORC1 gene variations among patients receiving warfarin**

| Author, year | Country Setting | Ethnic descent | N enrolled (N analyzed) | Age Mean (SD) or [range] | Male [%] | Study design | Target INR reported in the study | Stable dose as an inclusion criteria | Genotype method | Methodology replicated? Or quality control described? | Blinding | rs7294 | rs8050894 | rs9923231 | rs9934438 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D'Andrea, 2005[72] | Italy, Anticoagulation clinic | European | 203 (147)[1] | Med 43 [15-84] | 54 | Retros-pective | ND | ND | PCR | ND | ND | √ | | | √+ |
| Rieder, 2005[73] | US, Anticoagulation clinic | European | 1st cohort: 186 (186); Validation: 368 (368) | ND | ND | Retros-pective | ND | No | PCR | ND | ND | √- | √- | √- | √- |
| Sconce, 2005[74] | UK, anticoagulation clinic | ND | 1st cohort: 297 (297) Validation: 38 (38) | 1st cohort: 68 [26-90] Validation: 72 [39-91] | 54 50 | Prospe-ctive | 2-3 | Yes (3 visits, ≥3 mo) | PCR | ND | ND | | | √ | |
| Vecsler, 2005[75] | Israel, Anticoagualtion clinic | European-Jewish | 100 (100) | 62 [18-88] | 52 | Retros-pective | ND | Yes (for ≥3 mo) | PCR | Yes[2] | ND | | √ | | |
| Wadelius, 2005[76] | Sweden, Anticoagulation clinic | European | 201 (201) | 67 [28-88] | 67 | Retros-pective | ND | No | PCR | Yes[3] | ND | √ | | √ | √- |
| Aquilante, 2006[77] | US, Anticoagulation clinic | European (91%) African-American (7%) Other (2%) | 350 (350) | 69 [22-89] | 87 | Retros-pective | 2-3 (n=311); 2.5-3.5 (n=35); 3-4 (n=4) | Yes (3 visits) | PCR | No | ND | √- | | √+ | |
| Carlquist, 2006[78] | USA, Anticoagulation clinic | European | 213 (213) | 71 [28-100] | 49 | Prospe-ctive | 2-3 | Yes (for ≥1 mo) | PCR | ND | ND | | | | √+ |
| Herman, 2006[79] | Slovenia, Anticoagulation clinic | European | 165 (165) | 72 (9) | 50 | Retros-pective | 2-3 (n=162); 2.5-3.5 (n=3) | Yes (2 visits) | PCR | ND | ND | √ | | | √+ |
| Li, 2006[80] | US, | European | 93 (93) | 63 | 69 | Prospe- | 2-3 (n=77); | ND | Sequencing | ND | ND | √ | √ | | √+ |

1 Because of unavailable samples or other technical reasons.
2 Negative controls
3 Duplicate samples (n=4), excluded SNPs with less than 80% successful calls.

| Author, year | Country Setting | Ethnic descent | N enrolled (N analyzed) | Age Mean (SD) or [range] | Male [%] | Study design | Target INR reported in the study | Stable dose as an inclusion criteria | Genotype method | Methodology replicated? Or quality control described? | Blinding | rs7294 | rs8050894 | rs9923231 | rs9934438 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Anticoagulation clinic | | | [24-90] | | ctive | 2.5-3.5 (n=13); 1.5-2 (n=3) | | and PCR | | | | | | |
| Osman, 2006[81] | Sweden, Anticoagulation clinic | European | 98 (92)[4] | ND [22-89] | 67 | Retros-pective | 2-3 | ND | PCR | ND | ND | √ | | | √- |
| Takahashi, 2006[58] | Japan/US, ND | European (43%); Asian (57%)[5] | 365 (179)[6] | 61 (11) | 52 | Prospe-ctive | 1.5-2.5 (n=64); 2-3 (n=115) | Yes (for ≥1 mo) | PCR | ND | ND | √ | | | √+ |
| Schelleman, 2007[82] | US, Anticoagulation clinic | European (49%); African-american (51%) | 338 (317) | 59 (ND) | 69 | Prospe-ctive | 2-3 | No | PCR | ND | Yes (Geno-typing) | | | | √+ |
| Veenstra, 2005[48] | Hong Kong – China, Anticoagulation clinic | Asian | 69 (41) | 58 (10) | 46 | Prospe-ctive | 1.8-3.2 | ND | PCR | Yes | ND | √ | √ | | |
| Yuan, 2005[83] | Taiwan-China, Anticoagulation clinic | Asian[7] | 104 (104) | 58 (15) | 54 | Prospe-ctive | 1.4-3 | Yes (for ≥3 wk) | PCR | ND | ND | | | √ | |
| Tham, 2006[884] | Singapore, Anticoagulation clinic | European (Indian, 13%); Asian (87%) | 215 (215) | 56 (13) | 56 | Retros-pective | 2-3 | Yes (for ≥3 mo) | PCR | ND | ND | √- | √- | √- | √- |
| Obayashi, 2006[85] | Japan, CVD unit | Asian | 125 (125) | 63 (13) | 51 | Prospe-ctive | ND[9] | Yes (for ≥2 mo) | PCR | ND | ND | | √ | √ | √+ |

[4] 180 disease-free Swedish controls ("randomly selected") were also genotyped.
[5] Takahashi also reports genotyping 64 African American participants (they include disease-free people) – however no association with outcomes are reported for these people.
[6] Only those with combined *VKORC1* and *CYP2C9* genotypic information were analyzed.
[7] 95 disease free Chinese controls and 92 disease-free European controls from the US were also genotyped. This study also included a selected group of 11 Chinese patients with warfarin sensitivity and 5 with warfarin resistance, who are not part of the analyses.
[8] Same population as in Lee Clin Pharmacol Ther 2006;79:197-205.
[9] Mean INR 2.12 ± 0.44

| Author, year | Country Setting | Ethnic descent | N enrolled (N analyzed) | Age Mean (SD) or [range] | Male [%] | Study design | Target INR reported in the study | Stable dose as an inclusion criteria | Genotype method | Methodology replicated? Or quality control described? | Blinding | rs7294 | rs8050894 | rs9923231 | rs9934438 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mushiroda, 2006[46] | Japan, Hospital | Asian | 790 (752) | 68 [19-92] | 67 | Prospe-ctive | ND | ND | PCR | ND | ND | | √ | √ | √+ |
| Kimura, 2007[86] | Japan, Cerebrovascular (stroke) patients | Asian | 93 (93) | 68 (11) | 71 | Retros-pective | 1.6-2.6 | No | PCR | ND | ND | √ | | √ | √+ |

CVD: Cardiovascular disease; Med: median; mo: month(s); N: number; ND: not described; PCR: polymerase chain reaction; SD: standard deviation; wk: week(s)

