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Polymorphism of the human platelet antigen-5 system is a risk factor for occlusive vascular complications in patients with sickle cell anemia

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Vox Sanguinis

Background Polymorphisms of platelet membrane glycoproteins such as human platelet antigen (HPA)-1b, HPA-2b, the -5T/C Kozak sequence and C807T have been described as risk factors for vascular disease. Vaso-occlusion episodes are a common feature of sickle cell anaemia (SCA), leading to complications such as stroke, acute chest syndrome, avascular head femur necrosis and priapism. Complex interactions are involved in vaso-occlusion, and activated platelets may play an important role. These data raised the question of whether platelet polymorphisms could be implicated in occlusive vascular complications (OVC) of SCA.

Materials and Methods In this study, 97 patients with SCA were analysed in two groups: 34 patients presenting with OVC (SCA-VC) and 63 without these complications (SCA-N). The distribution of the HPA-1, -2 and -5 systems, as well as C807T dimorphism and -5T/C Kozak sequence alleles, was evaluated using DNA-based methods.

Results Patients of the SCA-VC group showed a higher frequency of the HPA-5b allele (0·324) compared with those of the SCA-N group (0·111) ($\chi^2 = 13{\cdot}19$, $P = 0{\cdot}0002$). None of the other polymorphisms, isolated or associated as haplotypes, demonstrated any correlation with the development of OVC in these patients.

Conclusions The findings of this study suggest that the HPA-5b allele is a genetic risk factor for the development of OVC in patients with SCA. This allele could be explored as a target for the development of new therapeutic approaches.

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Key words: glycoprotein, platelet polymorphism, risk factors, sickle cell, vaso-occlusion.

Introduction

Sickle cell anaemia (SCA) is a disease characterized by homozygozity for the β-globin S mutation [1]. Red cells containing haemoglobin S undergo complex interactions with endothelial cells, leucocytes, platelets and constituents of plasma, leading to vaso-occlusion, which is responsible for most of the clinical features and complications of the disease [2–5]. Serious complications related to vaso-occlusive crises include acute chest syndrome, ischaemic stroke, priapism and avascular necrosis of the femur head, and leg ulcers [1,3,4], which are all associated with high morbidity and/or mortality. Several studies have demonstrated that hypercoagulability, decreased fibrinolytic activity and increased platelet activity are common among individuals with SCA [6]. Antithrombotic primary prevention treatment of adults with SCA, for occlusive vascular complications (OVCs), has not been explored in clinical trials. It is possible that, for a

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specific group of patients with an underlying predisposition to OVCs, preventive therapies may have a favourable outcome. Early studies performed to identify those SCA subjects with an increased risk of vascular complication were based on the search for inherited risk factors for venous thrombosis, such as factor V Leiden or prothrombin variant 20210A [7–9]. Whilst these risk factors are prevalent among Caucasian descendants, they are present in fewer than 1% of African descendants [10–12]. Thus, a large number of affected subjects must be analysed to address the role of inherited hypercoagulability in the vascular complications of SCA.

Increasing evidence suggests that the augmented adhesion of sickle red cells to vascular endothelium has an important role in the phenomenon of vaso-occlusion [1,4]. Of the adhesive ligands identified in sickle red cell adhesion, some are related to platelet activation or are involved in platelet adhesion [13].

Platelet membrane glycoprotein (Gp) complexes mediate platelet adhesion, activation, aggregation and act as immunogenic targets [14]. Platelet receptors may be identified in other cells, such as epithelial and endothelial cells, lymphocytes and fibroblasts. Inherited polymorphisms of platelet Gps have been shown to influence their receptor density and function and these therefore may influence thrombus formation [15]. Some polymorphisms have been suggested as risk factors for occlusive arterial disease, such as the HPA_2 Met/VNTRB and the -5TC Kozak silent polymorphism in GpIb, the HPA-1b allele in GpIIIa, and HPA-5a and the C807T silent polymorphism in GpIa [16–20]. In addition, these glycoproteins may have a role in acute vascular rejection of renal transplants, acute idiopathic thrombocytopenic purpura [21,22], renal scarring and as virus receptors [23,24].

Therefore, we sought to determine whether OVC-related platelet polymorphic sequences allow the identification of individuals with SCA who are at high risk for these vascular complications.

