Beca
cause of the complex and multiorgan origin of hyperten-
sion, the genetic approach is the preferred strategy for dis-
covering genes predisposing to the disease, a strategy that was successful mostly for monogenic forms of the disease. Both linkage studies in families with informative markers and case-control studies with biallelic markers or putative functional variants on candidate genes were widely used.

Two studies published in this issue illustrate the interest and the limits of this approach on 2 genes responsible for the enzymatic generation of nitric oxide (%NO). Among the multiple actions of NO, those involving the cardiovascular system were extensively documented and designate the genes coding for NO-generating enzymes as candidate genes.

Because, in the mammalian genome, 3 genes encoding 3 different nitric oxide synthases (NOS) are responsible for enzymatic generation of NO from l-arginine in various cells and under various stimuli, the role of each gene in NO generation defines its place as a candidate gene in hypertension.

Transgenic experiments supply the best arguments that the endothelial NOS (eNOS) is a strong candidate since pharmacological inhibition of this isoform is not available. Homozygous mice for the knockout of the eNOS gene have a level of blood pressure =15 mm Hg higher than control mice, a result obtained independently by 2 groups. More recently, the mouse eNOS cDNA was transduced in mice under the transcriptional control of a heterologous endothelial cell-targeting promoter, the preproendothelin gene promoter. Basal systolic and diastolic blood pressure were both reduced by =20 mm Hg in male transgenic mice overexpressing eNOS, owing to the presence of several copies of this chimeric gene in the genome of transgenic mice. Although this experiment is far from physiology, it shows that blood pressure is sensitive to increased transcription of eNOS mRNAs.

The hypothesis that genetic variability of the eNOS gene could lead to hypertension through a decrease in the transcription level of the gene or a decreased enzymatic activity caused by a change in the amino acid sequence of the encoded enzyme was tested by several groups. An initial linkage and association study performed on Caucasian subjects was negative. The information content of the microsatellite marker (CA repeat) used in the affected sib-pair study was high as reflected by a 92% heterozygosity, and 2 biallelic neutral markers were used for the case-control study. However, one can argue that the negativity of these results was due to a lack of power in the family study and from an absence of linkage disequilibrium between the markers used and a putative functional variant that could lie in the gene. Three other reports corroborated these negative findings with the use of either the CA repeat or other polymorphisms in linkage or association studies. More recently, 2 association studies were performed with a Glu298Asp polymorphism as genotype, after it was found to be associated with coronary spasm and considered as a putative functional variant. In both studies, the Glu298 allele was the most frequent allele in the control population, but, as compared with their respective controls, the 298Asp allele was found more frequently in Japanese hypertensives and the Glu298 allele was found more frequently in Caucasian hypertensives. In the study presented in this issue by Kato et al., no difference in the allele frequency of the Glu298Asp polymorphism was found between Japanese hypertensives and normotensives, even by testing different control populations from Japan in which they did not observe any significant variation of the 298Asp allele frequency.

The age of the patients is the only marked difference in anthropometric variables, since, in the Miyamato study, patients are, on average, 10 years younger than in the Kato study but have mean diastolic blood pressure =10 mm Hg lower. However, under the hypothesis that the 298Asp allele is found more frequently in young hypertensives, the frequency found in older patients studied by Kato et al should be closer to that found in control subjects, but it is not the case. Indeed, in the latter study, the 298Asp allele has a frequency similar to what was found in hypertensives from the Miyamato study.

The absence of consistent results with the Glu298Asp polymorphism does not plead in favor of a risk of hypertension associated with this genotype. In addition, there is no experimental argument supporting the functionality of this marker, which could compensate for its low heterozygosity. A Glu-to-Asp change can induce a loss of activity if located in a critical residue of an enzyme. The Glu298 does not lie in a part of the molecule identified as
by gene markers and by demonstrating the effect of these functional variants on these intermediate phenotypes and the molecular mechanisms underlying this effect by biochemical techniques. A large variety of techniques will be required from computer-assisted modeling of structural changes induced by amino acid substitutions to site-directed mutagenesis of regulatory regions, allowing the effect of sequence variation in transcriptionally active regions to be assessed. Few examples of functionality of polymorphisms affecting gene regulation have been described, and results are sometimes difficult to interpret.22,23 Thus it is clear that assessing the effects of gene variability on gene function and on complex biological systems will be the bottleneck in the coming years for genetics of complex diseases.

References


a critical region.14 In this case, this conservative amino acid change, in terms of charge, should not alter the conformation or activity of the enzyme.

The iNOS gene is not a candidate, as strongly documented by experimental data than the eNOS gene. The expression of this gene was found in basal conditions at a very low level in vascular smooth muscle cells of the vascular wall in rats and humans and in the medulla of the kidney.15 Knockout experiments of this gene in mice do not lead to hypertension in nullizygous mice in contrast to those with the eNOS gene knockout.5,16 Congenic lines constructed with the aim of identifying the high blood pressure gene located on rat chromosome 10, near the angiotensin-converting enzyme locus, were able to exclude the iNOS gene and closely surrounding genes.17

Results form Glenn et al2 do not support a role of this gene, even if negative results raise the usual question of power, in view of the number of genes potentially implicated and the low risk ratio of the disease. A variation in allelic frequency of a biallelic polymorphism in older patients was found in this study. Such a slight variation restricted to a subgroup is not a strong argument for a biological support of this association.

The third NO-generating enzyme, neuronal NOS (nNOS), is also an interesting candidate because nNOS is expressed in the kidney, in particular in the macula densa, and is probably involved in renin regulation.18–20 Moreover, selective inhibitors of nNOS were recently shown to lower blood pressure in nullizygous mice for the eNOS gene, thus attributing to nNOS-derived NO the blood pressure increase observed in these particular mice under NOS inhibition.21

These two articles raise some questions in view of the new era of genetics of hypertension and other complex diseases, open by the high throughput DNA technologies and the increasing number of identified, sequenced, and mapped genes.

The first question is: Is this gene a good candidate? With the availability of the gene sequences and high throughput technologies for polymorphism detection and genotyping, several groups will be able to test hundreds of genes for hypertension, thus making this definition very wide.

The second question is: Is the hypothesis of its involvement well tested? The lack of power of sib-pair studies has encouraged several investigators to give their favor to association studies with single nucleotide polymorphisms, usually biallelic, easily found by recent technologies, such as denaturing high-pressure liquid chromatography. These techniques should allow intensive investigation of the gene sequence and, ideally, allow association studies to be performed with functional variants as marker genotypes. Although this investigation can be easily exhaustive for the coding regions, regulatory regions of the genes will be more difficult to delineate. In the case of the eNOS gene, mapping the regulatory regions will be of great interest for investigating its molecular variability, since its transcription appears to be of major importance for basal blood pressure regulation.

Discrepancies between results from association studies will be solved only by finding intermediate phenotypes influenced...


Nitric Oxide Synthase Genes: Candidate Genes Among Many Others
Florent Soubrier

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