## Appendix 2. Table 3.1. Study Characteristics

| First author: Year: | Country: | Journal | type of study | duration of folow up | Statin Used* | Dosage (mg) | hypolipidaemic diet used | Outcomes assesed# |
|---|---|---|---|---|---|---|---|---|
| Tavintharan,2007[1] | Singapore | Diab Obes Metab | Cohort | 12 weeks | SIMVA – LOVA | SIMVA 10-LOVA 20 | yes | TC,LDL,HDL,TGs |
| Eiriksdottir,2006[2] | Iceland | Atherosclerosis | cross-sectional | | Nr | Nr | nr | CRP |
| Fiegenbaum,2005 [3] | Brazil | Clinica Chimica Acta | Cohort | 6 months | SIMVA | 20 | nr | TC,LDL,HDL,TGs |
| Pedro-Botet,2001 [4] | USA | Atherosclerosis | cohort-RCT arm | 12 months | ATORVA | 10 | yes | TC,LDL,HDL,TGs |
| Kajinami,2005 [5] | USA | Atherosclerosis | cohort-RCT arm | 12 months | ATORVA | 10 | yes | TC,LDL,HDL,TGs |
| Takane,2006 [6] | Japan | J Hum genet | retrospective study | 12 months | PRAVA | 9.4 | Nr | TC,LDL |
| Pena,2002 [7] | Spain | J Int Med | Cohort | 16 weeks | PRAVA | 20 | yes | TC,LDL,HDL,TGs |
| Korhonen,2002 [8] | Finland | Eur J Clin Pharmacol | Cohort | | LOVA | 76.8 | yes | TC,LDL,HDL,TGs,ApoA1,CETP |
| Couture,1998 [9] | Canada | Arterioscler Thromb Vasc Biol | cohort-RCT arm | 6 weeks | SIMVA | 20 | yes | LDL,HDL |
| Vohl,2002 [10] | Canada | Atherosclerosis | cohort-RCT arm | 6 weeks | SIMVA | 20 | yes | LDL |
| Gerdes,2000 [11] | Finland, Denmark | Circulation | cohort-RCT arm | 5.4 years | SIMVA | 26.8 | yes | Death, Clinical events |
| Ballantyne,2000 [12] | USA | JACC | cohort-RCT arm | 12 weeks for lipids / 2.5 years for angiography | FLUVA | 40 | yes | TC,LDL,HDL,TGs, Clinical Events, Angiographical Change of CAD |
| Sanlehy,1998 [13] | Spain | Metabolism | Cohort | 12 weeks | LOVA | 40 | yes | TC,LDL,HDL, TGs,ApoA1,ApoB,ApoA2 |
| Ordovas,1995 [14] | USA | Atherosclerosis | cohort-RCT arm | 12 months | PRAVA | 40 | yes | TC,LDL,HDL,TGs |
| Heath,1999 [15] | UK | Atherosclerosis | Retrospective | 12 weeks | SIMVA | 10-20-40 | no | TC,LDL |
| Garcia-Otin,2002 [16] | Spain | Eur j Clin Invest | cohort-RCT arm | 12 months | ATORVA | 10-20 | yes | TC,LDL,HDL,TGs |
| Maitland van der Zee,2006 [17] | Netherlands | Acta Cardiol | cohort-RCT arm | 2 years | PRAVA | 40 | Nr | TC,LDL,HDL,TGs, Angiographical Change of CAD |
| Maitland van der Zee,2002 [18] | Netherlands | Pharmacogenetics | Cohort | 7.2 years/ 26,244.1 person-years | Statins | Nr | Nr | Mortality, Clinical Events |
| Marquez-Vidal,2003 [19] | France | Am J Cardiol | Cross-sectional | | Statins | Nr | Nr | TC,LDL,HDL,TGs,ApoA1,ApoB |
| Maitland van der Zee,2003 [20] | Netherlands | Pharmacogenetics | Cohort | 3 years | statins | Nr | Nr | Discontinuation of Statins |
| Ojala,1991 [21] | Finland | J Int Med | cohort-RCT arm | 6 and 12 weeks | LOVA | 20-40 | yes | TC,LDL,HDL,TGs |
| Christidis,2006 [22] | Greece | J Cardiovasc Pharmacol Therap | Cohort | 6 months | ATORVA | 20 | yes | TC,LDL,HDL,TGs, ApoA1,ApoB,ApoE |

## Appendix 2. Table3.1 (cont). Study Characteristics

| First author: Year: | Country: | Journal | type of study | duration of folow up | Statin Used* | Dosage (mg) | hypolipidaemic diet used | Outcomes assesed# |
|---|---|---|---|---|---|---|---|---|
| Drmanac,2001 [23] | USA | J Cardiovasc Pharmacol Therap | Retrospective | nr (at least two observations from baseline for each subject | SIMVA, ATORVA, LOVA, PRAVA | 10-20-40-80 | Nr | LDL,TGs |
| De Kniff,1990 [24] | Netherlands | Atherosclerosis | Cohort | 12 weeks | SIMVA | 40 | nr | TC,LDL,HDL,TGs |
| Ye,2003 [25] | China | Chin Med Sci J | Cohort | 12 weeks | SIMVA | 5 | yes | TC, LDL,TGs,ApoB |
| Watanabe,1993 [26] | Japan | Diab Res Clin Pract | Cohort | 12 weeks | PRAVA | 10 | yes | TC, LDL,HDL, TGs,ApoA1,ApoB,ApoE,ApoCII |
| Dallinga-Thie,2006 [27] | Netherlands | Diabetes | cohort-RCT arm | 30 weeks | ATORVA | 10,80 | nr | PLTP |
| Alaupovic,1999 [28] | USA | Atherosclerosis | cohort-RCT arm | 4.3 years | LOVA | 40,80 | yes | TC, LDL, HDL,TGs, ApoA1, ApoB,ApoCII,LP(a), |
| Carmena,1993 [29] | Canada | Metabolism | Retrospective | 12 weeks | LOVA | 80 | yes | TC, LDL, HDL,TGs |
| Dergunov,2003 [30] | Russia | Vasc Pharmacol | Cohort | 16 weeks | FLUVA | 40 | yes | TC, LDL, HDL,TGs |
| Kobayashi,2001 [31] | Japan | J ClinPharmacol | Cohort | 12 weeks | PRAVA | 20 | yes | TC, LDL, HDL,TGs, ApoA1, ApoB,ApoE |
| O'Malley,1990 [32] | USA | Metabolism | Cohort | 6 weeks | LOVA | 20/40 | yes | LDL, HDL,TGs |
| Zuccaro,2007 [33] | Italy | Pharmacol Res | Retrospective | nr | Statins | All | nr | TC, LDL, HDL,TGs |
| Rustemeijer,2001 [34] | Netherlands | Clin Sci | Cohort | 12 weeks | PRAVA | 40 | yes | TGs,Pche |
| Berglund,1993 [35] | Sweden | J Int Med | Cohort | 12 weeks | PRAVA | 10-20 | nr | TC, LDL, HDL,TGs,ApoE |
| Chaves,2001 [36] | Spain | J Clin Endoc Metab | Cohort | 6 weeks | SIMVA | 20 | yes | TC, LDL, ApoB |
| Leitersdorf,1993 [37] | Israel | Circulation | Cohort | 16 weeks | FLUVA | 18.75 | yes | LDL |
| Nestel,1997 [38] | Australia | Atherosclerosis | cohort, cross-over trial | 12 weeks | SIMVA | 20 | yes | TC, LDL, HDL,TGs |
| O'Neill,2001 [39] | UK | ATVB | Cohort | 4 weeks | ATORVA | 10 | yes | LDL |
| Woo,2004 [40] | USA | Stroke | Cross-sectional | - | Statins | Nr | no | ICH |
| Vega,2004 [41] | USA | Curr Alz Res | Cohort | 6 weeks | Statins | Nr | nr | Non-HDL-C |
| Leren,1995 [42] | Norway | Eur J Clin Invest | Cohort | 12 weeks | LOVA | 60 | yes | TC, LDL, ApoB |
| Chasman, 2004 [43] | USA | JAMA | Cohort-RCT arm | 24 weeks | PRAVA | 40 | Yes | TC, LDL, HDL |
| Thompson,2005 [44] | USA | Pharmacogenomics J | Cohort-RCT arm | 6 weeks | ATORVA (1483 patients) – [FLUVA, LOVA, PRAVA, SIMVA] (1252 patients) | ATORVA 10, FLUVA 20, LOVA 20, PRAVA 20, SIMVA 10 | nr | TC, LDL, HDL,TGs |

