Abstract—The role of fibrinogen in cardiovascular disease has been extensively studied, and meta-analyses have definitively confirmed that high levels of fibrinogen are associated with an increased risk of the disease. In recent years, several polymorphisms have been identified in the fibrinogen chain genes that contribute to determine the levels of fibrinogen in the general population. The fibrinogen \(\beta\)-chain gene has been more extensively studied because the \(\beta\)-chain synthesis is the limiting step in the production of mature fibrinogen. Overall, the studies show an association between \(\beta\)-fibrinogen chain polymorphisms and the levels of fibrinogen. In contrast, the majority of the studies did not find any relation with the risk of cardiovascular disease. The individual responses to gender or to environmental stimuli such as smoking, physical exercise, or infections may be genetically determined, and genetic variability underlies changes in biological reactions that contribute to differences in cardiovascular risk. In the future, gene-environment interactions should be considered in evaluating the relevance of genetic variations on the risk of cardiovascular disease. (Hypertension. 2001;38:1199-1203.)

Key Words: fibrinogen ■ polymorphisms ■ gene-environment interactions ■ risk factors ■ cardiovascular disease

After the first evidence made in the early 1980s by Meade et al
d in the Northwick Park Heart Study (NPHS), the role of fibrinogen as a predictor of primary and secondary ischemic coronary events has been demonstrated by several epidemiological studies.2-5 Three meta-analyses have been published on the association between fibrinogen and cardiovascular disease: all agree on the finding that high fibrinogen levels are associated with an increased risk of disease.6-8 The last of them8 reviewed 18 prospective studies on \(\approx\)4000 cases of coronary heart disease followed for a mean of 8 years: fibrinogen levels in the top third were associated with an odds ratio of 1.8 (95% confidence interval [CI], 1.6 to 2.0). These results definitively confirmed the importance of fibrinogen, because they assemble a large number of prospective studies, weighed for possible confounders.

Many pieces of evidence make plausible a causal contribution of fibrinogen to cardiovascular disease. This protein is crucial in the process of thrombus formation and evolution, and its levels are influenced by several conventional risk factors. Fibrinogen is the last target of coagulation: its lysis by thrombin produces soluble fibrin fragments that are then stabilized in a clot by factor XIII. It is a mediator of platelet aggregation and a determinant of plasma viscosity. Fibrinogen is an acute phase protein, synthesized in the liver, the plasma levels of which acutely rise during inflammatory conditions. Fibrinogen levels increase with age, pregnancy, use of oral contraceptives, menopause, and obesity. Smoking habits, infections, hypertension, and diabetes are associated with increased levels of fibrinogen, whereas physical activity, moderate alcohol consumption, and fish intake reduce fibrinogen levels.9 Furthermore, fibrinogen can be considered as a common pathway through which risk factors may act in promoting arterial disease.

More recently, the study of genetics added new insights to this complex picture of interactions, showing that the variation of fibrinogen levels in a general population can be explained in part by variations at fibrinogen gene loci.

Genetic Determinants of Fibrinogen Levels
Fibrinogen is 340-kDa dimeric glycoprotein; each dimer consists of 3 different polypeptide chains known as \(\alpha\)-, \(\beta\)-, and \(\gamma\)-chains, linked by disulfide bonds. The 3 polypeptide chains are encoded by 3 different genes clustered on chromosome 4 in region q28. The 3 genes, ordered as \(\gamma\), \(\alpha\), and \(\beta\), span a distance of \(\approx\)50 kb, with the direction of transcription of \(\beta\)-gene opposite that the \(\alpha\)- and \(\gamma\)-genes.10

Many polymorphisms of this cluster have been identified; however, the most studied have been those located in the \(\beta\)-chain fibrinogen gene (Figure). Indeed, in vitro studies have suggested that \(\beta\)-chain synthesis limits the rate of production of mature fibrinogen.11 As a consequence, polymorphisms affecting the production of this chain would more likely influence the levels of fibrinogen. Overall, the studies show a strong association between \(\beta\)-chain fibrinogen genotypes and its plasma concentrations.

A DNA variation of the \(\beta\)-fibrinogen gene has been detected at the 3′ region, by the \(BcI\) restriction enzyme (\(BcI\)
polymorphism), as 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% to 20% higher than that of the B1B1 genotype. However, because of its location on the gene, it would be difficult to consider a biological function for this polymorphism, which has been mainly considered as a marker for another locus on the fibrinogen gene.

**BclI polymorphism** is in linkage disequilibrium with the G/A sequence variation at position −455, detected in the promoter region, by the HaeIII restriction enzyme, which is more compatible with a modified transcription of the gene. It is, indeed, located in the promoter, close to responsive elements (IL-6 and the HNF-1 elements).

