ELSEVIER

Cardiovascular Research 33 (1997) 693–697

Cardiovascular Research

Glycoprotein IIIa polymorphism and risk of myocardial infarction

Nilesh J. Samani *, David Lodwick

Divisions of Cardiology and Medicine, Department of Medicine, University of Leicester, Leicester, UK

Received 8 August 1996; accepted 18 October 1996

Abstract

Objectives: To prospectively investigate whether the Pl^{A2} variant of the platelet adhesion molecule glycoprotein IIIa influences the risk of myocardial infarction. **Background:** The platelet glycoprotein IIb/IIIa receptor plays an important role in platelet aggregation. The IIIa polypeptide is polymorphic due to a single base change at position 1565 resulting in either proline Pl^{A1} or leucine Pl^{A2} at position 33 in the protein. It has recently been reported that the Pl^{A2} variant may be strongly associated with the risk of acute coronary syndromes, particularly in younger subjects. **Methods:** Pl^{A} genotypes of 242 prospectively collected cases of first myocardial infarction admitted to our Coronary Care Unit were compared with those of 209 community-based control subjects. **Results:** We found no difference in either Pl^{A} genotype ($P = 0.65$) or allele ($P = 0.64$) frequencies between cases and controls. The Pl^{A2} allele frequency was 18.2 and 19.4% in cases and controls, respectively. The age- and sex-stratified odds ratio for risk of myocardial infarction associated with the Pl^{A2} allele was 0.89 (95% CI 0.58–1.37, $P = 0.65$) and remained non-significant when the analysis was confined to subjects under the age of 60 (odds ratio 0.77, 95% CI 0.38–1.56, $P = 0.44$). There was no interaction between Pl^{A2} and other coronary risk factors. For cases, the age at myocardial infarction was not different between those carrying the Pl^{A2} allele and those not (66.3 ± 10.8 vs. 65.6 ± 11.7 years, $P = 0.63$). **Conclusions:** We conclude that, in our subjects, the Pl^{A2} variant of platelet glycoprotein IIIa is not an important risk factor for myocardial infarction.

Keywords: Myocardial infarction; Platelets; Integrins; Glycoprotein IIb/IIIa; Human; Genetics; DNA polymorphism

1. Introduction

Myocardial infarction (MI) usually occurs because of the development of an acute occlusive thrombus at the site of a ruptured atheromatous plaque in an epicardial coronary artery. Abnormalities in both the vessel wall and in blood constituents can, therefore, contribute to the development of MI [1,2]. At least part of the risk of MI is inherited [3] and recent studies have identified several genetic factors which may modify the risk [4–6].

In addition to the activation of the extrinsic clotting cascade, activation and aggregation of platelets also plays an important role in the formation of the occlusive thrombus [1,2]. Indeed, anti-platelet therapy with aspirin is a well-established part of the acute treatment of MI [7] as well as its primary and secondary prevention [8]. Platelet aggregation is mediated via the surface glycoprotein (GP) IIb/IIIa receptor complex [9], a member of the integrin family of adhesion receptors. When activated by agonists the receptor binds fibrinogen and von Willebrand factor, thereby linking platelets together into larger aggregates. An anti-GP IIb/IIIa antibody has been found to be a powerful anti-thrombotic and to be useful in prevention of ischaemic complications following percutaneous transluminal coronary angioplasty [10].

The GPIIb and GPIIIa polypeptides are coded by separate although closely linked genes [11,12]. Mutations in both genes have been shown to result in a rare moderately severe bleeding disorder called Glanzmann's thrombasthenia [13,14]. The GPIIIa gene also contains a more common polymorphism at position 1565 in exon 2 where the base can be either thymidine or cytosine. This results in a variable amino acid at position 33 in the polypeptide

* Corresponding author. Department of Cardiology, Clinical Sciences Wing, Glenfield General Hospital, Groby Road, Leicester LE3 9QP, UK. Tel.: (+44-116) 2563038; Fax: (+44-116) 2875792.

Time for primary review 19 days.

