Adducin Polymorphism
Detection and Impact on Hypertension and Related Disorders

Giuseppe Bianchi, Patrizia Ferrari, Jan A. Staessen

Abstract—Adducin is a heterodymeric cytoskeleton protein, the 3 subunits of which are encoded by genes (ADD1, ADD2, ADD3) mapping to 3 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 may cause hypertension through an enhanced constitutive tubular sodium reabsorption. Six human linkage studies showed positive results when a DNA marker mapping to 30 kb from the ADD1 locus or single-nucleotide polymorphisms (SNPs) of 1 of the 3 adducin genes were considered either alone or in combination with each other or angiotensin-converting enzyme (ACE) D allele or salt intake. When DNA markers mapping at much larger distance from the ADD1 locus were used, negative results were found by 4 studies. Positive results were also obtained in 18 of 20 association studies that, in addition to blood pressure, investigated variables reflecting body sodium or the renin-angiotensin system. Mixed results regarded case-control studies or studies in predominantly normotensive populations that did not consider the above-mentioned variables. Four of 5 studies showed a selective beneficial effect of diuretics in carriers of the mutated ADD1. Twelve of 16 studies found that ADD1 polymorphism alone or in combination with that of ACE positively associates with stroke or coronary heart disease or renal or vascular dysfunctions. In conclusion, when context is taken into account, the impact of adducin in hypertension and its related disorders is clear. (Hypertension. 2005;45:331-340.)

Key Words: genetics ■ hypertension, essential ■ human ■ rats, spontaneously hypertensive ■ diuretics

Primary and even renorph forms of hypertension are characterized by heterogeneity in terms of potential molecular mechanisms, pathophysiological and clinical patterns, and response to therapy. Furthermore, a network of feedback loops interacting with the renal, hormonal, hemodynamic, and nervous mechanisms that control blood pressure hampers the distinction between primary and secondary mechanisms. Therefore, appropriate animal models may facilitate elucidation of the hierarchical and temporal order of events linking a given molecular mechanism to hypertension.1 The usefulness of the animal model obviously depends on its similarity to the human condition. This implies a precise definition of the subset of patients whose pathophysiological and clinical profiles approximate to that of the model. We pursued this complex approach throughout a series of empirical observations that led us to propose adducin as a candidate gene for human hypertension. Adducin polymorphism is certainly one of the very few polymorphisms,2 if not the unique one,3 affecting blood pressure in 2 species (rat and human) that diverged ≈40 million years ago.

To discuss the adducin data within the framework of the multitude of interactive blood pressure–regulating mechanisms, this review is subdivided as follows: (1) comparison of experimental results obtained in the Milan hypertensive rat strain (MHS) and its normotensive control (Milan normotensive rat strain [MNS]) with observations in humans, focusing on renal intermediate phenotypes that are on the pathophysiological pathway linking a genetic mutation to the blood pressure phenotype; (2) description of the molecular mechanisms affected by adducin; and (3) characterization of those subsets of patients who are comparable to the animal models. This approach allows identification of genetic and environmental factors that favor expression of the cardiovascular phenotypes associated with a gene that influences renal sodium handling. Moreover, we reviewed those pharmacological studies with diuretics, which tested the hypothesis of a genetically programmed increase in renal tubular sodium reabsorption.

The most relevant primary pathophysiological changes occur at the transition from normotension to hypertension. Therefore, we compared animals and humans prone to develop hypertension with their respective controls at this early stage of the disease.4 For instance, we compared young normotensive subjects having 2 hypertensive parents with matched controls with 2 normotensive parents.4

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TABLE 1. Comparison of Humans Either Prone to or at the Early Hypertensive Stage With Rats of the Milan Strain

<table>
<thead>
<tr>
<th>Intermediate Phenotypes</th>
<th>Human Essential</th>
<th>MHS Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressor effect of kidney transplantation</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Renal blood flow</td>
<td>↑</td>
<td>↓ = or ↑ in isolated kidney</td>
</tr>
<tr>
<td>GFR</td>
<td>↑ = ↓</td>
<td>↑</td>
</tr>
<tr>
<td>Na excretion after load</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>24-hour urinary output</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Plasma renin</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Urine kallikrein</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Erythrocyte Na content</td>
<td>↓ = ↑</td>
<td>↓</td>
</tr>
<tr>
<td>Net erythrocyte membrane Na transport</td>
<td>↑ = ↓</td>
<td>↑</td>
</tr>
</tbody>
</table>

Higher (↑), lower (↓), or equal (=) in the hypertensive humans and rats with the appropriate controls.

