**in vivo** *23*: 177-182 (2009)

# Platelet GP IIIA Polymorphism HPA-1 (PLA1/2) Is Associated with Hypertension as the Primary Cause for End-stage Renal Disease in Hemodialysis Patients from Greece

THEODORE CHIRAS, EMMANUEL D. PAPADAKIS, AGGELIKI KATOPODI, EFROSINI CHATZIANESTI, KOSTAS FOURTOUNAS, STAMATINA PAPAKONSTANTINOU, ILIAS THEODOROPOULOS, ANASTASIOS DAKOURAS, NIKOLAS ZEREFOS, DIMITRIOS VALIS and HELEN TZANATOS-EXARCHOU

*Renal Unit, Ygeia Hospital, Athens, Greece*

**Abstract.** *Human platelets carry membrane glycoproteins that control platelet aggregation and activation. A number of clinical studies have suggested that certain polymorphisms of genes encoding these proteins increase the risk for cardiovascular disease. The frequency of gene polymorphisms for the four most common platelet glycoproteins (HPA 1, 2, 3 and 5) was examined and correlated with the primary cause of end-stage renal disease (ESRD) in Greek patients on HD. Fifty-five (55) patients on chronic maintenance haemodialysis (HD) (22 female, 33 male), aged from 23 to 87 years old, (mean age 66 years), being on dialysis for 53±34 months, were included in the study. HPA-1, -2, -3, and -5 genotyping was performed using polymerase chain reaction (PCR) amplification with sequence-specific primers (PCR-SSP). Calculated relative frequencies of the alleles were as follows: HPA-1a/b 0.81/0.19, HPA-2a/b 0.92/0.08, HPA-3a/b 0.62/0.38 and HPA-5a/b 0.93/0.07. There was a statistically significant association between the HPA-1b allele and hypertension as the primary cause of ESRD (65% of patients with hypertension vs 23% of all other patients carried the HPA-1b allele, p=0.02, Fisher's exact test). The results suggest that Greek carriers of the HPA-1b allele with hypertension may be at increased risk for developing end-stage renal disease.*

Platelets possess a central role in acute arterial occlusion and platelet hyperactivity on *in vitro* testing may confer an increased risk for cardiovascular disease. Platelets adhere to subendothelial structures by specific receptors such as the collagen receptor glycoprotein (GP) Ia/IIa, or the primary von Willebrand factor (vWF) receptor GPIb/IX. After adhesion, platelets become activated and finally aggregate by cross-linking *via* the fibrinogen receptor GPIIb/IIIa (1). The receptors for vWF (GPIb/IX), fibrinogen (GPIIb/IIIa) and collagen (GPIa/IIa) are encoded by polymorphic genes. Most of these polymorphisms are caused by single base-pair substitutions resulting in an amino acid replacement (2).

The membrane IIb–IIIa complex is a member of the integrin family and plays a key role in platelet aggregation and activation. The complex carries the human platelet antigen (HPA) 1 or PlA which is polymorphic and is the binding site for fibrinogen and (vWF), vitronectin and thrombospondin. Polymorphism in Gp IIIa results in a substitution of proline for leucine at position 33 (HPA-1 bp T196C, amino acid Leu33Pro) and is described as the PLA1/A2 alloantigen system (3). The GP IIb gene polymorphism consists of a substitution of serine for isoleucine at position 843 (HPA-3a/3b bp T2622G, amino acid Ile843Ser) (4). GPIba, is a transmembranous platelet glycoprotein, with a molecular weight of 143,000, which forms a noncovalent complex with GPIbb, GPIX and GPV. Two polymorphisms of GPIba have been described. One polymorphism has been shown to result from a variable number of tandem repeats (VNTR) of 39 bp in the macroglycopeptide region of GPIba. The second polymorphism within the GPIba coding region (HPA-2 polymorphism bp T524C, amino acid Met145Thr, a Thr in HPA-2a, Met in HPA-2b) (5, 6) is located close to the vWF and the high-affinity thrombin-binding sites and might therefore influence the receptor function of these variants. DNA typing has recently showed that the HPA-2 polymorphism is in linkage disequilibrium with the VNTR polymorphism. The collagen receptor GPIa/IIa carries the HPA-5 polymorphic system. The HPA-5 polymorphism (bp G1648A, amino acid Glu505Lys) (7) correlates with the number of GPIa molecules on the platelet surface (8). The HPA-5b allele is associated with increased numbers of GPIa molecules on the platelet surface.

