Letters to the Editor

Increased Platelet Reactivity Due to Platelet Receptor Polymporphisms? Not in the Real World

To the Editor:

We read with interest the article by Pontiggia et al,[1] discussing the possible functional implications of double homozygosity for two "prothrombothic" platelet glycoprotein receptor polymorphisms (Pl$^{A1/A2}$ of GpIIIa and C807T of GpIa) in a family with a strong history of premature cardiovascular events. We would like to report our experience in 90 stable coronary artery disease (CAD) patients, studied in the "real world" while taking their usual medications, in whom we tried to recognize environmental and genetic determinants of high-shear platelet aggregation, measured by collagen-ADP PFA-100 closure time (mimicking the high-shear conditions of diseased arteries).

Using a multivariate analysis approach, we recorded clinical variables such as age, sex, smoking, diabetes, previous myocardial infarction, and drug therapy, including use of thyenopiridines (aspirin use was not considered as it is known not to influence collagenADP PFA-100 values). We also measured laboratory variables (platelet and white blood cell count, mean platelet volume, cholesterol, triglycerides, von Willebrand factor activity as Ristocetin Cofactor Activity [vWf RCA]) and determined GpIIIa Pl$^{A1/A2}$ and GpIa C807T genotypes.

On univariate analysis, increased vWf RCA ($r=-0.372$, $P=0.0001$) and platelet count ($r=-0.207$, $P=0.04$), reduced triglyceride levels ($r=0.318$, $P=0.002$), male sex (89 ± 18 vs 105 ± 23 s, $P=0.003$) and nonusage of thyenopiridines (84 ± 16 vs 96 ± 20 s, $P=0.02$) were associated with increased platelet reactivity (ie, reduced PFA-100 closure times). In contrast, the genetic polymorphisms were not. No double homozygotes for the variant genotypes were observed in our population, and double heterozygotes did not differ from the general population (Table).

On stepwise multivariate linear regression, all the nongenetic predictors maintained their significant relation with closure time (with vWf RCA remaining the strongest predictor), with an R^2 (total explained variance) of 0.52, indicating a good predictive power. When the genetic predictors were forced into the model, no significant increase in the explained variance occurred.

Thus, in a general population of patients with stable CAD taking their usual medications, the presence of putative "prothrombotic" GpIa and GpIIIa polymorphisms is unlikely to exert important effects on platelet reactivity. We believe the results by Pontiggia et al[1] are very convincing. However, the authors do not claim universal applicability of their findings. Moreover, some old-fashioned predictors of platelet reactivity, such as platelet count, were not considered in their analysis, because these authors correctly used platelet-rich plasma (at a fixed concentration) for their functional tests and for PFA-100 closure times.

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1. Pontiggia L, Lassila R, Pederiva S, Schmid HR, Burger M, Beer JH. increased platelet-collagen interaction associated with double homozygosity for receptor polymorphisms of platelet GPIa and GPIIIa. *Arterioscler Thromb Vasc Biol*. 2002;22:2093–2098.

In Response:

We appreciate the contribution by Dr Porto et al, and we agree that "basic" parameters, such as the platelet count and the level of von Willebrand factor RCA, are of importance and need to be considered, particularly in the global tests, such as the PFA-100, as has been shown before.[1,2] The influence of the triglyceride levels is an interesting observation. The fact that thienopyridines (but not aspirin alone) prolong the closure times in the collagen-ADP-assays confirms previous studies. In combination with clopidogrel, however, aspirin seems to prolong the PFA-100-closure times significantly[3] and it may not really be justified not to consider it. The finding by Porto et al that single homozygous or double heterozygous patients for GPIIIa Pl^{A2} and GPIa 807T patients did not have statistically shorter PFAs than the GPIIIa Pl^{A1} or the GPIa 807Cs appears not surprising given the relatively small numbers and the huge variability (SDs of up to 28.5 s even in samples sizes of n=10), which reflects the multitude of genetic and acquired (and "procedural") risk factors. Unfortunately, a double homozygous case was not found.

