GENES ALTERATION IN FOUR FAMILIES OF VIETNAMESE DIOXIN VICTIM

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent molecule that belongs to Dioxin family, which is associated to the Agent Orange used by the U.S army. Although TCDD was classified as a human carcinogen by the WHO and the US National Toxicological Program, the potential carcinogen of TCDD is still controversial. In animal, acute exposure to Dioxin induces gastrointestinal haemorrhage, liver toxicity, weight loss and dead[16, 21]. Other studies focus on neurotoxic effects of Dioxin implicated that changes in behavior and long term cognitive deficiency were induced by TCDD in Rhesus monkey [18, 19]. Furthermore, neurodevelopment alterations were displayed in human after gestational exposure to TCDD [6, 25]. More recently, a research group investigated that TCDD toxicity in SHSY5Y neuroblastoma cells promoted the calcium homeostasis disruption, leading to apoptosis process [13]. As far as TCDD effects on immune system were concerned, there are data indicated that TCDD elevates resting intracellular calcium levels in murine B cells and selectively prohibit calcium dependent signaling pathways that related to surface Ig [8]. In addition, it was shown that TCDD inhibits $CD4^+$ T cell differentiation in to T helper (Th)1, Th2 and Th17 effector cells via AhR activation [10]. Regarding dioxin toxicity on teratogenesis, in 2006, a meta-analysis on 13 Vietnamese and 9 non-Vietnamese indicated that parental exposure to Agent Orange appears to be associated with an increased risk of birth defects[15].

TCDD bind to an intracellular receptor named AhR (Aryl hydrocarbon Receptor) with high potency, leading to modulation of genes expression. As a cascade of AhR signaling, AhR-Arnt complex binds to a specific DNA sequence called xenobiotic-response element (XRE), inducing a broad spectrum of biochemical toxic effects like teratogenesis, modulation of immune system and tumor promotion[12, 17]. *CYP1B1* belongs to the cytochrome P450 superfamily of enzymes which catalyze many reactions related to drug metabolism as well as synthesis of cholesterol, steroids and lipids. In 2014, *CYP1B1* was found responded to at least 10-1000 fold higher after TCDD exposure in rat liver[24]. Using hCMEC/D3 cell line, it was demonstrated that AhR was involved in elevation of *CYP1B1* expression mediated by TCDD in dose dependent manner[5]. Another gene was considered as TCDD target gene is *p53*, which has been known as most frequently mutated gene (>50%) in human cancer. In order to elucidate the effect of TCDD treatment on p53 characteristics, some studies have been published. A marked repression of p53 mRNA expression was found in fetal mouse liver[1] but not in Hepa1c7 mouse blastoma cells[20]. In human breast cancer cells, exposure to TCDD and hypoxia simultaneously triggered to a moderate inhibition of *p53* expression[22].

Materials and methods

Study subjects

Four families of dioxin victims of Vietnamese army veterans who have been exposed directly under sprays or carried out missions for at least 2 years in the heavily sprayed regions (Figure 1). All biological samples in this study were approved by the Institutional Review Board of Hanoi Medical University, Hanoi, Viet Nam.

2.2. DNA sequencing

Genomic DNA was isolated from peripheral blood samples using a DNeasy blood and tissue kit (Qiagen, Hilden, Germany). To determine mutations of the AhR (Aryl Hydrocarbon Receptor), CYP1B1 (Cytochrome P450 subfamily 1B1) and TP53 (Tumor Protein 53) genes, polymerase chain reaction (The Veriti® Thermal Cycler, Applied Biosystems, USA) and DNA sequencing (ABI Prism® 3100 and/or 3500 Genetic Analyzers, Thermo Scientific, USA) were performed with specific primer pair as below. All obtained PCR fragments were purified with a GeneJET PCR purification kit (Thermo Scientific, USA). The PCR products were sequenced on both strands with the same primers used for the PCR.

Gene symbol	Primer name	Primer sequences (5'-3')	Gene symbol	Primer name	Primer sequences (5'-3')
TP53	E24F	TGCTGGATCCCCACTTTTCC	**AHR**	AhR_F1	ACCAGCCTCAGGATGTGAAC
	E24R	GCCAGCCCCTCAGGGCAAC		AhR_R1	GAATCTTGGACATACGTCAG
	E56F	GACTTTCAACTCTGTCTCCTTCC		AhR_F2	TTTCCTGCCATAATGGATCC
	E56R	GCCCCCCTACTGCTCACCCGG		AhR_R2	TGCTGTGGACAATTGAAAGG
	E79F	TCTTGGGCCTGTGTTATCTCC	**CYP1B1**	Cyp1B1-F1	ACGCTCCTGCTACTCCTGTC
	E79R	GAGGTCCCAAGACTTAGTACC		Cyp1B1-R1	GTGAAGAAGTTGCGCATCA
				Cyp1B1-F2	TAAGAATTTTGCTCACTTGC
				Cyp1B1-R2	TTTACTCCTCATCTCCGAAG

DNA sequence variations were identified by comparing subject DNA sequence to the AhR, CYP1B1 and TP53 reference sequences with Genbank Accession Numbers NM_001621, AY393998 and X54156, respectively.

