Original Article / *Klinik Çalışma - Araştırma*

The Association of Hypertension and C825T Polymorphism of the Gene Encoding the G-protein Beta-3 subunit (GNB3) in a Group of Turkish Hypertensive Patients[*]

Hipertansiyonlu Türk Hasta Grubunda Hipertansiyon ile G Proteini Beta-3 Altbirimini Kodlayan Gende C825T Polimorfizmi Arasındaki İlişki

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Objectives: The C825T polymorphism of the gene encoding the G-protein beta-3 subunit (GNB3) is associated with enhanced signal transduction via G proteins which in turn results in an increased Na^+/H^+ exchanger activity. This ion transport system mediates sodium reabsorption in the proximal tubule. Increased activity of Na^+/H^+ exchanger was found in the cells of patients with arterial hypertension in different populations. We investigated this polymorphism in a Turkish group of patients with hypertension and compared them with normotensive subjects.

Patients and Methods: 99 hypertensive patients (57 males, 42 females) and 45 normotensive controls (33 males, 12 females) were genotyped for the GNB3 C825T polymorphism.

Results: Allele distribution of patient and control groups were CC: 35, CT: 51, TT: 13 and CC: 26, CT: 15, TT: 4, respectively. T-allele carriers (CT and TT) were significantly higher in hypertensive group. There was also a statistically significant (Chi-square test, p=0.041) relationship between 825T allele and hypertension.

Conclusion: These results suggest that hypertension is related with GNB3 polymorphism in Turkish patients and increased G protein activity may be the pathophysiologic link between C825T and hypertension.

Key Words: Hypertension; polymorphism; GNB3; G proteins; C825T.

Amaç: G proteini beta-3 (β3) altbirimini (GNB3) kodlayan genin C825T polimorfizmi G proteinleri yoluyla sinyal iletiminde artışla ve sonuçta artmış Na^+/H^+ değiştiricisinin aktivitesi ile ilişkilidir. Bu iyon taşıma sistemi proksimal tübülden sodyum geri emilimine aracılık eder. Değişik nüfuslardan hipertansif hastaların hücrelerinde Na^+/H^+ değiştiricisinin aktivitesinde artış bulunmuştur. Bu çalışmada hipertansiyonlu Türk hasta grubunda bu polimorfizm araştırıldı ve normotansif bireylerle karşılaştırıldı.

Hastalar ve Yöntemler: Doksan dokuz hipertansif hasta (57 erkek, 42 kadın) ve 45 normotansif kontrolde (33 erkek, 12 kadın) GNB3 C825T polimorfizmi için genotipleme yapıldı.

Bulgular: Hasta ve kontrol gruplarında alel dağılımı sırasıyla CC:35, CT:51, TT:13 ve CC:26, CT:15, TT:4 şeklindeydi. T aleli taşıyıcıları (CT ve TT) hipertansiyonlu grupta anlamlı şekilde daha yüksekti. Hipertansiyon ve C825T aleli arasında istatistiksel bakımdan anlamlı ilişki (Ki-kare testi, p=0.041) bulundu.

Sonuç: Bu bulgular Türk hastalarda hipertansiyon ve GNB3 polimorfizminin ilişkili olduğunu ve artmış G proteini aktivitesinin C825T ile hipertansiyon arasındaki patofizyolojik bağlantı olabileceğini düşündürmektedir.

Anahtar Sözcükler: Hipertansiyon; polimorfizm; GNB3; G proteini; C825T.

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GNB3 gene was located in 12p13 region of chromosome. Gene comprises of 11 exons and starts with ATG codon in the third exon and ends with TGA codon in the eleventh exon.[1] Recently, C/T polymorphism at position 825 in the cDNA encoding β3 subunit of heterotrimeric G protein.[2,3] T-allele is associated with Gβ3-s which is an additional variant of Gβ3 having 123 deleted-nucleotides between 498-620 nucleotides in the ninth exon.[2,4] Deletion of 123 base (41 amino acid) results in the change of 3D structure of trimeric G proteins.[3] C825T dimorphisms of the gene encoding G protein β3 subunit characterized by enhanced Na^+/H^+ exchanger activity in hypertensive subjects. Enhanced activity of G proteins was also suggested to be in association with obesity, salt collection, low plasma renin activity, insulin resistance and left ventricle hypertrophy.[1]

