α-Adducin 460Trp Allele Is Associated With Erythrocyte Na Transport Rate in North Sardinian Primary Hypertensives

Nicola Glorioso, Fabiana Filigheddu, Daniele Cusi, Chiara Troffa, Milena Conti, Marcella Natalizio, Giuseppe Argiolas, Cristina Barlassina, Giuseppe Bianchi

Abstract—Erythrocyte membrane alterations mirror those of vascular smooth muscle and renal tubular cell membrane. The interaction between adducin and Na-K pump is the most likely biochemical mechanism responsible for the increased tubular Na reabsorption and hypertension in Milan hypertensive strain (MHS) rats. To substantiate this hypothesis in humans, we tested to see if α-adducin Gly460Trp genotype is associated with erythrocyte sodium transport rate in a new cohort of n=268 never-treated North Sardinian primary hypertensives. Plasma renin activity and blood pressure response to hydrochlorothiazide were also measured to evaluate the relationship between sodium transport rate and two intermediate phenotypes with a higher degree of genetic complexity. Na-K pump, Na-K-Cl cotransport, and Li-Na countertransport at V_{max} were faster (P<0.0001), whereas intracellular Na concentration was lower (P<0.0001) in patients carrying one or two 460Trp alleles. Such behavior was mirrored by opposite changes of intracellular Na concentration. Plasma renin activity and blood pressure response to diuretic treatment, on the other hand, showed a weaker association with the sodium transport rate. In conclusion, our findings are consistent with the hypothesis that the Gly460Trp α-adducin polymorphism may affect renal Na handling through an alteration in ion transport across the cell membrane mirrored by erythrocytes. These results may also have clinical relevance because the Gly460Trp α-adducin polymorphism may explain, at least in part, the variability of blood pressure response to diuretics in primary hypertensive patients. (Hypertension. 2002;39[part 2]:357-362.)

Key Words: hypertension, essential • sodium pump • erythrocytes • genetics • diuretics

Abnormalities of erythrocyte ion transport across the cell membrane in primary hypertension1−3, together with data on vascular and renal tubular cells in experimental hypertension, provided the basis to hypothesize an important role of Na transport in primary-polygenic hypertension.4,5 Erythrocyte membrane alterations mirror those of vascular smooth muscle and tubular cell membrane.6,7 Starting from the erythrocyte membrane, polymorphisms in the adducin and G protein β3 subunit genes were identified, and their involvement in primary hypertension extensively studied.8,9 Adducin is a heterodimeric protein composed by α, β, and γ subunits coded by genes mapped on different chromosomes. The α subunit is present in almost all types of cells, whereas the presence of the β and γ subunit varies in different tissues. Adducin is involved in cell signal transduction, regulation of actin cytoskeleton, and ion transport across the cell membrane.10 The Gly460Trp missense mutation was found in human α-adducin, and the 460Trp allele was associated with (1) primary hypertension, with some contrasting results,11,12 (2) faster proximal tubular reabsorption,13 and (3) a greater fall in blood pressure (BP) after diuretic treatment and lower plasma renin activity (PRA). The association of the 460Trp allele with a greater response of BP to diuretic treatment and with lower PRA was found even in North Sardinian primary hypertensives in whom the 460Trp allele was not associated with hypertension.14

Trp α-adducin variant displays a greater binding affinity to Na-K pump when compared with the Gly460 allele in a cell free system.15 This functional alteration of the human 460Trp allele is similar to that shown in rats where the MHS 316Tyr allele showed a higher binding affinity to Na-K pump than the MNS 316 Phe.16 Moreover, transfection studies in tubular cells showed that Milan hypertensive strain (MHS) α-adducin cDNA increased Na-K pump cell surface expression and activity, the driving mechanism of tubular Na reabsorption.10 Therefore, we proposed that adducin could affect Na transport rate by interacting directly with the Na-K pump and that its variants associated with hypertension in some cohorts could contribute to the increased tubular Na reabsorption observed in a subset of primary hypertensives,13 thus explain-
ing the lower PRA and the better therapeutic response to diuretics.