*Correspondence to:* Theodore Chiras, Renal Unit, YGEIA Hospital, 4-6 Er. Stavrou St. Athens, Greece. Tel: +30 2106927019, Fax: +30 2106452044, e-mail: hiras@ath.forthnet.gr

*Key Words:* Platelets, platelet glycoproteins, human platelet antigens, hypertension, haemodialysis.

Table I. *Patient characteristics*.

| | |
|---|---|
| Number of patients | 55 |
| Gender (male/female) | 33/22 |
| Age (years) | 66 (range: 23-87) |
| Months on dialysis (years) | 53 (range: 19-87) |
| Hours on dialysis per week | 1±0.5 |
| Dose of erythropoietin-a (IU) | 8000±3000 |
| Type of dialysis HD/HDF | 30/25 |
| Type of dialyzer low/high flux | 30/25 |

Table II. *Primary disease for the study patients*.

| Primary disease | No. of patients |
|---|---|
| Hypertension | 16 |
| Diabetes mellitus | 14 |
| Glomerulonephritis | 9 |
| Polycystic disease | 6 |
| Obstructive nephropathy | 7 |
| Autoimmune disease | 3 |

In the present study, the distribution frequency of polymorphisms of genes that encode platelet GPs was examined in Greek haemodialysis (HD) patients. Analysis included the four most common HPA polymorphisms in Caucasians, *i.e.* HPA-1 and HPA-3 on the fibrinogen receptor (GPIIb/IIIa), HPA-2 on the vWF receptor (GPIb/IX) and HPA-5 on one of the platelet collagen receptors (GPIa/IIa). To the authors' knowledge, this is the first study that describes the frequency of HPA polymorphisms in Greek hemodialysis patients and suggests an association of the HPA-1b allele with hypertension as the primary cause of end stage renal disease (ESRD) in the population included in the study (9-12).

## Patients and Methods

*Patients*. The study was approved by the Institutional Ethics Committee. Fifty-five (55) patients on chronic maintenance haemodialysis (HD) (22 female, 33 male), aged 23 to 87 years old, (mean age 66 years), maintained on dialysis for 53±34 months, gave informed consent for participation in the study. Patient characteristics are presented in Table I. Primary disease for the HD patients in the study is presented in Table II.

*HPA genotyping*. DNA was isolated from peripheral blood collected from these patients. DNA was extracted from the buffy coat using the QIAamp DNA blood kit (QIAGEN GmbH, Germany) according to the manufacturer's instructions. The method of polymerase chain reaction (PCR) amplification with sequence-specific primers (PCR-SSP) was used for HPA-1, -2, -3 and -5 genotyping in Caucasians (13-14). The primers were supplied from Sigma Genosys, UK. The reaction tube for each allele (polymorphism) contained 5 μl of a four-primer mix at the concentration which is written in Table III. All tubes also contained 1.8 μl of distilled water, 1 μl of reaction buffer, 0.2 μl of dNTPSs (each at 0.2 mmol/l), 0.07 μl of Taq polymerase and 2 μl of DNA for a total reaction volume of 10.07 μl. The cycling conditions used were: 1 cycle × 96˚C for 1 min; 5 cycles × 96˚C for 25s, 68˚C for 45s, 72˚C for 30s; 1cycle x 72˚C for 3 min. Appropriate DNA controls of known HPA genotype were used in each PCR run.

*Statistical analysis*. SSPS version 11.5 software was used for statistical analysis. A value of $p \leq 0.05$ was considered statistically significant. Parametric and nonparametric tests were used for the analysis. Fisher's exact test was used for comparison of proportions, while analysis of variance (ANOVA) and method of multiple variances by Scheffe and Bonferroni were used where needed.

## Results

*Platelet genotyping*. Table IV summarizes the genotypic profiles for the patients in the study. The calculated relative frequencies of the alleles were as follows: HPA-1a/b 0.81/0.19, HPA-2a/b 0.92/0.08, HPA-3a/b 0.62/0.38 and HPA-5a/b 0.93/0.07. These results are similar to the findings of other studies including populations of various ethnic and racial origin (Table V).

