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Abstract

Keywords: Transitional Cell Carcinoma; Inorganic arsenic; multiplex family study

Inorganic arsenic has been well documented as a human carcinogen of skin and lung. A significant association between arsenic exposure and risk of urinary cancer has also been reported in many epidemiological studies carried out in many countries of the world. Our recent study had also found a significant dose-response relationship between risk of cancers of urinary organs, especially for transitional cell carcinoma (TCC), and arsenic exposure through drinking well water in Lanyang Basin. However, the major susceptible gene(s) of bladder cancer from arseniasis-endemic and non-endemic areas and their functional changes that make a person to be a victim of arsenic-induced bladder cancer is still unclear. The objective of this subproject is to identify and differentiate the major susceptibility gene(s) for arsenic-induced and non-arsenic-induced bladder cancers through the linkage analysis of genetic markers in members of multiplex families. It will include following specific aims: 1) the ascertainment of multiplex families through the telephone or home-visit interview, based on a family history questionnaire, of bladder cancer cases reported to the national cancer registry and cases diagnosed and treated in collaborative medical centers; 2) the recruitment of probands and families members through home-visit personal questionnaire interview and biospecimen collection; 3) the purification, depository and inventory of DNA samples in central biospecimen bank; 4) the typing of genetic markers on chromosomes in which loss or gain have been observed through previous comparative genomic hybridization and loss of heterozygosity studies; 5) the mapping of susceptibility gene(s) through linkage analysis of multiplex family data for bladder cancer cases in arseniasis -endemic and non-endemic areas; 6) the examination of the effects of possible candidate genetic marker(s) and their synergistic interactions with environmental factors on bladder cancer; and 7) the establishment of Genetic Epidemiology and Biostatistics Supporting Core to provide methodological support to other subprojects. A total of 150 multiplex families of bladder cancer including 150 probands and 1500 first-degree relatives from arseniasis -endemic and non-endemic area will be recruited by the end of three year grant period. An informed consent will be obtained from each participant for the collection of risk factor information through questionnaire interview. A 35 mL blood and buccal cell specimen will be obtained from each consenting participant. DNA samples will be extracted from peripheral lymphocytes and buccal cells, aliquoted and frozen at -70¢J. Polymorphisms of genetics markers closely linked to the major susceptible gene(s) of bladder cancer will be typed. Analysis of Lod score, and transmission disequilibrium

test will be carried out to map susceptible gene(s) of arsenic-induced and non-arsenic-induced bladder cancer based on multiplex family study.

Background and Significance

Our previous studies have shown that capability of arsenic methylation is associated with the risk of arsenic-induced skin cancer (12). Null genotypes of glutathione S-transferase (GST) M and T1 and variant genotype of GST P1 have been found to increase the risk of arsenic-induced skin cancer (13). Whether the genotypic and phenotypic polymorphisms of GSTs and other enzymes related to arsenic methylation may modify the risk of arsenic-induced TCC remain to be elucidated. Furthermore, genetic polymorphisms of some DNA repair enzymes have been observed to be associated with the risk of arsenic-induced skin cancer (14). It is also important to assess whether the DNA repair enzymes, either their genotypes or gene expression profiles, are related to the risk of arsenic-induced bladder cancer. The existence of genetic susceptibility to arsenic-induced bladder cancer may be explored through the microarray analysis of differential expression profiles and single nucleotide polymorphisms of related genes. It may also be investigated by the genetic epidemiological study on familial TCC through linkage analysis.

Tobacco smoke is another important etiological agent of urinary bladder cancer in Taiwan. It plays an important role in the induction of urinary bladder cancer in non-arseniasis-endemic areas. The tobacco-related bladder cancer risk is modified by the genetic polymorphisms of N-acetyltransferase 1 and 2 (15). The comparison of toxicological genomic characteristics of TCC induced by tobacco and arsenic may help the elucidation of the arsenic-induced carcinogenicity. This program project is organized to study the difference in toxicological genomic characteristics between TCC induced by arsenic and tobacco smoke.

