行政院國家科學委員會專題研究計畫成果報告

計畫名稱：有白袍高血壓之中國人的基因研究

Study on the gene of Chinese with white coat hypertension

計畫編號：NSC90-2314-B-002-200

執行期限：90 年 8 月 1 日至 91 年 7 月 31 日

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中文摘要

本研究在探討有白袍高血壓人之血管張力素原(angiotensinogen, AGT)之基因多態型(gene polymorphism)。目前共有 72 位有白袍高血壓者作為研究對象，其中男性 30 人、女性 42 人，年齡介於 22 至 72 歲。白袍高血壓之診斷依據隨機血壓及移動式高血壓。白袍高血壓之定義指隨機血壓異常昇高（收縮壓≥140mmHg 或舒張壓≥90mmHg 或兩者），而平均移動式血壓正常（收縮壓＜140mmHg 及舒張壓＜90mmHg）。血管張力素原基因之多形性由聚合酶鏈反應(polymerase chain reaction)來確定。

本文為初步結果，進一步分析等待完成。

關鍵詞：白袍高血壓，血管張力素原，基因多態型，聚合酶鏈反應

Abstract

This is to study the angiotensinogen gene polymorphism in subjects with white coat hypertension (WCH).

A total of 72 subjects with WCH were studied. They were 30 men and 42 women. Their age ranged from 22 to 72 years.

They were diagnosed by casual blood pressure and ambulatory blood pressure. The white coat hypertension is defined as an abnormally high causal blood pressure (SBP ≥ 140 mmHg or DBP ≥ 90 mmHg or both) and a normal ambulatory blood pressure average (SBP < 140 mmHg and DBP < 90 mmHg). The angiotensinogen gene polymorphism was identified by polymerase chain reaction.

Their genotype and polymorphism gene were analyzed.

This is a preliminary result. Further data analysis remains to complete.

Keywords: white coat hypertension, angiotensinogen, gene polymorphism, polymerase chain reaction.

Introduction

Hypertension has been one of the leading causes of morbidity and mortality of

adults in the developed countries. Anti-hypertensive strategy plays an important role on the prevention of the above outcomes. Nevertheless, there may be risks of therapy. To avoid labeling falsely and treating inappropriately the subjects is imperative. This is particularly true for the subjects with mild and labile hypertension. Correctly labeling individual as hypertension has medical, social and economic importance.

Some subjects are identified as having hypertension in the clinical setting but have normal ambulatory or self-measured blood pressure (BP) outside the physician's office. This type of hypertension is called white coat hypertension (WCH).[1-3] The long-term prognostic significance of WCH has been delated,[4-16] whether it needs to be treated is yet unclear.[2,4,9,13-18] Some reports showed that subjects with WCH do not differ from sustained hypertensive patients.[19,20] However, the etiology of WCH remains to be established.

Angiotensinogen (AGT) is a rennin substrate. It is converted into angiotensin I by rennin, and then into angiotensin II by angiotensin converting enzyme. This product exerts its physiologic effect on the regulation of vascular tone and salt-water homeostasis.[21] There are reports that the plasma concentration of AGT is correlated with blood pressure.[21,22] Transgenic animals carrying the AGT gene develop hypertension; the more copies of AGT gene the higher the blood pressure.[23] A molecular variant of AGT has been reported to be associated with preeclampsia.[24] Thus, the AGT gene is a good candidate for predisposition to human essential hypertension.

The AGT gene was recently cloned and its sequence was determined.[25] Fifteen molecular variants were found. Among them, the M235T and T174M variants have been reported to be associated with increased plasma levels of AGT and high blood pressure.[26] A linkage or association of the AGT gene with human essential hypertension in Caucasian was found by some investigation,[26-28] but Afro-Caribbean[29] and Japanese[30,31] population, others did not.[32-35] We also found the association of molecular variant M235T of the angiotensinogen gene with essential hypertension in Taiwanese.[36] Since no data has been reported on the association of angiotensinogen gene polymorphism with WCH, we carried out this study.

