Pharmacogenetic methods: an epidemiological perspective

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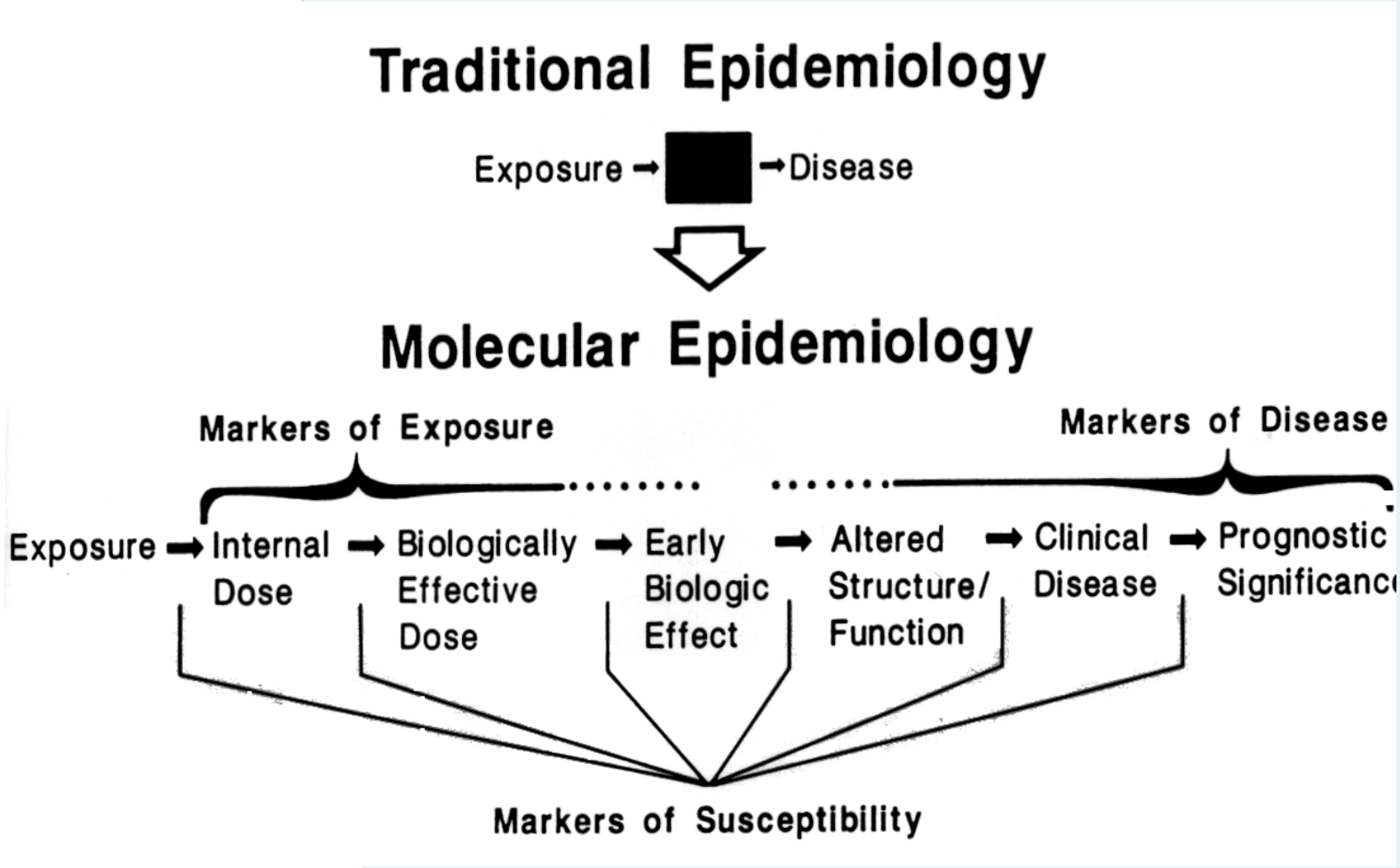


Schulte PA, Perera FP. Molecular Epidemiology, 1993: 6.

How to identify relevant genes ?

- *Candidate gene* / genome-wide screen
- *Study design*
  - RCT
  - Observational

Methodological quality in pharmacogenetic studies with binary assessment of treatment response: a review

Albert Cobos[a], Pilar Sánchez[b], Jaume Aguado[a] and Josep Lluís Carrasco[a]

Objective To evaluate the reporting of critical design issues and methods of statistical analysis in pharmacogenetic studies published in the medical literature.

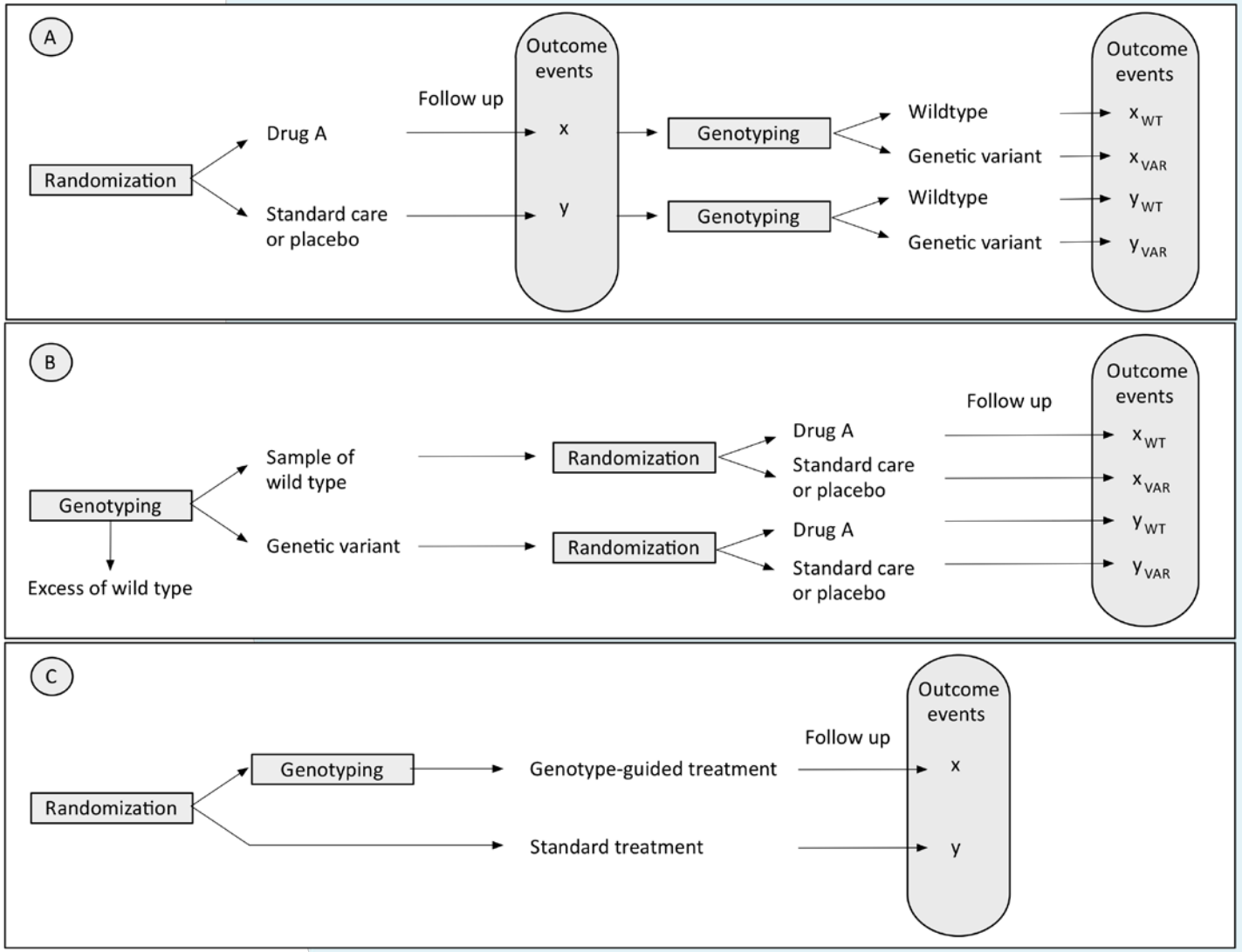
Study design and settings Systematic review of 65 original pharmacogenetic studies published in the literature over the last 15 years.

