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Minireview

# Adducin in essential hypertension

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**Abstract In Milan hypertensive rats (MHS) the sequence of events going from renal function to cell membrane ion transport abnormalities and finally to the molecular defect responsible of hypertension has been established. A polymorphism of the cytoskeletal protein adducin has been identified as a likely culprit for hypertension in these rats. Two point mutations in MHS α- (F316Y) and β- (Q529R) adducin genes have been shown to be associated with hypertension in genetic crosses of MHS and MNS rats.**

**Also in humans, a polymorphism of α-adducin gene (Gly460Trp) has been found to be significantly associated both to hypertension and salt sensitivity.**

**Studies aimed at clarifying the functional role of α-adducin variants have shown that adducin from the MHS rats is able to stimulate Na-KATPase activity both after transfection in renal tubular cells and after incubation with the enzyme in a cell-free system. Also the human hypertensive α-adducin variant displays the same activity of MHS adducin in a cell-free system.**

**Therefore, both in humans and in rats, adducin polymorphisms may affect blood pressure and kidney function by modulating the overall capacity of tubular epithelial cells to transport ions, through variations of the Na-KATPase activity.**

**However adducin polymorphisms account for only a portion of hypertension both in humans and rats. Therefore additive or epistatic interactions with other genes involved in renal sodium handling need to be studied.**



*Key words:* Cytoskeleton; High blood pressure; Na-KATPase; Genetic

## 1. Introduction

Hypertension is a significant risk factor for heart attack and stroke, and represents a major public health burden because of its high prevalence (e.g. 15–20% of the European and American populations). Although blood pressure is known to have a strong genetic determination, the genes responsible for susceptibility to essential hypertension are mostly unknown. From studies in human and animal models it is clear that several genetic loci are involved in regulation of blood pressure and hypertension [1]. The Milan hypertensive rats (MHS) and its control normotensive strain (MNS) have been validated as an appropriate model for at least a subgroup of patients with primary hypertension [2]. In these animals, the complex trait hypertension has been dissected from the primary organ abnormality to the molecular abnormality (candidate gene) through the following steps: (1) Kidney transplantation experiments provided the major and more consistent evidences that the kidney plays a causal role in the pathogenesis of rat genetic hypertension [3,4]. The pressor role of the kidney has also been demonstrated in humans [5,6]. (2) Na transport is faster [7,8] and cell volume is lower both in erythrocytes [9] and in renal tubular MHS cells than in the corresponding MNS cells [10]. (3) Erythrocyte functional differences are genetically determined within the stem cells and are genetically associated to hypertension in F2 hybrids [11]. (4) The difference in cell membrane ion transport disappears after removal of the membrane skeleton, suggesting the involvement of some of its components [12]. (5) Cross immunization between MHS and MNS with cytoskeletal extracts of the other strain were then performed and yielded to the production of antibodies against a protein subsequently identified as adducin [13].

The scope of this review is to briefly summarise: (1) The biochemical function of adducin; (2) the association of α-adducin to hypertension; (3) the relation between α-adducin polymorphisms and renal sodium handling in essential hypertension.

## 2. Biochemical functions of adducin

Adducin is a heterodimeric protein that consists of α, β and γ similar subunits. Adducin subunits have structurally distinct domains: an $NH_2$ terminal globular head (40 kDa), an 8 kDa neck domain and a COOH terminal domain, which is responsible for the association of actin and spectrin complexes and contains sites of phosphorylation [14,15].

Adducin promotes the binding of spectrin with actin and also directly binds actin and bundles actin filaments. It is present in many tissues and within regions of cell-cell contacts [16]. Its role is believed to be the regulation of the assembly of spectrin and actin [17]. As a consequence adducin can modulate the lattice structure of the cytoskeleton and the exposure of transmembrane proteins. Adducin can move from the cytoplasm to cell to cell contact sites under the control of intracellular $Ca^{2+}$ and phosphorylation.

