行政院國家科學委員會專題研究計畫　成果報告

探討 GSTT1 基因多型性與宜蘭地區砷暴露相關之糖尿病的關係

計畫類別：個別型計畫
計畫編號：NSC92-2320-B-002-156-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：國立臺灣大學醫學院內科

計畫主持人：曾慶孝

計畫參與人員：曾慶孝

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 94 年 2 月 2 日

行政院國家科學委員會專題研究計畫成果報告

計畫名稱：探討 GSTT1 基因多型性與宜蘭地區砷暴露相關之糖尿病的關係

計畫編號：NSC 92-2320-B-002-156

執行期限：92 年 08 月 01 日至 93 年 07 月 31 日

主持人：曾慶孝 台大醫院內科部

一、中文摘要

本研究以宜蘭縣曾由飲用水中暴露於無機砷的居民為對象，探討 glutathione S-transferase (GST) T1 基因型與糖尿病的可能相關性. 總計完成651位受檢者的GST T1 基因型分析，其中 78 位患有糖尿病，另外 573 位為無糖尿病之對照組。GST T1 基因型以聚合胸鏈鎖反應方式進行，且分為 null type 及 non-null type 二型。結果顯示在年齡、性別及身體質量指數匹配之情況下，對照組之 null type 盛行率為 58.3%，而糖尿病組則為 46.2% ($p<0.05$),顯示 GSTT1 non-null type 與糖尿病的發生有關。未來我們須要更大樣本之進一步分析，並針對砷代謝產物及解毒過程對疾病的發生相關性研究，將有助於進一步釐清砷引起之健康危害的影響。

關鍵詞：糖尿病，環境污染物，砷，聚合胸鏈鎖反應，分子生物學

Abstract

This study evaluated the possible association between diabetes mellitus and the glutathione S-transferase (GST) T1 genotype in residents of the Ilan county, who showed history of arsenic exposure from drinking well water. A total of 651 subjects were studied. Among them, 76 were diabetic and 573 were not diabetic. GST T1 genotyping was performed by a polymerase chain reaction technique and the genotypes were classified as null and non-null types. Results showed that with comparable age, sex and body mass index, GST T1 null type was present in 58.3% of the non-diabetic subjects and 46.2% of the diabetic patients ($p<0.05$), suggesting an association between non-null type GSTT1 and diabetes mellitus. Further studies with larger sample size or studies involving the metabolites of arsenic and the detoxification procedures are required to clarify the role of arsenic on the impact of human health hazards.

Keywords: diabetes mellitus, environmental pollutants, arsenic, polymerase chain reaction, molecular biology

Introduction

Diabetes mellitus is a heterogeneous disease. Although family history, obesity and physical inactivity, etc., are strong risk factors, exposure to or deficiency of various chemicals, metals, essential elements and micronutrients have been implicated as causes of diabetes mellitus [1-2]. In our previous cross-sectional and cohort studies carried out in blackfoot disease hyperendemic villages located on the southwestern coast of Taiwan, we have shown that both prevalence and incidence of diabetes mellitus in these arseniasis-hyperendemic villages are significantly higher than general population and control areas in Taiwan [3-5]. Dose-response relationship has also been found between arsenic exposure and prevalence and incidence of type 2 diabetes mellitus [3-5]. Studies have suggested that arsenic methylation capability and body retention was modified by genetic polymorphism of glutathione S-transferase (GST) [6]. Therefore, the genetic factor of GST may affect the development of arsenic-induced diabetes mellitus. However, no study has been performed to address this possibility. The purpose of this study was to evaluate whether GST T1 genotype polymorphism could be associated with arsenic-related diabetes mellitus.

Material and Methods

Study subjects--- A total of 651 residents of the Ilan county with history of exposure to

arsenic-containing well water were recruited in this study. Among them, 78 were identified as diabetic patients and 573 were not. The diagnosis of diabetes mellitus was based on a current treatment with anti-diabetic agents or under insulin injection or a fasting plasma glucose level ≥126 mg/dL. A baseline questionnaire was interviewed to obtain the information of duration of well water consumption, residential history, sociodemographic characteristics, cigarette smoking, alcohol consumption, physical activities, history of sun exposure, as well as personal and family history of hypertension, diabetes mellitus, cardiovascular disease, coronary artery disease, and various cancers.

Isolation of genomic DNA --- Genomic DNA was isolated as described with some modification [7,8]. Five ml whole blood was mixed with 5 ml TKM1 buffer (10 mM Tris-HCl, pH 7.6, 10 mM KCl, 10 mM $MgCl_2$, and 2 mM EDTA) and 125 µl Nondet P-40. The mixture was centrifuged at 2,200 rpm 10 min, room temperature. The pellets were washed several times with 5 ml TKM1 buffer until all the RBC are lysed. Then TKM2 buffer (10 mM Tris-HCl, pH 7.6, 10 mM KCl, 10 mM $MgCl_2$, 2 mM EDTA, and 0.4 M NaCl) and 10% SDS was added and incubated at 55^0C 10 min. After adding 0.3 ml 6 M NaCl, the mixture was centrifuged at 12,000 rpm for 5 min. The supernatant was precipitated with 2 volume of 100% ethanol. With two washes of 70% ethanol, the DNA pellets were resuspended in appropriated volume of TE buffer. The OD value of 260 nm was analyzed to determine the DNA concentration.