PII S0008-6363(96)00222-2

(proline or leucine) [15]. The two forms of GPIIIa, termed Pl^{A1} and Pl^{A2}, respectively, differ antigenically [15]. While no difference in platelet function has been attributed to the polymorphism, Weiss et al. [16] recently reported a strong association between the Pl^{A2} polymorphism and acute coronary syndromes. In a study of 71 cases with MI or unstable angina and 68 controls, they found the prevalence of Pl^{A2} to be 2.1 times higher in cases compared with controls. In subjects under 60 years of age, the difference was even greater with a 3.6 times higher prevalence of Pl^{A2} in cases. If true, Pl^{A2} would represent a major novel risk factor for MI whose mechanism of involvement would be important to elucidate, not least because it may offer new therapeutic and preventative opportunities. The purpose of our study was to further investigate the association of Pl^{A2} with MI in a larger cohort of subjects prospectively recruited specifically for genetic studies.

2. Methods

2.1. Case and control subjects

Cases and controls were recruited as previously described [17]. Briefly, patients admitted to the coronary care unit (CCU) at the Leicester Royal Infirmary, Leicester, who satisfied the World Health Organisation (WHO) criteria [18] for MI in terms of symptoms, enzyme elevations and electrocardiographic changes were recruited. At least two of the three WHO criteria had to be present. The CCU, serving a population of around 300 000, accounts for the majority (> 65%) of admissions with MI in Leicester. The period of recruitment was between July 1993 and April 1994, and > 97% of eligible subjects were recruited. For the purpose of this study, genotype analysis was restricted to Caucasian cases presenting with a first MI (see Section 4).

Control subjects were recruited from adult *visitors* to patients with non-cardiovascular illnesses on general medical and surgical wards at the Leicester Royal Infirmary to provide subjects likely to be representative of the source population from which the cases came. Controls were recruited concurrently with the MI cases, using information on the age and sex distribution of MI cases admitted to the CCU in the previous year, as a guide to recruit controls of a mix similar to the likely MI cases although no individual willing to participate was excluded.

Cases and controls filled in a standard questionnaire about their personal histories, had height and weights measured and provided blood samples for measurement of serum total and HDL-cholesterol and DNA analysis. The study was approved by the local clinical research ethics committee and conformed with the principles outlined in the Declaration of Helsinki.

2.2. DNA analysis

DNA was prepared from 1 ml of whole blood collected in EDTA using a standard extraction protocol [19]. Pl^A genotypes were determined as described [16] by PCR amplification of a 266 base pair region encompassing exon 2 of the gene followed by restriction enzyme digestion with *Msp*I and separation of the resulting fragments for identification on 2.5% agarose gels. The cytosine residue in (Pl^{A2}) allele causes an extra restriction site for *Msp*I resulting in three fragments of 177, 50 and 45 base pairs compared with two fragments of 221 and 45 base pairs with Pl^{A1} [16]. Briefly, 50 ng of genomic DNA was amplified in a 25 μl reaction containing 20 mM Tris HCl (pH 8.3), 25 mM KCl, 1.5 mM $MgCl_2$, 100 μg/ml bovine serum albumin, 0.2 mM each of dCTP, dGTP, dATP and dTTP, 2 units of Taq polymerase (Bioline, UK) and 10 pmol each of the upstream and downstream primers (see [20] for primer sequences). PCR was carried out for 38 cycles consisting of 1 min denaturation at 95°C, 45 s annealing at 60°C and 75 s extension at 72°C. After checking that the amplification had worked by running 1/5 of the product out on a 2% agarose gel, 15 μl of the remainder was digested with 20 units of *Msp*I for 16 h at 37°C. The digested DNA fragments were then run out 2.5% agarose gels containing 50 μg/ml ethidium bromide for 3 h at 80 V and visualised under ultraviolet light. Negative controls were included with every set of amplifications.

2.3. Statistical analysis

Distribution of Pl^A genotypes and alleles between cases and controls and qualitative risk factors between different genotype groups were compared using the χ^2 test. Quantitative sample means were compared by analysis of variance (ANOVA). Odds ratios and 95% confidence intervals (CI) estimating the relative risk of MI associated with the Pl^{A2} allele were calculated using logistic regression with adjustment for other factors as specified, using the GLIM 4.0 statistical package (NAG, Oxford, UK).

