Of the 61 black patients, one C/T heterozygote was found (Fig. 1B) whereas all the 64 black controls were of the wild type. A DNA sequence analysis was subsequently performed and confirmed heterozygosity for the C/T polymorphism, 1 bp upstream from the known 20210G/A mutation at position 20209. A review of the medical history of this patient revealed that he was a 79 year-old Jamaican male with prostatic carcinoma and proximal DVT. The C20209T polymorphism was not found in any of the 50 healthy Asian Indians or 69 healthy South East Asians tested.

PT20209C/T is a novel polymorphism previously reported in 4 unrelated African Americans but not in white Americans (4). We have demonstrated a low prevalence (1.6%) of this polymorphism in 61 black subjects with venous thrombosis and an absence of the polymorphism in healthy black controls, Asian Indians and SE Asians. The true prevalence of the PT20209C/T polymorphism cannot be defined in a small case-control study but it would appear to be lower in our study population (heterozygosity 0.8%) compared to African-Americans (heterozygosity 4.9%). Interestingly, in an unpublished study of 503 individuals in the UK (unknown ethnicity) referred for standard thrombophilia tests, only one C/T heterozygote was detected. Thus, it would appear that the frequency of this mutation in the UK population is not high. The clinical significance of the 20209C/T polymorphism among the black population remains unclear and there are as yet insufficient data to suggest a possible prothrombotic role. Whether the PT20209C/T polymorphism is associated with elevated prothrombin levels is unknown but this may be a possibility given its proximity to PT20210G/A. Further research is required to clarify the prevalence and significance of the PT20209C/T in the black population.

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A functional serotonin transporter (SLC6A4) polymorphism modifies the association of smoking and diabetes with asymptomatic carotid atherosclerosis

Dear Sir,

Serotonin (5-HT), a crucial mediator of platelet activation, is conveyed into the cells through a membrane transporter. There is a 44-bp insertion/deletion polymorphism (long [L] and short [S] allele) in the promoter region of the SLC6A4 gene, coding for the 5-HT transporter. The L allele is associated with higher promoter activity (1), increased 5-HT uptake in platelets (2) and with higher 5-HT plasma levels (3). Recently, three case-control studies described an association of the SLC6A4 polymorphism with coronary heart disease (CHD) and myocardial infarction (4–6). Carotid atherosclerosis (CA) measured by ultrasound correlates with existing CHD and is predictive of cardiac events in individuals without clinically evident disease (7). We consequently investigated the association of the SLC6A4 polymorphism with asymptomatic CA among participants of the Study of Health in Pomerania (SHIP).

For this purpose, a total of 629 subjects aged 45 to 79 years had completed genotyping by PCR and ultrasound investigation of the carotid arteries as previously described (8). CA was defined as presence of atherosclerotic plaques in the extracranial carotids on either side (common, internal, external carotids and carotid bifurcation). Hypertension was defined as a systolic blood pressure (BP) of ≥160 mmHg, a diastolic BP of ≥95 mmHg, or intake of antihypertensives. Smoking was defined as current smoking of at least one cigarette per day (mean daily consumption: 13.4 ± 8.8 cigarettes), diabetes as self-reported physician diagnosis of diabetes, or serum haemoglobin A_{1C} (HbA_{1C}) of >7.0%. Non-fasting blood samples were taken and lipids and HbA_{1C} were determined according to standard procedures. Data were analyzed using ANOVA and logistic regression. Gene-to-

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Table 1: ((author: add legend)) Odds ratios and confidence intervals were calculated by backward and forward stepwise regression analyses with CA as dependent variable and as independent variables: age (10-year increments), sex, diabetes, hypertension, smoking, BMI, total cholesterol/HDL ratio and genotype as independent variables. *p<0.05, †p<0.01, ‡p<0.001

	Subjects (%)	Odds ratio	95% CI
Age, per 10-year increment	-	2.74	2.10 – 3.59‡
Male sex, y/n	186 (29.6)	1.69	1.05 – 2.70*
Hypertension, y/n	375 (59.6)	1.87	1.22 – 2.87†
Smoking, y/n	95 (15.1)	1.37	0.78 – 2.36
BMI, per 1-SD	-	1.37	1.03 – 1.70*
Diabetes, y/n	97 (15.4)	1.27	0.66 – 2.46
Total Cholesterol/ HDL ratio, per 1-SD	-	1.36	0.97 – 1.62
LS genotype, y/n	290 (46.1)	1.06	0.62 – 1.83
LL genotype, y/n	235 (37.4)	1.17	0.66 – 2.07
L allele, y/n	525 (83.5)	1.11	0.66 – 1.85
LS genotype and smoking	38 (6.0)	6.70	1.31 – 34.37*
LL genotype and smoking	44 (7.0)	8.97	1.76 – 45.83†
L allele and smoking	82 (13.0)	7.85	1.25 – 28.65†
LS genotype and diabetes	47 (7.5)	5.95	1.07 – 33.08*
LL genotype and diabetes	35 (5.6)	5.86	0.98 – 35.45
L allele and diabetes	82 (13.0)	5.98	1.25 – 28.65*

environment interactions between genotype and cardiovascular risk factors were tested by backward and forward stepwise regression analyses.

Results

The percentages of the SLC6A4 LL, LS, and SS genotypes in the sample were 37.4%, 46.1%, and 16.5%. As shown in the table, age, sex, hypertension, and BMI , yet not SLC6A4 genotype, were independently related to the prevalence of CA. However, L allele carriership was associated with greatly increased odds ratios for CA among smokers and also among diabetics.

