Pharmacogenetics of aspirin resistance: a comprehensive systematic review

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- A genetic basis for aspirin resistance has been postulated to exist.
- The individual studies published have been too small to allow reliable conclusions to be drawn.
- In order to clarify what, if any, genetic basis exists for aspirin resistance, we have undertaken, to our knowledge, the largest and most comprehensive systematic review conducted to-date in this field.

WHAT THIS STUDY ADDS

- There is a significant association between the PIA1/A2 molecular variant and aspirin resistance in healthy subjects, with the effect diminishing in the presence of cardiovascular disease.
- However further studies are needed to confirm this.
- We argue that investigators now need to agree on a standard technique to measure and define aspirin resistance.

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AIMS

The aim was to perform a systematic review of all candidate gene association studies in aspirin resistance.

METHODS

Electronic databases were searched up until 1 December 2007 for all studies investigating any candidate gene for aspirin resistance in humans. Aspirin resistance was required to have been measured by a standardized laboratory technique to be included in the analysis.

RESULTS

Within 31 studies, 50 polymorphisms in 11 genes were investigated in 2834 subjects. The PIA1/A2 polymorphism in the GPIIb/IIIa platelet receptor was the most frequently investigated, with 19 studies in 1389 subjects. The PIA1/A2 variant was significantly associated with aspirin resistance when measured in healthy subjects [odds ratio (OR) 2.36, 95% confidence interval (CI) 1.24, 4.49; $P = 0.009$]. Combining genetic data from all studies (comprising both healthy subjects and those with cardiovascular disease) reduced the observed effect size (OR 1.14, 95% CI 0.84, 1.54; $P = 0.40$). Moreover, the observed effect of PIA1/A2 genotype varied depending on the methodology used for determining aspirin sensitivity/resistance. No significant association was found with aspirin resistance in four other investigated polymorphisms in the COX-1, GPIa, P2Y1 or P2Y12 genes.

CONCLUSIONS

Our data support a genetic association between the PIA1/A2 molecular variant and aspirin resistance in healthy subjects, with the effect diminishing in the presence of cardiovascular disease. The laboratory methodology used influences the detection of aspirin resistance. However, as heterogeneity was significant and our results are based on a limited number of studies, further studies are required to confirm our findings.

Introduction

Acetylsalicylic acid (aspirin) is the most commonly used antiplatelet drug worldwide in both primary and secondary

prevention of cardiovascular disease [1–4]. High-risk vascular patients treated with aspirin have a 34% reduction in nonfatal myocardial infarction (MI), 25% reduction in non-fatal stroke and 18% reduction in all-cause mortality [4].

Aspirin acts by irreversibly inhibiting the cyclooxygenase-1 (COX-1) enzyme through acetylating the serine residue at position 529. COX-1 catalyses the conversion of arachidonic acid to prostaglandins G_2 and H_2 , which are subsequently converted by thromboxane synthase to thromboxane A₂ (TXA₂), a potent vasoconstrictor and activator of platelet aggregation. However, the antiplatelet effects of aspirin may not be equal in all individuals. A proportion of patients prescribed aspirin suffer recurrent thromboembolic vascular events, giving rise to the term 'aspirin resistance'. This term, however, is probably misleading, because there may be a number of reasons why patients do not respond to aspirin, such as poor adherence to therapy, and it may be more appropriate for this situation to be defined as 'clinical treatment failure' rather than resistance [5].

Aspirin resistance is probably better defined biochemically and/or functionally using measures such as: (i) light transmission aggregometry [6], (ii) bleeding time [7], (iii) platelet function analyzer-100 (PFA-100) [8], (iv) VerifyNow Aspirin® system [9], and (v) levels of serum TXB₂ or urinary 11-dehydroTXB₂ (spontaneous degradation products of TXA₂) [10]. Each method has its own advantages and disadvantages [11, 12]. The problems with determining drug adherence [13] and the different methodologies available for determining the biochemical and functional effects of aspirin have compounded the problems of attempting to define aspirin resistance, with some investigators suggesting that the term should be used only when production of TXA₂ (or its breakdown products) is blocked regardless of platelet function [10]. Since there is no single definition of, or validated method to identify patients with, aspirin resistance, its reported prevalence varies greatly in different studies, from 5 to 45% [14], and in one study as high as 60% [15].

