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CLINICAL RESEARCH
Coronary artery disease

Genetic variation at the coronary artery disease risk locus *GUCY1A3* modifies cardiovascular disease prevention effects of aspirin

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Aims Efficacy of aspirin in primary prevention of cardiovascular disease (CVD) may be influenced by a common allele in guanylate cyclase *GUCY1A3*, which has been shown to modify platelet function and increase CVD risk.

Methods and results We investigated whether homozygotes of the *GUCY1A3* rs7692387 risk (G) allele benefited from aspirin in two long-term, randomized placebo-controlled trials of aspirin in primary CVD prevention: the Women's Genome Health Study (WGHS, $N = 23\,294$) and a myocardial infarction (MI, $N = 550$) and stroke ($N = 382$) case–control set from the Physician's Health Study (PHS, $N = 22\,071$). Bleeding risk was evaluated in the WGHS. In the placebo group of the WGHS, the *GUCY1A3* risk (G) allele was confirmed to increase CVD risk [hazard ratio 1.38; 95% confidence interval (CI) 1.08–1.78; $P = 0.01$]. Random-effects meta-analysis of the WGHS and PHS revealed that aspirin reduced CVD events among risk allele homozygotes [G/G: odds ratio (OR) 0.79; 95% CI 0.65–0.97; $P = 0.03$] but increased CVD events among non-risk allele carriers (e.g. G/A: OR 1.39; 95% CI 1.03–1.87; $P = 0.03$) thus implying an interaction between genotype stratum and aspirin intake ($P_{interaction} = 0.01$). Bleeding associated with aspirin increased in all genotype groups, with higher risks in heterozygotes.

Conclusion In two randomized placebo-controlled trials in the setting of primary prevention, aspirin reduced the incidence of CVD events in individuals homozygous for the *GUCY1A3* risk (G) allele, whereas heterozygote individuals had more events when taking aspirin.

Keywords Coronary artery disease risk gene • *GUCY1A3* • Guanylate cyclase • rs7692387 • Aspirin • Primary prevention

Introduction

Platelet inhibition with aspirin is a cornerstone for preventing recurrent ischaemic cardiovascular disease (CVD) events, but its use in primary prevention is controversial.[1] Aspirin irreversibly acetylates platelet cyclooxygenase-1 (COX-1) which inhibits formation of thromboxane A_2 and, thereby, blocks platelet activation and aggregation.[2] Inherent to its pharmacological mechanism, aspirin also slightly

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increases the risk of extracranial bleeding events and haemorrhagic stroke.[3] While the benefit-to-risk-ratio in patients who have experienced ischaemic stroke or myocardial infarction (MI) favours aspirin treatment,[4] a meta-analysis of primary prevention trials in healthy individuals found the decrease of ischaemic events to be only twice the increase in bleeding events.[5] Thus, because of uncertain net benefit, the US Preventive Task Force guidelines limited use of low-dose aspirin in primary prevention to adults, aged 50–59 years with a 10% or higher 10-year risk of CVD, who were not at increased risk for bleeding.[6] Nonetheless, millions of Americans and Europeans use aspirin in this indication.[7]

GUCY1A3 (according to a newly introduced nomenclature *GUCY1A1*)—together with *GUCY1B3* (*GUCY1B1*)—forms soluble guanylyl cyclase (sGC), an enzyme which, upon stimulation with nitric oxide, inhibits platelet aggregation.[8] Recent genome-wide association studies identified common variants in *GUCY1A3* that increased risk for coronary artery disease (CAD)/MI and impaired platelet inhibition upon nitric oxide stimulation.[8–15]

It is not known whether the *GUCY1A3* risk allele also influences aspirin therapy outcomes. As homozygous carriers of the *GUCY1A3* rs7692387 risk (G) allele, who represent ~63% of a Western European population,[16] are relatively less sensitive to platelet inhibition in response to the natural inhibitor nitric oxide,[8,13] we hypothesized that these individuals might uniquely benefit from aspirin in primary prevention. Thus, we examined whether *GUCY1A3* genotype rs7692387 interacts with randomized aspirin treatment in subsets from two large randomized aspirin trials, the Women's Genome Health Study (WGHS)[17] and an MI[18] and stroke[19] case–control set from Physician's Health Study (PHS).[20]