Materials and Methods

The subjects diagnosed to have mild hypertension without treatment at outpatient clinic and from a mass survey will be enrolled to this study. Signed informed consent will be obtained from all participators. Each participator will make 5 visits the study clinic over a 3-4 week periods. The following data will be collected: medical history with emphasis on cardiovascular diseases, height, weight, blood chemistry, standardized reading blood pressure measurement on sitting position, urinalysis, electrocardiogram, chest x-ray, echocardiogram, eye ground finding and 24-hour ABPM and polymorphism of gene.

Office BP will be measured in standardized fashion using appropriately sized cuff and a random-zero mercury sphygmomanometer. Systolic blood pressure (SBP) is recorded at Konotkoff phase 1, and diastolic blood pressure (DBP) at phase 5. The BP will be taken after at least 10 minutes of rest when subjects visit the clinic, and is defined as the average of two sitting blood pressure readings obtained at 2-minute intervals taken on the same arms.[37] All patients in this study will fulfill the following: (1) systolic blood pressure on at least three different clinic visits during a 3-4 week periods are 140 mmHg or higher, or diastolic blood pressure are 90 mmHg or higher or both; (2) no use of anti-hypertensive agents, psychotropic agents or sympathomimetics for at least one month prior to blood pressure measurement; (3) no use of caffeine containing materials and no smoking for at least 2 hours before blood pressure measurements, and (4) no DM, renal

disease, coronary or other organic heart disease or secondary hypertension.

24-hour ABPM will be carried out using a commercially available automated ambulatory BP recorder (Del Mar Avionics model 1990 pressureometer IV system). Monitoring will be done on a work day. All participants will be encouraged to pursue a variety or routine activities during monitoring. Each participant will keep a diary of his or her activities and sleep during monitoring. All participants will be instructed to stay still, with the forearm extended, during each reading. All ambulatory blood pressure (ABP) readings will be taken using the participant's nondominant arm, at a frequency of once every 15 minutes interval from 07:00 to 23:00 (daytime period) and 30 minutes interval from 23:00 to 07:00 (nighttime period). The accuracy of the recorder will be cross-checked against blood pressures measured manually through the same cuff system using a "Y" tube connected to a mercury sphygmomanometer at the beginning of the monitoring period. Only those data within 5 mmHg difference between these two measurements will be accepted as valid.

The data of ABPM will be analyzed by a microcomputer. Any ABP readings that showed an inconsistent increase or decrease in systolic/diastolic BP > 20 mmHg will be excluded in this analysis.[38] Tracings will be analyzed only if more than 85% of the maximal number of readings during the 24-hour period passes the deletion criteria. The respective mean ambulatory BP for wholeday, daytime and nighttime will be separately calculated. Blood pressure loads, blood pressure varieties and circadian blood pressure patterns will be analyzed. Seventy-two consecutive subjects who had abnormally elevated casual blood pressure (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or both) in the outpatient clinic but a normal ABP average (systolic ABP < 140 mmHg and diastolic ABP average < 90 mmHg) were included in this study.

Genomic DNA preparation and polymerase chain reaction amplification

Genomic DNA was extracted by a nonenzymatic method.[39] DNA fragments, including the M235T and T174M variants, were amplified by polymerase chain reaction (PCR). Forward and reverse primers were selected from the genomic sequence of AGT.[26] The forward primer sequence from +921 to +940 in exon 2 of the AGT gene was 5'-GAT GCG CAC AAG GTC CTG TC-3'; the reverse primer from +1225 to +1274 was 5'-GCC AGC AGA GAG GTT TGC CT-3'. The PCR mixture consisted of 0.5 μg DNA, 25 pmol of each primer, 0.15 nmol/l dNTP and 1U Taq polymerase in a final volume of 50 μl. The PCR was carried out in a Perkin-Elmer thermal cycler (Model 480; Perkin-Elmer, Norwalk, Connecticut, USA). The reaction condition was achieved first by denaturing for 3 min, and then repeating the following cycle: denaturing at 95°C for 1 min, annealing at 60°C for 45 s, and extension at 72°C for 1 min. This cycle was repearted 30 times with a final extension for 10 min. The 394 bp PCR product was revolved on a 2% ethidium bromidestained gel and purified by centrifugation through a paper slurry for sequencing.

