G-protein β3-subunit gene variant, blood pressure and erythrocyte sodium/lithium countertransport in essential hypertension

ESTEBAN POCH[1], DANIEL GONZÁLEZ-NÚÑEZ[1], MONTSERRAT COMPTE[2] and ALEJANDRO DE LA SIERRA[2].

[1]Servicio de Nefrología and [2]Unidad de Hipertensión, Servicio de Medicina Interna, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, Universidad de Barcelona, Spain.

Accepted: 18 October 2001

ABSTRACT

Recently, a C825T polymorphism in the gene coding for the β3 subunit of G proteins (*GNB3*) has been described in cells from patients with essential hypertension and enhanced Na^+/H^+ exchange activity. This study aims to evaluate the association between the 825T allele and activity of erythrocyte sodium/lithium countertransport (Na^+/Li^+ CT) and other sodium transport systems in red blood cells from patients with essential hypertension. A group of 77 patients (36 male, 41 female; aged 51.7 ± 1.1 years) was studied. The maximal rates (V_{max}) of Na^+/Li^+ CT, $Na^+/K^+/Cl^-$ cotransport and Na^+K^+ ATPase were evaluated in erythrocytes from all the patients. They were genotyped for the C825T polymorphism by a polymerase chain reaction (PCR) method, followed by digestion with BseDI. Body mass index (BMI) was higher in CT+TT patients than in CC patients (28.9 ± 0.5 *vs*. 27.0 ± 0.7 kg/m^2; P=0.023). Hypertensives with the T allele (CT+TT genotypes) showed significantly higher systolic blood pressure (BP) values (156.9 ± 2.1 *vs*. 148.9 ± 2.8 mmHg; P=0.024), whereas differences in diastolic BP did not reach statistical significance (96.4 ± 1.0 *vs*. 94.0 ± 1.1 mmHg; P=0.120). No differences in the V_{max} of Na^+/Li^+ CT between the genotypes was seen (CC: 236 ± 19 and CT+TT 277 ± 23 mmol/L cells per h; P=0.221). Similarly, no differences were detected in the V_{max} of erythrocyte $Na^+/K^+/Cl^-$ cotransport and Na^+K^+ ATPase among the genotypes. There was no appreciable association between the G-protein β3-subunit C825T polymorphism and erythrocyte Na^+/Li^+ CT and other sodium transport systems in the hypertensive patient sample studied; however, those with the T allele were more obese and had more severe systolic hypertension.

KEY WORDS: GTP-binding proteins. Hypertension. Lithium. Obesity. Polymorphism (genetics). Sodium.

Introduction

A single base substitution (C→T) at position 825 of the *GNB3* gene, which encodes the β3 subunit of G proteins, is related to alternative splicing of exon 9 and results in the loss of 41 amino acids.[1] The 825T allele has been associated with enhanced stimulated binding of labelled GTP in cell lines from hypertensive patients and in transfected insect cells.[1] This polymorphism appears to be responsible, at least in part, for enhanced Na^+/H^+ exchange activity, and is an intermediate phenotype displayed by 30-50% of patients with essential hypertension.[2]

Na^+/H^+ exchange is involved in the control of intracellular pH and cell volume, and may also participate in the initiation of cell growth and proliferation.[2,3] Recently, it has been reported that abnormal Na^+/H^+ exchanger kinetics are genetically fixed, as the abnormality persists in immortalised lymphocytes from patients with essential hypertension.[4] In addition, the enhanced Na^+/H^+ phenotype observed in the 'hypertensive' cell lines is associated with enhanced proliferation pattern and enhanced G-protein activation of the corresponding cell line.[4,5]

Recently, several case-control studies in different populations have reported an association between the 825T allele and essential hypertension, but the results are conflicting.[1,6-9] In addition, recent studies have associated the T allele of *GNB3* with body mass index (BMI) in population studies of several ethnic groups performed in different countries.[10]