*Platelet genotyping and primary disease for chronic renal failure*. There was a statistically significant association between the HPA-1b allele and hypertension as the primary cause of ESRD: 65% of patients with hypertension *vs*. 23% of patients with all other causes carried the HPA-1b allele, $p$=0.02, Fisher's exact test.

## Discussion

The main finding of this study was that the HPA-1b allele was associated with hypertension as the primary clinical condition leading to ESRD in Greek patients. To the authors' knowledge this is the first study to disclose a similar association (15, 16).

The calculated relative frequency for the HPA-1a/b polymorphism in this study is identical (*i.e.* HPA-1a/b 0.81/0.19) to the relative frequency found in the Lebanese population by Sabbagh *et al*. (17). This is a fact that increases the validity of the data, since Greek and Lebanese populations have resided together in the Eastern Mediterranean basin for millenia and it is logical to assume that they share common genetic origins.

*In vitro* studies have shown that the presence of one or two HPA-1b alleles is associated with a proportional increase in platelet responsiveness to adrenaline and fibrinogen binding. The latter may be due to an effect of the HPA-1b polymorphism on the function of the fibrinogen-binding site as well as by lowering the threshold for the GPIIb-IIIa complex activation (18).

Table III. *Characteristics of primers*.

| Primer | Nucleotide sequence | Product size (bp) | Final concentration (μM) |
|---|---|---|---|
| HPA-1a | 5' TCACAGCGAGGTGAGGCCA 3' | 90 | 0.35 |
| Common-1 | 5' GGAGGTAGAGAGTCGCCATAG 3' | | 0.35 |
| HPA-1b | 5' TCACAGCGAGGTGAGGCCG 3' | 90 | 0.35 |
| Common-1 | 5' GGAGGTAGAGAGTCGCCATAG 3' | | 0.35 |
| HPA-2a | 5' GCCCCCAGGGCTCCTGAC 3' | 258 | 0.35 |
| Common-2 | 5' TCAGCATTGTCCTGCAGCCA 3' | | 0.35 |
| HPA-2b | 5' GCCCCCAGGGCTCCTGAT 3' | 258 | 0.35 |
| Common-2 | 5' TCAGCATTGTCCTGCAGCCA 3' | | 0.35 |
| HPA-3a | 5' TGGACTGGGGGCTGCCCAT 3' | 267 | 0.50 |
| Common-3 | 5' TCCATGTTCACTTGAAGTGCT 3' | | 0.50 |
| HPA-3b | 5' TGGACTGGGGGCTGCCCAG 3' | 267 | 0.50 |
| Common-3 | 5' TCCATGTTCACTTGAAGTGCT 3' | | 0.50 |
| HPA-5a | 5' AGAGTCTACCTGTTTACTATCAAAG 3' | 246 | 0.50 |
| Common-5 | 5' CTCTCATGGAAAATGGCAGTACA 3' | | 0.50 |
| HPA-5b | 5' AGAGTCTACCTGTTTACTATCAAAA 3' | 246 | 0.50 |
| Common-5 | 5' CTCTCATGGAAAATGGCAGTACA 3' | | 0.50 |
| HGH | 5' GCCTTCCCAACCATTCCCTTA 3' | 429 | 0.10 |
| Controls | 5' TCACGGATTTCTGTTGTGTTTC 3' | | 0.10 |

Table IV. *Genotypic profile for study patients*.