Results The sample size determination and the planned sample size were lacking in 63 papers. The study design characterization was lacking in 43 papers. The number of patients analyzed ranged from 36 to 1400 (median=161 and interquartile range of 119–250). The Pearson's χ^2 test of association analyses were commonly reported as *P* values but rarely as estimates of an association measure (odds ratio or relative risk) and its accuracy.

Conclusions These results show that there is considerable room for improvement in the current standards of design, analysis, and reporting of pharmacogenetic research. ***Pharmacogenetics and Genomics*** 21:243–250 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

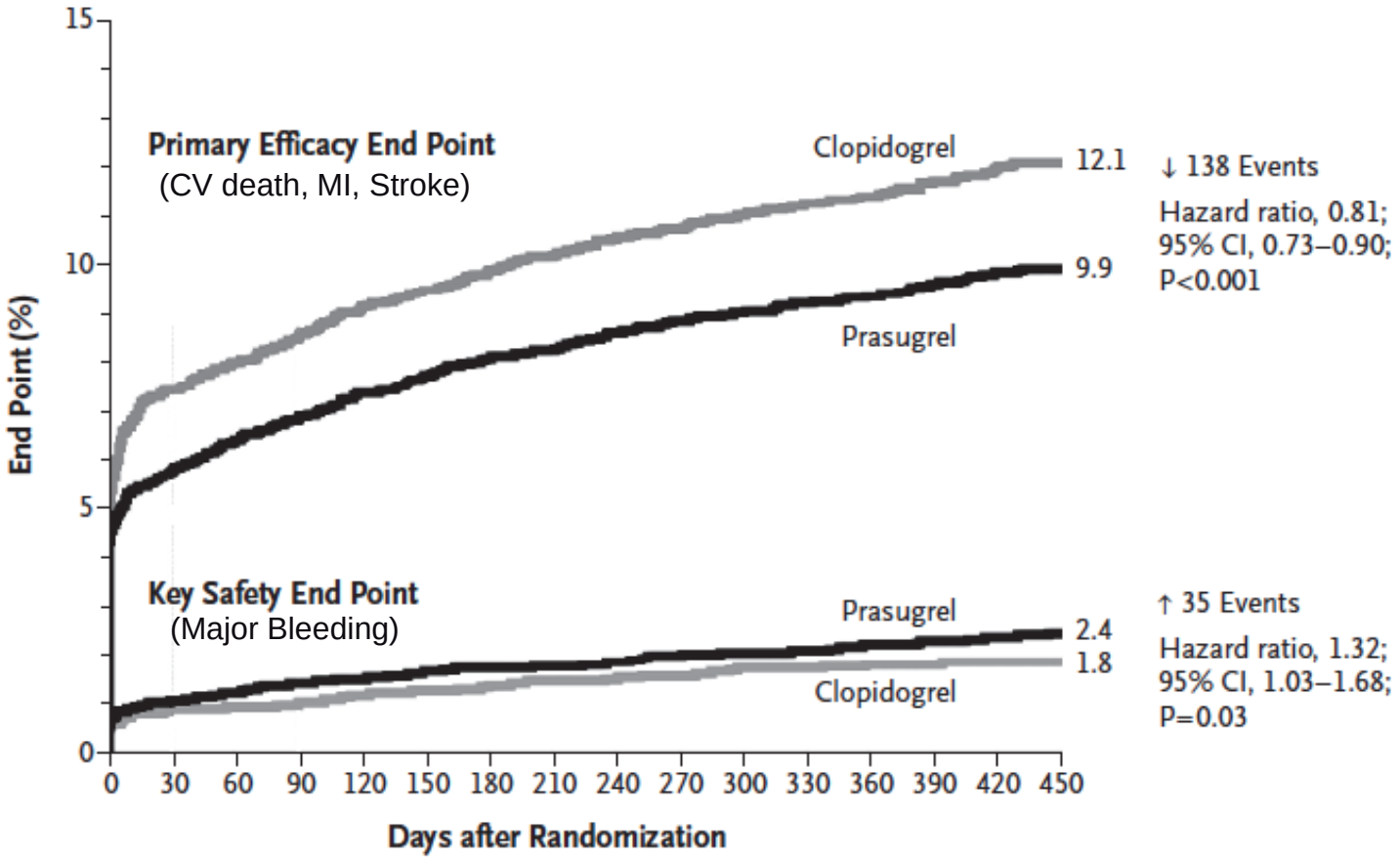
Pharmacogenetics and Genomics 2011, 21:243–250

