patients who developed agranulocytosis and tested positive for antineutrophil antibodies;[7] and (2) tumor necrosis factor (TNF)–mediated myelosuppression, in a setting of rituximab-induced TNF-α release.[8]

Finally, detection of del(20)(q11.2) is evidence for secondary myelodysplastic syndrome related to high-dose therapy (HDT)[9]; nevertheless, as previously shown, one cannot exclude the possibility of stem cell damage resulting from prior conventional dose chemotherapy and actually unrelated to HDT.[10]

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To the editor:

Vagaries of genetic association studies in myocardial infarction

Those who follow publications on genetic association studies are aware that none of the single nucleotide polymorphisms (SNPs) of genes encoding proteins involved in hemostasis and thrombosis have been consistently associated with an increased or decreased risk of such complex and multifactorial diseases as myocardial infarction or stroke.[1] Studies on the same gene SNPs have often given discrepant results, so that only 16% of initially identified associations was subsequently replicated.[2] Typically, a few large studies (those including 1000 cases or more) found weak associations or no association at all, with strong associations found by several small studies.[3] This situation is epitomized by 2 recent studies, those by Butt et al[4] and by the Atherosclerosis Thrombosis and Vascular Biology Italian Study Group[5] on the role of SNPs of coagulation factor genes such as 20210G>A factor II, 1691G>A factor V, and 185G>T factor XIII-A. In 1210 Italian patients who survived a first myocardial infarction at an age younger than 45 years compared with an equal number of matched controls, none of these SNPs nor 6 additional SNPs of genes encoding proteins involved in coagulation, platelet function, and fibrinolysis were associated.[5] In contrast, in 500 Canadians from Newfoundland, 20210G>A factor II was significantly more prevalent in patients with myocardial infarction than in controls (Table 1). Prevalence of mutant 1691G>A factor V (generally known as factor V Leiden) and of 185G>T factor XIII-A was not significantly different in cases and controls, but the prevalence of 1691G>A factor V was significantly higher when a subgroup of 46 patients who developed myocardial infarction at an age of 50 years or younger was analyzed (Table 1).[4] Furthermore, Butt et al[4] claim that their study gives evidence of strong gene-gene interaction, the prevalence of combined carriership of 20210G>A factor II and 185G>T factor

Table 1. Frequency of genotypes of single nucleotide polymorphisms of coagulation factor genes and relative risk of myocardial infarction (as measured by odds ratios) in Italians (aged 45 years or younger) and in Canadians (aged 50 years or older)

	Italians			Canadians		
Polymorphism and gene	Cases, n = 1210	Controls, n = 1210	Odds ratio	Cases, n = 500	Controls, n = 500	Odds ratio
20210 G > A, factor II						
0/0	96.7	96.8		96.8	99.0	
0/1	3.3	3.1	1.0	3.2	1.0	3.3 (*P* = .015)
1/1	0	0.1	(NS)	0	0	5.6* (*P* = .04)
1691G > A, factor V						
0/0	96.9	96.4		95.4	95.4	
0/1	3.1	3.6	1.1	4.6	4.6	1.0 (NS)
1/1	0	0	(NS)	0	0	3.0* (*P* = .007)
185G > T, factor XIII-A						
0/0	64.4	65.2		53.0	52.2	
0/1	31.0	30.0	1.1	38.6	41.4	0.97 (NS)
1/1	4.6	4.8	(NS)	8.4	6.4	0.8* (NS)

0/0 indicates genotypes characterized by the presence of 2 wild-type alleles (values are percentages); 0/1, the presence of 1 wild-type and 1 mutant allele; 1/1, the presence of 2 mutant alleles, and NS, not statistically significant.

*The odds ratios obtained in the subgroup of 46 patients 50 years or younger.

XIII-A being 12-fold higher in patients than in controls. In the Italian study, which was much larger than the Canadian one, no subgroup analysis was carried out because the sample size was judged to be insufficient for any such subanalysis. For the same reason, no gene-gene and gene-environment interactions were evaluated.

Why did the 2 studies give such different results? We purport that differences depend on the different sample sizes in the 2 studies. The sizes of association studies must be pre-established on the basis of the expected relative risk (as measured by odds ratio) carried by the mutant allele versus the wild-type allele, allelic frequency, and the desired power and statistical significance. Assuming that odds ratios higher than 1.5 are unlikely to be expected in the context a of multifactorial complex disease such as myocardial infarction, the size of the Italian study (1210 matched pairs) had a power no higher than 70% to detect an odds ratio of 1.5 with a statistical significance of 5% if the frequency of the mutant allele is approximately 1% to 2%, as is that of 20210G>A factor II and 1691G>A factor V. We calculated that the sample of the Canadian study, taking all the 500 pairs irrespective of age, had a power no higher than 30% to detect a relative risk of 1.5 for the same SNPs. The power becomes negligible for the 46 patients younger than 51 years, the only subgroup that gave statistically significant results for the factor V SNP[4] and that is comparable with the Italian study for the age of patients investigated.

