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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※　　**中國人之白袍高血壓的研究（第四年計劃）**　　※
※　　Study on White Coat Hypertension in Chinese (IV)　　※
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計畫類別： o**個別型計畫**　　□**整合型計畫**
計畫編號：NSC89－2314－B－002－407
執行期間：89 **年** 8 **月** 1 **日至** 90 **年** 7 **月** 31 **日**

計畫主持人：曾淵如　教授

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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：中國人之白袍高血壓的研究（第四年計劃）

Study on White Coat Hypertension in Chinese (IV)

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中文摘要

本研究在探討有白袍高血壓人之昇壓素轉化胺(angiotensin converting enzyme)**之基因多態型**(gene polymorphism)**，目前共有** 67 **位有白袍高血壓者作為研究對象，其中男性** 28 **人、女性** 39 **人，年齡介於** 22 **至** 72 **歲。白袍高血壓之診斷依據隨意血壓及移動式高血壓，昇壓素轉化胺基因之** I/D **多態型由聚合胺鏈反應**(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，昇壓素轉化胺，基因多態型，聚合胺鏈反應

Abstract

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

A total of 67 subjects with WCH were studied. They were 28 men and 39 women. Their age ranged from 22 to 72 years.

They were diagnosed by casual blood pressure and ambulatory blood pressure. The I/D polymorphism of the angiotensin converting enzyme (ACE) gene was identified by polymerase chain reaction.

Their genotype and polymorphism of ACE gene were analyzed.

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

Keywords: white coat hypertension, angiotensin converting enzyme, gene polymorphism, polymerase chain reaction.

Introduction

Hypertension has been one of major risk factors for premature death and disability of human. Anti-hypertensive strategy plays an important role on the prevention of the above outcomes. Nevertheless, there may be risks of therapy. It is necessary to avoid labeling falsely and treating inappropriately the subjects. This is particularly true for the subjects with mild and label 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.[2,4,9,13-16] Subjects with WCH do not differ from sustained hypertensive patients as regard demographic data.[17] However, the etiology of WCH remains to be established.

Renin-angiotensin system has been considered as one of the important mechanisms for hypertension. Angiotensin converting enzyme (ACE) is a key enzyme that converts angiotensin I to angiotensin II and catalyzes bradykin, which plays important roles in cardiovascular homeostasis.[18] An insertion/deletion (I/D) polymorphism has been

found in intron 16 of the ACE gene has been found to be linked with myocardial infarction[18,20,21] and other cardiovascular disease.[22-24]

The I-polymorphism of the ACE gene was first demonstrated to be associated with essential hypertension in an Australian population[25] but could not be proven in other Australian population,[26,27] American,[28] Japanese,[29] European population,[30-32] or Taiwanese.[33] On the other hand, the D-polymorphism was reported to be a risk factor for essential hypertension in Japanese[34] and African-American population,[35] but not in Taiwanese.[33] Since no data has been reported on the association of ACE 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.[36] 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.[37] 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.

Heparinized blood of participates is collected and leukocyte DNA is extracted by an automated extractor (Applied Biosystem, USA) or a DNA isolation kit (Gentra System, USA). The I/D polymorphism of ACE gene was determined by polymerase chain reaction (PCR) as described by Rigat *et al.*[38] Briefly, the PCR primers were: 5'CTGGAGACCACTCCCATCCTTTCT-3'

(sense primer) and 5‘GATGTGGCCATCACATTCGTCAGAT-3’ (antisense primer). The PCR was carried out in a Perkins-Elmer thermal cycler (model 480) with denaturing at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min. In total, 30 cycles with a final extension of 10 min were done. The I/D alleles were determined by 2% agarose gel electrophoresis and ethidium bromide stains. Each PCR reaction was controlled by a pair of known I- and D-allele samples. Unclear results were repeated until unequivocal.

Plasma ACE activity was assayed spectrophometrically using a commercially available ACE assay kit (Sigma, USA). Briefly, the principle is based on the hydrolysis of Furylacryloylphenylalanylglycylglycine to furylacryloylphenylalanine and glycylglycine which results in a decrease in absorbance at 340 nm. The ACE activity (U/L) in the heparinzed plasma is determined by comparing the sample reaction rate to that obtained with the ACE calibrator. Details of the procedure have been described by Holmquist *et al.*[39]

Preliminary Result

This preliminary result included 67 subjects with WCH. They were 28 men and 39 women. Their age ranged from 22 to 72 years. Most of them belonged to 40-59 years.

Their genotype and polymorphism of ACE gene will be analyzed.

Discussion

The results for the association of ACE gene polymorphism with essential hypertension were diverse.[25-35] Our previous report[33] showed lack of association of the ACE gene polymorphism with essential hypertension in a Chinese population.

Zee *et al.* first reported the association of ACE gene I allele with high blood pressure. In their study, the I allele was found in 56% of hypertensive and 41% of normotensive.[25] However, studies in other population[26-32] showed different results. In these reports, the I allele was found in about 40-50% of normotensive white populations and 60-70% of normotensive oriental populations.[29] In our previous study, this I allele frequency was 58%.[40] In this study, the I allele was found in 55% of subjects with WCH.

Morise *et al.* found that the D allele was more frequent in hypertensive Japanese.[34] Duru *et al.* had a similar result in an African-American population. Most other reports showed negative results.

Barley *et al.*[41] found that I allele frequency was higher in Samoan Polynesians and Yanomami Indians than white Europeans and black Nigerians and concluded that ACE gene polymorphism was associated with ethnic origin whether it is also true for WCH remains to elucidate.

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

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