Pharmacogenetics in RCTs



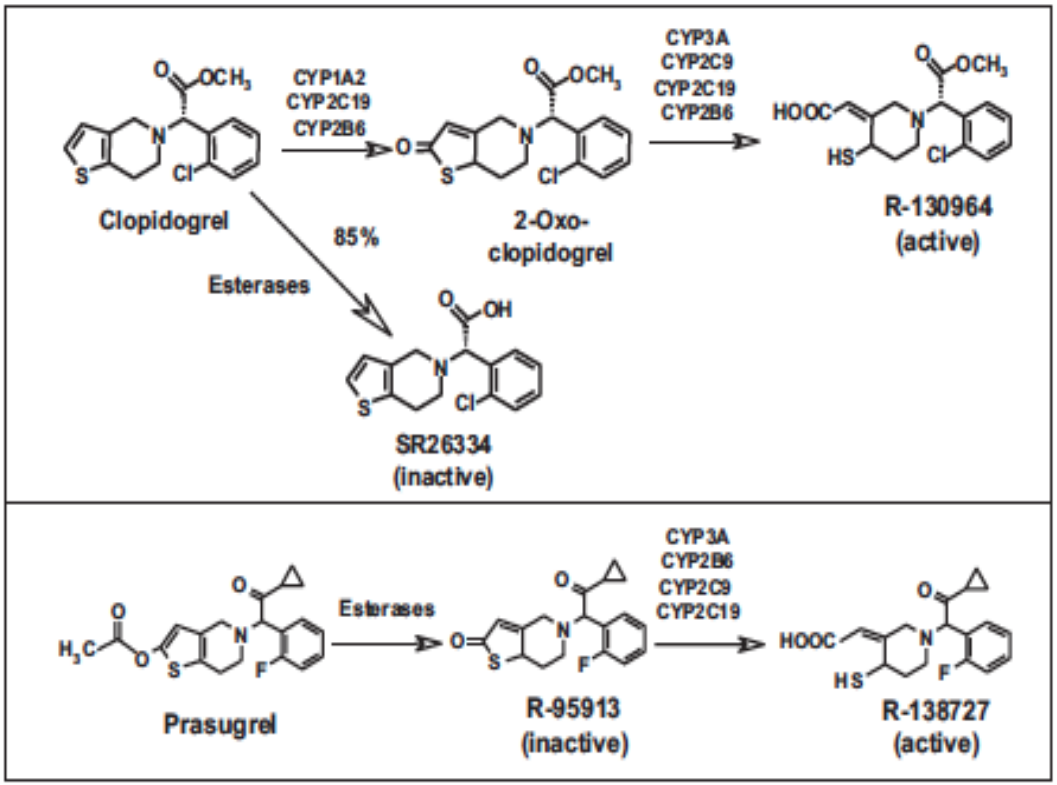
Van der Baan FH, Klungel OH, Egberts ACG, et al. Pharmacogenomics 2011;12:1485-92

A. Posthoc analysis of RCT (TRITON-TIMI 38)



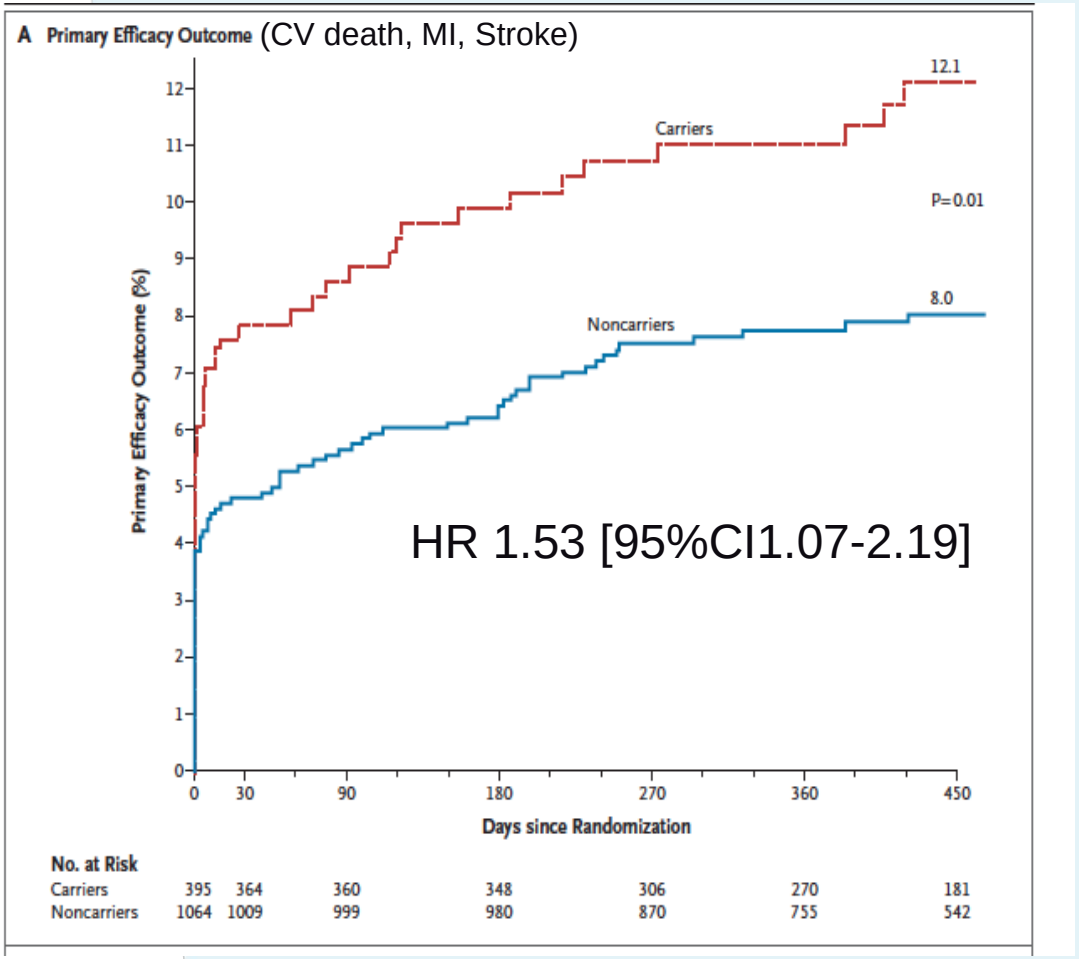
Wiviott SD, et al. NEJM 2007;357:2001-15