Out of 23 pertinent publications between 1991 and 2001, all the studies, but 4, 26, 29, 33, 37 are consistent with the finding that the A−455 allele, which is present in ~20% of the general population, is associated with increased levels of fibrinogen of ~0.30 g/L compared with homozygotes for the G allele (Table). Experimental studies have demonstrated that the GA substitution at −455 has a substantial effect on the basal and stimulated rate of transcription of the β-chain gene, the A allele being associated with a significant increase in the promoter activity. The ~854 G/A polymorphism has also been independently associated with increased transcription of β-fibrinogen in vitro and can also contribute to the regulation plasma fibrinogen levels. However, a recent study, reporting a different allelic association between −455 G/A and −148 C/T in different ethnic groups living in England, showed that the T allele rather than the A allele was associated with high fibrinogen levels.

**Fibrinogen Polymorphisms and Risk of Cardiovascular Disease**

Although the association between fibrinogen polymorphisms and fibrinogen levels has been consistently reported, their association with the risk of cardiovascular disease is still largely debated.

The involvement of BclI fibrinogen polymorphism in the risk of ischemic vascular disease has been confirmed by some studies, although others gave negative results. Fowkes et al described an association between the B2 allele and the risk of peripheral arterial disease. In the ECTIM (Etude Cas-Témoin de l’Infarctus du Myocarde) study, it was related with the severity of coronary artery disease rather than with myocardial infarction. In cohorts of Italian patients, carriers of the B2B2 genotype showed a 2.4-fold increased risk of myocardial infarction. However, the risk was present only when patients with a family history of ischemic vascular disease were selected.

Only few studies have found an association between −455 G/A polymorphism and the risk of ischemic vascular disease (Table). Out of 14 studies in which the relation between −455 G/A polymorphism and the risk was evaluated, only 5 reported the presence of an association. Of the latter were related to thrombosis in coronary arteries. However, 2 groups showed an association with the risk of cardiovascular disease in patients with non-insulin-dependent diabetes, one with peripheral arterial disease, one with deep venous thrombosis, and the last one with large vessel cerebrovascular disease. An additional study documented that the −455 G/A polymorphism was related to the progression of cardiovascular disease, in patients with hypercholesterolemia.

The number of subjects included in the different studies has never been sufficient to detect any statistically significant 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 in the risk for coronary heart disease. Nevertheless, homozygosity for the A−455 allele increases of ~0.30 g/L the levels of fibrinogen, which assuming linearity in logarithm of relative risk corresponds to a relative risk of ~1.20. To have the power of 80% to detect such an increase, a case control study should recruit ~11 600 cases and a corresponding number of controls. This size has never been reached in the studies published until now. However, it is relevant to consider the cost-effectiveness of performing such a large study with a single polymorphism to demonstrate an association that, in any case, would be of poor clinical relevance. New strategies, such as considering multiple haplotypes or identifying new polymorphisms in less explored fibrinogen gene sequences,
should be also undertaken to definitively define the role of fibrinogen polymorphisms in the risk of cardiovascular disease.

**Gene-Environment Interactions and Fibrinogen Levels**

Rather than directly affect the levels of proteins, polymorphisms can amplify the effect of environmental or intermediate conditions on the final phenotype. 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.

The fibrinogen genetic asset can also regulate the relationship between physical activity and fibrinogen levels. An inverse dose-response relationship has been described between regular physical exercise and fibrinogen levels, although an intensive, strenuous exercise is associated with an acute rise in fibrinogen levels. The increase lasts several days, probably because of a continuous fibrinogen production. However, this phenomenon varies according to the G/A −455 polymorphism of the fibrinogen β-chain gene. After 2 days of strenuous military exercise, an increase in fibrinogen levels (compared with the concentration at pretraining) was recorded and found maximal at the second day after the exercise. This effect was significantly stronger in the AA homozygotes compared with heterozygotes and GG homozygotes.

