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Hypertension and SNP Genotyping in Antihypertensive Treatment

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Abstract

Hypertension is prevalent, affecting approx 20–25% of the adult population in the Western world. Primary hypertension is a multifactorial, complex disorder where many genes and genetic variants are assumed to interact with environmental factors in order to produce the specific blood pressure level for a given individual. Family and twin studies show that between 30 and 60% of blood pressure variation is determined by genetic factors. Monogenic disorders of hypertension are rare and do not explain blood pressure variability in the population at large. Obvious candidate genes for the study of hypertension are those that encode components of a blood pressure regulating system targeted by an antihypertensive drug, or those that are involved in counterregulatory systems. In this review, we give a brief pathophysiological background to hypertension and the rational behind utilizing SNP genotyping in the study of hypertension and the antihypertensive response to treatment. We also discuss some of the novel results of pharmacodynamic studies in antihypertensive treatment, an area in its infancy.

Key Words: Hypertension; genotyping; single nucleotide polymorphisms; SNP; genetic variation; response to treatment; pharmacodynamics.

The Definition of Hypertension

As summarized by Pickering *(1)*, "arterial pressure [is] a quantity and the consequence numerically related to the size of the quantity," making the definition of hypertension somewhat arbitrary. However, the World Health Organization–International Society of Hypertension (WHO-ISH) Guidelines Committee *(2)*, and later the European Society of Hypertension and European Society of Cardiology *(3)* and the U.S. Department of Health *(4)*, have published guidelines for management of chronically elevated blood pressure based on evidence from epidemiological studies and clinical trials. According to these guidelines, hypertension is defined as a systolic blood pressure (SBP) of 140 mmHg or higher and/or a diastolic blood pressure (DBP) of 90 mmHg or greater. *Primary* hypertension (earlier referred to as "essential" or "benign"), hypertension of unknown cause, as distinguished from *secondary* hypertension whose cause is known, represents as much as 90–95% of all hypertension. Unless otherwise stated, *hypertension* refers to primary hypertension.

In the following text, we start by giving a brief background of the pathophysiological mechanisms involved in the development of hypertension. This is done in order to give an appreciation for the complexity of hypertension as a clinical entity.

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What Causes Hypertension?

The clinical presentation of hypertension is age dependent. In young borderline hypertensives, cardiac output is raised without change in vascular resistance (hyperkinetic circulation) *(5)*. This is believed to be caused by an increased sympathetic nerve activity, raising cardiac output by increasing both heart rate and myocardial contractility *(6)*. In addition to the direct pressor effect, catecholamines are trophic to vascular tissue *(7)*. In established hypertension, however, cardiac output is normal or even reduced, and blood pressure is elevated by increased vascular resistance *(8)*, the benchmark of established hypertension.

Blood pressure can be viewed from a hemodynamic perspective, since it is the product of stroke volume and heart rate. The blood volume filling the heart is a major determinant of stroke volume (through the Frank-Starling mechanism), and one of the key determinants of circulating blood volume is sodium balance and its regulation, effectuated by the kidney. A high sodium intake may increase blood volume, as shown from both epidemiological and experimental data. It has been shown that lowering dietary salt (sodium chloride) intake also lowers blood pressure *(9,10)*. As a result, maintaining a moderate sodium intake has become part of the dietary recommendations given by the American Heart Association *(11)*. The observation of varying responses of blood pressure to short periods of low- and high-sodium intake lead to the definition of salt sensitivity *(12)*. Salt-sensitive individuals (more common in hypertensives than in normotensives) will increase their blood pressure more in response to sodium than salt-resistant subjects. There are several theories that attempt to explain why the kidneys are unable to excrete excess sodium and restore sodium and fluid volume balance *(13–17)*. All of these support a defective sodium output in relation to blood pressure level in salt-sensitive subjects, resulting in chronically increased blood volume.

There are many hormonal systems involved in blood pressure regulation; the adrenergic system has been mentioned above. Another key system is the renin–angiotensin–aldosterone system (RAAS), which plays a central role by influencing both the salt/water homeostasis and vascular tone. Decreased intravascular volume presents a threat, necessitating a rapid response to support circulation. This is effectuated by the pressor actions of angiotensin II, resulting in vasoconstriction; increased aldosterone secretion, leading to salt retention; increased thirst and release of antidiuretic hormone to conserve water; increased cardiac contractility to maintain cardiac output; and the potentiation of the sympathetic nervous system, which synergizes with angiotensin II. The RAAS is also responsible for more long-term effects, such as structural remodeling of the cardiovascular system.

