Genetic polymorphisms in CYP1A1 and GST-T1 predispose PCBs/PCDFs-induced chloracne and arthritis

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Introduction

In 1979, over 2000 Taiwanese in central Taiwan exposed to polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) by ingesting contaminated rice oil. More medical problems, i.e., goiter, anemia, arthritis, skin disease, were reported by the Taiwanese exposed to high levels of PCBs and PCDFs.[1] Chloracne is one of the most prominent clinical features associated with the poisoning. Similar clinical condition of skin was found in the cases of Yusho in Japan[2]. The CYP1A1 polymorphisms are one of the important biomarkers determining personal susceptibility to exposure to environmental toxicants. Previous studies suggested that the cytochrome P450 1A1 (CYP1A1) polymorphisms are associated with lung cancer and liver function in exposed populations[3]. Coplanar PCB congeners and PCDFs share a common mechanism of action of toxicity to 2,3,7,8-TCDD by binding to the cytosolic aryl hydrocarbon receptor (AhR). The ligand-activated receptor forms a heterodimer with the nuclear protein ARNT and after binding to specific DNA elements increase transcription of dioxin-activated genes[4]. Different genotypes in AhR may be associated with different gene expressions induced by dioxin-like chemicals[5]. Glutathione S-transferases (GST) play an important role in the defense of the body against reactive compounds. They catalyze the conjugation of electrophiles with glutathione, thereby inactivating these often cytotoxic and genotoxic substances[6]. Two human GST isoenzymes, the ì-class enzyme GSTM1 and GSTT1 have been shown to be polymorphic[7,8]. Lack of GSTM1 had been reported to be associated with an increased susceptibility for tobacco smoke-induced lung, bladder, skin, breast, prostate and liver cancer[9]. Null genotype of GST T1 is associated with more reduction in fetal weight in smoking mothers. Therefore, the GST status of a person is also an important determinant of the individual risk toward chemical carcinogenesis. Thus, in this study, we would like to know if the CYP1A1, AhR and GSTM1/T1 polymorphisms modify the incidence of polychlorinated biphenyls-induced health effect.

Materials and Methods

In 1993, 476 subjects answered questions for review of systems had blood collected and genotypes analyzed. Among them, 261 had serum levels of PCB measured previously in 1980-2. The CYP1A1 polymorphisms were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), according to the method of Hayashi et al.[10] The DNA sample was amplified with 2 primers: 5'-CAGTGAAGAGGTGTAGCCGC-3' (upstream) and 5'-TAGGA GTCTTGTCTCATGCC-3' (downstream) (Perkin Elmer, Taipei, Taiwan). The PCR amplification was carried out with 1 ìg DNA in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 3 mM $MgCl_2$, 0.3 mM deoxyribonucleotide triphosphates (Boehringer Mannheim GmbH, Mannheim, Germany), 0.2 ìM of each primer and 1.5 U Taq polymerase (AmpliTaq; Perkin Elmer) in a total volume of 50 ìl. The PCR was performed in a GenAtaq thermal cycler (Pharmacia). Amplification was performed with an initial denaturation at 94 degree C for 5 minutes, followed by 30 cycles at 94 degree C for 1 minute, 61 degree C for 1 minute, and 72 degree C for 1 minute, and a final extension at 72 degree C for 7 minutes. The PCR-amplified DNA fragments including the polymorphic site will be digested with MspI and NcoI and subjected to electrophoresis in 1.8% agarose gel.

The GST polymorphisms were determined by PCR and RFLP by Michael Arand et al.[11]. A premix was prepared, containing 50 mM KCl, 2.5 mM $MgCl_2$, 20 mM Tris-HCl, pH 8.4, GSTM1 primers at 3ìg/ml each, GSTT1 primers at 1 ìg/ml each, albumin primers at 600 ng/ml each, and 50 U/ml AmpliTaq (Cetus) in a total volume of 50 ìl. After addition of 20-100 ng of genomic DNA

(volume 2 ìl), we isolated from whole blood with the aid of the IsoQuick DNA Isolation Kit (MicroProbe). GST multplex PCR was amplification with an initial denaturation at 95 degree C for 2 minutes, followed by 30 cycles at 94 degree C for 1 minute, 64 degree C for 1 minute, and 72 degree C for 1 minute, and a final extension at 72 degree C for 5 minutes. The DNA sample was separately amplified with 2 primers: GSTM1: 5'-GAACTCCCTGAAAAGCTAAAGC-3' (upstream), 5'-GTTG GGCTCAAATATACGG TGG-3' (downstream) GSTT1: 5'-TTCCTTACTGGTCCTC ACATCTC-3' (upstream) and 5'-TCACCGGATCATGGCCAGCA-3' and Albumin(internal control): 5'-GCCCTCT GCTAACAAGTCCT -AC-3' (upstream), 5'-GCCCTAAAAAGAAAATC-3' (downstream).