In subsets of patients.

Comparison Between MHS Model and Humans

Table 1 shows the pathophysiological similarities across the 2 species when prehypertensive or early hypertensive individuals are compared with their respective normotensive controls.

The glomerular filtration rate (GFR) of young prehypertensive MHS is definitely higher than that of MNS when measured at inulin concentrations of 0.1 mg/mL,4 whereas the opposite is true when 10× higher inulin concentrations are used.4 Definitely in adult MHS, GFR is similar to that of adult MNS.

In humans, GFR is higher, similar or lower in young prehypertensive or early hypertensive patients compared with normotensive controls.4,5 Variability may be accounted for by the experimental conditions under which GFR is measured6 and by genetic–molecular mechanisms, such as those operating in some rat strains showing a reduced GFR at the very early stage of hypertension.6 These findings highlight the importance of standardizing the experimental settings, the stage of hypertension, and the genetic and environmental backgrounds of the subjects to identify subsets of patients having a renal pathophysiological profile similar to that of the rat model.4,6,7

As summarized in Table 2, the higher GFR and the lower plasma renin activity (PRA) at the prehypertensive phase, together with the renal sodium retention during development of hypertension, point to a primary increase in tubular sodium reabsorption in MHS as the cause of hypertension. This hypothesis is further strengthened by the following observations: (1) hypertension may be transplanted with the kidney in rats as well as in humans;8 (2) in MHS, ion transport occurs at a faster rate across the membranes of renal tubular cells and erythrocytes than in MNS;9 and (3) bone marrow transplantation experiments from MHS to irradiated MNS suggest that the erythrocyte membrane abnormalities of MHS are transplanted with the stem cells.9 In addition, in the MHS×MNS F2 population, the blood pressure and erythrocyte phenotypes cosegregate.9

In a subset of hypertensive patients, erythrocyte Na-K-Cl cotransport and Na-Li countertransport occur at rates higher than the maximal values observed in normotensive controls.9 Furthermore, a subset of young offspring of hypertensive

TABLE 2. Major Differences Between MHS and MNS According to the Level of Biological Organization

<table>
<thead>
<tr>
<th>Biological Level</th>
<th>Intermediate Phenotypes (By Type of Experimental Performed)</th>
<th>Experimental Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body</td>
<td>Kidney cross-transplantation</td>
<td>Pressor effect of MHS kidney44,35</td>
</tr>
<tr>
<td></td>
<td>H2O and Na+ metabolism (during the development of hypertension)</td>
<td>Renal Na+ retention in MHS38</td>
</tr>
<tr>
<td></td>
<td>Kidney function in whole animal (prehypertensive stage)</td>
<td>GFR and Na+ tubular reabsorption ↑ in MHS34</td>
</tr>
<tr>
<td></td>
<td>Isolated kidney</td>
<td>GFR, RBF, Na+ tubular reabsorption and O2 consumption ↑ in MHS38,39</td>
</tr>
<tr>
<td>Cellular</td>
<td>Structure and function of erythrocytes*</td>
<td>Cell volumes and Na+ content ↓ in MHS40,41</td>
</tr>
<tr>
<td></td>
<td>Renal tubular cells (proximal)</td>
<td>Na+ cotransport ↑ in MHS45</td>
</tr>
<tr>
<td></td>
<td>Isolated tubuli (proximal-ascending)</td>
<td>Cell volume and Na+ content ↓ in MHS44</td>
</tr>
<tr>
<td></td>
<td>Resealed erythrocyte ghosts (with membrane skeleton)</td>
<td>Na+ transport ↑ and volume ↓ in MHS42</td>
</tr>
<tr>
<td></td>
<td>Inside-out erythrocyte vesicles (without membrane skeleton)</td>
<td>Na+ transport = in MHS and MNS45</td>
</tr>
<tr>
<td></td>
<td>Luminal renal membrane</td>
<td>Na-H exchange, Na-K cotransport ↑ in MHS29,30</td>
</tr>
<tr>
<td></td>
<td>Basolateral renal membrane</td>
<td>Na-K pump ↑ in MHS31,32</td>
</tr>
<tr>
<td>Subcellular</td>
<td>Na-K ATPase in basolateral renal membrane vesicles</td>
<td>↓ in MHS as activity and protein amount53</td>
</tr>
<tr>
<td>Biochemical</td>
<td>Cytoskeletal adducin†‡</td>
<td>Immunochemical difference between MNS and MHS Production of an antiadducin antibody46</td>
</tr>
<tr>
<td>Molecular</td>
<td>DNA Sequencing of the genes coding for α, β, and γ subunits of adducin genes (Figure 1)55,56</td>
<td></td>
</tr>
</tbody>
</table>

