Analysis of gene-environment interaction in coronary heart disease: fibrinogen polymorphisms as an example

Michela Vischetti, Francesco Zito, Maria Benedetta Donati, Licia Iacoviello

"Angela Valenti" Laboratory of Genetic and Environmental Risk Factors for Thrombotic Disease, Department of Vascular Medicine and Pharmacology, Consorzio Mario Negri Sud, S. Maria Imbaro (CH), Italy

Key words:
Fibrinogen; Gender; Gene-environment interaction; Infections; Physical activity; Polymorphisms.

Several epidemiological studies have shown that an increase in fibrinogen levels is associated with the risk of cardiovascular disease. Recently, it has been demonstrated that the levels of fibrinogen can be genetically determined. Overall the studies show a strong association between two polymorphisms of the fibrinogen β-chain gene and fibrinogen plasma concentration. Few studies have, in contrast, found an association between such polymorphisms and the risk of ischemic vascular disease.

Rather than directly affecting the levels of proteins or the risk of disease, polymorphisms can amplify the effect of environmental or intermediate conditions on the final phenotype. The genetic control of fibrinogen has to be considered together with environmental factors: fibrinogen genotypes may interact with cigarette smoking, gender, physical activity, use of drugs and infections in determining the increase in fibrinogen levels and perhaps the risk of ischemic heart disease. Three examples are presented supporting the concept that, in multifactorial diseases, genetic variability influences the risk of disease by determining a different individual susceptibility to environmental risk factors.

(Ital Heart J 2002; 3 (1): 18-23)

This work was supported by a grant from Telethon (Rome, Italy) contract n. E.1005 and by the Italian Ministry of Health.

Address:

Dr.ssa Licia Iacoviello

Laboratorio "Angela Valenti" dei Fattori Genetici e Ambientali di Rischio Trombotico Dipartimento di Medicina e Farmacologia Vascolare Consorzio Mario Negri Sud 66030 S. Maria Imbaro (CH) E-mail: iaco@cmns.mnegri.it

Fibrinogen and cardiovascular disease

Among the components of the coagulation system, high levels of fibrinogen have been strongly associated with occlusive vascular disorders[1-3].

A number of fibrinogen properties are relevant in the process that leads to thrombosis, such as its role as a substrate for fibrin formation, as a mediator of platelet aggregation[4,5] and as a determinant of plasma viscosity[6].

The first evidence of such association was provided in the early 80's by Meade et al.[1,7] in the Northwick Park Heart Study. Long-term prospective studies followed these findings. Ernst and Resch[8] published a meta-analysis of six of these studies on a total of 845 cases of coronary heart disease (CHD). Afterwards, several of these studies have reported additional follow-up results and many other studies have been performed. Danesh et al.[9] reviewed them more recently in a meta-analysis: fibrinogen levels in the top third were associated with an odds ratio of 1.8 (95% confidence interval 1.6-2.0). Increased fibrinogen level also clusters with other risk factors[10-12]. Furthermore, increased fibrinogen levels can be considered as one of the links between these environmental factors and the risk of CHD.

However, more recently the study of genetic determinants has offered a new perspective in this complex scenario of interactions.

Genetic polymorphisms and plasma fibrinogen levels

Fibrinogen is a 340-kD dimeric glycoprotein; each dimer consists of three different polypeptide chains known as α, β and γ chains, linked by disulfide bonds. The three polypeptide chains are encoded by three different genes clustered within a 50 kb region located in the distal third of the long arm of chromosome 4q23-32[13,14].

All three genes have an interleukin (IL)-6 responsive element[15,16] near to the site of the start of transcription. Fibrinogen levels arise following an acute phase response; it is therefore possible that changes in this region may affect the rate of transcription. *In vitro* studies have suggested that β-chain synthesis limits the rate of the production of mature fibrinogen[17]. As a consequence, most of the studies have focused on the β chain. Many polymorphisms have been

identified, however the two most studied were those detected by BclI and HaeIII restriction enzymes. Overall, the studies show a strong association between β-chain fibrinogen genotypes and its plasma concentrations[18-28]. On the other hand, the relation between these polymorphisms and the risk of CHD is still debated.

