Genetic risk assessment for workers in nuclear power plants using chromosomal aberrations and copy number variation

A dissertation submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy in Public Health

To the Faculty of the Graduate School of Public Health
at **Seoul National University**
by **Joong Won Lee**

Date approved : February, 2013

Joohon Sung

Sung-Il Cho

Hyojee Joung

Byung-Mu Lee

Hai Won Chung

원전근로자에서 염색체이상 및 유전자 복제수변이 지표를 이용한 유전학적 위해성 평가

지도교수 정 해 원

이 논문을 보건학박사 학위논문으로 제출함

2013년 2월

서울대학교 대학원

보건학과 분자역학 전공

이 중 원

이중원의 보건학박사 학위논문을 인준함

2013년 2월

위 원 장 성 주 헌 (인)

부 위 원 장 조 성 일 (인)

위 원 정 효 지 (인)

위 원 이 병 무 (인)

위 원 정 해 원 (인)

ABSTRACT

Joong Won Lee
Major in Molecular Epidemiology
Department of Public Health
The Graduate School of Public Health
Seoul National University

Nowadays, due to the strict radiation protection/safety program, current levels of exposure to ionizing radiation (IR) in occupation have decreased and are far below the regulatory limit established by the International Commission on Radiological Protection (ICRP), however personal cumulative doses in nuclear power plant workers are continuously increasing. The biological and genetic effects of low-dose level of IR exposure and its relationship to carcinogenesis have received great attention in the last years.

The aim of the present study is to assess occupationally induced chromosomal damage in relation to copy number variation in a large population of nuclear power plant workers exposed to chronic low dose IR. For this purpose, we used the Chromosome aberration (CA) and micronuclei (MN) assay in peripheral blood lymphocytes (PBL) of nuclear power plant workers never exceeded 20 mSv per year for their duration of work and compare the results with control individuals. And furthermore we investigated for the first time the influence of Glutathione S-Transferase mu 1 (GSTM1) and theta 1 (GSTT1) copy number variations (CNVs) on chromosomal damage in low dose IR-exposed workers.

In this study, the CA frequency was significantly higher in radiation exposed workers than controls (Mann-Whitney test, $p < 0.001$), and chromosome-type aberration frequency was significantly increase with the recent 1.5-year dose (Pearson's correlation coefficient test; $r = 0.17$, $p = 0.02$). The Poisson regression analyses revealed that chromosome-type aberration was significantly associated with recent 1.5-yr dose after adjusting for confounding variables such as age, smoking and alcohol intake. when exposure workers were divided into two groups based on the median split (high and low exposure groups), in relatively high exposure group, we found a negative correlation between the frequencies of Chromatid-type aberration and GSTM1 and GSTT1 copy number (Kendall's Tau = -0.17, $p = 0.058$ and Kendall's Tau = -0.17, $p = 0.054$, respectively),and the frequency of Chromatid-type aberration increased as the each copy number of GSTM1 and GSTT1 deletion variants increased from zero to one to two after adjusting for confounding variables such as age, smoking and alcohol intake. In addition, a significant negative association between combined copy number of GSTM1/GSTT1 and chromatid-type aberrations have shown in relatively high exposed group after considering confounding variables (Frequency ratio = 0.84, 95% CI 1.23 - 4.39, $p = 0.009$) and gene-environment interaction between radiation exposure and the sum of GSTM1 and GSTT1 gene copy number in occurrence of chromatid type aberratoins have also been observed

In conclusion, our current results reconfirm the usefulness of chromosome aberrations assay as a early effective markers for individual monitoring and diagnosis of occupationally exposed to low levels of IR. A clear inverse association between copy number of GSTM1 and GSTT1 and

the frequency of chromosomal aberrations in radiation workers present putative markers of individual susceptibility to IR-induced DNA damage.

Key words : Ionizing radiation, Biomarkers, Chromosome aberration, Copy number variation, Glutathione S-transferase mu-1 (GSTM1) and glutathione S-transferase theta-1 (GSTT1).

Student Number : 2009-30671

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

CA	Chromosome Aberration
CI	Confidence Interval
CNV	Copy Number Variation
FR	Frequency Ratio
GST	Glutathione-S-transferase
ICRP	International Commission on Radiological Protection
IR	Ionizing Radiation
MN	Micronuclei
PAH	Polycyclic Aromatic Hydrocarbons
PBL	Peripheral Blood Lymphocytes
ROS	Reactive Oxygen Species
SNP	Single Nucleotide Polymorphism

CHAPTER Ⅰ.

Introduction

1. Concern with occupational radiation risk from occupational chronic exposure to low levels of ionizing radiation

There are two broad types of radiation, ionizing and non-ionizing. Ionizing radiation (IR) is high-frequency radiation that has extremely high energy to break off electrons from (ionize) an atoms. This radiation is a well-known human carcinogen that can induce a large variety of biological responses depending on dose, duration, dose rate, and quality of the radiation. IR causes different lesions in human cells, thereby disturbing their normal functioning. IR induced-lesions include single- and double-strand DNA breaks, DNA base damage, apyrimidinic / apurinic sites formation, DNA-DNA and DNA-protein crosslink. In addition to DNA damage, IR also induces oxidative damage of cellular macromolecules due to reactive oxygen species (ROS) formation (Kryston et al., 2011).

Human exposure to IR occurs constantly in a wide range of occupational settings including medical field, manufacturing and service industries, in research field, and in the nuclear power industry (Chida, 2012). For populations receiving such exposures, the primary concern is that radiation could increase the risk of cancers or harmful genetic effects. Nowadays, due to the strict radiation protection / safety program, current levels of exposure to ionizing radiation in occupation have decreased and are far below the regulatory limit of 20 mSv (2 rem) per year, averaged

over five year (100 mSv / 5 years) established by the International Commission on Radiological Protection (ICRP 103, 2007), however personal cumulative doses in power plant workers are continuously increasing. Thus, there is currently great concern about an increase in a person's exposure to IR, even at low doses (Picano et al., 2012; Holmberg et al., 2010). The biological and genetic effects of low-dose level of IR exposure and its relationship to carcinogenesis have received great attention in the last years (Pierce and Preston, 2000; Mothersill and Seymour, 2001; Bonner, 2003; Brenner et al., 2003b; Morgan, 2003; Feinendegen, 2005; Dauer et al., 2010; Buonanno et al., 2011).

2. Possible Biological effect of low-level chronic radiation

The probability of IR-caused cancer or genetic effect is related to the total dose of IR accumulated by an individual. Based on current scientific evidence, any exposure to IR can be harmful. The most common long-term effect of chronic radiation exposure is an increased risk of cancer (Cohen, 2002). IR induced cancer in humans has long latent periods. Thus, there is a long period between IR exposure and the appearance of tumors. This implies that IR induced abnormalities (due to gene mutations and / or chromosomal damage) that can be detected immediately after IR exposures are not directly responsible for initiating carcinogenesis. However, such abnormalities induce genomic instability that make cells more sensitive to accumulation of additional mutations caused by exposure to additional IR exposure, chemical mutagens and carcinogens, or their combinations. One of the most studied, yet least understood effects of low-dose IR is genomic instability (Huang et al., 2007), which is defined as the increased rate of acquisition of mutations in the genome (Morgan et al., 1996) may play a key role in the early stages of cancer progression. Moreover, it has been shown that genomic instability occurs even in the progeny of exposed cells multiple generations (Little, 2003) and this transgenerational nature of genomic instability leads to multiple genetic alterations and affects chromosome structure and minisatellite instability (Dubrova, 2003). Other studies have also reported chromosomal and gene

mutations in non-irradiated cells and have recognized the bystander effect. Bystander effect is characterized as the effect, which IR induced damage signals might be transmitted from the irradiated to neighboring non-irradiated cells in the population, leading to the induction of genetic changes among these bystander cells (Morgan, 2012). Figure Ⅰ-1 shows the radiation-induced genomic instability and bystander effect.

At doses of 100 mSv or less, statistical issue and limitations make it difficult to estimate cancer risk in humans. However the lack of a scientific data does not exclude the possibility of the carcinogenesis. A critical review of available biological and biophysical data led the committee on the Biological Effects of IR to conclude that the risk would continue in a linear fashion at lower doses without a threshold and that there will be some risk, even at low doses (Tubiana et al., 2005; Brenner et al., 2003a).

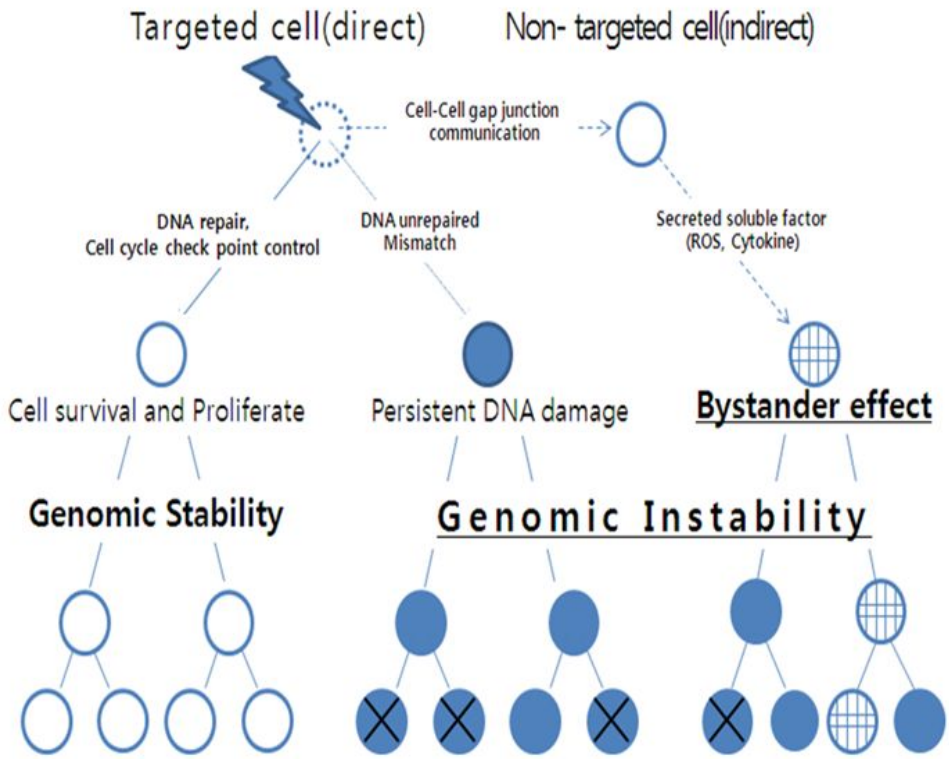


Figure Ⅰ-1. Ionizing radiation-induced genomic instability and bystander effect. Ionizing radiation induces direct DNA damage or indirect damage through the radiolysis of water. These DNA damage is either eliminated (white) or fixed in the cell as a mutation or chromosomal rearrangement by DNA repair processes. (A) Radiation-induced genomic instability. Delayed effects occur in the progeny of the irradiated cell, for example, delayed mutation (black), cell death (cross). (B) Bystander effect. The irradiated cell can communicate with non irradiated neighboring cells that result in an 'activated cell'(hatched) able to produce bystander damage in neighboring and progeny.

3. Biomarkers in molecular epidemiology studies for health risk prediction in radiation workers

Molecular epidemiology studies incorporating biomarker and bioassays make possible to integrate epidemiological, biological and genetic studies in human. A number of these have been used to investigate exposure, effects and susceptibility to radiation carcinogenesis. Recently, several epidemiological studies have reported an association between the high frequency of chromosomal alteration and increased cancer risk (Bonassi et al., 1995; Hagmar et al., 1994; Sram et al., 2006) suggesting that the monitoring of an increase in chromosome aberration in peripheral blood lymphocytes (PBL) may be used as a marker for susceptibility to cancer. Monitoring of radiation workers occupationally exposed to IR consists of regular physical dosimetry and periodic health examination. Cytogenetic studies as specific biomarkers provide additional information which complements physical dosimetry and enables better evaluation of radiation health effects. Analysis of chromosomal aberrations (CA) and other cytogenetic biomarkers such as micronuclei (MN) in PBL have also been used for a long time for the assessment of exposures to various mutagens or carcinogens and several cytogenic studies have been performed among workers occupationally exposed to chronic low doses of IR to evaluate the cyto- / genotoxic effects of these radiation exposures. Genetic variability as predictive biomarkers for intrinsic radiosensitivity of healthy individuals may allow the monitoring of occupational or environmental radiation exposure, as well as help in the identification of risk groups for occupational IR exposure (Pernot et al., 2012).

4. Biomarkers for genetic susceptibility to ionizing radiation

Many of current study to identify genetic variation associated with IR sensitivity have focused on identifying effects of single nucleotide polymorphism (SNP) in candidate genes, among which DNA repair and cell cycle regulation genes have been scrutinized because of their pivotal role in maintaining genome stability. However many of the candidate gene approach have often yielded conflicting result.

Human genomes vary from one to another at the genetic level. Some genetic variations are large structural or chromosomal variations, while others come out at the single-nucleotide (sequence) level. A structural variation that has been described in recent years is Copy Number Variation (CAV) (Figure Ⅰ-2).

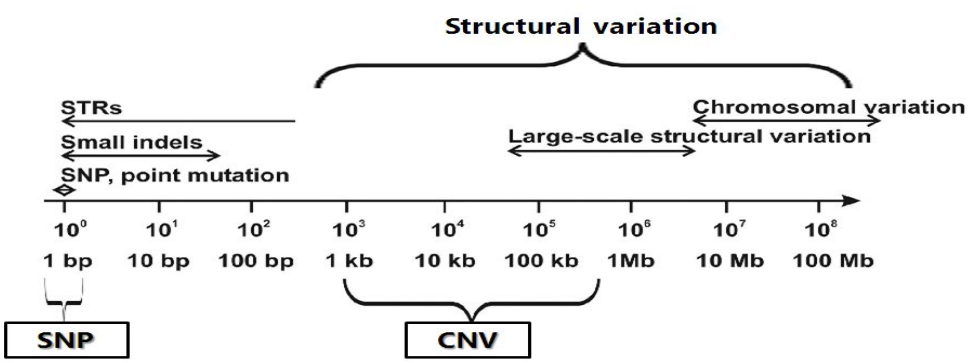


Figure Ⅰ-2. Types of genetic variations in the human genome. Common types of genetic variations can be categorized into two major groups—those that involve single base changes (e. g. SNPs) and those that alter more than one base (e. g. structural variants).

Broadly defined, a CAV is a DNA segment of at least 1 kb in size and is present at a variable copy umber in comparison with a reference genome (Feuk et al. 2006). This definition encompasses large stretches of genomic DNA that vary in copy number amongst individuals as a result of the variable occurrence of deletions (loss), duplications (gain), and tandem repeats of segments of genomic DNA (Figure Ⅰ-3). These CNVs can influence gene expression and can be associated with specific phenotypes and diseases. In 2006, a comprehensive map of CNVs was released based on 270 person from different ethnic groups of European, Asian and African descents, who were originally included in the International HapMap Project. Redon et al. reported a total of 1447 copy number variable regions (CNVRs), corresponding to 360 Mb of human DNA sequences or 12% of the human genome (Redon et al. 2006).

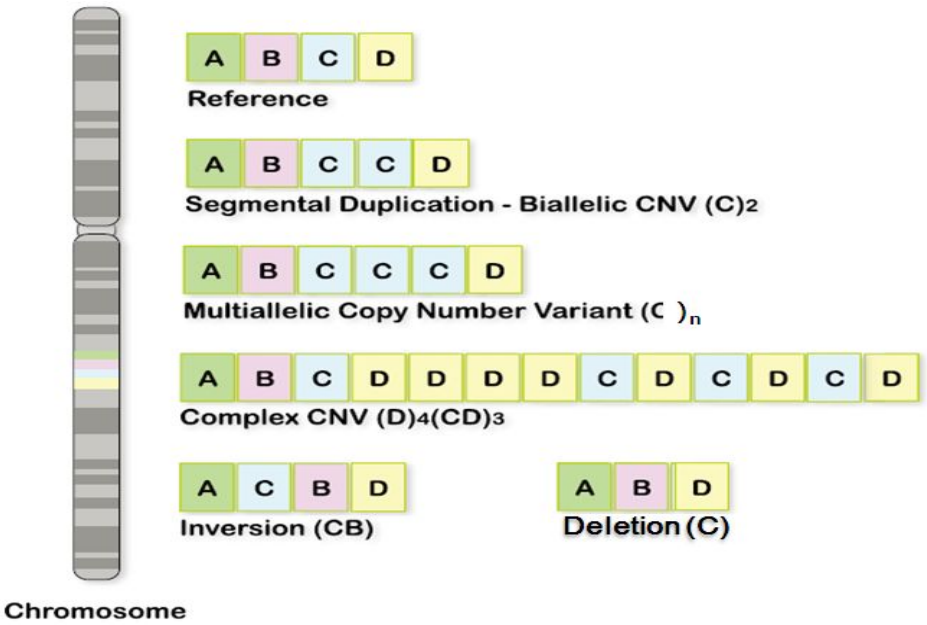


Figure Ⅰ-3. Types of genomic structural changes affecting segments of DNA. That lead to different types of variations (deletions, duplications, inversions, and complex CAV Changes)3

These variations may have a greater effect on disease susceptibility than previously thought. For instance, low copy number of the CCL3L1 gene (chemokine gene) has been associated with markedly enhanced HIV-1/AIDS infection, low copy number of the FCGR3 (the CD16 cell surface immunoglobulin receptor) can increase susceptibility to several types of autoimmune disorders such as systemic lupus erythematosus (SLE), different copy number of the complement component C4 (C4A and C4B) lead to different susceptibilities to SLE, variable copy number of DEFB4 also lead to different susceptibility to Crohn's disease etc (Estivill and Armengol, 2007). Copy number variation has also been associated with

autism, schizophrenia, and idiopathic learning disability (Cook and Scherer, 2008). Therefore, It is important for any genetic association study to consider the presence of structural variation in the region of interest and to have suitable technologies for detecting them (Feuk et al., 2006).

Recent genotyping technologies that made it possible to identify CNVs in large population based molecular epidemiological studies developing novel, low-cost and high-throughput processing. Prior to the availability of CAV approach, Single nucleotide polymorphism (SNP) studies could not classify individuals as having either complete absence of both gene copies or having 1 or more copies. Individuals with a single deletion, with reduced but not complete loss of enzyme activity, were grouped with those with normal enzyme activity. CAV analysis allow examination of gene dosage effect to provide information about gradation of risk and to further address the effect of gene presence on a biological pathway. However, up to now, the role of CNVs in particular after IR exposure has been little studied. A recent study have shown the ATM,p53 and CHEK2 copy number dependant increase in ATM,p53,CHEK2 enzyme activity before and after in vitro IR exposure (Kabacik et al., 2011)

5. Objectives of the study

Even when occupational exposure is far below the limits set by ICRP or legislation, personal cumulative doses in power plant workers are continuously increasing, and their monitoring is generally based on physical dosimetry so a health surveillance program using a cytogenetic assay for the early detection of diseases is needed. And studies on the effect of CNVs on the level of chromosomal damage in individuals occupationally exposed to IR may increase the sensitivity of cytogenetic biomarkers as indicators of genotoxic effects, as well as help in the identification of risk groups. The aim of the present study was to examine effects of low dose IR on different individual cytogenetic response measured by CA and MN frequency, Therefore, the aim of the present study is to assess occupationally induced chromosomal damage in relation to copy number variation in a large population of nuclear power plant workers exposed to low dose IR.

For this purpose, 1) we used the CA and MN assay in PBL of nuclear power plant workers never exceeded 20 mSv per year for their duration of work and compare the results with control individuals. 2) we investigated for the first time the influence of GSTM1 and GSTT1 copy number polymorphisms on chromosomal damage in low dose IR - exposed workers.

References

Bonassi, S., Abbondandolo, A., Camurri, L., Dal Pra, L., De Ferrari, M., Degrassi, F., Forni, A., Lamberti, L., Lando, C., Padovani, P. and et al. (1995) Are chromosome aberrations in circulating lymphocytes predictive of future cancer onset in humans? Preliminary results of an Italian cohort study. Cancer Genet Cytogenet, 79, 133-135.

Bonner, W. M. (2003) Low-dose radiation: thresholds, bystander effects, and adaptive responses. Proc Natl Acad Sci U S A, 100, 4973-4975.

Brenner, D. J., Doll, R., Goodhead, D. T., Hall, E. J., Land, C. E., Little, J. B., Lubin, J. H., Preston, D. L., Preston, R. J. and Puskin, J. S. (2003a) Cancer risks attributable to low doses of ionizing radiation: assessing what we really know. Proceedings of the National Academy of Sciences of the United States of America, 100, 13761.