Sodium/lithium countertransport (Na^+/Li^+ CT) is a membrane transport system involving a one-to-one exchange of sodium for lithium.[11] The Na^+/Li^+ CT activity, usually measured either as sodium-dependent lithium efflux in lithium-loaded red blood cells or as lithium-dependent sodium efflux in sodium-loaded cells,[12] is regarded as a marker of essential hypertension.[11] Recent studies demonstrate that the maximal rate (V_{max}) of the Na^+/Li^+ CT can predict the future development of hypertension in subjects with high-normal blood pressure (BP) levels.[13] Follow-up studies show that changes in Na^+/Li^+ CT activity over time are related to changes in BMI.[14,15] Moreover, Na^+/Li^+ CT has been shown to positively correlate with BMI in healthy subjects.[16]

Correspondence to: Dr. Esteban Poch
Servicio de Nefrología, Hospital Clinic, Universidad de Barcelona, Villarroel 170
08036 Barcelona, Spain.
Email: epoch@medicina.ub.es

Table 1. Clinical characteristics of essential hypertensive patients classified according to the G-protein β3-subunit C825T polymorphism genotype

Clinical parameter	CC (n=30)	CT+TT (n=47)	P
Age (years)	51.6 ± 1.8	51.8 ± 1.4	0.933
Sex (M/F)	13/17	23/24	0.637
BMI (kg/m^2)	27.0 ± 0.7	28.9 ± 0.5	0.023
Systolic BP (mmHg)	148.9 ± 2.8	156.9 ± 2.1	0.024
Diastolic BP (mmHg)	94.0 ± 1.1	96.4 ± 1.0	0.120

Table 2. Maximal rates of the erythrocyte sodium transport systems and hormonal parameters in the patients studied, classified according to the G-protein β3-subunit C825T polymorphism genotype

	CC (n=30)	CT+TT (n=47)	P
Na^+/Li^+ countertransport (mmol/L cells per h)	236 ± 19	277 ± 23	0.221
$Na^+/K^+/Cl^-$ cotransport (mmol/L cells per h)	519 ± 56	517 ± 45	0.978
Na^+K^+ ATPase (mmol/L cells per h)	7.38 ± 0.40	7.53 ± 0.31	0.770
PRA (ng/mL per h)	0.51 ± 0.14	0.57 ± 0.17	0.824
Aldosterone (ng/dL)	15.5 ± 2.7	13.6 ± 1.2	0.515
ANP (fmol/mL)	18.9 ± 5.4	14.7 ± 2.3	0.425

PRA: plasma renin activity

ANP: atrial natriuretic peptide

The pathological link between the Na^+/H^+ exchanger and Na^+/Li^+ CT is supported by several reports that demonstrate a relationship,[17,18] or even biochemical identity,[19] between these two membrane transporters. Erythrocyte Na^+/Li^+ CT activity is a very reproducible measurement that has been used in many studies of the pathophysiology of essential hypertension, related intermediate phenotypes and genetics.

This study aims to evaluate the possible association between the C825T *GNB3* polymorphism and Na^+/Li^+ CT activity, BMI, BP and other erythrocyte sodium transport systems, in a sample of patients with essential hypertension.

Materials and methods

Study subjects

Seventy-seven essential hypertensive out-patients were recruited consecutively from the nephrology service and the hypertension unit of the internal medicine service of the Hospital Clínic, Barcelona, Spain. A diagnosis of essential hypertension was considered when a cause for high BP could not be detected after complete clinical, biochemical and radiological examination. All patients had at least three office BP measurements above 140/90 mmHg after four weeks on an unrestricted salt diet and without antihypertensive medication. Informed consent was obtained from all the participants.

Mean arterial BP was calculated as the diastolic pressure plus one-third the pulse pressure. BMI was calculated as weight (kg)/height $(m)^2$. Gender distribution was 36 males and 41 females, aged 51.7 ± 1.1 years, with systolic BP 153.9 ± 1.7 mmHg and diastolic BP 95.5 ± 0.8 mmHg.

