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Circulating soluble E-selectin levels and the Ser128Arg polymorphism in individuals from different ethnic groups

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Summary *Background and aim:* An association between the Ser128Arg polymorphism and coronary heart disease (CHD) has been previously demonstrated in a white population. The aim of this study was to investigate whether the Ser128Arg polymorphism of the E-selectin gene is associated with soluble E-selectin levels in individuals from a multiethnic population.
Methods and results: Plasma sE-selectin levels and the Ser128Arg E-selectin gene polymorphism were determined in 244 white (109 females), 176 of African origin (90 females) and 208 South Asian (95 females) healthy individuals living in England selected from the Wandsworth Heart and Stroke Study (WHSS). The substitution of serine for arginine (A to C mutation) was more common in whites (9.6%) and South Asians (7.9%) compared to the people of African origin (3.7%); $p = 0.005$. The C mutation had no effect on sE-selectin levels in any ethnic group.
Conclusions: We found a lower frequency of this polymorphism in the people of African origin who have a low CHD risk. However, in this study the polymorphism was not associated with circulating sE-selectin levels. Whether it plays a role in determining ethnic differences in vascular disease via a mechanism affecting leukocyte recruitment remains to be determined.

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Introduction

Adhesion molecules are important in the development and formation of atheromatous plaques [1]. Selectins mediate leukocyte rolling on the endothelium and platelet–leukocyte interaction. E-selectin is only expressed in activated endothelial cells and acts as an adhesive reactant [2]. Endothelial activation is a characteristic of cardiovascular disease (CVD) and a role for E-selectin in CVD has been postulated.

On activation a soluble form of E-selectin (sE-selectin) is released into the circulation [3]. Increased levels of sE-selectin have been found in individuals with myocardial infarction (MI) [4] and sE-selectin levels are related to blood pressure [5,6]. Coronary heart disease (CHD) and CVD vary by ethnic origin [7]. In our own studies, we have shown that there are ethnic differences in sP-selectin, sICAM-1 and VCAM-1 levels but not in sE-selectin levels [8].

Selectins are glycoproteins, which have both an amino terminal lectin-like domain and an epidermal growth factor-like domain. The lectin-like domain plays an important role in mediating cell binding through interaction with cell surface carbohydrate ligands [9,10]. Wenzel et al. [11] described the polymorphism at codon 128 in the epidermal growth factor-like domain of E-selectin. This results in an adenine to cytosine substitution, which causes an amino acid exchange from serine (S) to arginine (R) [11]. The 128Arg allele exhibits decreased binding specificity and increased affinity for additional ligands [12] and, the range of lymphocytes recruited by E-selectin is extended [13]. These effects may provide a mechanistic link between this polymorphism and vascular inflammatory disease. Indeed, the 128Arg allele has been linked to the prevalence of atherosclerosis in young white individuals [11] and has been associated with increased restenosis following coronary angioplasty [14]. The frequency of the polymorphism has been shown to vary with age [15] possibly indicating selective mortality. Moreover, Bannan et al. [16] demonstrated an association between this polymorphism and E-selectin levels, the levels being higher in those individuals possessing the arginine allele. Therefore the purpose of our study was to determine whether the Ser128Arg polymorphism was related to plasma sE-selectin levels in a multiethnic population.

Materials and methods

Subjects

The Wandsworth Heart and Stroke Study (WHSS) population of 1577 individuals comprises approximately equal numbers of whites, black Africans (West African & Caribbean) and South Asians (40–59 years), recruited from the lists of general practices in South London [17,18]. For the present study, individuals were selected if they did not have diabetes, were not on hypertension or lipid lowering medication and not taking the oral contraceptive pill or hormone replacement therapy. Subjects were selected who did not have any previous medical history of ischaemic heart disease or stroke. Seven hundred and five individuals were identified and 628 had samples suitable for genetic analysis. The characteristics of the 628 were not significantly different from the 77 who did not have suitable samples. Of the individuals studied, 244 were whites (109 females), 176 were of African origin (90 females) and 208 were South Asians (95 females). People of African origin were all first generation immigrants. The Local Ethics Committee approved the study. All participants gave their informed consent to participate.

Methods

Subjects who had fasted overnight and had refrained from smoking or taking vigorous exercise were seen between 08:00 am and 12:00 noon the following day. A detailed questionnaire was administered and height and weight were measured [17,18]. Blood pressure was taken using standard methods and an automated recorder [17,18]. Fasting blood was taken in the seated position without stasis [18]. Age was used as a proxy for menopausal status with a cut-off of 50 years. The number of subjects in each ethnic group <50 years or ≥ 50 years of age were as follows (whites 136 vs 108, South Asians 73 vs 103, Africans 140 vs 68).

