

tions of Maharashtra and Gujrat, the two states from West India. Since this polymorphism has also been studied as a risk factor for premature myocardial infarction (MI), we also recruited 120 patients of young MI (age <40 years). Two hundred unrelated Parsi individuals were also screened. The reason for including Parsis is that it is an old immigrant population which migrated from Iran (old Persia) to India several years ago to evade religious persecution and has since then remained strictly endogamous. We also found a high prevalence of FV Leiden mutation in Parsis [5], unlike the local population where FV Leiden frequency is low. Hence, in order to study the founder effect of this polymorphism in Parsis, we screened 200 unrelated Parsis. To date, 750 deep vein thrombosis patients have also been screened. We did not find this polymorphism in any of these different individuals.

In conclusion, after screening a total of 1506 individuals, half belonging to the high-risk group, our data suggest that this polymorphism is absent in India. Therefore, we strongly feel that prothrombin gene polymorphism should not be a part of routine investigations for thrombophilia in our country.

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Effect of bezafibrate on plasma homocysteine concentration in men with lower extremity arterial disease

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In the Lower Extremity Arterial Disease Event Reduction (LEADER) trial [1], a randomized trial of bezafibrate compared with placebo in men with lower extremity arterial disease (LEAD), bezafibrate non-significantly reduced the combined end-points of coronary death, fatal and non-fatal myocardial infarction and stroke despite achieving the anticipated reductions in total and LDL-cholesterol, triglycerides and plasma fibrinogen and elevation of HDL-cholesterol [1]. This limited clinical benefit might be because bezafibrate had adverse effects on other variables, one candidate being homocysteine, a novel risk factor for cardiovascular disease. We have

therefore measured plasma homocysteine in participants in LEADER.

The participants have been described elsewhere [1]: 1568 patients with LEAD were recruited through 85 practices in the Medical Research Council's General Practice Research Framework and through nine hospital vascular clinics.

Blood samples were taken at baseline and after 3 and 6 months of treatment from one of the participating vascular clinics (Glasgow Royal Infirmary). Homocysteine samples were collected in three further vascular clinics only after the initiation of treatment. Samples were collected into dipotassium EDTA and placed on ice until centrifugation at 2000 × g. The samples were posted to the central laboratory and stored at –70 °C until analysis. Homocysteine was measured by fluorescence polarization immunoassay (Abbott Laboratories, Maidenhead, UK).

Plasma homocysteine was log-transformed and means are geometric with 1 SD specified as a multiple of the mean. Levels were adjusted for serum creatinine using the regression estimate

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Table 1 Effect of bezafibrate on mean (standard deviation expressed as multiple of the mean) serum creatinine and plasma homocysteine ($\mu\text{mol L}^{-1}$)

Baseline and follow-up samples	Bezafibrate <i>n</i> = 28	Placebo <i>n</i> = 30	<i>P</i> -value
Creatinine			
Baseline	96.4 (0.18)	96.5 (0.18)	
Follow-up	108.6 (0.21)	96.7 (0.18)	
Percent change	13.2	0.6	< 0.0001
(95% CI)	12.4, 14.1	0, 1.2	
Homocysteine			
Baseline	12.74 (0.33)	12.18 (0.38)	0.64
Follow-up	17.27 (0.36)	11.63 (0.39)	0.0002
Percent change	35.6	-4.6	< 0.0001
(95% CI)	(23.6, 48.6)	(-10.1, 1.1)	
Percent change*	25.9	0.7	< 0.0001
(95% CI)	(15.3, 37.4)	(-5.4, 7.2)	
<hr/>			
Follow-up samples only	Bezafibrate <i>n</i> = 76	Placebo <i>n</i> = 101	<i>P</i> -value
Homocysteine	17.4 (0.33)	13.0 (0.36)	< 0.0001
Homocysteine*	17.3 (0.35)	13.1 (0.34)	< 0.0001

*After adjustment for serum creatinine.

of the effect of creatinine. There was no evidence of a difference between on-treatment values taken at different times (3 or 6 months), so all on-treatment values were combined. Differences between groups were tested by two-sample *t*-tests using the change in the log-transformed values.

Table 1 shows that in those with both baseline and on-treatment measures there was a significant mean increase of 35.6% in plasma homocysteine with bezafibrate but not with placebo ($P < 0.0001$) and homocysteine was significantly higher in those receiving bezafibrate than in those receiving placebo ($P = 0.0002$). After adjustment for serum creatinine, the increase in homocysteine with bezafibrate remained highly significant, the mean value being 25.9% higher than baseline, whereas it was virtually unchanged with placebo ($P < 0.0001$). A very similar effect was observed when these on-treatment results were combined with those from the three clinics where only on-treatment samples were collected, assuming baseline samples were similar (the unequal numbers in the two groups were nevertheless based on random allocation).

The $4.5 \mu\text{mol L}^{-1}$ (35.6%) rise in homocysteine in men with LEAD taking bezafibrate may have prevented the favorable lipid and fibrinogen responses leading to a reduction in clinical outcomes. A recent meta-analysis of prospective studies [2] suggests that a $5 \mu\text{mol L}^{-1}$ increase in homocysteine increases the risk of ischemic heart disease by 34% (95% confidence interval 1.22, 1.47) and stroke by 59% (1.30, 1.95). Especially if this increase were manifest soon after the increase in homocysteine, the increase of $4.5 \mu\text{mol L}^{-1}$ with bezafibrate might be sufficient to counter any benefits of lipid and fibrinogen alterations. In LEADER there was a non-significant reduction in major coronary events (relative risk 0.81, 0.60, 1.08) but a non-significant increase in stroke (relative risk 1.34, 0.80, 2.01), which is consistent with an adverse effect of bezafibrate

mediated through homocysteine. Support for the clinical significance of short-term changes in plasma homocysteine comes from the finding that homocysteine lowering by vitamin supplementation reduced the risk of coronary re-stenosis within 6 months after angioplasty [3].

Effects of fibrates on homocysteine have previously been reported [4–6]. Changes in the levels of the vitamins involved in homocysteine metabolism (folic acid, vitamin B₁₂ and vitamin B₆) are unlikely to be the mechanism for the findings [5]. There is uncertainty as to whether or not the rise in serum creatinine with fibrates is due to a reduction in creatinine clearance or to increased synthesis of creatinine with normal creatinine clearance and therefore whether or not alteration in renal function is the explanation for the rise in homocysteine [5,6]. Adjustment of homocysteine for serum creatinine in our study did not materially alter the effect of bezafibrate on homocysteine, suggesting that alteration in renal function is an unlikely explanation for the effect of bezafibrate on homocysteine that we observed.

Two studies have shown that coadministration of fenofibrate and folic acid with or without vitamin B₆ and B₁₂ significantly reduced the fibrate-induced rise in homocysteine [7,8]. Further evaluation of the effect of folic acid given concurrently with bezafibrate is now required to see if, by lowering homocysteine, this allows the potentially beneficial effects of bezafibrate on coronary heart disease and stroke to be fully expressed. Alternatively, fibrates that do not cause elevation of plasma homocysteine could be developed.

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