Genotype determination for the GNB3 *C825T polymorphism*

DNA was extracted from whole blood using a standard procedure. The polymerase chain reaction (PCR) was conducted in a 25 µL reaction volume containing 20 mmol/L Tris HCl (pH 8.4), 50 mmol/L KCl, 1.1 mmol/L $MgCl_2$, 200 µmol/L dNTPs, 0.8 µmol/L of each primer, 1 unit *Thermus aquaticus* (*Taq*) polymerase (Boehringer Mannheim, Germany) and 125 ng genomic DNA. The primer pair used for PCR amplification was 5′-TGACCCACTTGCCACCCGTGC-3′ (sense) and 5′- GCAGCAGCCAGGGCTGGC-3′ (antisense), as previously reported.[1] After a denaturation step at 94°C for 5 min, PCR was conducted for 34 cycles with denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec and extension at 72°C for 30 sec, with a final extension step at 72°C for 5 min. The PCR product was digested with the restriction enzyme BseDI (Fermentas, Lithuania), electrophoretically resolved on 2.5% agarose and visualised under ultraviolet (UV) illumination.

The non-digested product (TT genotype) showed a band of 268 bp and the complete digestion of the PCR product (CC genotype) produced bands of 116 bp and 152 bp. Heterozygotes (CT) displayed all three bands. Genotype determination was confirmed by automated sequencing of several samples and was performed blind.

Simultaneous measurement of the maximal rate of erythrocyte Na^+/K^+ ATPase, $Na^+/K^+/Cl^-$ cotransport and Na^+/Li^+ countertransport

Maximal rates (V_{max}) of Na^+/K^+ ATPase, $Na^+/K^+/Cl^-$ cotransport and Na^+/Li^+ CT were measured in sodium-loaded erythrocytes using previously described methods.[20-22] V_{max} of Na^+/Li^+ CT was estimated as lithium-dependent Na^+ efflux in Na^+-loaded cells. Previously, it was reported that this measurement correlates with the more popular technique of Li^+ efflux in Li^+-loaded cells.[12] However, the former has the advantage that it permits the simultaneous measurement of Na^+/K^+ ATPase and $Na^+/K^+/Cl^-$ cotransport activities.

Briefly, 10 mL venous blood was collected in heparinised tubes. Plasma and the buffy coat were removed and erythrocytes washed twice with cold KCl (150 mmol/L). The internal Na^+ content was modified to 39.82 ± 2.54 mmol/L cells using the nystatin technique.[20] At the end of this procedure, Na^+ efflux was measured in red blood cells washed (x5) in cold $MgCl_2$ (110 mmol/L) and resuspended (duplicates) at a final haematocrit of 0.05 in four different Na^+-free media comprising $MgCl_2$ (75 mmol/L), sucrose (85 mmol/L), MOPS-Tris (10 mmol/L; pH 7.4) and glucose (10 mmol/L), to which were added: 2 mmol/L KCl (medium 1); 0.1 mmol/L ouabain (medium 2); 0.1 mmol/L ouabain and 0.02 mmol/L bumetanide (medium 3); and 0.1 mmol/L ouabain, 0.02 mmol/L bumetanide and 10 mmol/L lithium chloride (medium 4). They were incubated at 37°C for 30 min (medium 1) or 60 min (media 2, 3 and 4).

After incubation, tubes were transferred to ice and centrifuged at 1750 x*g* for 4 min at 4°C. The supernatant was removed, avoiding pellet contamination, and stored for subsequent Na^+ concentration measurement by flame photometry. The Na^+ flux depending on Na^+/K^+ ATPase (ouabain-sensitive Na^+ efflux) was estimated from the difference in Na^+ efflux measurement with (medium 2) and without (medium 1) ouabain. The Na^+ flux depending on $Na^+/K^+/Cl^-$ cotransport (bumetanide-sensitive Na^+ efflux) was obtained from the difference in Na^+ efflux in media containing ouabain with (medium 3) and without (medium 2) bumetanide. The Na^+ flux depending on Na^+/Li^+ CT (Li^+-stimulated Na^+ efflux) was estimated from the difference in Na^+ efflux in media containing ouabain and bumetanide with (medium 4) and without (medium 3) lithium chloride. V_{max} for Na^+/K^+ ATPase was expressed in mmol/L cells per h, and for $Na^+/K^+/Cl^-$ cotransport and Na^+/Li^+ CT as mmol/L cells per h.