In some contrast to Porto et al, di Paola et al[4] found that the collagen-receptor density of GPIa (which is regulated by the GPIa 807 C/T polymorphism) correlated well with closure times. Thus, other groups that used the same method did find indeed an measurable influence. In our study,[5] we did attempt to find a model case of double homozygosity with a clinical endpoint (family history), but without recognizable acquired risk factors or medications affecting the results. This case was then analyzed by a series of independent assays. As stated in our report, we cannot exclude other until present unknown prothrombotic factors affecting this family.

Overall, we believe that the findings of the two groups are not mutually exclusive. Whether the "real world" is better reflected by negative global test results affected by a multitude of acquired and genetic factors or by a selected model case without recognizable risk factors analyzed by several independent methods seems a rather philosophical question.

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1. Roller RE, Dorr A, Ulrich S, Pilger E. Effect of aspirin treatment in patients with peripheral arterial disease monitored with the platelet function analyzer PFA-100. *Blood Coagul Fibrinolysis*. 2002;13:277–281.
2. Carcao MD, Blanchette VS, Stephens D, He L, Wakefield CD, Butchart S, Christie DJ, Rand ML. Assessment of thrombocytopenic disorders using the Platelet Function Analyzer (PFA-100). *Br J Haematol*. 2002;117:961–964.
3. Grau AJ, Reiners S, Lichy C, Buggle F, Ruf A. Platelet function under aspirin, clopidogrel, and both after ischemic stroke: a case-crossover study. *Stroke*. 2003;34:849–854.
4. Di Paola J, Federici AB, Mannucci PM, Canciani MT, Kritzik M, Kunicki TJ, Nugent D. Low platelet alpha2beta1 levels in type I von Willebrand disease correlate with impaired platelet function in a high shear stress system. *Blood*. 1999;93:3578–3582.

Impact of Platelet GpIIIa Pl$^{A1/A2}$ and GpIa C807T Polymorphisms on Platelet Reactivity Measured as Collagen-ADP PFA-100 Closure Time in 90 Patients With Stable CAD

Gp IIIa	GP Ia 807	PFA-100 closure time, s	N
PIA1/PIA1	CC	88.8±17.8	19
	CT	88.1±17.2	37
	TT	94.6±28.5	10
PIA1/PIA2	CC	89.8±17	5
	CT	100.3±25.3	10
	TT	96.25±22.2	4
PIA2/PIA2	CC	112.5±17.7	2
	CT	104.0±12.1	3

All comparisons are nonsignificant by ANOVA.

Expanded Version of Table 2 (Brouilette et al.) Effects of CHD Risk Factors on Mean TRF Length Showing Data Separately for Cases and Controls and Any Interaction

	Adjusted Analysis		Cases Only (n=203)		Controls Only (n=180)		Interaction,
	Effect in BP (SE)	P Value	Effect in BP (SE)	P Value	Effect in BP (SE)	P Value	P Value
History of hypertension	−99 (93)	0.29	−161 (98)	0.10	+62 (202)	0.76	0.32
History of diabetes	+1 (147)	0.99	+3 (141)	0.98	−103 (531)	0.85	0.82
Smoking							
Ex-smoker vs nonsmoker	+8 (85)	0.92	+29 (117)	0.81	−85 (126)	0.50	0.52
Current vs nonsmoker	−64 (104)	0.54	−289 (152)	0.06	+226 (155)	0.15	0.03
Per pack year	−2 (2)	0.26	−2.6 (2.2)	0.25	−1.4 (4.1)	0.73	0.77
Per 1×10^6 higher white cell count	−17 (20)	0.40	−27 (22)	0.24	+17 (46)	0.71	0.35
Per mmol/L higher fibrinogen	−21 (55)	0.70	−15 (63)	0.82	−24 (100)	0.81	0.97
Per mmol/L higher CRP	−4 (8)	0.58	−1 (8)	0.88	−33 (28)	0.24	0.24
Per mmol/L higher homocysteine	+12 (9)	0.15	−4 (11)	0.72	+31 (13)	0.02	0.05