Abbreviations: nr: non-reported, RCT: Randomized Controlled Trial, ICH: Intracerbral Hemorrhage, CAD: Coronary Artery Disease.

**Appendix 2. Table3.2. Patient inclusion and exclusion criteria for each study**

| First author: Year: | Inclusion criteria | Criteria of Diagnosis: | Type of Disease: | exclusion criteria |
|---|---|---|---|---|
| Tavintharan,2007 | DM2 requiring LLT | Nr | Hyperlipidemia-DM2 | poor glycemic control (HbA1c >10.0%), unstable medical conditions that might interfere with the evaluation, impaired renal or thyroid function, TGs>4.5 mmol/L, recent MI |
| Eiriksdottir,2006 | Random selection - statin treatment | Nr | Unspecified hyperlipidemia | Nr |
| Fiegenbaum,2005 | hypercholesterolemia requiring LLT | Nr | Hypercholesterolemia | TGs>4.5 mmol/l, DM, unstable medical conditions that might interfere with the evaluation, impaired hepatic, renal or thyroid function. |
| Pedro-Botet,2001 | primary hypercholesterolemia, >18 years, BMI<32 kg/m$^2$, LDL>4.14 mmol/l at week-4 and >3.75 mmol/l at week-2,TGs<4.52 mmol/l. | Nr | Primary hypercholesterolemia, | DM1 or uncontrolled DM2, impaired hepatic or renal function, unstable medical conditions that might interfere with the evaluation, <80% compliance with study medication during the placebo baseline phase, or concurrent participation in another clinical trial. Patients with elevated SGOT,SGPT,CPK, taking any immunosuppressive agent: medications affecting lipid metabolism, possibly interact with study medications, or possibly affect clinical laboratory parameters; and drugs known to be associated with rhabdomyolysis or other lipid-regulating drugs. Patients with other LLT could be screened after a 4-week washout period, except for probucol, which required at least 6 months for washout. |
| Kajinami,2005 | primary hypercholesterolemia, >18 years, BMI<32 kg/m$^2$, LDL>4.14 mmol/l at week-4 and >3.75 mmol/l at week-2,TGs<4.52 mmol/l. | Nr | Primary hypercholesterolemia, | DM1 or uncontrolled DM2, impaired hepatic or renal function, unstable medical conditions that might interfere with the evaluation, <80% compliance with study medication during the placebo baseline phase, or concurrent participation in another clinical trial. Patients with elevated SGOT,SGPT,CPK, taking any immunosuppressive agent: medications affecting lipid metabolism, possibly interact with study medications, or possibly affect clinical laboratory parameters; and drugs known to be associated with rhabdomyolysis or other lipid-regulating drugs. Patients with other LLT could be screened after a 4-week washout period, except for probucol, which required at least 6 months for washout |

| First author: Year: | Inclusion criteria | Criteria of Diagnosis: | Type of Disease: | exclusion criteria |
|---|---|---|---|---|
| Takane,2006 | hypercholesterolemia requiring LLT | Nr | Hypercholesterolemia | uncontrolled DM, impaired hepatic or renal function, no drug compliance, other LLT |

**Appendix 2. Table3.2 (cont). Patient inclusion and exclusion criteria for each study**

| First author: Year: | Inclusion criteria | Criteria of Diagnosis: | Type of Disease: | exclusion criteria |
|---|---|---|---|---|
| Pena,2002 | hypercholesterolemia requiring LLT despite diet | NCEP-ATPIII guidelines | Hypercholesterolemia | TGs>4.5 mmol/L, DM, other LLT, unstable medical conditions that might interfere with the evaluation, impaired hepatic, renal or thyroid function., secondary hypercholesterolemias, hypersensitivy to statins. |
| Korhonen,2002 | TC>5 mmol/l during adequate diet and colestipol treatment. | FH:TC>8mmol/l with tendon xanthomata or a family history of severe hypercholesterolaemia premature CAD | Primary hyperlipidemia (type IIa, IIb) | DM, impaired hepatic function, symptomatic gallstones, unstable angina, MI or CABG within the previous 2 months, TGs>3 mmol/l,, premenopausal women, secondary hypercholesterolaemia (due to hypothyroidism or nephrotic syndrome) concomitant use of barbiturates, anticonvulsants, anticoagulants, theophylline, quinidine, corticosteroids, cimetidine or regularly used antacids) |
| Couture,1998 | FH patients with LDL> 95th percentile for age and sex despite diet | presence of three mutations Δ>15 kb, C646Y, W66G | FH | DM, anorexia nervosa, impaired hepatic, renal or thyroid function, constitutional or pathologically delayed puberty |
| Vohl,2002 | FH patients with LDL> 95th percentile for age and sex despite diet | presence of three mutations Δ>15 kb, C646Y, W66G | FH | DM, anorexia nervosa, impaired hepatic, renal or thyroid function, constitutional or pathologically delayed puberty |
| Gerdes,2000 | MI or angina, TC:5.5-8 mmol/l, TGs<2.5mmo/l, after diet | Nr | CAD-hyperlipidemia | Nr |
| Ballantyne,2000 | Males and postmenopausal females with recent coronary angiography documenting CAD, and LDL: 2.97-4.91 mmol/l after diet | quantitative coronary angiography | CAD-hyperlipidemia | MI within six months, TGs>3.39 mmol/L, DM, uncontrolled hypertension, previous CABG or PTCA |