One of the functions of actin-spectrin-based membrane skeleton is to organize certain integral membrane proteins and to couple them to cytoplasmic proteins [18]. Spectrin molecules are linked in a polygonal array, the vertices of which are formed by short actin filaments [19]. At these junctions, accessory proteins are found including adducin. In the cell, the interaction between cytoskeletal protein network and integral membrane proteins is fundamental for several functions such as maintenance of cell polarity [20,21] and regulation of ion transports [22,23]. It has been demonstrated that the actin-based cytoskeleton interacts with the band 3-anion

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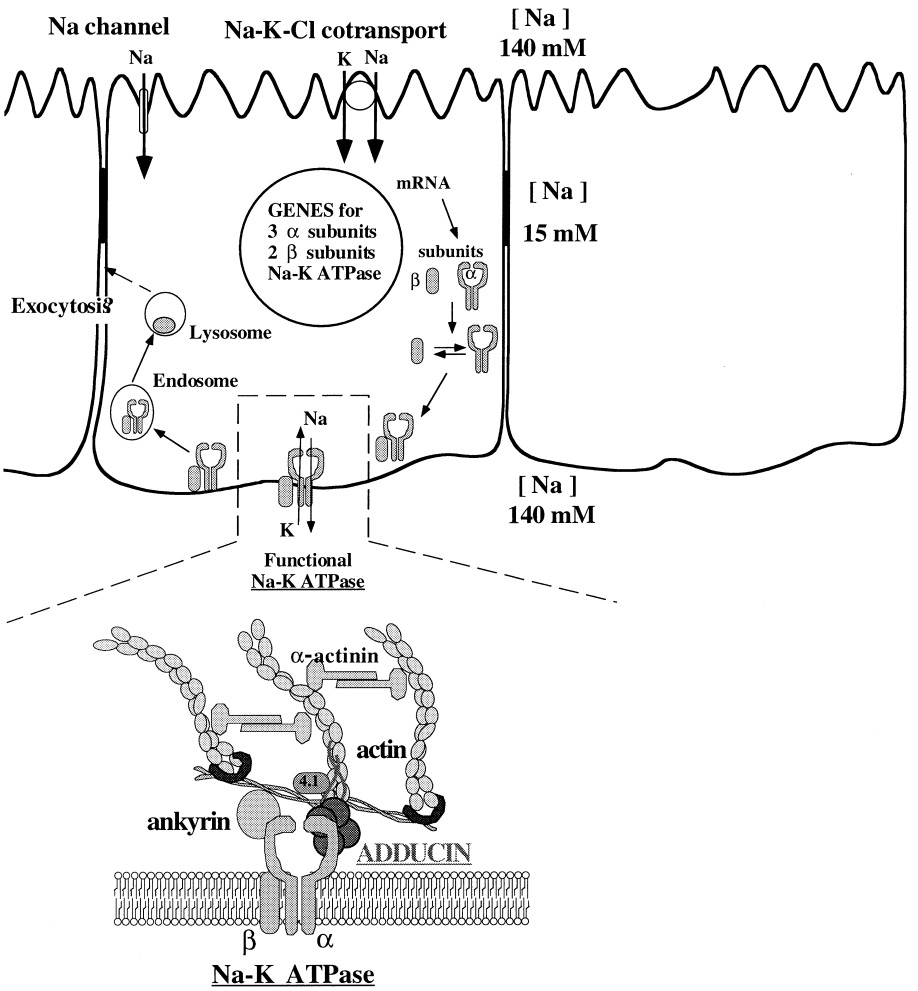


Fig. 1. A schematic model of adducin interaction with cytoskeleton and membrane protein in renal tubular cell. The cytoskeleton protein adducin is shown associated with actin filaments and a subunit of the Na-KATPase. The barbed ends of actin filaments are associated to spectrin filaments and other constituents of membrane skeleton as talin, fodrin and integrin. The Na-K pump is responsible of the Na gradient across the basolateral membrane which, in turn, allows the entrance of Na across the luminal membrane. Adducin polymorphisms may affect Na transport by modulating the Na-K pump.

exchanger [24], the epithelial Na channels [22], the Na-K-Cl cotransport [25] and the Na-KATPase [26,27].