3. Results

A total of 451 subjects (242 cases and 209 controls) were analysed. Table 1 shows the GPIIIa Pl^A genotype distributions for the two groups. There was no difference in either genotype distribution ($P = 0.64$) or allele fre-

Table 1
Distribution of GPIIIa Pl^A genotypes in MI cases and controls

Genotype	Pl^{A1}/Pl^{A1}	Pl^{A1}/Pl^{A2}	Pl^{A2}/Pl^{A2}
Cases	157 (64.9)	82 (33.9)	3 (1.2)
Controls	133 (63.6)	97 (34.0)	5 (2.4)

Pl^{A1}/Pl^{A1} = homozygote for GPIIIa Pl^{A1} allele; Pl^{A1}/Pl^{A2} = heterozygote; Pl^{A2}/Pl^{A2} = homozygote for GPIIIa Pl^{A2} allele. Number of subjects (% of group) are shown.

quencies ($P = 0.65$) between cases and controls. The allele frequencies of the PI^{A2} variant were 18.2 and 19.4% in cases and controls, respectively, and the three genotype frequencies in both cohorts were consistent with the frequencies predicted by the Hardy-Weinberg equilibrium.

Cases were significantly older than controls (65.8 ± 11.4 (s.d.) years versus 56.0 ± 12.5 years, $P < 0.001$). However, there was no age-dependent variation in the prevalence of the PI^{A2} allele in either cases or controls. Thus, the frequencies of the PI^{A2} allele in subjects < 55 years, 56–65 years and > 65 years were 12.2, 20.8 and 19.3% respectively, in cases ($P = 0.28$), and 18.0, 20.2 and 21.1% respectively, in controls ($P = 0.76$). 65.3% of cases were males compared with 58.9% of controls ($P = 0.16$). The age- and sex-stratified odds ratio (OR) for risk of MI associated with carriage of the PI^{A2} allele (i.e. PI^{A2} homozygotes + heterozygotes versus PI^{A1} homozygotes) was 0.89 (95% CI 0.58–1.37, $P = 0.65$). The odds ratio remained non-significant when the analysis was confined to subjects under the age of 60 ($n = 67$ for cases and 118 for controls) (OR = 0.77, 95% CI 0.38–1.56, $P = 0.44$). Likewise, the odds ratios were non-significant for the two sexes analysed separately (males OR = 0.85, 95% CI 0.50–1.46, $P = 0.62$; females OR = 0.97, 95% CI 0.45–2.08, $P = 0.92$).

Distribution of demographic variables and coronary risk factors in subjects grouped by their PI^{A} genotype are shown for both cases and controls in Table 2. As expected, several classical risk factors for CAD were significantly increased ($P < 0.01$) in cases compared with controls. However, there was no association of any of the risk factors with the PI^{A2} allele and in logistic regression analysis none of the risk factors either considered singly or in combination had any significant influence on the risk of MI associated with PI^{A2}.

Finally, in the MI group there was no difference in the age at time of MI between those subjects carrying a PI^{A2} allele ($n = 85$) and those not ($n = 157$) (66.3 ± 10.8 vs. 65.6 ± 11.7 years, $P = 0.63$).

4. Discussion

Epidemiological studies have established several common risk factors for coronary atherosclerosis and MI [21]. However, taken together these predict no more than 50% of the cases of MI. Family studies have repeatedly highlighted the importance of genetic factors in influencing the risk of coronary heart disease [3] and with developments in molecular biology, the potential use of genetic techniques to improve identification of subjects at increased risk has aroused interest. Studies have shown, for example, an association of specific polymorphisms in the genes for apolipoprotein B100 and angiotensin converting enzyme (ACE) and the risk of MI [4,5]. The list of polymorphisms in relevant genes such as those regulating vascular biology, haemostasis and lipid metabolism that require epidemiological evaluation is rapidly expanding.

In this study we have investigated the association with MI of a polymorphism in the gene for GPIIIa, recently reported to strongly predict the risk of acute coronary syndromes [16]. Given the important role of platelets in the pathogenesis of MI [1,2] and the central role of the GP IIb/IIIa complex in mediating platelet aggregation [9], the reported finding has biological plausibility. However, in contrast to the original study [16], in a population three-fold larger we found no evidence of an association between PI^{A2} form of GPIIIa and MI.