To substantiate this hypothesis in humans, we tested to see whether Gly460Trp α-α-adducin polymorphism is associated with erythrocyte sodium transport rate in never-treated primary hypertensives. PRA and BP response to thiazide (HCTZ) were also measured to evaluate the association between these 2 intermediate phenotypes with high degree of genetic complexity and the Na transport rate.

Methods

Patients

The study cohort comprised 337 mild-to-severe primary hypertensive patients enrolled at the Hypertension Center, University of Sassari Medical School, Sassari, Italy. The study protocol was approved by the local ethical committee. All patients were born in domains of North Sardinia previously demonstrated to have a high degree of genetic homogeneity, and all resided in Sardinia. None of these patients were included in previous studies.

During an 8-week run-in on their usual diet, patients underwent a complete diagnostic workout to exclude secondary hypertension. Twenty-four hour urine Na excretion and body mass index (BMI) were measured every 2 weeks. Seated systolic (SBP) and diastolic blood pressure (DBP), as well as heart rate, were assessed weekly from week 8 through week 0 (average of 3 measurements on each occasion at 2-minute intervals, made by the same nurse in a quiet room; automated electronic device, Hestia). The values recorded at the end of week 0 were taken as a baseline. At this time 24-hour urine and 25 mL of venous blood were collected to measure Na, K (flame photometry), aldosterone, PRA (Ria), erythrocyte cation transports (see below), creatinine, and lipids (autoanalyzer). White blood cell DNA was used to determine α-adducin genotype (see below).

Sixty-six patients were not included in the study for the following reasons: 23 showed a 24-hour urinary Na excretion 25% higher than the average of the first 2 urinary excretions; 11, because of erratic erythrocyte cation transport readings due to hemoglobin <12 g/dL and MCV <80 fL; 3, because of plasma potassium <3.5 mEq/L; 17, because of symptoms related to high blood pressure, such as persistent headaches requiring immediate treatment; 12, because of BP normalization; and 3, because of diabetes mellitus. Thus, 268 patients were actually included in the study and started HCTZ 25 mg daily for 4 more weeks. BP, heart rate, BMI, 24-hour urinary Na, and side effects were monitored at weeks 2, 4, and 8. The personnel handling patients or laboratory samples were unaware of patients’ genotype.

Erythrocyte Ion Transport Systems

Intraerythrocyte sodium concentration ([Na] i) and the rate constant of Na passive permeability (PP Na) were measured in fresh, unloaded cells. Na efflux mediated by Na-K pump (Na-K PUMP) and Na-K-Cl cotransport (Na-K COT) were measured in Na- and choline-loaded erythrocytes. Li efflux mediated by Li-Na countertransport (Li-Na CNT) was measured in Li-loaded erythrocytes as described.

Genotyping

The Gly460Trp polymorphism was investigated by PCR amplification of genomic DNA followed by allele specific oligonucleotide hybridization as described.

Statistical Analysis

Comparisons of the response to treatment as well as of erythrocyte Na transport rate among the three α-adducin genotypes were performed by one-way analysis of variance, with post hoc Student-Newman-Keuls (S-N-K) multiple comparison test. Differences attributable to possible confounders such as age, BMI, sex, and serum lipids were entered as covariates in the model. Except for Na-K pump, erythrocyte transport systems and [Na], were normally distributed (Kolmogorov- Smirnov test z=0.10 for [Na]; 1.12 for Na-K COT and 1.21 for Li-Na CNT). The distribution for the Na-K pump was significantly skewed to the right (z=1.72, P=0.005), but it became normal after logarithmic transformation (z=0.91, P=0.37). The cohort was then divided into 3 groups by tertiles of Na-K pump, Na-K COT, Li-Na CNT, PP Na, and [Na], BP changes after treatment, as well as basal PRA, were analyzed by tertile of all the erythrocyte transport systems. Two-tailed values of P<0.05 were taken as statistically significant. SPSS 10™ for Macintosh™ was used.

