行政院國家科學委員會補助專題研究計畫成果報告

血管張力素元與張力素轉化酶基因多態型在本態性高血壓疾病預測上之互相作用：haplotype 分析

Interaction of Angiotensinogen and Angiotensin-converting Enzyme Gene Polymorphisms in Predicting Essential Hypertension: a haplotype analysis

計畫類別： 個別型計畫

計畫編號： NSC90-2314-B-002-214

執行期間： 90 年 8 月 1 日 至 91 年 7 月 31 日

計畫主持人：許寬立

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一、中文摘要：

本研究目的在於探討血管張力素元、張力素轉化┊與第一型張力素受器基因之 haplotype 是否與本態性高血壓有關。方法：共有 408 位病人與 286 位正常人鑑定上述三種基因之多態型。利用 permutation 為基礎的假設分析三種基因 haplotypes 與高血壓之關連，並分析個別基因之互相作用。結果發現血管張力素元基因之 haplotype 與高血壓有關。而此種關連只見餘於張力素轉化┊基因為 II 型者。因此高血壓之關連研究，有基因之間的互相作用存在。

關鍵詞：血管張力素元，張力素轉化┊，高血壓，基因型，互相作用

二、英文摘要：

There are many reports demonstrating the association of renin-angiotensin system gene polymorphisms with hypertension in different populations. In the present study, we used haplotype analyses of the angiotensinogen gene with a new permutation-based hypothesis testing method to determine the association between multilocus angiotensinogen gene polymorphisms and hypertension in a relatively homogeneous Taiwanese population。 We also genotyped angiotensin converting enzyme gene insertion/ deletion polymorphism and angiotensin II type I receptor gene A1166C polymorphism to detect epistatic gene-gene interactions. There were 408 patients with hypertension (hypertensives) and 286 controls. The angiotensinogen gene haplotype frequencies were significantly different between hypertensives and controls, and this finding was only present in subjects with angiotensin converting enzyme gene II genotypes when the analysis was stratified by genotype of this polymorphism. In addition, the angiotensinogen gene hyplotype structure of hypertensives was more heterogeneous than that of controls. Our results showed that angiotensinogen gene haplotypes were associated with hypertension, and might play a synergistic action with I allele of angiotensin converting enzyme gene.

Key words:Angiotensinogen, Angiotensin-converting enzyme, Hypertension, Haplotype,Interaction

三、緣由與目的：

The renin-angiotensin system (RAS) genes have been most extensively studied as hypertension candidate genes. However, the results are different in different populations or studies. For example, the insertion/deletion (I/D)

polymorphism in intron 16 of the angiotensin converting enzyme (ACE) gene showed and association with hypertension in Japanese[1]， but not in Chinese[2]. A coding polymorphism of the angiotensinogen (AGT) gene (M235T) showed an association with hypertension in Chinese[3], but not in Japanese[4]. The A1166C transversion in the 3' untranslated region of the angiotensin II type I receptor (AT_1R) gene has also shown an association with hypertension in a Caucasian population[5], but not in another study with a different Caucasian population[6].

The causes of these discrepancies are multiple. The most important one is that hypertension is a polygenic disorder, but not a monogenic trait. One gene may be responsible for the disorder in one population, but not necessarily in another population. Other possible reasons include a spurious association caused by population stratification, epistatic gene-gene interactions when the effect of the studied gene is masked by the effect of other susceptible genes, or that a specific multilocus haplotype rather than any of the single loci which define the haplotype is more significant to determine the association[7].

Genotyping of multiple diallelic sites, especially dense single nucleotide polymorphism (SNP) sites, is now available for genetic studies of human disease. It is more powerful too focus on the transmission of multilocus haplotypes, as opposed to alleles at individual loci. Therefore, haplotype analysis is mandatory in this regard, and has become an increasingly popular tool for population-genetic studies and disease-gene discovery.

However, its application is limited by its complex statistical work and the requirement of either laboratory-based chromosome isolation or recruitment of family members for phase information, which is laborious and usually not available. Therefore, the wealth of haplotype information has created a need for efficient statistical methods for estimation haplotype frequencies from a sample of genotyped but unphased diploid individuals. A number of methods have been described for estimating haplotype frequencies. Among them, the maximum-likelihood estimation method based on expectation maximization (EM) algorithm[8] can accommodate several loci with an arbitrary number of alleles, and its accuracy has been validated by several studies[9,10].