Activation of clopidogrel and prasugrel



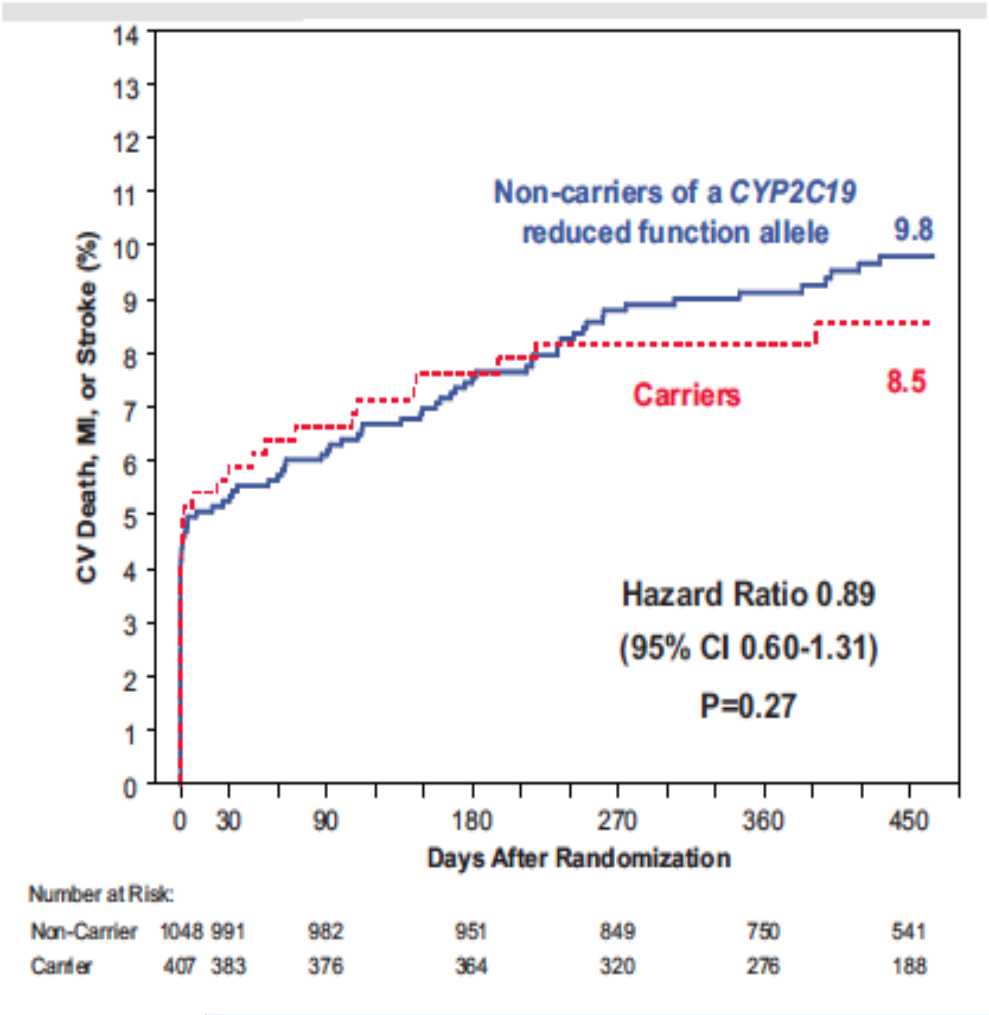
Mega JL, et al. Circulation 2009;119:2553-2560

Clopidogrel and CYP2C19 PM



Mega JL, et al. NEJM 2009;360:354-62

Prasugrel and CYP2C19 PM



Mega JL, et al. Circulation 2009;119:2553-2560

Exposure-only analysis

Exposure	Genotype	Cases	Controls	OR
0	0	A	E	1.0
1	0	B	F	BE /AF
0	1	C	G	CE/AG
1	1	D	H	DE/AH

Case-control in variant : ORvariant = DG/CH
Case-control in wildtype : ORwildtype = BE/AF
Synergy index : ORvariant/ORwildtype = DF/BH x GA/CE
Exposure only OR : DF/BH

If GA/CE=1 then exposure-only OR = case-control SI

Exposure-only analysis TRITON-TIMI 38

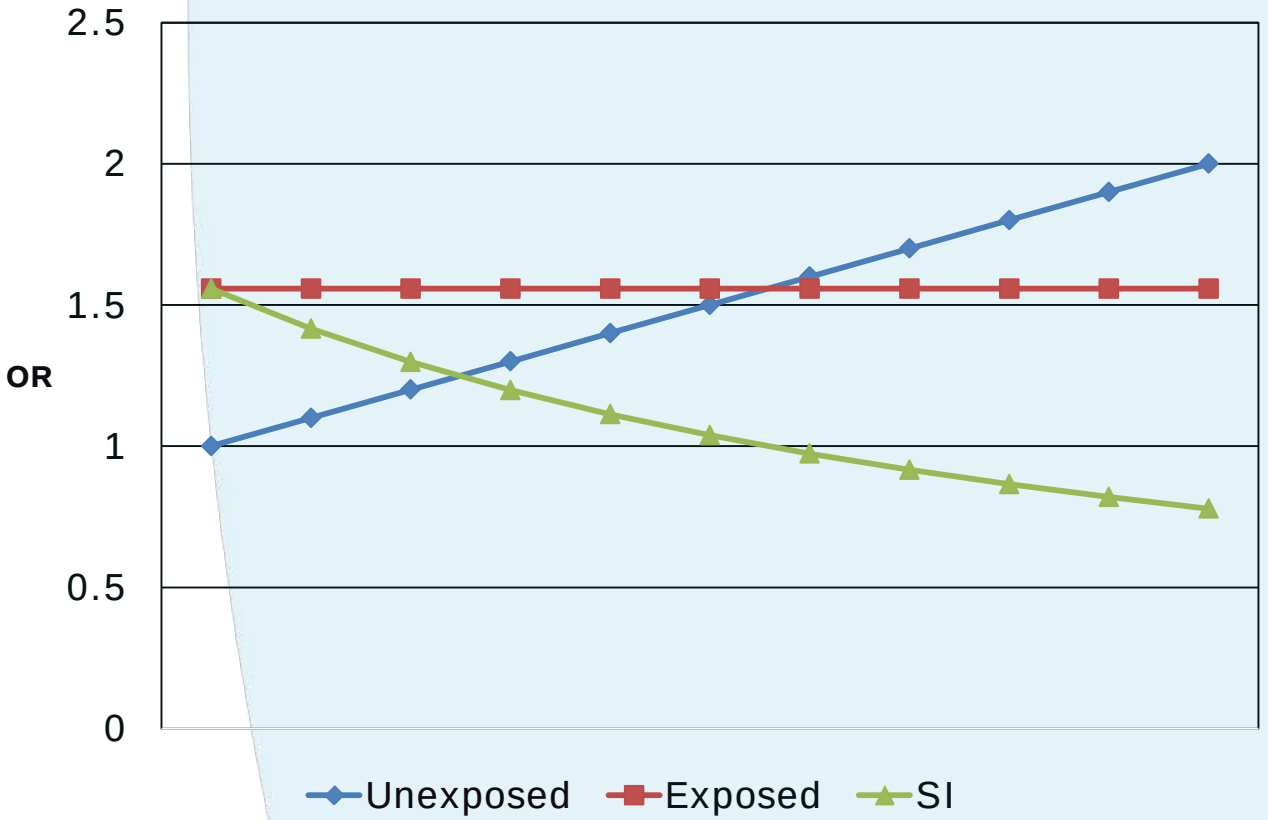
Exposure	Genotype	Cases	Controls	OR
Prasugrel	Wildtype	99	949	1.0
Clopidogrel	Wildtype	83	981	0.81
Prasugrel	Variant	34	373	0.87
Clopidogrel	Variant	46	349	1.26

Case-control in variant : ORvariant = 1.45
Case-control in wildtype : ORwildtype = 0.81
Synergy index : ORvariant/ORwildtype = 1.78
Exposure-only OR = 1.56

Note: OR in unexposed (prasugrel) = 0.87

Mega JL, et al. Circulation 2009;NEJM 2009

What if genotype related to outcome in unexposed ?