Aspirin resistance is likely to be multifactorial in origin. Reduced absorption and/or increased metabolism of aspirin may contribute, as may biosynthesis of TXA₂ from pathways not inhibited by aspirin as well as alternative pathways involved in platelet activation not blocked by aspirin [e.g. those involving adenosine diphosphate (ADP), collagen, epinephrine and thrombin]. Moreover, some of the literature suggests that a majority of aspirin resistance reported may be the result of poor adherence [16–18].

A genetic aetiology to aspirin resistance has also been proposed. A number of studies have examined the association of aspirin resistance with single nucleotide polymorphisms (SNPs) in the genes for COX-1 and for several receptors on the surface of platelets [19]. These individual studies have been too small to allow reliable conclusions to be drawn, and have rarely taken into account the different available biochemical and functional methodologies, thus giving rise to conflicting results.

In order to clarify what, if any, genetic basis exists for aspirin resistance, we have undertaken a comprehensive systematic review of all genetic studies on aspirin resis-

tance, encompassing both patients with cardiovascular disease and healthy subjects.

Methods

Data sources

Electronic databases (MEDLINE, EMBASE and Google Scholar) were searched up to 1 December 2007 for all case-control studies evaluating any candidate gene and aspirin resistance in humans. Letters and abstracts were included in the systematic review. The Medical Subject Headings terms and text words used for the search were 'aspirin', 'acetylsalicylic acid', 'aspirin resistance' and 'aspirin non-responder' in combination with 'genetic', 'polymorphism', 'mutation', 'genotype', or 'gene'. The search results were limited to human. All languages were searched and included. The references of all computer-identified publications were hand-searched for any additional studies, and the MEDLINE option *related articles* was used to identify other relevant articles. Studies were required to have measured aspirin resistance using validated laboratory methods described previously. Studies that defined aspirin resistance from a clinical perspective but did not confirm this using laboratory methods were excluded from the analysis.

Data extraction

The primary search generated 35 potentially relevant articles, of which 31 met the inclusion criteria. Data for analysis were extracted independently and entered into separate databases.

Statistical analysis

Data were analysed using software for preparing and maintaining Cochrane reviews (Review Manager, version 4.1; Cochrane Collaboration, Syracuse, NY, USA) and meta-analysis software (Comprehensive Meta-analysis, version 2; Biostat, Englewood, NJ, USA). For each genetic marker (polymorphism) for which data were available for at least two studies, a meta-analysis was carried out. For each gene variant, a pooled odds ratio (OR) was calculated using fixed- and random-effects models, along with the 95% confidence interval (CI) to measure the strength of the genetic association. Fixed-effects summary ORs were calculated using the Mantel-Haenszel method [20, 21] and the DerSimonian and Laird method was used to calculate random-effects summary ORs [22].

Tests for heterogeneity were performed for each meta-analysis (with significance set at $P < 0.05$) [23]. For assessment of publication bias, we used the funnel plot and the Egger regression asymmetry test [24]. In addition, the effect of individual studies on the summary OR was evaluated by re-estimating and plotting the summary OR in the absence of each study.

Results

Thirty-one candidate gene studies of aspirin resistance were found and analysed. In total, 50 polymorphisms in 11 genes were identified. Of these, data were available from at least three studies for 10 polymorphisms in six genes. For another seven polymorphisms, two studies per genetic marker were identified, and in the case of 33 polymorphisms only one study per genetic marker was identified. Table 1 summarizes all polymorphisms related to aspirin resistance that have had at least two studies published. Genotype frequency was required to be available for all groups studied. Table 2 summarizes the five polymorphisms that were analysed statistically, and the number of studies which could be used for our analysis.

The PIA1/A2 SNP present in the GPIIb/IIIa receptor, expressed on the surface of platelets, has been by far the

Table 1

Polymorphisms in genes related to aspirin resistance that have had at least two studies published

Gene	Polymorphism	Number of studies
COX-1	C22T	6
COX-1	C50T/A842G	8
COX-1	G128A	2
COX-1	C644A	4
COX-1	C714A	4
COX-1	C10427A	2
COX-1	G1446A	2
COX-2	G765C	2
GPIa	C807T	7
GPIb α	C5T	3
GPIIIa	T196C	19
GPVI	T13254C	2
FXIII	G34T	2
P2Y1	C893T	2
P2Y1	A1622G	3
P2Y12	H1/H2	4

most investigated, with 19 studies that included 1389 cases [25–43]. Ten of the 19 studies provided enough detailed information on genotype frequency to enable statistical analysis. The remaining nine studies are summarized in Table 3.