| Pt No. | HPA-1 | HPA-2 | HPA-3 | HPA-5 | Pt No. | HPA-1 | HPA-2 | HPA-3 | HPA-5 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ab | aa | bb | aa | 31 | aa | aa | ab | aa |
| 2 | ab | aa | bb | aa | 32 | ab | ab | bb | aa |
| 3 | bb | aa | ab | aa | 33 | aa | aa | ab | aa |
| 4 | aa | aa | ab | ab | 34 | aa | aa | ab | aa |
| 5 | aa | aa | aa | aa | 35 | aa | ab | ab | aa |
| 6 | aa | aa | bb | aa | 36 | aa | aa | ab | aa |
| 7 | aa | aa | ab | aa | 37 | aa | aa | ab | aa |
| 8 | aa | aa | ab | aa | 38 | ab | ab | ab | aa |
| 9 | ab | ab | bb | aa | 39 | aa | aa | aa | aa |
| 10 | aa | aa | ab | aa | 40 | aa | aa | ab | aa |
| 11 | aa | aa | aa | aa | 41 | aa | ab | ab | aa |
| 12 | ab | aa | bb | aa | 42 | ab | aa | aa | aa |
| 13 | ab | aa | aa | aa | 43 | aa | ab | ab | aa |
| 14 | ab | aa | ab | aa | 44 | ab | aa | bb | aa |
| 15 | aa | aa | aa | aa | 45 | aa | ab | aa | aa |
| 16 | ab | aa | ab | aa | 46 | aa | aa | ab | aa |
| 17 | ab | aa | bb | aa | 47 | aa | ab | bb | ab |
| 18 | aa | aa | aa | aa | 48 | ab | aa | ab | aa |
| 19 | aa | aa | aa | aa | 49 | ab | aa | ab | ab |
| 20 | aa | ab | aa | aa | 50 | aa | aa | aa | ab |
| 21 | aa | ab | aa | aa | 51 | ab | bb | aa | aa |
| 22 | aa | ab | ab | aa | 52 | aa | aa | aa | aa |
| 23 | aa | aa | ab | - | 53 | aa | aa | ab | aa |
| 24 | aa | ab | ab | aa | 54 | aa | aa | ab | aa |
| 25 | aa | ab | ab | aa | 55 | aa | aa | aa | aa |
| 26 | bb | aa | bb | aa | | | | | |
| 27 | bb | aa | ab | aa | | | | | |
| 28 | ab | aa | aa | aa | | | | | |
| 29 | aa | ab | bb | aa | | | | | |
| 30 | aa | aa | ab | aa | | | | | |

Table V. *Prevalence (%) of HPA in various populations*.

| Antigen | Netherlands | Finland | USA | Japan (Caucasians) | Korea | China |
|---|---|---|---|---|---|---|
| HPA-1a | 97.90 | 99.00 | 98.00 | 100.00 | 99.50 | >99.90 |
| HPA-1b | 28.80 | 26.50 | 20.00 | 0.30 | 2.00 | <0.15 |
| HPA-2a | 100.00 | 99.00 | 97.00 | 99.20 | 99.00 | |
| HPA-2b | 13.50 | 16.50 | 15.00 | 19.70 | 14.00 | |
| HPA-3a | 81.00 | 83.50 | 88.00 | 85.10 | 82.50 | 78.54 |
| HPA-3b | 69.80 | 66.50 | 54.00 | 66.20 | 71.50 | 71.85 |
| HPA-4a | 100.00 | | 100.00 | 100.00 | 100.00 | >99.99 |
| HPA-4b | 0.00 | | 0.00 | 2.00 | 2.00 | <0.17 |
| HPA-5a | 100.00 | 99.50 | 98.00 | 99.00 | 100.00 | 99.29 |
| HPA-5b | 19.70 | 10.00 | 21.00 | 7.00 | 4.50 | 17.73 |
| HPA-6aw | | 100.00 | | 99.70 | 100.00 | |
| HPA-6bw | | 2.40 | | 4,80 | 4,00 | |

An increased prevalence of the HPA-1b/PlA2 allele has been described among black patients with hypertension (19). The GPIIIa HPA-1b/PlA2 allele has also been associated with increased mean arterial pressure (MAP) and pulse pressure (PP) in women. Boudoulas *et al*. (20) showed that the GPIIIa HPA-1b/PlA2 polymorphism may function as a modifier for the effect of estrogen on platelet aggregation. Nevertheless, only a third of the patients included in the study were female and therefore association of HPA-1b with hypertension as a cause of ESRD is probably not attributed to gender-related factors.

The GPIIb–IIIa GP is also expressed in the endothelium and in vascular smooth muscle cells (VSMCs) (21). After

endothelial injury, the GPIIb–IIIa complex mediates VSMC growth (22). The association of the HPA-1b allele with hypertension may be therefore independent of platelet function and actually the result of altered VSMC growth in response to endothelial injury (23, 24).

In 1996, a significant association between the HPA-1b (PlA2) allele and the risk of myocardial infarction and unstable angina was reported (25, 26), but results of subsequent studies were conflicting (27-31). A number of clinical studies have also suggested the association of the low frequency HPA-1b allele with an increased risk for myocardial infarction and stroke among young adults (32-34) although it was not found in all reports (35-38). Consistent with the suggested platelet hyper-reactivity and higher thrombotic activity associated with this variant (39-41) is the finding that the HPA-1b allele (PlA2) of platelet GPIIIa significantly reduces the risk of subarachnoid hemorrhage (42).