Methods

Participants, clinical measurements, and outcomes

The WGHS[17] is a large subset of the Women's Health Study (WHS), including 23 294 women of verified European ancestry. Detailed study methods were previously published[17,21] and are available in the Supplemental Material online. In brief, the WHS was a randomized, placebo-controlled trial which examined the effect of aspirin (100 mg) and/or vitamin E (600 IU) on primary CVD and cancer prevention over 10 years among 39 876 initially healthy female health-care professionals ≥45 years at baseline.

Two nested case–control studies including individuals of European ancestry from PHS were used to validate WGHS findings. In PHS, 22 071 male physicians aged 40–84 and free from known MI, stroke, transient ischaemic attack, or cancer were randomized to aspirin (50 mg) or beta-carotene (325 mg) using a 2 × 2 factorial design to test CVD and cancer primary prevention.[22] As randomization was terminated in 1988 and study participants were allowed to continue regular aspirin use irrespective of treatment allocation, we focused on the randomization period, i.e. until 1988. Detailed study methods were previously published.[18–20]

Genotyping

Genotyping in WGHS was performed twice using DNA extracted from baseline blood samples. The original genotyping used Human-Hap300 Duo '+' (Illumina, San Diego, CA, USA) with Infinium II protocol.[17] This dataset included 23 294 WGHS participants of self-reported European ancestry confirmed by PLINK multi-dimensional scaling.[23] This genotyping array did not include rs7692387 which was imputed as maximum likelihood allele dose from HumanHap300 experimental genotype using minimac3[24] and Haplotype Reference Consortium reference panel.[25] Independently, 22 618 (97.1%) WGHS samples were genotyped using exome array (v.1.1) (Illumina, San Diego, CA, USA) which included rs7692387.[26,27] For rs7692387, correspondence between exome array and maximum likelihood allele dose was high ($R^2 = 99.8\%$). Experimentally determined genotypes were used for primary analysis while imputed data provided technical replication. For further corroboration, imputed *GUCY1A3* variants rs13139571 and rs7688323 in linkage disequilibrium with rs7692387 were also examined. Baseline demographics and CVD risk factors did not differ between exome array and whole WGHS European ancestry datasets (all $P > 0.1$).

Of the 595 PHS cases reported in the original trial,[20] our analysis was limited to those whose samples were available for genotyping, were successfully genotyped for the lead single nucleotide polymorphism (SNP) rs7692387, and self-reported their ancestry as European, $N = 409$. For PHS, rs7692387, rs13139571, and rs7688323 TaqMan SNP genotyping assays from Applied Biosystems (Foster City, CA, USA) were carried out on Applied Biosystems 7900HT instrument, using SDS version 2.4 software. Genotyping was successful in 95%, 95%, and 92% of all samples for rs7692387, rs7688323, and rs13139571, respectively. All three SNPs were consistent with Hardy–Weinberg Equilibrium among controls (all $P > 0.05$).[28]

Statistical analysis

GUCY1A3 CAD lead SNP rs7692387 was the primary SNP in all analyses. For estimates of aspirin effects in WGHS and PHS, the two arms with aspirin were combined and labelled 'Aspirin' and compared to the two arms without aspirin, labelled 'Placebo'.