Vascular remodeling (rearranging of existing vascular cells around a smaller lumen) of resistance arteries may represent an adaptation in response to chronically elevated blood pressure *(18,19)*, but, as pointed out above, may also develop in response to trophic hormones, such as angiotensin II *(20)* and endothelin *(21)*. Hypertension is thought to be perpetuated by vascular hypertrophy "making permanent" the increased peripheral resistance *(22)*. Chronically elevated blood pressure, trophic factors, and atherosclerosis affect the large central arteries, rendering them less compliant, which is manifested as isolated systolic hypertension.

In summary, hypertension involves three main factors: (1) abnormalities in blood volume and salt regulation, (2) increased peripheral vascular tone, and (3) vessel wall remodeling. Multiple pathophysiological pathways are involved, interwoven and interacting with each other in a complex balance. There is therefore no way to discern one specific pathophysiological mechanism that gives rise to hypertension in an individual hypertensive patient.

Is There a Genetic Component of Hypertension?

It is common clinical knowledge that hypertension and cardiovascular disease run in families; this is supported by several reports showing that blood pressure is more similar between relatives than between unrelated individuals *(23–27)*. The similarities in blood pressure within families are not restricted to hypertensives, but apply to all levels of blood pressure *(28,29)*. Twin studies *(30–35)* show that as much as 40–70% of blood pressure variance can be explained by genetic factors (heritability). Interestingly, it has recently been shown that this also holds true for the general population *(36)*.

The genetic makeup of monozygotic twins is virtually identical, but when raised together, environmen-

tal factors are also shared. Therefore, studies of monozygotic twins brought up in separate environments are of interest, and show that 34–44% of the variance in blood pressure can be explained by genetic factors *(37)*.

Blood pressure is a quantitative trait, and any characteristic dependent on the additive action of a large number of small, individually independent causes will show a normal distribution in the population. This is the case for the blood pressure distribution, of which hypertension represents the upper tail. There are, however, many factors that complicate the dissection of blood pressure as a genetic trait. In addition, the individual genetic contributions are most likely not independent, due to gene–gene and gene–environment interaction. Moreover, the same genetic background can be responsible for more than one phenotype (the observable characteristics). This is exemplified by the genetic clustering of hypertension, diabetes, and obesity *(35,38)*. The reverse may also occur, that different genetic make-ups may give rise to the same phenotype (phenocopies).

It is clear that hypertension in part can be explained by genetic factors; however, most of the knowledge of the genetics of common disease relates to rare families segregating high-risk alleles *(39)*. Such alleles are rare in the population and therefore explain little of the overall disease prevalence. The currently dominating common disease:common variant hypothesis speculates that the gene variation underlying susceptibility to common heritable disease, such as hypertension, existed within the founding population of contemporary humans *(40–43)*. Despite small effects of each individual gene or genotype on the overall phenotype, the magnitude of their attributable risk (the proportion of people affected due to them) may be large because they are frequent in the population.

In summary, primary hypertension is most likely not a singular disease but a clinical syndrome attributable to a variety of underlying pathophysiological mechanisms, genes, and genetic variants interacting with environmental factors. In light of this assumption, how do we then dissect this genetic susceptibility?

SNP Genotyping

Sequence variation in its most common form, the single nucleotide polymorphism (SNP), has been cataloged. More than 5 million SNPs are now validated and publicly accessible http://www.ncbi.nlm.nih.gov/SNP/. SNPs are estimated to represent approx 90% of all genetic variation, and lend themselves to rapid, automated analysis, making SNPs choice for studies of genetic variation. 300,000–600,000 SNPs are estimated to reflect the entire genome (http://www.genome.gov/11006929).

With the development of array-based genotyping technologies, research has shifted from the analysis of individual genes and genetic variants to highly parallel assays. As a consequence of the miniaturized format of array-based technologies and their possible automation, both the costs for reagents and the amount of work needed for each genotype are reduced.

Three major reaction principles form the basis of the currently available genotyping technologies: (1) primer extension, (2) ligation, and (3) hybridization (*see* Fig. 1). The DNA polymerase-assisted single nucleotide primer extension reaction is one of the most commonly used reaction principles. The primer extension reaction mimics the replication of genetic information during DNA synthesis in the cells and relies on the high sequence specificity in allele discrimination of the DNA polymerase. In the minisequencing assay, an oligonucleotide designed to anneal adjacent to the specific SNP position of interest is extended with a nucleotide by the action of the DNA polymerase. The primer extension reaction allows specific genotyping of most SNPs at similar reaction conditions using only a single primer per SNP. The simplicity of the reaction and the need for only one detection primer to identify the allelic variant, makes the reaction easy to establish and suitable for high-level multiplexing.