Statistical analysis

Values were expressed as mean ± standard error of the mean (SEM). Differences in parameters among G-protein β3-subunit C825T genotype polymorphisms were compared by Student's *t*-test, with the CT and TT genotypes pooled together. The influence of gender on the association between genotype and BMI or BP was analysed by two-way ANOVA. $P<0.05$ was considered significant. All statistical analyses were performed using the SPSS version 9 statistical software package (SPSS Advanced statistics, Chicago, IL).

Results

The genotype distribution in the sample studied was 30 (39%) CC, 39 (50.6%) CT and eight (10.4%) TT, which proved similar to that reported previously for Caucasian populations.[10] Owing, in part, to the low frequency of the TT genotype, patients with CT and TT genotypes were considered together for further analysis (CT+TT). There were no differences in age, gender or BP between CT and TT subjects (data not shown), and this approach was supported by the work of Siffert *et al*.,[1] who found that the associated cellular phenotype (PAF-stimulated binding of [^{35}S]GTPγS) did not differ between CT and TT genotypes. The clinical characteristics of the patients studied are shown in Table 1.

No differences in age or gender distribution among genotypes were seen; however, BMI was higher in CT+TT patients than in CC patients (28.9 ± 0.5 *vs*. 27.0 ± 0.7 kg/m^2; $P=0.023$; data not shown), irrespective of gender (two-way ANOVA, $P=0.570$). In addition, essential hypertensive patients with the T allele had significantly higher systolic BP values (156.9 ± 2.1 *vs*. 148.9 ± 2.8 mmHg; $P=0.024$) and slightly, but not significantly, higher diastolic BP values (96.4 ± 1.0 *vs*. 94.0 ± 1.1 mmHg; $P=0.120$) (Table 1). Association between systolic BP and C825T genotype was not influenced by gender ($P=0.557$; data not shown).

With respect to the sodium transport systems, there were no differences in V_{max} for Na^+/Li^+ CT between the different genotypes: 236 ± 19 and 277 ± 23 mmol/L cells per h for CC and CT+TT, respectively ($P=0.221$). Similarly, no differences were seen in the V_{max} for erythrocyte $Na^+/K^+/Cl^-$ cotransport and Na^+/K^+ ATPase among the different G-protein β3-subunit C825T genotype polymorphisms (Table 2).

Discussion

The G-protein β3-subunit C825T polymorphism is relevant to the pathogenesis of essential hypertension because it is related to functional changes linked to this disease. Previous investigations have shown that enhanced Na^+/H^+ exchange activity is associated with several phenotypes in hypertensive individuals such as left ventricular hypertrophy,[21] insulin resistance,[23] renal sodium retention[24] or obesity.[25]

There is evidence that the erythrocyte Na^+/H^+ exchange and Na^+/Li^+ CT are mediated by the same membrane transport protein. A proportion of patients with essential hypertension display higher V_{max} for erythrocyte Na^+/H^+ exchange and Na^+/Li^+ CT than do normotensive controls, and the V_{max} for these transport systems – as well as the K_m for internal H^+ (Na^+/H^+ exchange) and external Na^+ (Na^+/Li^+ CT) – correlate significantly.[18] A study using nuclear magnetic resonance has demonstrated biochemically that Li^+ efflux is dependent on the pH gradient and is inhibited with amiloride, suggesting a biochemical identity between Na^+/H^+ exchange and Na^+/Li^+ CT.[19]

Increased Na^+/Li^+ CT activity, measured by either Na^+ or Li^+ efflux, has been described in patients with essential hypertension,[11,18,20] and it seems to be a risk marker for the future development of hypertension in follow-up population studies.[13,15] In addition, increased activity of this transporter has been related to BMI in several studies.[15,16]

Therefore, we explored the relationship between the G-protein β3-subunit C825T polymorphism and the V_{max} of Na^+/Li^+ CT in essential hypertension. The maximal rate of Na^+/Li^+ CT was not statistically different in patients with or without the T allele, although the present study was limited by a low statistical power (70%) to detect differences in Na^+/Li^+ CT, because of the small sample size. However, with this sample size, we were able to find differences in BMI and BP, both of which were higher in patients with the T allele.