**Appendix 2. Table3.3 (cont). Patient inclusion and exclusion criteria for each study**

| | | | | |
|---|---|---|---|---|
| Sanlehy,1998 | primary lipid disorders (isolated hypercholesterolemia or combined hyperlipidemia) and known apoE after diet | FH:severe hypercholesterolemia,tendon xanthomas and/or a family history / isolated hypercholesterolemia-combined hyperlipidemia:WHO criteria | Primary hyperlipidemia | alcoholism, DM, impaired hepatic, renal or thyroid function |
| Ordovas,1995 | Males and postmenopausal females with recent coronary angiography documenting CAD, and LDL > 130 and < 190, TGs < 350 mg/dl. | quantitative coronary angiography | CAD-moderate hypercholesterolemia | uncontrolled hypertension, impaired hepatic, renal or thyroid function, type III hyperlipoproteinemia, congestive heart failure, chronic pancreatitis, dysproteinemia, porphyria, lupus erythematosus, poorly controlled or insulin-dependent DM, likelihood of CABG or PTCA to the qualifying coronary artery within 6 months, cerebrovascular disease, significant gastrointestinal disease, alcoholism, hypersensitivity to statins, other LLT |
| Heath,1999 | FH | 'definite' FH:LDL>4.9mmol/l and tendon xanthoma (TX) | FH | Nr |
| Garcia-Otin,2002 | combined hyperlipidemia: baseline TGs < 500 and > 200 mg/dl, LDL< 250 and > 190, 180, 160 or 135 mg dL according to CAD risk | combined hyperlipidemia: baseline TGs < 500 and > 200 mg/dl, LDL< 250 and > 190, 180, 160 or 135 mg dL according to CAD risk | Combined hyperlipidemia | Nr |

**Appendix 2. Table3.3 (cont). Patient inclusion and exclusion criteria for each study**

| | | | | |
|---|---|---|---|---|
| Maitland van der Zee,2006 | Males and postmenopausal females with recent coronary angiography documenting CAD, and TC:4-8mmol/l, TG<4mmol/l | at least one coronary artery with a stenosis of>50% | CAD-normal to moderate hypercholesterolemia | Nr |
| Maitland van der Zee,2002 | statin treatment in a large populatio-based cohort study | pharmacy records for statin use | Clinical event (MI, stroke, death) | No |
| Marquez-Vidal,2003 | case-control subjects treated with statins in a genetic association study for MI | previous MI before 6 months or healthy middle-aged men | MI or healthy men | Nr |
| Maitland van der Zee,2003 | initiation of statin use in a large population-based cohort study | pharmacy records for statin use | Discontinuation of statin therapy | initiation of statin therapy during the first 6 months after the start of the study |
| Ojala,1991 | TC>6.2mmol/l, TGs<4.0mmol/l despite diet | FH:severe hypercholesterolemia,tendon xanthomas and/or a family history / the rest classified as non-familial | Hypercholesterolemia | Nr |
| Christidis,2006 | Heterozygous FH | Nr | heterozygous FH | DM, impaired hepatic, renal or thyroid function, cardiovascular disease with medications affecting lipid metabolism, TG>4.52mmol/L |
| Drmanac,2001 | retrospective analysis of patients treated with statins for hyperlipidemia | Nr | Unspecified hyperlipidemia | DM, impaired renal or thyroid function, medications affecting lipid metabolism |
| De Kniff,1990 | Heterozygous FH | FH:severe hypercholesterolemia,tendon xanthomas and/or a family history | heterozygous FH | medications affecting lipid metabolism |

**Appendix 2. Table3.3 (cont). Patient inclusion and exclusion criteria for each study**

| | | | | |
|---|---|---|---|---|
| Ye,2003 | TC>5.7mmol/L, TGs>2.23mmol/L | Nr | hyperlipidemia | impaired hepatic or renal function, pancreatitis |
| Watanabe,1993 | TC>5.7mmo/l | TC>5.7mm/l for hypercholesterolemia, Nr for DM | hypercholesterolemia -DM2 | impaired hepatic, renal or thyroid function, alcoholism |
| Dallinga-Thie,2006 | men and women, aged 45–75 years with duration of DM>1 year, HbA1c<10%, TC:4-8mmol/L, TGs:1.5-6mmol/L | TC:4-8mmol/L, TGs:1.5-6mmol/L | hyperlipidemia-DM2 | nr (I have to look in the DALI study) |
| Alaupovic,1999 | subjects who had saphenous vein coronary bypass graft surgery 1–11 years prior to study entry | LDL:130-175mg/dl, TG<300mg/dl | CAD-CABG-hyperlipidemia | Nr |
| Carmena,1993 | retrospective analysis of FH patients having been treated with 80 mg LOVA for 12 weeks | FH:severe hypercholesterolemia,tendon xanthomas and/or a family history | heterozygous FH | nr |
| Dergunov,2003 | controlled HT patients needing LLT | nr | hyperlipidemia-HT | Nr |
| Kobayashi,2001 | patients with type II hyperlipidemia requiring LLT | LDL>150, TG<400mg/dl | type II hyperlipidemia | FH, TG>400 |

**Appendix 2. Table3.3 (cont). Patient inclusion and exclusion criteria for each study**

| | | | | |
|---|---|---|---|---|
| O'Malley,1990 | FH patients having been treated with LOVA in the previous 6 years | FH: persistent primary TC>260,g/dl, pripary hypercholesterolemia in other family members, absence of multiple phenotypes in these family members, tendon xanthomas | heterozygous FH | use of probucol in the past 6 months |
| Zuccaro,2007 | patients treated with statins: 50 had clinical symptoms of muscle pain and 50 patients without any significant subjective or objective disturbances | Muscle pain during statin treatment: pain at the upper girth after limited effort; pain to the lower limbs after minimal effort, e.g. uphill or protracted walking or running; diffuse muscle pain | hyperlipidemia-statin treatment with and without AE | chronic liver disease, alcoholism, renal disease, serious atherosclerotic disease limiting patient motility, malignant disease at any stage, insulin dependent DM as well as multiple associations with drugs and CYP3A4 inhibitors (azole antifungals, grapefruit juice, dihydropyridine calcium antagonists, others) |
| Rustemeijer,2001 | total cholesterol >5.0 mmol/l but <8.0 mmol}l ; TG >1.8 mmol/l but <5.0 mmol/l) with Type II diabetes | WHO for DMII | hyperlipidemia-DM2 | oral hypoglycemic agents, primary hyperlipidaemia, secondary hyperlipidaemia due to hypothyroidism or nephrotic syndrome, alcohol or drug use infuencing carbohydrate and lipid metabolism, the use of immunosuppressive drugs, hepatobiliary or endocrine diseases, pancreatitis, porphyria and malignancy |
| Berglund,1993 | FH patients | nr | heterozygous FH | Nr |