In the oligonucleotide ligation assay (OLA) *(44)*, the two SNP alleles are detected using allele-specific probes and a ligation probe, which hybridizes to its target sequence. The junction between the probes is closed by a DNA ligase when there is a perfect match between the allele-specific probe and its target.

The destabilizing effect of a single nucleotide mismatch between an oligonucleotide and its target is used to distinguish sequence variants in hybridization-based assays. Temperature, ionic strength, the sequence surrounding the SNP position, and the formation of secondary structures in the probe and target, all affect the stability of the probe-target hybrid. Therefore, careful optimization of reaction conditions and primer design is necessary when performing highly multiplexed hybridization assays.

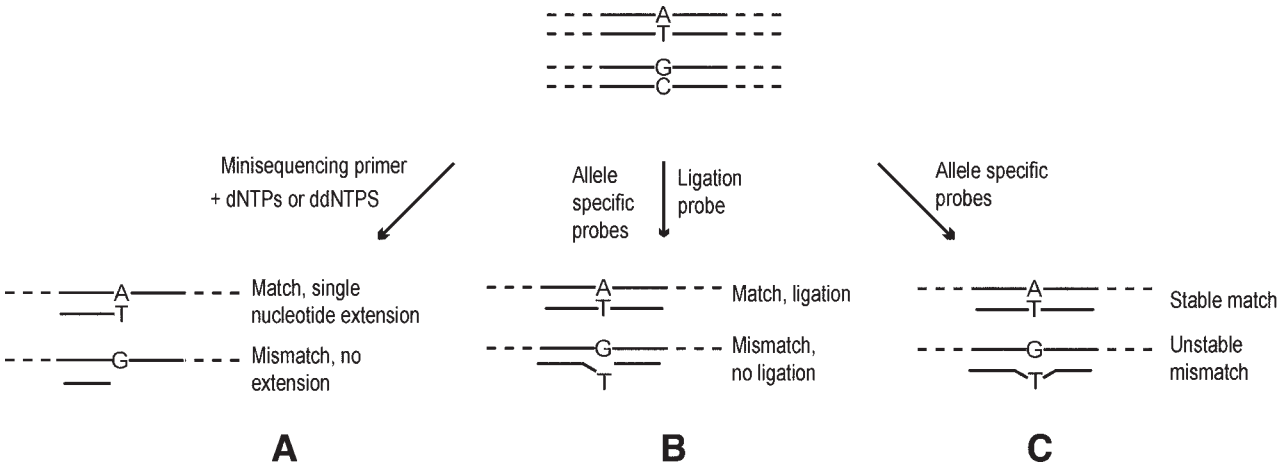

Fig. 1. Illustration of three major reaction principles. (**A**) The primer extension reaction here illustrated by the incorporation of a dideoxy thymidine nucleotide when there is a match with adenine on the complimentary strand. No incorporation will occur when there is a mismatch. (**B**) In the oligonucleotide ligation assay (OLA), an allele-specific probe and a ligation probe identify the specific SNP of interest. The junction between the probes is closed by a DNA ligase when there is a match between the allele specific probe and its target. (**C**) Hybridization occurs when there is a complementary match between the allele-specific probe and the target sequence.

Most of the genotyping methods used at present depend on prior amplification of the genomic region of interest, using the polymerase chain reaction (PCR) *(45–47)*. This is done in order to provide sufficient sensitivity and specificity for detection of the SNP of interest, as one base change among the 3×10^9 possible base pairs constituting the human genome. Due to the difficulty of performing multiplex amplification, PCR is at present the major bottleneck for high-throughput genotyping *(48)*.

New high-throughput genotyping systems are emerging where performing PCR subsequent to allele recognition circumvents the initial multiplex PCR reaction. One such example involves multiplex oligonucleotide ligation in the SNPlex™ Genotyping System (Applied Biosystems). The ligation of a perfectly matched allele-specific probe to the ligation probe is followed by amplification of the ligation products by PCR using a universal primer pair. A similar strategy is applied in the SNPWave™ technology for multiplex genotyping *(49)*. Here, padlock probes are used to identify the SNP allele of interest. Following the ligation step, the probes are selectively amplified in a multiplex strategy based on the amplified fragment length polymorphism technology using a single set of PCR primers.