BclI polymorphism of the β-fibrinogen gene. Several studies have reported a DNA variation of the β-fibrinogen gene, detected at the 3' region, by the BclI restriction enzyme (BclI polymorphism), as influencing the levels of fibrinogen. This is a biallelic polymorphism, with a frequency of the rare B2 allele of 0.18 in the general population.

The rare B2B2 genotype has been consistently associated with levels of fibrinogen 15-20% higher than B1B1 genotype[19,22,28,29]. The involvement of BclI fibrinogen polymorphism in the risk of ischemic vascular disease has been confirmed by some studies, although others gave negative results[30].

-455 G/A polymorphism of the β-fibrinogen gene. The BclI polymorphism was found to be in linkage disequilibrium with the G/A sequence variation at position -455, detected in the promoter region, by the HaeIII restriction enzyme[21,26].

Due to its position in the β-fibrinogen gene, it is conceivable that this polymorphism may have an effect on transcription. It is, indeed, located in the promoter, close to responsive elements (IL-6 and the hepatocyte nuclear factor-1 elements)[16]. Therefore, this polymorphism has been selected for study by many investigators[18,20-27,30-48].

Among 22 publications between 1991 and 2000, all the studies[22,23,26,27,35-48] but four[30-33], are consistent with the finding that the A^{-455} allele, which is present in about 20% of the general population, is associated with increased levels of fibrinogen of about 0.30 g/l as compared with homozygotes for the G allele. On the contrary, only few studies have found an association between -455 G/A polymorphism and the risk of CHD[32,39,44,46,47]. In particular, out of 13 studies in which the relation between -455 G/A polymorphism and the risk of CHD was evaluated, only 5 reported the presence of an association.

The inhomogeneity of the findings published suggests two types of considerations:
• the number of subjects included in the different studies is never sufficient to detect any rational association. Indeed, considering the results of the meta-analyses of fibrinogen, it can be assumed that an increase in 1 g/l of fibrinogen accounts for a relative risk of 1.8 for CHD. Nevertheless, homozygosity for the A^{-455} allele increases of about 0.30 g/l the levels of fibrinogen, which, assuming linearity in the logarithm of relative risk, corresponds to a relative risk of about 1.20. To have the power of 80% to detect such an increase, a case control study should recruit about 11 600 cases and a corresponding number of controls. This size has never been reached in the studies published until now;
• since the effects of genetics on multifactorial diseases could not be expected to be strong by definition, it would be difficult to reveal it by studying CHD as a bulk of patients not further subtyped. Studying more homogeneous subgroups of patients can help to better define the role of genetics. This could be indeed, the case of patients with familial myocardial infarction or at young age or with very well defined clinical phenotypes. Moreover, rather than directly affecting the levels of proteins or the risk of disease, polymorphisms can amplify the effect of environmental or intermediate conditions on the final phenotype. Stressing gene-environment interactions can be finally a suitable approach to highlight the role of genetics.

Gene-environment interaction and changes in plasma fibrinogen levels

Individuals have different ability to maintain homeostasis and this can be due to genetic variations: an individual with a genetic predisposition might have a stronger response when exposed to a specific stimulus, which translates in the synthesis of higher levels of fibrinogen. As a consequence, he can have a greater thrombotic risk in respect to one who makes a moderate response to environmental stimuli (Fig. 1).

Fibrinogen is an acute phase protein and its levels rise in response to infections, inflammations and trauma[49], and to other factors such as hypertension, age, obesity, serum cholesterol level, hormonal changes and diabetes[50-54]. The within-individual variation of fibrinogen levels is high because of its sensitivity to environmental factors; however, the genetic control of fibrinogen has to be considered together with environmental factors: it has been shown that fibrinogen genotypes may interact with cigarette smoking, gender, physical activity, use of drugs in determining the increase in fibrinogen levels[35,36,40,55] and perhaps the risk of ischemic heart disease.

We shall mention in the next paragraphs three different examples of gene-environment interaction, that can modulate either the level of fibrinogen or the risk of CHD.