MHS and MNS rats differ at the molecular level for two missense mutations in α (F316Y) and β (Q529R) subunits of adducin [28]. These polymorphisms are associated with blood pressure variation in the Milan rats [25], and they account for up to 50% of the total blood pressure difference between the two strains. Transfection experiments in rat kidney epithelial cells (NRK-52E) with MHS and MNS rat adducin cDNA have clearly demonstrated the functional role of adducin polymorphisms [29]. Cells transfected with MHS adducin show: (1) a significant increase in Na-K pump activity at $V_{max}$ and of Na-K pump units compared with cells carrying the MNS adducin [29]. (2) Studies in a cell-free system of the interaction between actin and normal or mutated adducin [29] have shown that mutated adducin leads to a higher final level of filamentous actin. Moreover actin bundling is favored by MHS adducin. Therefore the mutated protein interferes with a cellular fundamental biological function, that is the ability of actin monomers to polymerize into filaments.

Recently a series of evidences have further supported the concept of a functional interaction between adducin and the Na-K pump: (1) When compared to their MNS controls, MHS rats show an increased enzymatic activity of the outer medulla Na-KATPase. This increase precedes and follows the development of hypertension and it is associated to a higher number of functionally active pump sites on the cell membrane surface and to higher cellular levels of the complementary mRNA and protein [30]. Therefore, in vivo, renal Na-KATPase is up-regulated in MHS at all ages. (2) Experiments in a cell-free system have demonstrated that adducin purified from rat erythrocytes stimulates Na-KATPase activity [31]. This effect seems to be specific since it occurs at nanomolar concentrations, it is retained by a restricted portion of the COOH-terminal tail of 200 amino acids and is lost when the tail fragment is reduced to 31 amino acids or when native adducin is extensively proteolyzed. Moreover this modulation is related to the genetic variants of both α (F316Y) and β (Q529R) adducins, with a higher affinity for MHS adducin compared to the MNS one [31]. (3) Adducin and Na-KATPase co-precipitate both in a cell-free system [31] and in a cell system [32], using monoclonal antibodies either to adducin or to Na-KATPase. (4) Na-KATPase and adducin are co-local-

ized exclusively at the basolateral side of renal tubules [31] (Fig. 1).

Preliminary experiments have shown that also human α-adducin polymorphism is able to differently modulate the activity of rat purified Na-KATPase [31].

### 3. α-Adducin polymorphisms in human essential hypertension

The Milan hypertensive strain has been validated as an appropriate model for at least a subgroup of patients with primary hypertension. For this reason and since adducin gene has a very high homology (about 94%) in rat and man we tested if α-adducin polymorphism could also affect blood pressure level in humans. Three different studies were performed: (1) Four highly polymorphic markers, mapping at different distances from the α-adducin locus (4p16.3), were studied in a case-control study on the assumption that possible functional mutations could be in linkage disequilibrium with the markers mapping in close proximity to the α-adducin locus [33]; (2) linkage of these markers to hypertension in affected sib-pairs [34]; (3) variants of human α-adducin gene were identified and studied in normotensive and hypertensive populations [34].

The study with four polymorphic multiallelic markers showed that the marker mapping closest to the α-adducin locus (20 kb) was highly significantly associated to hypertension. The association with the other markers decreased with increasing distance from the α-adducin locus. Similarly, in the second study [34], a significant linkage was detected for all markers in affected sib-pairs, and the percentage of shared alleles decreased with increasing distance of the markers from the α-adducin locus. The third study demonstrated a linkage disequilibrium between a new α-adducin polymorphism (Gly460Trp) and hypertension in two Caucasian populations. The Gly460Trp polymorphism was tested in a case-control study in an Italian and in a French population. A significant association of the mutated allele (Trp) with hypertension was shown (20.7% in hypertensives vs. 13.6% in normotensives; $P = 0.001$). Recently the Gly460Trp variant has been found associated to hypertension also in a Japanese case-control study [35]. The frequency of the 460Trp allele is much higher in this study than that reported in our study on European populations (65.6% in Japanese hypertensives vs. 52.9% in Japanese normotensives, $P = 0.0087$). Moreover, in a sample of Italian general population the relationship of this polymorphism with blood pressure and cardiovascular structure has been investigated [36]. A trend towards higher blood pressure values was observed in patients carrying the 460Trp allele. These studies demonstrate a significant linkage of the α-adducin locus to hypertension in different population studies.