In AhR polymorphism were determined at similar method[12]. The DNA sample was amplified with 2 primers: 5'- GAATCTTGGACATACGTCAG ¯3' (upstream) and 5'- AGG-CAT-TGA-TTT-TGA-AGA-CATT -3' (downstream). The PCR amplification was carried out with 20 ìl genomic DNA, 5ìl of 10X reaction buffer, 3ìl of 2.5mM dNTP, 1ìl of 20 ìM of each primer and 0.5ìl of 2U/îl Taq polymerase in a total volume of 50 ìl. The PCR was performed in a GenAtaq thermal cycler (Pharmacia).Amplification was performed with an initial denaturation at 95 degree C for 3 minutes, followed by 30 cycles at 95 degree C for 30 seconds, 58 degree C for 30 seconds, and 72 degree C for 30 seconds, and a final extension at 72 degree C for 10 minutes. The AhR PCR product is a 126bp DNA fragment. The product contained two MseI cutting sites. One site can be digested in all individuals and can be as an internal control for restriction enzyme digestion. The other site is polymorphic. The polymorphic site will be digested with MseI and subjected to electrophoresis in 4% NuSieve (3:1).

Results and Discussion

A total of 312 Yucheng subjects participated in this study, including 148 men and 164 women. The mean (Range) of PCB concentration level in serum measured in 1980-2 is 85.2 (0-1150) ppb. The mean age is 46.6 years old and 28.8% are smokers. The education levels are: less than elementary school, 14%, elementary school, 51%, middle school 27%, high school, 5%. The genotype frequencies of AhR, CYP1A1 Msp1, Nco1, GST T1 and M1 are shown in Table 1. Health effects including chloracne, hyperkeratosis, abnormal nail, skin allergy, gum pigmentation, broken tooth, goiter, headache, and anemia are in association with PCB level[1]. Table 2 showed people exposed to higher level of PCB caused higher rate of chloracne (OR=22.2). AhR, Nco1, GST M1 and T1 were not significantly associated with the incidence of chloracne. Msp1 variant genotype (m1/m2 and m2/m2) was significantly associated with chloracne, adjusted odds ratio of 1.8 (adjusting for age, smoking and serum PCB concentration). Table 3 showed that people exposed to higher level of PCB caused higher rate of arthritis (OR=2.5). GSTT1 non-null genotype was significantly associated with arthritis, with adjusted odds ratio 3.0 (adjusting for age, smoking, and serum PCB concentration).

Table 1. The genotype of five genes in Yucheng population

	Wild	Heterozygous	Mutant
CYP1A1			
MspI	90 (30%)	161 (50%)	61 (20%)
NcoI	21 (10%)	117 (40%)	169 (50%)
AhR			
MseI	152 (50%)	126 (40%)	34 (10%)
GSTT1	146 (50%)		166 (50%)
GSTM1	141 (40%)		171 (60%)

Table 2. Chloracne and genetic polymorphism in Yucheng population
(Adjusted for age, smoking and serum PCB levels)

Chloracne	OR	95% CI of OR
CYP1A1-MspI		
Wild (m1/m1)	1	
Hetero (m1/m2) or Variant (m2/m2)	1.8	(1.0 – 3.4)
CYP1A1-NcoI	N.S.	
GST-M1	N.S.	
GST-T1	N.S	
AhR-MseI	N.S.	

Table 3. The arthritis and genetic polymorphism in Yucheng population
(Adjusted for age, smoking and serum PCB levels)

Arthritis	OR	95% CI of OR
CYP1A1-MspI	N.S.	
CYP1A1-NcoI	N.S.	
GST-M1	N.S.	
GST-T1	N.S	
Null	1	
Non-null	3.0	(1.2, 8.1)
AhR-MseI	N.S.	

Our findings showed that CYP1A1 Msp1 variant genotype is associated with chloracne in PCBs/PCDFs-exposed population. CYP1A1, with aryl hydrocarbon hydroxylase (AHH) activity, can catalyses the oxygenation of polyaromatic hydrocarbons. Kiyohara et al. (1996) have found increased AHH inducibility in variants of the CYP1A1 MspI gene[13]. It is possible that increased inducibility of CYP1A1 variant genotype mediated elevated activation of other environmental toxicants, and therefore increased chloracne. CYP1A1 polymorphisms therefore play a role as an effect modifier of health outcomes in people exposed to PCBs and dioxin-like chemicals. The exact mechanism by which CYP1A1 genotype affects the causation of chloracne warrants further investigation.

We found that GSTT1 non-null genotype is associated with arthritis, which has never been reported previously. GSTT1 null genotype is associated with increased susceptibility to cancers of the cervix, head/neck, skin, brain, liver, kidney, and prostate, as well as with more reduction in fetal weight in smoking mothers. The mechanism of protective effects of null genotype in GST-T1 in the development of arthritis is unknown. Further investigation of the mechanisms of arthritis in Yucheng people as well as the role of GST-T1 genotype in this disorder is under way.

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