Table 1 Frequencies of occlusive vascular complications (OVCs) in patients of the SCA-VC group

OVC complication	No. of patients	%
FHO	15	44·1
IS	9	26·5
ACS	5	14·7
IS and FHO	3	8·9
ACS and FHO	1	2·9
Priapism	1	2·9
Total	34	

ACS, acute chest syndrome; FHO, femur head ostenecrosis; IS, ischaemic stroke.

Table 2 Clinical characterization of patients with sickle cell anaemia (SCA) presenting vascular occlusion complications (group SCA-VC) and patients with SCA not presenting vascular occlusion complications (group SCA-N) enrolled in the study

	SCA-VC group	SCA-N group
Number of patients	34	63
Age[a]: mean (range)	29·4 (16–48)	27·8 (14–51)
Gender		
Male	15	34
Female	19	29
β^S haplotype distribution		
BEN/BEN	5	13
BEN/CAR	11	23
CAR/CAR	15	20
BEN/ATP	1	1
ATP/ATP	0	1
CAR/ATP	2	4
SEN/ATP	0	1
Fetal haemoglobin level[b]: mean (range)	6·6 (1·4–16·0)	6·3 (1·0–24)

[a]Years.

[b]%.

Materials and methods

Patient population

Ninety-seven patients of African descent with SCA (homozygous SS), who attended the outpatient clinics of three centres located in two cities (Campinas and São Paulo) in the southeast region of Brazil, were included in the study. The study was approved by the Ethical Committee on Research of the State University of Campinas School of Medicine. The median age of the patients was 27 years (range 14–66 years), with a gender distribution of 48 females and 49 males. The patients were divided into two groups: one group comprised 34 patients presenting one of the following OVCs (sickle cell anaemia with vascular complications – the SCA-VC group): acute chest syndrome, characterized by new pulmonary infiltrate in the radiographic examination, frequently accompanied by fever, chest pain, hypoxaemia and leucocytosis [25]; ischaemic stroke, characterized by imaging (computed tomography/magnetic resonance imaging) and clinical symptoms such as hemiparesis, focal seizures, altered level of consciousness, dysphasia, gait difficulty [26]; priapism, clinically characterized by persistent, usually painful, engorgement of the penis [27]; or femur head osteonecrosis, confirmed by imaging (radiography/magnetic resonance imaging) [28]. The number of patients presenting each complication is summarized in Table 1. The other group (the sickle cell anaemia non-complication group – SCA-N) comprised 63 patients without these complications. Clinical data of both groups of patients, including the β^S haplotype distribution, are presented in Table 2.

Methods

Genomic DNA was obtained from peripheral blood samples of the patients by using a salting-out procedure [29]. Platelet polymorphisms of the HPA-1, -2 and -5 systems and -5T/C Kozak sequence were analysed by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) using *Msp*I, *Bsa*HI, *Mnl*I and *Ppu*MI restriction enzymes, respectively, and the silent C807T polymorphism was analysed by PCR-sequence-specific primers (SSP), as previously described [30–32]. PCR reactions were carried out using known controls for the genotypes analysed. As the HPA-5 system and C807T polymorphisms have been described to be in linkage disequilibrium [33], haplotype frequencies (HPA-5bb/807CC; HPA-5aa/807TT; HPA-5ab/807CT; HPA-5aa/807CT; HPA-5ab/807CC; HPA-5aa/807CC) were also analysed.

Statistical analysis

Comparison of categorical data was performed using the χ^2-test with Yates' correction. The odds ratio was obtained to analyse the risk of vascular complications related to the presence of alleles (Epi-Info, version 6·04d software; Center for Disease Control, Atlanta, GA) [34]. Multivariate analysis (logistic regression) using S-Plus 2000® software (MathSoft Inc., Seattle, WA) was also performed to verify whether there was any association between risk alleles in the occurrence of OVC with odds ratio correction of the presence of each one.

Results

Based on the presence of at least one event of OVC, we divided the study population into two groups. The general characteristics of those subjects are shown in Table 1. The distribution of β^S haplotypes, the other clinical parameter, as well as age, gender distribution and fetal haemoglobin levels did not differ between the groups.