Biochemistry

Soluble E-selectin (sE-selectin) levels were determined using commercially available ELISA kits (R & D systems Europe Ltd, Abingdon, U.K.) on heparinized plasma, which had been stored at $-40\,^{\circ}C$ and defrosted at room temperature prior to analysis. We avoided using EDTA plasma samples because sE-selectin is a Ca^{++} dependent molecule, or serum samples because P-selectin is contained in platelets and their activation during the clotting process may lead to the release of P-selectin into the circulation. Intra- and inter-assay coefficients of variation were all $<2.5\%$. Biochemical measurements were performed with standardised methods, as described previously [17,18].

Genetic analysis

Genomic DNA was extracted from whole blood as previously described using Nucleon BACC DNA extraction kit [19]. In order to detect the A-128C Serine (S) to arginine (R) polymorphism, polymerase chain reaction (PCR) was performed in a total volume of 25 μL containing 100 ng of DNA, 12.5 pmol of each primer, 200 μmol/L dNTPs, 1.5 mmol/L $MgCl_2$ and 0.5 U Redhot DNA polymerase (Abgene EPSON, U.K.). The sequence of the sense oligonucleotide primer was 5′-AGTAATAGTCCTCCTCATCATG-3′ and that of the antisense primer was 5′-ACCATCTCAAGTGAA GAAAGAG -3′. After an initial denaturation at 94 °C for 5 min, amplification was carried out by 35 cycles of 94 °C for 30 s, 58 °C for 60 s and 72 °C for 60 s and a final extension at 72 °C for 10 min. The PCR product (357 bp) was then digested using *Pst*I (Fermentas), and the digested products run on a 2% agarose gel and visualised under UV light by ethidium bromide staining. Genotype was confirmed by direct sequence analysis of both strands on an ABI 377 automated sequencer. Samples with A-128A, A-128C and C-128C genotype confirmed by sequencing were used for internal controls for the verification of the digestion assay. To prevent observer bias, the investigator was unaware of the sample origin and a separate individual cross-checked all the gels. Another independent individual performed the sequencing.

Statistical analysis

Plasma levels of sE-selectin were positively skewed; therefore analyses were performed on log transformed data and the results are presented as geometric mean and 95% confidence intervals (C.I.). Differences between ethnic groups (adjusted for age and sex) were tested using analysis of covariance. Differences between ethnic groups in smoking were adjusted using age standardisation, a direct method. Associations between plasma levels and genotype were done using analysis of variance and covariance. Differences in genotype and allelic frequency between ethnic groups were evaluated with Fisher's exact test or χ^2 tests as appropriate. A p value of less than 0.05 was considered statistically significant.

Results

As reported previously, in a similar subset of the WHSS study [8] there were marked ethnic differences in the cardiovascular risk factors but no difference in soluble E-selectin levels (whites 45.5 (43.2—47.9) ng/mL; South Asians 46.0 (43.4—48.7) ng/mL; Africans 46.3 (43.4—49.3) ng/mL; $p = 0.904$). The C allele was more common in white (9.6%) and South Asian (7.9%) than in the people of African origin (3.7%) ($p = 0.0046$) (Table 1). However, homozygosity for the C allele was rare. There was no difference in allele frequency between the 102 Caribbean and the 74 West African individuals studied (3.4% vs 4.1%: $\chi^2 = 0.09$; $p = 0.76$ (df = 1)). The allele frequency did not vary by smoking status in white ($\chi^2 = 0.6$; $p = 0.72$ (df = 2)), in South Asian ($\chi^2 = 0.1$; $p = 0.95$ (df = 2)) or in the people of African origin ($\chi^2 = 1.1$; $p = 0.57$ (df = 2)). Likewise, the frequency of the polymorphism was not

Table 1 Gene and allele frequency of the Ser128Arg E-selectin gene polymorphism, in individuals of different ethnic origin from the Wandsworth Heart and Stroke Study

Ethnic origin	Ser128Arg genotypes				Allele frequency		
	AA	AC	CC	Total	A	C	Total
White	198 (81.1%)	45 (18.4%)	1 (0.4%)	244 (100%)	441 (90.4%)	47 (9.6%)	488 (100%)
South Asian	175 (84.1%)	33 (15.9%)	0 (0.00%)	208 (100%)	383 (92.1%)	33 (7.9%)	416 (100%)
African origin	165 (93.8%)	9 (5.1%)	2 (1.1%)	176 (100%)	339 (96.3%)	13 (3.7%)	352 (100%)