Brenner, D. J., Doll, R., Goodhead, D. T., Hall, E. J., Land, C. E., Little, J. B., Lubin, J. H., Preston, D. L., Preston, R. J., Puskin, J. S., Ron, E., Sachs, R. K., Samet, J. M., Setlow, R. B. and Zaider, M. (2003b) Cancer risks attributable to low doses of ionizing radiation: Assessing what we really know. Proc Natl Acad Sci U S A, 100, 13761-13766.

Buonanno, M., de Toledo, S. M., Pain, D. and Azzam, E. I. (2011) Long-Term Consequences of Radiation-Induced Bystander Effects Depend on Radiation Quality and Dose and Correlate with Oxidative Stress. Radiat Res, 175, 405-415.

Chida, K. (2012) Medical and occupational radiation exposure: present situation and countermeasures. Nippon Rinsho Jpn J Clin Med, 70, 479-484.

Cohen, B. L. (2002) Cancer risk from low-level radiation. American journal of roentgenology, 179, 1137-1143.

Cook Jr, E. H. and Scherer, S. W. (2008) Copy-number variations associated with neuropsychiatric conditions. Nature, 455, 919-923.

Dauer, L. T., Brooks, A. L., Hoel, D. G., Morgan, W. F., Stram, D. and Tran, P. (2010) Review and evaluation of updated research on the health effects associated with low-dose ionising radiation. Radiat Prot Dosim, 140, 103-136.

Dubrova, Y. E. (2003) Radiation-induced transgenerational instability. Oncogene, 22, 7087-7093.

Estivill, X. and Armengol, L. (2007) Copy number variants and common disorders: filling the gaps and exploring complexity in genome-wide association studies. Plos Genet, 3, 190.

Feinendegen, L. E. (2005) Evidence for beneficial low level radiation effects and radiation hormesis. Br J Radiol, 78, 3-7.

Feuk, L., Marshall, C. R., Wintle, R. F. and Scherer, S. W. (2006) Structural variants: changing the landscape of chromosomes and design of disease studies. Human molecular genetics, 15, R57-R66.

Hagmar, L., Brogger, A., Hansteen, I. L., Heim, S., Hogstedt, B., Knudsen, L., Lambert, B., Linnainmaa, K., Mitelman, F., Nordenson, I. and et al. (1994) Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer Res, 54, 2919-2922.

Holmberg, O., Malone, J., Rehani, M., McLean, D. and Czarwinski, R.

(2010) Current issues and actions in radiation protection of patients. Eur J Radiol, 76, 15-19.

Huang, L., Kim, P. M., Nickoloff, J. A. and Morgan, W. F. (2007) Targeted and nontargeted effects of low-dose ionizing radiation on delayed genomic instability in human cells. Cancer Res, 67, 1099-1104.

ICR Ppublication 103, I. p. (2007) The 2007 Recommendations of the International Commission on Radiological Protection. ICRP publication 103. In Ann ICRP, Vol. 37, pp. 1-332.

Kabacik, S., Ortega-Molina, A., Efeyan, A., Finnon, P., Bouffler, S., Serrano, M. and Badie, C. (2011) A minimally invasive assay for individual assessment of the ATM/CHEK2/p53 pathway activity. Cell cycle (Georgetown, Tex.), 10.

Kryston, T. B., Georgiev, A. B., Pissis, P. and Georgakilas, A. G. (2011) Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res, 711, 193-201.

Little, J. B. (2003) Genomic instability and radiation. J Radiol Prot, 23, 173-181.

Morgan, W. F. (2003) Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects in vivo, clastogenic factors and transgenerational effects. Radiat Res, 159, 581-596.

Morgan, W. F. (2012) Non-targeted and Delayed Effects of Exposure to Ionizing Radiation: I. Radiation-Induced Genomic Instability and Bystander Effects In Vitro. Radiat Res, 178.

Morgan, W. F., Day, J. P., Kaplan, M. I., McGhee, E. M. and Limoli, C. L. (1996) Genomic instability induced by ionizing radiation. Radiat Res, 146, 247-258.

Mothersill, C. and Seymour, C. (2001) Radiation-induced bystander effects: Past history and future directions. Radiat Res, 155, 759-767.

Pernot, E., Hall, J., Baatout, S., Benotmane, M. A., Blanchardon, E., Bouffler, S., Saghire, H. E., Gomolka, M., Guertler, A. and Harms-Ringdahl, M. (2012) Ionizing radiation biomarkers for potential use in epidemiological studies. Mutat Res, 751, 258-286.

Picano, E., Vano, E., Domenici, L., Bottai, M. and Thierry-Chef, I. (2012) Cancer and non-cancer brain and eye effects of chronic low-dose ionizing radiation exposure. Bmc Cancer, 12, 157.

Pierce, D. A. and Preston, D. L. (2000) Radiation-related cancer risks at low doses among atomic bomb survivors. Radiat Res, 154, 178-186.

Sram, R. J., Rossner, P., Rubes, J., Beskid, O., Dusek, Z., Chvatalova, I., Schmuczerova, J., Milcova, A., Solansky, I., Bavorova, H., Ocadlikova, D., Kopecna, O. and Musilova, P. (2006) Possible genetic damage in the Czech nuclear power plant workers. Mutat Res, 593, 50-63.

Tubiana, M., Aurengo, A., Averbeck, D., Bonnin, A., Le Guen, B., Masse, R., Monier, R., Valleron, A. and De Vathaire, F. (2005) Dose-effect relationships and estimation of the carcinogenic effects of low doses of ionizing radiation. Int J Low Radiat, 2, 134-151.

CHAPTER Ⅱ. Evaluation of cytogenetic effects in nuclear power plant workers exposed to low dose ionizing radiation using chromosome aberration and micronuclei assay

Abstract

Ionizing radiation (IR) is a well-known mutagen and carcinogen but the biological effects of chronic occupational IR exposure have not been fully understood due to a lack of direct human evidence. This study was performed to assess occupationally induced chromosomal damage in peripheral blood lymphocytes (PBL) of 180 nuclear power plant workers never exceeded 20 mSv per year for their duration of work and compare the results with 45 control individuals. Frequencies of chromosome-type and chromatid - type aberration were significantly higher in workers than in controls (Mann Whitney test; $p < .001$), and chromosome-type aberration frequency was significantly increase with the recent 1.5 year dose (Pearson's correlation coefficient test; r=0.17, p = 0.02). The Poisson regression analyses revealed that chromosome-type aberration was significantly associated with recent 1.5-yr dose after adjusting for confounding variables such as age, smoking and alcohol intake. The frequency of micronucleus in PBL was significantly higher in radiation exposed workers than in controls (Mann Whitney test; $p < .001$). However the present study did not find any association between micronucleus occurrences and total cumulative dose, recent 5.5 year dose, and recent 5.5 year dose.

In conclusion, there is a strong indication that chromosome damage could be induced in workers exposed to low dose of IR, even below occupational permissible dose limit. we suggest that the application of the chromosome (i.e. chromosome-type) aberrations test for individual monitoring and diagnosis of occupationally exposed workers to chronic low dose IR.

Key words : Ionizing radiatno, Biomarkers, Chromosome aberration, Micronuclei.

1. Introduction

Human exposure to ionizing radiation (IR) occurs in medical field, as well as in manufacturing and service industries, in research field, and in the nuclear power industry. Levels of exposure to ionizing radiation in occupation have decreased in recent decades and are now far below the regulatory limit of 20 mSv (2 rem) per year, averaged over five year (100 mSv/5years) established by the International Commission on Radiological Protection (ICRP 103, 2007), however personal cumulative doses in power plant workers are continuously increasing. Therefore, health and welfare of human population occupationally exposed to chronic low dose radiation are of great concern.

While the association between cancer and exposure IR has been well described from data on atomic bomb survivors and the Chernobyl accident (Zamostian et al., 2002; Little et al., 2010), the potential risk of chronic low dose IR exposure is still not clear (Ogata, 2011). Also protective effects such as hormesis and adaptive response have been also reported following exposure to low dose of radiation (Zhang et al., 2012). Thus, the health effect of chronic exposure to low dose of radiation is controversial.

IR causes different lesions in human cells. These lesions include single- and double-strand DNA breaks, DNA base damage, apyrimidinic /

apurinic sites formation and DNA-protein crosslinks. In addition to DNA damage, IR also induces oxidative damage of cellular macromolecules due to reactive oxygen species (ROS) formation. Many studies reported that the occupational exposure of a low dose of radiation, a few hundred mSv, can induce chromosomal damage such as micronuclei, sister chromatid exchange, and chromosome aberration (Evans et al., 1979; Lindholm, 2001). The biological and genotoxic effects of chronic low dose IR exposure and its relationship to carcinogenesis have received much attention in the recent years (Kovalchuk et al., 2004; Zhizhina, 2011; Laurent et al., 2010; Russo et al., 2012). As an initiating event in radiation carcinogenesis, it is generally accepted that mis - repaired double strand DNA breakage are the main lesions at the origin of both genomic instability and gene mutations (Natarajan et al., 1993; Morgan et al., 1996; Bishayee et al., 2001). Recently, several epidemiological studies have reported an association between the high frequency of chromosomal alteration and increased cancer risk (Bonassi et al., 1995; Hagmar et al., 1994; Sram et al., 2006) suggesting that the monitoring of an increase in chromosome aberration in peripheral blood lymphocytes (PBL) may be used as a marker for susceptibility to cancer.

Evaluating of occupationally exposed to IR consists of regular film dosimetric monitoring and periodic health examination. Cytogenetic studies as specific biomarkers provide additional information which complements

physical dosimetry and enables better evaluation of radiation health effects. Analysis of chromosomal aberrations (CA) and other cytogenetic biomarkers such as micronuclei (MN) in PBL have also been used for a long time for the assessment of exposures to various mutagens or carcinogens. Several cytogenic studies have been performed among workers occupationally exposed to chronic low doses of IR to evaluate the cyto- / genotoxcic effects of these radiation exposures.

Chromosome aberration analysis in PBL is wildly used for the surveillance of workers exposed to mutagens and carcinogens (Carrano and Natarajan, 1988), and especially, a dicentric chromosome has been accepted as a valid biological indicator for biological dosimetry in management of radiation accidents (Sasaki and Miyata, 1968). Although the presence of chromosomal aberrations itself in lymphocytes of workers exposed to IR does not necessarily lead to adverse health effect, high levels of chromosome aberration apparently indicates its potential health risk. Most studies have revealed that chromosome aberrations in lymphocytes are more frequent in radiation workers than in controls (Chung et al., 1996; Bonassi et al., 1997) suggesting CA assay is one of the important biological consequences of human exposure to IR. Until now, it has remained the most suitable assay for the evaluation of the genetic damage induced by IR. Analysis of frequency of chromosomal aberrations in PBL is

internationally standardized. Furthermore, contrary to alternative measures, the process of validation of this biomarker was fully accomplished.

Another cytogenetic assay used for biodosimetry is the scoring of micronucleus formation. The cytokinesis-blocked micronuleui (CBMN) analysis in human lymphocytes also represents a reliable test to assess radiation induced chromosome damage (Fenech et al., 1999a). It detects whole chromosome loss or break and reflects the level of non-repaired breaks at the time of cell division. Micronuclei arise either from acentric fragments (Countryman and Heddle, 1976) or from whole chromosome (Vig and Swearngin, 1986). Malfunction of the mitotic spindle or damaged kinetochores are the reason for lagging chromosomes (Scott and Zampettibosseler, 1980), which form MN containing whole chromosome. As the simplicity of MN scoring, analysis of micronuclei assay has been suggested as a valid and less laborious alternative to CAs analysis for large-scale studies (Fenech et al., 1999b) among human populations occupationally or environmental exposed to IR. Most of studies reported significantly higher MN rates in exposed populations than in controls (Cardoso et al., 2001; Joseph et al., 2004).

Even when occupational exposure is far below the limits set by ICRP or legislation, personal cumulative doses in power plant workers are continuously increasing, and their monitoring is generally based on physical

dosimetry so a health surveillance program using a cytogenetic assay for the early detection of diseases is needed. The aim of the present study was to assess occupationally induced chromosomal damage in a large population of nuclear power plant workers exposed to low dose IR. For this purpose, we used the CA and MN assay in PBL of 180 nuclear power plant workers never exceeded 20 mSv per year for their duration of work and compare the results with 45 control individuals.

2. Materials and Methods

2-1. Ethics statement

The study protocol was approved by the Research Ethics Review Board of Seoul National University, and written informed consent was obtained from each individual before their inclusion in the study.

2-2. Study population

The radiation - exposed group consisted of 180 male workers occupationally exposed to radiation more than 100 mSv of cumulative dose within the workers who had ever exceeded the international accepted acceptable permissible dose limit, 20 mSv per year and 100 mSv per 5 years. Radiation exposure and personal dosimetry records based on film badges were collected for each worker for the whole entire working period. The non-exposed (control) to radiation group consisted of 45 age - matched volunteers who had never been exposed to occupational ionizing radiation. All subjects were requested to complete a questionnaire regarding theire smoking habits, alcohol consumption, medical history, and duration of occupational exposure to radiation (years of employment). All subjects did not have a personal medical history of cancer, genetic or other chronic disease and had no drug intake in the months prior to the study.

2-3. Collection of blood samples and culture

Peripheral whole blood samples from each subject were drawn into heparin-containing tubes and processed for analysis immediately upon arrival in the laboratory. 1 ml of heparinized blood sample were seeded into 9 ml of culture medium, RPMI 1640 (Gibco BRL, Grand Island, NY) containing 10% fetal bovine serum (Sigma Chemical Co., St. Louis, MO), penicillin (100 U/ml), streptomycin (100 μL/mL) and phytohemagglutinin M (1%). The culutures wer incubated at 37 °C and in a humidified atmosphere of 5% CO_2.

2-4. Chromosome aberration (CA) Assay

The cultures were incubated for 48h from the start of the culture with PHA and colcemid (0.1 μg/ml) was added to the cultures 3 hours prior to harvesting. Chromosome preparations were performed according to the standard procedures (IAEA, 2001) The slides were dried in the air and stained with Giemsa. Slides were coded and 500 metaphases for each subject were scored blindly. After completing the chromosome aberration analysis, the radiation records were linked to the coded number for the data analysis.

2-5. Micronuclei (MN) Assay

The CBMN assay was performed as described by Fenech (2000). Briefly, peripheral blood (1 ml) was added to 9 ml of RPMI-1640 medium containing 10% FBS. After 44 h, cytochalasin-B (4.5 μg/ml; Sigma) was added to the culture, which was incubated for another 28 h (total incubation time, 72 h). The cells were collected, treated with 0.075 M KCl hypotonic solution for 2 min, and fixed in a mixture of methanol and acetic acid (3 : 1). The samples were air-dried and stained with Giemsa stain. All slides were coded and scored blindly according to the criteria of Fenech (2000). A total of 1000 binucleated lymphocytes were scored.

2-6. Statistical analysis

Statistical analyses were performed using a statistical package for Windows (SAS 9.1; SAS Institute, Cary, NC). The differences between radiaion exposed group and control in terms of general characteristics were tested using the χ^2-tests and Mann-Whitney U-test. The Mann-Whitney U-test was used to compare the the frequency of MN / CA in radiation - exposed workers with those of the control subjects. The relation of MN / CA yield with radiation expousre was tested by kendall rank correlation coefficient. A simple regression model was used to find the relationship between MN / CA yield and various variables. The correlation between

cytogenetic markers was tested by Pearson Correlation. Poisson regression analysis was applied to evaluate the independent association between the frequency of chromosome aberrations and various variables including age, smoking status, alcohol intake, and radiation doses. The criterion for significance was set at $p < .05$.

3. Results

3-1. General characteristics of the study population

The general characteristics of the study population are listed in Table Ⅱ-1. The ages of the radiation exposed workers and controls ranged from 29 to 59 yr with a mean of 47.46 ± 5.94 yr and 24 to 65 yr with a mean of 40.89 ± 10.01 yr, respectively. Current smokers were 38.33% of the radiation exposed workers and 44.44% of the control subjects. There was no significant difference in the patterns for age and smoking status between the radiation exposed workers and the control subjects indicating that radiation exposed workers and control subjects were closely matched (Table Ⅱ-1). The mean duration of employment for the radiation exposed workers was 20.06 ± 6.19 yr and the cumulative radiation dose ranged from 12.67 to 400.25 mSv.

Table Ⅱ-1. General characteristics of the study population

Variables	No. of subjects (%)		*p*-value
	Controls (n=45)	Exposed (n=180)	
Age (mean ± SD, years)	40.89 ± 10.01	47.46 ± 5.94	<0.001[a]
<50	36 (80)	117 (65)	0.05[b]
≧50	9 (20)	63 (35)	
Smoking status			
Current smoker	20 (44.44)	69 (38.33)	0.45[b]
Former or never smoker	25 (55.56)	111 (61.67)	
Alcohol consumption			
Yes	30 (66.67)	147 (81.67)	0.03[b]
No	15 (33.33)	33 (18.33)	
Duration of employment (mean ± SD, years)		20.06 ± 6.19	
<20		58 (32.22)	
20≦-<25		90 (50)	
≧25		32 (17.78)	
Cumulative dose (mean ± SD, years, mSv)		157.31 ± 85.24	
<100		38 (21.11)	
100≦-<150		61 (33.89)	
150≦-<200		32 (17.78)	
≧200		49 (27.22)	
Recent 1.5-yr radiation dose (mean ± SD, years, mSv)		8.19 ± 7.08	
<1.5		45 (25)	
1.5≦-<7.5		44 (24.44)	
7.5≦-<15		56 (31.11)	
≧15		35 (19.44)	

SD, Standard deviation.

[a] Determined by Mann-Whitney U-test.

[b] Determined by x^2test.

3-2. Chromosome aberration

The differences in chromosome aberration frequencies between the radiation exposed workers and the control group were statistically significant for all chromosome-type aberrations (Table Ⅱ-2, p <.001). The mean values of chromosome-type aberration were 3 / 500 metaphase cells in the radiation exposed workers and 0.6 / 500 metaphase cells in the control subjects. The baseline frequency of chromosome aberration (0.8% ; 4.09 / 500 metaphase cells) was lower than the frequency observed in our previous study (1.16%; Chung et al., 1996), but was similar to the data observed by Maddileti et al. (2002) and Samavat and Mozdarani (2004).

As shown in Fig. Ⅱ-1, no significant relation between chromosome-type aberration and cumulative dose and recent 5.5 year dose was found, whereas positive relation between the frequency of chromosome-type aberration and recent 1.5 year dose is revealed (r^2 = 0.03, p = 0.021). The correlation between years of employment and three different radiation doses such as total cumulative dose, recent 5.5 year dose, and recent 1.5-year is shown in Fig. Ⅱ-2. There was a positive correlation between work duration and total cumulative radiation dose (r^2 = 0.22, p <. 0001), but an inverse correlation with the recent 5 - year (r^2 = 0.03 p = 0.02) and recent 1.5-year emerged (r^2 = 0.08 p = 0.0001). The workers with longer duration of work tend to be exposed to radiation for recent 1.5 year less than those with short duration of work (Fig. Ⅱ-2). Table Ⅱ-6

showed that the frequency of CA did not increase by the duration of work.

The frequency of CA induced by cumulative radiation dose, recent 5.5 year dose and 1.5 - year dose are shown in Table Ⅱ-3,5. The frequency of CA increased according to the recent 1.5 year dose but the frequency of CA decreased according to the recent 5.5 year dose and cumulative radiation dose. Table Ⅱ- 7 shows the results of multiple Poisson regression analysis for CA frequency, after adjusting for the potential impact of demographic and lifestyle factors (e.g. exposure dose of IR, age, smoking status and alcohol consumption). recent 1.5 year dose was significantly associated with altered CA frequencies ($\beta = 0.022$, $p < 0.001$).

Poisson regression analysis applied to the overall study population revealed that recent 1.5 year dose was significantly associated with the frequency of chromosome aberration after adjusting for age, smoking status and alcohol intake (Table Ⅱ-8). However no significant association was found when only the exposed group was considered. Meanwhile, when Poisson regression analysis was performed to estimate the association with the frequency of different chromosomal type aberration and recent 1.5 year dose, the frequency of chromosome-type aberration is significantly increased with recent 1.5 year dose after adjusting for age, smoking status and alcohol intake (Table Ⅱ-9).

Table Ⅱ-2. Frequency of chromosomal aberrations in radiation exposed workers and controls

Types of chromosome aberration	Controls			Exposed			
	Mean/ 500cells	SD	Range	Mean/ 500cells	SD	Range	*p*-value*
No. of individuals	45			180			
Chromatid-type deletion	3.53	2.21	0-10	5.41	2.76	0 - 17	<0.0001
Chromatid-type exchange	0.04	0.21	0-1	0.06	0.23	0 - 1	0.7697
Chromosome-type deletion	0.51	0.82	0-3	2.08	1.98	0 - 14	<0.0001
Chromosome-type exchange	0.13	0.40	0-2	0.93	1.02	0 - 6	<0.0001
Total aberration	4.09	2.51	1-12	8.39	3.53	1 - 19	<0.0001

SD, Standard deviation.