Results

Baseline values of the 268 patients who completed the study are reported in Table 1 as a whole and divided according to α-adducin genotype. No significant differences were found in the major pretreatment parameters except for circulating cholesterol. Although no substantial differences were found in possible confounders such as age, BMI, sex, and serum lipids according to the α-adducin genotype, they were entered in the analysis as covariates. Indeed, a slightly higher serum cholesterol concentration was seen in Gly460Trp patients (Table 1). We tend to interpret this as a chance finding in consideration of the multiple comparisons performed. Whatever the case, these slightly higher values of circulating cholesterol (not significantly higher than Gly460Gly or Trp460Trp with S-N-K test) are of doubtful biological significance.

The α-adducin polymorphism strongly affected erythrocyte cation transport rates: Na transport rate increased progressively and significantly in patients having from 0 to 2 gly460trp alleles, whereas this did not affect PP Na. The statistics indicated an additive effect of the 460Trp allele (statistics of the model in Table 1; statistics of S-N-K post hoc test summarized in Figure 1). The α-adducin Gly460Trp polymorphism alone explained 18.3%, 15.1%, 13.0%, and 19.0% of the variance of the Na-K PUMP, Na-K COT, Li-Na CNT and [Na], respectively. Consistently, this faster transport rate is accompanied by a decrease of [Na], (Figure 1).

Confirming previous studies, pretreatment PRA was lower, and the fall in mean blood pressure (MBP) after 8 weeks of diuretic treatment was greater in individuals carrying at least one 460Trp allele. In this case, the 460Trp allele appeared dominant because no significantly greater effect was seen when individuals homozygous for the 460Trp allele were compared with the heterozygous ones (S-N-K test; Gly460Gly versus Gly460Trp and Gly460Gly versus Trp460Trp, P<0.005) for MBP decrease and P<0.0001 for pretreatment PRA. No significant differences were found between Gly460Trp and Trp460Trp: P>0.25 in either case for both MBP decrease and pretreatment PRA. Basal PRA was lower, and the MBP decrease after treatment was greater, moving from the lower to the higher tertile of all the transport systems. Accordingly, such behavior was mirrored by opposite changes of [Na], although the analysis did not always reach the statistical significance (Table 2). The analysis for PP Na did not show significant results.
Discussion

Our data show an association of the Gly460Trp α-adducin polymorphism with erythrocyte sodium transport rate in a large cohort of never-treated North Sardinian primary hypertensive patients who were relatively homogeneous from a genetic viewpoint. These findings help to explain the genetics of the previously shown 18, 19 erythrocyte abnormalities of Na-K pump, Na-K COT, Li-Na CNT, and [Na]. As in previous studies, these patients also show lower PRA and a greater fall in BP after diuretics. None of the patients included in this cohort were considered in previous studies. 11, 14

In the present study, the total variance of Na transport rates explained by α-adducin genotype ranged from 13% to 19%. Such values should be compared with previous heritability estimates of Na-K COT (40%), [Na], and Li-Na CNT (34%). 21, 22 Therefore, our study indicates that the α-adducin Gly460Trp polymorphism may explain approximately one third of the genetic variance of erythrocyte transport systems by a likely major gene effect. 21, 22 Familial and longitudinal

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<th>TABLE 1. Baseline Clinical and Laboratory Characteristics of the Study Cohort According to α-Adducin Genotype</th>
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HDL, high density lipoprotein; HCTZ, hydrochlorothiazide; RBC, red blood cells.

Erythrocyte sodium transport systems (Na-K pump, Na-K COT, Li-Na CNT) and intraerythrocyte sodium concentration [Na] according to α-adducin genotype in never-treated North Sardinian patients with primary hypertension. Values are represented as mean±SEM. *, **, and *** indicate the significant levels for Student-Newman-Keuls multiple comparisons test. *P<0.01; **P<0.001; ***P<0.0001.
studies showed an association between elevated erythrocyte Na-K cot and Li-Na CNT with the development of hypertension.

