Pharmacogenomics and Pharmacogenetics of Hypertension: Update and Perspectives—The Adducin Paradigm

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There is a growing literature on the potential prospective use of genome information to enhance success in finding new medicines. An example of a prospective efficacy of pharmacogenetic and pharmacogenomics is the detection and impact of adducin polymorphism on hypertension. Adducin is a heterodimeric cytoskeleton protein, the three subunits of which are encoded by genes (ADD1, ADD2, and ADD3) that map to three different chromosomes. A long series of parallel studies in the Milan hypertensive rat strain model of hypertension and humans indicated that an altered adducin function might cause hypertension through an enhanced constitutive tubular sodium reabsorption. In particular, six linkage studies, 18 of 20 association studies, and four of five follow-up studies that measured organ damage in hypertensive patients support the clinical impact of adducing polymorphism. As many modulatory genes and environment affect the adducin activity, the context must be taken into account to measure the clinical effect size of adducins. Pharmacogenomics is giving an important contribution to this end. In particular, the selective advantages of diuretics in preventing myocardial infarction and stroke over other antihypertensive therapies that produce a similar BP reduction in carriers of the mutated adducin may support new strategies that aim to optimize the use of antihypertensive agents for the prevention of hypertension-associated organ damage.


The enormous variation in the individual response to antihypertensive treatment has led to acceleration of the interest in pharmacogenomic and pharmacogenetic studies with the consequent technological developments (1). Publications on these fields have increased sharply in the past 5 yr with the emergence of molecular genetics and genotyping technologies in clinical investigations. The terms pharmacogenomics and pharmacogenetics have been used interchangeably in the literature; therefore, it is useful to define them here. Pharmacogenomics refers to the application of genome-wide approaches to understanding genetic influences on drug response and to developing novel drugs. It has the potential to uncover additive or even synergistic influences of multiple genes on drug response. Historically, pharmacogenetic studies were developed initially to understand individual differences in drug pharmacokinetics and metabolism, often focused on single gene polymorphisms (SNP), especially in drug metabolism genes (1,2).

The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (3), the guideline for the management of hypertension of both the European Society of Hypertension (4) and the British Hypertension Society (5), delineates specific high-risk conditions that are compelling indications for the use antihypertensive drug classes that will be required to achieve goal BP (140/90 mmHg, or 130/80 mmHg for patients with diabetes and chronic kidney disease). Moreover, lowering BP to the optimal goal levels seems to be more important than specific drug selection. Indeed, the results of different meta-analyses (6,7) concluded that the treatment with any commonly used regimen (angiotensin-converting-enzyme inhibitors, calcium antagonists, angiotensin-receptor blockers, diuretics, and β blockers) reduces the risk for total major cardiovascular events, and larger reductions in BP produce larger reductions in risk. Although the percentage of patients who had hypertension and received treatment increased from 31 to 59% in the period from 1976 to 1980 and to 70% in 2000, the percentage of patients with high BP controlled to <140/90 mmHg is only approximately 25% in Western industrialized countries, and this values has been constant over the past 15 yr. Despite the great number of antihypertensive drugs that are available the poor BP control, adverse effects represent approximately 80% of the causes for the discontinuity of the antihypertensive therapy (8). Pharmacogenomics may reduce the interindividual variation to antihypertensive therapy by tailoring therapy to individual genetic make-up.

However, a recent systematic review (9) demonstrated that there is a lack of consistency in the findings of different pharmacogenomic studies. Once again, this inconsistency is due mainly to the failure to take into account the various factors that modulate the genotype–drug response phenotype (1). The same picture concerns genetics of essential hypertension. Among thousands of association studies that have used polymorphisms in candidate genes, few studies demonstrated consistent findings. This may be due to the variability of the current statistical genetic method to define causation in complex polygenic multifactorial disease such as primary hypertension (10). Most of the genetic and pharmacogenomic studies have major weaknesses related to three major problems:
1. **Selection criteria**: The accuracy and the amount of detailed phenotypic data that can be compared in case-control studies are crucial to the interpretation of the data. For example, in case-control studies, the appropriate control subjects must be above the age of onset of "primary" hypertension. Gender, body mass index, ethnic origin, and other biologic variables must be taken into account.

2. **Inadequate statistical power** of small studies to address complex genetic questions (11,12): Indeed, in case-control studies, the methods of statistical genetics are based on the assumption that a genetic variant is involved in hypertension when a higher frequency of this variant is present in hypertensive individuals (12). However, the biologic complexity of polygenic-multifactorial diseases challenges these assumptions. Differences in evolutionary history, population stratification and admixture, epistatic interaction, context dependency, etc. all may weaken the scientific validity of methods that are based on allelic frequency (10). This may explain why the case-control studies on candidate gene have provided inconsistent findings across populations in polygenic-multifactorial disease (13,14).