Gene frequency statistics	Allele frequency statistics
Total group: $\chi^2 = 18.62$; $p < 0.0009$ (df = 4)	Total group: $\chi^2 = 0.77$; $p = 0.0046$ (df = 2)
White and South Asian origin: $\chi^2 = 1.41$; $p = 0.4951$ (df = 2)	White and South Asian origin: $\chi^2 = 0.8$; $p = 0.3702$ (df = 1)
White and African origin: $\chi^2 = 16.76$; $p = 0.0002$ (df = 2)	White and African origin: $\chi^2 = 10.87$; $p = 0.001$ (df = 1)
South Asian and African origin: $\chi^2 = 13.44$; $p = 0.0012$ (df = 2)	South Asian and African origin: $\chi^2 = 6.09$; $p = 0.0136$ (df = 1)

Table 2 Allele frequency of the Ser128Arg E-selectin gene polymorphism, in individuals of different ethnic origin from the Wandsworth Heart and Stroke Study according to age <50 or ≥ 50 years

Ethnic origin	Allele frequency							
	White		South Asian		African origin		Total	
	A	C	A	C	A	C	A	C
Women								
<50 Years	119 (89)	15 (11)	121 (92)	11 (8)	81 (96)	3 (4)	321 (92)	29 (8)
≥ 50 Years	77 (92)	7 (8)	54 (93)	4 (7)	91 (95)	5 (5)	222 (93)	16 (7)
	$\chi^2 = 0.52$; $p = 0.47$ (df = 1)		$\chi^2 = 0.07$; $p = 0.79$ (df = 1)		$\chi^2 = 0.12$; $p = 0.73$ (df = 1)		$\chi^2 = 0.07$; $p = 0.79$ (df = 1)	
Men								
<50 Years	126 (91)	12 (9)	137 (93)	11 (7)	31 (100)	0 (0)	325 (93)	23 (7)
≥ 50 Years	119 (90)	13 (10)	71 (91)	7 (9)	105 (95)	5 (5)	295 (92)	25 (8)
	$\chi^2 = 0.06$; $p = 0.81$ (df = 1)		$\chi^2 = 0.27$; $p = 0.60$ (df = 1)		$\chi^2 = 0.51$; $p = 0.02$ (df = 1) but χ^2 test invalid Fisher's exact test $p = 0.06$ (2 sided)		$\chi^2 = 0.07$; $p = 0.79$ (df = 1)	
Women and men								
<50 Years	245 (90)	27 (10)	258 (92)	22 (8)	143 (98)	3 (2)	646 (93)	52 (7)
≥ 50 Years	196 (91)	20 (9)	125 (92)	11 (8)	196 (95)	10 (5)	517 (93)	41 (7)
	$\chi^2 = 0.06$; $p = 0.81$ (df = 1)		$\chi^2 = 0.00$; $p = 1$ (df = 1)		$\chi^2 = 1.33$; $p = 0.25$ (df = 1)		$\chi^2 = 0.00$; $p = 1$ (df = 1)	

Allele frequencies are given as % in brackets.

affected by gender in white ($\chi^2 = 0.1$; $p = 0.76$ (df = 1)), in South Asian ($\chi^2 = 0.0$; $p = 0.98$ (df = 1)) or in the people of African origin ($\chi^2 = 0.58$; $p = 0.45$ (df = 1)). In each ethnic group the frequency of the C allele was not significantly different between those <50 or ≥ 50 years of age (whites 10% vs 9%; South Asians 8% vs 8%, Africans 2% vs 5% (all not significant by χ^2)) (Table 2). The C mutation was not associated with changes in sE-selectin levels in any of the ethnic groups (Table 3).

Discussion

Our study shows that the Ser128Arg polymorphism of the E-selectin gene is rarer in the people of African origin than the white and South Asians. Moreover, the presence of the C allele does not seem to be associated with higher levels of circulating soluble E-selectin levels. The Ser128Arg polymorphism is in the coding region of the gene. Polymorphisms in this region do not normally affect gene expression levels and consistent with Rauchhaus et al. [14] we did not find an association between plasma E-selectin levels and the Ser128-Arg polymorphism. However, a study by Bannan et al. [16] had previously demonstrated an association between this polymorphism and sE-selectin levels. One possible explanation for this is that the S128R mutation may be linked to other E-selectin mutations. Indeed, Wenzel et al. [15] found a correlation between the Ser128Arg polymorphism and the G98T mutation and although Zheng et al. [20] did not find a significant correlation between the two mutations, they noted that 16% of the patients with premature CAD had both mutations compared with 4% of controls. Since our study was performed in relatively healthy individuals it is likely that these individuals have a low frequency of the G98T mutation and hence,