Recently, this method used with permutation-based hypothesis testing has been documented to be a powerful method to identify disease-predisposing haplotypes in a case/control design form individuals inn large, freely mixing populations[11]. Accordingly, we applied this powerful method to demonstrate the association of specific AGT gene haplotypes with hypertension in a relatively homogeneous Taiwanese population with a large sample size. In

addition, we also genotyped ACE gene I/D polymorphism and AT_1R gene A1166C polymorphism simultaneously to detect any epistatic gene-gene interaction between these genes.

四、結果與討論：

The results of the single-locus analyses showed that the ACE gene I allele and AGT gene C-20 variant were significantly associated with hypertension by both genotype and allele frequency analyses.

Haplotypes of the AGT gene and their association with hypertension

Six-locus haplotype frequency for the AGT gene in the hypertensives and normotensives was analyzed. The omnibus haplotype profile test[11] was significant ($\div^2$=53.96, p=0.001), which indicated the overall haplotype frequency profiles were different between the hypertensives and normotensives, and thus there might be some disease-predisposing or protecting haplotypes in the hypertensives.

Accordingly, in the individual haplotype analyses, we identified five haplotypes (GGAGCC,GGCACC,GGCATC,GACACC and GAAACC) with significantly higher or lower haplotype frequency in the hypertensives than in the normotensives at the significance level $p<0.05$, but the differences were not significant after the use of stringent Bonferroni's correction for multiple tests ($p<8\times10^{-4}$). There were also several haplotypes which were only found in the hypertensives, such as GGAATT and GGAGTT although their frequencies were not as high as too a significant level by permutation test. In other words, the haplotype structure of the hypertensives was more heterogeneous than that of the normotensives.

Gene-gene interactions

In the single-locus analysis, ACE gene I allele and AGT gene C-20 allele were associated with hypertension. No significant difference of odds ratio ofr hypertension with at least on copy of I allele was noted when stratified on AGT gene A-20C polymorphism. There was also no significant difference of the analysis of A-20C polymorphism with hypertension when stratified on ACE gene I/D polymorphism (II and ID+DD). The significance level was $p<0.025$ (0.05/2) since 2 stratifications were performed.

When the patients were stratified according to ACE gene I/D genotype, the number of patients with II genotype was greatly decreased, and was lower than that with II+DD genotypes. However, the association of the AGT haplotypes with hypertension was only found in patients with II genotype at the significance level $p<0.05$, but not at $p<0.025$. No similar finding was noted when stratified by AT_1R gene A1166C genotype.

There were several advantages in our study. First we selected the polymorphism in the promoter region of AGT gene based on functional transcription studies, although many of

the other polymorphisms may serve as only markers without functional significance. Second, we developed and used rapid mini-sequencing method to detect diallelic polymorphisms of AGT gene in every subject[3]. This PCR direct-sequencing method decrease inaccuracy of genotyping by PCR-RFLP owing to incomplete cutting. Furthermore, we used stepdown PCR, which increased the detection accuracy of ACE gene I/D heterozygotes[12]. Third, the accuraacy of haplotype frequencies estimation by EM-based maximum likelihood method has been validated in many studies[9,10]. The accuracy of this estimation depends on several conditions, such as sample size, the number of locus, and dispersion of haplotype frequency values. Many of these conditions were fulfilled in our study. Nevertheless, there are also limitations in our studies. First, the normotensive subjects were relatively young compared with the hypertensives. This age difference might reduce the power of our study due to some younger age normotensives might develop hypertension later in life. Second, many LDs between unlinked loci were found in hypertensives. Although they most likely resulted from epistatic gene-gene interaction[13], the problem of selective population stratification in hypertensives could not be definitely excluded. The use of microsatellite markers unlinked to hypertension candidate genes are necessary to exclude this possibility[14] However, our study was conducted in a relatively homogenous Taiwanese population, and we did not find any LD in pairs of unlinked loci in the normotensives. The possibility of selective population stratification solely in the hypertensives was low in a relatively homogenous population. Third, we only demonstrated the epistasis of RAS gene statistically and obtained a p value that was only borderline significant after multiple-test correction.

五、計畫成果自評：

本計畫未達原計畫目標。因為冠動脈支架術之再狹窄，有許多病例無法再以原來之血管攝影片重新評估，而新病例之收集緩慢。故另以本同步研究繳交成果。本報告為一系列 RAS 系統與心血管病(包括高血壓與冠動脈硬化)之關連。本成果已投稿中。

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