Fibrinogen polymorphisms and gender. Among others, a stronger association has been found between -455G/A or BclI polymorphisms and fibrinogen levels in women than in men[26,35,36].

Fibrinogen levels vary according to sex and menopausal status. They are lower in premenopausal women with respect to men of the same age, and increase after menopause. Hormone replacement therapy, however, may revert the effect of menopause on fibrinogen levels. The overall effect of the -455 G/A polymorphism is also different in males and females.

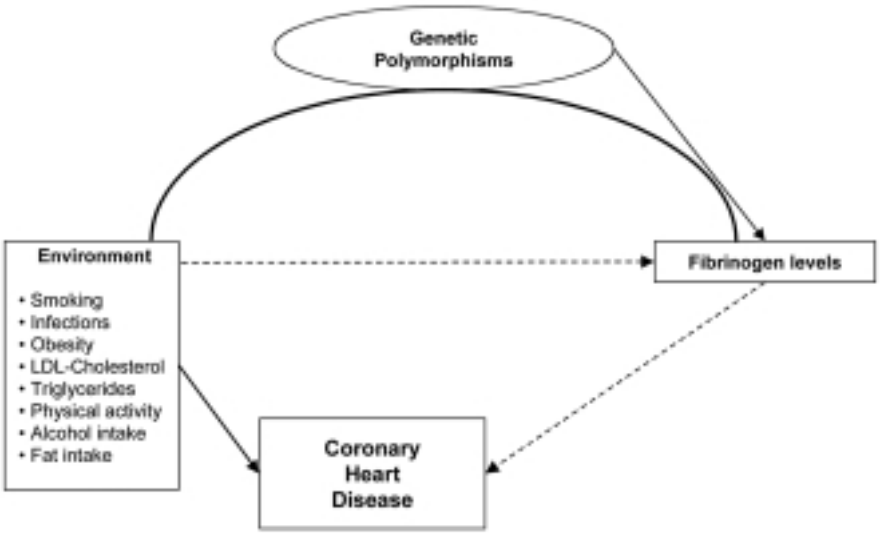

Figure 1. *Interaction between genetic and environmental factors in determining levels of fibrinogen and the risk of coronary heart disease.*

Indeed, the effect of the A allele on fibrinogen levels is additive in men, while it appears to be dominant in women with no additional effect on fibrinogen levels in the homozygotes. After stratification for menopausal status and hormone replacement therapy, the dominant effect of the A allele was evident only in postmenopausal women not taking hormones. In both premenopausal and postmenopausal women treated with hormone replacement therapy, the effect of the A allele was additive, as observed in men[36].

These findings suggest that hormones or other gender-specific factors modulate the increase in fibrinogen probably by down-regulating hepatic fibrinogen synthesis. The presence of genetic variance at the promoter levels can differently regulate the repression of the fibrinogen gene transcription. On the basis of these results, the interaction with gender should be always taken into consideration in studying the effect of fibrinogen polymorphisms on the risk of CHD.

Fibrinogen polymorphisms and physical training. Several studies have shown that a moderate physical activity decreases the levels of fibrinogen. A decrease in plasma fibrinogen concentration has also been reported in elderly men after physical training programs and in physically active postmenopausal women[56].

An inverse dose-response relationship has been described between regular physical exercise and CHD risk. Reduction in fibrinogen could, at least in part, be responsible for such an association. Some of the benefits of regular exercise, however, are lost under very intensive training, especially in those with a sedentary lifestyle.

The association between physical activity and plasma fibrinogen may vary according to fibrinogen genotypes.

Physical exercise may acutely enhance the fractional catabolic rate of fibrinogen and the degradation rate of the α-chain. However, these effects are evident only in carriers of the B2 allele of BclI β-chain polymorphism. Vaisanen et al.[57] showed that physical activity levels explained up to 9% of variance in the carriers of the B2 genotype, while the effect was only marginal in the carriers of the common B1 fibrinogen genotype. Similar results were shown for polymorphisms in the α-chain gene of fibrinogen. This is a polymorphic site within codon Aa312 which can be detected by RsaI restriction enzyme, resulting in a more frequent Thr or less common Ala phenotype.