The 10 studies on the PIA1/A2 SNP analysed statistically included 673 subjects sensitive, and 295 subjects resistant, to aspirin. An OR of 1.14 (95% CI 0.84, 1.54; $P = 0.40$) was observed for aspirin resistance in subjects carrying the PIA2 allele (PIA1A2 + PIA2A2) (Figure 1a). Significant interstudy OR heterogeneity was observed ($\chi^2 = 24.36$; $P_{\text{Het}} = 0.004$). The distribution of the OR in relation to its standard deviation in the funnel plot was symmetrical, suggesting a low probability of publication bias, although this possibility cannot be discounted completely.

The measurement technique used to define aspirin resistance and the population studied varied greatly from one study to another; therefore a further subanalysis was undertaken. Out of the 10 studies, four investigated aspirin resistance in healthy subjects [33, 34, 36, 40] and six used subjects who had cardiovascular diseases of different types [35, 37–39, 41, 42]. Carriers of the PIA2 allele in healthy subjects were 2.36 times more likely to be resistant to aspirin when calculated using the fixed effects model (95% CI 1.24, 4.49; $P = 0.009$). Significant OR heterogeneity was observed ($\chi^2 = 9.34$; $P_{\text{Het}} = 0.03$). This significance was lost when the data were analysed using the random effects model (OR for aspirin resistance 2.83; 95% CI 0.62, 12.80; $P = 0.18$). By contrast, there was no significant association between carriage of the PIA2 allele and aspirin resistance in subjects with cardiovascular disease (OR for aspirin resistance 0.92; 95% CI 0.65, 1.30; $P = 0.64$), and again significant OR heterogeneity was observed ($\chi^2 = 11.19$; $P_{\text{Het}} = 0.05$) (Figure 1b).

The methods used to measure aspirin resistance were PFA-100 (five studies) [35–37, 39, 40], light transmission aggregometry (three studies) [38, 40, 42], bleeding time (one study) [33] and thrombin generation (one study) [34].

Table 2

Candidate genes relating to aspirin resistance which were analysed statistically

Gene (no of studies)	Variant	Genetic model	Total no: aspirin resistant	Total no: aspirin sensitive	OR (95% CI)	P-value
GPIIIa (10)	PIA1/A2	Dominant	295	673	1.14 (0.84, 1.54)	0.4
GPIa (4)	C807T	Dominant	90	204	1.37 (0.81, 2.31)	0.24
COX-1 (3)	A842G (C50T)	Dominant	27	152	1.07 (0.41, 2.77)	0.89
P2Y12 (3)	H1/H2	Dominant	66	226	0.90 (0.48, 1.68)	0.73
P2Y1 (2)	A1622G	Dominant	101	267	0.80 (0.48, 1.33)	0.39

Table 3

Summary of studies investigating the association of the PIA/1A2 SNP in the GPIIIa receptor gene and aspirin resistance which could not be included in the statistical analysis