DNA sequencing

Sequencing for molecular variants M235T and T174M was conducted by using a dye-terminator cycle sequencing method (ABD; Perkin-Elmer, Cetus, California, USA). Single-strand sequencing was carried out by PCR with sense primer as the sequencing primer. The PCR reagents and cycling conditions were the same as described above, except that dye-labeled ddNTP replaced unlabeled ddNTP and AmpliTaq-FS enzyme was used. The product was run in an automatic sequencing apparatus (ABI 373A sequencer) in a 6% denatured polyacrylamide gel at 1500 V and 40°C. The results were analyzed using incorporated sequencing analysis software (Version 2.01, ABD; Perkin-Elmer).

Statistics

The between-group demographic and laboratory data were compared with Student's unpaired *t* test for parametric data and with the χ^2 test. $P < 0.05$ was considered statistically significant. The odds ratio and 95% confidence interval were calculated using Woolf's method.

Preliminary Result

This preliminary result included 72 subjects with WCH. They were 30 men and 42 women. Their age ranged from 22 to 72 years. Most of them belonged to 40-59 years.

Their genotype and polymorphism of angiotensinogen gene will be analyzed.

Discussion

Jeunemaitre *et al*.[26,27] were the first to report the linkage of the molecular variants M235T and T174M with hypertension. Caulfield *et al*.[28,29] found an association between a CA repeat polymorphism of the AGT gene and essential hypertension, but not with the M235T and T174M variants. Kamitani *et al*.[30] and Hata *et al*.[31] also documented a positive association. A negative association has also been reported in several other studies.[32-35] The frequency of the 235T variant among normotensive Caucasians ans 13-16%; it was 45-63% among Japanese and 84% among Afro-Caribbeans. In our study,[36] the 235T frequency was 67% among normotensive Taiwanese.

The allele and genotype frequencies of the 235T variant were high among normotensives from Taiwanese population and the frequencies among hypertensives were even higher, approaching those of the Afro-Caribbeans and contrasting with those of the Caucasians.[36] The M235 genotype was rare both among normotensives and among hypertensives in our study. Whether this association of M235 genotype with ethnic origin is true for WCH remains to elucidate.

This is a preliminary report. Further data analysis remains to complete.

References

1. Cardillo C, De Felice F, Campia U, Folli G: Psychophysiological reactivity and cardiac end organ Pickering TG, James GD, Boddie C, Harshfield GA, Blank S, Laragh JH: How common is white coat hypertension? JAMA 259; 225-228, 1988.
2. White WB, Schulman P, McCabe EJ, Dey HM: Average daily blood pressure, not office blood pressure, determines cardiac function in patients with hypertension. J Am Med Assoc 261; 873-877, 1989.
3. Julius S, Mejia A, Jones K, *et al.*: "White coat" versus "sustained" borderline hypertension in Tecumseh, Michigan. Hypertension 617-623, 1990.
4. Cardillo C, De Felice F, Campia U, Folli G: Psychophysiological reactivity and cardiac end organ changes in white coat hypertension. Hypertension 21; 836-844, 1993.
5. Hoegholm A, Kristensen KS, Bang LE, Nielsen JW, Nielsen WB, Madsen NH: Left ventricular mass and geometry in patients with established hypertension and white coat hypertension. Am J Hypertens 6; 282-286, 1993.
6. Pierdomenico SD, Lapenna D, Guglielmi MD, Antidormi T, Schiavone C, Cuccurullo F, et al: Target organ status and serum lipids in patients with white coat hypertension. Hypertension 26; 801-807, 1995.
7. Cavallini C, Roman MJ, Pickering TG, Schwartz JE, Pini R, Devereux RB: Is white coat hypertension associated with arterial disease or left ventricular hypertrophy? Hypertension 26; 413-419, 1995.