The analysis of GpIa-IIa polymorphisms demonstrated that 19 patients (55·8%) in the SCA-VC group presented at least one HPA-5b allele, which was homozygous in three patients (8·8%). In the SCA-N group, fewer patients (n = 14; 22·2%, χ^2 = 11·15, P = 0·0008) carried the HPA-5b allele compared with the SCA-VC group, and no patient was homozygous. Analysis of allelic distribution of the HPA-5 system polymorphism showed a higher prevalence of the HPA-5b allele among SCA-VC patients, compared with the SCA-N group (0·324 and 0·111, respectively, χ^2 = 13·19, P = 0·0002). The prevalence of this allele in both groups of patients was compared with that previously described among healthy Brazilian Blacks [30]. A higher prevalence of the HPA-5b allele was also seen among the SCA-VC group of patients (0·324 and 0·124, respectively, χ^2 = 16·50, P = 0·00005). The presence of this allele in patients with SCA led to a 3·83-fold higher risk of OVCs (1·70 < OR < 8·7, P = 0·0006). The analysis of GpIa haplotypes 1, 2 and 3 (HPA-5a/807T, HPA-5a/807C and HPA-5b/807C, respectively) showed a higher frequency of haplotype 3 in the SCA-VC group than in the SCA-N group (0·324 vs. 0·111, respectively, χ^2 = 13·19, P = 0·000282). The frequencies of HPA-5aa/807TT; HPA-5ab/807CT; HPA-5aa/807CT; HPA-5ab/807CC; HPA-5aa/807CC and HPA-5bb/807TT genotypes did not differ between groups (8·8%, 14·7%, 14·7%, 32·4%, 20·6%, 8·8% and 17·5%, 6·3%, 30·2%, 15·9%, 30·2%, 0%, respectively). Multivariate analysis, including all high-risk alleles, showed that the HPA-5b allele was still correlated with a higher risk of OVC (OR = 4·2, 1·3 < OR < 14·0, P = 0·020). Evaluation of C807T allele frequencies alone showed a similar distribution between the groups (0·750 and 0·250, 0·643 and 0·357, respectively).

The distribution of platelet HPA-1a and HPA-1b (of GpIIb-IIIa) alleles was similar in SCA-VC and SCA-N groups (0·809 and 0·191, 0·857 and 0·143, respectively).

The analysis of polymorphisms of GpIb showed no significant differences in allele *a* and *b* distributions of the HPA-2 system between the groups (0·868 and 0·132, 0·778 and 0·222, respectively). In addition, there was no correlation between the presence or absence of OVC and allele distribution of the -5T/C Kozak sequence (0·208 and 0·792, 0·220 and 0·780, respectively). Kozak polymorphism could not be analysed in 23 patients (10 of the SCA-VC group; 13 of the SCA-N group), because of refusal to donate further blood samples.

No differences in allele distribution of the polymorphisms studied were found when OVC were stratified according to each of the complications, although the number of samples in each analysis was too small to permit conclusive results.

These results are summarized in Tables 3 and 4.

Discussion

The present study investigated two groups of patients in which clinical data such as fetal haemoglobin level and β-globin S haplotypes were similar, suggesting that other factors may affect the development of OVC in these patients.

The study demonstrated that the presence of the HPA-5b (Br^a) allele of GpIa in patients with SCA led to an approximately fourfold higher risk of the development of vascular complications. The frequency of HPA-5b was higher among patients of the SCA-VC group than among patients of the SCA-N group, as previously described among Brazilian African descendants [30]. The HPA-5 system has been described to be in genetic linkage with the C807T polymorphism of GpIa [33,35,36]. The HPA-5/C807T haplotype frequencies were also evaluated and analysis demonstrated no significant differences between SCA-VC or SCA-N groups. The HPA-5 system is located in the GpIa (α_2) of the $\alpha_2\beta_1$ complex, which

Table 3 Genotypes, number of alleles and allelic frequencies of human platelet antigen (HPA)-1, -2 and -5 systems in patients with sickle cell anaemia with (SCA-VC) or without (SCA-N) occlusive vascular complications

	SCA-VC (n = 34)							SCA-N (n = 63)							
	Genotype (%)			No. of alleles		Allele frequency		Genotype (%)			No. of alleles		Allele frequency		
	aa	ab	bb	a	b	a	b	aa	ab	bb	a	b	a	b	P[a]
HPA-1	64·7	32·4	2·9	55	13	0·809	0·191	73·0	25·4	1·6	108	18	0·857	0·143	NS
HPA-2	76·5	20·6	2·9	59	09	0·868	0·132	60·3	34·9	4·8	98	28	0·778	0·222	NS
HPA-5	44·1	47·1	8·8	46	22	0·676	0·324	77·8	22·2	0·0	112	14	0·889	0·111	$\chi^2 = 13{\cdot}19$ $P = 0{\cdot}0002$

[a]χ^2 P-values were obtained by comparing allele frequencies of each polymorphism between the groups.