G proteins interact with cell surface receptors, which span cell membrane sevenfold, and activate different intracellular pathways. Therefore, C825T polymorphism of GNβ3 gene enhances activation of G proteins and alters many physiologic process. C825T polymorphism may also play an important role in the pathogenesis of hypertension.[3] Alteration in signal transduction pathways allows abnormal movement of ions and change intracellular conditions.[5] 825T variant, by enhancing Na^+/H^+ exchanger activity, may obtain several mechanisms that have potential for development of hypertension.[2] Enhanced Na^+/H^+ exchanger activity stimulates vascular tonus and cell growth as well as sodium reabsorption from epithelial cells of proximal tubule. All these effects play important role in the pathogenesis of hypertension.[5] There is growing evidence that the enhanced Na^+/H^+ exchanger activity observed in 30% to 50% of patients with essential hypertension is mediated by this genetically fixed G protein activation.[6] Previous studies of different ethnic groups demonstrated conflicting results on the relationship of GNB3 and hypertension. For instance, Dong et al.[2] suggested 825T allele frequency is high in black people and T allele might account for 44% of the cases of hypertension in blacks. Conversely, Kato et al.[7] reported that the T825 variant of the G protein β3 subunit gene is unlikely to constitute major susceptibility for essential hypertension in the Japanese population studied. In addition, Brand et al.[8] failed to find any relation between GNB3 and hypertension in a French study group. These results suggest that genetic tendency of hypertension resulting from GNB3 polymorphism may vary among different ethnic groups. Therefore, we investigated the C825T polymorphism of GNB3 in relation to hypertension in hypertensive and normotensive groups of Turkish population.

PATIENTS AND METHODS

Subjects

Newly diagnosed 99 hypertensive patients of Nephrology Department, Trakya University School of Medicine, and 45 healthy controls were enrolled in the study. Resting blood pressure measurements were performed by using sphygmomanometer. Participants with systolic blood pressure ≥140 mmHg, and/or diastolic blood pressure ≥90 mmHg were accepted as hypertensive.

Genotyping

DNA was extracted from blood samples (white blood cells) of patient and control groups by salting-out precipitation method. 5` - TGA CCC ACT TGC CAC CCG TGC - 3` and 5` - GCA GCA GCC AGG GCT GGC - 3` primer pair were used for enhancement of isolated genomic DNAs. In the mixture prepared for PCR, 16 μl dH_2O, 2.5 μl $MgCl_2$, 2.5 μl buffer, and 0.5 μl Primer I, 0.5 μl Primer II, 1 μl dNTP, 0.25 μl Taq Polymerase (Sigma, Saint Louis, Missouri, USA) was used for one patient sample. 1.5 μl patient's DNA was added into the mixture. After an initial denaturation step at 94 °C for 5 minutes, 35 cycles of 94 °C for 1 minute, 69.3 °C for 45 seconds, and 72 °C for 1 minute were performed. The PCR products were then electrophoresed on a 2.5% agarose gel and visualized under UV light by 5μg/mL ethidium bromide staining. A representative example of appearance of an ethidium bromide-stained gel on which digests of PCR products were run is shown in Figure 1.

In order to search for genotype of C825T polymorphism, 268 bp PCR products were incu-

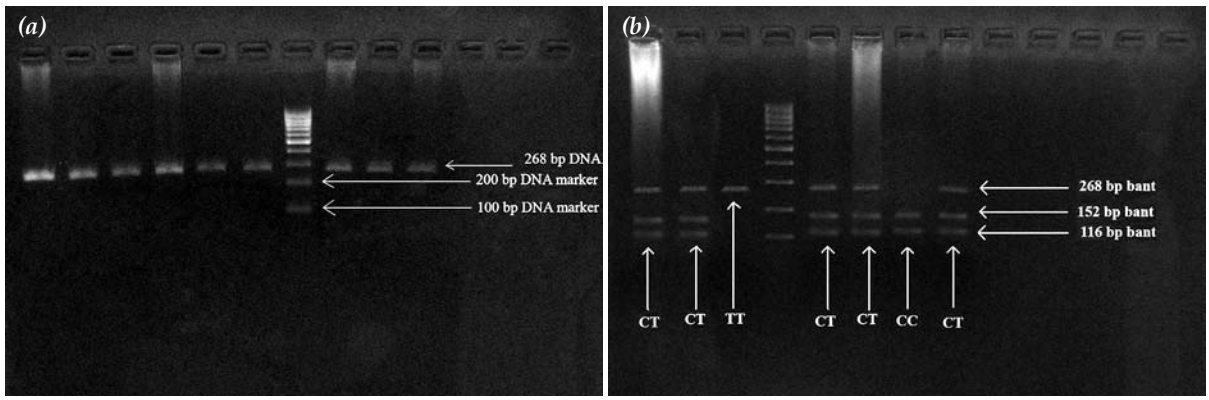

Fig. 1. *PCR products under UV light.* ***(a)*** *Visualization of 268 bp bands of PCR products under UV light by ethidium bromide staining following electrophoresing on 2.5% agarose gel.* ***(b)*** *Visualization of 116, 152, and 268 bp bands of PCR products following restriction.*

