Haplotypes of the fibrinogen gamma gene do not affect the risk of myocardial infarction

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Table 1 Variant allele and haplotype group frequencies for *VKORC1* and *CYP2C9* in selected populations (actual SNP locations in parentheses). Haplotype groups A and B are based on classifications from Reider *et al.* [1] where haplotype A represents individuals at risk for excessive anticoagulation with standard warfarin dosing, and haplotype B represents individuals at risk for subtherapeutic anticoagulation from standard warfarin dosing. Overall low dose group defines individuals with at least one haplotype A and/or at least one *CYP2C9* variant allele. Combined haplotype A and *CYP2C9* group defines individuals with at least one haplotype A in combination with at least one *CYP2C9* variant allele. The functional significance of haplotypes not in group A or group B (other) is currently unknown. IVS is standard nomenclature for intronic sequence

	Peruvian	Mexican	European–American	African–American	African	Asian
VKORC1 861 C > A (−4451C > A)	0.04	0.15	0.33	0.12	0.04	0.02
VKORC1 5808 T > G (IVS1 + 324T > G)	0.02	0.13	0.21	0.06	0.00	0.00
VKORC1 6853 G > C (IVS2 + 124G > C)	0.31	0.49	0.37	0.22	0.25	0.91
VKORC1 9041 G > A (626G > A)	0.62	0.46	0.45	0.49	0.46	0.13
*CYP2C9**2 (3608C > T)	0.00	0.06	0.14	0.02	0.01	0
*CYP2C9**3 (42614A > C)	0.01	0.02	0.06	0.01	0.01	0.04
VKORC1 haplotype group A	0.27	0.38	0.42	0.21	0.23	0.85
VKORC1 haplotype group B	0.71	0.57	0.57	0.58	0.49	0.14
VKORC1 haplotype group – other	0.02	0.05	0.01	0.21	0.28	0.01
Overall low dose group	0.28	0.45	0.55	0.22	0.23	0.86
Combined haplotype A and *CYP2C9* group	0.00	0.03	0.18	0.01	0.01	0.06

Haplotypes of the fibrinogen gamma gene do not affect the risk of myocardial infarction

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As the precursor of fibrin, fibrinogen plays an important role in hemostasis [1]. Of all the components of the coagulation system, elevated plasma fibrinogen levels have most consistently been shown to be associated with occlusive vascular disorders [2–5]. Several polymorphisms in the fibrinogen genes (*FGA*, *FGB*, *FGG*) have been reported to be associated with fibrinogen levels. Most studies focused on FGB polymorphisms. However, results are not consistent and none of these polymorphisms has been found to be associated with an increased risk of venous [6,7] or arterial thrombosis [7]. Recently, we reported that a specific haplotype (H2) of the fibrinogen gamma gene (*FGG*) was associated with a 2.4-fold increased risk of deep venous thrombosis [95% confidence intervals (CI): 1.5–3.9] [6]. In the same study, we found that another haplotype (FGG-H3) was associated with a slight reduction in risk [odds ratio (OR) = 0.8, 95% CI: 0.6–1.0]. None of these haplotypes was associated with total fibrinogen levels. However, the FGG-H2 haplotype was associated with reduced levels of fibrinogen γ', a product of alternative splicing of the *FGG* gene. In a recent report, Mannila *et al.* [8] studied the effect of haplotypes across the fibrinogen gene cluster on the risk of myocardial infarction (MI). They determined the *216C > T polymorphism, which is identical to FGG-H2 tagging SNP 10034 C/T [rs2066865]. In their study, the FGG-H2 haplotype was not associated with the risk of MI. However, they found a significant difference in the frequency distribution of the minor allele of the 1299 + 79T > C polymorphism between patients and controls (0.294 vs. 0.342, $P = 0.04$), which suggests that this haplotype might be protective against the development of MI. This polymorphism is identical to FGG-H3 tagging SNP 9340 T/C [rs1049636].