Association studies are an important first step in identifying new risk factors. However, they can be prone to several biases [22]. A strict phenotype definition is important. In this study a single phenotype (MI) was analysed and its diagnosis was unequivocal. Further, cases were recruited prospectively and as soon as possible (all within 24 h) after diagnosis. Ethnic differences exist for many genetic polymorphisms and indeed this has been reported for PI^{A} [23,24] and we have preliminary evidence for a lower frequency of PI^{A2} amongst Indian Asians (~ 10%). For this reason we confined our analysis to Caucasian cases and controls only and the overall frequency of PI^{A2}

Table 2
Prevalence of coronary risk factors in MI cases and controls grouped by PI^{A} carrier status

	MI cases			Controls		
	PI^{A2} −ve $n = 157$	PI^{A2} +ve $n = 85$	P	PI^{A2} −ve ($n = 133$)	PI^{A2} +ve ($n = 76$)	P
Age	65.6 (11.7)	66.3 (10.8)	0.63	55.8 (12.3)	56.5 (13.1)	0.81
Males (%)	66.2	63.5	0.67	57.1	61.8	0.51
Hypertension (%) [a]	29.2	37.8	0.18	15.8	18.4	0.62
Diabetes (%) [a]	9.6	9.6	0.98	3.8	2.6	0.66
Current smokers (%) [a]	38.3	33.3	0.45	19.5	19.7	0.97
BMI (kg/m^2) [b]	26.1 (3.9)	25.4 (4.7)	0.28	25.3 (3.6)	25.5 (3.9)	0.71
Total cholesterol (mmol/l) [b]	5.6	5.8 (1.2)	0.64	5.5 (1.0)	5.8 (1.1)	0.14
HDL cholesterol (mmol/l) [b]	1.27 (0.34)	1.26 (0.33)	0.86	1.21 (0.36)	1.19 (0.31)	0.73

PI^{A2} −ve, non-carriers of PI^{A2}; PI^{A2} +ve, carriers of PI^{A2}; BMI, body mass index. [a] These risk factors all significantly ($P < 0.01$) higher in cases compared with controls; [b] BMI, total cholesterol and HDL-cholesterol were available for 76.0, 78.9, 68.6% of cases respectively, and for 100, 97.1, 96.2% of controls respectively. Values in parentheses are standard deviations.

we observed (~ 18%) agrees well with that previously reported in other European populations [24] and also the control subjects in the study by Weiss et al. [16] Finally, to avoid any bias due to a possible influence of the Pl^{A2} polymorphism on prognosis after MI we confined our analysis to only subjects presenting with a first MI. Despite these measures aimed at trying to obtain as homogeneous a group for analysis as possible, we could not confirm the finding of Weiss et al. [16] More specifically, while our study cannot exclude a small effect of Pl^{A2} (as defined by the 95% CI to the odds ratios), it rules out the magnitude of effect reported by them.

There was a significant age difference between our cases and controls. However, we observed no age-related decline in the frequency of the Pl^{A2} allele in either cases or controls, adjustment for age made no difference to the association of MI with Pl^{A2}, and the association remained similarly non-significant when analysis was confined to subjects under the age of 60. Therefore the age difference is very unlikely to have confounded the results, and the absence of a greater association of Pl^{A2} in younger subjects also argues against it being a major risk factor where thrombotic factors may be more important. Indeed none of the MI cases under the age of 60 was homozygote for Pl^{A2}. Weiss et al. [16] also found a significant difference of more than 7 years in the age at onset of disease between their Pl^{A2}-positive and Pl^{A2}-negative patients. However, in keeping with our overall findings, we saw no such difference in our cases. Finally, we found no association of the Pl^{A2} allele with several other risk factors for coronary artery disease or any evidence of interaction between Pl^{A2} and these factors in determining the risk of MI.

A critical feature of any association study is the selection of control subjects. They need to provide a reliable estimate of the prevalence of putative risk factor in the base population from which the cases are recruited. We therefore chose to recruit our controls from individuals *visiting* patients on non-cardiac wards in the same hospital, rather than from other patients with non-cardiovascular diseases where unrecognised selection biases could exist.

In summary, in a cohort of prospectively recruited cases of first MI we found no evidence of an excess of the Pl^{A2} allele of the GPIIIa polypeptide compared with a community-based control population. Our findings raise serious doubts as to whether Pl^{A2} is a novel and important risk factor for MI.

Acknowledgements

We are grateful to Mr. Daniel Martin for collection of samples, Mrs. Pamela Smith for technical assistance, and Dr. John Thompson for statistical advice.