*, Significantly different from control subjects (determined by Mann-Whitney U-test, $p < .05$).

Table Ⅱ-3. Frequency of chromosomal aberrations in relation to cumulative radiation dose in exposed workers

Dose (mSv)	No. of subjects	Chromatid aberration		Chromosome aberration		Total aberration
		Deletion	Exchange	Deletion	Exchange	
<100	38	7.13	0.16	2.37	1.05	10.32
100≦-<150	61	5.07	0.02	1.93	0.79	7.74
150≦-<200	32	5.41	0.03	2.25	1.19	8.88
≧200	49	4.51	0.04	1.92	0.82	7.41
Kendall's τ		-0.20780	-0.12959	-0.05866	-0.03945	-0.19379
p		0.0004	0.0585	0.3371	0.5386	0.0009

Chromosome aberration frequencies are given per 500 cells.

Kendall's τ was calculated on individual bases.

p, Tested by Kendall rank correlation coefficient (τ).

Table Ⅱ-4. Frequency of chromosomal aberrations in relation to recent 5.5-yr radiation dose in exposed workers

Dose (mSv)	No. of subjects	Chromatid aberration		Chromosome aberration		Total aberration
		Deletion	Exchange	Deletion	Exchange	
<10	42	5.88	0.05	1.83	0.88	8.62
10≦-<25	43	6.07	0.02	2.28	1.09	9.23
25≦-<50	53	5.06	0.08	1.77	0.82	7.66
≧50	42	4.71	0.07	2.50	0.88	8.24
Kendall's τ		-0.15722	0.05665	0.04445	0.02193	-0.09896
p		0.0074	0.4067	0.4655	0.7317	0.0886

Chromosome aberration frequencies are given per 500 cells.

Kendall's τ was calculated on individual bases.

p, Tested by Kendall rank correlation coefficient (τ).

Table Ⅱ-5. Frequency of chromosomal aberrations in relation to recent 1.5-yr radiation dose in exposed workers

Dose (mSv)	No. of subjects	Chromatid aberration		Chromosome aberration		Total aberration
		Deletion	Exchange	Deletion	Exchange	
<1.5	45	5.16	0.02	1.80	0.80	7.76
1.5≦-<7.5	44	5.48	0.02	1.91	0.75	8.16
7.5≦-<15	56	5.25	0.05	2.21	1.13	8.38
≧15	35	5.91	0.14	2.43	0.97	9.54
Kendall's τ		0.02650	0.15606	0.11047	0.10419	0.10201
p		0.6521	0.0224	0.0844	0.0875	0.0795

Chromosome aberration frequencies are given per 500 cells.

Kendall's τ was calculated on individual bases.

p, Tested by Kendall rank correlation coefficient (τ).

Table Ⅱ-6. Frequency of chromosomal aberrations in relation to duration of work in radiation exposed workers

Duration of Work (Year)	No. of subjects	Chromatid aberration		Chromosome aberration		Total aberration
		Deletion	Exchange	Deletion	Exchange	
<20	58	6.21	0.14	2.26	0.88	9.22
20≦-<25	90	4.94	0.02	1.86	0.89	7.73
≧25	32	5.28	0.00	2.38	1.09	8.75
Kendall's τ		-0.11149	-0.22460	0.02486	0.01983	-0.05786
p		0.0685	0.0016	0.6953	0.7661	0.3394

Chromosome aberration frequencies are given per 500 cells.

Kendall's τ was calculated on individual bases.

p, Tested by Kendall rank correlation coefficient (τ).

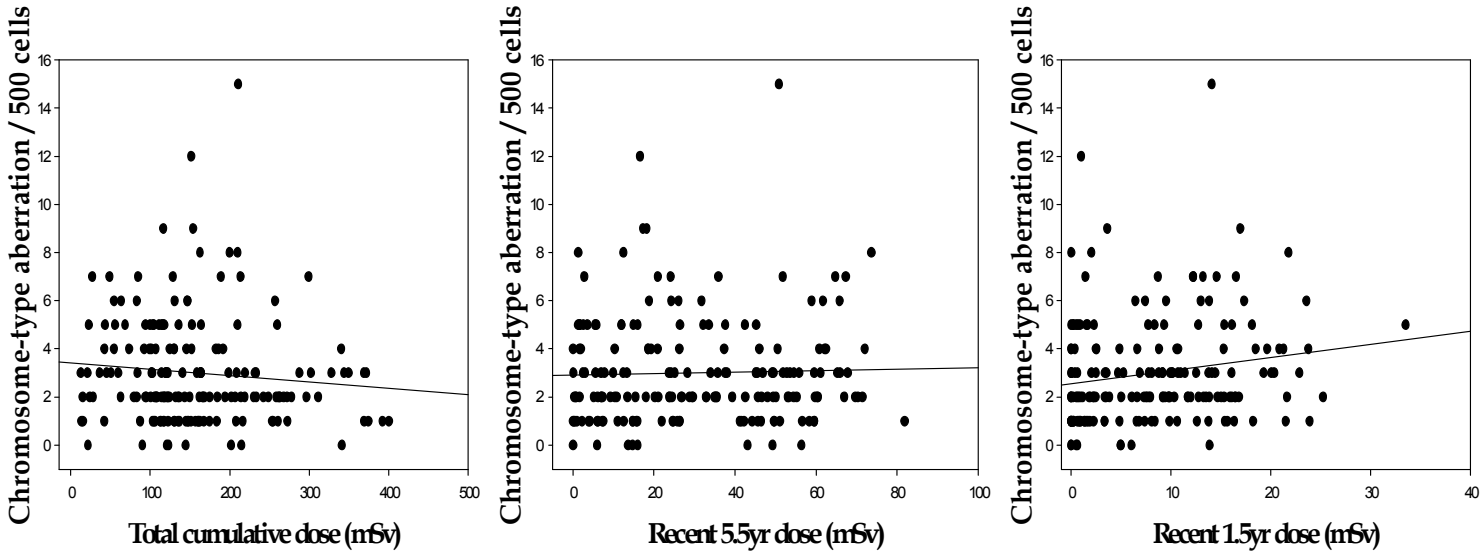


Figure Ⅱ-1. Correlation between the frequency of chromosome-type aberration and radiation doses. cumulative dose, recent 5.5-yr dose and recent 1.5-yr dose

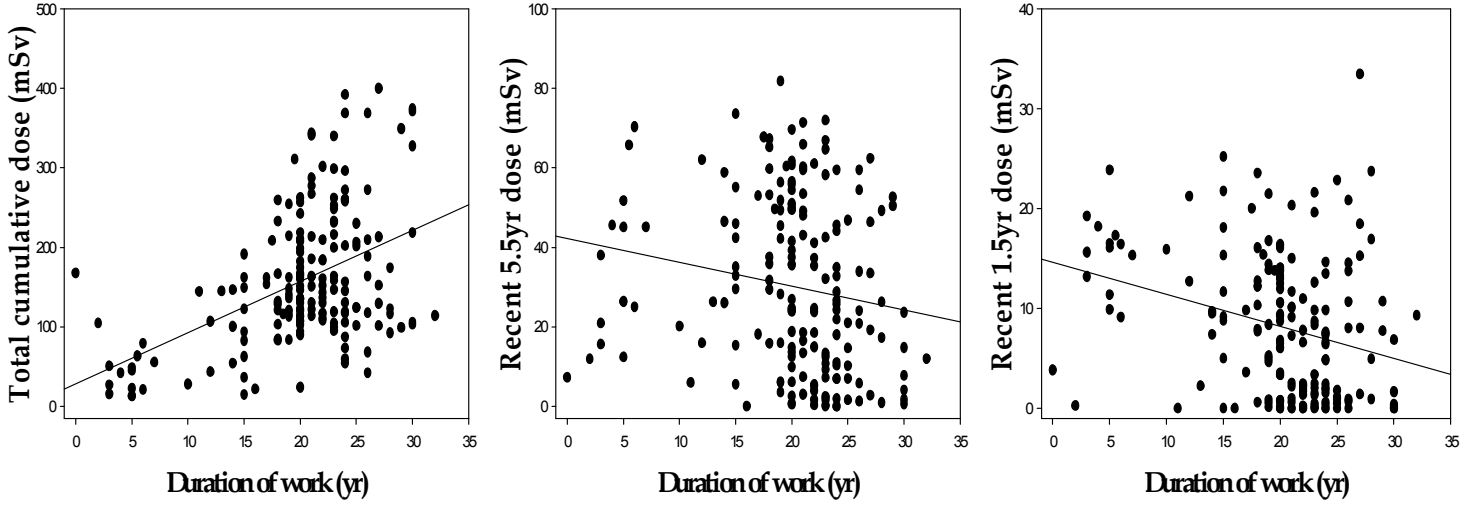


Figure Ⅱ-2. Correlation between the radiation dose and years of employment cumulative dose. cumulative dose, recent 5.5-yr dose and recent 1.5-yr dose

Table Ⅱ-7. Poisson regression analysis for total chromosome aberrations with respect to age, smoking status, alcohol intake, and radiation dose

Variable	Model 1			Model 2			Model 3		
	β	coefficient	*p*-value	β	coefficient	*p*-value	β	coefficient	*p*-value
Intercept		1.78	<.0001		2.011	<.0001		2.22	<.0001
Age (in years)		0.0039	0.27		0.0011	0.74		-0.0032	0.41
Smoking status (0,1)[a]		0	1.00		-0.0086	0.87		-0.017	0.74
Alcohol consumption (0,1)[b]		-0.082	0.19		-0.11	0.09		-0.099	0.11
Recent 1.5-yr radiation dose (mSv)		0.022	<.0001		NI	-		NI	-
Recent 5.5-yr radiation dose (mSv)		NI	-		0.0039	0.0002		NI	-
Cumulative dose (mSv)		NI	-		NI	-		0.0007	0.01

[a], Smoking status: 0, never smoking; 1, smoking.

[b], Alcohol intake: 0, never; 1, current.

Abbreviation: NI, variable not included in this run.

Table Ⅱ-8. Poisson regression analysis for total chromosome aberrations associated with age, smoking status, alcohol intake, and recent 1.5-yr radiation dose

	Variable	β coefficient	95% CI low	95% CI Upper	*p*-value
Total chromosome aberrations in overall population	Intercept	1.6164	1.2645	1.9612	<.0001
	Age (in years)	0.0039	-0.0029	0.0107	0.2679
	Smoking status (0,1)[a]	-0.0000	-0.1016	0.1010	0.9998
	Alcohol consumption (0,1)[b]	0.0817	-0.0392	0.2054	0.1904
	Recent 1.5-yr radiation dose (mSv)	0.0223	0.0160	0.0286	<.0001
Total chromosome aberrations in radiation exposed workers	Intercept	2.5422	2.0447	3.0350	<.0001
	Age (in years)	-0.0104	-0.0197	-0.0010	0.0301
	Smoking status	0.0448	-0.0645	0.1535	0.4200
	Alcohol consumption	0.0064	-0.1246	0.1410	0.9246
	Recent 1.5-yr radiation dose (mSv)	0.0061	-0.0017	0.0138	0.1246

[a], smoking status: 0, never smoking;1, smoking.

[b], alcohol intake: 0, never; 1, current.

Table Ⅱ-9. Poisson regression analysis for chromosome-type aberrations associated with age, smoking status, alcohol intake, and recent 1.5-yr radiation dose

	Variable	β coefficient	95% CI low	95% CI Upper	*p*-value
Chromosome-type aberrations in overall population	Intercept	-0.0004	-0.6396	0.6180	0.9990
	Age (in years)	0.0121	0.0000	0.0244	0.0524
	Smoking status (0,1)[a]	0.0015	-0.1750	0.1761	0.9870
	Alcohol consumption (0,1)[b]	0.1034	-0.1065	0.3222	0.3437
	Recent 1.5-yr radiation dose (mSv)	0.0377	0.0271	0.0481	<.0001
Chromosome-type aberrations in radiation exposed workers	Intercept	0.8011	-0.0399	1.6285	0.0598
	Age (in years)	0.0025	-0.0132	0.0183	0.7604
	Smoking status	0.0597	-0.1239	0.2414	0.5217
	Alcohol consumption	0.0061	-0.2117	0.2339	0.9573
	Recent 1.5-yr radiation dose (mSv)	0.0177	0.0051	0.0301	0.0055

[a], smoking status: 0, never smoking;1, smoking.

[b], alcohol intake: 0, never; 1, current.

3-3. Micronuclei

MN frequency in radiation exposed workers and controls are shown in table Ⅱ- 10. The frequency of MN in the radiation exposed workers significantly higher compared to the controls. The frequency of MN in radiation exposed workers was 20.79 ± 11.67 per 1000 binucleated cells whereas 9.20 ± 3.09 per 1000 binucleated cells in the controls. The baseline MN frequency was within the range of 3 - 12% reported by the HUman MicroNucleus project, which gathered data from 25 labs representative of many countries and populations (Bonassi et al., 2001) .

The frequencies of MN induced by cumulative radiation dose, recent 5.5-year dose and 1.5-year dose are shown in Table Ⅱ-11-13. The frequency of MN increased according to the recent 1.5-year but the frequency of MN decreased according to the cumulative radiation dose and recent 5.5-year dose.

Table Ⅱ- 14 showed that the frequency of MN increased by the duration of work. However there was no statistically significant relationship between duration of work and the increased frequency of MN.

Poisson regression analysis applied to the overall study population revealed that recent 1.5-year dose was significantly associated with the frequency of MN after adjusting for age, smoking status and alcohol intake (Table Ⅱ- 15). However no significant association was found when only the exposed group was considered.

Table Ⅱ-10. Frequency of micronuclei in radiation exposed workers and controls

	No. of subjects	No. of total MN/ 1000 BN cells		*p*-value*
		Mean ± SD	Range	
Controls	45	9.20 ± 3.09	3 - 15	<.0001
Exposed workers	180	20.79 ± 11.67*	4 - 80	

SD, Standard deviation.

BN, binucleated cells.

MN, micronuclei.

*, significantly different from control subjects (Determined by Mann-Whitney U-test).

Table Ⅱ-11. Frequency of micronuclei in 1000 binucleated cells in relation to cumulative radiation dose in radiation exposed workers

Dose (mSv)	No. of subjects	No. of total MN/ 1000 BN cells		
		Mean ± SD	Range	
<100	38	23.37 ± 14.25	4 - 67	
100≦-<150	61	21.54 ± 13.05	4 - 80	
150≦-<200	32	17.91 ± 9.48	4 - 46	
≧200	49	19.76 ± 8.26	5 - 49	
Kendall's τ				-0.05670
p				0.3189

BN, binucleated cells.

MN, micronuclei.

SD, Standard deviation.

p, Tested by Kendall rank correlation coefficient (τ).

Table Ⅱ-12. Frequency of micronuclei in 1000 binucleated cells in relation to recent 5.5-yr radiation dose in the radiation exposed workers

Dose (mSv)	No. of subjects	No. of total MN/ 1000 BN cells		
		Mean ± SD	Range	
<100	42	20.57 ± 10.46	4 - 46	
100≦-<150	43	21.91 ± 15.35	4 - 80	
150≦-<200	53	20.66 ± 11.24	4 - 67	
≧200	42	20.05 ± 9.05	8 - 52	
Kendall's τ				-0.00116
p				0.9836

BN, binucleated cells.

MN, micronuclei.

SD, Standard deviation.

p, Tested by Kendall rank correlation coefficient (τ).

Table Ⅱ-13. Frequency of micronuclei in 1000 binucleated cells in relation to recent 1.5-yr radiation dose in the radiation exposed workers

Dose (mSv)	No. of subjects	No. of total MN/ 1000 BN cells	
		Mean ± SD	Range
<1.5	45	19.31 ± 10.40	4 - 49
1.5≦-<7.5	44	21.70 ± 13.94	5 - 80
7.5≦-<15	56	21.84 ± 11.72	4 - 67
≧15	35	19.89 ± 10.15	4 - 52
Kendall's τ			0.03300
p			0.5610

BN, binucleated cells.

MN, micronuclei.

SD, Standard deviation.

p, Tested by Kendall rank correlation coefficient (τ).

Table Ⅱ-14. Frequency of micronuclei in 1000 binucleated cells in relation to duration of work in radiation exposed workers

Duration of Work (Year)	No. of subjects	No. of total MN/ 1000 BN cells		
		Mean ± SD	Range	
<20	58	21.22 ± 13.76	4-67	
20≦-<25	90	20.30 ± 11.45	4-80	
≧25	32	21.41 ± 7.81	6-47	
Kendall's τ				0.05559
p				0.3468

BN, binucleated cells.

MN, micronuclei.

SD, Standard deviation.

p, Tested by Kendall rank correlation coefficient (τ).

Table Ⅱ-15. Poisson regression analysis for micronuclei associated with age, smoking status, alcohol intake, and recent 1.5-year dose

	Variable	β coefficient	95% CI low	95% CI Upper	*p*-vale
Overall population	Intercept	1.9849	1.7501	2.2167	<.0001
	Age (in years)	0.0166	0.0121	0.0212	<.0001
	Smoking status (0,1)[a]	-0.1645	-0.2308	-0.0987	<.0001
	Alcohol consumption (0,1)[b]	0.1138	0.0368	0.1919	0.0040
	Recent 1.5-yr radiation dose (mSv)	0.0186	0.0145	0.0227	<.0001
Exposed workers	Intercept	3.0683	2.7417	3.3928	<.0001
	Age (in years)	-0.0006	-0.0067	0.0056	0.8518
	Smoking status	-0.1969	-0.2685	-0.1258	<.0001
	Alcohol consumption	0.0026	-0.0292	0.1391	0.2063
	Recent 1.5-yr radiation dose (mSv)	0.0061	-0.0024	0.0075	0.3047

[a], smoking status: 0, never smoking;1, smoking.

[b], alcohol intake: 0, never; 1, current.

3-3. Correlations between markers

Figure Ⅱ-3 shows the relation between the frequency of MN and CA. There was a positive correlation between the frequency of MN and CA (r^2 = 0.03, p = 0.0139). Table Ⅱ- 16 shows the correlation among recent 1.5 year dose, CA, chromosome-type aberration, chromatid-type aberration and MN. CA, chromosome-type aberration, chromatid-type aberration and MN were significantly correlated with recent 1.5 year dose. There was significantly and positively correlated with each marker except non-significant correlation between the frequency of MN and chromatid-type aberration.

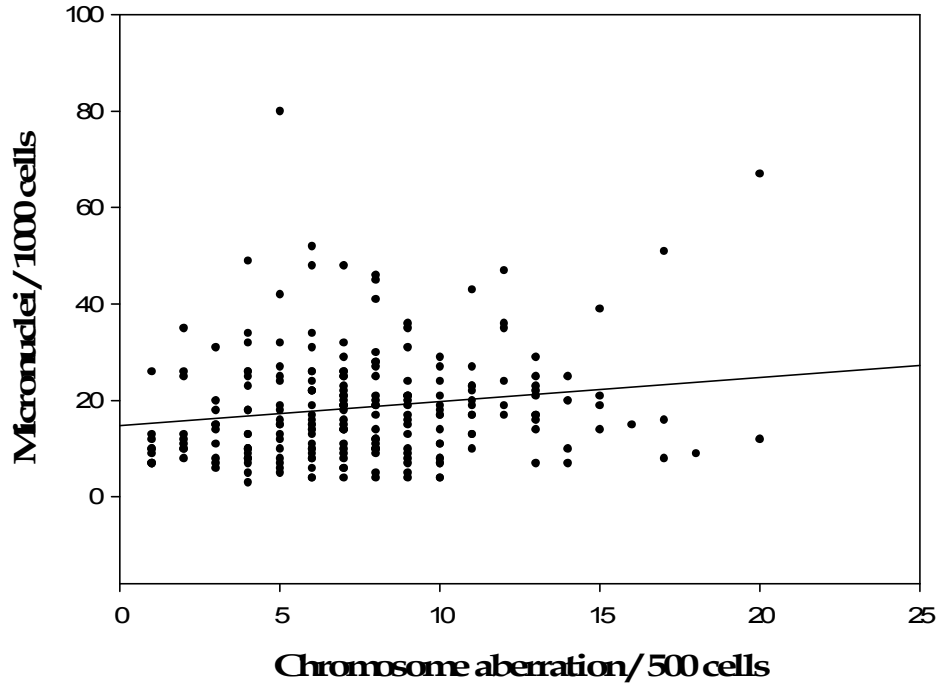


Figure Ⅱ-3. Correlation between the frequency of micronuclei and chromosome aberrations.

Table Ⅱ-16. Pearson correlation coefficients among recent 1.5-yr radiation dose, CA, chromosome-type aberration, chromatid-type aberration and MN.