↑, ↓, and = indicate higher, lower, or equal in MHS than in MNS.

*Erythrocyte characteristics and blood pressure are highly correlated in (MHS×MNS) F2 hybrids. Bone marrow transplantation between MHS and MNS in irradiated F1 hybrids demonstrates that MHS erythrocyte characteristics originate in the stem cells.

†Identified by cross-immunizing MNS and MHS with the reciprocal erythrocyte membrane skeletons.

‡Identified by screening an expression library with the antiadducin antibody.
parents shows a lower intracellular sodium content than controls (Table 1). When the renal tubular function of hypertensive patients with high erythrocyte Na-K-Cl cotransport is compared with that of normotensive subjects or patients with normal cotransport, the former shows lower fractional excretion of uric acid, lower PRA, and a larger natriuretic response to furosemide than the latter.9

Detection of Adducin as a Candidate Gene for Hypertension

The widespread constitutive cellular abnormalities in sodium handling and volume observed in MHS and in a subgroup of hypertensive patients, both characterized by a common defect of renal tubular function, were suggestive of a protein alteration in the actin cytoskeleton. Indeed, the removal of the actin membrane skeleton from erythrocytes nullifies the alteration in the actin cytoskeleton. Indeed, the removal of the actin membrane skeleton from erythrocytes nullifies the differences in membrane ion transport between MHS and MNS erythrocytes.9 To detect subtle protein differences between the membrane cytoskeleton of the 2 strains, we cross-immunized MHS and MNS rats with the membrane skeleton. The antibodies raised in these experiments recognize a protein that we later identified as adducin, via screening of a cDNA expression library.9

Molecular Mechanism of Adducin

Adducin is highly conserved through the different species, thus suggesting a role in basic cellular functions. The analysis of the full-length adducin cDNA sequence in the MHS and MNS strains revealed the presence of point mutations causing an amino acid substitution on the α- (F316Y) and the β- (Q529R) adducin subunits.10 We also detected a point mutation in the γ-adducin subunit of MHS (Q572K) (Figure 1). In the MHS×MNS F2 hybrid population, mutation of the Add1 gene accounts for the 50% blood pressure difference between MHS and MNS.11 Add2 and Add3 gene mutations are not, per se, associated with hypertension but epistatically interact with that of Add1 in determining the blood pressure level of the F2 hybrids.11 Moreover, the transfer of a short chromosomal region including Add1 locus from MHS to MNS and vice versa raises the blood pressure in the MNS genetic background and reduces it in the reciprocal strain.14

In humans, 2 polymorphisms of the ADD1 gene lead to amino acid substitutions: G460W and S586C.15 Other polymorphisms occur in the human ADD2 and ADD3 (Figure 1). The first linkage and case-control studies demonstrated an association of the ADD1 W allele with hypertension.12 Moreover, carriers of the ADD1 W allele, when compared with homozygotes for the ADD1 G allele, have a decreased erythrocyte sodium content and faster Na-K cotransport,16 in analogy with the findings in MHS.

