Susceptibility-conferring polymorphic genotypes in cardiovascular multifactorial syndromes

See page 325, doi:10.1053/euhj.2001.2776 for the article to which this Editorial refers

Determining polymorphic genotypes that could be major risk factors for multifactorial diseases such as myocardial infarction or arterial hypertension is a challenge that gave rise to a tremendous number of contradictory articles. Such a flood is not a priori detrimental to scientific knowledge, providing it leads progressively to certitude — science needs replication — and also to clinical applications.

Such a challenge, highlighted in the report by Benze et al.[1] in this issue, concerns the association between myocardial infarction, in 287 young men (first myocardial infarction <45 years) and two polymorphisms, C807T and PtA1/A2 on two platelet glycoproteins, respectively GPIa/IIa (integrin α2β1) and GPIIb/IIIa (integrin αIIbβ3). These polymorphisms are functional and can influence thrombogenicity: (i) GPIa/IIa mediates adhesion of platelets to collagen and C807T correlates with the rate of platelet attachment to collagen; (ii) GP IIb/IIIa is the functional receptor for fibrinogen, and PtA1/A2 is associated with platelet aggregability.

Benzé’s contribution is one of the rare genetic testings that really takes into account the other well-documented risk factors (body mass index, smoking habits, hypertension, lipidaemia, fibrinogen, plasminogen, C-reactive protein). The statistical tool utilized was logistic regression analysis that appropriately allows determination of whether the genetic linkage is independent or, in fact, it goes through one of the other risk factors. Interestingly, the authors found, first, that the linkage between myocardial infarction and the PtA1/A2 polymorphism on GPIIb/IIIa, was no more significant when other established risk factors were taken into account. In addition, and as usual, they did not confirm the linkages between myocardial infarction and the C807T polymorphism on GPIa/IIa that have been previously published.

Candidate gene analysis in complex diseases, the limits

In 2001, the search for gene polymorphism has become a popular challenge in every cardiovascular journal and congress, particularly concerning hypertension and myocardial infarction. These studies were aimed at detecting markers for drug efficacy and risk factors and more generally at better understanding the determinants of these complex diseases that are both multifactorial and multigenic[2–4]. For the moment, however, such an approach induces more confusion than clarification.

The best example of such confusion is the endless debate around the linkage with an Insertion, I/Deletion, D, polymorphism DD/DD/I of the angiotensin converting enzyme, ACE, gene[5]. The ID polymorphism is located in position 287 on the intron 16. It follows Mendelian laws and is in linkage disequilibrium[6] with the gene locus involved in the control of plasma ACE levels. Several studies showed that the plasma levels of ACE were significantly lower in II genotype than in DD. Since the pioneer work of F. Cambien[5], conflicting data regarding the association between this polymorphism and myocardial infarction have been reported almost monthly[7]. Currently 12 articles support the association, and five, including a large meta-analysis, found no or little influence from the different genotypes[8]. In addition, unsuccessful attempts were made by several authors to correlate this polymorphism with hypertension, restenosis or drug effects[7]. The story is roughly the same for the two integrins[1], and also for other polymorphisms located on endothelial NO synthase, paraoxonase-1, aldolsynthase, plasminogen activator inhibitor-1 genes, and probably several others.

The search for a candidate gene in hypertension is still more depressing. The Gly460Trp polymorphism, located on ADDA, the α-adducin gene, is a functional polymorphism linked to hypertension in several studies, while others were unable to confirm the linkage[9,10]. Attempts to link hypertension with several functional polymorphisms of the adrenergic system, including the Gly16 variant of the β2-receptor (which exhibited enhanced isoproterenol-induced down-regulation), the 825T allele of the β3-subunit of the G-protein (which is associated with enhanced signal transduction) also resulted in pros and cons in equal measure[10–12]. Two candidates have been studied in depth, the angiotensinogen, and epithelial amiloride-sensitive sodium channel (ENaC) genes. It is now clear that the 235T variant of angiotensinogen is in linkage disequilibrium with an A-6C functional variant located on the promoter of angiotensinogen.
Nevertheless although this variant was linked with hypertension in most studies, there are, still, major negative reports\textsuperscript{13}. The Liddle’s syndrome is a rare disease that is caused by mutations in ENaC, and several reports tried to link essential hypertension and functional mutations of different ENaC subunits, again the results are still debatable\textsuperscript{13}.

As far as myocardial infarction is concerned, the definition of the phenotype requires comment. The linkage between myocardial infarction and integrins, if any, has to go through coagulation factors and thrombosis, so there is a need for a better patient selection. Of course, the confinement to young males, as in the Benze paper, is progress, but it would probably still be better to try to select patients with thrombosis, against, for example, patients without thrombosis or with coronary spasm. The same can be said for the association between the alleles of apolipoprotein E and the severity of myocardial infarction. The routine determination of the other associated risk factors is indeed rarely done, except, of course, in the Benze paper\textsuperscript{1}. The need for better phenotyping in a hypertensive population has also been highlighted. Longitudinal blood pressure determination is better than averaged instant measurements\textsuperscript{14}, and, genetic studies on hypertension that are limited to a particular subgroup, such as the obese\textsuperscript{15}, should a priori be more informative than reports based on a wider cohort. Better clinical and biochemical descriptions of study populations would allow studies on more homogeneous groups and, then, improve statistical power\textsuperscript{13}. Such a comment is still more valid for myocardial infarction that can be caused by numerous well-identified risk factors which are rarely reported.