CYP2C19 Genotype, Clopidogrel Metabolism, Platelet Function, and Cardiovascular Events

A Systematic Review and Meta-analysis

Michael V. Holmes, MBBS, MSc
Pablo Perel, PhD
Tina Shah, PhD
Aroon D. Hingorani, PhD
Juan P. Casas, PhD

CLOPIDOGREL IS AN ANTIplatelet drug used by approximately 40 million patients worldwide[1,2] to treat or prevent atherothrombotic events and after percutaneous coronary revascularization. An overview of randomized trials including 7384 cardiovascular events in 79 613 patients with acute or stable coronary heart disease (CHD) or with multiple CHD risk factors demonstrated an association of clopidogrel therapy with reduced rates of cardiovascular events (odds ratio [OR], 0.88; 95% CI, 0.83-0.93) compared with placebo. Clopidogrel is also associated with a mechanism-based increase in major

Context The US Food and Drug Administration recently recommended that *CYP2C19* genotyping be considered prior to prescribing clopidogrel, but the American Heart Association and American College of Cardiologists have argued evidence is insufficient to support *CYP2C19* genotype testing.

Objective To appraise evidence on the association of *CYP2C19* genotype and clopidogrel response through systematic review and meta-analysis.

Data Sources PubMed and EMBASE from their inception to October 2011.

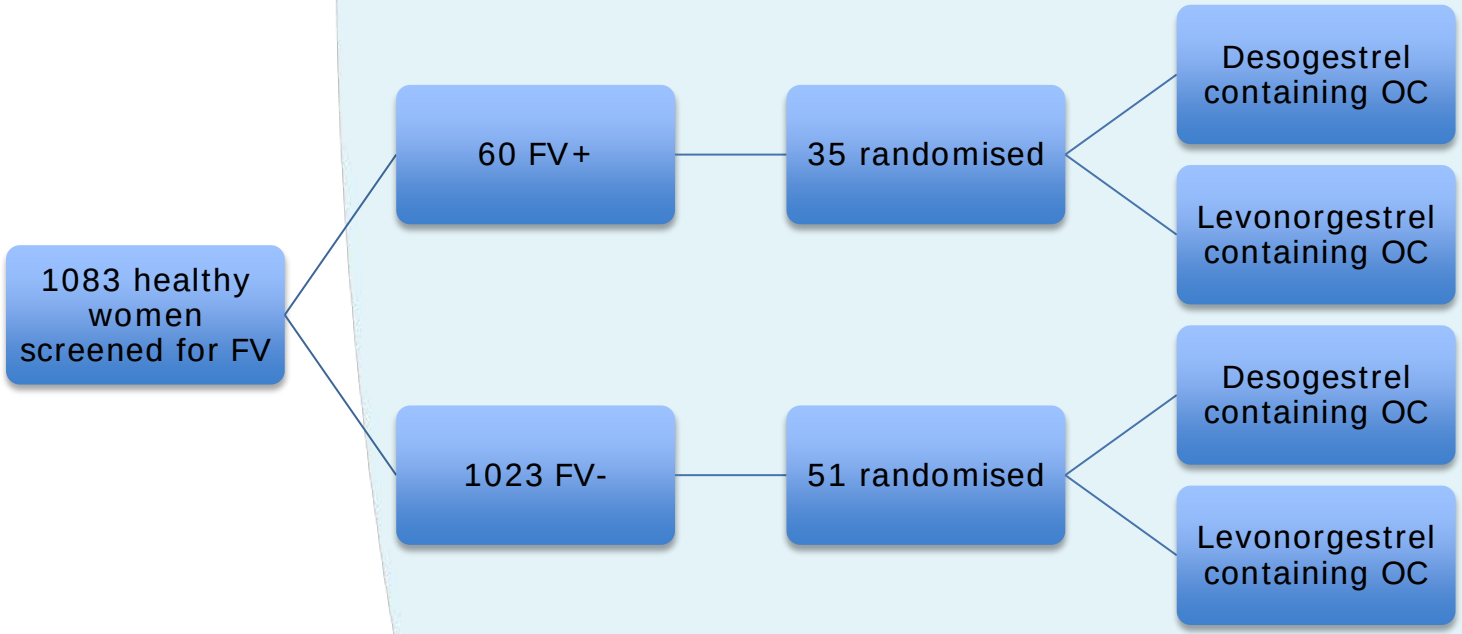
Study Selection Studies that reported clopidogrel metabolism, platelet reactivity or clinically relevant outcomes (cardiovascular disease [CVD] events and bleeding), and information on *CYP2C19* genotype were included.

Data Extraction We extracted information on study design, genotyping, and disease outcomes and investigated sources of bias.

Results We retrieved 32 studies of 42 016 patients reporting 3545 CVD events, 579 stent thromboses, and 1413 bleeding events. Six studies were randomized trials ("effect-modification" design) and the remaining 26 reported individuals exposed to clopidogrel ("treatment-only" design). In treatment-only analysis, individuals with 1 or more *CYP2C19* alleles associated with lower enzyme activity had lower levels of active clopidogrel metabolites, less platelet inhibition, lower risk of bleeding (relative risk [RR], 0.84; 95% CI, 0.75-0.94; absolute risk reduction of 5-8 events per 1000 individuals), and higher risk of CVD events (RR, 1.18; 95% CI, 1.09-1.28; absolute risk increase of 8-12 events per 1000 individuals). However, there was evidence of small-study bias (Harbord test $P=.001$). When analyses were restricted to studies with 200 or more events, the point estimate was attenuated (RR, 0.97; 95% CI, 0.86-1.09). In effect-modification studies, *CYP2C19* genotype was not associated with modification of the effect of clopidogrel on CVD end points or bleeding ($P>.05$ for interaction for both).