Factor	Recent 1.5-yr dose	Total chromosome aberration	Chromosome-type aberration	Chromatid-type aberration	Micronuclei
Recent 1.5-yr dose	1				
Total chromosome aberration	0.34**	1			
Chromosome-type aberration	0.33**	0.71**	1		
Chromatid-type aberration	0.19**	0.80**	0.20**	1	
Micronuclei	0.20**	0.16*	0.17*	0.11	1

** $p < 0.01$, * $p < 0.05$

4. Discussion

IR is a known human carcinogen that can induce a variety of biological effects depending on the physical nature, duration, doses and dose - rates of exposure. However, the magnitude of health risks at low doses and dose-rates (below 100 mSv) remains controversial due to a lack of direct human evidence (Radiation, 2009).

This study was performed to validate as biomarkers for monitoring and obtain the baseline data for a surveillance program of workers occupationally exposed to chronic low doses of IR. In this study, although the level of exposure was below the accepted annual limit of 20 mSv, the yields of both chromosome-type and chromatid - type aberrations were significantly higher in the radiation workers than in the controls. The frequency of chromosome-type aberration (Dicentric chromosome) was 0.93 / 500 cells in the radiation exposed workers and 0.13 / 500 cells in control subjects. The control value in this study was a little lower compared to the published data (generally in the order of 0.25 – 0.5 / 500 cells scored) (Pernot et al., 2012). But it is within the range of earlier studies. Our data were consistent with previous studies that have shown a rise in chromosome alterations among occupational workers (Braselmann et al., 1994; Lindholm, 2001; Chung et al., 1996b). The influence of age on the incidence of chromosome aberration was not found, although the correlation

of increased chromosomal damage with age was reported in published data (Cremer et al., 1990). Also, we found no association of smoking status and alcohol intake with any type of chromosome aberrations. In this study, current exposure dose for radiation showed an inverse correlation with duration of employment, while cumulative dose was increased according to the duration of work. A possible explanation of these fact that worker's working conditions may have improved now and workers were rotated so as to not exceed 100 mSv (avg 20 mSv). Therefore, the duration of work is not proper as a surrogate of IR exposure. To determine the importance of recent exposure to radiation, we compared the chromosome-type aberration yields according to total cumulative dose, recent 5.5-year and recent 1.5-year. The frequency of chromosome-type aberration was significantly increase with the recent 1.5-yr dose (r = 0.17, p = 0.02, Fig. Ⅱ-1), whereas it was inversely correlated with total cumulative radiation dose. A possible explanation of this finding is that the level of chromosome-type aberration in PBL reduces over the time, because the cells with unstable chromosome aberration such as dicentric chromosome decay over time with an average half-life of about 3 years (Buckton et al., 1967) because the presence of two centromeres in one chromosome interferes with cell division, thus recent exposure could be more effective in inducing chromosomal damage for the radiation exposed workers. Multiple Poisson regression analysis for CA frequency, after adjusting for the potential

impact of demographic and lifestyle factors (e.g. exposure dose of IR, age, smoking status and alcohol consumption) also revealed that recent 1.5-year dose was significantly associated with altered CA frequencies ($\beta = 0.022$, $p < 0.001$) (Table Ⅱ-7). It also demonstrates that recent exposure to radiation, within the last 1.5 years, had contributed more to the observed chromosome aberration than earlier exposure. This result is similar to Bender et al. (1988), Chung et al. (1996) and Balakrishnan and Rao (1999) that the recent exposure was more effective in inducing aberrations than cumulative exposure.

Poisson regression analysis revealed significant association between the frequency of chromosome-type and cumulative dose, recent 5.5 year dose and recent 1.5 year dose for overall population, and a dose dependant increase in chromosome aberration frequency with only recent 1.5 year dose was found when only the radiation exposed workers were included. Even though chromosome aberration is uncertain for accurate dose reconstruction at the individual level, a rise of chromosome aberration apparently indicates an increase of potential health risk related to radiation exposure. Radiation exposure is known to induce specific types of cancer, particularly leukemia, and chromosome aberrations were frequently found in cases of hematological disorders (Erdoğan and Aksoy, 1973). Recent studies showed a positive association between high frequency of chromosomal aberrations, in particular of chromosome-type aberration, and increased cancer risk

(Rossner et al., 2005), indicate that the frequency of chromosomal aberrations in PBL could be predictive of cancer risk. Therefore, it can be an early effective marker of cancer risk in addition to being a biomarker of IR exposure at the population level through a long term prospective study.

In this study, occurrences of chromosome damage and their association with the exposure to low level of IR have been also evaluated using the micronucleus assay in human lymphocytes. The frequency of micronucleus in peripheral blood lymphocytes was significantly higher in radiation exposed workers than in controls. However the present study did not find any association between micronucleus occurrences and total cumulative dose, recent 5.5 year dose, and recent 1.5 year dose, concordant with the results from some studies, although this association has been described by others. The micronucleus test have shown a limitation to low doses of IR because of the significant inter-individual variability of spontaneous MN exists in human lymphocytes (Thierens et al., 1991). It is known that cytogenetic damage accumulates in humans with age, either due to the prolonged exposure to oxidative stress, other environmental chemicals as well as occupational, therapeutic radiation (Ramsey et al., 1995). Micronuclei formation is also known to be affected by factors such as age, gender, diet, alcohol intake and smoking status (Fenech et al., 1999a). Cytokinesis-block micronucleus test endpoints are not specific to the biological effects of low dose of radiation and are related to both other

environmental exposures and individual characteristics. Thus, it cannot be determined whether the enhanced micronucleus formation observed in this study is due to the chronic low-dose radiation exposure,

In conclusion, there is a strong indication that chromosome damage could be induced in workers exposed to low dose of radiation, even below occupational permissible dose limit. we suggest that the application of the chromosome (i.e. chromosome-type) aberrations test for individual monitoring and diagnosis of occupationally radiation exposed workers to chronic low dose IR at risk for cancer might be a efficient approach. However, to establish meaningful dose-response relationship between chromosome aberration and radiation dose in occupationally chronic exposure, studies of genetic variation of DNA repair enzymes reducing individual variability are needed.

5. References

Balakrishnan, S. and Rao, S. B. (1999) Cytogenetic analysis of peripheral blood lymphocytes of occupational workers exposed to low levels of ionising radiation. Mutat Res, 442, 37-42.

Bender, M. A., Preston, R. J., Leonard, R. C., Pyatt, B. E., Gooch, P. C. and Shelby, M. D. (1988) Chromosomal aberration and sister-chromatid exchange frequencies in peripheral blood lymphocytes of a large human population sample. Mutat Res, 204, 421-433.

Bishayee, A., Hill, H. Z., Stein, D., Rao, D. V. and Howell, R. W. (2001) Free radical-initiated and gap junction-mediated bystander effect due to nonuniform distribution of incorporated radioactivity in a three-dimensional tissue culture model. Radiat Res, 155, 335-344.

Bonassi, S., Abbondandolo, A., Camurri, L., Dal Pra, L., De Ferrari, M., Degrassi, F., Forni, A., Lamberti, L., Lando, C., Padovani, P. and et al. (1995) Are chromosome aberrations in circulating lymphocytes predictive of future cancer onset in humans? Preliminary results of an Italian cohort study. Cancer Genet Cytogenet, 79, 133-135.

Bonassi, S., Fenech, M., Lando, C., Lin, Y. P., Ceppi, M., Chang, W. P., Holland, N., Kirsch-Volders, M., Zeiger, E., Ban, S. Y., Barale, R., Bigatti, M. P., Bolognesi, C., Jia, C., Di Giorgio, M., Ferguson, L. R., Fucic, A., Lima, O. G., Hrelia, P., Krishnaja, A. P., Lee, T. K., Migliore, L., Mikhalevich, L., Mirkova, E., Mosesso, P., Muller, W. U., Odagiri, Y., Scarfi, M. R., Szabova, E., Vorobtsova, I., Vral, A. and Zijno, A. (2001) HUman MicroNucleus Project: International database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. Environ Mol Mutagen, 37, 31-45.

Bonassi, S., Forni, A., Bigatti, P., Canevarollo, N., De Ferrari, M., Lando, C., Padovani, P., Bevegni, M., Stella, M., Vecchio, D. and Puntoni, R. (1997) Chromosome aberrations in hospital workers: evidence from surveillance studies in Italy (1963-1993). Am J Ind Med, 31, 353-360.

Braselmann, H., Schmid, E. and Bauchinger, M. (1994) Chromosome aberrations in nuclear power plant workers: the influence of dose accumulation and lymphocyte life-time. Mutat Res, 306, 197-202.

Buckton, K.E., W.M. Court Brown and P.G. Smith (1967) Lymphocyte survival in men treated with X-rays for ankylosing spondylitis, Nature, 214, 470-473.

Cardoso, R. S., Takahashi-Hyodo, S., Peitl, P., Ghilardi-Neto, T. and Sakamoto-Hojo, E. T. (2001) Evaluation of chromosomal aberrations, micronuclei, and sister chromatid exchanges in hospital workers chronically exposed to ionizing radiation. Teratogen Carcin Mut, 21, 431-439.

Carrano, A. V. and Natarajan, A. T. (1988) International Commission for Protection Against Environmental Mutagens and Carcinogens. ICPEMC publication no. 14. Considerations for population monitoring using cytogenetic techniques. Mutat Res, 204, 379-406.

Chung, H. W., Ryu, E. K., Kim, Y. J. and Ha, S. W. (1996) Chromosome aberrations in workers of nuclear-power plants. Mutat Res, 350, 307-314.

Countryman, P. I. and Heddle, J. A. (1976) Production of Micronuclei from Chromosome-Aberrations in Irradiated Cultures of Human Lymphocytes. Mutat Res, 41, 321-331.

Cremer, T., Popp, S., Emmerich, P., Lichter, P. and Cremer, C. (1990) Rapid metaphase and interphase detection of radiation-induced chromosome aberrations in human lymphocytes by chromosomal suppression in situ hybridization. Cytometry, 11, 110-118.

Erdoğan, G. and Aksoy, M. (1973) Cytogenetic studies in thirteen patients with pancytopenia and leukaemia associated with long-term exposure to benzene. New Istanbul contribution to clinical science, 10, 230.

Evans, H. J., Buckton, K. E., Hamilton, G. E. and Carothers, A. (1979) Radiation-induced chromosome aberrations in nuclear-dockyard workers. Nature, 277, 531-534.

Fenech, M. (2000) The in vitro micronucleus technique. Mutat Res, 455, 81-95.

Fenech, M., Crott, J., Turner, J. and Brown, S. (1999a) Necrosis, apoptosis, cytostasis and DNA damage in human lymphocytes measured simultaneously within the cytokinesis-block micronucleus assay: description of the method and results for hydrogen peroxide. Mutagenesis, 14, 605-612.

Fenech, M. and Crott, J. W. (2002) Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes - evidence for breakage-fusion-bridge cycles in the cytokinesis-block micronucleus assay. Mutat Res, 504, 131-136.

Fenech, M., Holland, N., Chang, W. P., Zeiger, E. and Bonassi, S. (1999b) The HUman MicroNucleus Project - An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. Mutat Res, 428, 271-283.

Hagmar, L., Brogger, A., Hansteen, I. L., Heim, S., Hogstedt, B., Knudsen, L., Lambert, B., Linnainmaa, K., Mitelman, F., Nordenson, I. and et al. (1994) Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer Res, 54, 2919-2922.

ICRP 103 (2007) The 2007 Recommendations of the International Commission on Radiological Protection. ICRP 103. In Ann ICRP, Vol. 37, pp. 1-332.

Joseph, L. J., Patwardhan, U. N. and Samuel, A. M. (2004) Frequency of micronuclei in peripheral blood lymphocytes from subjects occupationally exposed to low levels of ionizing radiation. Mutat Res, 564, 83-88.

Kovalchuk, O., Burke, P., Besplug, J., Slovack, M., Filkowski, J. and Pogribny, I. (2004) Methylation changes in muscle and liver tissues of male and female mice exposed to acute and chronic low-dose X-ray-irradiation. Mutat Res, 548, 75-84.

Laurent, O., Metz-Flamant, C., Rogel, A., Hubert, D., Riedel, A., Garcier, Y. and Laurier, D. (2010) Relationship between occupational exposure to ionizing radiation and mortality at the French electricity company, period 1961-2003. Int Arch Occup Environ Health, 83, 935-944.

Lindholm, C. (2001) Stable and unstable chromosomal aberrations among Finnish nuclear power plant workers. Radiation protection dosimetry, 93, 143-150.

Little, M. P., Tawn, E. J., Tzoulaki, I., Wakeford, R., Hildebrandt, G., Paris, F., Tapio, S. and Elliott, P. (2010) Review and meta-analysis of epidemiological associations between low/moderate doses of ionizing

radiation and circulatory disease risks, and their possible mechanisms. Radiat Environ Biophys, 49, 139-153.

Maddileti, U., Padmaja, T., Hema Prasad, M. and Reddy, P. (2002) Analysis of chromosomal aberrations in the peripheral lymphocytes of workers exposed to diagnostic X-rays. Int J Hum Genet, 2, 265-268.

Morgan, W. F., Day, J. P., Kaplan, M. I., McGhee, E. M. and Limoli, C. L. (1996) Genomic instability induced by ionizing radiation. Radiat Res, 146, 247-258.

Natarajan, A. T., Darroudi, F., Jha, A. N., Meijers, M. and Zdzienicka, M. Z. (1993) Ionizing radiation induced DNA lesions which lead to chromosomal aberrations. Mutat Res, 299, 297-303.

Ogata, H. (2011) A Review of Some Epidemiological Studies on Cancer Risk from Low-Dose Radiation or Other Carcinogenic Agents. Radiat Prot Dosim, 146, 268-271.

Pernot, E., Hall, J., Baatout, S., Benotmane, M. A., Blanchardon, E., Bouffler, S., Saghire, H. E., Gomolka, M., Guertler, A. and Harms-Ringdahl, M. (2012) Ionizing radiation biomarkers for potential use in epidemiological studies. Mutat Res, 751, :258-286.

Radiation, U. N. S. C. o. t. E. o. A. (2009) Effects of Ionizing Radiation: United Nations Scientific Committee on the Effects of Atomic Radiation-UNSCEAR 2006 Report, Volume II-Report to the General Assembly, with Scientific Annexes C, D, and E, United Nations Pubns.

Ramsey, M. J., Moore, D. H., Briner, J. F., Lee, D. A., Olsen, L. A., Senft, J. R. and Tucker, J. D. (1995) The Effects of Age and Life-Style Factors on the Accumulation of Cytogenetic Damage as Measured by

Chromosome Painting. Mutat Res, 338, 95-106.

Rossner, P., Boffetta, P., Ceppi, M., Bonassi, S., Smerhovsky, Z., Landa, K., Juzova, D. and Šrám, R. J. (2005) Chromosomal aberrations in lymphocytes of healthy subjects and risk of cancer. Environ Health Persp, 113, 517.

Russo, G. L., Tedesco, I., Russo, M., Cioppa, A., Andreassi, M. G. and Picano, E. (2012) Cellular adaptive response to chronic radiation exposure in interventional cardiologists. Eur Heart J, 33, 408-414.

Samavat, H. and Mozdarani, H. (2004) Chromosomal aberrations in Iranian radiation workers due to chronic exposure of X-irradiation. International Journal of Low Radiation, 1, 216-222.

Sasaki, M. S. and Miyata, H. (1968) Biological dosimetry in atomic bomb survivors. Nature, 220, 1189-1193.

Scott, D. and Zampettibosseler, F. (1980) Relationship between Cell Killing, Chromosome-Aberrations, Spindle Defects and Mitotic Delay in Mouse Lymphoma-Cells of Differential Sensitivity to X-Rays. Int J Radiat Biol, 37, 33-47.

Sram, R. J., Rossner, P., Rubes, J., Beskid, O., Dusek, Z., Chvatalova, I., Schmuczerova, J., Milcova, A., Solansky, I., Bavorova, H., Ocadlikova, D., Kopecna, O. and Musilova, P. (2006) Possible genetic damage in the Czech nuclear power plant workers. Mutat Res, 593, 50-63.

Thierens, H., Vral, A. and Deridder, L. (1991) Biological Dosimetry Using the Micronucleus Assay for Lymphocytes - Interindividual Differences in Dose-Response. Health Phys, 61, 623-630.

Vig, B. K. and Swearngin, S. E. (1986) Sequence of Centromere Separation - Kinetochore Formation in Induced Laggards and Micronuclei. Mutagenesis, 1, 461-465.

Zamostian, P., Moysich, K. B., Mahoney, M. C., McCarthy, P., Bondar, A., Noschenko, A. G. and Michalek, A. M. (2002) Influence of various factors on individual radiation exposure from the Chernobyl disaster. Environ Health, 1, 4.

Zhang, J., Buonanno, M., Gonon, G., Li, M., Galdass, M., Shim, G., Toledo, S. M. and Azzam, E. I. (2012) Bystander Effects and Adaptive Responses Modulate In Vitro and In Vivo Biological Responses to Low Dose Ionizing Radiation. Radiobiology and Environmental Security, 71-86.

Zhizhina, G. P. (2011) [Effect of low doses of low-intensity ionizing radiation on the DNA structure and functions]. Radiats Biol Radioecol, 51, 218-228.

CHAPTER Ⅲ.

Association of copy number variation in glutathione S-transferase M1 and T1 with the frequency of chromosomal aberrations in nuclear power plant workers exposed to low dose ionizing radiation

Abstract

The adverse health effects of occupational exposure to low dose ionizing radiation (IR) are of great concern. This cross - sectional study aimed to examine whether copy number of glutathione S-transferase mu (GSTM1) and theta (GSTT1) influence on the frequency of chromosome aberrations (CA) in peripheral lymphocytes of 180 low level IR-exposed nuclear power plant workers and 45 control subjects. The distribution of copy number of GST gene was evaluated by quantitative real - time PCR well devised to distinguish between one copy (GST*1/0) and two copies (GST*1/1) with the endogenous calibrator known to have two copies of the test sequence for comparative results.

The CA frequency was significantly higher in radiation exposed workers than controls (Mann-Whitney test, $p < 0.001$). Among the relatively high exposure gorup (Recent - 1.5 yr exposure dose, ≧ 7.5 mSv; n= 91 / 180), the frequencies of Chromatid - type aberration and GSTM1 and GSTT1 copy number have shown the overall negative relationship (Kendall τ correlation coefficient -0.17, $p = 0.058$; -0.17, $p = 0.054$) and the frequency of Chromatid - type aberration increased as the each copy number of GSTM1 and GSTT1 deletion variants increased from zero to one to two after adjusting for confounding variables such as age, smoking and alcohol intake. In addiation, a significant negative association between combined

copy number of GSTM1 / GSTT1 and chromatid - type aberrations have shown in relatively highly exposed group after considering confounding variables (Frequency ratio = 0.84, 95% CI 1.23 - 4.39, p = 0.009) and gene-environment interaction between radiation exposure and the sum of GSTM1 and GSTT1 gene copy number in occurrence of chromatid type aberrations have also been observed.

Our finding suggest that copy nubmer variations of GSTM1 and GSTT1 may be a putative markers of individual susceptibility to IR-induced DNA damage. Furthermore these results emphasize the need of true genotyping strategy based on gene copy number in the studies of genetic susceptibility to environmental carcinogens because of the gene dosage effect associated with having two, one, or no alleles.

Key words : Ionizing radiatno, Chromosome aberration, Copy number variation, Glutathione S-transferase mu-1 (GSTM1) and glutathione S-transferase theta-1 (GSTT1).

1. Introduction

Human exposure to ionizing radiation (IR) occurs constantly in medical field, as well as in manufacturing and service industries, in research field, and in the nuclear power industry under the International Commission on Radiological Protection (ICRP)'s occupational limits. Therefore, adverse health effects of human population occupationally exposed to low dose radiation are of great concern (Mullenders et al., 2009; Eken et al., 2010). Several epidemiological studies using cytogenetic markers demonstrated that even low levels of IR exposure increase the frequency of chromosome aberration (CA) and micronuclei (MN) in radiation workers (Cho et al., 2009a; Zakeri and Hirobe, 2010). In the recent years, the identification of predictive biomarker for inherent radio-sensitivity (Inherited susceptibility) has received much attention to enhance monitoring of occupational IR exposure (Straume et al., 2008).

It have been well described that IR induces DNA damage through direct ionization of DNA or through free reactive oxidative species (ROS) formed by radiolysis of water (Little, 1998). ROS produced by IR occur in an increase of oxidative stress, leading to the formation of DNA-reactive products that could induce mutations in target tissues (Trachootham et al., 2009).