Although no association was found between such polymorphism and plasma fibrinogen, in control populations low levels of fibrinogen were described in postmenopausal women, homozygous for the Thr allele, if physically most active, but not in carriers of the Ala allele[58]. Moreover, in a controlled randomized study, an interaction between physical activity and fibrinogen genotype was also demonstrated in middle-aged men[56]. Among the men assigned to a regular exercise training of low intensity for 3 years, individual changes in anaerobic threshold, an index of fitness, explained 48% of fibrinogen variance only in the Thr homozygotes.

Montgomery et al.[40] have demonstrated that an acute rise in fibrinogen levels occurs in response to intensive exercise. The increase lasted several days, probably due to a continuous fibrinogen production. This phenomenon is strongly influenced by the G/A -455 polymorphism of the fibrinogen gene. In particular, a gradient of increasing acute fibrinogen response was seen across the GG, GA, and AA genotypes, respectively. After 2 days of strenuous military exercise, 156 male British army recruits showed an increase in fibrinogen levels as compared to the concentration at pre-training. Levels were maximally increased at the second day after exercise. This effect, however, was stronger in the AA homozygotes as compared with heterozygotes and GG homozygotes.

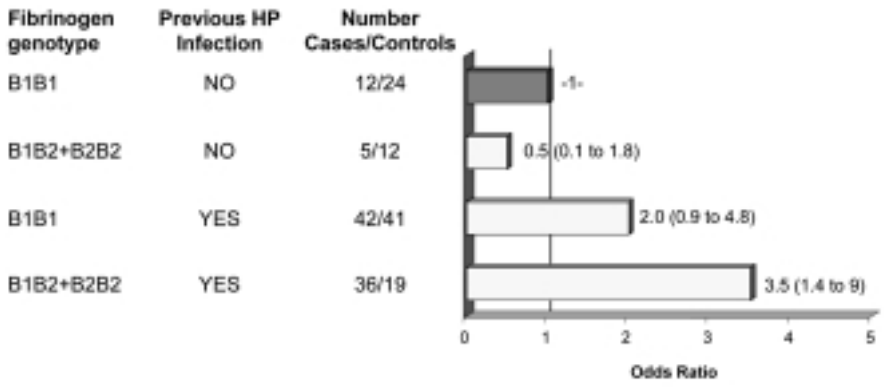

Figure 2. *Combined effect of fibrinogen genotype and* Helicobacter pylori *(HP) infection on myocardial infarction risk.*

This finding suggests the possibility of a greater response of β-chain promoter to cytokines, whose increase can also be induced by a strenuous physical training. The G/A change at position -455, indeed, is in an almost complete linkage disequilibrium with a T/C polymorphism at position 148 of the promoter. The latter is close to a consensus sequence that confers responsiveness to IL-6 in *in vitro* experiments.

More recently, however, it has been demonstrated that the G to A substitution at position -455 increases by itself the basal rate of transcription in HepG2 cells.

Fibrinogen polymorphism and *Helicobacter pylori* infection. *Helicobacter pylori* (HP) infection has recently been associated with a higher risk to develop ischemic heart disease, although the results are controversial[59-64]. Zito et al.[29] confirmed such an association in a case-control study. They found that HP infection accounts for a 4-fold increase in the risk of myocardial infarction. In that study the cases, patients who had suffered from a first episode of myocardial infarction, showed fibrinogen levels higher than controls. A clear association was found between HP infections and fibrinogen levels both in cases and in controls. Fibrinogen levels were also associated with the BclI fibrinogen polymorphism, carriers of B2 allele showing higher fibrinogen levels as compared to B1 homozygous subjects.

HP is a life-long bacterial infection and it is conceivable that the infectious stimulus could chronically increase the plasma levels of acute phase reactants, such as fibrinogen, with the possible mediation of certain cytokines. In fact, IL-6 and tumor necrosis factor-α levels were found increased in patients with HP-associated gastritis[59,60,64,65].