The aim of the present study was to investigate the effect of the four most common haplotypes of the *FGG* gene on the risk of MI. For this study, a large population-based case–control study, 'Study of Myocardial Infarctions Leiden' (SMILE) was used. Full details of the SMILE study have been described

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elsewhere [9]. The subjects used in our previous study on venous thrombosis [6] were all of Dutch origin and four common haplotypes of the fibrinogen gamma gene were present in this population. Because the subjects of the SMILE study were all Dutch men, we assumed that in the SMILE population only these four common haplotypes would be present. To tag these four common haplotypes, all subjects were genotyped for three haplotype-tagging polymorphisms by the 5′ nuclease/TaqMan assay [10]. Polymorphism 10034 C/T [rs2066865] tagged FGG-H2, polymorphism 9340 T/C [rs1049636] tagged FGG-H3 and polymorphism 5836 G/A [rs2066860] tagged FGG-H4 [6]. FGG-H1 consisted of the common alleles of the three-mentioned polymorphisms. (Position numbering according to SeattleSNPs [11], GenBank Accession number AF350254).

The distribution of the genotypes among control subjects was as expected for a population in Hardy–Weinberg equilibrium. No haplotypes could be assigned to four patients and two control subjects, because of intragenic recombination. These samples were excluded from the analyses. As shown in Table 1, overall none of the haplotypes of the *FGG* gene was associated with the risk of MI, including FGG-H2, which we found to be a risk factor for venous thrombosis [6]. The allele frequency of FGG-H2 was 0.276 in cases and 0.277 in control subjects. This is in agreement with the data of Mannila *et al*. [8], who did not find an association in 377 MI patients and 387 control subjects aged < 60 years in Sweden. However, we could not confirm the reduction in risk for FGG-H3 found by Mannila *et al*. We found allele frequencies of 0.298 and 0.284 for patients and control subjects, respectively (Table 1).

Most patients with MI are middle-aged or elderly. Those suffering MI at a younger age usually have an excess of risk factors, including genetic factors. In men below the age of 50 (150 patients and 158 control subjects), the risk of MI was slightly decreased for FGG-H1 (Table 1) and slightly increased for FGG-H2. There was no effect on risk for FGG-H3 and FGG-H4. For men aged 50 or above, there was no effect on risk in any of the haplotypes. So, we only found a slight increase in risk for FGG-H2 in men below the age of 50. This might be an indication that FGG-H2 not only affects venous thrombosis risk, but also the risk of myocardial infarction, although the effect was weak (OR for FGG-H2H2 carriers 1.5) with a wide CI (0.7–3.5). The sample size of this young subgroup was relatively small and it would be useful to investigate the role of FGG-H2 in a larger study of patients with myocardial infarction at a young age.

Furthermore, we investigated the combined effect of FGG haplotypes and cardiovascular risk factors such as smoking (345 patients and 214 control subjects) or metabolic risk factors (203 patients and 197 control subjects), in which having a metabolic risk factor was defined as the presence of obesity, diabetes, hypertension or hypercholesterolemia [9]. The risks of the four haplotypes in the different subgroups with or without the cardiovascular risk factors did not change compared with the risks in the overall population. In fact, the risks did not exceed that of the single effects of the cardiovascular risk factors, so we did not find evidence for synergistic effects between FGG haplotypes and cardiovascular risk factors.

In addition, we studied the relation between the FGG haplotypes and fibrinogen levels. In the control subjects, fibrinogen levels were measured in plasma using the Clauss thrombin time method as described previously [12]. We did not observe an association of FGG haplotypes with fibrinogen

Table 1 The risk of myocardial infarction in the presence of the four most common haplotypes of the fibrinogen gamma gene

					< 50 years		≥50 years	
Haplotype (htSNP)	Patients (%) $n = 556$	Controls (%) $n = 644$	OR	95% CI	OR	95% CI	OR	95% CI
H1 (all common)								
HxHx	210 (37.8)	228 (35.4)	1*		1*		1*	
H1Hx	265 (47.7)	318 (49.4)	0.9	0.7–1.2	0.7	0.4–1.2	1.0	0.7–1.3
H1H1	81 (14.6)	98 (15.2)	0.9	0.6–1.3	0.7	0.4–1.4	1.0	0.6–1.5
Frequency H1	0.384	0.399						
H2 (10034 C/T)								
HxHx	290 (52.2)	336 (52.2)	1*		1*		1*	
H2Hx	225 (40.5)	259 (40.2)	1.0	0.8–1.3	1.3	0.8–2.1	0.9	0.7–1.2
H2H2	41 (7.4)	49 (7.6)	1.0	0.6–1.5	1.5	0.7–3.5	0.8	0.5–1.4
Frequency H2	0.276	0.277						
H3 (9340 T/C)								
HxHx	266 (47.8)	322 (50.0)	1*		1*		1*	
H3Hx	249 (44.8)	278 (43.2)	1.1	0.9–1.4	1.1	0.7–1.7	1.1	0.8–1.5
H3H3	41 (7.1)	44 (6.8)	1.1	0.7–1.8	1.1	0.4–2.9	1.2	0.7–1.9
Frequency H3	0.298	0.284						
H4 (5836 G/A)								
HxHx	509 (91.5)	593 (92.1)	1*		1*		1*	
H4Hx	47 (8.5)	51 (7.9)	1.1	0.7–1.6	0.9	0.4–2.1	1.2	0.7–1.8
H4H4	–	–	–	–	–	–	–	–
Frequency H4	0.042	0.040						