Cell culture and cell-free system experiments helped to elucidate the molecular mechanisms that, in humans and rats, make adducin mutation responsible for the abnormal cell sodium handling and ultimately for hypertension. In renal cells, transfection with the MHS Add1 Y increases the Na-K pump activity and causes a rearrangement of the actin cytoskeleton.17 In a cell-free system, rat-mutated adducin accelerates actin polymerization,17 and rat- and human (ADD1 W)-mutated adducins bind to and activate the Na-K pump with higher affinity than the respective normal proteins.18 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.19 Cells transfected with either the human or rat hypertensive...
α-adducin compared with cells transfected with the wild-type variant show a higher Na-K pump activity and an impaired Na-K pump endocytosis in basal conditions as well as in response to natriuretic signals such as dopamine (Figure 2). Clathrin-dependent endocytosis of membrane proteins is initiated by adaptor proteins (AP2), which simultaneously bind to cargo proteins, recruit clathrin, and promote formation of clathrin-coated vesicles (CCV), with the cooperation of many other proteins. This protein interaction is reduced by mutated α-adducin (Figure 2). Deficient endocytosis of the Na-K pump might therefore be an important factor contributing to the increased renal tubular reabsorption observed in rats as well as in humans 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.

Impact of Adducin Polymorphism on Human Hypertension and Related Disorders

According to a PubMed literature search, after our initial report, at least 63 articles addressed the association between human hypertension and the ADD1 W allele. Four of these report our results in the Milan population. 15 concern populations studied in different centers and blindly genotyped in Milan, and 44 describe the results obtained by others groups in European, white American, African American, Chinese, and Japanese populations. As with other candidate genes, the genetic background, environmental factors, and lifestyle differed across the human adducin studies so that, not surprisingly, their conclusions are contradictory. In an attempt to better account for the context dependency and possible methodological limitations, we reviewed the studies, grouping them as follows: (1) adducin in relation to genetic, hormonal, dietary determinants of body sodium, blood pressure, and renin-angiotensin system (RAS); (2) adducin in family studies; (3) adducin in general predominately normotensive populations; (4) adducin in hypertensive case-control studies; and (5) adducin in cardiovascular or renal diseases.

Adducin in Relation to Variables (Genetic, Hormonal, Dietary) Involved in Regulation of Blood Pressure, Body Sodium (Sodium Excretion and Intake), and RAS

We grouped these findings together because of the close interrelationship among these variables throughout feedback regulatory mechanisms. In fact, the role of any variables should always be evaluated within the context given by the others. Context dependency also applies to the effects of genes, such as adducin, affecting the constitutive capacity of the tubular cells to retain sodium.

Among hypertensive patients, plasma renin is lower in carriers of the ADD1 W allele than in wild-type homozygotes. Patients with low renin hypertension have higher blood pressure in the presence of mutated α-adducin, and ADD1 W/W homozygotes experience the largest increase in blood pressure. In normotensives or hypertensives treated up to 3 weeks before the study, blood pressure changes with low-salt diet are not associated with the 3 ADD1 genotypes. However, in carriers of the ADD1 W allele, a significant 3-fold increase in the urinary excretion of NO metabolites occurs that may be attributable to their peculiar abnormal renal sodium handling. Carriers of ADD1 W allele, compared with the G/G homozygotes, show an increased proximal tubular reabsorption measured by lithium clearance and a larger increase of blood pressure after a saline infusion.
Adducin and Blood Pressure Variation After Diuretics

Two studies in our population,15,24 1 study in a Sardinian population27 that we blindly genotyped, and 2 studies46,47 in populations composed of whites and African Americans examined the association between the ADD1 polymorphism and the response to diuretics. The rationale for these studies was that in the presence of constitutive enhanced tubular sodium reabsorption, drugs such as diuretics should trigger less counter-regulatory mechanisms, thus yielding a more beneficial therapeutic effect. Indeed, all these studies but 147 showed a positive association between the ADD1 W allele and the diuretic effect.