An endogenous antioxidant defense system constitutively express a number of detoxifying enzymes and protecting against oxidative damage enzymes such as the GST family (Tiwari et al., 2009). The Glutathione-S-transferase (GSTs) is the multigene family of enzymes that detoxify electrophilic compounds, including carcinogens, therapeutic drugs as well as catalyze the neutralization of free radicals by their conjugation with glutathione (Hayes et al., 2005) and thus reduce radiation-induced oxidative stress within cells. In humans, cytosolic GSTs are divided into seven classes: α (GSTA), μ (GSTM), π (GSTP), θ (GSTT), τ (GSTZ), σ (GSTS), and ω (GSTO). Particularly, members of the family μ (GSTM) and a member of the family θ (GSTT) enzymes detoxify numerous molecules resulting from reactive oxidant damage (Hayes and McLellan, 1999).

Both GSTM1 and GSTT1 have high prevalence of deletion polymorphisms (Table Ⅲ-1) (He et al., 2011), and these common deletion polymorphism present with different frequencies in various population (Table Ⅲ-10) (Piacentini et al., 2011b). Polymorphism of these genes lead to decrease or absence of enzyme activity in individuals with one or both the alleles deleted. Given the crucial role of GSTs in cellular protection and the high prevalence of GSTM1 and GSTT1 deletions in the human

population, deletion polymorphisms of these genes have been considered as inherited risk factors for various types of cancer (Alexandrie et al., 2004; Rajagopal et al., 2005), asthma and other respiratory diseases (Brasch-Andersen et al., 2004; Tamer et al., 2004). In addition, various studies have been performed to identify the association between the GSTM1 and GSTT1 deletion polymorphisms and the susceptibility of exogenous carcinogens such as polycyclic aromatic hydrocarbons (PAHs) among occupational workers (Wan et al., 2002; Aguilera et al., 2010; Dhillon et al., 2011).

In contrast, despite the clear concept (Figure Ⅲ-1) that GSTM1 and GSTT1 enzymes regulate DNA damage via reduction of ROS generated by IR, only studies on the association between the polymorphic nature of GSTM1 and GSTT1 genes and the possibility of developing biomarkers or predictive assays for radio- susceptibility of tissues treated with IR in various cancer patients have been done. Meanwhile there is a lack of clear literature on the associations between the polymorphisms of GSTM1 and GSTT1 genes and susceptibility to IR in occupational worker (Lima Sombra et al., 2011; Sal'nikova et al., 2011; Sram et al., 2006; Vasil'eva et al.).

Table Ⅲ-1. Common polymorphisms of cytosolic GSTs in human

Class	Chromosome	Gene accession no.	Alleles	Position of polymorphism	Protein alteration
Alpha	6p12	GSTA1_2938	GSTA1*A	-567T, -69C, -52G	Reference
			GSTA1*B	-567G, -69T, -52A	Low protein levels
		GSTA2_2939	GSTA2*A	328C, 335G, 588G, 629A	Pro^{110}, Ser^{112}, Lys^{196}, Glu^{210}
			GSTA2*B	328C, 335G, 588G, 629C	Pro^{110}, Ser^{112}, Lys^{196}, Ala^{210}
			GSTA2*C	328C, 335C, 588G, 629A	Pro^{110}, Thr^{112}, Lys^{196}, Glu^{210}
			GSTA2*D	328C, 335G, 588T, 629C	Pro^{110}, Ser^{112}, Asn^{196}, Ala^{210}
			GSTA2*E	328T, 335G, 588G, 629A	Ser^{110}, Ser^{112}, Lys^{196}, Glu^{210}
Mu	1q13.3	GSTM1_2944	GSTM1*A	519G	Lys^{173}
			GSTM1*B	519C	Asn^{173}
			GSTM1 null	Gene deletion	No protein
		GSTM3_2947	GSTM3*A	Wildtype	Reference
			GSTM3*B	3 bp deletion in intron 6	Protein unchanged
Pi	11q13.3	GSTP1_2950	GSTP1*A	313A, 341C	Ile^{105}, Ala^{114}
			GSTP1*B	313G, 341C	Val^{105}, Ala^{114}
			GSTP1*C	313G, 341T	Val^{105}, Val^{114}
			GSTP1*D	313A, 341T	Ile^{105}, Val^{114}
Theta	22q11.23	GSTT1_2952	GSTT1*A	310A	Thr^{104}
			GSTT1 null	Gene deletion	No protein
			GSTT1*B	310C	Pro^{104}
Omega	10q24.3	GSTO1_9446	GSTO1*A	419C	Ala^{140}
			GSTO1*C	419A	Asp^{140}
		GSTO2_119391	GSTO2*A	424A	Asn^{142}
			GSTO2*B	424G	Asp^{142}

Adapted from (Hayes et al., 2005)

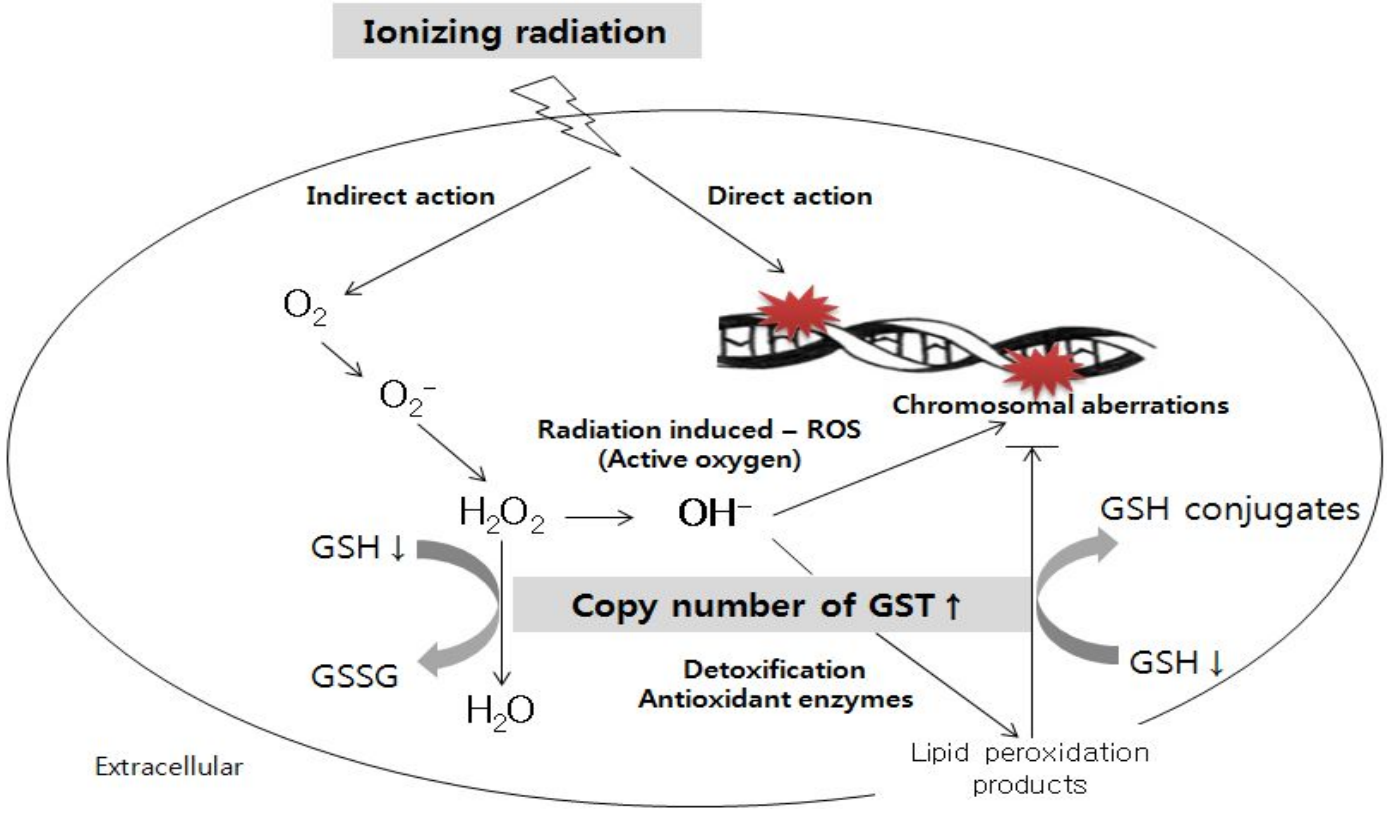


Figure Ⅲ-1. Schematic representation for the role of copy number variation of GSTs in radiation-induced DNA damage.그

Sram et al. (2006) have shown no association between homozygosity for GSTM1 and GSTT1 deletions and CA frequency in workers of Czech nuclear power plants. In later studies, lack of association between each separate null genotype (GSTM1 and GSTT1) and high frequency of CA among uranium workers and Chernobyl liquidators were confirmed by Vasil'eva et al. (2010) and Sal'nikova et al. (2011). A possible explanation for these studies showed no relationship between the level of chromosomal aberrations and genotypes (GSTM1 and GSTT1) is that previous studies only distinguished between "zero copies" (GST*0/0; null genotype) and "non-zero copies" (GST*1/0 or GST*1/1; positive genotype). And another reason may be the small sample sizes in othre studies, which failed to offer sufficient power for statistically significant results.

Complicate genotyping is important because the zero copy or "non-conjugator" and non-zero copy or "conjugator" are associated with loss of catalytic activity (Sprenger et al., 2000; Pemble et al., 1994) and gene dosage effect associated with the copy number of functional alleles for both genes (GSTM1 and GSTT1) has been exhibited (Covault et al., 2003; Buchard et al., 2007). In this regard, It is expected that the effect of these two deletions (GSTM1 and GSTT1) on the various biological and clinical outcomes might also best be described in a dose-dependent manner and expected that previously published results may tend to underestimate the true associations of GSTM1 and GSTT1 in relation to IR induced DNA damage because misclassification of integer copy number of these genes can lead to biased results. Recently, GSTM1 and GSTT1 genotyping to

determine allele copy numbers are available using either long-range PCR or quantitative real-time PCR. However, Due to the technical difficulty, time consuming and labor intensive process, long-range PCR is not suitable for large scale epidemiological and clinical studies, while due to the advantage of being fast, semi-automated with accurate quantification and minimal amount of input DNA requirements, quantitative real-time PCR method have been well established as a high-throughput, low cost assay enabling reliable estimates in large epidemiological samples. More recently, quantitative real-time PCR method have been well devised to determine gene copy number of GSTM1 and GSTT1 with the endogenous calibrator known to have two copies of the test sequence for comparative results (Hoebeeck et al., 2007).

Studies on the effect of copy number polymorphisms on the level of chromosomal damage in individuals occupationally exposed to IR may increase the sensitivity of cytogenetic biomarkers as indicators of genotoxic effects, as well as help in the identification of risk groups. Therefore, the aim of the present study was to examine effects of low dose IR on different individual cytogenetic response measured by CA and MN frequency, and furthermore to investigate for the first time the influence of GSTM1 and GSTT1 copy number polymorphisms on chromosomal damage in low dose IR-exposed workers. The quantitative real-time PCR and the ΔΔCt method were used for distinguish between one copy (GST*1/0) and two copies (GST*1/1) in 180 radiation workers and 45 controls from the General Population in Korea.

2. Materials and Methods

2-1. Ethics statement

The study protocol was approved by the Research Ethics Review Board of Seoul National University, and written informed consent was obtained from each individual before their inclusion in the study.

2-2. Study population

The radiation-exposed group consisted of 180 male workers occupationally exposed to radiation more than 100 mSv of cumulative dose within the workers who had ever exceeded the international accepted acceptable permissible dose limit, 20 mSv per year and 100 mSv per 5 years. Radiation exposure and personal dosimetry records based on film badges were collected for each worker for the whole entire working period. The non-exposed (control) to radiation group consisted of 80 age-matched volunteers who had never been exposed to occupational ionizing radiation. All subjects were requested to complete a questionnaire regarding theire smoking habits, alcohol consumption, medical history, and duration of occupational exposure to radiation (years of employment). All subjects did not have a personal medical history of cancer, genetic or other chronic disease and had no drug intake in the months prior to the study.

2-3. Collection of blood samples and culture

Peripheral whole blood samples from each subject were drawn into heparin-containing tubes and processed for analysis immediately upon arrival in the laboratory. 1 ml of heparinized blood sample were seeded into 9 ml of culture medium, RPMI 1640 (Gibco BRL, Grand Island, NY) containing 10% fetal bovine serum (Sigma Chemical Co., St. Louis, MO), penicillin (100 U/ml), streptomycin (100 μL/mL) and phytohemagglutinin M (1%). The culutures wer incubated at 37 °C and in a humidified atmosphere of 5% CO_2.

2-4. Isolation of DNA

All genomic DNA samples were isolated from 2 mL of Peripheral whole blood samples using Wizard™ DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. In brief, Whole blood sample was mixed with a threefold volume of cell lysis solution and incubated at room temperature for at least 30 minutes. The lysate was then centrifuged at 2000 (g), and pellet of intact leukocytes was resuspended in nuclei lysis solution and protein precipitation. The mixture was shaken vigorously and then after centrifuged at 2000 (g) for 10 minutes to pellet the cellular proteins. DNA in the supernatant was then precipitated with 2 volume of isopropanole, washed in 70% ethanol and resuspended in rehydrated solution. Exact quantification of the DNA content was assessed with the NanoDrop spectrophotometer (Celbio NanoDrop Technologies, Wilmington, DE). The extracted DNA was stored at -20 °C until the Copy Number anaysis.

2-5. GSTM1 and GSTT1 copy number assays

Copy number assays were performed by quantitative real-time polymerase chain reaction (PCR) on an 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA).

Copy numbers for GSTM1 and GSTT1 were quantified using both FAM® dye-labeled TaqMan® Copy Number Assay (Hs02575461_cn probe for GSTM1 and Hs00010004_cn probe for GSTT1; Applied Biosystems, Foster City, CA) and VIC® dye-labeled TaqMan® Copy Number Reference assay(Product # 4403328, Applied Biosystems, Foster City, CA). The Copy Number assay detects the target gene and the Reference assay detects a sequence (RNase p gene) that is known to exist in two copies in a diploid genome.

This relative qauntitation was used to determine the relative copy number of the target of interest in a genomic DNA (gDNA) sample, normalized to the known copy number of the reference sequence, and compared to a calibrator sample (NA10851) obtained from the Coriell Institute for Medical Research (http://www.coriell.org/) known to have two copy numbers of GSTM1/T1 genes.

Table Ⅲ-2. Detail information for GSTM1 and GSTT1 copy number assay

Gene	Assay ID[a]	Genomic Location[b]	DGV IDs[C]	Reporter dye	Amplicon Length	Description (Allele information)
GSTM1	Hs02575461_cn	Chr.1:110230486 (1p13.3b)	18 DGV IDs[*]	FAM (target gene) VIC (RNaseP)	82	Gene deletion Gene duplication
GSTT1	Hs00010004_cn	Chr.22:24383886 (22q11.23b)	25 DGV IDs[**]	FAM (target gene) VIC (RNaseP)	81	Gene deletion

[a] TaqMan® Copy Number Assay ID

[b] Genomic location on NCBI build 37.1

[c] DGV Version37,hg19.v10

[*] DGV Locus location : Chr1:110152918-110260150

[**] DGV Locus location : Chr22:24257337-24722933

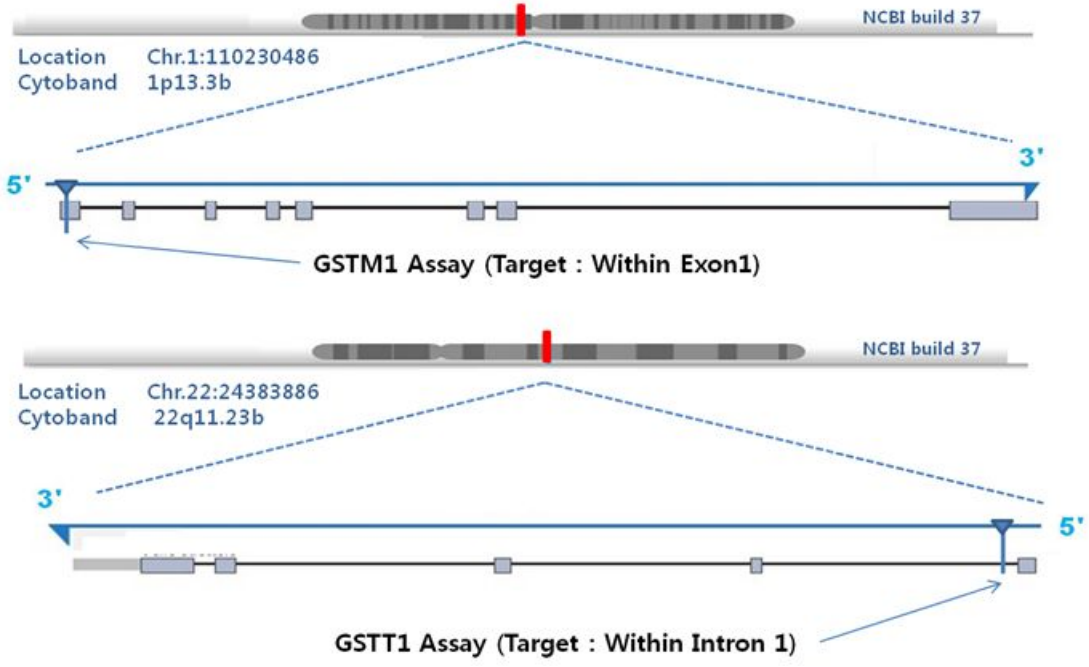


Figure Ⅲ-2. Detail location of CNV assay in GSTM1 and GSTT1

The number of copies of the target in each sample was determined by relative quantification using the comparative Ct (ΔΔCt) method. First Ct value difference (ΔCt) between target and reference was determined for each well and then averaged across sample triplicates, and then compared the ΔCt values of target samples to a calibrator samples (NA10851). So ΔCt's from all other DNA samples were normalized to NA10851 to determine the ΔΔCt. The relative quantity is $2^{-\Delta\Delta Ct,}$ and the copy number of the target was calculated to be two times the relative quantity.

$$\Delta\Delta Ct = \Delta Ct_{\text{target}} - \Delta Ct_{\text{reference}}$$

$$\text{Copy number} = 2^{-\Delta\Delta Ct} \times 2$$

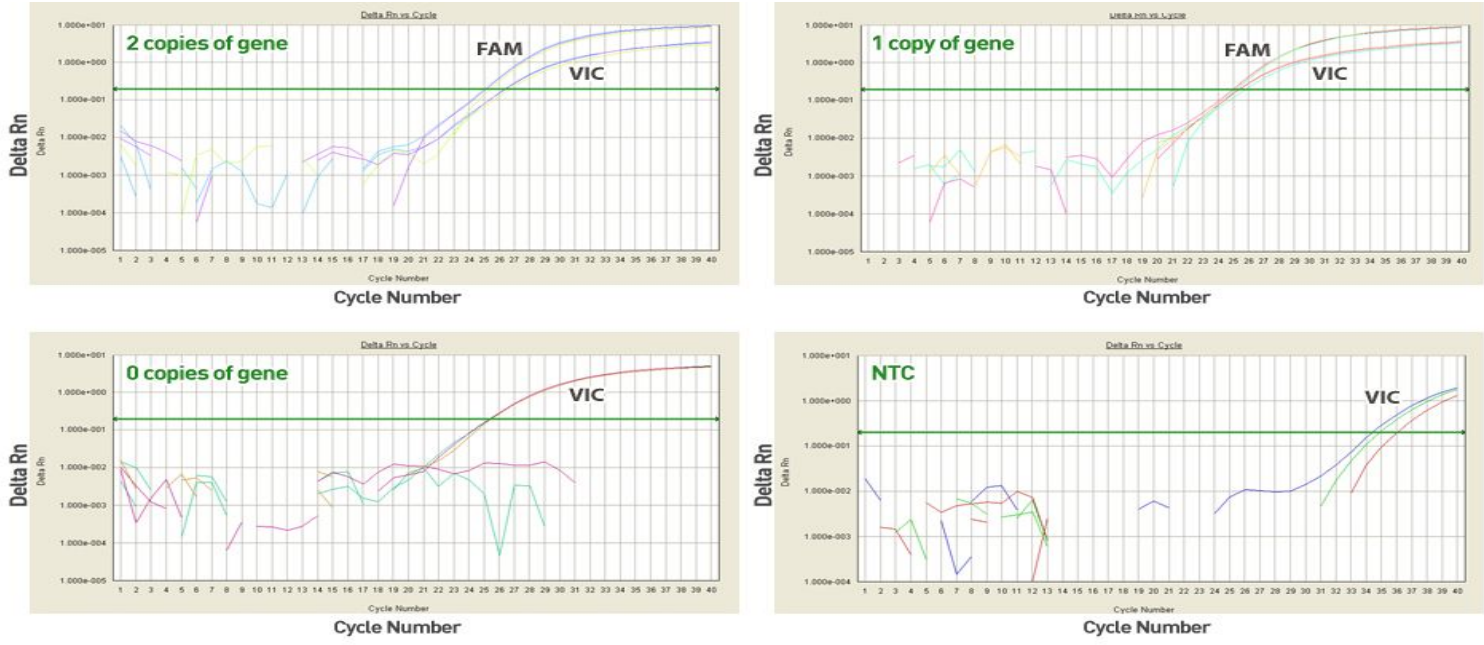
$\Delta Ct_{\text{target}}$ = difference in Ct value between target and reference gene for target samples.

