Clinical studies show that almost 10% of acute coronary events occur in patients under 45 years of age and it has been estimated that genetic factors contribute to between 20% and 60% of these events.\(^1\) In young individuals with acute myocardial infarction (AMI),\(^2\) an apparent reduction in plasma fibrinolytic activity could play a causal role in acute coronary events. High plasma concentrations of plasminogen activator inhibitor type 1 (PAI-1) are associated with thrombotic events.\(^3\) An association between the 4G/5G polymorphism in the PAI-1 gene and ST elevation acute myocardial infarction (STEMI) has been described in young patients. This polymorphism consists of the insertion/deletion of a guanine base in the promoting region in position –675, resulting in the presence of the 4G or 5G allele; the polymorphism exerts a regulatory action on the plasma concentration of PAI-1.\(^4,5\) The frequency with which the 4G/5G polymorphism presents varies in different parts of the world leading to variations in PAI plasma concentrations across populations. The increased concentration of PAI-1 in plasma is associated with increased mortality and AMI in subjects under 45 years of age. Individuals homozygous for the 4G allele (4G/4G) are also reported to have PAI-1 concentrations which are higher than those in subjects who are homozygous for the 5G allele (5G/5G).\(^6\) We would therefore expect individuals who are homozygous for the 4G/4G allele to have increased thrombogenic activity and a subsequent higher incidence of AMI. However, previous studies that have investigated this association have provided contradictory results.

In this issue of the Revista Española de Cardiología, Isordia-Salas et al\(^7\) present the results of an interesting study assessing the relationship between the 4G/5G polymorphism in the PAI-1 gene in young patients (age ≤45 years) with STEMI and the possible influence of the polymorphism in regulating PAI-1 plasma levels. The researchers consecutively recruited 127 patients aged ≤45 years with STEMI admitted to the Intensive Cardiovascular Care Unit of the Cardiology Hospital in the Centro Médico Nacional Siglo XXI (Mexico) between January 2006 and March 2007. They also recruited 127 blood donors, who were assumed to be free of cardiovascular risk factors, as a control group. Determination of PAI-1 plasma levels were performed in blood samples taken 6 weeks after STEMI using an immunoenzymatic technique (ELISA) (Coaliza PAI-1, Chromogenix, Milan, Italy). Genotype determination was performed on DNA obtained from leukocyte concentrate using polymerase chain reaction techniques.

The study’s most important findings were that:

- The most common genotype in patients with STEMI was 4G/5G, with the 4G/4G genotype being found in only 7% of cases. No statistically significant differences in allelic frequency were observed between the control group and patients with AMI.

- The multivariate logistic regression analysis for AMI risk showed that the presence of the 4G allele was associated with increased risk for myocardial infarction (odds ratio [OR] = 2.29), but that the risk was lower than that associated with conventional cardiovascular risk factors such as smoking, high blood pressure, and a family history of heart disease. The risk associated with smoking was >15 times greater than that associated with the polymorphism. From a clinical point of view, then, the polymorphism’s contribution to cardiovascular risk was small in comparison with more established risk factors; further research is required to better define
its clinical utility in the context of other risk markers.

In this study, the highest concentrations of PAI-1 occurred in subjects with the 4G/4G polymorphism, intermediate level concentrations occurred in subjects with the 4G/5G polymorphism, and the lowest concentrations were observed in the 5G/5G group. Nevertheless, the percentage of 4G/4G subjects in the group with AMI was lower than in the control group (7.1% vs 13.4%), whilst the largest group was that with a 4G/5G polymorphism (50.4%). This suggests that the presence of the 4G allele per se is insufficient for the development of STEMI and that interaction with other, traditional cardiovascular risk factors is required to produce a harmful outcome. This finding may have a clinical application if, as the authors note, the results are verified in studies with larger numbers of patients and controls strictly matched for cardiovascular risk factors.

Subjects homozygous for 4G had higher concentrations of PAI-1 in plasma than those who were homozygous for 5G. The authors suggest that the 4G allele is a cardiovascular risk factor for STEMI in subjects over 45 years of age, and represents a moderate risk for AMI in persons with a history of smoking.

The authors' final conclusion, that the 4G allele is an independent risk factor for STEMI in young subjects and that its identification, like that of other risk factors, could be useful in primary prevention strategies, requires confirmation in future studies.

The study by Isordia-Salas et al, published in this issue of the Journal,7 investigating the association between PAI-1 and STEMI is typical of many studies which analyze the association between a given clinical problem and polymorphisms in a "candidate" gene. In this case, the initial findings were positive, although the number of cases and controls was small. The finding is confirmed by the fact that PAI-1 concentrations were higher in 4G/4G individuals. This finding should be treated with caution, however, as it is based on only 9 cases who were carriers of the genotype. The results require validation in studies that replicate the model and include a higher number of cases and controls.

**Concluding Remarks**

The field of genetic association studies has evolved remarkably over the last 5 years, with the advent of techniques that allow analysis of up to one million genetic variants in single experiments. In the light of this huge step forward, the "humble" study focused on a single genetic variant of a "candidate" gene, appears today to be a relatively low-powered research tool. However, such studies have merit when applied correctly and in the appropriate circumstances, for example when they deal with a very specific population, such as that investigated in this study. Still, in these cases it is important to obtain objective evidence of the functionality of the genetic variant studied.

Replication studies are still the simplest way to confirm the significance of such genetic findings. However, this can be difficult when dealing with very specific groups with a small number of subjects. In such cases, replication of the findings in 2 distinct groups often produces more valuable scientific data than simply replicating the findings in a larger number of subjects.

Functional studies are also important in this context and help to provide answers to questions that support the validity of the information obtained. Among these questions are whether the variant found increases gene expression, whether the amount of circulating protein changes in direct proportion to the number of variants, whether there is a logical progression between homozygous and heterozygous individuals. It is also important to establish the real significance and statistical power of previously published evidence. Many of these issues require the appropriate use of data from studies involving genomics, proteomics, and metabolomics. This trend will increase as our knowledge of genetics evolves and as the functional consequences of genetic variants, such as that studied by Isordia-Salas et al,7 become more apparent. One thing is clear, though, and that is that the humble study of "candidate" genes requires a major evolutionary shift in order to survive. That shift also needs to be rapid if such studies are to escape their status as an endangered species.

**REFERENCES**