Owing to the fact that these two factors have been related to Na^+/Li^+ CT,[11,16,18,20] possible differences among C825T genotypes in a larger sample could be important. Moreover, recent data from a prospective population study supports the participation of environmental factors (e.g. lifestyle) and metabolic parameters in the activity of erythrocyte Na^+/Li^+ CT.[15]

Case-control study has shown a significant association between the T allele of the G-protein β3-subunit C825T polymorphism and essential hypertension,[1] which was confirmed subsequently by population-based study.[6] However, studies performed in different ethnic groups failed to show an association with blood pressure[7] or hypertension status.[8,26] In addition, a recent report failed to show association between the *GNB3* polymorphism and severity of hypertension, assessed by ambulatory BP monitoring.[9]

Differences in allele frequency among populations have been advocated as a cause of this discrepancy. For example, in a negative study performed in Japan,[8] the frequency of the T allele was considerably higher (49%) than that reported for Caucasian populations (approximately 30%).[1] In contrast, a positive association between the T allele and hypertension has been reported in a Black population, with a T-allele frequency as high as 79.2%.[27]

In the present study, genotype frequency among the

subjects was similar to that reported for white populations,[10] and was in Hardy-Weinberg equilibrium. Moreover, we found a significant difference in systolic BP between genotypes, confirming previous observations.[1,6,27,28]

As reported previously by others,[10,29] the T allele was associated with higher BMI values in our sample of patients, which was independent of gender. The pathophysiological link between this polymorphism and obesity relies on the important role played by pertussis-sensitive G proteins in adipogenesis.[10,29] It has been proposed that the effects of the G-protein β3-subunit C825T polymorphism on BP may be mediated by an increase in BMI, because the association with BMI has been found to be stronger than that with BP.[10]

In conclusion, the G-protein β3-subunit C825T polymorphism did not to have an quantitatively important influence on the V_{max} for Na^+/Li^+ CT in the sample of essential hypertensive patients studied. However, it confirmed that essential hypertensive patients with the T allele are more obese and suffer a more severe systolic hypertension. □

This work was supported by a grant to EP from the Fondo de Investigaciones Sanitarias FIS 98/0255.

References

1 Siffert W, Rosskopf D, Siffert G et al. Association of a human G-protein β3 subunit variant with hypertension. *Nat Genet* 1998; **18**: 45-8.

2 Siffert W, Düsing R. Sodium-proton exchange and primary hypertension. An update. *Hypertension* 1995; **26**: 649-55.

3 Grinstein S, Rotin D, Mason M. Na^+/H^+ exchange and growth factor-induced cytosolic pH changes. Role in cellular proliferation. *Biochim Biophys Acta* 1989; **988**: 73-97.

4 Rosskopf D, Frömter E, Siffert W: Hypertensive sodium-proton exchanger phenotype persists in immortalized lymphoblasts from essential hypertensive patients. *J Clin Invest* 1993; **92**: 2553-9.

5 Siffert W, Rosskopf D, Moritz A *et al*. Enhanced G-protein activation in immortalized lymphoblasts from patients with essential hypertension. *J Clin Invest* 1995; **96**: 759-66.

6 Schunkert H, Hense HW, Döring A, Riegger GAJ, Siffert W. Association between a polymorphism in the G-protein b3 subunit gene and lower renin and elevated diastolic blood pressure levels. Hypertension 1998; 32: 510-3.

7 Hegele RA, Harris SB, Hanley AJG, Cao H, Zinman B. G-protein β3 subunit gene variant and blood pressure variation in Canadian Oji-Cree. *Hypertension* 1998; **32**: 688-92.

8 Kato N, Sugiyama T, Morita H, Kurihara H, Yamori Y, Yazaki Y. G-protein β3 subunit variant and essential hypertension in Japanese. *Hypertension* 1998; **32**: 935-8.

9 Beige J, Hohenbleicher H, Distler A, Sharma AM. G-protein β3 subunit C825T variant and ambulatory blood pressure in essential hypertension. *Hypertension* 1999; **33**: 1049-51.

10 Siffert W, Forster P, Jöckel K-H *et al*. Worldwide ethnic distribution of the G- protein β3 subunit 825T allele and its association with obesity in Caucasians, Chinese, and Black African individuals. *J Am Soc Nephrol* 1999; **10**: 1921-30.