3. **Pharmacogenomics analyses** should be used to differentiate phenotypic heterogeneity, to segment populations that seem to be responsive or unresponsive to a medicine, or to define accurately individuals who might be at higher personal risk for an adverse event: To address this important point, it is crucial to enroll patients who have never treated before. For instance, during a chronic treatment with an angiotensin-converting enzyme inhibitor or angiotensin II antagonist, a great amount of plasma renin, angiotensinogen, angiotensin I, or angiotensin II is available both in the circulation and at the tissue level. After 3 or 4 wk from therapy withdrawal, BP eventually increases also because these substances are increased both in plasma and in the tissues. This pressor mechanism certainly is different from what is responsible for the gradual increase in BP with age that underlies the development of "primary" hypertension. Conversely, a long (years) period of treatment with diuretics may favor the development of an insulin-resistant status that, per se, may increase the risk for organ damage that may appear in the subsequent years (15). Therefore, patients who are already on antihypertensive therapy should be enrolled only for a particular type of study. Furthermore, when BP is measured as office BP, it should be recorded several times, or, alternatively, 24-h BP monitoring should be used.

Our research group approached the dissection of this complexity throughout a series of studies on animal models and patients at different levels of biologic organization: Whole body, organ, cell, subcellular structures, protein, and genes (16). These studies led to the identification of adducin polymorphism as one of the genetic mechanism that, by modulating the constitutive capacity of tubular cell to reabsorb sodium, may favor the development of hypertension with its organ complications.

**Molecular Mechanisms of a Candidate Gene: Adducin**

Adducin is a heterodimeric cytoskeleton protein and consists of an α subunit (103 kDa) and either a β (97 kDa) or a γ subunit (90 kDa). Three human genes (ADD1, ADD2, and ADD3) that map to different chromosomes encode these subunits (17). Adducin is highly conserved through the different species, thus suggesting a role in basic cellular functions. Adducin is a ubiquitously expressed cytoskeleton protein that is involved in the formation of actin-spectrin lattice, actin polymerization, and cell signal transduction (18,19), including an effect on Na-K ATPase. Figure 1 shows ADD1, ADD2, and ADD3 gene structure and SNP identified in human and rat hypertension. In humans, two polymorphisms of the ADD1 gene lead to amino acid substitutions: Gly460Trp and S586C (20). Other polymorphisms occur in the human ADD2 and ADD3. The first linkage and case-control studies demonstrated an association of the ADD1 Trp allele with hypertension (20).

Cell culture and cell-free system experiments helped to elucidate the molecular mechanisms that make adducin mutation responsible for the abnormal cell sodium handling:

1. In renal cells, transfection with the rat-mutated α-adducin increases the Na-K pump activity and causes a rearrangement of the actin cytoskeleton (21).
2. In a cell-free system, rat-mutated adducin accelerates actin polymerization (21), and rat-mutated and human-mutated (ADD1 Trp) adducins bind to and activate the Na-K pump with higher affinity than the respective normal proteins (22).
3. Studies on the dynamics of the endocytotic processes in transfected cells have provided an interpretation for the increased cellular expression and activity of the Na-K pump caused by the expression of the α-adducin mutants (23). Cells that are transfected with either the human or the rat hypertensive α-adducin compared with cells that are transfected with the wild-type variant show a higher Na-K pump activity and an impaired Na-K pump endocytosis in basal conditions (23) as well as in response to natriuretic signals such as dopamine. Deficient endocytosis of the Na-K pump therefore might be an important factor contributing to the increased renal tubular reabsorption observed in humans (24,25), carrying the mutated adducin variant. In fact, an efficient endocytosis of the sodium transporting proteins is crucial for blunting the rise in systemic arterial pressure when body sodium increases.

**Impact of Adducin Family Genes on Human Hypertension**

In support of these findings are the data collected in human hypertension: Among hypertensive patients, plasma renin is lower in carriers of the ADD1 Trp allele than in wild-type homozygotes (20,26). Patients with low renin hypertension have higher BP in the presence of mutated α-adducin, and ADD1 Trp/Trp homozygotes experience the largest increase in BP (26–28). Furthermore, carriers of ADD1 Trp allele, compared with the Gly/Gly homozygotes, show an increased prox-
imal tubular reabsorption measured by lithium clearance (25) and a larger increase of BP after a saline infusion (24).

In most tissues that are involved in cardiovascular homeostasis (kidney, brain, heart, and vessels), adducin is expressed as a heterodimeric protein that consists of $\alpha$ and $\gamma$ subunits. Therefore, this protein structure justifies the search for an interaction between ADD1 and ADD3. Recently (29), we described an epistatic interaction between the adducin genes (ADD1 and ADD3) in a large cohort of never-treated hypertensive individuals ($n = 512$), in which BP was determined with 24-h ambulatory BP monitoring. Patients who carried both the mutated ADD1 Trp allele and ADD3 G/G had the higher systolic BP and diastolic BP values (approximately 8 mmHg; $P = 0.002$).

Furthermore, in a study (30) that was conducted in 642 participants who were randomly recruited from three European populations, peripheral and central pulse pressure (PP), an index of vascular stiffness, was measured. Among carriers of the ADD1 Trp allele, peripheral and central PP were 5.8 and 4.7 mmHg higher in ADD3 G/G had the higher systolic BP and diastolic BP values (approximately 8 mmHg; $P = 0.002$).