Renal tubular cell and erythrocyte Na transport systems are encoded by different genes and show several functional differences, although similar abnormalities were reported. Our data parallel those on the G protein β3 subunit gene polymorphism, which affects the Na-H renal antport, renal tubular Na handling, and PRA, and response to diuretic treatment.

The molecular mechanisms by which α-adducin may act on blood pressure and renal cells remain to be elucidated. The mutation sites of rat and human adducin are different; nevertheless, they both stimulate the Na-K pump more than the wild type. Differences between the hypertensive and normotensive strains of rats and mice were also detected on actin polymerization and bundling. Through changes in the actin cytoskeleton, adducin may also affect the regulation of other transport systems such as Na channel, Na-H antiport, and Na dependent cotransporters.

It is likely that other tissues display similar Na transport activation as observed in the erythrocytes, thus making the patients “sicker” than having just hypertension. However, because of different tissue regulation and isoform-specific affinity for Na and K, this widespread alteration must be tested by appropriate methods.

From the present data, it is not clear whether there is a primary alteration of one transport system driving all the others, or whether there is a common mechanism affecting simultaneously all the erythrocyte Na transport systems. The evidence of an increased Na-K pump with lower [Na], favors the hypothesis of a primary effect on the Na-K pump driving all the other cellular changes. However, whatever the sequence of events, the present findings further support the notion that, even in humans, genetic alterations of α-adducin accelerate Na transport—thus favoring the development of hypertension—by increasing the Na transport across the cell membrane. As cation transports were measured at Vmax, mutated adducin either may cause a faster turnover of the Na-K pump or may increase the number of the pump units on the cell membrane. All these considerations are based on the assumption of a direct effect of Gly460Trp—adducin polymorphism on the erythrocyte Na transport systems. However, we must leave open the possibility that these effects may be secondary to some as yet unknown humoral change triggered by adducin.

The faster ion transport in erythrocytes of patients with the 460Trp allele is associated with a greater fall in BP after diuretics and lower PRA, suggesting a common genetic background for such changes. However, the association of Gly460Trp α-adducin polymorphism with these intermediate phenotypes is weaker than that with erythrocyte transport systems. As the genetic complexity increases when moving from cell ion transport to the regulation of whole body BP level, the regulation of PRA and BP response to HCTZ has a degree of genetic complexity (ie, influence of modifier genes) that is higher than ion transports but lower than the blood pressure level. Discovering the presence of genetic variants underlying the blood pressure response to drugs is one of the major goals of pharmacogenomics, although it remains still controversial.

Effects of the 460Trp allele on erythrocytes and on renal Na handling-related variables (ie, BP response to diuretics and PRA), consistent with a pressor effect, could be present.
in North Sardinian hypertensives despite the lack of association with hypertension.14 This is not surprising because the association between α-adducin Gly460Trp polymorphism and hypertension was found in some cohorts but not confirmed in others.11,34–38 Such apparently contrasting findings may be explained as follows: the theory of evolution by bricolage postulates that different populations “use” the alleles available to modulate a given cellular function needed to optimize their biological fitness for their specific environment.39 Selective pressure may favor the genetic mechanisms devoted to body sodium conservation, either by decreasing the frequency of alleles that depress the rate of reabsorption or by increasing the frequency of those that enhance it. This evolutionary force may affect the distribution of any given allele among cases and controls but will not affect its intrinsically specific effect on cell- or whole-body functions.

Therefore, according to this theory, these apparently contrasting findings may simply be due to the fact that some other yet unknown gene variants affecting body Na retention are distributed differently among hypertensives and normotensives causing a shift in the overall genetic network toward a larger body-sodium conservation in hypertensives. Of course, this hypothesis must be proven by genotyping all the known genes affecting body Na handling and blood pressure regulation through an alteration in ion transport across the cell membrane mirrored by erythrocytes. Although preliminary, these findings may also have clinical implications that help to explain the variability of blood pressure response to diuretics in primary hypertensive patients and help to predict this variability in the individual patient.

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**References**


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