Table 4 Genotypes, number of alleles and allelic frequencies of the Gpla C807T polymorphism and -5T/C Kozak sequence in patients with sickle cell anaemia with (SCA-VC) or without (SCA-N) occlusive vascular complications

	SCA-VC (n = 34)							SCA-N (n = 63)							
	Genotype (%)			No. of alleles		Allele frequency		Genotype (%)			No. of alleles		Allele frequency		
	CC	CT	TT	C	T	C	T	CC	CT	TT	C	T	C	T	
C807T	61·8	26·5	11·8	51	17	0·750	0·250	46·0	36·5	17·5	81	45	0·643	0·357	NS
Kozak	0·0	41·7	58·3	10	38	0·208	0·792	6·0	32·0	62·0	22	78	0·220	0·780	NS

NS, not significant.

is a collagen receptor on the platelet surface and is important for platelet adhesion under both static and flow conditions [37]. This receptor is essential for stable thrombus formation and generates intracellular signals when coupled with its ligand [38] that may have a significant role in pathological thrombosis, especially in individuals exposed to increased prothrombotic risk, such as that found in sickling events. The HPA-5a (Br^b) allele has been described as a risk factor for arterial vascular occlusion [20,39], in contrast to the finding of this study, in which the HPA-5b allele was correlated to OVC in patients with SCA. A role for this allele has been previously described in the development of acute idiopathic thrombocytopenic purpura (ITP) [22] and with acute vascular rejection of renal transplants [21].

Indeed, the $\alpha_2\beta_1$ complex is also expressed in endothelial cells, fibroblasts, epithelial cells and activated lymphocytes [36]. This complex mediates the cell–matrix interaction and is involved in collagen-induced angiogenesis, mediating fibroblast–collagen interactions, among other functions [23,40]. The HPA-5 polymorphism and the higher incidence of OVC in SCA may not involve platelet-mediated mechanisms. The effect of the HPA-5 polymorphism on the level of vessel stenosis (intimal hyperplasia, fragmentation and duplication of internal elastical lamina) seen in patients with SCA [41] has not been studied. However, the $\alpha_2\beta_1$ complex is known to participate in vascular modelling [40]. Agents that prevent signalling by $\alpha_2\beta_1$ may therefore represent a potential novel therapy that could lead to a better long-term outcome in patients with this disease.

None of the other vascular occlusion-related platelet polymorphisms (HPA-1b, HPA-2b, -5TC Kozak) studied here showed any correlation with OVC among patients with SCA, suggesting that they are not markers of risk for these complications in these patients.

In conclusion, the results presented herein suggest that HPA-5b is a genetic risk factor for the development of vascular complications in patients with SCA. Further studies with a larger number of patients and of different ethnical backgrounds, presenting each of the complications studied, may be of interest to evaluate whether this polymorphism can influence their occurrence as well as their outcome. In this setting, the development of agents directed against this target could lead to a new approach in long-term follow-up, with a lower incidence of OVCs in patients with SCA.