### 4. α-Adducin polymorphism and renal sodium handling

As discussed above, α-adducin polymorphism affects the activity of the Na-KATPase. These data provide the genetic molecular basis for a type of primary hypertension caused by a faster renal tubular Na reabsorption, in view of the key role of this enzyme in transtubular Na transport. Based on this hypothesis, we investigated the effect of α-adducin polymorphism on blood pressure response to acute salt sensitivity test, according to Weinberger's protocol [37], in hypertensive patients. Blood pressure variation after sodium loading and depletion was greater in heterozygotes Gly460Trp than in wild-type homozygotes. 88% of the heterozygotes were salt sensitive compared to 41% of the homozygotes. In spite of similar sodium intake the heterozygotes had significantly lower basal plasma renin activity [34]. In another group of hypertensive patients we tested the blood pressure response to chronic diuretic treatment [34]. Blood pressure decrease was about double in heterozygotes than in wild-type homozygotes. The relative risk of being salt sensitive or responder to the chronic diuretic treatment was respectively 9 and 4.8 for patients bearing the 460Trp allele. We also evaluated the pressure-natriuresis relationship during acute variation of body sodium [38]. The slopes obtained were significantly different according to the genotype (Gly/Trp $0.029 \pm 0.004$ vs. Gly/Gly $0.007 \pm 0.002$ mm Hg/mmol/min, $P = 0.0001$). Fig. 2 shows the change in mean blood pressure (MAP) going from volume depletion to volume load in 28 Gly/Trp and 80 Gly/Gly patients. For the same degree of variation in body sodium, the magnitude of blood pressure changes is greater in Gly/Trp than in Gly/Gly patients clearly demonstrating that the former have a regulation of blood pressure that is very sensitive to variations in total body sodium. Moreover, patients bearing the 460Trp allele had significantly lower plasma renin activity. Also in the Japanese study [35] the 460Trp allele is significantly associated to a lower plasma renin activity. These findings suggest that in humans adducin is associated to hypertension, seems to modulate sodium reabsorption and could be involved in a low renin hypertension with normal potassium and aldosterone, indistinguishable from 'essential' hypertension.

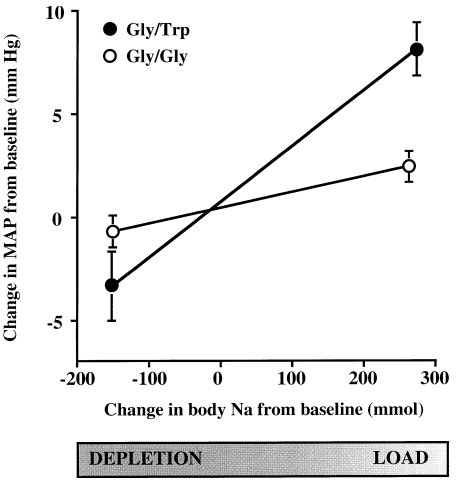


Fig. 2. Changes in mean arterial pressure (MAP) and changes in body sodium from baseline during sodium depletion or load in 28 Gly/Trp (closed circle) and 80 Gly/Gly (open circle) hypertensive patients. The changes in MAP were calculated from the baseline of each test. Body sodium was calculated either by subtracting the sodium excretion from the sodium load or simply by subtracting the amount of sodium excretion after furosemide. For the same degree of variation in body sodium, the magnitude of blood pressure changes is greater in Gly/Trp than in Gly/Gly patients ($P = 0.0001$) (from [38] with some modifications).

Since essential hypertension is a complex genetic trait, we tested if this peculiar modulation of renal sodium handling shown by the Gly460Trp patients could be determined also by the synergistic effect between α-adducin and some other gene involved in sodium homeostasis. Preliminary data show that α-adducin and angiotensin converting enzymes have a

synergistic effect in causing blood pressure changes following acute modifications of Na balance [39].

## 5. Conclusions

The findings discussed in this review demonstrate that: (1) Adducin mutations and blood pressure levels are associated both in rats and humans. (2) The affinity for the Na-K pump of the 'hypertensive' adducin variant is greater than that of the 'normotensive' one in both species. (3) A pathophysiological link exists between the adducin variants and the events leading to hypertension. To our knowledge the adducin polymorphism is the first genetic mechanism shown to be relevant in blood pressure regulation of both rats and humans.

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