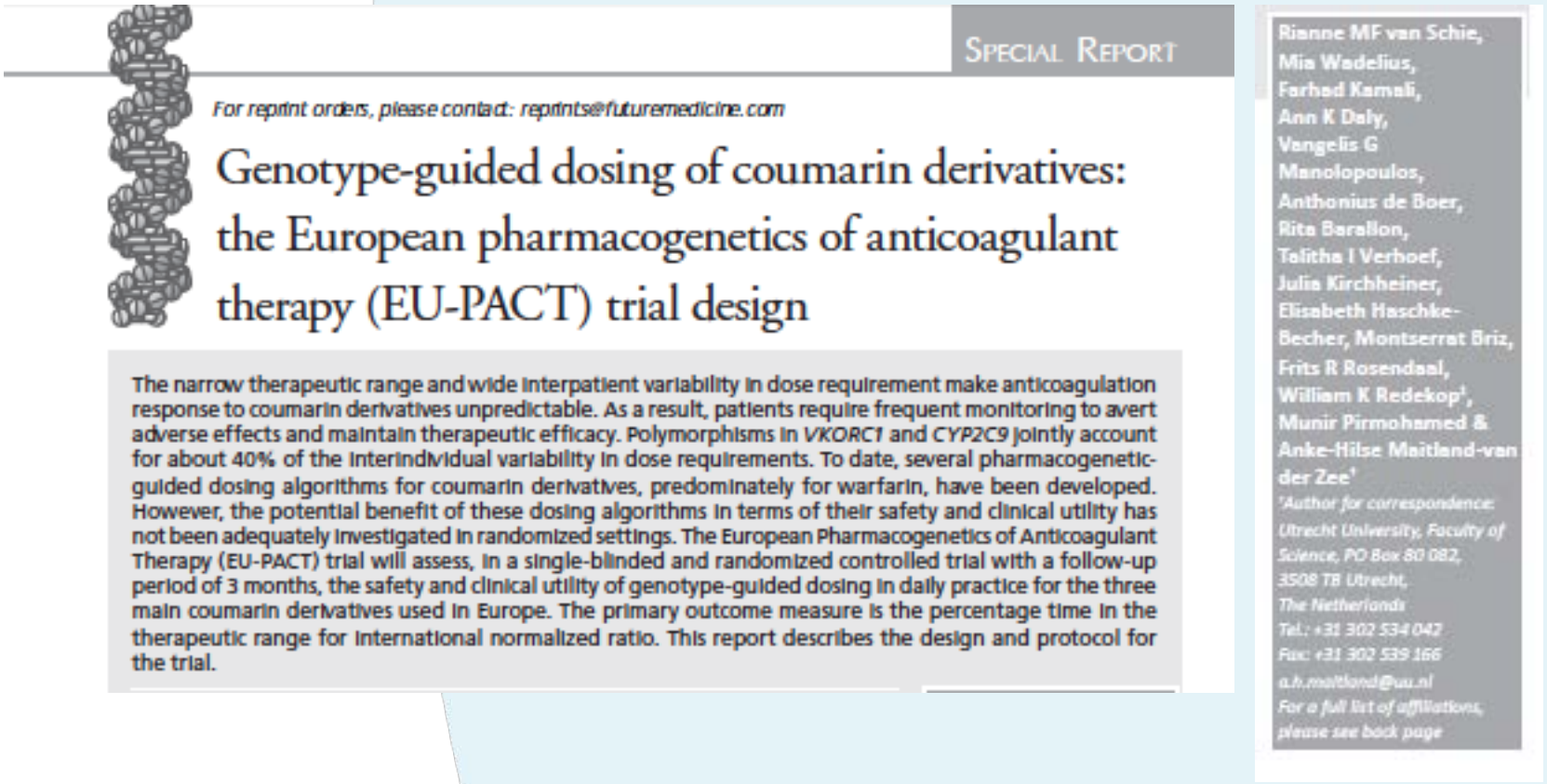
Holmes MV, et al. JAMA 2011;306:2704-14

B. Genotyping before enrollment



Kemmeren JM, et al. Blood 2004;103:927-33

C. Added value of genotyping in clinical practice



SPECIAL REPORT

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Genotype-guided dosing of coumarin derivatives: the European pharmacogenetics of anticoagulant therapy (EU-PACT) trial design

The narrow therapeutic range and wide interpatient variability in dose requirement make anticoagulation response to coumarin derivatives unpredictable. As a result, patients require frequent monitoring to avert adverse effects and maintain therapeutic efficacy. Polymorphisms in *VKORC1* and *CYP2C9* jointly account for about 40% of the interindividual variability in dose requirements. To date, several pharmacogenetic-guided dosing algorithms for coumarin derivatives, predominately for warfarin, have been developed. However, the potential benefit of these dosing algorithms in terms of their safety and clinical utility has not been adequately investigated in randomized settings. The European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) trial will assess, in a single-blinded and randomized controlled trial with a follow-up period of 3 months, the safety and clinical utility of genotype-guided dosing in daily practice for the three main coumarin derivatives used in Europe. The primary outcome measure is the percentage time in the therapeutic range for international normalized ratio. This report describes the design and protocol for the trial.

Rianne MF van Schie, Mia Wadelius, Farhad Kamali, Ann K Daly, Vangelis G Manolopoulos, Anthonius de Boer, Rita Barallon, Talitha I Verhoef, Julia Kirchheiner, Elisabeth Haschke-Becher, Montserrat Briz, Frits R Rosendaal, William K Redekop†, Munir Pirmohamed & Anke-Hilse Maitland-van der Zee†

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Van Schie RMF, et al. Pharmacogenomics 2009;10:1287-95

