Angiotensinogen as a Risk Factor for Essential Hypertension in Japan

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Abstract

A common molecular variant of angiotensinogen (*AGT*), the precursor of the potent vasoactive hormone angiotensin II, has been incriminated as a marker for a genetic predisposition to essential hypertension in Caucasians (Jeunemaitre, X., F. Soubrier, Y. V. Kotelevtsev, R. P. Lifton, C. S. Williams, A. Charru, S. C. Hunt, P. N. Hopkins, R. R. Williams, J. M. Lalouel, and P. Corvol. 1992. *Cell.* 71:169–180). We now show that the same variant, T235, is associated with essential hypertension in Japanese patients. The observation of this association in a distinct, ethnically homogeneous population further substantiates an involvement of angiotensinogen in the pathogenesis of essential hypertension and has physiological, epidemiological, and evolutionary implications. (*J. Clin. Invest.* 1994. 93:1285–1287.) Key words: hypertension • genetics, Japan • angiotensinogen • genetic marker

Introduction

Essential hypertension is a major public health concern in Japan, where its high prevalence accounts for one of the highest rates of cerebrovascular accidents in the world (1, 2). It is often advanced that the particular features of the epidemiology of essential hypertension and its vascular complications in Japan result from the high level of sodium intake in this population (3, 4).

The well-documented familial aggregation of essential hypertension supports the hypothesis that genetic factors contribute to its determination (5). The renin-angiotensin system is a prime suspect for harboring genetic determinants of hypertension, as it plays a cardinal role in salt and water homeostasis and in the maintenance of vascular tone (6). Renin cleaves angiotensinogen to generate angiotensin I, which, after further cleavage by the angiotensin-converting enzyme, yields angiotensin II, a peptide hormone that promotes vasoconstriction and sodium retention.

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An involvement of angiotensinogen in the pathogenesis of essential hypertension was suggested recently (7). This conclusion rested on the observation of genetic linkage in hypertensive siblings, of a higher occurrence of two molecular variants of the angiotensinogen gene (*AGT*) in hypertensive cases compared with controls, and of higher plasma concentrations of angiotensinogen in subjects carrying the most common *AGT* variant, T235. These findings were established in two independent series of Caucasian subjects, one from Salt Lake City and the other from Paris. In a separate study, T235 was found to occur at higher frequency among preeclamptic women than among women with normal pregnancies (8). The association with preeclampsia was observed in two independent samples: Caucasian women ascertained in Utah, and Japanese women ascertained in Japan. Variant T235 corresponds to the presence of a threonine instead of a methionine at residue 235 of the mature protein and occurs at a frequency of 35–40% in Caucasians (7). Another variant, M174, is characterized by the presence of a methionine instead of a threonine at residue 174; occurring at a frequency of 8–10% in Caucasians, it also exhibits significant association with hypertension (7). However, it is found only in genes encoding the T235 variant and does not behave as an independent predictor of risk (1, 7).

We now report that the *AGT* variant T235 occurs at higher frequency in essential hypertensive subjects than in normotensive controls in Japan. Furthermore, its population frequency is higher among Japanese than among Caucasian subjects. Possible implications of these observations are discussed.

Methods

Subjects. Patients with essential hypertension were ascertained at the Hypertension Clinics of the Yamanashi Medical University and the Nagoya City University Hospitals. The following selection criteria were applied: (*a*) established hypertension defined by a systolic blood pressure (BP)[1] > 160 mmHg and/or a diastolic BP > 95 mmHg; (*b*) absence of secondary hypertension; (*c*) absence of renal disease and renal insufficiency; and (*d*) absence of diabetes mellitus. This yielded a series of 105 cases with a mean current age of 61 yr. The 81 normotensive controls were collected in Yamanashi Medical University. They included healthy college students, physicians, and other staff members or patients who visited the same clinic but presented no serious illness. They had systolic and diastolic BP < 140 and 90 mmHg, respectively, no previous history of high blood pressure, and no renal disease or diabetes. Their age varied from 16 to 78 yr, with a mean of 34 yr.