Due to the lack of animal models for arsenic carcinogenicity, arsenic is a unique carcinogen in humans. Therefore, the high prevalence of urinary TCC in arseniasis-endemic area in Taiwan is a unique chance to investigate the carcinogenicity of arsenic. Since cancer development is mainly attributable to environmental factors and genes, it is of fundamental importance to understand the interaction between environment and genes. In this PPG, epidemiological components will establish the association of environmental factors and incidences of urinary TCC. Molecular, cellular and genetic components will identify the genetic factors involved in formation of urinary TCC. The joint efforts of epidemiological and molecular genetic studies will successfully dissect the interactions between environmental factors and genes. Novel findings of this PPG will allow us designing new strategy for prevention, diagnosis, and treatment of urinary TCC.

Though arsenic is a human carcinogen, there is no good animal model for the carcinogenicity of inorganic arsenic. Arsenic is inactive or extremely weak to induce gene mutations at specific loci (1,16). The modes of action for arsenic-induced carcinogenicity might include the induction of chromosome abnormality, inhibition of DNA repair, induction of oxidative stress, and increase of cell proliferation (17). Inorganic arsenic has several genotoxic effects including the induction of changes in chromosome structure and number, increases in sister chromatid exchanges and micronuclei, gene amplification, cell transformation, aneuploidy, and chromosome-type chromosome aberration (1,17-22). The role of inorganic arsenic in the carcinogenesis has also been hypothesized as a

co-carcinogen such as promoter or progressor rather than an initiator (23,24). However, the evidence is far from adequate to draw a definite conclusion on the exact mechanism of inorganic arsenic to induce various cancers in humans.

Most of the genetic variants which have been studied using the design of case-control study were only associated with a modest increased genetic risk for developing cancer, although the magnitude of their attributable risk is large because there are quite frequent in the population. Many rare cancer syndromes due to a mutated gene which confer very high risk of cancer have been well-documented, including the rare Li Fraumeni symdrom in which persons inhriting a gerlime mutation in the p53 gene are at almost 100 % risk of breast caner and other cancers by the age of 60 years (25,26), the familial adenomatous polyposis symdrome associated with mutation in the APC gene (27), and the breast-ovary cancer syndrome associated with an inherited mutation in BRCA1 (28).

Human genome sequence have been cloned successfully by "Human Genome Project". A total of 3 x 10^5 of an estimated 3-10 x 10^6 single nucleotide polymorphisms which distinguish individuals and their disease traits and risk will be identified by 2002 (29). Classical family, twin and adoptee studies have shown substantial heritabilities for many disease traits, but except in rare instances the pattern is polygenic rather than monogenic. Knowledge and technology have become sufficient to enable molecular geneticists to study megaphenic disorders in single families, with the exceptation of isolating 'the genes' understanding the pathology and deriving clinically applicable test of status (30,31).

For polygenic traits, knowledge is sufficient to initiate tests of hypotheses, but the technology is yet insufficient to measure the contribution of genetic diversity to disease liabilities. Linkage analysis of multiplex family has an important role in genetic epidemiology because it identifies a biologic mechanism for transmission of a trait or disease (31). A large family displaying a clear-cut segregation pattern for a disease is examined at polymorphic sites representing each part of each chromosome (30). Recently, most sites of cancer has been recognized as multigene disorders. It means that many regions of each chromosome have been examined intensively to identify susceptible genes and then to differentia their functions on risk of various cancers. With a availability of a fairly complete human genome sequence, this approach is reducing to searching the sequence for the possible culprit gene on criteria such as its tissue expression pattern, apparent function predicted from sequence, etc. For example, haemochromatosis was shown by positional cloning to be attributable to an HLA-related genes and it has immediately been possible to examine the relationship between genetic and iron status diagnostics, population prevalence of mutations, and prevalence of HFE mutations in haemochromatosis-associated disease groups such as diabetics (32). In addition, given the widespread roles of iron, for various phenotypes and investigation of HFE interactions in other disorders such as porphyries, haemoglobinopathies and coronary disease and environmental variables such as diet, lead poisoning and infections, is now

proceeding to explore gene-environment and gene-gene interactions through epidemiological design (33-35).

The transformation of a normal cell into a malignant cell is a multistep mechanism, which involves various alterations on the molecular and genetic level. These molecular alterations occur spontaneously or are induced by carcinogens. As in most other malignancies the development of bladder cancer is caused by the accumulation of various molecular changes. The expression of oncogenes (ras, erbB-2 and EGF receptor), tumor-suppressor genes (Rb, p53), cell-cycle genes (p15, p16) and DNA-repair genes is altered mostly by mutation or chromosomal aberration. Loss of heterozygosity of chromosome 9p and 9q has been shown to be a crucial event in the transition of normal urothelium to papillary transitional cell carcinoma while p53 is primarily involved in the development of carcinoma in situ (36). A study based on 44788 pairs of twin from Sweden, Denmark, and Finland showed that heritability was estimated to account 31 percent of the variation in susceptibility to the risk of bladder cancer. It was implied that genetic component might not be ignored in the carcinogeneicity of bladder cancer (37).