The negative study47 involved 291 unrelated non-Hispanic African Americans and 294 unrelated non-Hispanic white adults aged 30 to 59.9 years. The blood pressure value recorded after at least 4 weeks from discontinuation of the previous therapy was used as baseline to evaluate the blood pressure response to 25 mg of hydrochlorothiazide (HCTZ) given for 1 month. Certainly, this study was large enough to conclude that, in that context, no relationship exists between adducin genotypes and the blood pressure response to the diuretics. However, the important difference with the other similar but positive studies15,24,27 is that the latter were performed in newly discovered and never-treated hypertensive subjects. After at least 1 month of run-in and 3 measurements of blood pressure on 3 different occasions, HCTZ was given for 2 months, and blood pressure was measured after 1 and 2 months of treatment. In these patients, the genotype–blood pressure relationship was not influenced by a previous therapy, different phases of hypertension (because they were all in a relatively early hypertensive phase), and the variety of counter-regulatory mechanisms that come into play with sudden therapy withdrawal. In fact, even after 1 month from therapy withdrawal, the renin response to a standard dose of diuretic is still different from that observed in the never-treated hypertensive status.74 Psaty et al46 reviewed the data of 1038 hypertensives followed for several years. They showed a selective advantage of diuretics in preventing myocardial infarction (MI) and stroke (almost halved by this treatment) over other antihypertensive therapies that produced similar blood pressure levels in carriers of the ADD1 W allele but not in homozygous for the ADD1 G allele.

Adducin and Erythrocyte Ion Handling in Human

Erythrocyte sodium content was found to be lower, whereas Na-K-Cl cotransport, Na-H countertransport, and Na-K pump are faster in carriers of the ADD1 W allele than in homozygotes for the G allele.16 The Na-K pump and Na-K-Cl cotransport characteristics are also associated to lower plasma renin levels and to a greater blood pressure fall after diuretics, whereas Na-Li countertransport and erythrocyte sodium content associate, respectively, only with plasma renin or the diuretics effect. However, according to Grant et al,41 erythrocyte sodium content and Na-Li countertransport are lower in W/W carriers compared with the other ADD1 genotypes. The concordance of intracellular sodium and the discordance of Na-Li countertransport are not easy to explain. The only clear-cut difference between these 2 studies is that the former was performed on never-treated hypertensive patients, whereas in the latter, the therapy was withdrawn 4 weeks before the measurements. This period is shorter than the erythrocyte life span, and Na-Li countertransport may be affected by therapy or dietary sodium variation.75 Increased cell osmotic fragility has been described in MHS9 and in β-adducin–null mice, together with other erythrocyte abnormalities.76 In men who consume alcohol, the β-adducin polymorphism is associated with decreased values of red blood cell count, hemoglobin concentration, and hematocrit.30

To conclude, in 18 of 20 association studies, an influence of ADD1 polymorphism on the considered variables and basal blood pressure of low-renin hypertensives has been demonstrated.

Adducin Studies in Families

In families, transmission of ADD1-, ADD2-, or ADD3-mutated alleles, alone or in combination with other alleles, or of the angiotensin-converting enzyme (ACE) D alleles in ADD1-mutated allele carriers, was associated to an increase in blood or pulse pressure.33,34,36,48

Two additional studies15,37 measuring the shared alleles of a marker mapping at 30 kb from the ADD1 locus in sibling pairs furnished strong evidence for linkage with systolic blood pressure (P<0.001) or hypertension (P=0.0006). The other 4 linkage studies with DNA markers mapping at a distance >440 kb failed to find any linkage.37,50–52

Recent data (Science 2002, www.hapmap.org)77 show that between the ADD1 locus and the D4S43 and D4S126 markers used by Niu et al,50 which map above 570 kb from the ADD1 locus, there are 9 and 14 haplotype blocks, respectively. Therefore, the clinical relevance of negative linkage studies using markers at such a distance from the ADD1 locus is of limited value.

Studies on Normotensives and in Predominantly Normotensive General Populations

As discussed previously,37 none of the 5 studies performed in normotensive populations found an association between the adducin genotypes or alleles and blood pressure. These findings are in keeping with the data on pressure natriuresis. The cellular effects of adducin, described above, are addressed to blunt the compensatory increase in sodium excretion when the renal perfusion pressure rises.