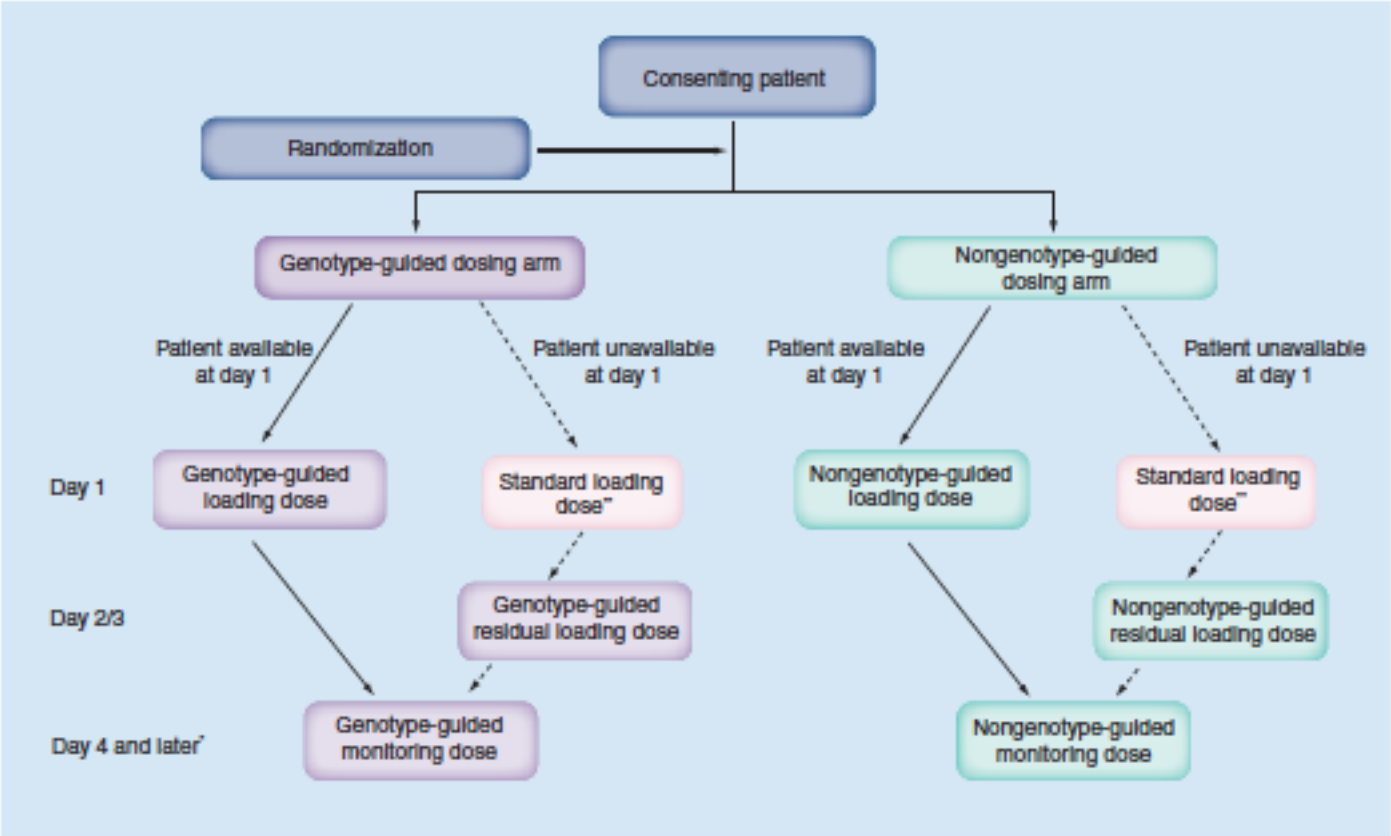


Figure 1. Schematic presentation of the study design as for each coumarin derivative.
*The loading dose algorithm is optimally followed for 3 days, and the start of the monitoring dose algorithm on day 4. However, due to weekends the monitoring dose algorithm may also start on day 3, 5 or 6.
**In exceptional cases (shown by the dashed arrow), if the patient is randomized to either the intervention or the control group later than day 1, and the patient has received coumarin dosing according to usual clinical care only at day 1, the patient may receive a loading dose according to one of the loading dose algorithms for the two days remaining until the next planned international normalized ratio test.

Observational studies

- Family studies
  - Twin, sib pair studies
- Population-based studies
  - Cohort
    - Case-cohort
    - *Exposure only*
  - Case control
    - *Case only*

Pharmacogenetic case-control studies based on enrichment of EHR databases

SLCO1B1 Genetic Variant Associated With Statin-Induced Myopathy: A Proof-of-Concept Study Using the Clinical Practice Research Datalink

DF Carr[1], H O'Meara[1], AL Jorgensen[2], J Campbell[3], M Hobbs[3], G McCann[3], T van Staa[3–5] and M Pirmohamed[1]

- Safety
- EHR GP database (CPRD)
- Recruitment through GPs

Clin Pharmacol Ther 2013, advance online publication

Pharmacogenetic case-control studies based on enrichment of EHR databases

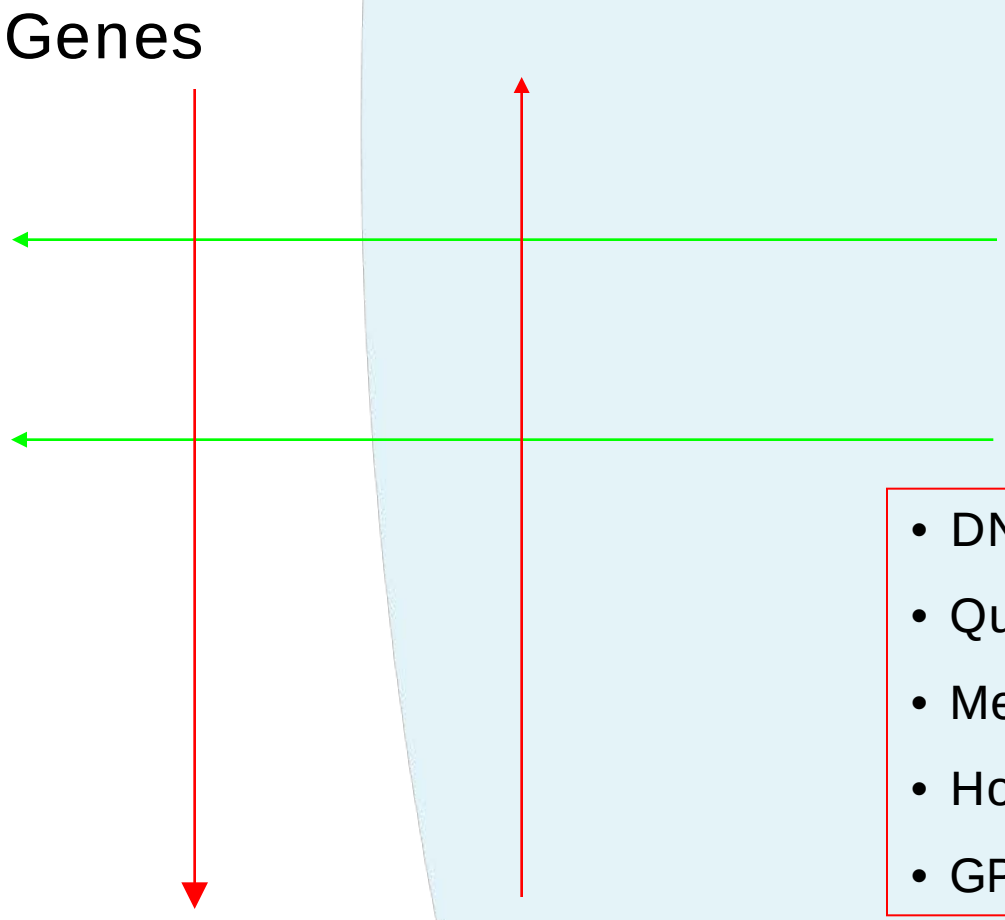
Interaction between the Gly460Trp α-adducin gene variant and diuretics on the risk of myocardial infarction

Diane B.M.A. van Wieren-de Wijer[a,b], Anke-Hilse Maitland-van der Zee[a], Anthonius de Boer[a], Abraham A. Kroon[c], Peter W. de Leeuw[c], Paul Schiffers[d], Rob G.J.H. Janssen[e,*], Bruce M. Psaty[f], Cornelia M. van Duijn[b], Bruno H. Ch. Stricker[b] and Olaf H. Klungel[a]

- Effectiveness
- Utrecht Cardiovascular Pharmacogenetic (UCP) study
- EHR Pharmacy/Hospitalisation database (PHARMO)
- Recruitment through Pharmacists

J Hypertens 2009;27:61-8

Pharmacogenetic case-control study



- DNA (buccal swab)
- Questionnaire
- Medication (160 pharmacies)
- Hospitalisations
- GP records