Genotyping. Genotypes at residues 174 and 235 of *AGT* were determined by hybridization of enzymatically amplified DNA with allele-

1. *Abbreviation used in this paper:* BP, blood pressure.

specific oligonucleotides previously described (7). Genomic DNA was extracted from 1.0 ml of whole blood using GENOMIX kits (Talent, Trieste, Italy) and resuspended with 200 μl of water. After enzymatic amplification of genomic DNA following published conditions (7), each product was spotted in duplicate onto nylon membranes (Hybond-N; Amersham Corp., Arlington Heights, IL). Spotted membranes were subjected to alkali denaturation, neutralized, and cross-linked with ultraviolet light. Each membrane was thereafter hybridized with oligonucleotide probes labeled with digoxigenin-ddUTP (Boehringer Mannheim Biochemicals, Indianapolis, IN), which corresponded to wild-type and mutant sequences (7). After hybridization in 5× SSC containing 5% (wt/vol) blocking reagent, 0.1% *N*-lauroyl-sarkosine, and 0.02% (wt/vol) SDS, for 2–3 h, the membranes were washed in 6× SSC with a stringency corresponding to the calculated melting temperature of the probe. Then the membranes were treated with anti–digoxigenin-AP and Fab fragments; chemiluminescent detection was performed according to the manufacturer's protocol.

Results

The *AGT* gene was examined in a sample of 105 subjects with a systolic BP > 160 mmHg and/or a diastolic BP > 95 mmHg at three or more visits at least 2 wk apart, and in 81 control subjects with normal blood pressure (see Methods). Allele T235 was significantly more common among hypertensive subjects than in controls (0.89 vs. 0.75, $\chi_1^2 = 11.3$, $P < 0.001$; Table I); 79% of cases and 54% of controls were homozygous for this allele. The frequency of allele T235 in normotensive Japanese was much higher than observed in normotensive Caucasians (0.75 vs. 0.36, $\chi_1^2 = 62.1$). There was no significant difference in the frequency of allele M174 among cases and controls (0.11 vs. 0.09, $\chi_1^2 = 0.77$); its frequency was similar to that observed in Caucasians. Genotypic proportions were in Hardy-Weinberg equilibrium in controls ($\chi_1^2 = 1.34$) as well as in cases $\chi_1^2 = 0.37$); however, the power to test deviations from such expected proportions is low, particularly when one of the homozygous class is rare.

Discussion

Retrospective case-control studies are notoriously susceptible to experimental bias (9). In particular, contrasts involving genetic variables such as genotypes or alleles are liable to cryptic ethnic or genetic stratification. However, genetic heterogeneity resulting from population subdivision, founder effects, or random drift is unlikely to account for the difference observed among cases and controls sampled in two large Japanese cities < 150 miles apart. The younger age of the normotensive controls compared with the hypertensive patients would be expected to deflate, rather than inflate, the observed association, as young normotensive subjects are still liable to develop hypertension later in life. Lastly, the association observed between T235 and pregnancy-induced hypertension in a sample of 41 Japanese women (8) provides independent corroboration.

A previous report (7) documented an association between plasma angiotensinogen and *AGT* genotypes at residue 235. Regrettably, the protocols for sample collection and storage used in this study precluded the accurate determination of plasma angiotensinogen concentrations by the indirect method. Although the pathophysiological significance of this association remains unclear, it would be of interest to test whether it is also observed among Japanese hypertensive subjects.

The association of T235 with hypertension in Japanese as well as in Caucasians suggests that this allele may behave as a global risk factor in humans. From the evidence obtained thus far, however, one cannot determine whether T235 exerts this effect directly, or whether it serves as a marker for one or more molecular variants of *AGT* that remain to be identified. The variant T235 was initially denoted M235T (7) because a methionine occurred at residue 235 in the first published DNA sequence of *AGT* (10) and because it occurred at higher frequency in the Caucasian subjects examined at the time. We have now observed that T235 is more common than M235 in all other ethnic groups examined, including Japanese, African-Americans, American Hispanics, and Native American (our unpublished observations). When a multiallelic DNA marker of *AGT* was examined (7), it was found that M235 occurred in association with a much more restricted range of marker alleles than T235. Together, these observations raise the possibility that T235 marks the original form of the gene.

The high frequency of T235 and the low prevalence of other risk factors such as obesity and atherosclerosis in Japan suggest that an *AGT*-related predisposition may contribute to a greater proportion of hypertensive cases among Japanese than among Caucasians. Under this hypothesis, cerebrovascular accidents may be a feature of the clinical course of *AGT*-dependent hypertension.

Because of the involvement of angiotensinogen in salt homeostasis, T235 may be a marker for a salt-sensitive form of essential hypertension. Epidemiological studies have documented a striking gradient of increasing prevalence of hypertension and stroke mortality from South to North Japan (11), which correlates with a parallel rise in average daily salt intake (4). In Japan as elsewhere, attempts to correlate individual salt intake with blood pressure within populations have yielded inconsistent results (12, 13). However, such studies have too little power to reveal an effect of the magnitude predicted by contrasts among populations (14), and commonly used assessments such as a single 24-h urine collection are poor indices of usual sodium intake (15, 16). Physiological studies with molec-

Table I. AGT Genotypes and Alleles at Residue 235 among Hypertensive (H) and Normotensive (N) Subjects

	Total	*AA*	*Aa*	*aa*	Total	*A*	*a*
H	105	2 (2%)	20 (19%)	83 (79%)	210	24 (11%)	186 (89%)
N	81	3 (4%)	34 (42%)	44 (54%)	162	40 (25%)	122 (75%)
			$\chi_2^2 = 12.92$			$\chi_1^2 = 11.29$	

Alleles are designated as *A* (M235) and *a* (T235), respectively.

ular markers of angiotensinogen may resolve these fundamental issues.