Discussion

In this study, we observed that, among smokers and diabetics, the SLC6A4 L allele is associated with an increased prevalence of CA. Although it has naturally to be emphasized that these interactions are effective for the definitions of smoking (i.e. only current smoking) and diabetes (previously established diagnosis of diabetes and/or HbA_{1C} of >7.0%) as specifically applied in this study.

To date, the SLC6A4 polymorphism has been related to CHD and myocardial infarction in three case-control studies (5–7). In one of them, it was shown that the association between the L allele and CHD was particularly pronounced among smokers (6). However, no such interaction could be shown for diabetics. In another investigation (7), individuals with the SS genotype were protected from early-onset myocardial infarction, especially those who were smokers. Protective properties of the SS genotype among diabetics, though, were likewise not found in this study. Thus, although no direct association between genotype and CA was established, our results add support to the findings from these studies and to the hypothesis that the 5-HT transporter polymorphism is related to cardiovascular disease. It seems important to note that both smoking and diabetes are conditions associated with increased platelet activation. Smoking augments platelet-dependent thrombin generation and thrombus formation (10), platelets from smokers are refractory to the anti-aggregating impact of nitroglycerin (11) and they exhibit a greater $5\text{-}HT_{2A}$- and GPIIb/IIIa receptor density (12). Similarly, platelets from diabetics show an enhanced response upon stimulation with collagen and thrombin (13) which is to some extent mediated through 5-HT (14).

In summary, we have shown in a non-clinical epidemiological cohort that the L-allele of the SLC6A4 polymorphism is associated with a 7.85-fold risk in smokers and a 6-fold risk in diabetics of CA. This association is mediated through an interaction with smoking and diabetes and may be attributable to a modification of 5-HT dependent platelet activation.

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Inverse correlation between phenylacetate hydrolase activity of the serum PON1 protein and homocysteinemia in humans

Dear Sir,

Hyperhomocysteinemia is an independent risk factor for cardiovascular disease and stroke, while also being a risk factor for neurodegenerative and renal disorders. However, the actual pathological mechanisms of increased serum levels of Homocysteine (Hcy) or its metabolites in human remain elusive.

The determinants of plasma Hcy concentration are both genetic as well as environmental. Moderate Hcy elevations can be due to homozygosity of the 677T allele of the MTHFR gene or nutritional deficiencies in the cofactors (vitamin B6 or B12) or substrate (folate) of the enzymes involved in Hcy metabolism. Severe hyperhomocysteinemia is often caused by inborn error of metabolism such as in Cystathionine Beta Synthase (CBS) deficiency.

Hcy is synthesized during the conversion of dietary methionine to cysteine. Once synthesized, Hcy can be recycled to methionine by the remethylation pathway or converted into cystathionine by the transulfuration pathway. Hcy is also metabolically converted to form homocysteine thiolactone (HTL) by a metabolic error-editing process in which Hcy is inactivated by methionyl-tRNA synthethase and is released as free HTL. HTL has been implicated in the pathology of hyperhomocysteinemia probably through increased protein homocysteinylation (1).

Paraoxonase 1 (PON1) is secreted by the liver and is a protein component of the high-density lipoproteins. Paraoxonase displays esterase activity and can hydrolyse various exogenous and endogenous substrates (2). It degrades oxidized lipids and is thus considered as a protective anti-oxidant enzyme. Several studies have shown increased susceptibility to cardiovascular disease in individuals carrying polymorphisms in the PON1 gene (3). Interestingly, paraoxonase has been recently shown to degrade HTL (4) and thus could contribute to the detoxification of this metabolite of Hcy. Moreover, in the murine model of severe hyperhomocysteinemia due to CBS deficiency, the activity of PON1 in the liver was downregulated 3-fold (5). The moderate to severe hyperhomocysteinemia in mice, caused by a hyperhomocysteinemic diet or by a genetic deficiency in CBS, is associated with reduced liver PON1 activity, with a downregulation of PON1 mRNA in the liver (6). We therefore hypothesized that homocysteine serum levels could influence serum paraoxonase activity in humans.

Blood samples from 112 subjects with various homocysteinemia were recruited from the Hospital Europeen Georges Pompidou (HEGP-France) in the different clinical units of hos-

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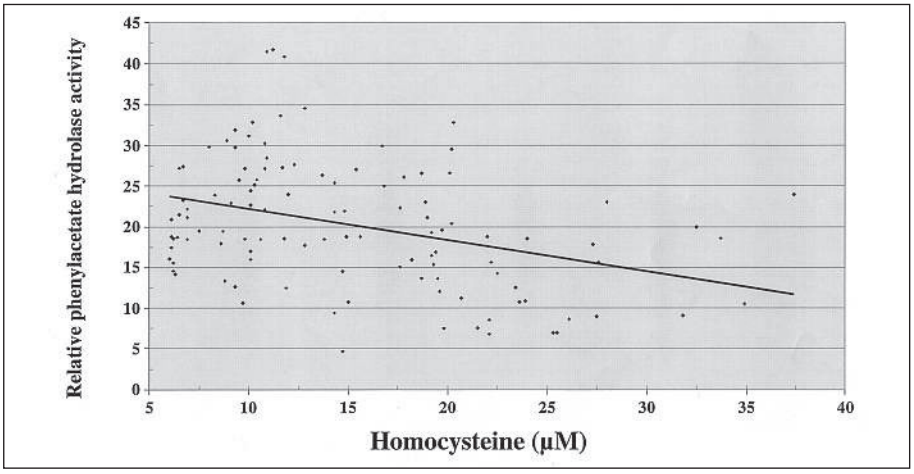

Figure 1: Relation between homocysteine concentration and relative phenylacetate hydrolase activity.