References

[1] Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. New Engl J Med 1992;326:242–250.
[2] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801–809.
[3] Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. New Engl J Med 1994;330:1041–1046.
[4] Hegle RA, Huang L-S, Herbert PN, Blum CB, Buring JE, Hennekens CH, Breslow JL. Apolipoprotein B-gene DNA polymorphisms associated with myocardial infarction. New Engl J Med 1986;315:1509–1515.
[5] Cambien F, Poirier O, Lecerf L, Evans A, Cambou J-P, Arveiler D, Luc G, Bard J-M, Bara L, Ricard S, Tiret L, Amouyel P, Alhenc-Gelas F, Soubrier F. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992;359:641–643.
[6] Scarabin PY, Bara L, Ricard S, Poirier O, Cambou JP, Arveiler D, Luc G, Evans A, Samama MM, Cambien F. Genetic variation at the beta-fibrinogen locus in relation to plasma fibrinogen concentrations and risk of myocardial infarction. The ECTIM Study. Arterioscl Thromb 1993;13:886–891.
[7] ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Randomized trial of intravenous streptokinase, oral aspirin, both or neither among 17,187 cases of suspected acute myocardial infarction. Lancet 1988;2:349–360.
[8] Antiplatelet Trialists' Collaboration. Collaborative overview of randomised trials of antiplatelet therapy. I. Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Br Med J 1994;308:81–106.
[9] Lefkovits J, Plow EF, Topol EJ. Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine. New Engl J Med 1995;332:1553–1559.
[10] The EPIC Investigators. Use of a monoclonal body directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. New Engl J Med 1994;330:956–961.
[11] Heidenreich R, Eisman R, Surrey S, Delgrosso K, Bennett JS, Schwartz E, Poncz M. Organisation of the gene for platelet glycoprotein IIb. Biochemistry 1990;29:1232–1244.
[12] Zimrin AB, Gidwitz S, Lord S, Schwartz E, Bennett JS, White GC III, Poncz M. The genomic organisation of platelet glycoprotein IIIa. J Biol Chem 1990;265:8590–8595.
[13] Bray PF, Schuman MA. Identification of an abnormal gene for the GPIIIa subunit of the platelet fibrinogen receptor resulting in Glanzmann's thrombasthenia. Blood 1990;75:881–888.
[14] Burk CD, Newman PJ, Lyman S, Gill J, Coller BS, Poncz M. A deletion in the gene for glycoprotein IIb associated with Glanzmann's thrombasthenia. J Clin Invest 1991;87:270–276.
[15] Newman PJ, Derbes R, Aster RH. The human platelet alloantigens, Pl^{A1} and Pl^{A2}, are associated with a leucine33/proline 33 amino acid polymorphism in membrane glycoprotein IIIa, and are distinguishable by DNA typing. J Clin Invest 1989;83:1778–1781..
[16] Weiss EJ, Bray PF, Tayback M, Schulman SP, Kickler TS, Becker LC, Weiss JL, Gerstenblith G, Goldschmidt-Clermont PJ. A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. New Engl J Med 1996;334:1090–1094.
[17] Adams M, Smith PD, Martin D, Thompson JR, Lodwick D, Samani NJ. Genetic analysis of thermolabile methylenetetrahydrofolate reductase as a risk factor for myocardial infarction. Q J Med 1996;89:437–444.
[18] WHO (Report of the Joint International Society and Federation of Cardiology/World Health Organisation Task Force on Standardization of Clinical Nomenclature). Nomenclature and criteria for diagnosis of ischaemic heart disease. Circulation 1979;59:607–609.

[19] Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1989.
[20] Jin Y, Dietz HC, Nurden A, Bray PF. Single-strand confirmation polymorphism analysis is a rapid and effective method for the identification of mutations and polymorphisms in the gene for glycoprotein IIIa. Blood 1993;82:2281–2288.
[21] McGill HC. Risk factors for coronary atherosclerosis. Adv Exp Med Biol 1978;104:273–280.
[22] Lander ES, Schork NJ. Genetic dissection of complex traits. Science 1994;265:2037–2048.
[23] Kim HO, Jin Y, Kickler TS, Blakemore K, Kwon OH, Bray PF. Gene frequencies of the five major human platelet antigens in African American, white and Korean populations. Transfusion 1995;35:863–867.
[24] Simsek S, Faber NM, Bleeker PM, Viekke ABJ, Huiskes E, Goldschmeding R, von dem Borne AEGKr. Determination of human platelet antigen frequencies in the Dutch population by immunophenotyping and DNA (allele-specific restriction enzyme) analysis. Blood 1993;81:835–840.