Citation	Population studied	Method	Comment
Andrioli G <i>et al.</i> Br J Haematol 2000; 110: 911–8 [25]	Healthy (n = 16)	AA- induced aggregation	PIA1A1 homozygotes associated with reduced response to aspirin.
Cooke GE <i>et al.</i> The Lancet 1998; 351 (9111): 1253 [26]	Healthy (n = 26)	ADP + epinephrine induced aggregation	PIA1A1 homozygotes associated with reduced responses to aspirin
Cooke GE <i>et al.</i> J Am Coll Cardiol 2006; 47: 541–6 [27]	Coronary artery disease (n = 20)	ADP + collagen induced aggregation. Collagen-stimulated α -granule release measurements. Measurement of fibrinogen binding	PIA2 associated with reduced responses to aspirin
Lepantalo A <i>et al.</i> Thromb Haemost 2006; 95: 253–9 [28]	Elective percutaneous coronary intervention (n = 101)	AA, collagen, epinephrine and ADP-induced aggregation. PFA-100 (CEPI and CADP). Plasma TXB2	PIA2 associated with aspirin sensitivity
Lim E <i>et al.</i> Ann Thorac Surg 2007; 83: 134–9 [29]	Patients who have undertaken a cardiopulmonary bypass (n = 63)	ADP, collagen epinephrine induced aggregation	PIA2 associated with reduced responses to aspirin
Dropinski J <i>et al.</i> Thromb Res 2007; 119: 301–3 [30]	Subjects suffered MI within 6 months (n = 28)	Thrombin generation, bleeding time	PIA2 associated with reduced responses to aspirin
Morawski W <i>et al.</i> J Thorac Cardiovasc Surg 2005; 130: 791–6 [31]	Patients undergoing a coronary artery bypass (n = 102)	Bleeding time PFA-100	PIA2 associated with aspirin sensitivity
Stepien E <i>et al.</i> Pol Arch Med Wewn 2007; 117: 33–40 [43]	Patients with stable angina (n = 31)	Thrombin generation and sCD40L release	PIA2 allele associated with thrombin generation but not sCD40L release
Undas A <i>et al.</i> Circulation 2001; 104: 2666–72 [32]	Healthy (n = 24)	Bleeding time. Prothrombin, thrombin–antithrombin III complex, factor V/Va, factor XII/XIIa, fibrinogen and fibrinopeptides A + B measurements	PIA2 associated with reduced responses to aspirin

Thrombin generation was measured from bleeding time wounds, thus these two measurements were grouped together for analysis. Kranzhofer *et al.* [41] was not included in the analysis, as both light transmission aggregometry and PFA-100 were undertaken with the results being combined. Fontana *et al.* [40] was included twice in the analysis, as both PFA-100 and light transmission aggregometry were undertaken in this study, and results were displayed separately for each technique used.

Studies which used light transmission aggregometry showed that PIA2 carriage conferred an OR of 1.44 (95% CI 0.90, 2.30; $P = 0.12$; $\chi^2 = 0.87$; $P_{\text{Het}} = 0.65$) for aspirin resistance. By contrast, studies which used PFA-100 suggested that PIA2 carriage might confer aspirin sensitivity, although this did not reach significance (OR 0.71; 95% CI 0.44, 1.14; $P = 0.16$; $\chi^2 = 8.64$; $P_{\text{Het}} = 0.07$). In the two remaining studies which used bleeding time or thrombin generation, PIA2 carriers were 11.76 times more likely to be aspirin resistant, and this was highly significant (95% CI 3.05, 45.37; $P = 0.0003$; $\chi^2 = 0.43$; $P_{\text{Het}} = 0.51$) (Figure 1c).

Four other polymorphisms were analysed statistically, each with a small sample size. No significant association was observed with any of these polymorphisms studied (Figure 2). Seven studies [28, 29, 35, 36, 39, 40, 44] (585 cases) examined the C807T polymorphism present on the GPIa receptor gene, although due to lack of relevant information, outlined above, only four studies (294 cases) [35, 36, 39, 40] were statistically analysed (OR for aspirin resistance 1.37; 95% CI 0.81, 2.31; $P = 0.24$; $\chi^2 = 2.51$; $P_{\text{Het}} = 0.47$). The A842G and C50T SNPs in the COX-1 gene are in linkage disequilibrium, and eight studies [28, 29, 36, 41, 44–47] examined the association of these with aspirin resistance, although only three could be statistically analysed [28, 36, 41] (OR for aspirin resistance 1.07; 95% CI 0.41, 2.77; $P = 0.89$; $\chi^2 = 5.55$; $P_{\text{Het}} = 0.06$). Four studies [39, 40, 42, 48] examined aspirin resistance in relation to the H1/H2 SNP in the P2Y12 receptor gene, three studies [39, 40, 42] were examined statistically which included 292 cases (OR for aspirin resistance 0.90; 95% CI 0.48, 1.68; $P = 0.73$; $\chi^2 = 0.76$; $P_{\text{Het}} = 0.68$). The A1622G SNP in the P2Y1 gene had three

Figure 1

Association between the PIA1/A2 polymorphism and aspirin resistance. Odds ratios (OR) are shown for the outcome comparing carriers of PIA2 (PIA1/A2 or PIA2A2) vs. wild-type (PIA1/A1). (a) All subjects. (b) Subanalysis in subgroups of subjects who were healthy or who had cardiovascular disease. (c) Subanalysis according to type of measurement used to define aspirin resistance. CI, confidence interval

C

Figure 1

Continued

studies [42, 49, 50] published in relation to aspirin resistance. Two studies [42, 49] could be analysed statistically with 368 cases (OR for aspirin resistance 0.80; 95% CI 0.48, 1.33; $P = 0.39$; $\chi^2 = 0.02$; $P_{\text{Het}} = 0.87$).