**Appendix 2. Table.3.3 (cont). Patient inclusion and exclusion criteria for each study**

| | | | | |
|---|---|---|---|---|
| Chaves,2001 | heterozygous FH with detected mutation on the LDLR gene, age 18 65 yr, and male or female (postmenopausal or adequate anticonceptive method and a negative pregnancy test | FH: genetic diagnosis | heterozygous FH | homozygous FH; presence of metabolic, hepatic, renal, or endocrine disease; acute coronary events in the preceding 3 months; treatment with drugs that could affect lipid metabolism (corticosteroids, E, androgens, theophyline, coumarin derivates, barbiturates, antiacids, fish oil preparations, thiazides, or blockers), and ethanol consumption more than 30 g/d. |
| Leitersdorf,1993 | heterozygous FH with detected mutation on the LDLR gene, age 18 | FH: genetic diagnosis, LDL>190mg/dl, TGs<300, tendon xanthomas, CAD | heterozygous FH | other forms of genetic hyperlipidemia, obese patients, pregnant or lactating women, evidence of any mental, medical, or surgical condition that might influence evaluation of the efficacy, safety, or tolerability of the compound, including surgical impairment of the gastrointestinal tract, liver, or kidneys, or any condition compromising the function of these systems and thereby possibly altering the absorption, distribution, or accumulation or causing impairment of the metabolism or excretion of either fluvastatin or its metabolites. history of MI, angioplasty, or major surgery during the 6 months preceding the study,congestive heart failure, severe or unstable angina pectoris, poorly controlled HT, history of drug abuse or alcohol consumption of more than 50 mL/week, Patients who were taking other investigational drugs, lipid-lowering medications, anticoagulants, hormones , antacids, insulin or oral hypoglycemic agents, erythromycin, ketoconazole, or cyclosporin A |
| Nestel,1997 | patients with combined hyperlipidemia | nr | combined hyperlipidemia | Nr |

**Appendix 2. Table3.3 (cont). Patient inclusion and exclusion criteria for each study**

| | | | | |
|---|---|---|---|---|
| O'Neill,2001 | patients with refractory heterozygous FH cases with statin treatment in a case-control study for ICH | Hyperlipidemia refractory to treatment was defined as having an LDL-C level of >5 mmol/L despite taking SIMVA (40 mg/d) plus at least 1 other drug, ie, cholestyramine, bezafibrate, or nicotinic acid | heterozygous FH | Nr |
| Woo,2004 | | nr | ICH | Nr |
| Vega,2004 | AD patients starting statin treatment | NINCDS/ADRDA criteria for probable AD | probable AD | TC<160mg/dl |
| Leren,1995 | FH patients | FH: genetic diagnosis + Goldstein&Brown criteria | heterozygous FH | Nr |
| Chasman, 2004 | Participants in the PRINCE trial who completed the full protocol and provided informed consent for genomic analysis | Nr | Hyperlipidemia | Nr |
| Thompson,2005 | Hypercholesterolemic patients | nr | Hypercholesterolemia | Nr |

Abbreviations: DM: Diabetes Mellitus, FH: Familial Hyperlipidemia, LLT: Lipid Lowering Therapy, AD: Alzheimer's Disease, MI: Myocardial Infarction, HT: Hypertension, LDLR: LDL-receptor, AE:Adverse Events, CABG: Coronary Artery By-pass Grafting, PTCA: Percutaneous Transluminal Coronary Angioplasty.

## Appendix 2. Table3.4. Cohort characteristics

| First author: Year: | Number of patients (M/F) | Racial descent: | Age: | Age at onset: | Hyperlipidemic population | Severity of hyperilipidemia | Primary hyperlipidemia included only | % of FH cases | Concomitant conditions | concomitant therapy |
|---|---|---|---|---|---|---|---|---|---|---|
| Tavintharan,2007 | 96 (nr) | East Asians (chinese) | nr | nr | Yes | Nr | Yes | Nr | 100% DM, 81.3% HT, 21% CAD | 7.3% insulin |
| Eiriksdottir,2006 | 449 (189/260) | Caucasians | 76±6 | nr | ± | Nr | Nr | Nr | Nr | nr |
| Fiegenbaum,2005 | 99 (25/74) | Caucasians | 59.2 (10.7) | nr | Yes | Nr | Yes | Nr | 11.1% DM, 61.6 %HT, 17.2 % SM | 23% HRT |
| Pedro-Botet,2001 | 328 (195/133) | Caucasians (Am whites 96%) | 57.8 (11.3) | >18 | Yes | moderate | Yes | Nr | Nr | No medications affecting lipid metabolism |
| Kajinami,2005 | 328 (195/133) | Caucasians (Am whites 96%) | 57.8 (11.3) | >18 | Yes | moderate | Yes | Nr | Nr | No medications affecting lipid metabolism |
| Takane,2006 | 33 (14/19) | East Asians (japanese) | 62.3 (34-83) | >34 | Yes | nr | Nr | Nr | Nr | nr |
| Pena,2002 | 398 (174/124) | Caucasians | 57.3 (11.6) | Nr | Yes | moderate | Yes | Nr | No DM | nr |

**Appendix 2. Table3.4 (cont). Cohort characteristics**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Korhonen,2002 | 133 (67/66) | Caucasians | 45.5 (18-63) | >18 | Yes | severe | Yes | yes (39%) | No DM | previous therapy with colestipol for 4 weeks, no other medications affecting lipid metabolism |
| Couture,1998 | 47 (28/19) | Caucasians (Am whites) | 12.6 (2.3) | >8 | Yes | severe | Yes | 100% | No DM, no HT | nr |
| Vohl,2002 | 47 (28/19) | Caucasians (Am whites) | 12.6 (2.3) | >8 | Yes | severe | Yes | 100% | No DM, no HT | nr |
| Gerdes,2000 | 488 (401/87) | Caucasians | 35-70 | >35 | Yes | nr | Nr | Nr | 5% DM, 22% HT, 100% CAD, 31% SM | Nr |
| Ballantyne,2000 | 324 (269/55) | mixed (mainly whites) | 58.8 (7.9) | >35 | Yes | moderate | Nr | Nr | O% DM, 100% CAD, 20%SM | Nr |
| Sanlehy,1998 | 106 (68/38) | Caucasians | 54 (12) | nr | Yes | severe | Yes | 15% | 37.7% CAD | 25.4% B-blockers, 13.2% diuretics |
| Ordovas,1995 | 97 (87/10) | Caucasians (Am whites) | 56.8 | <75 | Yes | moderate | Yes | 0% | 100% CAD | No medications affecting lipid metabolism |