These epistatic interactions, described in two separate studies, are biochemically consistent with the heterodimeric structure of the cytoskeleton protein adducin.

According to a PubMed literature search, after our initial report (16,26), at least 67 articles addressed the association between human hypertension and the ADD1 Trp allele. Six human (20,30–34) linkage studies showed positive results when a DNA marker that mapped to 30 kb from the ADD1 locus or SNP of one of the three adducin genes was considered either alone or in combination with each other or angiotensin-converting enzyme D allele or salt intake. When DNA markers that mapped at a much larger distance from the ADD1 locus were used, negative results were found by four studies (35–38). Within this large distance, many haplotype blocks were included (39). Positive results were also obtained in 18 of 20 association studies that, in addition to BP, investigated variables that reflect body sodium or the renin-angiotensin system (16,26). Four of five studies (40–44) showed an association between $\alpha$-adducin polymorphism and organ damage in hypertensive individuals. There were mixed results from case-control studies or studies in predominantly normotensive populations that did not consider the above-mentioned variables (see [16,26] for a detailed discussion of this issue).

**Pharmacogenomics Studies of Role of Adducin**

The association between the ADD1 polymorphism and the response to diuretics has been evaluated in five studies (20,41,45–47). The rationale for these studies was that in the presence of constitutive enhanced tubular sodium reabsorption, drugs such as diuretics should trigger less counterregulatory mechanisms, thus yielding a more beneficial therapeutic effect. Indeed, three studies (20,45,46) that were designed to test this hypothesis and conducted in never-treated hypertensive patients demonstrated a greater BP response to hydrochlorothiazide (HCTZ) among carriers of the 460Trp allele.

A negative study (47) involved 291 unrelated non-Hispanic black and 294 unrelated non-Hispanic white adults aged 30 to 59.9 yr. The BP value recorded after at least 4 wk from discontinuation of the previous therapy was used as baseline to evaluate the BP response to 25 mg of HCTZ given for 1 mo. Certainly, this study was large enough to conclude that, in that
context, no relationship exists between adducin genotypes and the BP response to the diuretics. However, the important difference with the other similar but positive studies (20,45,46) is that the latter were performed in newly discovered and never-treated hypertensive individuals. After at least 1 mo of run-in and three measurements of BP on three different occasions, HCTZ was given for 2 mo, and BP was measured after 1 and 2 mo of treatment. In these patients, the genotype–BP relationship was not influenced by a previous therapy, different phases of hypertension (because they all were in a relatively early hypertensive phase), and the variety of counterregulatory mechanisms that come into play with sudden therapy withdrawal. In fact, even after 1 mo from therapy withdrawal, the renin response to a standard dose of diuretic still is different from that observed in the never-treated hypertensive status (48).

In accordance with our findings are the results of an observational study (41) on 1038 hypertensive patients who were followed for approximately 10 yr and treated with a variety of antihypertensive drugs. These results show that in carriers of the 460Trp ADD1 allele (38% of the population), the administration of diuretics halves the incidence of myocardial infarction and stroke when compared with other antihypertensive treatments that produce a similar reduction of BP. The selective beneficial effect of diuretics over the other drugs was not present in carriers of the Gly/Gly ADD1 genotype. These data support the notion that matching of the genetic mechanism with the drug mechanisms of action produces a clear benefit probably because the magnitude of the counterregulatory mechanism and, hence, the global cardiovascular risk may be minimized. These results bring us to the heart of the issue regarding the application of pharmacogenomics to improve our ability to prevent organ damage in hypertension in the subset of patients who carry a specific genotype or combination of genotypes.

Conclusions

The potential of pharmacogenomics to improve treatment of hypertensive patients is enormous. It will not reduce the size of the population in whom drugs are effective but may reduce the waste associated with empirical optimization of treatment regimens. The added benefit of being able to screen out individuals who will experience unpleasant side effects should improve compliance. The incorporation of SNP haplotype characterization into the development pipeline for novel drugs would provide an excellent opportunity to determine efficacy haplotypes. Subsequently, this would be used to streamline treatment of patients with these novel drugs.

A practical way to “measure” the overall clinical impact of the ADD1 Trp allele and then to estimate the size of the population that may be affected by this genetic mechanism is to apply a very selective pharmacologic tool that is able to interfere with the sequence of events that are triggered by this allele. Among the available drugs, diuretics are those that better approximate this tool. The selective beneficial effects of these drugs in reducing BP and preventing myocardial infarction and stroke in carriers of the ADD1 Trp allele might be even greater if drugs that interfere with adducin but avoid the widely known side effects of diuretics are developed. However, the major problem to overcome in the exploitation of the full potential of pharmacogenomics is to abandon the “old,” “traditional” paradigms regarding the definition of causation of a given gene variant in a complex multifactorial disease such as hypertension (16,26). Almost all of the reviews concluded that the available data on genetics or pharmacogenomics of “primary” hypertension are conflicting and do not discuss the implications of the complexity of these diseases that arise from the genetic, environmental, or biologic interactions.

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