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References

1. Hirota, Y. 1969. Epidemiological study on cerebrovascular diseases and ischemic heart disease in Japan, based on mortality statistics, autopsy cases and population survey. *Jpn. Circ. J.* 33:1467–1471.

2. Menotti, A., A. Keys, H. Blackburn, C. Aravanis, A. Dontas, F. Fidanza, S. Giampaoli, M. Karvonen, D. Kromhout, S. Nedeljkovic, et al. 1990. Twenty-year stroke mortality and prediction in twelve cohorts of the Seven Countries Study. *Int. J. Epidemiol.* 19:309–315.

3. Watanabe, S. 1959. Outline of gerontology in Japan and some aspects of life span and causes of death in Japanese. *J. Gerontol.* 14:299–304.

4. Sasaki, N. 1964. The relationship of salt intake to hypertension in the Japanese. *Geriatrics.* 19:735–744.

5. Ward, R. 1990. Familial aggregation and genetic epidemiology of blood pressure. *In* Hypertension: Pathophysiology, Diagnosis and Management. J. H. Laragh and B. M. Brenner, editors. Raven Press, Ltd., New York. 81–100.

6. Sealey, J., and J. H. Laragh. 1990. The renin-angiotensin-aldosterone system for normal regulation of blood pressure and sodium and potassium homeostasis. *In* Hypertension: Pathophysiology, Diagnosis and Management. L. H. Laragh and B. M. Brenner, editors. Raven Press, Ltd., New York. 1287–1318.

7. Jeunemaitre, X., F. Soubrier, Y. V. Kotelevtsev, R. P. Lifton, C. S. Williams, A. Charru, S. C. Hunt, P. N. Hopkins, R. R. Williams, J. M. Lalouel, and P. Corvol. 1992. Molecular basis of human hypertension: role of angiotensinogen. *Cell.* 71:169–180.

8. Ward, K., A. Hata, X. Jeunemaitre, C. Helin, L. Nelson, C. Namikawa, P. F. Farrington, M. Ogasawara, K. Suzumori, S. Tomoda, S. Berrebi, et al. 1993. A molecular variant of angiotensinogen associated with preeclampsia. *Nature Genetics.* 4:59–61.

9. Feinstein, A. 1988. Scientific standards in epidemiologic studies of the menace of daily life. *Science (Wash. DC).* 242:1257–1263.

10. Kageyama, R., H. Ohkubo, and S. Nakanishi. 1984. Primary structure of human preangiotensinogen deduced from the cloned cDNA sequence. *Biochemistry.* 23:3603–3609.

11. Takahashi, E., N. Sasaki, J. Takeda, and H. Ito. 1957. The geographic distribution of cerebral hemorrhage and hypertension in Japan. *Hum. Biol.* 29:139–166.

12. Kihara, M., J. Fujikawa, M. Ohtaka, M. Mano, Y. Nara, R. Horie, T. Tsunematsu, S. Note, M. Fukase, and Y. Yamori. 1984. Interrelationship between blood pressure, sodium, potassium, serum cholesterol, and protein intake in Japanese. *Hypertension (Dallas).* 6:736–742.

13. Beilin, L. J. 1992. Dietary salt and risk factors for cardiovascular disease. *Kidney Int.* 41(Suppl. 37):90–96.

14. Frost, C. D., M. R. Law, and N. J. Wald. 1991. By how much does dietary salt reduction lower blood pressure? II. Analysis of observational data within populations. *Br. Med. J.* 302:815–818.

15. Liu, K., R. Cooper, J. McKeever, P. McKever, R. Byington, I. Soltero, R. Stamler, F. Gosch, E. Stevens, and J. Stamler. 1979. Assessment of the association between habitual salt intake and high blood pressure: Methodological problems. *Am. J. Epidemiol.* 110:219–226.

16. Luft, F. C., G. R. Aronoff, and N. S. Fineberg. 1986. Intra- and interindividual variability in sodium intake in normal subjects and in patients with renal insufficiency. *Am. J. Kidney Dis.* 7:375–380.