Nine studies dealing with predominantly normotensive general populations have been published so far.25,29,32,33,37 These populations also contain hypertensives, but in 2 negative studies,37 these patients were removed from the analysis. Moreover, in other 2 negative studies,37 the age of the subjects was <30 years.

Of the remaining 5 studies, 1 showed that ADD1 W allele is more frequent in hypertensive subjects,39 and 215,29 showed that ADD1 polymorphism alone is not associated to blood pressure levels, but in 1,25 it interacts with the ACE I/D polymorphism and aldosterone synthase on the incidence of hypertension during a follow-up study of 9.1 years on a population initially normotensive.25 In the other study,29 a significant interaction between ADD1 and ADD2 poly-
morphism on blood pressure was found in postmenopausal women. The other studies evaluated the influence of ADD2 polymorphism alone in 3 populations. In the 2 populations with high salt intake (241 and 206 mmol/L per day), the mutated ADD2 T allele is associated to a higher blood pressure. No association is found in the population at a lower salt intake. Interestingly, in all 3 populations, carriers of the mutated ADD2 T allele have a lower sodium excretion than the carriers of the wild allele. On the assumption that all the subjects were in dietary balance and each population had similar lifestyle factors, this observation may indicate a lower salt intake, probably because of a decreased sodium appetite.

Classical Association Studies Comparing Normotensive and Hypertensive Adult Subjects

Among the many confounding factors that may weaken the significance of these studies, the age of the control normotensive group is crucial because young normotensive subjects used as controls may develop hypertension later in their life. A minimum age of 40 years is generally accepted, but an age of 60 should be better. Nineteen studies have been published with the average age of a control group ranging from 44 to 72 years on a total of 23 populations. Association was present in 11 populations and absent in 12 populations. It should be noted that in 2 studies, positive and negative associations were observed in different populations. The proportion between positive and negative studies is similar to that discussed in our previous review. In 1 of the negative populations that we blindly genotyped, hypertensives with and without the mutated ADD1 W allele were compared. The W allele carriers have lower salt intake. Interestingly, in all 3 populations, the proportion between positive and negative studies is similar to that discussed in our previous review.

In 1 of the negative populations that we blindly genotyped, hypertensives with and without the mutated ADD1 W allele were compared. The W allele carriers have lower plasma renin and larger blood pressure fall, with diuretics associated to the erythrocyte abnormalities sodium handling discussed above. This clearly suggests that in spite of the absence of a protective association with blood pressure, carriers of the ADD1 W allele display the expected characteristics of cellular and renal body fluid regulation.

Cardiovascular and Renal Diseases

Many studies investigated the association between cardiovascular or renal disease and the ADD1 W allele, but most of them had a cross-sectional or retrospective design, which makes the interpretation of the results extremely difficult. Two studies on cerebrovascular disease revealed association with the ADD1 W allele for hemorrhagic stroke considered alone or in combination with ischemic stroke. When only ischemic stroke was considered, no association was found.

Coronary Heart Disease or MI

Two of 3 reports demonstrated an association between coronary events and the ADD1 W allele in hypertensive patients, whereas in a fourth study, a lower frequency of the W allele was reported in survivors of a MI aged <75 years compared with controls of the same age. The latter findings might be attributable to the premature death of high-risk W allele carriers or to a protective effect of this allele.

Winnicki et al described an association between left ventricular hypertrophy and the ADD1 W/W genotype and cardiac hypertrophy.

A Belgian population study demonstrated that intima-media thickness of the muscular femoral artery, but not of the elastic carotid artery, increases with the number of ACE D alleles. However, the effect of the ACE genotype on femoral intima-media thickness was confined to carriers of the ADD1 W allele or CYP11B2 -344T alleles. An interaction between the ACE and ADD1 polymorphism was also found in relation to femoral artery stiffness.