*Reference category.

levels in these control subjects. This is consistent with a previous study in which we did not find an association of these haplotypes with fibrinogen levels in the 473 control subjects of the Leiden Thrombophilia Study [6].

We conclude that none of the four common haplotypes of the FGG gamma gene has a strong effect on the risk of MI. Homozygosity for FGG-H2, which we found previously to be a risk factor for venous thrombosis, might slightly increase the risk of MI in patients younger than 50 years. These findings are in line with the studies on other prothrombotic factors that affect the risk of venous thrombosis but have small or no effects on arterial disease [13].

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References

1 Mosesson MW, Siebenlist KR, Meh DA. The structure and biological features of fibrinogen and fibrin. *Ann NY Acad Sci* 2001; **936**: 11–30.
2 Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WR, Haines AP, Stirling Y, Imeson JD, Thompson SG. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet* 1986; **2**: 533–7.
3 Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. The Framingham Study. *JAMA* 1987; **258**: 1183–6.
4 Yarnell JW, Baker IA, Sweetnam PM, Bainton D, O'Brien JR, Whitehead PJ, Elwood PC. Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell collaborative heart disease studies. *Circulation* 1991; **83**: 836–44.
5 Koster T, Rosendaal FR, de Ronde H, Briët E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet* 1993; **342**: 1503–6.
6 Uitte de Willige S, de Visser MC, Houwing-Duistermaat JJ, Rosendaal FR, Vos HL, Bertina RM. Genetic variation in the fibrinogen gamma gene increases the risk of deep venous thrombosis by reducing plasma fibrinogen {gamma}′ levels. *Blood* 2005; **106**: 4176–83.
7 Lane DA, Grant PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. *Blood* 2000; **95**: 1517–32.
8 Mannila MN, Eriksson P, Lundman P, Samnegard A, Boquist S, Ericsson CG, Tornvall P, Hamsten A, Silveira A. Contribution of haplotypes across the fibrinogen gene cluster to variation in risk of myocardial infarction. *Thromb Haemost* 2005; **93**: 570–7.
9 Doggen CJM, Cats VM, Bertina RM, Rosendaal FR. Interaction of coagulation defects and cardiovascular risk factors: increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A. *Circulation* 1998; **97**: 1037–41.
10 Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. *Genet Anal* 1999; **14**: 143–9.
11 Nickerson D. *SeattleSNPs. NHLBI Program for Genomic Applications*. [WWW document] UW-FHCRC, Seattle, WA. Available at: http://pga.gs.washington.edu. (accessed 15 April 2003).
12 Doggen CJM, Bertina RM, Cats VM, Rosendaal FR. Fibrinogen polymorphisms are not associated with the risk of myocardial infarction. *Br J Haematol* 2000; **110**: 935–8.
13 Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group. No evidence of association between prothrombotic gene polymorphisms and the development of acute myocardial infarction at a young age. *Circulation* 2003; **107**: 1117–22.

Statins modulate expression of components of the plasminogen activator/plasmin system in human cardiac myocytes *in vitro*

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Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors or statins represent an established class of drugs for the treatment of hypercholesterolemia and large-scale clinical trials have emphasized their benefits in the primary and secondary prevention of myocardial infarction (MI) through their positive effects on atherosclerosis and its complications [1–3]. Recent findings that the statins attenuate myocardial hypertrophy *in vitro* and *in vivo* provide evidence that these