In the WGHS, Cox proportional-hazard models were used to evaluate rs7692387 effects on rates of major CVD, stroke, and MI assuming a standard additive (on log scale) genetic model; results are presented here in terms of risk alleles. Major CVD, the primary WGHS outcome, was a composite of MI, stroke, or death from cardiovascular causes. Because the PHS cases used to replicate our findings in WGHS consisted of only stroke and MI cases, we also examined a composite endpoint of WGHS stroke and MI events. This outcome had 96 fewer events than the primary outcome major CVD. Models were adjusted for age and smoking or fully adjusted for cardiovascular risk factors which included age, systolic blood pressure, diastolic pressure, LDL-cholesterol, HDL-cholesterol, body mass index (BMI), family history of MI, family history of diabetes, and smoking. The proportionality assumption was verified for each model. The interaction of SNP with aspirin was tested by inclusion of a term corresponding to the product of SNP genotype (0, 1, or 2) and an indicator variable for aspirin use (0 = placebo/1 = aspirin). *P*-values for interaction were verified empirically by permutation procedure. For 10 000 iterations, the regressions were performed using genotypes that were resampled at random without replacement. An empirical two-sided *P*-value was computed as the fraction of the beta-coefficients of the interaction term from the permutations with absolute value greater than the absolute value of the beta-coefficient from the unpermuted data. The effect of randomized aspirin allocation was also examined within genotype strata. Kaplan–Meier curves were generated stratified by genotype determined using exome array. The rs7688323 and rs13139571 were examined in parallel analyses. The threshold for statistical significance for both main and interaction effects was *P*-value <0.05.

In PHS, conditional logistic regression models based on matching for age and smoking were evaluated to estimate odds ratios (ORs) of cardiovascular events and interaction with aspirin for each genotype assuming

additive allele encoding (on the log scale). Associations with major CVD for PHS were examined by combining MI and stroke case/controls sets. Models were adjusted for randomized treatment assignment and either adjusted for age and smoking alone or with cardiovascular risk factors (hypertension history—140/90 mmHg or on antihypertensive medication, cholesterol, diabetes, and BMI). Primary analyses were performed among matched case/control pairs of European ancestry from randomization period (before 25 January 1988). Secondary analyses included additional case/control pairs occurring after this date when participants were allowed to cross-over. Logistic regression models were used to determine ORs of CVD outcomes within separate genotype strata of randomized aspirin or placebo allocation. Because there were so few participants homozygous for the non-risk A-allele, we also conducted secondary analyses combining G/A with A/A participants (A-carriers). The results of these analyses are presented in the Supplementary Material online. Number needed to treat for aspirin was estimated for women in the WGHS where we had over 10 years of follow-up in a population randomized to aspirin treatment. Analyses were conducted using R.

Given that the WGHS and PHS were conducted in populations of uniformly different sex (women vs. men) and a decade apart, we used a random-effects models to estimate the mean effect size and confidence interval (CI) across the populations. Meta-analyses of *GUCY1A3* effect estimates from logistic regression age/smoking and fully adjusted models for WGHS and PHS were meta-analysed using Comprehensive Meta-Analysis (Englewood, NJ, USA). Authors K.T.H. and D.I.C. had full access to all the data in the study and take responsibility for its integrity and the data analysis.

Study oversight

Women's Health Study, WGHS, and PHS analyses were approved by the Institutional Review Board of Brigham and Women's Hospital, Boston, and performed in accordance with the Declaration of Helsinki.

Results

Baseline and demographics of participants

Demographics and baseline characteristics of the WGHS women and PHS men are presented in Supplementary material online, *Tables S1 and S2*, respectively. The *GUCY1A3* rs7692387 risk (G) allele frequency was 81% in the WGHS and 84% in PHS.

Replication of *GUCY1A3* as a cardiovascular disease locus

Consistent with the original studies that identified *GUCY1A3* as a CVD risk locus,[10,12] but did not include the WGHS (or PHS), in a gene dosage model, the rs7692387 risk (G) allele was associated with higher incidence of major CVD events in the WGHS placebo arm [hazard ratio 1.38; 95% CI 1.08–1.78; $P = 0.01$, *Table 1*]. Similar effects were observed with independently imputed rs7692387 genotype and two other *GUCY1A3* variants in linkage disequilibrium with rs7692387, rs13139571, and rs7688323 (Supplementary material online, *Table S3*). Adjustment for a panel of CVD risk factors did not modify these effects (*Table 1*).