Carriership of the B2 allele of the BclI polymorphism amplifies the effect of seropositivity for HP[29] on the risk of myocardial infarction (Fig. 2). Indeed, patients with both seropositivity for HP and carriers of the B2 genotype showed an additional increase in the risk of myocardial infarction as compared to subjects affected by HP but homozygous for the B1 allele.

Since both seropositivity for HP and BclI polymorphism are associated with fibrinogen levels and an interaction between them has been described in determining fibrinogen levels, the gene-environment interaction reported to increase the risk of myocardial infarction could be probably explained by cumulative effects of both factors on fibrinogen levels.

Conclusions

The role of a typically acute phase reactant such as fibrinogen in CHD risk has been deeply studied in recent years through the evaluation of some of its genetic determinants: as highlighted in the three above examples, the interindividual responses to variables like gender or to environmental stimuli like physical exercise or infections may be genetically determined and genetic variability underlies the differences in biological reactions which plausibly contribute to the differences in CHD risk. These examples strongly support the concept that, in multifactorial diseases (like CHD) genetic variability, rather than playing any causal role, influences the risk of disease by determining a different individual susceptibility to environmental risk factors.

Acknowledgments

We thank Dr. Giovanni de Gaetano for critical reading of the manuscript.

References

1. Meade TW, Brozovic M, Chakrabarti RR, et al. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. Lancet 1986; 2: 533-7.
2. Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. N Engl J Med 1984; 311: 501-5.
3. Heinrich J, Balleisen L, Schulte H, Assmann G, van de Loo J. Fibrinogen and factor VII in the prediction of coronary

