人類 C-C 趨化激素 RANTES/CCL5 Promoter 區域之單一核苷;酸變異情形與僵直性脊椎炎之探討

Single Nucleotide Polymorphism of the Human C-C Chemokine RANTES/CCL5 Promoter Region in Ankylosing Spondylitis

中文摘要

背景：僵直性脊椎炎(Ankylosing spondylitis, AS)爲慢性發炎性自體免疫疾病，其致病原因至今尚未完全明瞭，但與多重基因以及環境因素有著密切關係。目的：探討 C-C 趨化激素（chemokines）中 RANTES/CCL5[Regulated on Activation Normal T-cell Expressed and Secreted/ Chemokine (C-C motif) ligand 5]基因 promoter 區域-403 及-28 位置的單一核苷酸變異（Single nucleotide polymorphism, SNP）與僵直脊椎炎之間的關聯，以及分析此二 SNPs 在台灣漢人（正常對照組）與台灣原住民種族之間的差異性。方法：1)利用直接定序（direct sequencing）的方式來建立 200 名正常台灣漢人以及 58 名台灣原住民的 RANTES promoter 基因之 SNPs 基因型資料庫；2)以 Real-Time PCR 方式來進行 118 名 AS 患者 RANTES promoter 之單一核甘酸變異的確認工作，並以 Pearson χ 2 test 以及 Fisher’ s exact test 統計學方法評估-28 及-403 位置之 SNPs 是否與僵直性脊椎炎有關，以及確認不同種族及不同性別之間的 SNPs 的分布是否有差異。3)將三種族群之血漿進行 ELISA 測定血中 RANTES 蛋白質的濃度分布，並以 Student’ s t test 評估不同種族之間的差異性是否有意義。結果：僵直性脊椎炎患者血漿中 RANTES 蛋白質（103.58±7.46 ng/ml）的表現比正常對照組（8.04±0.83 ng/ml）濃度高出許多（p=0.001），然而在 RANTES 基因 promoter 區域-403 及-28 位置之基因型之分布均無統計學上差異（p>0.05）。在台灣漢人與台灣原住民之間 RANTES 基因 promoter 區域-403 位置 GG（ χ 2=5.694，p=0.017）及 AA（p=0.006）之基因型分布上是有差異的，此外 A allele frequency 也是有分布差異的（ χ 2=2.951，p=0.043），然而-28 位置之基因型則無分布上的差異。結論：台灣漢人與台灣原住民由於種族上的不同造成 RANTES 基因 promoter 區域-403 位置基因型的分布有所差異，但不影響血漿中蛋白質的濃度分布；依目前實驗結果，僵直性脊椎炎與 RANTES 基因 promoter 區域 SNPs 似乎是無關連的，但血漿中 RANTES 蛋白質的濃度顯著上升顯然是受到其他因子調控而在慢性發炎反應中扮演一定角色。

英文摘要

Background: Ankylosing spondylitis(AS) is a chronic inflammatory autoimmune disease that is found associated with multiple genetic and environment risk factors. The cause for AS is yet to be identified. Objective: To test whether the SNPs in C-C chemokine RANTES/CCL5 [Regulated on Activation Normal T-cell Expressed and

Secreted/ Chemokine (C-C motif) ligand 5] gene promoter region —403 and —28 positions are associated with AS or not, and whether the SNP genotypes of Taiwanese population (control subjects) is different from Aboriginal Taiwanese. Methods：i)To establish the RANTES/CCL5 gene promoter region SNP genotype database of 200 Taiwanese population(control subjects) and 58 Aboriginal Taiwanese by auto-sequencing, ii)to validate the SNP genotypes of 118 AS patients using real-time PCR. Pearson$\chi 2$ test and Fisher's exact test are performed to assess if there is significant genotype distribution between different races, and between normal and disease subjects, iii)to analyze the RANTES/CCL5 protein level in plasma of Taiwanese population (control subjects), Aboriginal Taiwanese, and AS patients by ELISA. Student's t test is performed to assess if there is statistical significant difference among different populations. Results: The genotype GG($\chi 2$=5.694, p=0.017) and AA(Fisher's exact test p=0.006)distribution in RNATES/CCL5 gene promoter region -403 position are strikingly different between Taiwanese population (control subjects) and Aboriginal Taiwanese. The A allele frequency ($\chi 2$=2.951, p=0.043)is also significantly different. However there is no statistical significant difference($p>0.05$) at —28 position. There is also no statistical significant difference in —403 and —28 SNP genotype distributions between Taiwanese population (control subjects) and AS patients. Yet the RANTES/CCL5 protein level of plasma in AS patients(103.58±7.46 ng/ml) is higher than that of Taiwanese population (control subjects) (8.04±0.83 ng/ml, Student's t test =0.001).Conclusion: The RANTES/ CCL5 gene promoter region —403 position genotype distribution is different between Taiwanese population (control subjects) and Aboriginal Taiwanese, but there is no difference in protein level in plasma. Although the SNPs in RANTES/CCL5 gene promoter region is found not likely associated with AS, we suggest that there is/are other factor(s) regulating the RANTES/CCL5 protein secretion, which is possibly playing an important role in this chronic inflammatory disease.