Benefit from aspirin in homozygous *GUCY1A3* risk (G) allele carriers

In the WGHS, there was a significant interaction effect between rs7692387 and randomized aspirin allocation for incidence of major CVD, such that benefit of aspirin varied depending on the number of rs7692387 risk (G) alleles, $P_{interaction} = 0.01$ (*Table 1* and *Take home figure*). The interaction *P*-value was verified by empirical permutation procedure (empirical $P_{interaction} = 0.009$). Interaction effects with randomized aspirin allocation were similar for rs13139571 ($P_{interaction} = 0.04$) and rs7688323 ($P_{interaction} = 0.008$) (Supplementary material online, *Table S3*).

In WGHS analyses of rs7692387 genotype strata, among the 65% women with the rs7692387 homozygous risk allele (G/G) genotype, risk of major CVD was lower but non-significant with randomized assignment to aspirin compared to placebo (OR 0.83; 95% CI 0.66–1.03; $P = 0.09$, *Table 3* and *Take home figure*). In contrast, CVD rates were higher among the 31% of women with the G/A (OR 1.38; 95% CI 1.00–1.91; $P = 0.048$) or the 4% with the A/A (OR 1.91; 95% CI 0.48–7.55; $P = 0.36$) genotypes, and A-allele carriers combined (OR 1.39; 95% CI 1.03–1.89; $P = 0.03$, Supplementary material online, *Table S4*). Results were similar for a composite endpoint consisting of just stroke and MI events. The direction of the effects for the individual MI and stroke endpoints were similar to that of major CVD but were non-significant (*Table 3*). Adjustment for a panel of CVD risk factors did not modify these effects.

In PHS, gene dosage response models based on case–control replication sets matched on age and smoking status, and controlled for CVD risk factors, the association of the rs7692387 risk (G) allele with the combined endpoint of MI and stroke (major CVD), was directionally concordant with previously observed increased risk (OR 1.31; 95% CI 0.66–2.61; $P = 0.44$, *Table 2*). The interaction between randomized aspirin allocation and rs7692387 genotype was significant for MI ($P_{interaction} = 0.02$), but not significant for major CVD ($P_{interaction} = 0.06$), or stroke ($P_{interaction} = 0.23$). Furthermore, the significant interaction for MI persisted through the post-randomization observational period from 1988 to 1990 ($P_{interaction} = 0.02$).