5. Pontiggia L, Lassila R, Pederiva S, Schmid HR, Burger M, Beer JH. Increased platelet-collagen interaction associated with double homozygosity for receptor polymorphisms of platelet GPIa and GPIIIa. *Arterioscler Thromb Vasc Biol*. 2002;22:2093–2098.

Telomere Length: An Independent Risk Factor for Premature MI?

To the Editor:

We read with great interest the recent report by Brouilette et al[1] on white cell telomere length and risk of premature myocardial infarction. We agree with the authors that the findings have the potential to be of great importance in understanding the etiology of coronary heart disease and in distinguishing biological from chronological aging. We were, however, concerned about two points in the analysis.

First, in Table 2, the effect of CHD risk factors on telomere length is displayed with adjustment for case status. Each of these variables differs between cases and controls, as does telomere length. The analysis controls for case status, and the results are presented as evidence for lack of a significant relationship between each of these factors and telomere length. We are concerned that, because the CHD risk factors are so different between cases and controls, adjusting for case status may artificially attenuate the effect of the risk factor on telomere length. We would like the authors to present a stratified analysis so that the effect of CHD risk factors on telomere length can be assessed in cases and controls separately.

Second, Figure 2 and the corresponding analysis are presented without apparent adjustment for age, sex, and the other CHD risk factors. While we are confident that the relationship will remain the same, we feel that it is necessary to present the adjusted analysis to fully support the authors' claim of the independence of effects of telomere length and age.

We look forward to the authors' reply and to more research on the concept of biological aging.

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1. Brouilette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol*. 2003;23:842–846.

In Response:

We thank Paynter and Watkins for their interest in our article. They request an expanded version of our original Table 2, which we are happy to provide (see Table). The expanded version contains the original analysis adjusted for case-control status and the separate analyses for cases and for controls (excluding those related to cholesterol and family history, which were carried out in only one group). It will be evident that the adjusted figures quoted in our article are in effect weighted averages of the results for cases and for controls. The final column is a test of difference between cases and controls in their effect on TRF length.

The full analysis of this study produced a vast number of probability values, so we must guard against over-interpreting every one that gets close to 0.05. The only difference between cases and controls that is perhaps notable is that associated with current smokers compared with nonsmokers (interaction P=0.03). In cases, current smokers have shorter TRF lengths (P=0.06), whereas in controls, current smokers have longer TRF lengths (P=0.15). There is no corresponding pattern in ex-smokers or when smoking is analyzed by number of pack years. While one can always invent reasons behind an observed difference in a data set, we had no prior reason to expect this particular pattern and feel that it is probably just a chance effect. Although further works needs to be done on the effect of acquired cardiovascular risk factors on telomere length, we would like to emphasize that in our study the relationship between telomere length and risk of premature myocardial infarction (MI) was independent of the measured risk factors.

Regarding Figure 2, after adjustment for age, sex, and the other risk factors, the odds ratios of MI are as follows: compared with subjects in the highest quartile for TRF, the odds ratios were 1.98 (95% CI, 0.92 to 4.29, P=0.082), 3.09 (95%CI, 1.40 to 6.80, P=0.003), and 3.34 (95%CI, 1.50 to 7.43, P=0.002), respectively in subjects in the second, third, and lowest quartile of mean TRF length. As anticipated by Paynter and Watkins, the adjustment makes little difference to the association.

We hope this additional information is helpful. We agree with Paynter and Watkins that the concept of biological ageing has considerable potential for understanding the etiology of coronary heart disease.

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Arteriosclerosis, Thrombosis, and Vascular Biology

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