Of the HD patients included in the study, carriers of the HPA-1b allele showed no statistically significant difference in the frequency of a major vascular event (defined as stroke, myocardial infarction or an episode of deep vein thrombosis) for an observation period of three years (unpublished data) in comparison to other HD patients. It is therefore probable that the effect of the HPA-1b allele in the pathophysiology of ESRD is mainly expressed in early stages and in later stages is offset by the metabolic derangement of uremia.

Although this study is limited by the sample size, data suggest that the presence of the HPA-1b allele together with hypertension may contribute to an accelerated decrease in renal function and the development of ESRD in Greek patients.

## Acknowledgements

We thank Dr. Dimitrios Valis for his valuable assistance with our study

## References

1 Metcalfe P, Watkins NA, Ouwehand WH, Kaplan C, Newman P, Kekomaki R, M de Haas, Aster R, Shibata Y, Smith J, Kiefel V and Santoso S: Nomenclature of human platelet antigens. Vox Sanguinis *85*: 240-245, 2003.

2 Beer JH, Pederiva S and Pontiggia L: Genetics of platelet receptor singlenucleotide polymorphisms: clinical implications in thrombosis. Ann Med *32*: 10-14, 2000.

3 Newman PJ, Derbes RS and Aster RH: The human platelet alloantigens, $Pl^{a1}$ and $Pl^{a2}$, are associated with a leucine33/proline33 amino acid polymorphism in membrane glycoprotein IIIa and are distinguishable by DNA typing. J Clin Invest *83*: 1778-1781, 1989.

4 Lyman S, Aster RH, Visentin GP and Newman PJ: Polymorphism of human platelet membrane glycoprotein IIb associated with the $Bak^a$/$Bak^b$ alloantigen system. Blood *75*: 2343-2348, 1990.

5 Kuijpers RWAM, Faber NM, Cuypers HTM, Ouwehand WH and von dem Borne AEGK: The *N*-terminal globular domain of the human platelet glycoprotein Iba has a methionine 145/threonine 145 amino acid polymorphism which is associated with the HPA-2 (Ko) alloantigens. J Clin Invest *89*: 381-384, 1992.

6 Mazzucato M, Pradella P, de Angelis V, Steffan A and de Marco L: Frequency and functional relevance of genetic threonine145/methionine145 dimorphism in platelet glycoprotein Iba in an Italian population. Transfusion *36*: 891-894,1996.

7 Santoso S, Kalb R, Walka M, Kiefel V, Mueller-Eckhardt C and Newman PJ: The human platelet alloantigens, $Br^a$ and $Br^b$, are associated with a single amino acid polymorphism on glycoprotein Ia (integrin a2). J Clin Invest *92*: 2427-2432, 1993.

8 Antoniades C, Tousoulis D, Vasiliadou C, Stefanadi E, Marinou K and Stefanadis C: genetic polymorphisms of platelet glycoprotein Ia and the risk for premature myocardial infarction: effects on the release of sCD40L During the Acute Phase of Premature Myocardial Infarction. J Am Coll Cardiol *47*: 1959-1966, 2006.

9 Kluter H, Fehlau K, Panzer S, Kirchner H and Bein G: Rapid typing for human platelet antigen systems-1, -2, -3 and -5 by PCR amplification with sequence-specific primers. Vox Sanguinis *71(2)*: 121-125, 1996.

10 Prozialeck WC and Edwards JR: Cell adhesion molecules in chemically-induced renal injury. Pharmacol Ther *114(1)*: 74-93, 2007.

11 Holden RM, Harman GJ, Wang M, Holland D and Day AG: Major bleeding in hemodialysis patients. Clin J Am Soc Nephrol *3(1)*: 105-10, 2008.

12 Ho SJ, Gemmell R and Brighton TA: Platelet function testing in uremic patients. Hematology *13(1)*: 49-58, 2008

13 Salobir B, Sabovic M, Zupn IP and Ponikvar JB: Platelet (dys)function and plasma plasminogen levels in hemodialysis patients. Ther Apher Dial *12(2)*: 133-6, 2008.