**Appendix 2. Table3.4 (cont). Cohort characteristics**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Heath,1999 | 76 (31/45) | Caucasians | 48.5 (1.72) | nr | Yes | severe | Yes | 100% | 15% HT, 36% CAD, 11% SM | Nr |
| Garcia-Otin,2002 | 56 (45/11) | Caucasians | 52 (11) | nr | Yes | severe | Yes | | 2.7% CAD | Nr |
| Maitland van der Zee,2006 | 307 (307/00) | Caucasians | <75 years | <75 | ± | normal to moderate | Nr | Nr | 100% CAD | Nr |
| Maitland van der Zee,2002 | 1394 person-years (494/900 person years) | Caucasians | nr | >55 | ± | nr | Nr | nr | 11.4% DM, 47.6% HT, 47.7% CAD, 20.4% SM | Nr |
| Marquez-Vidal,2003 | 154 (154/00) | Caucasians | 55 (35-64) | >35 | ± | nr | Nr | nr | 87.5% CAD | Nr |
| Maitland van der Zee,2003 | 798 (334/464) | Caucasians | nr | nr | ± | nr | Nr | nr | Nr | Nr |
| Ojala,1991 | 212 (89/123) | Caucasians | 55.2 (9.3) | nr | Yes | severe | Yes | 36.8% | Nr | 39.1% BBs, 18.4% diuretics, 5.1% HRT |
| Christidis,2006 | 134 (68/66) | Caucasians | 52.5 (13.8) | nr | Yes | severe | Yes | 100% | No DM, 22.4% SM | No medications affecting lipid metabolism |
| Drmanac,2001 | 142 (69/73) | nr | 58.5 | nr | Yes | nr | Nr | nr | No DM | No medications affecting lipid metabolism |

**Appendix 2. Table3.4 (cont). Cohort characteristics**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| De Kniff,1990 | 120 (75/45) | Caucasians | 45.4 | >18 | Yes | severe | Yes | 100% | Nr | No medications affecting lipid metabolism |
| Ye,2003 | 88 (56/32) | East Asians (Chinese) | 62.3 (3.9) | nr | Yes | severe | nr | 0% | | Nr |
| Watanabe,1993 | 42 (18/24) | East Asians (japanese) | 51.2 (41-70) | >41 | Yes | severe | nr | Nr | 100%DM | 28% oral hypoglymeci s agents, 14% insulin, no drugs affecting lipid metabolism |
| Dallinga-Thie,2006 | 72 (nr) | Caucasians | 60 (8) | >45 | Yes | Moderate to severe | nr | Nr | 100%DM | Nr |
| Alaupovic,1999 | 489 (449/40) | Nr | 61.5 | nr | Yes | Moderate | Nr | Nr | 100%CAD | warfarin and placebo |
| Carmena,1993 | 94 (54/40) | Caucasians (Am Whites) | 46.9 (10.5) | >18 | Yes | severe | Yes | 100% | | Nr |
| Dergunov,2003 | 67 (67/00) | Caucasians | nr | nr | Yes | moderate | Yes | Nr | 100%HT | BBs, CCB |
| Kobayashi,2001 | 123 (40/83) | East Asians (japanese) | 59,7 (11.9) | nr | Yes | Nr | nr | 0% | 9.3%DM, 42.7%HT, 10.7%CAD,1 6.7%SM | Nr |
| O'Malley,1990 | 134 (59/75) | Caucasians (Am Whites) | 42 (12.6) | >18 | | severe | Yes | 100% | | Nr |

**Appendix 2. Table3.4 (cont). Cohort characteristics**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Yes | | | | | BBs (24%), CCB (1%), APLT (42%), diuretics (14%), ACEi (20%), HRT (4%) |
| Zuccaro,2007 | 100 (55/45) | Caucasians | 61.2 (9.0) | nr | | Nr | Nr | Nr | 6%DM,35% HT, | |
| | | | | | Yes | | | | | 100% on |
| Rustemeijer,2001 | 45 (nr) | Caucasians | nr | nr | | moderate | No | 0% | 100%DM, | insulin |
| | | | | | Yes | | | | | |
| Berglund,1993 | 120 (nr) | Caucasians | nr | >18 | | severe | Yes | 100% | | Nr |
| | | | | | Yes | | | | 0%DM, | |
| Chaves,2001 | 42 (18/24) | Caucasians | 44.5 (16.0) | >18 | | severe | Yes | 100% | 21%CAD | Nr |
| | | | | | Yes | | | | | |
| Leitersdorf,1993 | 64 (38/26) | Jewish | 43.0 (12.1) | >18 | | severe | Yes | 100% | | Nr |
| | | | | | Yes | | | | | |
| Nestel,1997 | 66 (44/22) | Caucasians (Australians) | 54 (10) | nr | | severe | Nr | Nr | 27%HT, 36%CAD | Nr |
| | | | | | Yes | | | | | |
| O'Neill,2001 | 21 (15/6) | Caucasians | 51.4 (9.4) | >18 | | severe | Yes | 100% | 61.9%CAD | Nr |
| | | | | | Yes | | | | | |
| Woo,2004 | 78 (39/9) | mixed | 65 | nr | | nr | Nr | Nr | | Nr |
| | | | | | ± | | | | | |
| Vega,2004 | 39 (nr) | mixed | 73 (1.5) | nr | | moderate | nr | no | 0%CAD | Nr |
| | | | | | Yes | | | | | |
| Leren,1995 | 58 (45/13) | Caucasians | 48.3 | >18 | | severe | Yes | 100% | | Nr |
| Chasman, 2004 | 1536 (1002/534) | Mixed (88.7% Caucasians) | 60.2 (12.5) | nr | Yes | nr | nr | nr | 19.6% DM 62% SM | 15.5% HRT |
| Thompson,2005 | 1483 (901/582) | Mixed (88% Caucasians) | nr | nr | Yes | severe | nr | nr | nr | Nr |

**Abbreviations: CAD: Coronary Artery Disease, HT: Hypertension. DM: Diabetes Mellitus, SM: Smoking, HRT: Hormone Replacement Therapy, BB: Beta-Blockers, CCB: Calcium Channel Blockers, ACEi: Angiotensin Converting Enzyme Inhibitors, APLTs: Antiplatelet agents,**

## Appendix 2. Table3.5. Results reported from each study

| First author: Year: | gender interaction assesed? | gene-gene interactions assesed? | baseline differences in lipid profile phenotypes | genetic contrasts used | Description of major results |
|---|---|---|---|---|---|
| Tavintharan,2007 | Yes | no | yes (TC, LDL) | e2 carriers vs e3e3 vs e4 carriers | Baseline TC and LDL levels differed significantly amongst genotypes (adjusted p-values: p=0.017 and p=0.029, respectively). E4 carriers had the highest levels of TC and LDL. E4 carriers had greater percent reduction in TC and LDL compared with e2 carriers (adjusted p values: p=0.038 and p=0.04, respectively) |
| Eiriksdottir,2006 | no | no | Yes | e2e2+e2e3+e3e3 vs e3e4 vs e4e4 | Carriers of one or two *ε4* alleles have significantly lower CRP levels than non-carriers and this effect was observed in a dose-dependent manner. Carriers of one or two *ε4* alleles had higher TC and lower TGs levels |
| Fiegenbaum,2005 | no | no | Nr | e2 carriers vs e3e3 vs e4 carriers | No significant association between ApoE genotype and response to treatment was observed |
| Pedro-Botet,2001 | yes | no | yes (TC, TGs) | e2 carriers vs e3e3 vs e4 carriers | E2 carriers had higher baseline TC levels and e3e3 subjects had lower TGs levels. In men, e2 carriers had greater percent reduction in TC and LDL while e4 carriers had greater reduction in TG levels, compared with e3e3 subjects. In women, no significant effect was observed |
| Kajinami,2005 | yes | yes | yes (TC, TGs) | e2 carriers vs e3e3 vs e4 carriers | In male wild type allele homozygotes of the *CYP7A1* A–204C polymorphism, e2 and e4 carriers showed larger and smaller reductions in LDL, as compared to e3e3 homozygotes. In addition, in *CYP7A1* mutant allele homozygotes, subjects with E4 showed the smallest reduction. |