Table 3 Age and sex adjusted soluble adhesion molecule levels according to Ser128Arg E-selectin genotype

	sE-selectin levels (ng/mL) by Ser128Arg E-selectin genotype		
	AA	AC + CC	*p*
White	46.2 (43.5–49.0); $n = 198$	42.2 (37.4–47.8); $n = 46$	0.199
South Asian	46.1 (43.5–48.8); $n = 175$	44.9 (39.4–51.2); $n = 33$	0.722
African origin	46.0 (43.0–49.2); $n = 165$	49.2 (37.8–63.9); $n = 11$	0.626

Results are geometric means (95% C.I.). *p* values are for test of heterogeneity between different genotypes by analysis of covariance.

consistent with the previous studies we do not show an association between the Ser128Arg polymorphism and plasma levels. Alternatively, the Ser128Arg polymorphism could code for an E-selectin molecule with different susceptibility to the cleavage of the native form expressed on endothelial cell surface after cell activation, thus leading to low circulating soluble E-selectin levels.

Ellsworth et al. [21] demonstrated that the E-selectin polymorphism was significantly associated with coronary artery calcification but only in women who were younger than 50 years of age. Wenzel et al. [15] found that the mutation was increased in patients who were <40 years (frequency of mutation according to age: 8.7% (unselected population) 15.7% (<50) vs 21.6% (<40)). Our individuals were in the age range 40–59, however, we did not find any significant difference in the allele frequency according to the age in any of our ethnic groups.

The 128Arg allele is associated with decreased binding specificity and increased affinity for additional ligands [12] and with an extension in leukocytes recruitment [13]. Since these are potentially deleterious effects it is interesting that we have found a decreased frequency of this polymorphism in black individuals who have a lower incidence of CHD than whites or South Asians.

External validity and comparison with other studies

In one study Wenzel et al. [22] reported that the allele frequencies in 102 Caucasians were 91.0% and 9.0%. This is comparable to that found in our study in whites (90.4% and 9.6%). In a separate study, Wenzel et al. [11] reported that the frequency of the 128Arginine allele in patients with angiographically proven severe atherosclerosis was increased (15.5%) compared to that in an unselected population (8.8%). In our study, although the frequencies in relatively healthy white and South Asian individuals were comparable (9.6% and 7.9%, respectively), the allele frequency was significantly reduced in African individuals (3.7%). However, there was no difference in frequency between those of Caribbean origin (3.4%) and those from West Africa (4.1%).

Wenzel et al. [11] reported that in only three cases out of 199 persons (cases and controls) were both alleles mutated. In our total group of 628 healthy individuals we also found that the occurrence of two mutated alleles was rare and was present in only three individuals in this multiethnic population. For this reason, detailed analysis was performed comparing homozygous AA with pooled CC homozygotes and AC heterozygotes.

Strengths and limitations

Our study is population-based and used random sampling from the general population coresident in an inner city borough with a high proportion of ethnic minority populations. The participants lived within the same geographical area and this might have mitigated some potential effects of differences in environmental background including differences in socio-economic status. The study examined first generation immigrants of ethnic minority groups with both parents born in the country of origin and belonging to the same ethnic background, thus markedly reducing the possible impact arising from an unknown degree of admixture. We used standardised methods across all ethnic groups, thus minimising systematic bias. Moreover, our selection criteria excluded possible effects due to disease status or pharmacological treatment.

Potential limitations of our study include its cross-sectional design, which means it cannot establish cause–effect relationship. Moreover the decision to exclude diabetics, treated individuals and women on the oral contraceptive pill or hormone replacement therapy has led to exclusions which varied by ethnic group. Whilst this limits the generalisability of our findings to a rather healthy population it does provide a valid assessment of the relationship between circulating sE-selectin levels and the genetic polymorphism.

Implications

Genetic factors in association or combination with the environment play an important role in CHD pathogenesis. Adhesion molecules are important in atherosclerotic plaque development. In this study we have investigated the Ser128Arg polymorphism of the E-selectin gene, which has been previously demonstrated to be associated with atherosclerosis and increased coronary artery calcification [11,21], restenosis following coronary angioplasty [14,21], early-onset CAD [23] and early severe CHD [24]. In this study we have demonstrated that the arginine allele frequency was significantly reduced in individuals of African origin compared to the whites and South Asians, which is consistent with the reduced CHD observed in blacks compared to the white and South Asian individuals. Our study was performed in healthy individuals, therefore the possibility that African individuals with early severe atherosclerosis [11] might have an

increased allele frequency cannot be excluded. Detailed prospective studies in individuals of different ethnic origins are required to establish the importance of this polymorphism in CHD and atherosclerosis. This is especially important as the reduced frequency of the 128Arg allele, which is known to modulate leukocyte binding, might be contributing to the decreased CHD in blacks, although more likely through a mechanism independent of the circulating sE-selectin.

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