bated with BssEC I (Bio. Basic. Inc, Canada) restriction enzyme. For each patient sample, 8 μl H_2O, 1 μl buffer, 0.5 μl BssEC I (Sec I) restriction enzyme were added into the mixture. Then, 4 μl PCR product was added and incubated at 65 °C for 15-20 hours. Following incubation period, the digests were electrophoresed on a 2.5% agarose gel and visualized under UV light by 5 μg/mL ethidium bromide staining. BssEC I cuts at 5`-C ↓ CNNGG-3` to give bands of 116 and 152 bp (C allele) or does not cut, leaving a 268 bp band (T allele). One band (268 bp) for homozygote TT genotype, two bands (116 and 152 bp) for homozygote CC genotype, and three bands (268, 152, and 116 bp) for heterozygote CT genotype are seen.

Statistical analyses

Comparisons of patient and control groups were made by using t-test. Genotype frequency comparisons were made by using χ^2 test. P value of 0.05 or higher was accepted as statistically significant.

RESULTS

Demographic, anthropometric characteristics and blood pressure measurements of both groups were given in Table 1. Both groups were age- and sex-matched. Hypertensive group and control group were similar in terms of body mass index. Systolic and diastolic blood pressure measurements indicated that study group had significantly hypertensive when compared to normotensive control group.

The relationship between gender and genotype was given in Table 2. We failed to find any significant relation between genotype and gender. Male/female ratios were similar in CC, CT, and TT allele groups. TT allele frequency was similar in male (n=9; 10%) and female (n=8;

Table 1. General characteristics of patient and control groups

	Hypertensive group (n=99)	Normotensive group (n=45)	*p*
Demographic			
Sex, M/F	42/57	12/33	>0.05
Age, year	44±7	41±7	>0.05
Anthropometric			
Body mass index, kg/m^2	28.17±3.47	26.78±3.66	>0.05
Hemodynamic			
Systolic blood pressure, mmHg	157±12	119±8	<0.001
Diastolic blood pressure, mmHg	98±8	76±6	<0.001

Table 2. Gender and genotype relationship

	Male		Female		*Total*
	n	%	n	%	
CC	41	45	20	37	61
CT	40	44	26	48	66
TT	9	10	8	14	17

Chi-square, P=0.511.

Table 3. Disease and genotype relationship

	Hypertensive		Normotensive		*Total*
	n	%	n	%	
CC	35	35	26	57	61
CT	51	51	15	33	66
TT	13	13	4	9*	17
CT+TT	64	64	19	42**	83

Chi-square, *P=0.041; ** P=0.018

14%) subjects. We further analyzed the relationship between the existence of hypertension and T allele (Table 3). There was a statistically significant relationship between T allele and hypertension.

DISCUSSION

The main finding of this study is that there was a significant association of the C825T polymorphism of GNB3 with essential hypertension. To the best of our knowledge, this is the first study performed on a Turkish hypertensive patient group. This result confirms and extends the findings of Siffert et al.[9] Essential hypertension is commonly accepted as a multifactorial disorder. The attempt to study gene alterations causing or contributing to hypertension is driven by several promises of molecular medicine: Identification of involved loci will lead to a better understanding of the pathophysiological mechanisms leading to high blood pressure and, potentially, to the development of novel drugs for causal treatment.[1] The ubiquitously expressed pH-regulating ion transport system, Na^+/H^+ exchanger (NHE), mediates an electroneutral change of extracellular Na^+ against intracellular H^+ ions.[10] Enhanced NHE activity is due to enhanced G-protein activation in cells from subjects with hypertension.[11] Siffert et al.[9] were able to localize a polymorphism at position 825 (C→T) of the cDNA that encodes the β3 subunit (GNB3) of the pertussis toxin-sensitive G-protein and demonstrated that the 825T allele was significantly associated with hypertension. There is strong evidence that frequency of C825T allele in different ethnicities is different. All black African populations and black Americans showed the highest 825T allele frequencies in the range of 80-90%. On the contrary, Caucasian populations showed lowest 825T allele frequencies averaged 30%.[12] T allele frequency in our normotensive group was 0.42 whereas it was 0.64 in hypertensive group. In a previous study T allele frequency for Turkish people was reported as 0.38 which is similar with our normotensive group.[12] These results suggest that T allele could be a predisposing factor to hypertension in Turkish people as well as other ethnic groups. In addition, there was no difference in distribution of allele frequency between the two genders.