Renal Diseases or Abnormalities

In 2 studies on polycystic renal disease (autosomal dominant polycystic kidney disease), the frequency of the ADD1 W allele did not differ among small (<100 patients) subgroups with varying duration of renal replacement therapy. We cannot interpret a third negative study because the number of patients belonging to each genotype was not reported. Nicod et al found that the average time lag between diagnosis and end-stage renal failure in 260 patients with nephropathy of various origins was 11.6 and 4.6 years (P<0.003) in ADD1 GG and ADD1 WW homozygotes, respectively. Other investigators reported an interaction between ADD1 GW and ACE ID polymorphisms in the progression of renal failure.

Role of ADD3 Polymorphisms

In most tissues involved in cardiovascular homeostasis (kidney, brain, heart, and vessels), adducin is expressed as a heterodimeric protein consisting of α- and γ-subunits. Therefore, this protein structure justifies the search for epistatic interactions between ADD1 and ADD3. We found a missense mutation (Q572K) in Add3 in MHS as well as in spontaneously hypertensive rats (SHR). Taken alone, this mutation is not associated with hypertension in the cosegregating MHS×MNS F2 population. However, the Add3 mutation epistatically interacts with the Add1 polymorphism in relation to the blood pressure level in the F2 population. Compared with Wistar-Kyoto rats, Yang et al found a 22% decrease in the Add3 expression in the brain stem of adult SHR. In cultured neurons, these investigators even highlighted a consistent and more pronounced (60%) reduction in the protein level. Further experiments demonstrated that inhibition of Add3 by intracellular delivery of Add3-specific antibodies increases the neuronal firing rate to a similar extent as angiotensin II. However, the effects of these 2 interventions are not additive. Furthermore, administration of angiotensin II and other manipulations that increase blood pressure reduce the Add3 content in the brain stem. Based on these findings, the hypothesis has been put forward that a reduction in the Add3 expression might favor, or mediate, the increased
basal firing rate of neurons in cardiovascular regulatory brain areas as observed in SHR.

As in SHR, Add3 expression is also reduced in the same brain areas in MHS (G. Tripodi G. personal communication, 2004). This suggests that missense Add3 mutations might be associated with alterations in protein expression and neuronal firing rate in MHS as well as SHR.

We also found an epistatic interaction between ADD1 and ADD3 in never-treated hypertensive patients\(^8\) and in European populations in relation to pulse pressure.\(^6\) In the latter study, the association was consistent in family-based analyses, which made use of the quantitative transmission disequilibrium test.

**Relationship Between Adducin and Endogenous Ouabain**

In MHS, the increased renal interstitial pressure normalizes as hypertension develops.\(^4\) In line with Blaustein’s\(^8\) hypothesis, we speculated that an endogenous ouabain (EO)–like substance via inhibition of the Na-K pump might limit the tubular sodium reabsorption and, hence, reduce renal interstitial pressure, on the other hand, facilitating peripheral vasoconstriction. We demonstrated that EO levels in plasma and urine and in the hypothalamus are higher in MHS than MNS.\(^8\) In congenic normotensive rats carrying the MHS Add1 locus, a low-salt diet increases the plasma EO level, unlike in the control MNS.\(^8\) In isolated tubular cells, the long-term incubation with subnanomolar ouabain concentrations increases the Na-K pump expression on the cell membrane,\(^8\) similarly to what occurs when the same cells are transfected with the mutated MHS Add1.\(^17\) The mechanisms underlying this intriguing parallelism between the effects of mutated adducin and ouabain remain to be elucidated.

In a Belgian population study,\(^31\) plasma EO correlates independently and positively with male gender, smoking, urinary potassium excretion, and mutation and the ADD1 GW polymorphism. Before and after adjustment for covariables, continuous as well as categorical analyses revealed a significant interaction between plasma EO and urinary sodium excretion (mean 194 mmol/L per day) in relation to blood pressure. In individuals with plasma EO values below the median value, blood pressure increases by 2.2 mm Hg systolic and 1.4 mm Hg diastolic for each 50 mmol/L per day increment in urinary sodium excretion. No blood pressure increase with sodium was found when plasma EO exceeds the median. We also found that plasma EO concentration increases on a low-sodium diet or on treatment with diuretics.\(^8\) Together, EO seems to behave as a blood pressure–modulating factor, possibly released in response to potassium or sodium, either inhibiting the pressor effect of an excessive salt intake or counteracting the depressor action of sodium depletion. The mechanisms by which mutations in the adducin genes affect the interaction between plasma EO concentration and urinary sodium excretion in relation to blood pressure remain to be clarified.