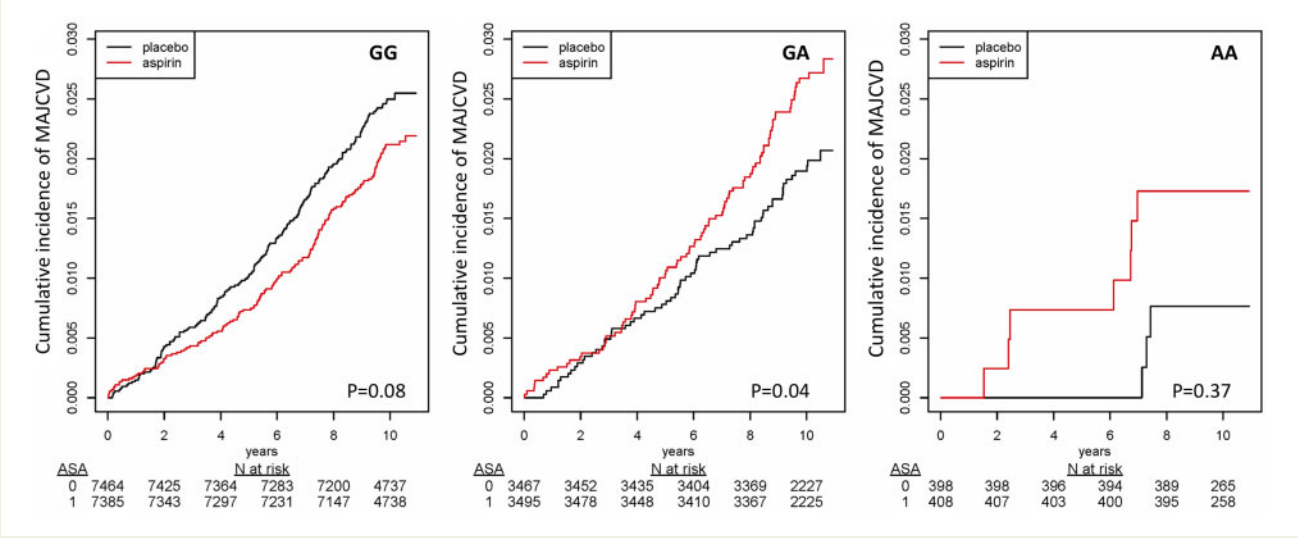
In PHS, the direction of the randomized aspirin effects in the genotype strata was directionally consistent with that observed in WGHS (*Table 3*), with lower risk of major CVD among individuals homozygous for the risk allele (G/G) in fully adjusted analysis (OR 0.49; 95% CI 0.27–0.89; $P = 0.02$, Supplementary material online, *Table S4*). A significant association with MI but not with ischaemic stroke appeared to drive the association such that in fully adjusted models, men homozygous for the risk allele (G/G) had lower risk of MI (OR 0.41; 95% CI 0.19–0.87; $P = 0.02$) with aspirin allocation, while heterozygous individuals (G/A) displayed higher risk (OR 3.28; 95% CI 1.08–9.95; $P = 0.04$) (data not shown), but the combined effect among A-allele carriers was not significant (OR 2.36; 95% CI 0.85–6.57; $P = 0.10$). Meaningful effects for MI in carriers of the A/A genotype could not be estimated due to a very small number of cases ($n = 13$), but the direction was concordant with heterozygous individuals in WGHS. Similar results were observed for the two other *GUCY1A3* variants in linkage disequilibrium (Supplementary material online, *Table S5*).

To evaluate the overall effect of aspirin compared to placebo in both sexes and to increase the statistical power, random-effects meta-analyses of the WGHS and PHS effect estimates in genotype strata were performed and both resulted in a significant major CVD risk reduction in individuals homozygous for the rs7692387 risk allele (OR 0.79; 95% CI 0.65–0.97; $P = 0.03$; $I^2 = 0$) in age-adjusted models (*Table 3*). In contrast, in heterozygous individuals, the effect was

Table 1 Gene dosage model effects of *GUCY1A3* rs7692387 risk (G) allele[a] on incident cardiovascular disease among Women's Genome Health Study women randomized to aspirin or placebo

		Aspirin (N = 11 288)			Placebo (N = 11 329)			
Model[b]	Outcome	Events	HR (95% CI)	P-value	Events	HR (95% CI)	P-value	$P_{interaction}$
Age-adjusted	Major CVD[c]	251	0.90 (0.72–1.11)	0.31	253	1.38 (1.08–1.78)	0.01	0.01
	MI	104	0.84 (0.61–1.16)	0.29	104	1.28 (0.87–1.87)	0.20	0.10
	Stroke	96	1.01 (0.71–1.44)	0.96	113	1.41 (0.97–2.06)	0.07	0.21
Fully adjusted	Major CVD	208	0.87 (0.69–1.10)	0.25	210	1.39 (1.05–1.84)	0.02	0.02
	MI	89	0.82 (0.57–1.18)	0.28	86	1.22 (0.80–1.87)	0.36	0.17
	Stroke	77	0.91 (0.62–1.34)	0.62	95	1.54 (1.00–2.36)	0.05	0.08

BMI, body mass index; HR, hazard ratio; MI, myocardial infarction.
[a]Allele key: rs7692387 coded (risk allele) = G, reference = A. Genotype data from the exome array, N = 22 617 (see Methods section).
[b]Cox gene dosage models were adjusted for age or fully adjusted for standard cardiovascular risk factors: age, systolic blood pressure, diastolic pressure, LDL-cholesterol, HDL-cholesterol, BMI, family history of MI, family history of diabetes and smoking. Observations with incomplete data were not included in the analyses.
[c]Major CVD, the primary WGHS outcome is a composite of MI, stroke, or death from cardiovascular causes.