In this study, by the aid of linkage analysis, we plan to map the susceptibility gene(s) through multiplex family data of bladder cancer cases from arseniasis-endemic and non-endemic areas, to differentiate the difference between gene profiles of residents from arseniasis-endemic and non-endemic areas and to clarify the genetic and functional changes that make a person affected with arsenic-induced bladder cancer.

Specific Aims.

The objective of this subproject is to identify and differentiate the major susceptibility gene(s) for arsenic-induced and non-arsenic-induced bladder cancers through the linkage analysis of genetic markers in members of multiplex families. This subproject will also set up a Genetic Epidemiology and Biostatistics Supporting Core for this program project.

It will include following specific aims:

1)The ascertainment of multiplex families through the telephone or home-visit interview, based on a family history questionnaire, of bladder cancer cases reported to the national cancer registry and cases diagnosed and treated in collaborative medical centers

2)The recruitment of probands and families members through home-visit personal questionnaire interview and biospecimen collection;

3)The purification, depository and inventory of DNA samples in central biospecimen bank;

4)The typing of genetic markers on chromosomes in which loss or gain have been observed through previous comparative genomic hybridization and loss of heterozygosity studies;

5)The mapping of susceptibility gene(s) through linkage analysis of multiplex family data for bladder cancer cases in arseniasis-endemic and non-endemic areas;

6)The examination of the effects of possible candidate genetic marker(s) and their synergistic interactions with environmental factors on bladder cancer;

7)The establishment of Genetic Epidemiology and Biostatistics Supporting Core to provide methodological support to other subprojects.

Studies and Results

¢„ Multiplex families ascertainment

A total of 558 urothelial carcinoma (UC) consenting participants have been recruited. Among them, 154 consenting participants from Taiwan University Hospital, 110consenting participants from Chia-Yi Christian Hospital and 469 consenting participants from Chi-Mei Hospital. An informed consent will be obtained from each participant for the collection related study information. through personal interview in centers for outpatient and inpatient by well trained interviewer. Risk factor questionnaire (RFQ) was used to collect risk factors of UC including cigarette smoking, alcohol drinking, well water consumption, nutrition status, and usage of dye and drug. Family history questionnaire (FHQ) was used to obtain disease history of various cancers especially for UC and chronic diseases including diabetes mellitus (DM) and hypertension. Flow chart for multiplex families recruitment was shown in Figure 1.

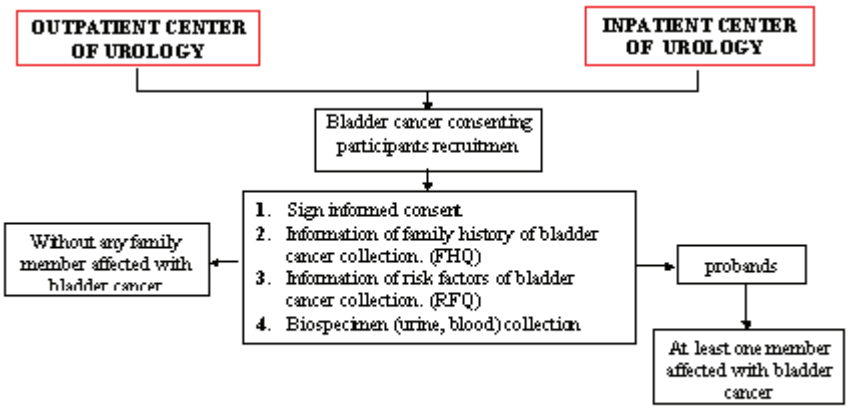

Figure1. Flow-chart for Multiplex Family Asertainment (Phase I)

¢". Recruitment of proband and families members

A total of 17 probands whose first degree relatives with UC have been ascertained through FHQ. Now we have mailed invited letters to family members of probands to invite them to participate this study. Among consenting family members, we decided to recruit ten members of each multiplex family including parents, spouse, sibling, and offspring. Questionnaire interview and biospecimen (blood, buccal cell, urine) collection will be executed either in outpatient center of collaborative medical center or household of consenting family members. The flow chart of multiplex families recruitment was shown in figure 2. 4 pedigrees newlyidentified from Chia-Yi

Christian Hospital were illustrated in Figure 3-1 to Figure 3-4.