Discussion

Genetic determinants of aspirin resistance

In this comprehensive systematic review, the laboratory tests used to measure aspirin resistance suggest that the commonly studied PIA1/A2 variant in the GPIIIa receptor gene was highly significantly associated with aspirin resistance when measured in healthy subjects. Combining genetic data from all studies (including studies with patients with cardiovascular disease) reduced the observed effect size markedly. One possible explanation for this is that patients with cardiovascular disease were likely to be on a variety of medications that influence platelet function, such as organic nitrates and statins, contrasting with healthy subjects who were on no medication and had not taken any medication for at least 10 days prior to

study. It is possible that such medication might cause a degree of platelet inhibition, thereby obscuring the presence of aspirin resistance; however, this is highly speculative and cannot be inferred from the data available in the literature reviewed. Alternatively, subjects with cardiovascular disease may differ statistically from those free of such disease. Delineation of the true reasons for the difference found between healthy subjects and those with cardiovascular disease needs to be done within the context of a future prospective randomized study.

The GPIIb/IIIa receptor is a key regulator of platelet aggregation. Upon platelet activation the receptor is able to bind to fibrinogen and link adjacent platelets to one another. Consequently, polymorphisms within the GPIIb/IIIa receptor have been of great interest with regard to aspirin resistance, the most commonly investigated being the PIA1/A2 SNP.

Some studies have suggested an association between the presence of the PIA2 allele and increased platelet activity, as determined by platelet aggregation and/or fibrinogen binding [51–54]. On the other hand, other studies have found no effect of PIA2 carriage on platelet activity [25, 55,

Figure 2

Association between the GP1a (C807T), COX-1 (A842G/C50T), P2Y12 (H1/H2) and P2Y1 (A1622G) polymorphisms and aspirin resistance. Odds ratios (ORs) are shown for the outcome comparing carriers of the mutant alleles vs. wild type. CI, confidence interval

56]. This lack of consistency in the literature makes it difficult to conclude whether the observed effect of carriage of the PIA2 allele on aspirin sensitivity in healthy subjects is in fact due to effects on platelet activity.

Further analysis suggested that the effect of the PIA1/A2 polymorphism appears to differ depending on the technique used to measure aspirin resistance. Studies which used thrombin generation or bleeding time as a measurement showed, in a highly significant manner, that aspirin resistance is related to carriage of PIA2. However, this subanalysis included only 220 subjects. Studies that used PFA-100 and light transmission aggregometry showed no significant association. Indeed, our analysis suggests that when PFA-100 is used to identify aspirin resistance, the PIA2 allele may actually confer

aspirin sensitivity, although this was not significant; by contrast, results from studies using light transmission aggregometry suggest that subjects may be more likely to be aspirin resistant when carrying the PIA2 allele.

This lack of concordance between the different methods used to measure aspirin resistance was highlighted by Fontana *et al.* [40]. Both light transmission aggregometry and PFA-100 were used in a cohort of 96 healthy subjects. One subject was identified resistant using arachidonic acid-induced aggregation, whereas 28 subjects were defined as resistant using PFA-100. From this study and others, the PFA-100 technique appears to be far less discriminating in defining subjects who are aspirin resistant compared with light transmission aggregometry. Thus, the PFA-100 technique might erroneously define

subjects as aspirin resistant, diluting the apparent effect of the PIA1/A2 polymorphism on aspirin resistance. Conversely, light transmission aggregometry may be too insensitive in detecting aspirin resistance. Arachidonic acid aggregation directly measures the degree of inhibition of COX-1, whereas PFA-100 measures the activation of platelets through ADP, collagen and epinephrine, pathways that are not specifically inhibited by aspirin. It could therefore be argued that PFA-100 is a measurement of platelet activity rather than of aspirin resistance.