Take home figure Kaplan–Meier curves showing cumulative incidence of major cardiovascular disease (MAJCVD) events across the 10 years of the Women's Genome Health Study (N = 22 617) stratified by randomized aspirin (ASA, red) or placebo (black) allocation and *GUCY1A3* rs7692387 genotype: (*A*) GG stratum; (*B*) GA stratum; and (*C*) AA stratum.

reversed resulting in significantly increased risk of major CVD with aspirin treatment (OR 1.39; 95% CI 1.03–1.87; P = 0.03; I^2 = 0). Results were essentially unchanged when A-allele carriers were combined (OR 1.39; 95% CI 1.03–1.87; P = 0.03; I^2 = 0, Supplementary material online, *Table S4*).

GUCY1A3 genotype and bleeding risk

In the WGHS, rates of a composite endpoint of all bleeding events increased significantly with aspirin use in all rs7692387 genotype groups ('all bleeds', Supplementary material online, *Table S6*). However, with increasing numbers of the CVD risk (G) alleles, 'all bleeds' showed weak trends toward decreased bleeding within the aspirin stratum and increased bleeding within the placebo stratum with higher rates of transfusion, gastrointestinal bleeds, and peptic ulcers observed among G/A heterozygotes compared to G/G homozygotes. These divergent trends, although individually non-significant, nevertheless were significantly different for the 'all bleeds' ($P_{interaction}$ = 0.04) and transfusion ($P_{interaction}$ = 0.03) endpoints. Omitting the A-allele carriers yielded similar results with significant interactions for gastrointestinal bleeds ($P_{interaction}$ = 0.03) and peptic ulcers ($P_{interaction}$ = 0.02).

Discussion

Here, we demonstrate an interaction between the outcome of aspirin therapy and a genetic variant in the *GUCY1A3* gene. In two major primary CVD prevention trials, aspirin was found to confer a significant (21%) reduction in risk of major CVD amongst the 66% of

Table 2 Gene dosage effects of *GUCY1A3* rs7692387 risk (G) allele on incident cardiovascular disease among Physician's Health Study men randomized to aspirin or placebo[a]

Model[a]	Outcome	Cases/controls	Aspirin OR (95% CI)	*P*-value	Placebo OR (95% CI)	*P*-value	$P_{interaction}$
Before 1988[b]	Major CVD	228/260	0.43 (0.17–1.04)	0.06	1.31 (0.66–2.61)	0.44	0.06
	MI	163/192	0.29 (0.09–1.00)	0.05	2.04 (0.91–4.54)	0.08	0.02
	Stroke	65/68	0.46 (0.10–2.23)	0.34	0.08 (0.00–1.26)	0.07	0.23
Through 1990	Major CVD	409/523	0.51 (0.28–0.92)	0.03	0.88 (0.52–1.51)	0.65	0.19
	MI	250/300	0.36 (0.15–0.88)	0.02	1.42 (0.72–2.77)	0.31	0.02
	Stroke	159/223	0.67 (0.28–1.59)	0.36	0.40 (0.14–1.12)	0.08	0.43

[a]Estimates from conditional logistic regression gene dosage models including terms for additive genetic effect (on the log scale) of the 'G' risk allele, randomized drug allocation, and interaction between the genetic effect and drug allocation. The genetic effect in aspirin or placebo strata, respectively, was estimated by encoding drug allocation as 0 = placebo/1 = aspirin or 1 = placebo/0 = aspirin. Controls were matched on age and smoking history were used only once.
[b]Models were adjusted for CVD risk factors: history of diabetes, cholesterol, BMI, systolic and diastolic blood pressure. Models: Before 1988 = white cases before 25 January 1988 when randomization to aspirin was terminated; through 1990 = all white cases and controls through 1990 when the trial ended.