11 Canessa M, Adragna N, Solomon HS, Connoly TM, Tosteson DC. Increased sodium-lithium countertransport in red cells of patients with essential hypertension. *N Engl J Med* 1980; **302**: 772-6.

12 Hannaert PA, Garay RP. A kinetic analysis of Na-Li countertransport in human red blood cells. *J Gen Physiol* 1986; **87**: 353-68.

13 Strazzullo P, Siani A, Cappuccio FP *et al*. Red blood cell sodium-lithium countertransport and risk of future hypertension. The Olivetti prospective heart study. *Hypertension* 1998; **31**: 1284-9.

14 Hunt SC, Williams RR, Owen KA. Changes in sodium-lithium countertrnasport correlate with changes in triglycerides levels and body mass index over 2½ years of follow-up in Utah. *Cardiovasc Drugs Ther* 1990; **4**: 357-62.

15 Cirillo M, Laurenzi M, Panarelli W, Trevisan M, Stamler J, for the Gubbio population study research group. Prospective analysis of traits related to 6-year change in sodium-lithium countertransport. *Hypertension* 1999; **33**: 887-93.

16 Trevisan M, Strazzullo P, Cappuccio FP *et al*. Sodium-lithium countertransport and body fat distribution. *Life Sci* 1992; **51**: 687-93.

17 Kahn AM, Allen JC, Cragoe EG Jr, Shelat H. Sodium-lithium exchange and sodium-proton exchange are mediated by the same transport system in sarcolemmal vesicles from bovine superior mesenteric artery. *Circ Res* 1989; **65**: 818-28.

18 Semplicini A, Ceolotto G, Felice M *et al*. Kinetic properties of erythrocyte Na^+-Li^+ and Na^+-H^+ exchange in hypertensive patients. *J Hypertens* 1995; **13**: 1566-70.

19 Chi Y, Mo S, Mota de Freitas D. Na^+-H^+ and Na^+-Li^+ exchange are mediated by the same membrane transport protein in human red blood cells: an NMR investigation. *Biochemistry* 1996; **35**: 12433-42.

20 De la Sierra A, Coca A, Aguilera MT, Urbano-Márquez A. Na^+-Li^+ countertransport in essential hypertension. *J Hypertens* 1988; **6**: 931-7.

21 De la Sierra A, Coca A, Paré JC, Sánchez M, Valls V, Urbano-Márquez A. Erythrocyte ion fluxes in essential hypertensive patients with left ventricular hypertrophy. *Circulation* 1993; **88**:1628-33.

22 Lluch MM, de la Sierra A, Poch E *et al*. Erythrocyte sodium transport, intraplatelet pH and calcium concentration in salt-sensitive hypertension. *Hypertension* 1996; **27**: 919-25.

23 Canessa M, Falkner B, Hulman S. Red blood cell sodium-proton exchange in hypertensive blacks with insulin-resistant glucose disposal. *Hypertension* 1993; **22**: 204-13.

24 Díez J, Alonso A, Garciandía A *et al*. Association of increased erythrocyte Na^+/H^+ exchanger with Na^+ retention in patients with essential hypertension. *Am J Hypertens* 1995; **8**: 124-32.

25 Delva P, Pastori C, Provoli E *et al*. Erythrocyte Na^+-H^+ exchange activity in essential hypertensive and obese patients: role of excess body weight. *J Hypertens* 1993; **11**: 823-30.

26 Larson N, Hutchinson R, Boerwinkle E. Lack of association of 3 functional gene variants with hypertension in African Americans. *Hypertension* 2000; **35**: 1297-1300.

27 Dong Y, Zhu H, Sagnella GA, Carter ND, Cook DG, Cappucio FP. Association between the C825T polymorphism of the G-protein β3-subunit gene and hypertension in blacks. *Hypertension* 1999; **34**: 1193-6.

28 Benjafield AV, Jeyasingan CL, Nyholt DR, Griffiths LR, Morris BJ. G-protein β3 subunit gene (GNB3) variant in causation of essential hypertension. *Hypertension* 1998; **32**: 1094-7.

29 Siffert W, Naber C, Walla M, Ritz E. G-protein β3 subunit 825T allele and its potential association with obesity in hypertensive individuals. *J Hypertens* 1999; **17**: 1095-8.