Variance between different measurements of aspirin resistance has been further confirmed by Lordkipanidze *et al.* [57]. These authors investigated the prevalence of aspirin resistance in 201 subjects with stable coronary artery disease. Prevalence of aspirin resistance varied greatly depending on the technique used, being relatively low with arachidonic acid-induced aggregation (4%) and VerifyNow Aspirin® (6.7%), whereas it was very high with ADP-induced aggregation (51.7%) and PFA-100 (59.5%). These findings make it especially difficult to ascertain whether the PIA1/A2 polymorphism, and indeed other molecular variants, is associated with platelet activation or whether they are truly genetic determinants of the inhibitory effect of aspirin on platelets.

Analysis of four other polymorphisms, namely GP1a (C807T), COX-1 (A842G/C50T), P2Y12 (H1/H2) and P2Y1 (A1622G), revealed no apparent association with aspirin resistance. However, the number of studies and of subjects used was small, making it difficult to exclude definitively any contribution of these polymorphisms to aspirin resistance. Due to the inhibitory action of aspirin on COX-1, this would be the most obvious gene to study with regard to aspirin resistance. However, our analysis has provided little evidence for such an association. Recent studies by Frelinger *et al.* [18] and Meen *et al.* [58] have shown that aspirin resistance in a number of subjects may be independent of both COX-1 and COX-2, although the precise mechanism is still unknown.

A recent systematic review by Krasopoulos *et al.* has concluded that aspirin-resistant patients are at greater risk of having further cardiovascular events than patients who respond to aspirin [59]. Studies such as ours are needed to understand the mechanisms behind aspirin resistance, since such an understanding will help to address how excess cardiovascular risk can be reduced in aspirin-resistant patients.

Strengths and limitations of the study

Pooling all published data has maximized the statistical power of our study to detect the genetic association of aspirin resistance. Despite this, the number of subjects included within this systematic review is limited. This is because most studies of this type are small in size. The number of published papers also remains relatively low, as this is still a comparatively new research area. In addition, many studies could not be included within this statistical

analysis, because insufficient detailed information on genotype frequency was given. These included nine studies of the PIA1/A2 polymorphism. It also meant that a number of other polymorphisms could not be included within this analysis. This included the C893T SNP in the P2Y1 receptor, documented by Li *et al.* [50] amongst others.

Furthermore, the optimal laboratory method to define aspirin resistance has yet to be standardized. Until such time as a definitive test can be established, it is difficult to determine with certainty the role of genes in aspirin resistance. The lack of standardization between the laboratory methods increases the heterogeneity and reduces the chances of finding a genetic association. When this is taken into account, the number of subjects in each analysis becomes smaller, also reducing the likelihood of determining a genetic association with aspirin resistance. All results shown were analysed using the fixed effects model. However, when the random effects model was applied, significance was lost. This may be explained by the significant heterogeneity within the studies. Thus, larger and more robust studies are needed to truly understand whether the PIA2 allele is a risk factor for aspirin resistance in healthy subjects.

At present, the number of studies published is too small to match according to gender, ethnicity, age and methodology used to define aspirin resistance [60]. These factors will need to be taken into account in future studies in order truly to understand whether a genetic aetiology can partly explain the phenomenon of aspirin resistance.

The interpretation of any systematic review must be made within the context of its limitations, including study selection, publication bias, and variability in the methodological quality of the included studies. Many of the individual studies included in our systematic review showed no statistical significance and were interpreted by their authors as negative studies. In addition, a funnel plot on all included studies showed no substantial evidence of publication bias in the five polymorphisms analysed, but clearly such bias cannot be completely excluded. There was no language restriction and meeting abstracts were included if found through the search strategy. Moreover, rigorous selection criteria (definition of aspirin resistance and population included) enriched the meta-analyses for studies with comparable selection of participants. Thus, lack of specificity by the inclusion of studies with no clear definition of aspirin resistance or the inclusion of studies in noncardiovascular diseases was avoided.

Conclusions

Our data support a possible genetic basis for the association between the PIA1/A2 polymorphism and aspirin resistance in healthy subjects, with the effect diminishing in the presence of cardiovascular disease. However, further larger studies are needed to confirm our findings. These data strongly reinforce the argument that, in order truly to understand the genetic contribution to aspirin resistance,

investigators need to agree on a standard technique to measure and define aspirin resistance.

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