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References

1 Beutler E: The sickle cell diseases and related disorders; in Ernest Beutler MAL, Barry SC, Thomas JK, Uri S (eds): *Williams Hematology*, edn 6. New York, McGraw-Hill Companies Inc., 2001; 581–605
2 Castro O: Management of sickle cell disease. recent advances and controversies. *Br J Haematol* 1999; **107**:2–11
3 Steinberg MH: Management of sickle cell disease. *N Engl J Med* 1999; **340**:1021–1030
4 Serjeant GR: The emerging understanding of sickle cell disease. *Br J Haematol* 2001; **112**:3–18
5 Prengler M, Pavlakis SG, Prohovnik I, Adams RJ: Sickle cell disease: the neurological complications. *Ann Neurol* 2002; **51**: 543–552
6 Wun T, Paglieroni T, Rangaswami A, Franklin PH, Welborn J, Cheung A, Tablin F: Platelet activation in patients with sickle cell disease. *Br J Haematol* 1998; **100**:741–749
7 Andrade FL, Annichino-Bizzacchi JM, Saad ST, Costa FF, Arruda VR: Prothrombin mutant, factor V Leiden, and thermolabile variant of methylenetetrahydrofolate reductase among patients with sickle cell disease in Brazil. *Am J Hematol* 1998; **59**:46–50
8 Kutlar A, Kutlar F, Turker I, Tural C: The methylene tetrahydrofolate reductase (C677T) mutation as a potential risk factor for avascular necrosis in sickle cell disease. *Hemoglobin* 2001; **25**:213–217
9 Zimmerman SA, Ware RE: Inherited DNA mutations contributing to thrombotic complications in patients with sickle cell disease. *Am J Hematol* 1998; **59**:267–272
10 Arruda VR, Annichino-Bizzacchi JM, Goncalves MS, Costa FF: Prevalence of the prothrombin gene variant (nt20210A) in venous thrombosis and arterial disease. *Thromb Haemost* 1997; **78**: 1430–1433
11 Arruda VR, von Zuben PM, Chiaparini LC, Annichino-Bizzacchi JM, Costa FF: The mutation Ala677→Val in the methylene tetrahydrofolate reductase gene: a risk factor for arterial disease and venous thrombosis. *Thromb Haemost* 1997; **77**:818–821
12 Arruda VR, Annichino-Bizzacchi JM, Costa FF, Reitsma PH: Factor V Leiden (FVQ 506) is common in a Brazilian population. *Am J Hematol* 1995; **49**:242–243
13 Rosse WF, Narla M, Petz LD, Steinberg MH: New views of sickle cell disease pathophysiology and treatment [American Society of Hematology Education Program]. *Hematology* 2000; 2–17
14 Santoso S: Clinical impact of platelet glycoprotein polymorphism. *Vox Sang* 2000; **78**:121–124
15 Santoso S: Platelet polymorphisms in thrombotic disorders. *Transfus Clin Biol* 2001; **8**:261–266
16 Mikkelsson J, Perola M, Penttila A, Karhunen PJ: Platelet glycoprotein Ibalpha HPA-2 Met/VNTR B haplotype as a genetic predictor of myocardial infarction and sudden cardiac death. *Circulation* 2001; **104**:876–880
17 Vijayan KV, Goldschmidt-Clermont PJ, Roos C, Bray PF: The Pl (A2) polymorphism of integrin beta(3) enhances outside-in signaling and adhesive functions. *J Clin Invest* 2000; **105**:793–802
18 Streifler JY, Rosenberg N, Chetrit A, Eskaraev R, Sela BA, Dardik R, Zivelin A, Ravid B, Davidson J, Seligsohn U, Inbal A: Cerebrovascular events in patients with significant stenosis of the carotid artery are associated with hyperhomocysteinemia and platelet antigen-1 (Leu33Pro) polymorphism. *Stroke* 2001; **32**:2753–2758
19 Afshar-Kharghan V, Bray PF: Platelet polymorphisms; in Michelson AD (ed.): *Platelets*. San Diego, CA, USA, Academic Press, Elsevier Science, 2002:157–180
20 Kunicki TJ, Nugent DJ: The influence of platelet glycoprotein polymorphisms on receptor function and risk for thrombosis. *Vox Sang* 2002; **83**:85–90
21 Kekomaki S, Salmela K, Partanen J, Koskimies S, Kekomaki R: Alloimmunization against platelet antigens in renal transplant patients with acute vascular rejection. *Clin Transplant* 1997; **11**:19–24
22 Castro V, Oliveira GB, Origa AF, Annichino-Bizzacchi JM, Arruda VR: The human platelet alloantigen 5 polymorphism as a risk for the development of acute idiopathic thrombocytopenia purpura. *Thromb Haemost* 2000; **84**:360–361
23 Kelynack KJ, Hewitson TD, Nicholls KM, Darby IA, Becker GJ: Human renal fibroblast contraction of collagen I lattices is an integrin-mediated process. *Nephrol Dial Transplant* 2000; **15**: 1766–1772
24 Hewish MJ, Takada Y, Coulson BS: Integrins alpha2beta1 and alpha4beta1 can mediate SA11 rotavirus attachment and entry into cells. *J Virol* 2000; **74**:228–236
25 Haynes J, Jr, Manci E, Norbert V: Pulmonary complications; in Steinberg MH (ed.): *Sickle Cell Disease: Basic Principles and Clinical Practice*. New York, Raven Press Ltd, 1994:623–631
26 Adams RJ: Neurologic complications; in Steinberg MH (ed.): *Sickle Cell Disease: Basic Principles and Clinical Practice*. New York, Raven Press Ltd, 1994:599–621
27 Hakim LS, Hashmat AI, Macchia RJ: Priapism; in Steinberg MH (ed.): *Sickle Cell Disease: Basic Principles and Clinical Practice*. New York, Raven Press Ltd, 1994:633–643
28 Milner PF, Joe C, Burke GJ: Bone and joint disease; in Steinberg MH (ed.): *Sickle Cell Disease: Basic Principles and Clinical Practice*. New York, Raven Press Ltd, 1994:645–661
29 Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**:1215
30 Castro V, Origa AF, Annichino-Bizzacchi JM, Soares M, Menezes RC, Goncalves MS, Costa FF, Arruda VR: Frequencies of platelet-specific alloantigen systems 1–5 in three distinct ethnic groups in Brazil. *Eur J Immunogenet* 1999; **26**:355–360
31 Carlsson LE, Santoso S, Spitzer C, Kessler C, Greinacher A: The alpha2 gene coding sequence T807/A873 of the platelet collagen receptor integrin alpha2beta1 might be a genetic risk factor for the development of stroke in younger patients. *Blood* 1999; **93**: 3583–3586
32 Afshar-Kharghan V, Li CQ, Khoshnevis-Asl M, Lopez JA: Kozak sequence polymorphism of the glycoprotein (GP) Ibalpha gene is a major determinant of the plasma membrane levels of the platelet GP Ib-IX–V complex. *Blood* 1999; **94**:186–191