Table 3 Combined effect of aspirin compared to placebo on risk of cardiovascular disease, myocardial infarction, and stroke by *GUCY1A3* rs7692387 genotype strata in Women's Genome Health Study and Physician's Health Study[a]

Outcome	Trial	rs7692387	Events/*N* cases/controls	OR (95% CI)[b]	*P*-value	*I2*
CVD	WGHS	G/G	335/14 849	0.83 (0.66–1.03)	0.09	
	PHS	G/G	154/195	0.63 (0.38–1.03)	0.07	
	Overall[c]	G/G		0.79 (0.65–0.97)	0.03	0
	WGHS	G/A	99/6962	1.38 (1.00–1.91)	0.05	
	PHS	G/A	68/81	1.42 (0.66–3.05)	0.37	
	Overall	G/A		1.39 (1.03–1.87)	0.03	0
MI	WGHS	G/G	137/14 849	0.85 (0.61–1.20)	0.36	
	PHS	G/G	110/136	0.58 (0.31–1.05)	0.07	
	Overall	G/G		0.77 (0.55–1.07	0.12	13
	WGHS	G/A	64/6962	1.28 (0.78–2.11)	0.33	
	PHS	G/A	48/53	2.24 (0.90–5.59)	0.08	
	Overall	G/A		1.48 (0.92–2.38)	0.11	10
Stroke	WGHS	G/G	138/14 849	0.74 (0.53–1.04)	0.08	
	PHS	G/G	44/59	0.76 (0.30–1.91)	0.56	
	Overall	G/G		0.74 (0.54–1.02)	0.07	0
	WGHS	G/A	67/6962	1.09 (0.67–1.77)	0.72	
	PHS	G/A	20/28	0.32 (0.06–1.82)	0.20	
	Overall	G/A		0.99 (0.62–1.59)	0.98	46

[a]White cases and controls before 25 January 1988.
[b]Odd ratios were estimated from logistic models. Models were adjusted for randomized treatment assignment, age, and smoking.
[c]Random effects meta-analysis.

participants who were homozygous for the rs7692387 risk (G) allele. Conversely, among the 31% of participants who were heterozygous (G/A), rates of major CVD significantly increased by 39% with randomization to aspirin. The aspirin effects in non-risk allele homozygotes were similar to the heterozygotes, but there were too few participants to estimate the overall effect in this group. Hence, these results suggest a significant benefit of aspirin in primary prevention among homozygotes of the *GUCY1A3* CVD risk allele (G), but potential increased risk among carriers of the non-risk allele (A).

Loss-of-function alleles in *GUCY1A3* had been found to enhance platelet aggregation and to co-segregate with MI in families.[11,29] Our study focused on a common *GUCY1A3* rs7692387 risk allele because—in addition to its broadly replicated effects on coronary risk and blood pressure[14,15]—functional assessment revealed that this variant results in reduced expression of the alpha1-subunit of sGC,[13] an enzyme that counterbalances pro-thrombotic stimuli in platelets.[30] Due to reduced cGMP-mediated signalling, platelet aggregation in homozygotes of the risk allele is less inhibited by nitric oxide which could lead to greater benefit from antiplatelet treatment in primary prevention.[13] Consistent with our hypothesis, in the combined analysis of both studies, homozygous *GUCY1A3* risk allele carriers experienced a marked reduction in CVD risk with randomization to aspirin.

