行政院國家科學委員會專題研究計畫　成果報告

有白袍高血壓之中國人的基因研究（II）

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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 （II）

Study on the gene of Chinese with white coat hypertension

(II)

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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)**之基因的分子變體（**molecular variant**）。目前共有** 64 **位有白袍高血壓人納入研究對象，其中有男性** 30 **位、女性** 34 **位，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血壓。白袍高血壓之定義是隨機血壓之收縮壓** ≥140mmHg **或舒張壓**

≥90mmHg，或兩者，而移動式平均血壓之收縮壓＜140mmHg 及舒張壓＜90mmHg。我們用染劑-終結物週期定序（dye-terminator cycle sequencing）之快速縮小定序法（mini-sequencing），同時測定每一受檢者之 AGT gene 之 M235T，T174M，G-217A，A-6G 分子變體。

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，分子變體，染劑-終結物週期定序。

Abstract

This is to examine the molecular variant of the angiotensinogen (AGT) gene in subjects with white coat hypertension (WCH).

A total of 64 subjects with WCH were studied. They were 30 men and 34 women. Their age ranged from 22 to 72 years. There were diagnosed by casual blood pressure and ambulatory blood pressure. The WCH 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). We created a rapid mini-sequencing method based on dye-terminator cycle sequencing to detect the M235T, T174M, G-217A and A-6G variants of the AGT gene for each subject.

The genotype and allele distribution of the M235T variant, the T174M, G-217A, and A-6G were analyzed.

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

Keywords: white coat hypertension, angiotensinogen, molecular variant, dye-terminator cycle sequencing.

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. 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 debated,[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.

There are reports that the plasma concentration of AGT is correlated with blood pressure.[20,21] Transgenic animals carrying the AGT gene develop hypertension; the more copies of AGT gene the higher the blood pressure.[22] A molecular variant of AGT has been reported to be associated with preeclampsia.[23] 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.[24] Fifteen molecular variants were found. Among them, the M235T, T174M, A-6G and G-217A variants have been reported to be associated with increased plasma levels of AGT and high blood pressure.[25] A linkage or association of the AGT gene with human essential hypertension in Caucasian was found by some investigation,[25-27] but Afro-Caribbean[28] and Japanese[29-31] population, others did not.[32-35] Genetic diversity also exists among different ethmic population.[36] Liu et al reported the association of the A-6G variant of the angiotensinogen gene and Chinese hypertensive.[37] We also found the association of molecular variant M235T and G-217A of the angiotensinogen gene with essential hypertension in Taiwanese.[38,39] Since no data has been reported on the

molecular variants of angiotensinogen gene in subjects with WCH, we carried out this study.

Materials and Methods

The subjects diagnosed to have mild hypertension without treatment at outpatient clinic. 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.[40] 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.[41] 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.[42] 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 sec, 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 64 subjects with WCH. They were 30 men and 34 women. Their age ranged from 22 to 72 years. Most of them belonged to 40-59 years.

Their genotype and polymorphism of molecular variants of angiotensinogen gene will be analyzed.

Discussion

Many association studies of G-6A and M235T variants with essential hypertension in various races were examined, but the results were discordant, especially in Asian and African populations compared with Caucasian population.[43-45] The allele frequency of A-6G has big difference the Asians and the whites.[45,46]

Jeunemaitre et al[25,26] were the first to report the linkage of the molecular variants M235T and T174M with hypertension. Caulfield et al[27,28] 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[29] and Hata et al[30] 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,[38] 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.[38] The M235 genotype was rare both among normotensives and among hypertensives in our study. Whether the association of the molecular variant of the

angiotensinogen gene with ethnic origin is true for WCH remains to elucidate..

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

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