Combining a primer extension reaction with a ligation step is another promising approach for increasing the specificity of direct SNP genotyping in genomic DNA. Two examples of such methods are the BeadArray technology from Illumina *(50)* and the use of molecular inversion probes in the system from ParAllele BioScience *(51)*. The ligation product is amplified using a single set of universal PCR primers, which allows for an even amplification of many fragments in a highly multiplex fashion, without the need for optimization of the amplification reaction.

An additional approach aiming to increase the selectivity of the target sequences prior to large-scale genotyping is to perform a restriction enzymatic cleavage of the genomic DNA. A subset of the fractionated genome is amplified using universal primers in a process similar to the amplified fragment-length polymorphism method *(52,53)*. Callow et al. have recently described a similar technique, where adapter sequences on the target sequence allow the formation of a circular structure during digestion of linear fragments, thus enriching the sequence of interest *(54)*. A single pair of amplification primers complementary to the adapter sequences is then used for multiplex amplification. Both these techniques are restricted to the analysis of SNPs located within the restriction fragments, limiting the selection of markers.

In the mid-1970s, it took several days to analyze a single genotype. Today, we can analyze more than 1000 genotypes overnight *(51)*, and with continued

technological advances, genome-wide approaches are within reach. We suggest that technology is no longer our greatest limitation when it comes to studies of genetic variation.

SNP Genotyping in Antihypertensive Treatment

The concept of a familial component modulating drug response was described in the 1950s, often in connection with case reports of unexpected drug response *(55–58)*. The term *pharmacogenetics* (the study of heritability of drug response) was coined prior to current knowledge in molecular biology. The observed variation in drug metabolism (pharmacokinetics) was ascribed to different metabolic rates in the enzymes either activating or inactivating the drug. Today we have achieved a basic understanding of a number of interactions and side effects resulting from genetic variation in drug metabolizing enzymes. These are predominantly single-gene effects. For recent reviews *see* refs. *59–63*.

Genotyping has become more commonly accessible as a result of technological advances. Consequentially, during the 1990s, it became possible to pose clinical questions relating to cardiovascular disease risk, such as the role of angiotensinogen gene polymorphisms and hypertension *(64)* and the angiotensin-converting enzyme (ACE) insertion/deletion polymorphisms and risk for myocardial infarction *(65)*. Obvious candidate genes for studies of hypertension are those that encode components of blood pressure regulating systems targeted by antihypertensive drugs, and genes corresponding to components in the counter-regulatory systems. The response of most commonly used antihypertensive drugs shows an approximately unimodal distribution, consistent with the effect of multiple genes, each individual contribution being small. Our knowledge of pharmacodynamics (genetic factors influencing the effect of a drug) in antihypertensive response is, however, in its infancy. An overview of pharmacogenetic studies in antihypertensive response is shown in Table 1; for recent reviews, *see* refs. *66–68*. Although pivotal, to our knowledge, there is no actual calculation of the heritability of antihypertensive drug response.

Studies on single candidate gene polymorphisms suggest that the contribution of genetics to the interindividual variation in blood pressure response to antihypertensive therapy is approx 3–5% *(69,70)*. Analyzing integrated sets reflecting a pathway, such as the renin-angiotensin-aldosterone system, should be more informative; however, there is little supporting evidence *(71)*. When studying 74 SNPs (single nucleotide polymorphisms) in 25 candidate genes, combinations of four to five SNPs were shown to explain 44–56% of the variation in blood pressure response to treatment *(72)*. It is probable that the more genetic variation we can take into account, the greater is the explanatory power. Moreover, it is interesting to recognize that this degree of explanation can exceed the degree of explanation from traditional risk factors, as exemplified by Liljedahl et al., where the predictive power of SNP combinations was shown to be greater than that of blood pressure reduction when predicting the change in left ventricular mass in response to antihypertensive treatment *(73)*.

How do we explain the conflicting results of genetic association studies in hypertension? As mentioned above, the contribution of individual genotypes is most likely small, rendering the outcome of the studies especially sensitive to ethnic heterogeneity (used as a surrogate for genetic composition) and varying and sometimes imprecise definitions of clinical phenotype. Again, assuming that each individual contribution from a specific polymorphism is small, an association with a disease may not be unmasked until provoked. Pharmacological treatment may be one way of standardizing the environmental impact and disclosing the effect of gene polymorphisms that would otherwise not be seen.

