Haemostatic gene polymorphisms are potential risk factors for thrombosis. Considerable attention has been focussed on identifying risk alleles. Progress has undoubtedly been made in venous thrombosis. Factor V Leiden and the prothrombin G20210A substitution are now established risk factors, and a number of other polymorphisms are candidates. The initial promise that genetic risk factors might contribute appreciably to an explanation of the development of arterial thrombotic disorders has largely been unfulfilled and the expectations raised by early reports of positive associations have been tempered by inconsistent results with almost all genes studied. The problems seen in arterial disease are replicated in investigations of other complex diseases. In the optimistic rush to show positive associations of genetic factors with diseases, sight has been lost of the need for stringent study design. Furthermore, the scale of studies needed to produce reproducible conclusions has been underestimated. The lessons learnt from accumulated experience should now enable progress to be made.

There has been substantial recent interest in risk assessment for thrombosis using haemostasis genes. The number of papers that have been produced on venous and arterial thrombosis reflects the early optimism that the genetic basis of vascular disorders could be established and used as part of individualised therapies. An appreciable number of polymorphisms have been repeatedly investigated in venous and arterial thrombosis (reviewed in [1]). The progress made in relation to venous thromboembolic disease has been appreciable [2,3]. Venous thrombotic disease occurs in the context of a low pressure, low flow system in which atheroma does not occur. Two common polymorphisms are known to contribute appreciably. Factor V Leiden is a “gain of function” polymorphism that modifies the protein C anticoagulant pathway, a pathway known to be involved in protection against thrombosis [4,5]. The prothrombin gene polymorphism alters the level of this protein. The challenge remaining with these two polymorphisms is to translate the information there is on risk into benefit for clinically affected and unaffected carriers. There are a number of other candidate haemostasis gene polymorphisms in venous thrombosis (factor V HR2, factor XIII Val34Leu, EPCR gene insertion, tissue factor –I1208D, protease activated receptor 1 –I1506D), but it remains unclear if these contribute risk. Several suggested risk polymorphisms remain on the candidates bench, as claims are variously made, confirmed then refuted. If the risks associated with these genetic variations are small there will inevitably be problems in establishing their roles.
In contrast to the progress in venous disease, there is little clarity in the role of haemostatic polymorphism in arterial disease [1,6]. The initial promise that genetic risk factors might contribute appreciably to an explanation of the development of arterial thrombotic disorders has largely been unfulfilled and the expectations raised by early reports of positive associations have been tempered by inconsistent results with almost all genes studied. Arterial disease occurs in a high pressure, high flow system with atheromatous disease as a dominant feature. The investigation of polymorphisms (genotypes) was preceded by numerous studies of the levels (phenotypes). Following the lead of the Northwick Park Heart Study [7], levels or activities of haemostasis factors have been extensively studied as possible risk determinants for disease. The most consistent associations have been found for fibrinogen. Fibrinogen may be merely a marker of another underlying process, such as the acute phase reaction, but a strong case can be made that it is causally involved in disease. While a causal role for a number of other coagulation and fibrinolytic factors (such as factor VII, PAI-1, tPA) is highly plausible, the accumulated evidence is less strong. It is interesting to note that these potential haemostatic risk factors for atherothrombotic disorders are largely those that have been associated with classical risk markers such as those of the syndrome of insulin resistance. This observation indicates both the importance of environmental influences and emphasises the complexity of the processes involved in arterial thrombotic disease. When individual haemostatic factors do contribute to risk it will be in concert with a myriad of other metabolic factors. Failure to appreciate the importance of environmental interactions may account for some of the inconsistencies in studies of risk in arterial disease.

It was hoped that in arterial disease confounding problems (such as reproducible measurement of haemostasis factor levels) would be circumvented when their polymorphisms were studied. This turned out not to be so [6]. Although polymorphisms do not in general suffer so much from the technical problems that beset their respective phenotypes, there remain important issues associated with their use. A major shortcoming in determining the risk of a polymorphism is that it may have a small effect only on its associated phenotype. A single polymorphism may contribute only a portion of the total heritability of a factor. If the effects of a phenotype (level or activity) on disease cannot be shown readily, then it is highly unlikely that studying a polymorphism will make this task easier. As explained above, the relationship between phenotype and arterial disease is not established for most clotting and fibrinolytic factors. In some cases, for example, the platelet surface glycoproteins, there is little or no information on the relationship between genetically determined density/activity of these receptors and disease. Polymorphisms in these latter genes have largely been studied by gene-disease association studies without any determination of phenotype. This type of study is particularly prone to artefact and error.

From recent accumulated experience, it is clear that haemostatic gene polymorphisms cannot now be thought to be causing arterial thrombotic disease. If these polymorphisms do present risks, they will be mediated through the interactions of encoded phenotypes with environmental factors. It can be concluded that the reported studies of the risks of haemostasis polymorphisms in arterial diseases have generally been too small to be informative. Additionally, some of the problems in identifying causal genetic markers are related to difficulties associated with the precise definition of the clinical phenotype under study. These have been compounded by inadequate consideration of issues surrounding the distribution of polymorphisms in the population. Examples of factors that may be responsible for some of the observed inconsistencies between numerous reports include (i) The effects of common single gene changes are often minor: study size and power are therefore critical issues (ii) There is plurality in clinical endpoints (MI, unstable angina, coronary artery disease, progression of arterial disease, stroke) and these have been often selected in a post-data collection search for significance. (iii) Genetic polymorphisms may vary appreciably within and between racial groups (iv) Numerous studies have paid insufficient attention to minimising recruitment bias in selection of patients and/or controls (v) It is likely that the greatest effect of the gene polymorphism will most often be in association with (a) specific (set of) environmental changes which may vary between study cohorts and which will require very large studies to define. It can be noted that similar problems of lack of reproducibility of genetic association studies have been documented when polymorphisms have been studied in other common complex diseases [8]. It is now clear that sample sizes must increase greatly above those (~100s of cases and controls) often currently used [9]. A recent editorial [10] provides guidance on what might be required for a reproducible genetic association study. The studies should be characterised by "large sample sizes, small P values, reported associations that make biological sense and alleles that affect the gene product in a physiologically meaningful way. In addition, they should contain an initial study as well as an independent replication, the association should be observed both in family-based and population-based studies...".

Because it is implausible that single genetic polymorphisms will be sole determinants of arterial disease, future studies of risk should be designed specifically to investigate interactions between potential genetic polymorphisms and environmental risk factors. They should be designed at their outset to minimise recruitment bias and have clear and precisely defined clinical endpoints. Because of these requirements, they should be substantially larger than are currently used. Studies in excess of 1000’s of patients and controls may be needed to provide more secure and reproducible results than have been obtained from contemporary studies. Such studies should enable us to move forward to a position from which we can use the knowledge of risk to minimise the development or ameliorate the effects of disease.
References

1. Lane DA, Grant PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombosis. Blood 2000;95:1517-1532