8. Ferrara LA, Guida L, Pasanisi F, Celentano AI, Palmieri V, Iannuzzi R, et al: Isolated office hypertension and end organ damage. J Hypertens 15; 979-985, 1997.
9. Pickering TG, Friedman R: The white coat effect: a neglected role for behavioral factors in hypertension. In: McCabe PM, Schniderman N, Field TM, et al, eds. Stress, Coping and Disease. Hillsdale: LR Erlbaum, p. 35-49, 1991.
10. Soma J, Aakhus S, Dahl K, Slordahl S, Wiseth R, Wideroe TE, Skjaerpe T: Hemodynamics in white coat hypertension compared to ambulatory hypertension and normotension. Hypertension 10; 17-20, 1996.
11. Weber MA: Is white coat hypertension truly a benign condition? Presented at the American Society of Hypertension. Eighth Scientific Meeting;; 19-23 May 1993, New York.
12. Kuwajima I, Suzuki Y, Fujisawa A, Kuramoto K: Is white coat hypertension innocent? Structure and function of the heart in the elderly. Hypertension 22; 826-831, 1993.
13. Verdecchia P, Schillaci G, Boldrini F, Zampi I, Porcellati C: Variability between current definitions of "normal" ambulatory blood pressure. Implications in the assessment of white coat hypertension. Hypertension 20; 555-562, 1992.
14. Rizzo V, Cicconetti P, Bianchi A, Lorido A, Morelli S, Vetta F, Salza MC, Marigliano V: White-coat hypertension and cardiac damage in elderly subjects. J Human Hypertens 10; 293-298, 1996.
15. Flores L, Recasens M, Gomis R, Esmatjes E: White coat hypertension in type 1 diabetic patients without nephropathy. Am J Hypertens 13; 560-563, 2000.
16. Muldoon MF, Nazzaro P, Sutton-Tyrrell K, Manuck SB: White-coat hypertension and carotid artery atherosclerosis: a matching study. Arch Int Med 160; 1507-1512, 2000.
17. Kario K, Shimada K, Schwartz JE, Matsuo T, ToshideS, Pickering TG: Silent and clinically overt stroke in old Japanese subjects with white-coatand sustained hypertension. J Cardiol (Suppl) 39; 52-54, 2002.
18. Kristen KS, Hoegholm A, Bang LE, Gustavsen PH, Poulsen CB: No impact of blood pressure variability on microalbuminuria and left ventricular geometry: analysis of daytime variation, diurnal variation and "white coat" effect. Blood Pressure Monitoring 6; 125-131, 2001.
19. Pierdomenico SD, Mezzetti A, Lapenna D, Guglielmi MD, Mancini M, Salvatore L, Antidormi T, Costantini F, Cuccurullo F: White coat hypertension in patients with newly diagnosed hypertension: evaluation of prevalence by ambulatory monitoring and impact on coat of health care. Eur Heart J 16; 692-697, 1995.
20. Grandi AM, Broggi R, Colombo S, Santillo R, Inperiale D, Bertolini A, Guasti L, Venvo A: Left ventricular changes in isolated office hypertension. Arch Intern Med 161; 2677-2681, 2001.
21. Gardes J, Bouhnik J, Clauser E, Corvol P, Menard J: Role of angiotensinogen in blood pressure homeostasis. Hypertension 4; 185-189, 1989.
22. Menard J, El Amrani AK, Savoie F, Bouhnik J: Angiotensinogen: an attractive but underrated participant in hypertension and inflammation. Hypertension 18; 705-706, 1991.
23. Kim HS, Krege JH, Kluckman KD, Hagaman JR, Hodgin JB, Best CF, et al.: Genetic control of blood pressure and the angiotensinogen locus. Proc Natl Acad Sci USA 92; 2735-2739, 1995.
24. Ward K, Hata A, Jeunemaitre X, Helin C, Nelson L, Namikawa C, et al.: A