| First author: Year: | gender interaction assesed? | gene-gene interactions assesed? | baseline differences in lipid profile phenotypes | genetic contrasts used | Description of major results |
|---|---|---|---|---|---|
| Takane,2006 | No | yes | Nr | NA* | heterozygotes for both CYP7A1 -204C and e4 alleles showed significantly poorer LDL reduction |
| Pena,2002 | Yes | no | yes (TGs) | e2 carriers vs e3e3 vs e4 carriers | E2 carriers had higher TGs baseline levels. No significant association between ApoE genotype and response to treatment was observed |

**Appendix 2. Table3.5 (cont). Results reported from each study**

| | | | | | |
|---|---|---|---|---|---|
| Korhonen,1999 | No | no | No | e2e3 vs e3e3 vs e3e4 vs e4e4 | No significant association between ApoE genotype and response to treatment was observed |
| Couture,1998 | No | yes | nr | e2e3 vs e3e3+e3e4 | E2 carriers had greater mean LDL response |
| Vohl,2002 | No | yes | nr | e2e3 vs e3e3+e3e4 | in FH heterozygotes for a LDL receptor-negative mutation, e2 carriers had greater mean LDL response |
| Gerdes,2000 | No | no | no | e4 carriers vs non carriers | Simvastatin treatment reduced the mortality risk to 0.33 (0.16-0.69) in e4 carriers and to 0.66 (0.35-1.24) in other patients |
| Ballantyne,2000 | No | no | no | e2 carriers vs e3e3 vs e4 carriers | e3 homozygites had greater reductions in TC and LDL than e4 carriers. E2 carriers had a greater increase in HDL than the other groups. CAD progression or clinical events did not differ significantly among genotypes. |
| Sanlehy,1998 | Yes | no | no | e2 carriers vs e3e3 vs e4 carriers | No significant association between ApoE genotype and response to treatment was observed |
| Ordovas,1995 | Yes | no | yes (LDL) | e2 carriers vs e3e3 vs e4 carriers | e2 carriers had greater percent reduction in LDL |
| Heath,1999 | No | no | no | e4 carriers vs e3e3 | No significant association between ApoE genotype, gender and response to treatment was observed |
| Garcia-Otin,2002 | No | no | no | e4 carriers vs non carriers | No significant association between ApoE genotype and response to treatment was observed |
| Maitland van der Zee,2006 | No | no | yes (LDL,HDL,TC,TGs) | e2 carriers vs e3e3 vs e4 carriers | E2 carriers exhibited the largest improvement in TC, HDL and LDL levels. No significant association between ApoE genotype and "Angiographical Change of CAD" was observed |
| Maitland van der Zee,2002 | No | no | nr | e4 carriers vs non carriers | The protective effect of statins on "clinical events" was independent of ApoE genotype |
| Marquez-Vidal,2003 | No | no | Nr | e2 carriers vs e3e3 vs e4 carriers | Statin-treated e4 carriers had increased LDL and ApoB levels in a cross-sectional observation and a lower frequency of adequately controlled subjects in this group |

**Appendix 2. Table3.5 (cont). Results reported from each study**

| | | | | | |
|---|---|---|---|---|---|
| Maitland van der Zee,2003 | Yes | No | yes (HDL) | (e2e4 or e3e3 or e3e4 or e4e4) vs e2e3 | Subjects with e2e2 and e4e4 genotypes showed a trend (though not stastistically significant) towards higher dosages of statin therapy. Relative to subjects with the e2e3 genotype, those with the e4e4 genotype had a risk of 2.28 (95%CI, 1.02-5.12) to discontinue statins within 3 years. In women the relative risk not significant versus 3.18 (05% CI, 1.01-10.03) in men |
| Ojala,1991 | No | No | yes (HDL) | | E4 carriers had higher HDL baseline levels. No significant association between ApoE genotype and response to treatment was observed |
| Christidis,2006 | Yes | No | yes (TGs, ApoE) | e2 carriers vs e3e3 vs e4 carriers | E2 carriers had higher TGs and ApoE baseline levels. No significant association between ApoE genotype and response to treatment was observed |
| Drmanac,2001 | Yes | No | nr | e4 carriers vs non carriers | E4 carriers had a trend toward greater reduction in TGs levels and lower reduction in LDL levels |
| De Kniff,1990 | Yes | No | no | e2 carriers vs e3e3 vs e4 carriers | Overall, no significant association between ApoE genotype and response to treatment was observed. Female patients with e3e3 genotype responded better in LDL reduction. |
| Ye,2003 | No | No | yes (TG, ApoB) | e2 carriers vs e3e3 vs e4 carriers | E2 carriers had significantly higher baseline levels of ApoB andTGs. E2 carriers showed significantly greater ApoB reduction |
| Watanabe,1993 | No | No | yes (TGs) | e2 carriers vs e3e3 vs e4 carriers | E2 carriers had significantly higher baseline levels of TGs. E2 carriers showed significantly lower ApoB reduction |
| Dallinga-Thie,2006 | No | No | nr | e3e3 vs others | no significant association between ApoE genotype and response to treatment was observed |
| Alaupovic,1999 | No | No | nr | nr | no significant association between ApoE genotype and response to treatment was observed |
| Carmena,1993 | Yes | Yes | no | e2 carriers vs e3e3 vs e4 carriers | Male e4 carriers respond less efficiently with respect to TC and LDL levels than e3e3 or e2 carriers or women of any ApoE genotype. However, they respond more efficiently with respect to increasing HDL levels. These effects were idependent of the nature of the LDL receptor defect |