Is genome-wide linkage analysis better?

Should priority be given to genome-wide searches for susceptibility loci based on linkage analysis in large families, or, even better, in twin cohorts, such as the Scandinavian cohort that recently quantified the role of the genetic factor in cancerogenesis\textsuperscript{16}? Such studies are less ambitious, more difficult and require a sophisticated genetic background. They do not provide any immediate explanations for the disease, nevertheless they have the advantage of identifying chromosomal regions that are linked to the trait. This can then be followed by candidate gene research by according priority to genes that are located within the loci of linkage, and even by suggesting loci that may contain orphan or unexpected genes.

In such multigenic diseases, several polymorphisms related to the disease are expected both in large populations and in individuals. So far, genome-wide research has never been performed on myocardial infarction, despite the fact that the genetic determinants of myocardial infarction are more numerous than those of hypertension (hypertension itself being one of the determinants of myocardial infarction as everybody knows!). By contrast, several well-documented studies on genome-wide analysis in hypertension or hyperlipidaemia have been recently reported\textsuperscript{14,15,17–19}. Nevertheless, they do not allow better targeting of positional cloning or candidate gene analysis. For hypertension, highly significant linkages were, indeed, found on chromosome 11q in Cambridge, U.K.\textsuperscript{17}, on chromosomes 2p, 5q, 6q and 15q in Houston, U.S.A.\textsuperscript{18} and, with two markers, on chromosome 17 in Framingham\textsuperscript{14}. The story is roughly the same for hyperlipidaemia\textsuperscript{19}. Several papers have identified multiple loci involved in variations of plasma concentrations in cholesterol or triglycerides, on chromosome 1q, on chromosomes 6, 10 or 11. For the moment, the only consensus that has been reached is that both hypertension and hyperlipidaemia are associated with multiple susceptibility loci that have both a complex and multigenic trait. Clearly, clinical genetics in cardiology is still at a very early stage.

Why a search for genetic linkage in such complex diseases?

There are several goals that could have important clinical consequences for clinicians and that justify better targeting of future research. The first is to adapt therapy better to a given patient\textsuperscript{20}. The therapeutic ratio is a polygenic trait which is determined by drug-metabolizing enzymes such as CYP2D6, which metabolizes several drugs, including metoprolol\textsuperscript{21}, and targets for drug action such as the ACE\textsuperscript{9}. Several reports have suggested polymorphisms which influence either drug effect or drug toxicity\textsuperscript{20}. The second is to identify new risk factors. Assuming that the incidence of myocardial infarction in young males is around 1%–2%, the relative risk is ≈3, and the frequency of a susceptibility-conferring genotype, such as the integrin polymorphism, is between 10%–25%, the calculated predictive value of such a genotype should be very low, around 10%–13%, and the risk attributable to such a polymorphism should be less than 4\%\textsuperscript{22}. For the moment, such low values have no real clinical applications. Of course, the simultaneous utilization of several polymorphic markers could improve the statistical power, but, as also shown in Benze’s paper\textsuperscript{1}, such a combination rarely provides a real benefit.
To conclude

Needless to say, the purpose of these comments was not to downplay the importance of basic knowledge on genetic determinants of coronary disease. On the contrary, there is need for more research, but a need for more complete and more carefully conducted studies and finally an urgent demand for a better selection in the cardiology journals. The studies, based on wide genome research, were all performed by experienced groups, nevertheless, they are not beyond reproach, from a pure methodological point of view. Indeed, they frequently showed linkages with a LOD score below 3 and even below 2 and strongly suggested that such linkages are indicative, while they are simply not significant. These studies also differed in the definition of the phenotype, some dealing with blood pressure as a single trait [14,18], others focusing on hypertension [17], or even on more homogeneous groups, such as the obese [19].

It is obviously too early to draw any conclusion; nevertheless, it is important to remember that the candidate gene method of analysis — even when two or more candidates were analysed together — supposes: (i) that candidate genes are functional — if not, they are only markers — and that functional mutations should be fully documented by animal experiments, since, for the moment, genome-wide analysis does not provide any solid direction; (ii) a high statistical power — N>200–400, a LOD score, if any, above 3 and P<0·001; (iii) a clear and limited definition of the phenotype with randomly selected homogeneous cohorts of patients; (iv) preferably, knowledge of the heritability of the trait (λs) and the allele frequency in the population studied. (v) Finally, as usual in science, replication in other laboratories working in the same conditions is mandatory [3,4,13].

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References


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