33 Corral J, Rivera J, Gonzalez-Conejero R, Vicente V: The number of platelet glycoprotein Ia molecules is associated with the genetically linked 807 C/T and HPA-5 polymorphisms. *Transfusion* 1999; **39**:372–378

34 Dean AG, Dean JA, Burton AH, Dicker RC: Epi Info: a general-purpose microcomputer program for public health information systems. *Am J Prev Med* 1991; **7**:178–182

35 Reiner AP, Aramaki KM, Teramura G, Gaur L: Analysis of platelet glycoprotein Ia (alpha2 integrin) allele frequencies in three North American populations reveals genetic association between nucleotide 807C/T and amino acid 505 Glu/Lys (HPA-5) dimorphisms. *Thromb Haemost* 1998; **80**:449–456

36 Kritzik M, Savage B, Nugent DJ, Santoso S, Ruggeri ZM, Kunicki TJ: Nucleotide polymorphisms in the alpha 2 gene define multiple alleles that are associated with differences in platelet alpha 2beta 1 density. *Blood* 1998; **92**:2382–2388

37 He L, Pappan LK, Grenache DG, Li Z, Tollefsen DM, Santoro SA, Zutter MM: The contributions of the $\alpha 2\ \beta 1$ integrin to vascular thrombosis *in vivo*. *Blood* 2003; **102**:3652–3657

38 Inoue O, Suzuki-Inoue K, Dean WL, Frampton J, Watson SP: Integrin alpha2beta1 mediates outside-in regulation of platelet spreading on collagen through activation of Src kinases and PLCgamma2. *J Cell Biol* 2003; **160**:769–780

39 Kroll H, Gardemann A, Fechter A, Haberbosch W, Santoso S: The impact of the glycoprotein Ia collagen receptor subunit A1648G gene polymorphism on coronary artery disease and acute myocardial infarction. *Thromb Haemost* 2000; **83**:392–396

40 Sweeney SM, DiLullo G, Slater SJ, Martinez J, Iozzo RV, Lauer-Fields JL, Fields GB, San Antonio JD: Angiogenesis in collagen I requires alpha2beta1 ligation of a GFP*GER sequence and possibly p38 MAPK activation and focal adhesion disassembly. *J Biol Chem* 2003; **278**:30516–30524

41 Adams RJ, Ohene-Frempong K, Wang W: Sickle cell and the brain. *Hematology* 2001; **2001**:31–46