Database:31.000

906 cases not approached

- 11 nursing home
- 32 unknown in pharmacy
- 384 pharmacy not participating
- 119 moved
- 360 died

14.131 controls not approached

- 12 nursing home
- 114 unknown in pharmacy
- 3525 pharmacy not participating
- 551 moved
- 995 died
- 8934 case not approached

15.973 approached

BMA van Wieren-de Wijer, et al. Pharm World Sci 2009;31:158-164

Characteristics of cases and controls

	Cases	Controls
Number	794	4997
Age, yr	64.8	64.5
Female	33%	33%
Consent DNA	88%	89%
Thiazide	17%	25%*
Smoking	21%	15%*
BMI>30 kg/m^2	24%	20%*
High cholesterol	66%	49%*
Diabetes mellitus	23%	19%*
Sedentary	31%	27%*
Caucasian	98%	98%

Gene-exposure interaction analysis in a case-control study

Exposure	Genotype	Cases	Controls	Odds Ratio
0	0	A	E	1.0
0	1	B	F	BE/ AF
1	0	C	G	CE/ AG
1	1	D	H	DE/ AH

Case control in variant $OR_{variant}$=DF/ BH
Case control in wild-type $OR_{wild\text{-}type}$=CE/AG
Synergy index (SI) = $OR_{variant}/OR_{wild\text{-}type}$ = DA/CB x FG/HE
Case-only OR=DA/CB
If FG/HE=1 then Case-only OR=case-control SI
RERI=OR_{11}-OR_{10}-OR_{01}+1

Am J Epidemiol 1996; 144: 207-13.

Farmacogenetic study antihypertensives

Exposure	Genotype	Cases	Controls	Odds Ratio [95%BI]
Other Ahyp	GG	305	1597	
Diuretic	GG	76	650	0.62 [0.44-0.87]
Other Ahyp	GT/TT	170	975	
Diuretic	GT/TT	62	415	0.88 [0.58-1.33]

SI = $OR_{GT/TT}/OR_{GG}$ = 1.41 [0.91-2.17]

Case-only OR = 1.46 (in controls OR=1.05)

RERI 0.26 [-0.07 – 0.59]

J Hypertens 2009;27:61-8

Case control study

$OR_{GT/TT} = 0.43$

$OR_{GG} = 1.05$

$SI = OR_{GT/TT}/OR_{GG} = 0.41$

Case-only OR =0.47

Psaty B, et al. JAMA 2002;287:1680-9

GenHAT Trial

$RR_{GT/TT} = 1.03$

$RR_{GG} = 0.97$

$SI = RR_{GT/TT}/RR_{GG} = 1.06$ Men SI=0.85 Women SI=1.51

Case-only OR = 1.03

Davis et al, The Pharmacogenomics J 2006

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doi:10.1093/ije/dyr088
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Bias in the case-only design applied to studies of gene–environment and gene–gene interaction: a systematic review and meta-analysis

Jessica Dennis,[1,2]* Steven Hawken,[1] Daniel Krewski,[1,2] Nick Birkett,[1,2] Mihaela Gheorghe,[1] Julia Frei,[1] Gail McKeown-Eyssen[3] and Julian Little[1,2]

Results The search yielded 365 unique articles of which 38 met the inclusion criteria. Potential sources of bias in the case-only design included non-independence of genotype and exposure in the source population. Meta-regression analysis, based on 24 evaluations, produced a mean IOR_{CC}/IOR_{CO} of 1.06 [95% confidence interval (95% CI) 0.93–1.22], suggesting that bias in case-only designs is not common in practice. The I^2 statistic indicated that 23.9% (95% uncertainty interval 0–53.9%) of the observed variation was due to heterogeneity between studies, which was not explained by any methodological characteristics of the included studies.

Conclusion As understanding of the relationships between genes and environmental exposures in the population improves, the case-only design may prove to be of considerable value.

Cholesteryl … morphisms, Statin Use, … terol Levels and Cardiov…

Table 1 Characteristics of po…

Study/ reference	Analysis	Partici… inclu…	Geographical location	Design
FAST-MI[31]	MI	2,…	France	Cohort
Go-DARTS[33]	MI + cholesterol	9,…	Scotland	Cohort
JUPITER[36]	Cholesterol	3,…	1,315 Sites in 26 countries	Multicenter RCT
PREVEND-IT[b35]	MI + cholesterol[c]	3,…	The Netherlands	Cohort/RCT
UCP[27]	MI	1,…	The Netherlands	Nested case–control

Participants included maximum numbe…

HDLc, high-density lipoprotein choleste…

[a]In the interaction analysis, both the sta… cholesterol analysis.

…trial.

…-IT. [c]Only PREVEND-IT included in

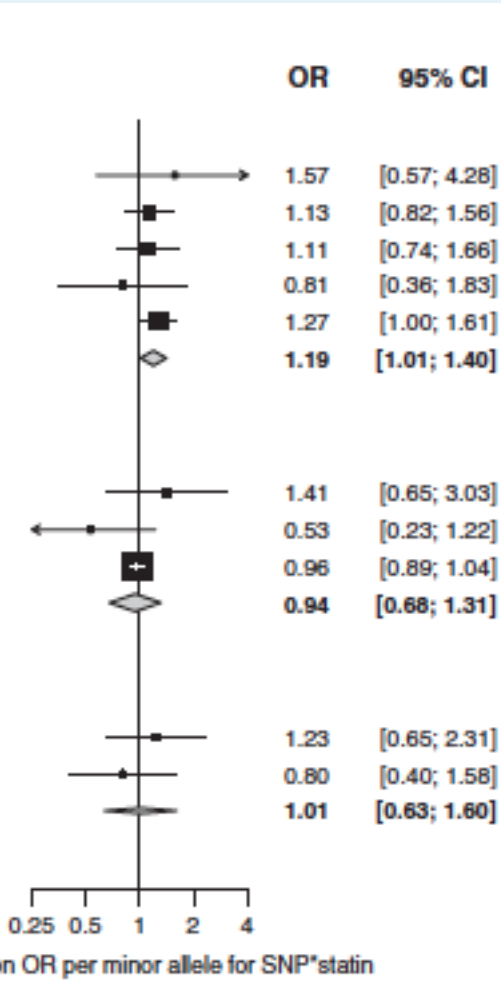

Figure 4 Meta-analysis of per-allele SNP × statin interaction ORs for MI outcome. An interaction OR > 1 implies less statin benefit. MI, myocardial infarction; OR, odds ratio; RE, random effects; SNP, single-nucleotide polymorphism.

Conclusion

- RCTs and observational studies complementary
- Analyses focus on effect *measure* modification
- Enrichment of EHR databases potential for PGx
- Collaboration in multi-centre studies to increase sample size and replicate PGx interactions