行政院國家科學委員會補助專題研究計畫成果報告

血管張力素元基因啟動區之多態型與轉譯活性之關係

Polymorphisms at the promoter region of angiotensinogen and its transcriptional activity

計畫類別： 個別型計畫

計畫編號： NSC90-2314-B-002-234

執行期間： 90 年 8 月 1 日 至 91 年 7 月 31 日

計畫主持人： 江福田

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中華民國 91 年 12 月 30 日

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一、中文摘要：

目的：研究血管張力素元基因啟動區 G-6A 與 A-217G 之多態型與轉譯能力之關係。方法：將血管張力素元基因 -614~+41 之片段選殖，放入 pSEAP2 之表現載体內，在細胞株 HuH7 及 HePG2 內進行体外表現試驗。結果：-217A 比-217G 表現較強的轉譯能力($p=2.58\times10^{-4}$ 在 HuH7;$p=1.2\times10^{-8}$ 在 HePG2);而-6G 比-6A 表現較強的轉譯能力($p=1.46\times10^{-7}$ 在 HuH7;$p=3.89\times10^{-9}$ 在 HePG2)

關鍵詞：血管張力素元，啟動區，多態型，轉譯活性

二、英文摘要：

Object: To examine the basal transcription activity of G-6A and G-217A variants of AGT gene in mammalian cell system. Methods: The promoter activity was studied by cloning promoter region (-614~+41) of AGT into pSEAP2-basic reporter vector and performing a transient transfection assay in HuH7 and HepG2 cells. Results: The –217A expressed higher transcription activity than the –217G in both HuH7 and HepG2 cells; while the –6G expressed higher activity than the –6A.

Keywords: Angiotensinogen, Promoter, Polymorphism, Transcription activity

三、緣由與目的：

Angiotensinogen(AGT), produced mainly in the liver, is cleaved by renin to form aangiotensin I, the precursor to angiotensinII.. The level of AGT is the rate-limiting for the generation of angiotensin II[1,2] and has been shown directly related to arterial blood pressure[3-5]. Many previous studies showed an association between AGT polymorphism and hypertension, but such a relationship remains controversial.[6-11] Among the polymorphisms of AGT gene, G-6A and M235T have been extensively studied. Both polymorphisms are in complete linkage dysequilibrium among the different ethnic populations. The molecular mechanism leading to increased plasma AGT level cannot explained solely on the M235T variance. A recent study suggests that the G-6A variants can affect the basal ttranscription.[12] We have previously found a significant association between G-217A polymorphism and hypertension in a Taiwanese population.[13] We thus plan to investigate the functional significance of this variant by performing a transient transfection assay

in mammalian cell system.

四、結果與討論：

To investigate the basal transcriptional activity of G-217A, 4 ug of each reporter constructs with 1 ug of internal control plasmids(pCAT) were cotransfected into HuH7 and HepG2 cells under the same condition. The –217A construct expressed higher activity than the –217G ($p<0.001$) in HuH7 cell. Similar results were obtained in HepG2 cell($p<0.001$). The relatively increased expression of –217A than –217G in independent experiments was 17% and %!% in HuH7 and HepG2 cell respectively. The –6G promoter variant expressed higher activity than the –6A in both cell lines($p<0.0001$).

Our results showed that the –6G variant expressed higher transcription than the –6A, which is different from a previous report.[12] The possible reason might be due to different expression construct and assay system. Our constructs are longer. Some previous studies have found that the existence of multiple positively or negatively cis-acting control elements affect the basal constitutive expression.[14-17] The promoter length indeed affect the basal transcription. The functional significance of G-217A was also found in a previous report.[13] A study suggested that there is a potential hormone response element located between –203 and –217 positions in the human AGT gene.[16] Jain et al found a C/EBP family transcriptional factor strongly bound to –217A promoter of AGT gene. Those evidences support that the –217A variant has functional meanings and can be a risk marker for cardiovascular diseases.

五、計畫成果自評：

本計畫成果已達計畫預期目標，並已寫成論文投稿。本計畫 G-217A 之變異為一新發現，當繼續研究其與心血管疾病之關連。

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