There are several limitations of the study that deserve comment. We did not have information about the amount of sodium intake of participants. High or low sodium intake may be a determining factor to rising blood pressure. Yamagishi et al.[13] reported that association of GNB3 and systolic blood pressure was more evident among subjects with lower sodium intake/excretion, but not with higher intake/excretion. Furthermore, they suggested high salt intake might mask the potential effect of GNB3 on blood pressure levels. However, it is not easy to classify participants into low or high sodium intake groups, as the variation of sodium intake may be very large from day to day. It is worthy to note that in the study of Yamagishi et al.,[13] 66% of subjects remained in the same group of higher or lower sodium intake after one-year re-collection of urinary sodium excretion. Our study population consisted of normotensive or hypertensive hospital outpatients. This study group, more or less, may not reflect the characteristics of general population. However, Ishikawa et al.[14] determined similar T allele distribution between hospital outpatients and general population.

Altered G-protein signaling may lead to hypertension by several putative mechanisms. First, expression of T allele leads to deletion of the GNB3 protein and eventually enhanced sensitivity of Gi proteins to receptor activation.[2] Hence, a higher blood pressure may result from increased sensitivity to vasoactive pressor hormones. Second, increased activity of renal Na^+/H^+ exchanger increase sodium reabsorption which then lead increase in blood pressure.[9]

In conclusion, this study shows a significantly higher frequency of the 825T allele in hypertensive Turkish people when compared to normotensive control subjects. Together with previous work, these findings highlight the possible contribution of variants in genes regulating renal sodium handling in the development of hypertension in Turkish.

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REFERENCES

1. Siffert W. G protein beta 3 subunit 825T allele, hypertension, obesity, and diabetic nephropathy. Nephrol Dial Transplant 2000;15:1298-306.
2. Dong Y, Zhu H, Sagnella GA, Carter ND, Cook DG, Cappuccio FP. Association between the C825T polymorphism of the G protein beta3-subunit gene and hypertension in blacks. Hypertension 1999; 34:1193-6.
3. Wüthrich RP, Cicvara S, Booy C, Widmer U, Binswanger U. The 825C/T polymorphism of the G-protein subunit beta3 does not influence blood pressure and renal function in kidney transplant recipients. Nephrol Dial Transplant 2000;15:1663-6.
4. Meirhaeghe A, Bauters C, Helbecque N, Hamon M, McFadden E, Lablanche JM, et al. The human G-protein beta3 subunit C825T polymorphism is associated with coronary artery vasoconstriction. Eur Heart J 2001;22:845-8.
5. Sağlam K, Yılmaz Mİ, Sönmez A, Baykal Y, Koçar İH. Hipertansiyon (Yüksek kan basıncı). 2003. Available from: http://www.gata.edu.tr/dahilibilimler/ichastaliklari/files/kitaplar/146.pdf
6. Siffert W. G proteins, hypertension, and coronary heart disease-novel findings and hypotheses. Kidney Blood Press Res 1996;19:71-80.
7. Kato N, Sugiyama T, Morita H, Kurihara H, Yamori Y, Yazaki Y. G protein beta3 subunit variant and essential hypertension in Japanese. Hypertension 1998;32:935-8.
8. Brand E, Herrmann SM, Nicaud V, Ruidavets JB, Evans A, Arveiler D, et al. The 825C/T polymorphism of the G-protein subunit beta3 is not related to hypertension. Hypertension 1999;33:1175-8.
9. Siffert W, Rosskopf D, Siffert G, Busch S, Moritz A, Erbel R, et al. Association of a human G-protein beta3 subunit variant with hypertension. Nat Genet 1998;18:45-8.
10. Mahnensmith RL, Aronson PS. The plasma membrane sodium-hydrogen exchanger and its role in physiological and pathophysiological processes. Circ Res 1985;56:773-88.
11. Siffert W, Rosskopf D, Moritz A, Wieland T, Kaldenberg-Stasch S, Kettler N, et al. Enhanced G protein activation in immortalized lymphoblasts from patients with essential hypertension. J Clin Invest 1995;96:759-66.
12. Siffert W, Forster P, Jöckel KH, Mvere DA, Brinkmann B, Naber C, et al. Worldwide ethnic distribution of the G protein beta3 subunit 825T allele and its association with obesity in Caucasian, Chinese, and Black African individuals. J Am Soc Nephrol 1999; 10:1921-30.
13. Yamagishi K, Tanigawa T, Cui R, Tabata M, Ikeda A, Yao M, et al. G-protein beta-3 subunit C825T polymorphism, sodium and arterial blood pressure: a community-based study of Japanese men and women. Ann Hum Genet 2006;70:759-66.
14. Ishikawa K, Imai Y, Katsuya T, Ohkubo T, Tsuji I, Nagai K, et al. Human G-protein beta3 subunit variant is associated with serum potassium and total cholesterol levels but not with blood pressure. Am J Hypertens 2000;13:140-5.