14 Metcalfe P and Waters AH: HPA-1 typing by PCR amplification with sequence-specific primers (PCR-SSP): a rapid and simple technique. Br J Haematol *85(1)*: 227-229, 1993.

15 Mann JFE: What's new in hypertension? Nephrol Dial Transplant *22*: 47-52, 2007.

16 Bernardo RI and Nosratola DV: Salt-sensitive hypertension- update of novel findings. Neprol Dial Transplant *22*: 992-995, 2007

17 Sabbagh AS, Taher AT, Zaatari GS and Mahfouz RA: Gene frequencies of the HPA-1 platelet antigen alleles in the Lebanese population. Transfus Med *17(6)*: 473-8, 2007.

18 Theodoropoulos I, Christopoulos C, Metcalfe P, Dimitriadou E, Economopoulos P and Loukopoulos D: The effect of human platelet alloantigen polymorphisms on the *in vitro* responsiveness to adrenaline and collagen. Br J Haemtol *114*: 387-393, 2001.

19 Wagner KR, Giles WH, Johnson CJ, Ou CY, Bray PF, Goldschmidt-Clermont PJ, Croft JB, Brown VK, Stern BJ, Feeser BR, Buchholz DW, Earley CJ, Macko RF, McCarter RJ, Sloan MA, Stolley PD, Wityk RJ, Wozniak MA, Price TR and Kittner SJ: Platelet glycoprotein receptor IIIa polymorphism P1A2 and ischemic stroke risk: the Stroke Prevention in Young Women Study. Stroke *29*: 581-585, 1998.

20 Boudoulas KD, Cooke GE, Roos CM, Bray PF and Goldschmidt-Clermont PJ: The PlA polymorphism of glycoprotein IIIa functions as a modifier for the effect of estrogen on platelet aggregation. Arch Pathol Lab Med *125*: 112-115, 2001.

21 Clemetson KJ and Clemetson JM: Integrins and cardiovascular disease. Cell Mol Life Sci *54*: 502-513, 1998.

22 Stouffer GA, Hu Z, Sajid M, Li H, Jin G, Nakada MT, Hanson SR and Runge MS: Beta-3 Integrins are up-regulated after vascular injury and modulate thrombospondin- and thrombin-induced proliferation of cultured smooth muscle cells. Circulation *97*: 907-915, 1998.