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**Studies on the Association Between −455 G/A (HaeIII) Polymorphism of β-Fibrinogen Gene, Fibrinogen Levels, and Risk of Cardiovascular Disease**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Fibrinogen Association</th>
<th>Cardiovascular Disease Association</th>
<th>No., Case/Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behague et al 13</td>
<td>FR+NI</td>
<td>+</td>
<td>MI, CAD</td>
<td>565/668</td>
</tr>
<tr>
<td>Scarabin et al 19</td>
<td>FR+NI</td>
<td>+</td>
<td>MI, CAD</td>
<td>533/648</td>
</tr>
<tr>
<td>Green et al 20</td>
<td>Sweden</td>
<td>+</td>
<td>MI, CAD</td>
<td>123/86</td>
</tr>
<tr>
<td>Humphries et al 21</td>
<td>Europe</td>
<td>+</td>
<td>MI, CAD</td>
<td>585/1106</td>
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<tr>
<td>Tybjærg-Hansen et al 22</td>
<td>Sweden</td>
<td>+</td>
<td>CVD</td>
<td>174/3937</td>
</tr>
<tr>
<td>Gardemann et al 23</td>
<td>Germany</td>
<td>+</td>
<td>MI, CAD</td>
<td>923</td>
</tr>
<tr>
<td>Wang et al 24</td>
<td>Australia</td>
<td>+</td>
<td>CAD</td>
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<tr>
<td>Koster et al 25</td>
<td>The Netherlands</td>
<td>+</td>
<td>DVT</td>
<td>199/199</td>
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<tr>
<td>Carter et al 26</td>
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<td>−</td>
<td>NIDDM</td>
<td>38/149</td>
</tr>
<tr>
<td>Montgomery et al 27</td>
<td>United Kingdom</td>
<td>+</td>
<td>ND</td>
<td>...</td>
</tr>
<tr>
<td>Henry et al 28</td>
<td>United Kingdom</td>
<td>±</td>
<td>ND</td>
<td>...</td>
</tr>
<tr>
<td>Thomas et al 27</td>
<td>United Kingdom</td>
<td>+</td>
<td>ND</td>
<td>...</td>
</tr>
<tr>
<td>Margaglione et al 29</td>
<td>Italy</td>
<td>−</td>
<td>ND</td>
<td>...</td>
</tr>
<tr>
<td>de Maat et al 16</td>
<td>Inuit</td>
<td>+</td>
<td>ND</td>
<td>...</td>
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<td>Heinrich et al 30</td>
<td>Germany</td>
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<tr>
<td>Lee et al 32</td>
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<td>+</td>
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<td>Doggen et al 33</td>
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<td>−</td>
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<tr>
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<td>+</td>
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<tr>
<td>Lam et al 35</td>
<td>China</td>
<td>+</td>
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<tr>
<td>Kessler et al 36</td>
<td>Germany</td>
<td>+</td>
<td>CVD of large vessels</td>
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<tr>
<td>Vaisanen et al 37</td>
<td>Finnland</td>
<td>−</td>
<td>ND</td>
<td>...</td>
</tr>
<tr>
<td>Folsom et al 38</td>
<td>USA</td>
<td>+</td>
<td>...</td>
<td>398/498</td>
</tr>
</tbody>
</table>

FR+NI indicates France and North Ireland; MI, myocardial infarction; CAD, coronary artery disease; DVT, deep venous thrombosis; NIDDM, non–insulin-dependent diabetes mellitus; PAD, peripheral arterial disease; ND, not determined; and CVD, cardiovascular disease.
Vaisanen et al\textsuperscript{33} showed that physical activity levels explained up to 9\% of fibrinogen variance in carriers of the B2 allele of the Bcl\textsubscript{I} \-\-\-\- chain gene polymorphism, whereas it had only a marginal effect in carriers of the common B1 fibrinogen genotype. Similar results were shown for polymorphisms in the $\alpha$-chain gene of fibrinogen in postmenopausal women\textsuperscript{44} and in middle-aged men.\textsuperscript{45}

**Gene-Environment Interactions and Risk of Cardiovascular Disease**

Beside their effect on fibrinogen levels, fibrinogen polymorphisms can also modulate the relationship between environmental risk factors and the risk of cardiovascular disease. *Helicobacter pylori* (HP) infection has been associated with a higher risk to develop ischemic heart disease, although the results are controversial.\textsuperscript{46} Zito et al\textsuperscript{16} showed that the Bcl\textsubscript{I} polymorphisms of $\beta$-fibrinogen gene modulated the effect of HP on both fibrinogen levels and the risk of myocardial infarction.

HP, a life-long bacterial infection, was found to be associated with chronically increased plasma levels of fibrinogen. However, the effect was stronger in carriers of B2 allele. Carriership of the B2 allele also amplified the effect of seropositivity for HP\textsuperscript{46} on the risk of myocardial infarction. Indeed, patients with both seropositivity for HP and carriers of B2 genotype showed an additional increase in the risk of myocardial infarction compared with that of subjects affected by HP but homozygous for the B1 allele.

**Conclusions**

The prognostic role of fibrinogen on the risk of cardiovascular disease has been extensively proved. Genetic determinants, through a modulation of fibrinogen levels, might contribute to the risk of cardiovascular disease. Epidemiological studies, however, failed to demonstrate this relation, probably because the effect, if present, is relatively small and needs larger and very selected populations to be detected. Moreover, it becomes more evident that gene-environment interactions are determinant in understanding the role of genetic polymorphisms on the risk of disease. Indeed, genetic variability, rather than playing any direct role, influences a different individual susceptibility to environmental risk factors.

In the future, efforts should be made in defining the mechanism of these interactions and their role in determining the risk of cardiovascular disease.

**Acknowledgments**

We thank Dr Giovanni de Gaetano for critical reading this manuscript. This work was supported by a grant from Telethon (Rome, Italy), contract No. E.1005, and by the Italian Ministry of Health.

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Genes Encoding Fibrinogen and Cardiovascular Risk
Licia Iacoviello, Michela Vischetti, Francesco Zito and Maria Benedetta Donati

_Hypertension_. 2001;38:1199-1203
doi: 10.1161/hy1101.099478

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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