molecular variant of angiotensinogen associated with preeclampsia. Nature Genet 4; 59-61, 1993.
25. Gaillard I, Clauser E, Corvol P: Structure of human angiotensinogen gene. DNA 8; 87-99, 1989.
26. Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, et al.: Molecular basis of human hypertension: role of angiotensinogen. Cell 71; 169-180, 1992.
27. Jeunemaitre X, Charru A, Chatellier G, Dumont C, Saassano P, Soubrier F, et al.: M235T variant of the human angiotensinogen gene in unselected hyeprtensive patients. J Hypertens 11 (Suppl 1); S80-S81, 1993.
28. Caulfield M, Lavender P, Farrall M, Munroe P, Lawson M, Turner P, et al.: Linkage of the angiotensinogen gene to essential hypertension. N Engl J Med 330; 1629-1633, 1994.
29. Caulfield M, Lavender P, Newell-Price J, Farral M, Kamdar S, Daniel H, et al.: Linkage of the angiotensinogen gene locus to human essential hypertension in African Caribbeans. J Clin Invest 96; 687-692, 1995.
30. Kamitani A, Rakugi H, Higaki J, Yi Z, Mikami H, Miki T, et al.: Association analysis of a polymorphism of the angiotensinogen gene with essential hypertension in Japanese. J Hum Hypertens 8; 521-524, 1994.
31. Hata A, Namikawa C, Sasaki M, Sato K, Nakamura T, Tamura K, et al.: Angiotensinogen as a risk factor for essential hypertension in Japan. J Clin Invest 93; 1285-1287, 1994.
32. Rotimi C, Morrison L, Cooper R, Oyejide C, Effiong E, Ladipo M, et al.: Angiotensinogen gene in human hyeprtension. Lack of an association of the 235T allele among the African Americans. Hyeprtension 24; 591-594, 1994.
33. Morise T, Takeuchi Y, Takeda R: Rapid detection and prevalence of the variants of the angiotensinogen gene in patients with essential hyeprtension. J Int Med 237; 175-180, 1995.
34. Bennett CL, Schrader AP, Morris BJ: Cross section analysis of Met235→Thr variant of angiotensinogen gene in severe family hyeprtension. Biochem Biophys Res Commun 197; 833-839, 1993.
35. Fornage M, Turner ST, Sing CF, Boerwinkle E: Variation at the M235T locus of the angiotensinogen gene and essential hyeprtension: a population-based case-control study from Rochester, Minnesota. Hum Genet 96; 295-300, 1995.
36. Chiang FT, Hsu KL, Tseng CD, Hsiao WH, Lo HM, Chern TH, Tseng YZ: Molecular variant M235T of the angiotensinogen gene is association with essential hypertension in Taiwanese. J Hypertens 15; 607-611, 1997.
37. National high blood pressure education program coordinating committee. Fifth Joint National Committee Report on the Detection, Evaluation and Treatment of High Blood Pressure (JNCV). Arch Intern Med 153; 154-183, 1993.
38. Drayer JIM, Weber MA, DeYoung JL, Wyle FA: Circadian blood pressure patterns in ambulatory hypertensive patients. Am J Med 93; 493-499, 1982.
39. Lahri DK, Nurnberger JI Jr: A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucl Acid Res 19; 5444, 1991.

附件：封面格式

行政院國家科學委員會補助專題研究計畫成果報告

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計畫類別：☑個別型計畫　　□整合型計畫
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中　華　民　國　91　年　10　月　22　日