In association studies, several thousands of patients are needed to evaluate SNPs with low frequency alleles such as 20210G>A factor II and 1691G>A factor V. None of the studies done so far (including the Italian study) is adequately powered (80% or more) for low frequency alleles; studies with a few hundred cases such as the Canadian one are greatly underpowered. Even worshipers of genetic association studies will agree that the role of SNPs of candidate genes so far considered is weak in itself and much weaker than that of traditional nongenetic risk factors such as family history, smoking habit, diabetes, hypertension, body mass index, and hypercholesterolemia.[5]

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Response:

Gene-gene interactions in myocardial infarction

The author of the letter entitled, "Vagaries of genetic association studies in myocardial infarction," attempted to evaluate the role of 3 SNPs of coagulation factor genes (20210G>A factor II, 1691G>A factor V, and 185G>T factor XIII) in myocardial infarction (MI) by comparing 2 recently published studies from Butt et al[1] (a Canadian group) and the Atherosclerosis Thrombosis and Vascular Biology Italian Study Group.[2] In the Canadian study, the 3 SNPs were genotyped on 500 MI patients and the same number of healthy controls from a genetically isolated population. A coeffect of gene-gene interaction between 20210G>A factor II and 185G>T factor XIII was strongly associated with MI. The 20210G>A factor II allele was independently and weakly associated with MI. Furthermore, early onset of MI (onset age 50 years or younger) was associated with 20210G>A factor II and 1691G>A factor V based on the data generated from a small subgroup in the study. In the Italian study, 1210 MI patients with an onset age younger than 45 years and the same number of age- and sex-matched controls were genotyped for 9 SNPs, including the 3 mentioned previously. No significant difference in the prevalence of the 9 analyzed SNPs between the patients and controls was observed.

It is not surprising to see conflicting results in the prevalence of each individual SNP between the 2 studies. Both studies differed greatly in the age and ethnic genetic background, as well as other genetic and important environmental factors of subjects examined. The different prevalence of 20210G>A factor II in the 2 studied control populations indicates the heterogeneity of the ethnic genetic background of these 2 populations. There were even differences in the age of onset that was classified as early in the 2 studies. However, data from the Italian study can only suggest that the 20210G>A factor II may not be independently associated with MI in the Italian population that occurs before 45 years of age. This is not sufficient to conclude no association between 20210G>A factor II and MI. MI typically has onset later in life. The vast majority of patients suffer their first MI after the age of 45 years. Those suffering MI at younger than 45 years tend to have a single or a few very strong risk factors. The results of the Canadian study suggest that if the allele is found alone it is weak. Nevertheless, the design of the Italian study is not appropriate to effectively assess the risk association of 20210G>A with MI. Carriers of 20210G>A in the control group of the Italian study may be at higher risk of developing MI later in life compared with individuals without the allele. Unlike other studied SNPs of coagulation factor genes, the majority of previous studies showed a trend toward increased prevalence of 20210G>A factor II in MI (although some did not reach statistical significance).[3-6]

The focus of the Canadian study was the identification of a gene-gene interaction between 20210G>A factor II and 185G>T factor XIII-A in the pathogenesis of MI. The coeffect of the gene-gene interaction was determined based on a disequilibrium distribution of combined carriers among the MI patients. Of 13 identified combined carriers of the 20210G>A factor II and 185G>T factor XIII-A (500 patients and 500 controls), 12 (92.3%) of those belonged to the MI patient group. Further study using larger sample numbers will be helpful in further validating this interesting finding. This is the goal of our ongoing studies in this

area. Due to the low prevalence of 20210G>A factor II, identification of a large number of combined carriers is a practical challenge. Unfortunately, the Italian study did not explore any differences in combined carrier status and any potential difference between the 2 groups.

Based on the data from other studies, it appears that SNPs of coagulation factor genes may either have no independent effect or have only a small effect on MI risk. Coeffect from gene-gene interaction among these alleles may be the major cause of genetic hypercoagulation predisposition in MI. Low prevalence of certain SNPs may indicate a nature of pathogenesis in MI that each coeffect may account for only a small portion of patients with MI. Therefore, investigation of gene-gene interaction should be continued although the number of combined carriers may be limited.

The focus of the 2 papers is different, and it is therefore difficult to make a simple comparison. The Italian paper focuses on the association of individual SNPs of coagulation factor genes with MI in young individuals. The focus of the Canadian study was the association of gene-gene interactions among individual SNPs in all patients with biochemical evidence of MI. The finding of a difference between the individual gene frequencies in the 2 studies is neither new nor surprising. A valid comparison of the 2 studies should involve using similar patient groups and looking at gene-gene interactions rather than concentrating on the prevalence of single SNPs, on which there is much published information already.[7-18]

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