Results and discussion

Family ThB3 have 3 children with birth defects in which one was died at 9 months of age. Three children of family ThB4 have abnormal phenotype: 1 daughter with hypopituitarism, bone deformities hand, dwarf (VT21); 1 daughter with porphyria (ThB4.VT25) and 1 son have Spina bifida. In the family ThN12, two children have birth defects. The father of of family DoN20 was diagnosed with type II diabetes and prostate tumor (Figure 1).

In this study, we sequenced partial DNA sequence of 3 genes (include AhR, CYP1B1, and TP53) in all members of 4 families of victim.

Family ThB3

After aligned with reference sequences we found 21 nucleotide variants in TP53 gene. Of which 5 nucleotide variants leading to amino acid change. G12099A → G59S and T13068A → L130H in ThB3.VT14 are new variants.

We found 13 nucleotide variants in AhR gene. All of theme leading to amino acid change. We also found R554K in ThB4.VT16 and ThB4.VT17. This mutation changes activity for CYP1A1 induction in lymphocytes [3, 26].

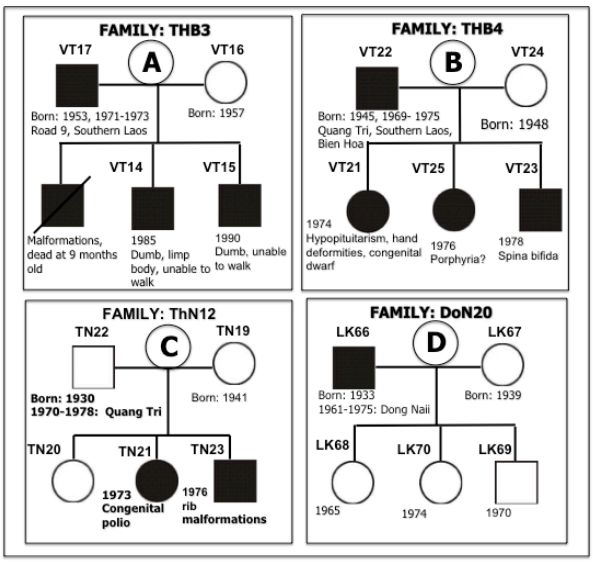

Figure 1: Information of 5 families of dioxin victim

Family ThB4

We identified 29 nucleotide variants in TP53 gene with 9 nuclotide variants leading to change amino acid. G12139C → R72P appear in all 5 individuals in the pedigree; A12127C → E68A (ThB4.VT24); T12207C → S95P (ThB4.VT22); T12285C → S121P (ThB4.VT21); G14052A → C242Y (ThB4.VT23); A14531C → N288H (ThB4.VT24); C14553T → P295L (ThB4.VT23); T14565C → L299P (ThB4.VT24); G14505A → G279E (ThB4.VT23 and ThB4.VT21).

Although we could not found any variant associated with phenotype of the family members but previous studies have found P295L associated with gastric carcinoma [7], L299P associated with pancreatic cancer [2], G279E associated with colorectal carcinoma [11] and cancer of male sex cells [4].

In CYP1B1 gene, we found 09 nucleotide variants. G2488A → V35M and G2492A → G36D appear in children with congenital malformations ThB4.VT21 (hypopituitarism, bone deformities hand, dwarf). C2527G → R48G, G2740T → A119S, T2764C → S127P and A7084G appear in person as CCB announced ThB4.VT22. A119S related to breast cancer and squamous carcinoma of the lung [23]. G2556A → W57Stop, T2659C → C92R, L432V and C6714G → C6767T appear in ThB4.VT25.

Family ThN12

In TP53 gene, we found 22 nucleotide variants in which 04 variants leading amino acid change. G12139C R72P appear in 4 individuals in the pedigree (except ThN12.TN21). A13388G N210D appear in ThN12.TN22 cause oligodendrogliomas. A14066G N247D cause prostate cancer [14]. A14541G K291R appear in ThN12.TN19. This mutation causes glioma [27], liver carcinoma [9].

In CYP1B1 gene, we found 9 nucleotide variant. Seven of which appear in individual ThN12.TN21 (G2519A R45Q, C2527G R48G, T2549C F55S, G2707C V108L, G2740T A119S, T6683C and A6795G R459G).

Family DoN20

We could not found any nucleotide variant in the CYP1B1 in all members of this family. In AhR gene, we found 3 nucleotide variants, A1883G in DoN20.LKh66, A1389G in LKh67 and A1464G in LKh68. However, only 1 nucleoitde variant leading to amino acid change (A1383G → K629R in LKh66).

In TP53 gene, we found 11 nucleotide variants, G12139C → R72P appear in LKh66; R72P and V31I appear in 3 remaining individuals.

Acknowledgements

This work is funded by the Program KHCN-33/11-15 (Grant KHCN-33.06/11-15), Ministry of Natural Resources and Environment.

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