$\Delta Ct_{\text{reference}}$ = difference in Ct value between target and reference gene for calibrator sample.

According to the manufacturer's recommendations, the DNA samples were diluted to a concentration of 5 ng/μL. Amplication reactions (20 μL) were carried out using 4 μL of template gDNA, 10 μL of 2 × TaqMan® Genotyping Master Mix (Applied Biosystems, Foster City, CA), 1 μL of the TaqMan Copy Number Assay, 1 μL of 20 × TaqMan Copy Number Reference Assay, and 4 μL of Nucluease-free water (Applied Biosystems, Foster City, CA). PCR conditions were as follows : 95 °C for 10 min hold and 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. Each sample was run in triplicate. A sample that does not contain a DNA template were aapplied on each 96-well plate to shows the background fluorescence and allows for the detection of contamination.

Ct values for the target and reference assay were collected and processed by SDS v1.4 software (Applied Biosystems, Foster City, CA) and then imported to CopyCaller™ Software v1.0. (Applied Biosystems, Foster City, CA) for duplex real-time PCR data analysis.

Figure Ⅲ-3. Representative amplification plots for quantitative copy number determination and calculations for determining GSTs copy number



Sample ID	Replicate analyzed	FAM Ct Mean	VIC Ct Mean	ΔCt Mean	ΔΔ Ct	RQ ($2^{-\Delta\Delta Ct}$)	Copy number calculated
A (NA10851)	3	25.16	26.45	-1.29	0.00	1	2
B	3	25.06	25.46	-0.41	0.88	0.54	1
C	3	-	25.36	-	-	-	0
D	3	-	-	-	-	-	Undetermined

2-6. Validation by generic PCR

Generic PCR were performed as a quality control for detection of GSTM1/T1 genes.

The primers of GSTM1 were

F:5'-CTGCCCTACTTGATTGATGGG-3' and

R:5'-CTGGATT- GTAGCGATCATGC-3'.

The primers of GSTT1 were

F:'5-TTCCTTACTGGTCCTCACATCTC-3' and

R:5'-TCACCGGATCATGGCCAGCA-3'.

The human coagulation factor XIII A subunit gene (382 bp) was also amplified in each reaction as a positive control to confirm the presence of amplifiable DNA in the samples. The primers of factor XIII were F: 5'-TCATCCCAGCAACTGGTTGC-3', R: 5'-CTGGCTCATAGGGTGCAGG-3'. For GSTM1 amplification, we performed with initial denaturation at 94 °C for 5 min and 30 cycles of 94 °C for 2 min, 60 °C for 1 min and 72 °C for 1 min 30 s. For GSTT1 amplification, we performed with initial denaturation at 94 °C for 5 min and 30 cycles of 94 °C for 1 min, 65 °C for 1 min and 72 °C for 1 min 15 s. The PCR products were

electrophoresed on 2% agarose gels stained with ethidium bromide to determine the GSTM1 and GSTT1 positive samples. Individuals with one or more GSTM1 alleles had 273 bp fragments and GSTT1 alleles had 480 bp fragments. Each genotype assay was repeated on 5% of DNA samples randomly chosen from all the samples and the replicates were 100% concordant.

Table Ⅲ-3. The list of primers and sequence information and generic PCR condition

		Sequence (5' ->3')	Anneling temp (℃)	Products sizes (bp)
GSTM1	Forward	CTGCCCTACTTGATTGATGGG	60	273
	Reverse	CTGGATTGTAGCAGATCATGC		
	Initial denaturation: 94℃, 5m, 30cycles:denaturation(94℃, 2m), annealing(60℃, 1m), extension(72℃, 1m30s)			
GSTT1	Forward	TTCCTTACTGGTCCTCACATCTC	65	480
	Reverse	TACCCGGATCATGGCCAGCA		
	Initial denaturation: 94℃, 5m, 30cycles:denaturation(94℃, 1m), annealing(65℃, 1m), extension(72℃, 1m15s)			

Table Ⅲ-4. Agreement between GSTM1 and GSTT1 genotypes results made by real time qPCR and generic PCR

	General PCR	
Real time qPCR	**Positive**	**Null**
Positive (1,2 and 3 Copies)	90	0
Null (0 copies)	0	90

2-7. Chromosome aberration (CA) Assay

The cultures were incubated for 48h from the start of the culture with PHA and colcemid (0.1 μg/ml) was added to the cultures 3 hours prior to harvesting. Chromosome preparations were performed according to the standard procedures (IAEA, 2001) The slides were dried in the air and stained with Giemsa. Slides were coded and 500 metaphases for each subject were scored blindly. After completing the chromosome aberration analysis, the radiation records were linked to the coded number for the data analysis.

2-8. Micronuclei (MN) Assay

The CBMN assay was performed as described by Fenech (2000). Briefly, peripheral blood (1 ml) was added to 9 ml of RPMI-1640 medium containing 10% FBS. After 44 h, cytochalasin-B (4.5 μg/ml; Sigma) was added to the culture, which was incubated for another 28 h (total incubation time, 72 h). The cells were collected, treated with 0.075 M KCl hypotonic solution for 2 min, and fixed in a mixture of methanol and acetic acid (3:1). The samples were air-dried and stained with Giemsa stain. All slides were coded and scored blindly according to the criteria of Fenech (2000). A total of 1000 binucleated lymphocytes were scored.

2-9. Statistical analysis

Statistical analyses were performed using a statistical package for Windows (SAS 9.1; SAS Institute, Cary, NC). The differences between radiaion exposed group and control in terms of general characteristics using the χ2-tests and Mann-Whitney U-test. The Kruskall-willis test and Mann-Whitney U test were performed to examine the difference of the frequency of CA and MN according to the copy number of GSTM1/T1. All analyses were first done in the total population and thereafter stratified by occupational exposure. Adjusted frequency ratio (FR) with 95% confidence interval was calculated to compare the mean CA frequencies for the different copy number group, using a Poisson regression model adjusting for potential confounding factors, including age, smoking status, and alcohol use. FR indicated a proportional increase/decrease of the CA frequency in a comparison group relative to the reference. For categorical variables, the FR represents the proportional increase of the CA frequency in the study tgroup. For continuous variables, the FR represents the proportional increase of CA frequency according to the increase of one copy number of GSTs.

3. Results

3-1. Characteristics of radiation exposed workers and controls

General characteristics of study populations are listed in Table Ⅲ-5. We have collected data from 180 radiation exposed workers and 45 controls. The ages of radiation exposed workers and controls ranged from 29 to 59 years with a mean of 47.46 ± 5.94 and 24 - 65 years with a mean of 40.89 ± 10.01, respectively. There was no significant difference in the patterns for age and smoking status between the radiation exposed workers and the control subjects indicating that radiation exposed workers and control subjects were closely matched. The only significant differences between radiation exposed workers and controls were found once the two groups were categorized according to alcohol intake. The dosimetry records over the duration of work showed that the last 1.5 - year and the cumulative radiation doses of exposed workers ranged from 0 - 33.49, 12.67 - 400.25 mSv, respectively.

Table Ⅲ-5. General characteristics of the study population

Variables	No. of subjects (%)		*p*-value
	Controls	Exposed	
Age (mean ± SD, years)	40.89 ± 10.01	47.46 ± 5.94	<0.001[a]
<50	36 (80)	117 (65)	0.05[b]
≧50	9 (20)	63 (35)	
Smoking status			
Current smoker	20 (44.44)	69 (38.33)	0.45[b]
Former or never smoker	25 (55.56)	111 (61.67)	
Alcohol consumption			
Yes	30 (66.67)	147 (81.67)	0.03[b]
No	15 (33.33)	33 (18.33)	
Duration of employment (mean ± SD, years)		20.06±6.19	
<20		103 (45.78)	
≧20		122 (54.22)	
Cumulative dose (mean ± SD, years, mSv)		157.31 ± 85.24	
<100		38 (21.11)	
100≦-<150		61 (33.89)	
150≦-<200		32 (17.78)	
≧200		49 (27.22)	
Recent 1.5-yr radiation dose (mean ± SD, years, mSv)		8.19 ± 7.08	
<1.5		45 (25)	
1.5≦-<7.5		44 (24.44)	
7.5≦-<15		56 (31.11)	

SD, Standard deviation.

[a] Determined by Mann-Whitney U-test.

[b] Determined by x^2test.

3-2. CA frequency in radiation exposed workers and controls

CA frequency in radiation exposed workers and controls are shown in Table Ⅲ- 6. The differences in chromosome aberration frequencies between the radiation exposed workers and the controls were statistically significant for all chromosome-type aberrations. The mean values of chromosome-type aberration were 3 / 500 metaphase cells in the radiation exposed workers and 0.6 / 500 metaphase cells in the control subjects. The baseline frequency of chromosome aberration (0.8%) was lower than the frequency observed in our previous study (1.16%; (Chung et al., 1996b)), but was similar to the data observed by other studies (Maddileti et al., 2002; Samavat and Mozdarani, 2004). Table Ⅲ- 8 shows the results of multiple Poisson regression analysis for CA frequency, after adjusting for the potential impact of demographic and lifestyle factors (e.g. exposure dose of IR, age, smoking status and alcohol consumption). recent 5.5 year dose was significantly associated with altered CA frequencies (β=0.022, $p < 0.001$). It was also found that recent exposure to radiation, within the last 1.5 - years, had contributed more to the observed chromosome aberration than earlier exposure.

3-3. MN frequency in radiation exposed workers and controls

MN frequency in radiation exposed workers and controls are shown in Table Ⅲ- 7. The frequency of MN in the radiation exposed workers significantly higher compared to the controls. The frequency of MN in radiation exposed workers was 20.79 ± 11.67 per 1000 binucleated cells whereas 9.20 ± 3.09 per 1000 binucleated cells in the controls. The baseline frequency of MN (9.2‰) was similar to the frequency observed in our previous study (9.3‰; (Cho et al., 2009b)) and was within the range of 3 - 12% reported by the HUman MicroNucleus project, which gathered data from 25 labs representative of many countries and populations (Bonassi et al., 2001). We performed a multiple Poisson regression analysis for MN frequencies, while adjusting for the potential impact of demographic and lifestyle factors (e.g. exposure dose of IR, duration of work, smoking status and alcohol consumption). There were no factor that significantly altered CBMN frequencies.

Table Ⅲ-6. Frequency of chromosomal aberrations in radiation exposed workers and controls

Types of chromosome aberration	Controls			Radiation exposed workers			
	Mean/ 500cells	SD	Range	Mean/ 500cells	SD	Range	*p*-value*
No. of individuals	45			180			
Chromatid-type deletion	3.53	2.21	0 - 10	5.41	2.76	0 - 17	<0.0001
Chromatid-type exchange	0.04	0.21	0 - 1	0.06	0.23	0 - 1	0.7697
Chromosome-type deletion	0.51	0.82	0 - 3	2.08	1.98	0 - 14	<0.0001
Chromosome-type exchange	0.13	0.40	0 - 2	0.93	1.02	0 - 6	<0.0001
Total aberration	4.09	2.51	1 - 12	8.39	3.53	1 - 19	<0.0001

SD, Standard deviation.

*, Significantly different from control subjects (determined by Mann-Whitney U-test, $p < .05$).

Table Ⅲ-7. Frequency of micronuclei in radiation exposed workers and controls

	No. of subjects	No. of total MN/ 1000 BN cells		*p*-value*
		Mean ± SD	Range	
Controls	45	9.20 ± 3.09	3 - 15	<.0001
Exposed workers	180	20.79 ± 11.67*	4 - 80	

SD, Standard deviation.

BN, binucleated cells.

MN, micronuclei.

*, significantly different from control subjects (Determined by Mann-Whitney U-test).

Table Ⅲ-8. Poisson regression analysis for total chromosome aberrations with respect to age, smoking status, alcohol intake, and radiation dose

Variable	Model 1		Model 2		Model 3	
	β coefficient	*p*-vale	β coefficient	*p*-vale	β coefficient	*p*-vale
Intercept	1.78	<.0001	2.011	<.0001	2.22	<.0001
Age (in years)	0.0039	0.27	0.0011	0.74	-0.0032	0.41
Smoking status (0,1)[a]	0	1.00	-0.0086	0.87	-0.017	0.74
Alcohol consumption (0,1)[b]	-0.082	0.19	-0.11	0.09	-0.099	0.11
Recent 1.5-yr radiation dose (mSv)	0.022	<.0001	NI	-	NI	-
Recent 5.5-yr radiation dose (mSv)	NI	-	0.0039	0.0002	NI	-
Cumulative dose (mSv)	NI	-	NI	-	0.0007	0.01

[a], Smoking status: 0, never smoking; 1, smoking.

[b], Alcohol intake: 0, never; 1, current.

Abbreviation: NI, variable not included in this run.

3-3. Distribution of GSTM1 and GSTT1 copy number in the study populations

The distribution of GSTM1 and GSTT1 copy number among both radiation exposed workers and controls are presented in Table Ⅲ- 9. There were no statistically significant differences in the copy number distribution between exposure workers and controls ($p > 0.05$, chi-square test). Among the whole study population, the prevalence of GSTM1 and GSTT1 null genotypes (zero copies) was 50.67 and 49.78%, respectively, a finding similar to previous described prevalence in East Asians including Japanese (Fujihara et al., 2009), Taiwan (Tsai et al., 2006) and Korean (Kim et al., 2000; Uhm et al., 2007; Piao et al., 2009) populations (Table Ⅲ- 10). Among the whole study population, the frequencies observed for GSTM1 wild-type (2 copies), hemizygous deletion (1 copy) genotype were 8.89% and 39.56% respectively (Table Ⅲ- 9). For GSTT1, the distribution was 9.78% and 40.44% for wild-type (2 copies), hemizygous deletion (1 copy) genotype, respectively. These copy number frequencies of the both GSTM1 and GSTT1 are in the Korean populations in our study was close to that of Chinese populations, but different from other ethnic groups, especially in the frequencies of the GSTT1 (Table Ⅲ- 11).

Table Ⅲ-9. Distribution of GSTM1 and GSTT1 copy number in radiation-exposed workers and controls

	Copy number	Total, N(%)	Controls, N(%)	Exposed, N(%)	*p*-value*
No. of Subjects		225	45	180	
GSTM1	0[a]	114 (50.67)	25 (55.56)	89 (49.44)	0.648
	1[b]	89 (39.56)	17 (37.38)	72 (40.00)	
	2[c]	20 (8.89)	3 (6.67)	17 (9.44)	
	3+	2 (0.89)	0 (0.00)	2 (1.11)	
	0	114 (50.67)	25 (55.56)	89 (49.44)	0.4633
	1+	111 (49.33)	20 (44.44)	91 (50.56)	
	0 or 1	203 (90.22)	42 (93.33)	161 (89.44)	0.4321
	2+	22 (9.78)	3 (6.67)	19 (10.56)	
GSTT1	0	112 (49.78)	24 (53.33)	88 (48.89)	0.7492
	1	91 (40.44)	16 (35.56)	75 (41.67)	
	2	22 (9.78)	5 (11.11)	17 (9.44)	
	0	112 (49.78)	24 (53.33)	88 (48.89)	0.5938
	1+	113 (50.22)	21 (49.67)	92 (51.11)	
	0 or 1	203 (90.22)	40 (88.89)	163 (90.56)	0.7364
	2	22 (9.78)	5 (11.11)	17 (9.44)	

* The difference of copy number between radiation exposed workers and controls, x^2test

[a] (0/0) Homozygous deletion (null genotype).

[b] (0/1) Hemizygous deletion.

[c] (1/1) Wild-type (Positive genotye, two functional genes).

Table Ⅲ-10. GSTM1 and GSTT1 null genotype frequencies in different ethnic populations

Populations	GSTM1 deletion	GSTT1 deletion	Reference
North America			
USA(Caucasian)	54.3	27.6	(Garte et al., 2001)
USA(African American)	23.7	17.5	(Lavender et al., 2009)
Mexico(Mestizo)	33.5	12.1	(Pérez-Morales et al., 2008)
Africa			
Cameroon	27.8	46.8	(Piacentini et al., 2011)
Ethiopia	43.8	37.3	(Piacentini et al., 2011)
Ghana	19.3	73.7	(Dash et al., 2006)
East Asia			
China(Han)	52	38.7	(Liu et al., 2009)
Taiwan	50	44	(Tsai et al., 2006)
Japan	50.8	45.8	(Fujihara et al., 2009)
Korea	53	50.8	(Kim et al., 2000)
Korea	51.4	51.6	(Uhm et al., 2007)
Korea	54.3	50.3	(Piao et al., 2009)
Europe			
France	49	26	(Abbas et al., 2004)
Germany	51.6	19.5	(Garte et al., 2001)
Middle East			
SaudiArabia	8.3	4.2	(Abu-Amero et al., 2009)

Table Ⅲ-11. Comparison of GSTM1 and GSTT1 copy number frequencies among present study and studies in different ethnic populations

Populations	GSTM1 copy number				GSTT1 copy number			
	0 N (%)	1 N (%)	2 N (%)	3 N + (%)	0 N (%)	1 N (%)	2 N (%)	3 N + (%)
East Asia								
Korea (The present study)	50.7	39.6	8.9	0.9	49.78	40.4	9.8	-
China (Luo et al., 2011)	58.6	34.6	6.8	-	52.7	39.1	8.2	-
NorthAmerica								
USA(Caucasian) (Lam et al., 2009)	54.5	34.1	6.3	-	20.3	52.5	21.7	-
Europe								
Germany (Timofeeva et al., 2009)	51.6	41.3	7.1	-	17.2	49.5	33.3	-
Spain (Rodríguez-Santiago et al., 2009)	49.4	42.2	8.4	-	17.7	48.9	33.4	-
Denmark (Christiansen et al., 2006)	53.6	40	6.9	-	11.9	52.4	35.7	-

3-3. Relationship between the GSTM1 and GSTT1 copy number polymorphism and susceptibility to radiation-induced chromosome aberration

Table Ⅲ- 12 shows the frequency of chromosome aberrations (CA) in radiation-exposed workers and controls according to GSTM1 and GSTT1 copy number. No significance effects of the three different GSTM1 copy number were noted on the decreased frequency of CA in control group. However, among exposed group, a significant decreased in the frequency of CA observed according to increase in GSTM1 copy number (Kendall's Tau coefficient= -0.13, p = 0.04). when we classified individuals with either one copy or two copies into one group (positive genotype) due to the low frequencies for individuals possessed two copies (or more) of GSTM1, the frequencies of CA were also significantly low in individuals with GSTM1 positive genotypes compared to with null genotypes (7.84 ± 3.49 vs. 8.97 ± 3.50, p = 0.02; Table Ⅲ- 12). On the other hand, individuals with zero copies of GSTT1 in a control group showed high significant higher frequency of CA than one copy (or more) of GSTT1.

For further analyses, IR exposure worker were divided into two classes according to their recent 1.5-year radiation exposure levels (relatively low exposure group, recent 1.5-year ≤ 7.5 mSV, n= 89/180; relatively high exposure group, recent 1.5-year ≥ 7.5 mSV, n= 91/180) Since CA frequency

was significantly associated with recent 1.5-year radiation dose (Table Ⅲ-8).

When different types of chromosomal aberration were viewed separately, in relatively high exposure group, we found a negative correlation between the frequencies of Chromatid-type aberration and GSTM1 and GSTT1 copy number (Kendall's Tau = -0.17, p = 0.058 and Kendall's Tau = -0.17, p = 0.054, respectively), but only with borderline significance ($0.05 < p < 0.1$). Among relatively high exposure group, Individuals with GSTM1 null genotype showed significant high frequency of chromatid type aberration compared to those with GSTM1 positive genotype (6.37 ± 3.47 vs. 4.96 ±2.93, p=0.048; Table Ⅲ- 13). On the other hand, in control group, GSTT1 copy number showed significant positive correlation with the frequencies of Chromatid-type aberration (Kendall's Tau = 0.29, p = 0.02)

When further multivariate analyses were performed to clarify the relationship between CA and copy number of GSTM1 and GSTT1, We found a significant negative relation between GSTM1 and GSTT1 copy number and frequencies of chromatid type aberration in relatively high exposure group after adjustment for smoking, age, alcohol intake and recent 1.5-year IR exposure dose (Table Ⅲ- 14, 15). For GSTM1, individuals with one copy and at least two copies (or more) have shown significantly lower

chromatid-type aberrations frequencies than zero copies subjects in a clear copy number-dependent manner (FR = 0.77, 95% CI = 0.64 - 0.9 and FR = 0.71, 95% CI = 0.51 - 0.98 respectively; derived from Table Ⅲ- 14). Also compared to those with GSTM1 null genotype, FR of CA was 0.76 (95% CI = 0.64 - 0.91) for those with GSTTM1 positive genotype (Table Ⅲ- 14). Consistent relations were observed between the GSTT1 copy number and the CA frequencies. Individuals with one copy and at least two copies of GSTT1 have shown copy number-dependent decrease effect of chromatid-type aberration frequencies (FR = 0.93, 95% CI = 0.77-1.13 and FR = 0.70, 95% CI = 0.49 - 0.99; respectively; derived from Table Ⅲ- 15) compared with zero copies of GSTT1, though the decrease for those in individuals with one copy was not statistically significant.