risk. Results from the PROCAM study in healthy men. Arterioscler Thromb 1994; 14: 54-9.
4. Meade TW, Vickers MV, Thompson SG, Seghatchian MJ. The effects of physiological levels of fibrinogen on platelet aggregation. Thromb Res 1985; 38: 527-34.
5. Marguerie G, Ginsberg MH, Plow EF. The role of fibrinogen in platelet aggregation. In: Lane DA, Henschen A, Jasani MK, eds. Fibrinogen-fibrin formation and fibrinolysis. Vol 4. Berlin: Walter de Gruyter, 1986: 175-83.
6. Lowe GDO, Forbes CD. Blood rheology and thrombosis. Clin Lab Haematol 1981; 10: 343-67.
7. Meade TW, Ruddock V, Stirling Y, Chakrabarti R, Miller GJ. Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. Lancet 1993; 342: 1076-9.
8. Ernst E, Resch KL. Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature. Ann Intern Med 1993; 118: 956-63.
9. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA 1998; 279: 1477-82.
10. Haines AP, Howarth D, North WR, et al. Haemostatic variables and the outcome of myocardial infarction. Thromb Haemost 1983; 50: 800-3.
11. Lee AJ, Lowe GD, Woodward M, Tunstall-Pedoe H. Fibrinogen in relation to personal history of prevalent hypertension, diabetes, stroke, intermittent claudication, coronary heart disease, and family history: the Scottish Heart Health Study. Br Heart J 1993; 69: 338-42.
12. Folsom AR, Wu KK, Shahar E, Davis CE. Association of haemostatic variables with prevalent cardiovascular disease and asymptomatic carotid artery atherosclerosis. Arterioscler Thromb 1993; 13: 1829-36.
13. Henry I, Uzan G, Weil D, et al. The genes coding for Aα, Bβ-, and γ-chains of fibrinogen map to 4q2. Am J Hum Genet 1984; 36: 760-8.
14. Kant JA, Fornace AJ, Saxe D, Simon MI, McBride OW, Crabtree GR. Evolution and organization of the fibrinogen locus on chromosome 4: gene duplication accompanied by transposition and inversion. Proc Natl Acad Sci USA 1985; 82: 2344-8.
15. Courtois G, Morgan JG, Campbell LA, Fourel G, Crabtree GR. Interaction of a liver-specific nuclear factor with the fibrinogen and alpha 1-antitrypsin promoters. Science 1987; 238: 688-92.
16. Dalmon J, Laurent M, Courtios G. The human β-fibrinogen promoter contains a hepatocyte nuclear factor 1-dependent interleukin-6 responsive element. Mol Cell Biol 1993; 13: 1183-93.
17. Yu S, Sher B, Kudryk B, Redman CM. Fibrinogen precursors. Order of assembly of fibrinogen chains. J Biol Chem 1984; 259: 10574-81.
18. Humphries SE, Cook M, Dubowitz M, Stirling Y, Meade TW. Role of genetic variation at the fibrinogen locus in determination of plasma fibrinogen concentrations. Lancet 1987; 1: 1452-5.
19. Fowkes FGR, Connor JM, Smith FB, Wood J, Donnan PT, Lowe GDO. Fibrinogen genotype and risk of peripheral atherosclerosis. Lancet 1992; 339: 693-6.
20. Snowden C, Houlston R, Laker MF, Kesteven P, Alberti KG, Humphries SE. Plasma fibrinogen levels and fibrinogen genotype in non-insulin dependent diabetics. Dis Markers 1992; 10: 159-67.
21. Thomas A, Lamlum H, Humphries S, Green F. Linkage disequilibrium across the fibrinogen locus as shown by five genetic polymorphisms, G/A_{-455} (HaeIII), C/T_{-148} (HindIII/AluI), T/G_{+1689} (AvaII), and Bc1I (β-fibrinogen) and TaqI (α-fibrinogen), and their detection by PCR. Hum Mutat 1994; 3: 79-81.
22. Behague I, Poirier O, Nicaud V, et al. β-fibrinogen gene polymorphisms are associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction. The ECTIM Study. Etude Cas-Temoin sur l'Infarctus du Myocarde. Circulation 1996; 93: 440-9.