Unexpectedly, in both studies, individuals who were not homozygous for the *GUCY1A3* risk allele displayed increased CVD event rates when treated with aspirin. The biological mechanisms underlying the increase in risk observed in non-risk allele carriers are not known. One hypothesis is that non-risk allele carriers taking aspirin display more pronounced platelet inhibition, and this may in turn increase their risk of bleeding. Indeed, in a recent study we found a lower on-aspirin platelet reactivity in non-risk allele carriers which translated into a lower incidence of cardiovascular death/stent thrombosis within 30 days after coronary stenting.[31] Whereas lower on-aspirin platelet reactivity might reduce the risk for ischaemic events in such patients, it could increase risk of bleeding in primary prevention. Our findings of higher rates of transfusion, gastrointestinal bleeding, and peptic ulcers among G/A heterozygotes are consistent with this hypothesis. Bleeding, in particular gastrointestinal bleeding can induce anaemia,[32] hypotension, and reduce oxygen-carrying capacity, which are in turn associated with myocardial injury[33] and subsequent ischaemic events including MI and stroke.[34] Nevertheless, these hypotheses remain untested and further studies should address the exact mechanism and magnitude of aspirin counterbalancing sGC activity depending on the number of *GUCY1A3* risk alleles.[35,36]

In addition to contributing to CVD risk assessment, knowledge of *GUCY1A3* genotype might help discriminate low- and intermediate-risk individuals who may—or may not—benefit from aspirin. In the WGHS, where women were free from any CVD at entry, the number of homozygous risk allele carriers to be treated to avoid one major CVD event was 121. Likewise, in the PHS, aspirin completely neutralized the risk increase otherwise seen in subjects homozygous for the risk allele. While benefit of aspirin was accompanied by increased rates in bleeding in WGHS, the rates of bleeding were relatively lower for risk allele homozygotes ($P_{interaction} = 0.04$).

Our study has several limitations. First, this is a *post hoc* analysis of two randomized clinical trials. Besides the inherent limitations of this approach, both studies were conducted several years ago and may not reflect current prevalence of risk factors and treatments. Nevertheless, both studies are unique and have made major contributions to current guidelines on aspirin use in primary prevention. Second, measures of sGC availability and activity or platelet function were not available in these studies making it impossible to correlate genotype and outcome to intermediate phenotypes. Third, while effects were similar for both MI and ischaemic stroke in the WGHS, a comparable effect among men in PHS was observed only for MI (and overall CVD) but not stroke, which cannot be explained using the available data and warrants further exploration. Interestingly, the original publication of PHS reported a reduction of incidence of MI but a trend towards increased incidence of stroke with aspirin.[20] Fourth, our data apply only to subjects of Western European descent in whom the risk allele was identified and functionally characterized. Fifth, these results have to be regarded as preliminary until validated by prospective trials and, importantly, this study does not question the role of aspirin in secondary prevention of CAD. Finally, whereas the direction of effect was the same, the effect size of the risk allele in the placebo stratum was larger in WGHS than in the published data. This may reflect the specific aspects or homogeneous nature of the WGHS or its prospective design as compared to the clinical case–control samples that dominate the CARDIoGRAMplusC4D meta-analysis.[10]

Interestingly, overall neutral results of aspirin in primary prevention were recently reported for the ASPREE,[37] and ASCEND[35] trials, which support reducing use in primary prevention at this time. This is in agreement with the current European guidelines on CVD prevention, in which aspirin is recommended in individuals with established CVD (IA) but not without CVD (IIIA).[38] Despite recommendations for limiting aspirin in US and European guidelines, it remains amongst the most extensively used medications for primary prevention of CVD, and is regarded as widely overused in this indication.[7] US surveys report that ~40% of men over 40 years old take aspirin for CVD prevention.[39] About 37% of these individuals will carry a *GUCY1A3* non-risk allele, suggesting millions of Americans taking aspirin for primary CVD prevention may have a genotype that was found to have increased risk in both the WGHS and PHS. However, individuals taking aspirin for primary prevention should consult their physician if they consider stopping aspirin as this might increase CVD risk.[40]

In conclusion, our findings present an example of the potential for genetics in precision medicine to differentiate between potential benefit and harm. Prospective, randomized, placebo-controlled trials studies of aspirin stratified by *GUCY1A3* genotype will be needed to further evaluate the extent to which aspirin may be useful for reducing incidence of CVD in primary prevention.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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