Genotypes of GST T1 --- Polymerase chain reaction was used to amplify DNA for GST T1. Briefly, PCR was performed with 1 µg genomic DNA, 100 ng of each primer, 200 µM dNTPs, 1X PCR reaction buffer, 1.5 units of Taq DNA polymerase (Life Technologies, Rockville, MD), 2 mM $MgCl_2$ and distilled water in a final volume of 20 µl. The reaction mixture was placed in a thermal cycle for 5 min at 94^0C, and then subjected to 35 cycles of 94^0C for 30 s, 60^0C for 30 s, and 72^0C for 1 min followed by a final step at 72^0C for 5 min. The 2 primers for the PCR are: 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and 5'-TCA CCG GAT CAT GGC CAG CA-3'.

Statistical analysis --- Allele frequency means the number of occurrences of the test allele in the population divided by the total number of alleles. Results obtained from the diabetic and non-diabetic groups were compared using the x^2-test to show statistical significance. A *P* of less than 0.05 was regarded as statistically significant.

Results

With comparable age, sex and body mass index, GSTT1 null type was present in 58.3% of the non-diabetic subjects and 46.2% of the diabetic patients ($p<0.05$), suggesting an association between non-null type of GSTT1 and diabetes mellitus.

Discussion

The onset of clinical diabetes mellitus is a consequence of interaction between genetic and environmental factors. Besides family history, obesity and physical inactivity, trace elements and micronutrients have been implicated as environmental factors that might contribute to its development. Arsenic has been recently suggested as diabetogenic in high exposure group from epidemiological studies. However, the role of interaction between arsenic and the role of GST gene polymorphsim on the development of diabetes mellitus is not known. The confirmation of its diabetogenic effect and elucidation of its mechanism will surely contribute to the implementation of preventive measures in the grounds of public health and scientific interest.

GST is a large family of detoxification enzymes that catalyze the conjugation of reduced glutathione to a wide spectrum of hydrophobic and electrophilic compounds. They play important roles in protection

mechanisms against chemical carcinogenesis [6]. Previous studies have shown that homozygous null genotype of GST M1 is more common among patients affected with cancers of the lung, liver, bladder and colorectum. Patients with skin cancer unrelated to chemical carcinogen exposure were found to have a high frequency of null genotype of GST M1 than healthy controls. The association between the GST M1 gene polymorphsim and arsenic-related diabetes mellitus were not observed in our previous study carried out in the residents living in the Ilan county, but this study showed that GSTT1 non-null type could be associated with diabetes mellitus. A larger sample size and the inclusion of other subclasses of the GST superfamily such as the GST P are required to further clarify the effect of GST on the impact of arsenic on human health.

References

1. Mooradian AD, Failla M, Hoogwerf B, Maryniuk M, Wylie-Rosett J. Selected viatmins and minerals in diabetes. Diabetes Care 1994; 17: 464-79.
2. Alberti KGMM, Zimmet PZ for the WHO Consultation. Definition, diagnosis and classification of diabetes mellitus and its complications, part 1: diagnosis and classification of diabetes mellitus, provisional report of a WHO consultation. Diabetic Med 1998; 15: 539-53.
3. Lai MS, Hsueh YM, Chen CJ, Shyu MP, Chen SY, Kuo TL, Wu MM, Tai TY. Ingested inorganic arsenic and prevalence of diabetes mellitus. Am J Epidemiol 1994; 139: 484-92.
4. Tseng CH, Tai TY, Chong CK, Tseng CP, Lai MS, Lin BJ, Chiou HY, Hsueh YM, Hsu KH, Chen CJ. Long-term arsenic exposure and incidence of non-insulin-dependent diabetes mellitus: a cohort study in arseniasis-hyperendemic villages in Taiwan. Environ Health Perspect 2000;108:847-51.
5. Tseng CH, Tseng CP, Chiou HY, Hsueh YM, Chong CK, Chen CJ. Epidemiologic Evidence of Diabetogenic Effect of Arsenic. Toxicol Lett 2002;133:69-76.
6. Board P, Coggan M, Johnston P, Ross V, Suzuki T, Webb G. Genetic heterogeneity of the human glutathione transferase: a complex of gene families. Pharmacol Ther 1990; 48:357-369.
7. Chiou HY, Hsueh YM, Hsieh LL, Hsu LI, Hsu YH, Hsieh FI, Wei ML, Chen HC, Yang HT, Leu LC, Chu TH, Chen-Wu C, Yang MH, Chen CJ. Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. Mutational Res 1997; 386:197-207.
8. Tseng CH, Tseng CP. Lack of association between angiotensin-converting enzyme gene polymorphism and peripheral vascular disease in Type 2 diabetic patients in Taiwan. Circ J 2002;66:1014- 1018.