23. Thomas A, Kelleher C, Green F, Meade TW, Humphries SE. Variation in the promoter region of the β-fibrinogen gene is associated with plasma fibrinogen levels in smokers and non-smokers. Thromb Haemost 1991; 65: 487-90.
24. Connor JM, Fowkes FGR, Wood J, Smith FB, Donnan PT, Lowe GDO. Genetic variation at fibrinogen loci and plasma fibrinogen levels. J Med Genet 1992; 29: 480-2.
25. Iso H, Folsom AR, Winkelmann JC, et al. Polymorphisms of the beta fibrinogen gene and plasma fibrinogen concentration in Caucasian and Japanese population samples. Thromb Haemost 1995; 73: 106-11.
26. de Maat MPM, de Knijff P, Green FR, Thomas AE, Jespersen J, Kluft C. Gender-related association between β-fibrinogen genotype and plasma fibrinogen levels and linkage disequilibrium at the fibrinogen locus in Greenland Inuit. Arterioscler Thromb Vasc Biol 1995; 15: 856-60.
27. Scarabin PY, Bara L, Ricard S, et al. Genetic variation at the β-fibrinogen locus in relation to plasma fibrinogen concentrations and risk of myocardial infarction. The ECTIM Study. Arterioscler Thromb 1993; 13: 886-91.
28. Zito F, Di Castelnuovo A, Amore C, D'Orazio A, Donati MB, Iacoviello L. BclI polymorphism in the gene of the fibrinogen β-chain is associated with the risk of familial myocardial infarction by increasing plasma fibrinogen levels: a case-control study in a sample of GISSI-2 patients. Arterioscler Thromb Vasc Biol 1997; 17: 3489-94.
29. Zito F, Di Castelnuovo A, D'Orazio A, et al. Helicobacter pylori infection and the risk of myocardial infarction: role of fibrinogen and its genetic control. Thromb Haemost 1999; 82: 14-8.
30. Doggen CJ, Bertina RM, Cats VM, Rosendaal FR. Fibrinogen polymorphisms are not associated with the risk of myocardial infarction. Br J Haematol 2000; 110: 935-8.
31. Margaglione M, Di Minno G, Grandone E, et al. Raised plasma fibrinogen concentrations in subjects attending a metabolic ward - relation to family history and vascular risk factors. Thromb Haemost 1995; 73: 579-83.
32. Carter AM, Mansfield MW, Stickland MH, Grant PJ. Beta-fibrinogen gene -455 G/A polymorphism and fibrinogen levels. Risk factors for coronary artery disease in subjects with NIDDM. Diabetes Care 1996; 19: 1265-8.
33. Vaisanen S, Rauramaa R, Penttila I, et al. Variation in plasma fibrinogen over one year: relationships with genetic polymorphisms and non-genetic factors. Thromb Haemost 1997; 77: 884-9.
34. Green F, Hamsten A, Blomback M, Humphries S. The role of beta-fibrinogen genotype in determining plasma fibrinogen levels in young survivors of myocardial infarction and healthy controls from Sweden. Thromb Haemost 1993; 70: 915-20.
35. Humphries SE, Ye S, Talmud P, Bara L, Wilhelmsen L, Tiret L. European Atherosclerosis Research Study: genotype at the fibrinogen locus (G-455-A β-gene) is associated with differences in plasma fibrinogen levels in young men and women from different regions in Europe. Evidence for gender-genotype-environment interaction. Arterioscler Thromb Vasc Biol 1995; 15: 96-104.
36. Tybjaerg-Hansen A, Agerholm-Larsen B, Humphries SE, Abildgaard A, Schnohr P, Nordestgaard BG. A common mutation in the β-fibrinogen promoter is an independent predictor of plasma fibrinogen, but not of ischaemic heart