23 Kimoto E, Shoji T, Shinohara K, Hatsuda S, Mori K, Fukumoto S, Koyoama H, Emoto M, Okuno Y and Nishizawa Y: regional arterial stiffness in patients with type 2 diabetes and chronic kidney disease. J Am Soc Nephrol *17*: 2245-2252, 2006
24 Loncar R, Stoldt V, Hellmig S, Zotz RB, Mihalj M and Scharf RE: HPA-I polymorphism of αIIbβ3 modulates platelet adhesion onto immobilized fibrinogen in an *in vitro* flow system. Thrombosis J *5*: 2, 2007
25 Weiss EJ, Bray PF, Tayback M, Schulman SP, Kickler TS, Becker LC, Weiss JL, Gerstenblith G and Goldschmidt-Clermont PJ: A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. N Engl J Med *334*: 1090-1094, 1996.
26 Ting HH, Yang EH and Rihal CS: Narrative Review: reperfusion strategies for ST-segment elevation myocardial infarction. Ann Intern Med *145*: 610-617, 2006.
27 Carter AM, Ossei-Gerning N, Wilson IJ and Grant PJ: Association of the platelet Pl(A) polymorphism of glycoprotein IIb/IIIa and the fibrinogen Bbeta 448 polymorphism with myocardial infarction and extent of coronary artery disease. Circulation *96*: 1424-1431, 1997.
28 Garg UC, Arnett DK, Folsom AR, Province MA, Williams RR and Eckfeldt JH: Lack of association between platelet glycoprotein IIb/IIIa receptor PlA polymorphism and coronary artery disease or carotid intima-media thickness. Thromb Res *89*: 85-89, 1998.
29 Zhu MM, Weedon J and Clark LT: Meta-analysis of the association of platelet glycoprotein IIIa PlA1/A2 polymorphism with myocardial infarction. Am J Cardiol *86*: 1000-1005, 2000.
30 Aleksic N, Juneja H, Folsom AR, Ahn C, Boerwinkle E, Chambless LE and Wu KK: Platelet PlA2 allele and incidence of coronary heart disease: results from the Atherosclerosis Risk In Communities (ARIC) Study. Circulation *102*: 1901-1905, 2000.
31 Di Castelnuovo A, De Gaetano G, Donati MB and Iacoviello L: Platelet glycoprotein receptor IIIa polymorphism PLA1/PLA2 and coronary risk: a meta-analysis. Thromb Haemost *85*: 626-633, 2001.
32 Zotz RB, Winkelmann BR, Müller C, Boehm BO, März W and Scharf RE: Association of polymorphisms of platelet membrane integrins alpha IIb(beta)3 (HPA-1b/Pl) and alpha2(beta)1 (alpha807TT) with premature myocardial infarction. J Thromb Haemost *3(7)*: 1522-1529, 2005.
33 Mikkelsson J, Perola M, Laippala P, Savolainen V, Pajarinen J, Lalu K, Penttila A and Karhunen PJ: Glycoprotein IIIa Pl(A) polymorphism associates with progression of coronary artery disease and with myocardial infarction in an autopsy series of middle-aged men who died suddenly. Arch Pathol Lab Med *19*: 2573-2578, 1999.
34 Goldschmidt-Clermont PJ, Coleman LD, Pham YM, Cooke GE, Shear WS, Weiss EJ *et al*: Higher prevalence of GPIIIa PlA2 polymorphism in siblings of patients with premature coronary heart disease. Arch Pathol Lab Med *123*: 1223-1229, 1999.
35 Carlsson LE, Greinacher A, Spitzer C, Walther R and Kessler C: Polymorphisms of the human platelet antigens HPA-1, HPA-2, HPA-3, and HPA-5 on the platelet receptors for fibrinogen (GPIIb/IIIa), von Willebrand factor (GPIb/IX), and collagen (GPIa/IIa) are not correlated with an increased risk for stroke. Stroke *28(7)*: 1392-5, 1997.
36 Carter AM, Catto AJ, Bamford JM and Grant PJ: Association of the platelet glycoprotein IIb HPA-3 polymorphism with survival after acute ischemic stroke. Stroke *30(12)*: 2606-11, 1999.
37 Meiklejohn DJ, Vickers MA, Morrison ER, Dijkhuisen R, Moore I, Urbaniak SJ and Greaves M: *In vivo* platelet activation in atherothrombotic stroke is not determined by polymorphisms of human platelet glycoprotein IIIa or Ib. Br J Haematol *112(3)*: 621-31, 2001.
38 Saidi S, Mahjoub T, Slamia LB, Ammou SB, Al-Subaie AM and Almawi WY: Polymorphisms of the human platelet alloantigens HPA-1, HPA-2, HPA-3, and HPA-4 in ischemic stroke. Am J Hematol *83(7)*: 570-573, 2008.
39 Feng D, Lindpaintner K, Larson MG, Rao VS, O'Donnell CJ, Lipinska I, Schmitz C, Sutherland PA, Silbershatz H, D'Agostino RB, Muller JE, Myers RH, Levy D and Tofler GH: Increased platelet aggregability associated with platelet GPIIIa PlA2 polymorphism: the Framingham Offspring Study. Arterioscler Thromb Vasc Biol *19*: 1142-1147, 1999.
40 Goodall AH, Curzen N, Panesar M, Hurd C, Knight CJ, Ouwehand WH and Fox KM: Increased binding of fibrinogen to glycoprotein IIIa-proline33 (HPA-1b, PlA2, Zwb) positive platelets in patients with cardiovascular disease. Eur Heart J *20*: 742-747, 1999.
41 Michelson AD, Furman MI, Goldschmidt-Clermont P, Mascelli MA, Hendrix C, Coleman L, Hamlington J, Barnard MR, Kickler T, Christie DJ, Kundu S and Bray PF: Platelet GP IIIa Pl(A) polymorphisms display different sensitivities to agonists. Circulation *101*: 1013-1018, 2000.
42 Iniesta JA, González-Conejero R, Piqueras C, Vicente V and Corral J: Platelet GP IIIa polymorphism HPA-1 (PlA) protects against subarachnoid hemorrhage. Stroke *35*: 2282-2286, 2004.

*Received July 31, 2008*
*Revised December 3, 2008*
*Accepted December 8, 2008*