To characterize the relation between the chromatid - type aberrations and combined copy number of GSTM1 and GSTT1, Poisson regression analysis with adjustment for age, and smoking and drinking status were used. In relatively high exposure group, the frequency of chromatid - type aberrations was significantly decreased according to the increase of combined copy numbers of GSTM1 and GSTT1 (FR= 0.84, p = 0.0006 derived from Table Ⅲ- 17).

Table Ⅲ- 18 showed the interaction between radiation exposure and the sum of GSTM1 and GSTT1 gene copy number in the occurrence of

chromosomal aberration. The relative FR was estimated using unexposed and low exposed workers with the highest genes copy number as reference. After adjusting for age, smoking status, and alcohol intake, relatively high exposed workers with the lowest genes copy number (zero copies) showed significant 1.36 fold (95% CI 1.03-1.81) increase in chromatid type aberration frequency compared to reference.

Table Ⅲ-12. Frequency of chromosome aberrations (CA) in radiation-exposed workers and controls according to GSTM1 and GSTT1 copy number

	Copy number	Controls			Exposure group		
		N	CA ± SD	*p*-value[b]	N	CA ± SD	*p*-value
GSTM1	0 [i]	25	4.04 ± 2.76	0.75	89	8.97 ± 3.50	0.0418
	1 [ii]	17	4.29 ± 2.28		72	7.75 ± 3.62	
	2+ [iii]	3	3.33 ± 2.08		19	8.16 ± 3.04	
	Kendall's τ			0.033[a] (0.79)			-0.13[a] (0.04)
	0	25	4.04 ± 2.76	0.69	89	8.97 ± 3.50	0.0169
	1+	20	4.15 ± 2.23		91	7.84 ± 3.49	
	0 or 1	42	4.41 ± 2.55	0.65	161	8.42 ± 3.59	0.9497
	2+	3	3.33 ± 2.08		19	8.16 ± 3.04	
GSTT1	0	24	3.50 ± 2.73	0.07	88	8.22 3.35	0.4294
	1	16	4.81 ± 2.20		75	8.75 3.46	
	2	5	4.60 ± 1.95		17	7.76 4.70	
	Kendall's τ			0.27[a] (0.03)			0.02[a] (0.76)
	0	24	3.50 ± 2.73	0.03	88	8.22 3.35	0.5252
	1+	21	4.76 ± 2.10		92	8.57 3.71	
	0 or 1	40	4.03 ± 2.59	0.44	163	8.46 3.40	0.3831
	2+	5	4.60 ± 1.95		17	7.76 4.70	

CA, chromosom aberration frequency per 500 cells.

SD, standard Deviation.

Kendall's τ was calculated on individual bases.

[a] Tested by kendall rank correlation coefficient (τ).

[b] Tested by Mann-Whitney u test, Kluskal-Wallis Test.

[i] (0/0) Homozygous deletion (null genotype).

[ii] (0/1) Hemizygous deletion.

[iii] (1/1) Wild-type (Positive genotye, two functional genes).

Table Ⅲ-13. Frequency of chromatid-type and chromosome-type CAs according to GSTM1 and GSTT1 copy number in controls, relatively low and high exposure workers group

	Copy number	Controls					Low expose					High expose				
		No.	Chromatid type		Chromosome type		No.	Chromatid type		Chromosome type		No.	Chromatid type		Chromosome type	
			Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD
GSTM1	0[i]	25	3.60	2.31	0.60	0.87	48	5.69	2.29	3.08	2.48	41	6.37	3.47	3.51	2.11
	1[ii]	17	3.47	2.10	0.82	0.88	31	4.96	2.59	2.11	1.75	41	5.02	3.05	3.15	2.53
	2+[iii]	3	4.00	2.65	0.00	0.00	10	5.00	1.87	2.89	2.15	9	4.67	2.40	3.67	1.66
	Kendall's τ[a]			0.02		0.01			0.14		0.13			0.17		0.04
	p			0.87		0.93			0.15		0.27			0.06		0.67
	p-value[b]			0.93		0.23			0.30		0.16			0.14		0.27
	0	25	3.60	2.31	0.60	0.87	48	5.69	2.29	3.08	2.48	41	6.37	3.47	3.51	2.11
	1+	20	3.55	2.11	0.70	0.86	41	4.97	2.41	2.30	1.85	50	4.96	2.93	3.24	2.39
	p-value			0.94		0.67			0.12		0.13			0.05		0.37
GSTT1	0[i]	24	2.96	2.26	0.67	0.87	38	4.88	2.20	2.41	1.65	50	6.00	3.29	3.38	2.41
	1[ii]	16	4.25	2.08	0.75	0.93	43	5.88	2.43	2.94	2.54	32	5.38	2.79	3.19	2.01
	2+[iii]	5	4.40	1.67	0.20	0.45	8	4.83	2.64	2.83	3.13	9	4.11	4.26	3.89	2.42
	Kendall's τ			0.29		0.08			0.11		0.02			0.17		0.01
	p			0.02		0.58			0.26		0.83			0.05		0.88
	p-value[b]			0.06		0.48			0.21		0.89			0.09		0.73
	0	24	2.96	2.26	0.67	0.87	38	4.88	2.20	2.41	1.65	50	6.00	3.29	3.38	2.41
	1+	21	4.29	1.95	0.62	0.86	51	5.72	2.46	2.92	2.59	41	5.10	3.15	3.34	2.09
	p-value			0.67		0.80			0.13		0.75			0.37		0.98

SD, standard Deviation.

Kendall's τ was calculated on individual bases.

[a] Tested by kendall rank correlation coefficient (τ).

[b] Tested by Mann-Whitney u test, Kluskal-Wallis Test.

[i] (0/0) Homozygous deletion (null genotype).

[ii] (0/1) Hemizygous deletion.

[iii] (1/1) Wild-type (Positive genotye, two functional genes).

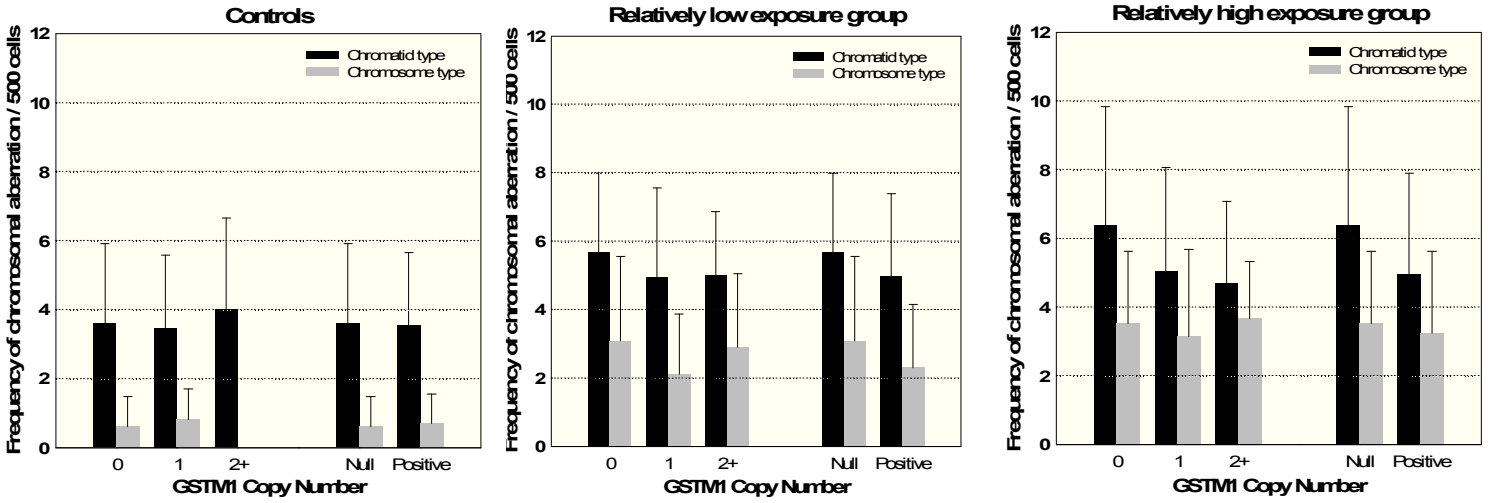


Figure Ⅲ-4. Frequency of chromatid-type and chromosome-type CAs according to GSTM1 copy number in controls, according to GSTM1 copy number in controls, relatively low and high exposure workers group

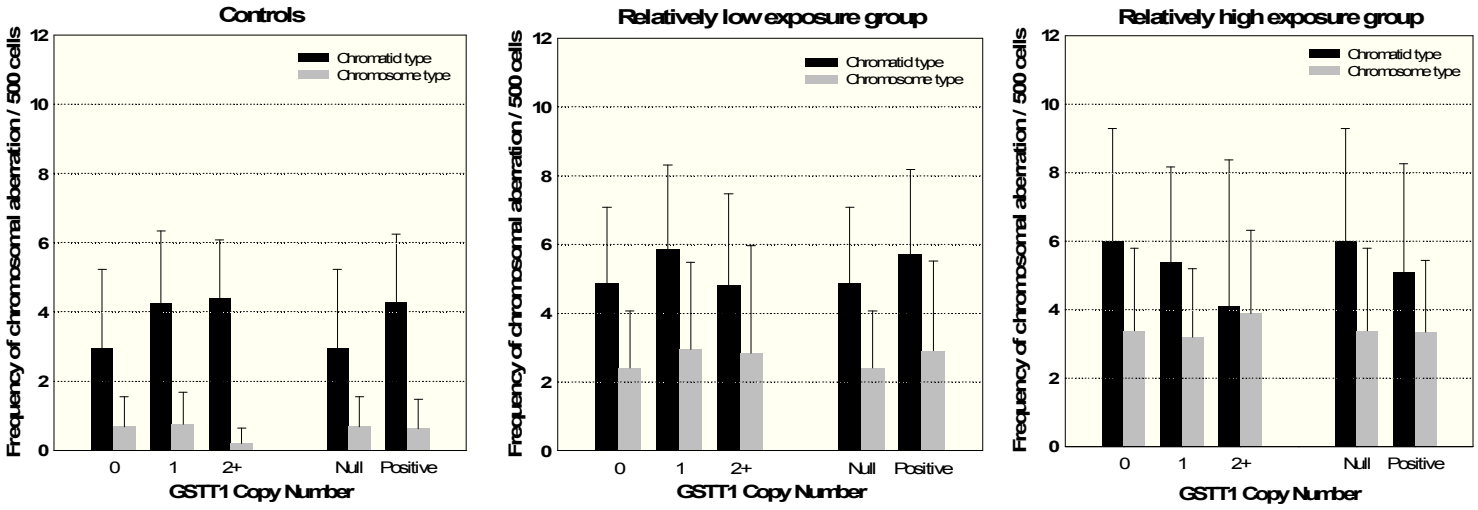


Figure Ⅲ-5. Frequency of chromatid-type and chromosome-type CAs in radiation-exposed workers and controls according to GSTT1 copy number in controls, relatively low and high exposure workers group

Table Ⅲ-14. Poisson regression analysis of chromosome aberrations (CA) : Effect of GSTM1 copy number and radiation exclosure in the relatively highly exposed group

	Copy number	Total CA frequency			Chromosome-type aberration frequency			Chromatid-type aberration frequency		
		FR[a]	95% CI	*p*-value	FR	95% CI	*p*-value	FR	95% CI	*p*-value
GST M1	0	1.00	reference		1.00	reference		1.00	reference	
	1	0.87	0.75 1.01	0.06	0.92	0.72 1.17	0.48	0.77	0.64 0.93	0.01
	2+	0.86	0.66 1.09	0.23	1.02	0.68 1.47	0.93	0.71	0.51 0.98	0.04
	0	1.00	reference		1.00	reference		1.00	reference	
	1+	0.87	0.75 1.00	0.05	0.94	0.75 1.18	0.57	0.76	0.64 0.91	0.01
	0 or 1	1.00	reference		1.00	reference		1.00	reference	
	2+	0.92	0.72 0.82	0.48	1.06	0.72 1.50	0.75	0.80	0.58 1.09	0.18

Relatively high exposure group. (Recent 1-5 yr dose, ≧7.5 mSv; n= 91/180)

[a], Frequency ratio adjusted for age, smoking status, and alcohol intake.

Table Ⅲ-15. Poisson regression analysis of chromosome aberrations (CA) : Effect of GSTT1 copy number and radiation exposure in the relatively highly exposed group

	Copy number	Total CA frequency			Chromosome-type aberration frequency			Chromatid-type aberration frequency		
		FR[a]	95% CI	*p*-value	FR	95% CI	*p*-value	FR	95% CI	*p*-value
GST T1	0	1.00	reference		1.00	reference		1.00	reference	
	1	0.97	0.83 1.12	0.65	0.97	0.75 1.24	0.79	0.93	0.77 1.13	0.48
	2	0.90	0.69 1.15	0.41	1.17	0.79 1.69	0.42	0.70	0.49 0.99	0.05
	0	1.00	reference		1.00	reference		1.00	reference	
	1+	0.95	0.83 1.10	0.49	1.01	0.80 1.27	0.94	0.89	0.74 1.06	0.19
	0 or 1	1.00	reference		1.00	reference		1.00	reference	
	2	0.91	0.70 1.16	0.46	1.19	0.81 1.69	0.36	0.72	0.50 1.01	0.07

Relatively high exposure group. (Recent 1-5 yr dose, ≧7.5 mSv; n= 91/180)

[a], Frequency ratio adjusted for age, smoking status, and alcohol intake.

Table Ⅲ-16. Poisson regression analysis of Chromosome Aberrations (CA) : Interaction between GSTM1 and GSTT1 copy number in the occurrence of chromosomal aberrations in the relatively highly exposed group

GSTM1 + GSTT1 copy number	Total CA frequency				Chromosome-type aberration frequency				Chromatid-type aberration frequency			
	FR*	95% CI		*p*-value	FR	95% CI		*p*-value	FR	95% CI		*p*-value
0[a]	1.00	reference			1.00	reference			1.00	reference		
1[b]	0.94	0.79	1.12	0.49	0.94	0.71	1.26	0.67	0.83	0.68	1.03	0.09
2[c]	0.87	0.70	1.07	0.19	1.05	0.74	1.47	0.80	0.69	0.52	0.89	0.01
3[d]	0.80	0.60	1.05	0.12	1.00	0.64	1.52	1.00	0.61	0.42	0.88	0.01
0	1.00	reference			1.00	reference			1.00	reference		
1[i]	0.94	0.79	1.12	0.49	0.94	0.71	1.26	0.67	0.83	0.67	1.03	0.09
2+[ii]	0.85	0.70	1.03	0.10	1.03	0.76	1.41	0.85	0.66	0.52	0.85	0.001

Relatively high exposure group. (Recent 1-5 yr dose, ≧7.5 mSv; n= 91/180)

*, Frequency ratio adjusted for age, smoking status, and alcohol intake. FR for each variable was computed by exponentiation of the coefficient after adjust for the other factors.

[a], (GSTM1/GSTT1 copy number; 0/0). [b], (GSTM1/GSTT1 copy number; 1/0 or 0/1). [c], (GSTM1/GSTT1 copy number; 1/1 or 2/0 or 0/2). [d], (GSTM1/GSTT1 copy number; 2/1 or 1/2 or 2/2).

[i], (GSTM1/GSTT1 copy number; 1/0 or 0/1). [ii],(GSTM1/GSTT1 copy number; 1/1 or 1/2 or 2/1 or 2/0 or 0/2 or 2/2).

Table Ⅲ-17. Poisson regression analysis of chromatid type aberration : Interaction between GSTM1 and GSTT1 copy number in the occurrence of chromosomal aberrations

	Variable	FR*	95% CI		*p*-value
			low	Upper	
Controls	Intercept	1.93	0.80	4.57	0.13
	Age (in years)	1.01	0.99	1.02	0.41
	Smoking status (0,1)[a]	1.35	0.97	1.90	0.08
	Alcohol consumption (0,1)[b]	0.83	0.58	1.16	0.29
	GSTM1 + GSTT1 copy number	1.08	0.91	1.28	0.32
Low exposure group	Intercept	17.12	4.10	70.11	<.0001
	Age (in years)	0.98	0.95	1.00	0.09
	Smoking status (0,1)[a]	0.92	0.75	1.15	0.47
	Alcohol consumption (0,1)[b]	1.12	0.88	1.39	0.36
	GSTM1 + GSTT1 copy number	0.98	0.88	1.08	0.68
High exposure group	Intercept	21.33	11.47	39.25	<.0001
	Age (in years)	0.98	0.96	0.99	0.0007
	Smoking status (0,1)[a]	1.01	0.83	1.22	0.96
	Alcohol consumption (0,1)[b]	0.91	0.69	1.19	0.51
	GSTM1 + GSTT1 copy number	0.84	0.76	0.92	0.0006

Relatively high exposure group. (Recent 1-5 yr dose, ≧7.5 mSv; n= 91/180)

*, Frequency ratio adjusted for age, smoking status, and alcohol intake. FR for each variable was computed by exponentiation of the coefficient after adjust for the other factors.

[a], Smoking status: 0, never smoking; 1, smoking.

[b], Alcohol intake: 0, never; 1, current.

Table Ⅲ-18. Poisson regression analysis of chromatid type aberrations : Interaction between GSTs copy number and radiation exposure in the occurrence of chromosomal aberrations

GSTM1 + GSTT1 copy number	Controls + Low exposure group					High expose group				
	No.	RF*	95% CI		*p*-value	No.	RF	95% CI		*p*-value
3[a]	15	1.00	reference			9	0.83	0.55	1.22	0.35
2[b]	27	1.03	0.78	1.38	0.82	20	0.96	0.70	1.30	0.78
1[c]	59	0.95	0.74	1.24	0.71	41	1.13	0.87	1.48	0.37
0[d]	33	0.87	0.66	1.16	0.33	21	1.36	1.03	1.81	0.035

*, Relative frequency ratio of chromatid type aberrations adjusted for age, smoking status, and alcohol intake.
[a], (GSTM1/GSTT1 copy number; 2/1 or 1/2 or 2/2). [b], (GSTM1/GSTT1 copy number; 1/1 or 2/0 or 0/2).
[C], (GSTM1/GSTT1 copy number; 1/0 or 0/1). [d], (GSTM1/GSTT1 copy number; 0/0).