disease. A study of 9127 individuals based on the Copenhagen City Heart Study. J Clin Invest 1997; 99: 3034-9.
37. Gardemman A, Schwartz O, Haberbossch W, et al. Positive association of the beta fibrinogen variation to basal fibrinogen levels and to the increase in fibrinogen concentration during acute phase reaction but not to coronary artery disease and myocardial infarction. Thromb Haemost 1997; 77: 1120-6.
38. Wang XL, Wang J, McCredie RM, Wilckeb DEL. Polymorphisms of factor V, factor VII, and fibrinogen genes. Relevance to severity of coronary artery disease. Arterioscler Thromb Vasc Biol 1997; 17: 246-51.
39. Koster T, Rosendaal FR, Reitsma PH, van der Velden PA, Briet E, Vandenbroucke JP. Factor VII and fibrinogen levels as risk factors for venous thrombosis. A case-control study of plasma levels and DNA polymorphisms - the Leiden Thrombophilia Study (LETS). Thromb Haemost 1994; 71: 719-22.
40. Montgomery HE, Clarkson P, Nwose OM, et al. The acute rise in serum fibrinogen concentration with exercise is influenced by the G-453-A polymorphism of the beta-fibrinogen gene. Arterioscler Thromb Vasc Biol 1996; 16: 386-91.
41. Henry JA, Bolla M, Osmond C, Fall C, Barker DJ, Humphries SE. The effect of genotype and infant weight on adult plasma levels of fibrinogen, factor VII and LDL cholesterol are additive. J Med Genet 1997; 34: 553-8.
42. Heinrich J, Funke H, Rust S, et al. Impact of the polymorphisms in the alpha- and beta-fibrinogen gene on plasma fibrinogen concentrations of coronary heart disease patients. Thromb Res 1995; 77: 209-15.
43. Margaglione M, Cappucci G, Colaizzo D, et al. Fibrinogen plasma levels in an apparently healthy general population - relation to environmental and genetic determinants. Thromb Haemost 1998; 80: 805-10.
44. Lee AJ, Fowkes FG, Lowe GD, Connor JM, Rumley A. Fibrinogen, factor VII and PAI-1 genotypes and the risk of coronary and peripheral atherosclerosis: Edinburgh Artery Study. Thromb Haemost 1999; 81: 553-60.
45. Van't Hooft FM, von Bahr SJF, Silveira A, Iliadou A, Eriksson P, Hamsten A. Two common, functional polymorphisms in the promoter region of the β-fibrinogen gene contribute to regulation of plasma fibrinogen concentration. Artrioscler Thromb Vasc Biol 1999; 19: 3063-70.
46. Lam KS, Ma OC, Wat NM, Chan LC, Janus ED. Beta-fibrinogen gene G/A-455 polymorphism in relation to fibrinogen concentrations and ischaemic heart disease in Chinese patients with type II diabetes. Diabetologia 1999; 42: 1250-3.
47. Kessler C, Spitzer C, Stauske D, et al. The apolipoprotein E and beta-fibrinogen G/A-455 gene polymorphisms are associated with ischemic stroke involving large-vessel disease. Arterioscler Thromb Vasc Biol 1997; 17: 2880-4.
48. de Maat PM, Kastelein JJP, Jukema JW, et al. The -455 G/A polymorphism of the β-fibrinogen gene is associated with the progression of coronary atherosclerosis in symptomatic men: proposed role for an acute phase reaction pattern of fibrinogen. Arterioscler Thromb Vasc Biol 1998; 18: 265-71.
49. Fey GH, Fuller GM. Regulation of acute phase gene expression by inflammatory mediators. Mol Biol Med 1987; 4: 323-38.
50. Balleisen L, Assmann G, Bailey J, Epping PH, Schulte H, van de Loo J. Epidemiological study on factor VII, factor VIII and fibrinogen in an industrial population - II. Baseline data on the relation to blood pressure, blood glucose, uric acid, and lipid fractions. Thromb Haemost 1985; 54: 721-3.
51. Meade TW, Imeson J, Stirling Y. Effects of changes in smoking and other characteristics on clotting factors and the risk of ischaemic heart disease. Lancet 1987; 2: 986-8.
52. Meade TW, North WR, Chakrabarti R, Haines AP, Stirling Y. Population-based distributions of haemostatic variables. Br Med Bull 1977; 33: 283-8.
53. Meade TW, Chakrabarti R, Haines AP, North WR, Stirling Y. Characteristics affecting fibrinolytic activity and plasma fibrinogen concentrations. BMJ 1979; 1: 153-6.
54. Fuller JH, Keen H, Jarrett RJ, et al. Haemostatic variables associated with diabetes and its complications. BMJ 1979; 2: 964-6.
55. Thomas AE, Kelleher CC, Green F, Humphries SE. Association of genetic variation at the β-fibrinogen locus and plasma fibrinogen levels: interaction between allele frequency of the G/A -455 polymorphism, age, smoking. Clin Genet 1996; 50: 184-90.
56. Rauramaa R, Vaisanen SB, Kuhanen R, Penttila I, Bouchard C. The RsaI polymorphism in the alpha-fibrinogen gene and response of plasma fibrinogen to physical training - a controlled randomised clinical trial in men. Thromb Haemost 2000; 83: 803-6.
57. Vaisanen S, Rauramaa R, Rankinen T, Gagnon J, Couchard C. Physical activity, fitness, and plasma fibrinogen with reference to fibrinogen genotypes. Med Sci Sports Exerc 1996; 28: 1165-70.
58. Rauramaa R, Vaisanen S, Nissinen A, et al. Physical activity, fibrinogen plasma level and gene polymorphisms in postmenopausal women. Thromb Haemost 1997; 78: 840-4.
59. Patel P, Mendall MA, Carrington D, et al. Association of Helicobacter pylori and Chlamydia pneumoniae infections with coronary heart disease and cardiovascular risk factors. BMJ 1995; 311: 711-4.
60. Mendall MA, Goggin PM, Molineaux N, et al. Relation of Helicobacter pylori infection and coronary heart disease. Br Heart J 1994; 71: 437-9.
61. Lip GH, Wise R, Beevers G. Association of Helicobacter pylori infection with coronary heart disease. Study shows association between H pylori infection and hypertension. BMJ 1996; 312: 250-1.
62. Morgando A, Sanseverino P, Perotto C, Molino F, Gai V, Ponzetto A. Helicobacter pylori seropositivity in myocardial infarction. (letter) Lancet 1995; 345: 1380.
63. Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? Lancet 1997; 350: 430-6.
64. Murray LJ, Bamford KB, O'Reilly DP, McCrum EE, Evans AE. Helicobacter pylori infection: relation with cardiovascular risk factors, ischaemic heart disease, and social class. Br Heart J 1995; 74: 497-501.
65. Patel P, Carrington D, Strachan DP, et al. Fibrinogen: a link between chronic infection and coronary heart disease. Lancet 1994; 343: 1634-5.