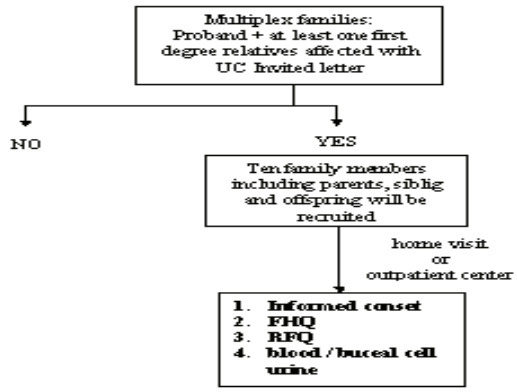

Figure2. Flow-chart for Multiplex families recruitment (Phase II)

Figure-3.1

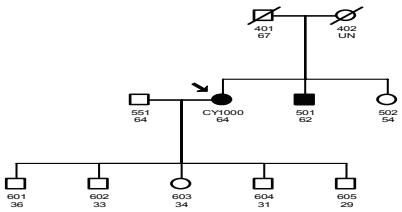

Figure-3.2

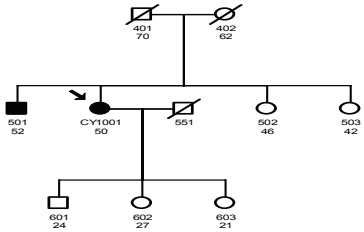

Figure-3.3

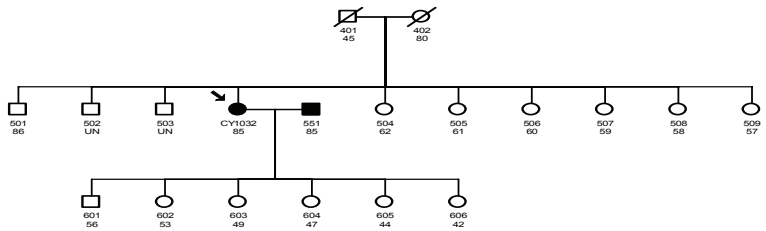

Figure-3.4

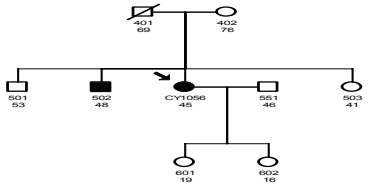

Table 1. The age and sex of 17 UC probands

Family ID	Sex	Age (year)
NTU10222	M	61
NTU10263	F	67
NTU10310	M	61
NTU10264	M	66
CM1010	M	71
CM1012	M	85
CM1071	M	57
CM1059	F	62
CM1123	M	73
CM1356	F	98
CM1352	M	64
CM1355	F	65
CM1485	M	58
CY1001	F	50
CY1032	F	85
CY1000	F	64
CY1056	F	45

mean age:66.58 years old; sex ratio (M:F)=9:8

Table 2. The first degree relatives among 17 UC multiplex families

		First-degree relatives					
Family ID	**members**	**Father**	**mother**	**brother**	**sister**	**son**	**daughter**
NTU10222	11	1(1)	1	4	4	1	0
NTU10263	10	1(1)	1	1	2	4	1
NTU10310	6	1(1)	1	0	0	3	1
NTU10264	12	1(1)	1	1	5	3	1
CM1010	9	1(1)	1	2	1	3	1
CM1012	14	1	1	2(1)	2	2	6
CM1071	14	1	1	6(1)	2	2	2
CM1059	10	1	1(1)	3	3	1	1
CM1123	9	1	1(1)	2	1	1	3
CM1356	14	1	1	1	3	4(1)	4
CM1352	10	1	1	3	2	2	1
CM1355	11	1	1	3(1)	2(1)	1	3

CM1485		1(1)	1	7	3	1	2
CY1001	8	1	1	1(1)	2	1	2
CY1032	17[a]	1	1	3	6	1	5
CY1000	9	1	1	1(1)	1	4	1
CY1056	7	1	1	2(1)	1	0	2

* Number of first-degree relatives affected with UC are shown in parenthesis

a The husband of this proband was TCC case, but he is not the first degree relative.

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