Limitations in SNP Analysis of Complex Disease

Linkage analysis is more powerful than association studies for identifying rare high-risk alleles, but association studies are more powerful for the detection of common disease alleles that confer moderate disease risks *(39,41)*. Another advantage of association analysis is that it is easier to recruit large numbers of unrelated affected individuals, especially for diseases with late onset such as hypertension *(74)*. Many studies performed to date are association studies based on genetic polymorphisms in candidate genes, but with insufficient study power due to limited sample size. The estimated risk of developing hypertension in siblings of affected probands (λ) is ap-

Table 1
Overview of Pharmacogenetic Studies

RAAS + kallikrein-kinin	Adrenergic system	Endothelial system	Renal sodium handling	Oxidative stress	Metabolic abnormalities	Drug metabolism
ACE 20	β_1-adrenoceptor 4	Endothelin 1	Epithelial sodium channel 1	None	Apolipoprotein B 2	CYP2C9 1
Angiotensinogen 7	α_2 adrenergic receptor 1		α-adducin 4		LDL receptor 1	CYP2D6 1
AT_1-receptor 7	β_2 adrenergic recpetor 1		11 β hydroxysteroid			
Aldosterone synthase 3	G-proteins 3		dehydrogenase 1			
B2 bradykinin receptor 1						
AT_2 receptor 1						
Mineralcorticoid receptor 1						
Renin 1						
TGF-β_1 1						
ALAP 1						
Total: 43	Total: 9	Total: 1	Total: 6	Total: none	Total: 2	Total: 2

This table includes pharmacogenetic studies published until April 2005, adapted from ref. *68*. So far, as can be seen in the table, the focus has been on the renin–angiotensin–aldosterone system. For specific references of publications until January 2004, *see* ref. *68*, and additional publications from January 2004 through March 2005 are refs. *77–81*.

proximately 4, leading to an estimated number of 500 case control pairs in order to reach statistical significance *(41)*. These conditions are, however, rarely met.

Although approx 5 million SNPs have been cataloged, our knowledge of the functionality of these SNPs is limited. SNPs in the coding regions of genes may alter the function or structure of the encoded protein and cause most of the known recessively or dominantly inherited monogenic disorders. These SNPs can be analyzed for diagnostic purposes. Other SNPs in the coding regions may lead to changes in the amino acid sequence, in turn leading to altered functionality of drug-metabolizing enzymes. These SNPs may be used as targets for pharmacogenetic analysis. Missense SNPs in the coding regions of genes may contribute to common disease, thus being relevant to use in association studies of hypertension. However, most SNPs are located in the non-coding region of the genome and have no known impact on the (hypertensive) phenotype. These SNPs are commonly used as genetic markers in population genetics and evolutionary studies *(75)*. In addition, the range and structure of linkage disequilibrium has yet to be determined. Thus, SNP selection is still a limitation in studies of genetic variation.

It is also imperative to overcome the statistical limitations. Microarray technology is a suitable instrument for the dissection of the hypertensive phenotype due to the assumption that a large number of gene polymorphisms are involved. However, since a microarray-based approach will invariably lead to multiple comparisons, it is important to avoid a type I error. A Bonferroni correction assumes that the tests are independent of one another, which is incorrect due to linkage disequilibrium, and would lead to an over-correction with the risk of a type II error. A test such as the permutation test, which is designed to correct for a type I error and maintain an overall probability of <0.05, may be suitable *(76)*. This test has been shown to be especially useful in genetic investigations.

Conclusion

As we learn more about the pathophysiological mechanisms and the genes that are connected with hypertension, it is likely we will find well-defined subcategories of hypertension. In some cases, we may learn of yet unknown monogenetic causes, although the majority will likely remain multifactorial and complex. In clinical studies, it is imperative to stringently define the individual hypertensive phenotype.

Disease-related genetic polymorphisms may come to be used in combination with other factors to define populations and individuals at risk. Complex traits, such as hypertension and the antihypertensive response to treatment, can at best be predicted in part. If, however, it becomes feasible to identify high-risk individuals through genotyping, intervention is possible by lifestyle changes and pharmacotherapy. Since successful intervention will in turn reduce these individuals' risk for the development of hypertension and cardiovascular disease, genotype-based screening tests are ethically defensible both for the individual and our society. In the near future, genetic studies will lead to a gradual dissection of complex traits, leading to an understanding of disease susceptibility and response to treatment based on the many genes involved.

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