**Limits and Perspectives**

Although the evidence that relates mutations in the adducin genes with hypertension and other cardiovascular renal disorders is overwhelming, space constraints and the rather small number of published results limit the discussion on the following important issues that are currently under investigation: (1) the effect of interaction among the 3 adducin subunits at molecular, cellular, renal, and blood pressure levels. Because adducin functions in the cell as a heterodimer composed of these subunits, the biological effect of adducin genes must be clearly assessed on the heterodimer; (2) the composition of the adducin heterodimer in the different tissues with possible tissue-specific splicing isoforms; (3) the molecular mechanism through which the rat and human adducin variants affects the Na-K pump endocytosis; (4) the haplotype blocks and the various splicing isoforms of the 3 adducin subunits; (5) the interaction of adducin with hormones regulating body sodium, and, particularly, with EO, which, as adducin, interacts with the Na-K pump; (6) congenic rats with different combinations of the DNA segments containing the 3 adducin loci; (7) mice with knockout of the Add1 gene; and (8) the effect of adducin on vascular tone and vascular Na-K pump.

**Conclusions**

The association between adducin polymorphism and hypertension has been found positive in all 6 linkage studies in which stringent methodological criteria have been applied. Positive associations were also detected in 18 of 20 studies in which, besides blood pressure, also the variables reflecting body sodium or RAS status have been taken into account. The consistency among these positive results and those on the action mechanism of adducin in the MHS model contrasts with the mixed results obtained in human studies in which the variables mentioned above were not considered. The mixed results of human studies may also be attributable to the opposite cardiovascular and renal effects of ADD1 W according to the ACE I/D polymorphism. In fact, compared with the population mean values, in ADD1 W carriers, the incidence of hypertension,\(^25\) the thickness of the femoral arterial wall,\(^28\) the increase of blood pressure after saline infusion,\(^23\) and the plasma creatinine and urinary proteins\(^26\) are all higher in the ACE D/D carriers and lower in ACE I/I carriers. Epistatic interactions between ADD1 and ACE variants are ignored in most of the case-control and population studies. Therefore, the negative findings in these settings are not surprising.

The existence of gene modifiers (either enhancing or blunting the “pathological” effect of another gene variant) is a well-established fact in monogenic diseases\(^86,87\) and applies also to genes affecting renal sodium handling and blood pressure.\(^88\) Indeed, it is unlikely that a single gene polymorphism underlies a very heterogeneous syndrome such as primary hypertension, affecting up to 40% of the adult population of industrialized countries. Variation in the intrarenal formation of angiotensin II associated to ACE D/D allele may produce a synergistic effect with the ADD1 W allele on renal sodium excretion. Indeed, carriers of these allele combinations, compared with carriers of the W allele alone, have a more marked decrease of plasma renin for any level of sodium intake\(^23,34\) associated to a larger increase of blood pressure during a saline load,\(^23\) a larger femoral intima-media thickness with a consequent variation in arterial stiffness,\(^28,35\) a lower renal...
function with a larger increase in urine protein excretion for the same phase of hypertension or age, and a greater fall in blood pressure with diuretics. The ACE/D allele seems to potentiate the clinical impact of ADD1 W allele. Clearly, if confirmed, these characteristics ranging from ethiopathogenetic mechanisms to clinical profiles and response to therapy support the proposal of a new clinical entity. This proposal is strengthened by the consistency among data collected in a variety of animal and human contexts, but it is weakened by the lack of appropriate longitudinal studies in an adequate sample size of patients, in which all these variables are measured in the same subject.

A practical way to “measure” the overall clinical impact of the ADD1 W allele, and then to estimate the size of the population that may be affected by this genetic mechanism, is to apply a very selective pharmacological tool able to interfere with the sequence of events 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 blood pressure and preventing MI and stroke in carriers of the ADD1 W allele might be even greater if drugs interfering with adducin but devoid of the well-known side effects of diuretics are developed.

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