**Appendix 2. Table3.5 (cont). Results reported from each study**

| | | | | | |
|---|---|---|---|---|---|
| Dergunov,2003 | No | No | yes (HDL) | e2 carriers vs e3e3 vs e4 carriers | E2 carriers had higher baseline HDL levels. No significant association between ApoE genotype and response to treatment was observed |
| Kobayashi,2001 | No | No | yes (ApoE) | e2 carriers vs e3e3 vs e4 carriers | E2 carriers had higher baseline ApoE levels. E2 carriers had significantly greater reductions in TC, LDL and ApoB levels. |
| O'Malley,1990 | Yes | No | yes (TG) | e2 carriers vs e3e3 vs e4 carriers | E2 carriers had higher baseline TGs levels. No significant association between ApoE genotype, gender and response to treatment was observed |
| Zuccaro,2007 | No | No | yes (TG) | e2 carriers vs e3e3 vs e4 carriers | E2 carriers had higher baseline TGs levels. E2 carriers had significantly greater reductions in TGs lavels and significantly greater increase in HDL. |
| Rustemeijer,2001 | No | No | no | e2 carriers vs e3e3 vs e4 carriers | No significant association between ApoE genotype and response to treatment was observed |
| Berglund,1993 | No | No | yes (ApoE) | e2e3 vse2e4 vs e3e3 vs e3e4 vs e4e4 | E2 carriers had higher baseline ApoE levels. No significant association between ApoE genotype and response to treatment was observed |
| Chaves,2001 | Yes | Yes | no | e4 carriers vs e3e3 | No significant association between ApoE genotype, gender or LDLR mutation and response to treatment was observed |
| Leitersdorf,1993 | No | Yes | nr | e4 carriers vs e3e3 | E4 carriers had significantly greater reduction in LDL, irrespective of the LDLR mutation. |
| Nestel,1997 | No | No | nr | e2 carriers vs e3e3 vs e4 carriers | E2 carriers responded better in TC and TGs lowering |
| O'Neill,2001 | No | No | nr | e2 carriers vs e3e3 vs e4 carriers | E3e3 homozygotes showed a significantly greater reduction in LDL. Possession of an e4 allele was significantly more frequent among poor responders (6 of 8) than among good responders (3 of 11, $P<0.05$). |
| Woo,2004 | No | No | no | e2carriers+e4carriers vs e3e3 | E3e3 homozygotes had a reduced risk for ICH |
| Vega,2004 | No | No | nr | e4e4 vs e3e4 vs e3e3 | No significant association between ApoE genotype and response to treatment was observed |

**Appendix 2. Table3.5 (cont). Results reported from each study**

| | | | | | |
|---|---|---|---|---|---|
| Leren,1995 | No | No | no | e4 carriers vs non-carriers | E4 carriers had a significantly lower reduction in TC. |
| Chasman, 2004 | Yes | Yes | Yes (LDL) | Nr | SNP 17 in the *Apo E* gene was associated with a greater reduction in LDL cholesterol among individuals heterozygous or homozygous for the minor allele (corrected *P*=.047). Its effect was attenuated and no longer fully significant in the analysis limited to Caucasians (corrected *P*=0.22) |
| Thompson,2005 | no | no | nr | E2 carriers vs e3e3 vs e4 carriers | E2 Caucasians carriers have a 39.9% lowering of LDL following treatment with ATORVA, compared to 36.4% lowering among caucasiand wth e3e3 genotype (p=0.0015). The results for Caucasians treated with the other statins did not show any significant association with ApoE genotype. |

Non-Applicable*: The APOE genotypes were presented in combination with CYP7A1 gene polymorphisms

**Table 4.** Characteristics of eligible studies considered in the report.

| Author | Year | Country | Ethnicity | No | Median age (range) yrs | Male/ Female | Gene polymo-rphism | Type of cancer | Type of malig-nancy | Clinical outcomes investigated | Responders defined | Non-responders defined | Type of chemotherapy | Tumor stage (Tumor size, lympoma Nodes, Metastasis) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Terrazzino [87] | 2006 | Italy | Caucasian | 125 | 60 (31–79) | 80/45 | C677T and A1298C | primary adenocarcinoma of the mid and low rectum (Rectal) | solid | Tumor Regression Grade (TRG) | TRG(1-2) | TRG(3-5) | 5-FU and external beam radiotherapy (median dose 48.4 Gy) | I, II, III, IV (T2-T4, N0-N1, M0-M1) |
| Suh [88] | 2006 | Korea | Asian | 54 | 58 | 30/24 | C677T | metastatic colorectal cancer | solid | CR, PR, SD and DP | CR+PR+SD | DP | FOLFOX chemotherapy (folinic acid, 5-FU and oxaliplatin) | II, III, IV |
| Ott [89] | 2006 | Germany | Caucasian | 135 | 56 (23-70) | 97/38 | C677T | advanced gastric cancer | solid | Histopathological Response Evaluation* | <10% residual tumor cells | all that is not responders | cisplatin and 5-FU | II, III (cT3-4, any N, cM0) |
| Chung [90] | 2006 | South Korea | Asian | 36 | 51 | nr | C677T and A1298C | bulky cervical cancer | solid | Tumor Shrinkage (CR, PR, SD and DP) | CR+PR | SD+DP | 5-FU/platinum agent with or without ADRIA and ETP | Ib2, IIa, IIb |
| Wu [91] | 2006 | USA | Mixed (90% whites) | 210 | 61 (32-79) | 182/28 | C677T and A1298C | esophageal cancer | solid | Recurrence | No Recurrence | Recurrence | FU | IIa-IVb |
| Ruzzo [92] | 2006 | Japan | Caucasian | 175 | 61 (38-79) | 99/76 | C677T | Advanced gastric cancer | solid | CR, PR, SD and DP | CR+PR | SD+DP | FU/cisplatin | |
| Sarbia [93] | 2006 | Germany | Caucasian | 68 | 57 (37-70) | 60/8 | C677T | Advanced esophageal squamous cell cancer | solid | CR, PR, SD and DP | CR+PR | SD+DP | FLEP (folinic acid, ETP, 5-FU, cisplatin) | (cT3-4, cN0-1, cM0) |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jakobsen [94] | 2005 | Denmark | Caucasian | 139 | 63 | 50/38 | C677T and A1298C | Metastatic colorectal cancer | solid | | | | FU | |
| Etienne [95] | 2004 | France | Caucasian | 98 | 64 (40-82) | 57/41 | C677T and A1298C | advanced colorectal cancer | solid | CR, PR, SD and DP | CR+PR | SD+DP | 5-FU and folinic acid | IV (Tx, Nx, M1) |
| Cohen [96] | 2003 | Canada | Caucasian | 43 | 59 (43-70) | 27/16 | C677T | advanced colorectal cancer | solid | CR, PR, SD and DP | CR+PR | DP | 5-FU | |
| Marcuello [97] | 2006 | Spain | Caucasian | 94 | 68 (43–83) | 68/26 | C677T and A1298C | colorectal cancer | solid | CR, PR, SD and DP | CR+PR | SD+DP | 5-FU | |

*The macroscopically identifiable tumor bed of the resected tumors was completely examined histologically.

TRG-1=absence of viable cancer cells in the resected specimen;
TRG-2=presence of residual cancer cells;
TRG-3=fibrosis outgrowing residual cancer cells;
TRG-4=residual cancer cells outgrowing fibrosis;
TRG-5=absence of response.
CR=Complete Response (disappearance of the disease)
PR= Partial Response (at least 50% reduction in tumor load of the lesions)
SD= Stable Disease (≤25% progression or <50% shrinkage)
DP=Disease Progression (size enlargement >25% or appearance of new lesions)
FU=fluorouracil
ADRIA=Adriamycin
ETP=Etoposide
T=Tumor size
N=Node involvement
M=metastasis
Tx=any T
Nx=any N

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