附件：封面格式

行政院國家科學委員會補助專題研究計畫成果報告

探討砷引起糖尿病的分子機轉

計畫類別：☑個別型計畫　□整合型計畫

計畫編號：NSC　90-2320-B-002-197

執行期間：90 年 08 月 01 日至 91 年 07 月 31 日

計畫主持人：曾慶孝 台大醫院內科部

共同主持人：曾慶平　長庚大學醫事技術學系

計畫參與人員：

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：台大醫院內科部

中　華　民　國　　年　　月　　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：探討砷引起糖尿病的分子機轉
計畫編號：NSC 90-2320-B-002-197
執行期限：90 年 08 月 01 日至 91 年 07 月 31 日
主持人：曾慶孝 台大醫院內科部
共同主持人：曾慶平 長庚大學醫事技術學系

一、中文摘要

本研究擬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) M1 基因型與糖尿病的可能相關性。總計完成 651 位受檢者的 GST M1 基因型分析，其中 76 位患有糖尿病，另外 573 位為無糖尿病之對照組。GST M1 基因型以聚合酶鏈鎖反應方式進行，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 55.2%，而糖尿病組則為 43.6% (p=NS)。在理則迴歸分析中，控制了可能干擾因子後，non-null type 對應於 null type 之糖尿病危險性，以對比值表示為 1.598 (0.974-2.621) (p=NS)。以上結果雖然並未發現糖尿病與 GST M1 之基因型有統計上顯著之意義，但我們認為更大樣本之進一步分析，並包含 GST 其他次分級如 GST T1 之研究，將有助於進一步釐清 GST 對砷引起之健康危害的影響。

關鍵詞：糖尿病，環境污染物，砷，聚合酶鏈鎖反應，分子生物學

Abstract

This study evaluated the possible association between diabetes mellitus and the glutathione S-transferase (GST) M1 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 M1 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 M1 null type was present in 55.2% of the non-diabetic subjects and 43.6% of the diabetic patients (p=NS). In logistic regression analysis, after controlling for confounders, the adjusted odds ratio for diabetes mellitus in subjects with GST M1 non-null type versus null type was 1.598 (0.974-2.621) (p=NS). Although no significant association between the GST M1 genotype and arsenic-related diabetes mellitus was observed in this study, further studies with larger sample size and including other subclasses such as the GST T1 are required to clarify the role of this detoxification enzyme on the impact of health hazards associated with arsenic exposure.

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 M1 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, 76 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. in each subject

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 M1 --- Polymerase chain reaction was used to amplify DNA for GST M1. 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'-GAA CTC CCT GAA AAG CTA AAG C-3' and 5'-GTT GGG CTC AAA TAT ACG GTG G-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. Odds ratios and their corresponding confidence intervals were calculated from the logistic regression parameter estimates. A *P* of less than 0.05 was regarded as statistically significant.

Results

With comparable age, sex and body mass index, GSTM1 null type was present in 55.2% of the non-diabetic subjects and 43.6% of the diabetic patients ($p>0.05$). In logistic regression analysis, after adjusting for the effect of age, sex and body mass index, the odds ratio for diabetes mellitus in subjects with GSTM1 non-null type versus null type was 1.598 (0.974-2.621) ($p>0.05$).

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 this preliminary study carried out in a group of residents living in the Ilan county, but it was not safe to conclude that GST would not play any role on the arsenic-related health hazards. A larger sample size and the inclusion of other subclasses of the GST superfamily such as the GST T1 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.