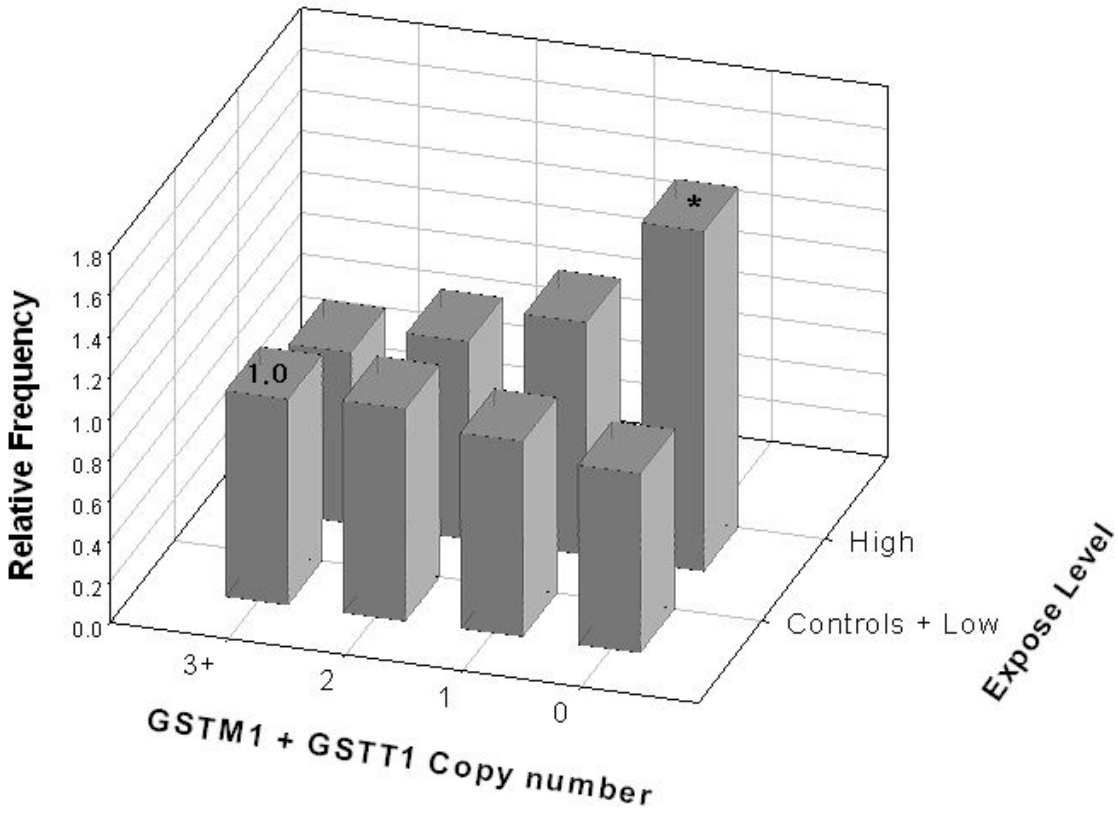


Figure Ⅲ-6. Interaction between GSTs copy number and radiation exposure in the occurrence of chromatid type aberrations
*, $p < 0.05$ compared to the reference (for which the relative frequency ratio is 1.0)

4. Discussion

Nowadays, human beings are exposed to low-dose level of IR inevitably as a result of nuclear research, radiation accidents and diagnostic, therapeutic and occupational exposures, though IR exposures have been minimized and regulated well. Therefore, the biological and genetic effects of low-dose level of IR exposure and its relationship to carcinogenesis have received great attention in the last years (Pierce and Preston, 2000; Mothersill and Seymour, 2001; Bonner, 2003; Brenner et al., 2003; Morgan, 2003; Feinendegen, 2005; Dauer et al., 2010; Buonanno et al., 2011). Also Studies on individuals occupationally exposed to IR has been performed to find out the carcinogenic effects of low-dose level of IR.

GSTM1 and GSTT1 are enzymes that involved in detoxification of products of oxidative stress caused by IR. Despite these crucial roles of GSTs in cellular protection, only a few studies on the relationship between GSTs polymorphisms and the susceptibility to occupational exposure of IR have been addressed (Sal'nikova et al., 2011; Vasil'eva et al., 2010), and overall weak or no association have been observed.

In this study, I investigated the frequencies of CA in low level of IR-exposed workers at power plant (less than the ICRP's occupational limits of 20 mSv per year; n = 180) and control subjects (n = 45) from the

general population in Korea to assess if GSTM1 and GSTT1 influenced individual levels of genotoxic damage.

The value of this study was the use of complicate GSTs genotyping method to discriminate between the three genotype of wild-type, hemizygous deletion and homozygous deletion that provide more insight into the dosage effect of the copy number of GSTM1 and GSTT1 on IR susceptibility, whereas most previous studies could only discriminate the null (GST*0/0) and non-null (GST*1/0 or GST*1/1) genotype. Here we provide the first demonstration of the effect of copy number variations of GSTM1 and GSTT1 on chromosomal damage in low dose IR-exposed workers.

This study also provide a basic database for further clinical and genetic studies as analyzing the frequencies of the major copy number polymorphisms of GSTM1 and GSTT1 in a Korean male population. Our observed GSTM1 and GSTT1 null genotypes frequency is similar to other studies in East Asians (Fujihara et al., 2009; Tsai et al., 2006; Kim et al., 2000; Uhm et al., 2007)and observed distributions of GSTM1 and GSTT1 copy umber was close to that of Chinese populations (Luo et al., 2011) but different from other ethnic groups (Lam et al., 2009; Timofeeva et al., 2009; Rodríguez-Santiago et al., 2009; Christiansen et al., 2006) especially in the frequencies of the GSTT1.

In this study, the CA frequency was significantly higher in radiation exposed workers than controls and the CA frequency was significantly associated with cumulative dose, recent 5.5-year, and recent 1.5-year radiation exposure dose after adjusting age, smoking status and alcohol consumption. recent 1.5-year exposure was more effective in inducing chromosomal damage (β=0.022, p<0.001). These data are consistent with many epidemiological studies in radiation workers (Cho et al., 2009a; Zakeri and Hirobe, 2010; Chung et al., 1996a). The results of these investigations confirmed the increased frequencies of chromosomal aberrations in nuclear power plant workers occupationally exposed to low dose of IR. There is a strong indication that chromosome damage could be induced in workers exposed to low dose of radiation, even below occupational permissible dose limit. In addition, our data represent the issue of the reliability of current dose, and whether or not chromosome aberrations are actually revealed by this measure.

In this study, I found that chromosomal aberrations were predominantly expressed as chromatid-type aberrations. In fact, Different type of chromosome aberrations occurs at low and high doses of IR (Maffei et al., 2004), the aberrations induced by low level of IR were prevalently chromatid-type aberration (Ballardin et al., 2007). The proposed molecular mechanisms that lead to the formation of chromatid-type in individual exposed to low dose IR are the indirect effect of IR, such as

genomic instability and the inaccurate recombinogenic exchange at the chromatid level initiated by IR induced double strand breaks (Ma et al., 2010; Ballardin et al., 2007) .

When exposure workers were divided into two groups based on the median split (high and low exposure groups), the frequencies of Chromatid-type aberration and GSTM1 and GSTT1 copy number have shown the overall negative relationship in high exposure group, in addition the frequency of Chromatid-type aberration increased as the each copy number of GSTM1 and GSTT1 deletion variants increased from zero (wild-type) to one (hemizygous deletion) to two (homozygous deletion). These observations are conceivable, because recent radiation biology studies demonstrate a possible mechanism that IR induces chromosomal damage by producing ROS (in cytoplasm or nucleus) (Little, 1998). Therefore, these results suggest that GSTM1 and GSTT1 copy number may be involved in detoxification of IR-induced ROS.

In this study, further multivariate analyses were performed to clarify the relationship between CA and either each copy number or combined copy number of GSTM1 and GSTT1. Multiple Poisson regression analysis showed that the frequency of chromatid-type aberration was inversely and significantly associated with GSTM1 copy number after adjusting for age, smoking status, and alcohol intake among high exposure

group. And consistent relationship was observed between the GSTT1 copy number and the CA frequency. Individuals with two copy number of GSTT1 had significant decreased among high exposure group. Notably, in addition, we found a significant negative association between combined copy number of GSTM1/GSTT1 and chromatid type aberration in the relatively highly exposed group. One possible explanation for the strong negative association is gene dosage effects of GSTM1 and GSTT1 copy number. Current study have shown that GSTM1 and GSTT1 enzymatic activity is directly proportional to the number of gene copies (Sprenger et al., 2000; Rotunno et al., 2012). As reduced GST activity may lower an individual's ability to protect against oxidative stress generated by IR, individuals with fewer GST1 and GSTM1 copy number may be at higher level of radiation induced chromosomal aberration. Our data also confirmed that gene-environmental interaction between radiation exposure and phase II detoxification genes copy number show a model 2 relationship (Ottman, 1990), indicating that the response of a phenotype to an environmental risk factor is modified by the individual genotype.

In our knowledge, none of studies assessed potential GSTM1 and GSTT1 copy number dependent relationships with chromosomal aberrations in peripheral blood lymphocytes of nuclear power plant workers occupationally exposed to low dose of IR. In addition, none of studies explored putative interactions between GST1 and GSTT. Therefore, our

current study provides better understanding of the relationship between GSTM1 and GSTT1 copy number and susceptibility for radiation induced chromosomal aberration by assessing potential gene dosage effect and gene-environmental interactions.

In conclusion, our current results highlights the usefulness of chromosome aberrations assay as a early effective markers for assessing DNA damage in population occupationally exposed to low levels of IR. A clear inverse association between copy number of GSTM1 and GSTT1 and the frequency of chromosomal aberrations in radiation workers present putative markers of individual susceptibility to IR-induced DNA damage. Furthermore these results emphasize the need of complicate genotyping strategy based on gene copy number in the studies of genetic susceptibility to environmental carcinogens because of the gene dosage effect associated with having two, one, or no alleles.

References

Abbas, A., Delvinquière, K., Lechevrel, M., Lebailly, P., Gauduchon, P., Launoy, G. and Sichel, F. (2004) GSTM1, GSTT1, GSTP1 and CYP1A1 genetic polymorphisms and susceptibility to esophageal cancer in a French population: different pattern of squamous cell carcinoma and adenocarcinoma. World J Gastroenterol, 10, 3389-3393.

Abu-Amero, K. K., Milcarek, B. and Bosley, T. M. (2009) GSTM1 and GSTT1 deletion genotypes in various spontaneous optic neuropathies in Arabs. Br J Ophthalmol, 93, 1101.

Aguilera, F., Mendez, J., Pasaro, E. and Laffon, B. (2010) Review on the effects of exposure to spilled oils on human health. J Appl Toxicol, 30, 291-301.

Alexandrie, A. K., Nyberg, F., Warholm, M. and Rannug, A. (2004) Influence of CYP1A1, GSTM1, GSTT1, and NQO1 genotypes and cumulative smoking dose on lung cancer risk in a Swedish population. Cancer Epidem Biomar, 13, 908-914.

Ballardin, M., Antonelli, A., Cipollini, M., Fallahi, P., Scarpato, R., Tomei, A., Traino, C. and Barale, R. (2007) Induction of chromatid-type aberrations in peripheral lymphocytes of hospital workers exposed to very low doses of radiation. Mutat Res, 626, 61-68.

Bonassi, S., Fenech, M., Lando, C., Lin, Y. P., Ceppi, M., Chang, W. P., Holland, N., Kirsch-Volders, M., Zeiger, E., Ban, S. Y., Barale, R., Bigatti, M. P., Bolognesi, C., Jia, C., Di Giorgio, M., Ferguson, L. R., Fucic, A., Lima, O. G., Hrelia, P., Krishnaja, A. P., Lee, T. K., Migliore, L., Mikhalevich, L., Mirkova, E., Mosesso, P., Muller, W. U., Odagiri, Y., Scarfi, M. R., Szabova, E., Vorobtsova, I., Vral, A. and Zijno, A.

(2001) HUman MicroNucleus Project: International database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. Environ Mol Mutagen, 37, 31-45.

Bonner, W. M. (2003) Low-dose radiation: thresholds, bystander effects, and adaptive responses. Proc Natl Acad Sci U S A, 100, 4973-4975.

Brasch-Andersen, C., Christiansen, L., Tan, Q. H., Haagerup, A., Vestbo, J. and Kruse, T. A. (2004) Possible gene dosage effect of glutathione-S-transferases on atopic asthma: Using real-time PCR for quantification of GSTM1 and GSTT1 gene copy numbers. Hum Mutat, 24, 208-214.

Brenner, D. J., Doll, R., Goodhead, D. T., Hall, E. J., Land, C. E., Little, J. B., Lubin, J. H., Preston, D. L., Preston, R. J., Puskin, J. S., Ron, E., Sachs, R. K., Samet, J. M., Setlow, R. B. and Zaider, M. (2003) Cancer risks attributable to low doses of ionizing radiation: Assessing what we really know. Proc Natl Acad Sci U S A, 100, 13761-13766.

Buchard, A., Sanchez, J. J., Dalhoff, K. and Morling, N. (2007) Multiplex PCR detection of GSTM1, GSTT1, and GSTP1 gene variants: simultaneously detecting GSTM1 and GSTT1 gene copy number and the allelic status of the GSTP1 Ile105Val genetic variant. The Journal of molecular diagnostics: JMD, 9, 612.

Buonanno, M., de Toledo, S. M., Pain, D. and Azzam, E. I. (2011) Long-Term Consequences of Radiation-Induced Bystander Effects Depend on Radiation Quality and Dose and Correlate with Oxidative Stress. Radiat Res, 175, 405-415.

Cho, Y. H., Kim, Y. J., An, Y. S., Woo, H. D., Choi, S. Y., Kang, C. M. and Chung, H. W. (2009a) Micronucleus-centromere assay and DNA repair gene polymorphism in lymphocytes of industrial radiographers. Mutat Res, 680, 17-24.

Christiansen, L., Brasch-Andersen, C., Bathum, L., Kruse, T. and Christensen, K. (2006) A longitudinal study of the effect of GSTT1 and GSTM1 gene copy number on survival. Mechanisms of ageing and development, 127, 597-599.

Chung, H. W., Ryu, E. K., Kim, Y. J. and Ha, S. W. (1996a) Chromosome aberrations in workers of nuclear-power plants. Mutat Res, 350, 307-314.

Chung, H. W., Ryu, E. K., Kim, Y. J. and Ha, S. W. (1996b) Chromosome aberrations in workers of nuclear-power plants. Mutat Res, 350, 307-314.

Covault, J., Abreu, C., Kranzler, H. and Oncken, C. (2003) Quantitative real-time PCR for gene dosage determinations in microdeletion genotypes. Biotechniques, 35, 594-599.

Dash, B., Afriyie-Gyawu, E., Huebner, H., Porter, W., Wang, J., Jolly, P. and Phillips, T. (2006) Determinants of the Variability of Aflatoxin–Albumin Adduct Levels in Ghanaians. Journal of Toxicology and Environmental Health, Part A, 70, 58-66.

Dauer, L. T., Brooks, A. L., Hoel, D. G., Morgan, W. F., Stram, D. and Tran, P. (2010) Review and evaluation of updated research on the health effects associated with low-dose ionising radiation. Radiat Prot Dosim, 140, 103-136.

Dhillon, V. S., Thomas, P., Iarmarcovai, G., Kirsch-Volders, M., Bonassi, S. and Fenech, M. (2011) Genetic polymorphisms of genes involved in DNA repair and metabolism influence micronucleus frequencies in human peripheral blood lymphocytes. Mutagenesis, 26, 33.

Eken, A., Aydin, A., Erdem, O., Akay, C., Sanal, H. T., Soykut, B., Sayal, A. and Somuncu, I. (2010) Cytogenetic analysis of peripheral blood lymphocytes of hospital staff occupationally exposed to low doses of ionizing radiation. Toxicol Ind Health, 26, 273-280.

Feinendegen, L. E. (2005) Evidence for beneficial low level radiation effects and radiation hormesis. Br J Radiol, 78, 3-7.

Fenech, M. (2000) The in vitro micronucleus technique. Mutat Res, 455, 81-95.

Fujihara, J., Yasuda, T., Iida, R., Takatsuka, H., Fujii, Y. and Takeshita, H. (2009) Cytochrome P450 1A1, glutathione< i> S</i>-transferases M1 and T1 polymorphisms in Ovambos and Mongolians. Legal Medicine, 11, S408-S410.

Garte, S., Gaspari, L., Alexandrie, A. K., Ambrosone, C., Autrup, H., Autrup, J. L., Baranova, H., Bathum, L., Benhamou, S. and Boffetta, P. (2001) Metabolic gene polymorphism frequencies in control populations. Cancer Epidem Biomar, 10, 1239-1248.

Hayes, J. D., Flanagan, J. U. and Jowsey, I. R. (2005) Glutathione transferases. Annu. Rev. Pharmacol. Toxicol., 45, 51-88.

Hayes, J. D. and McLellan, L. I. (1999) Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free radical research, 31, 273-300.

He, Y. J., Hoskins, J. M. and McLeod, H. L. (2011) Copy number variants in pharmacogenetic genes. Trends Mol Med, 17, 244-251.

Hoebeeck, J., Speleman, F. and Vandesompele, J. (2007) Real-time quantitative PCR as an alternative to Southern blot or fluorescence in situ hybridization for detection of gene copy number changes. Methods Mol Biol, 353, 205-226.

Kim, J. W., Lee, C. G., Park, Y. G., Kim, K. S., Kim, I. K., Sohn, Y. W., Min, H. K., Lee, J. M. and Namkoong, S. E. (2000) Combined analysis of germline polymorphisms of p53, GSTM1, GSTT1, CYP1A1, and CYP2E1. Cancer, 88, 2082-2091.

Lam, T. K., Ruczinski, I., Helzlsouer, K., Shugart, Y. Y., Li, K. E., Clipp, S., Strickland, P. T. and Alberg, A. J. (2009) Copy number variants of GSTM1 and GSTT1 in relation to lung cancer risk in a prospective cohort study. Annals of epidemiology, 19, 546-552.

Lavender, N., Benford, M., VanCleave, T., Brock, G., Kittles, R., Moore, J., Hein, D. and Kidd, L. C. (2009) Examination of polymorphic glutathione S-transferase (GST) genes, tobacco smoking and prostate cancer risk among Men of African Descent: A case-control study. BMC Cancer, 9, 397.

Lima Sombra, C. M., Coêlho Cavalcanti, B., de Moraes, M. O., Santos, S., Ribeiro-dos-Santos, Â., Rodríguez Burbano, R. and Pessoa, C. (2011) Genetic biomonitoring of inhabitants exposed to uranium in the north region of Brazil. Ecotoxicology and environmental safety.

Little, J. B. (1998) Radiation-induced genomic instability. Int J Radiat Biol, 74, 663-671.

Liu, L., Li, C., Gao, J., Li, K., Gao, L. and Gao, T. (2009) Genetic polymorphisms of glutathione S-transferase and risk of vitiligo in the Chinese population. Journal of Investigative Dermatology, 129, 2646-2652.

Luo, J., Gao, Y. T., Chow, W. H., Shu, X., Li, H., Yang, G., Cai, Q., Li, G., Rothman, N. and Cai, H. (2011) Urinary polyphenols, glutathione S-transferases copy number variation, and breast cancer risk: Results from the shanghai women's health study. Mol Carcinog.

Ma, S., Liu, X., Jiao, B. and Yang, Y. (2010) Low-dose radiation-induced responses: focusing on epigenetic regulation. Int J Radiat Biol, 86, 517-528.

Maddileti, U., Padmaja, T., Hema Prasad, M. and Reddy, P. (2002) Analysis of chromosomal aberrations in the peripheral lymphocytes of workers exposed to diagnostic X-rays. Int J Hum Genet, 2, 265-268.

Maffei, F., Angelini, S., Forti, G. C., Violante, F. S., Lodi, V., Mattioli, S. and Hrelia, P. (2004) Spectrum of chromosomal aberrations in peripheral lymphocytes of hospital workers occupationally exposed to low doses of ionizing radiation. Mutat Res, 547, 91-99.

Morgan, W. F. (2003) Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects in vivo, clastogenic factors and transgenerational effects. Radiat Res, 159, 581-596.

Mothersill, C. and Seymour, C. (2001) Radiation-induced bystander effects: Past history and future directions. Radiat Res, 155, 759-767.

Mullenders, L., Atkinson, M., Paretzke, H., Sabatier, L. and Bouffler, S.

(2009) Assessing cancer risks of low-dose radiation. Nat Rev Cancer, 9, 596-U589.

Ottman, R. 1990. An epidemiologic approach to gene–environment interaction. Genet. Epidemiol. 7:177–185.

Pérez-Morales, R., Castro-Hernández, C., Gonsebatt, M. and Rubio, J. (2008) Polymorphism of CYP1A1* 2C, GSTM1* 0, and GSTT1* 0 in a Mexican Mestizo population: A similitude analysis. Hum Biol, 80, 457-465.

Pemble, S., Schroeder, K., Spencer, S., Meyer, D., Hallier, E., Bolt, H., Ketterer, B. and Taylor, J. (1994) Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J. 300, 271-276

Piacentini, S., Polimanti, R., Porreca, F., Martínez-Labarga, C., De Stefano, G. F. and Fuciarelli, M. (2011a) GSTT1 and GSTM1 gene polymorphisms in European and African populations. Mol Biol Rep, 38, 1225-1230.

Piacentini, S., Polimanti, R., Porreca, F., Martinez-Labarga, C., De Stefano, G. F. and Fuciarelli, M. (2011b) GSTT1 and GSTM1 gene polymorphisms in European and African populations. Mol Biol Rep, 38, 1225-1230.

Piao, J. M., Shin, M. H., Kweon, S. S., Kim, H. N., Choi, J. S., Bae, W. K., Shim, H. J., Kim, H. R., Park, Y. K. and Choi, Y. D. (2009) Glutathione-S-transferase (GSTM1, GSTT1) and the risk of gastrointestinal cancer in a Korean population. World journal of gastroenterology: WJG, 15, 5716.

Pierce, D. A. and Preston, D. L. (2000) Radiation-related cancer risks at low doses among atomic bomb survivors. Radiat Res, 154, 178-186.

Rajagopal, R., Deakin, M., Fawole, A., Elder, J., Elder, J., Smith, V., Strange, R. and Fryer, A. (2005) Glutathione S-transferase T1 polymorphisms are associated with outcome in colorectal cancer. Carcinogenesis, 26, 2157.

Rodríguez-Santiago, B., Brunet, A., Sobrino, B., Serra-Juhé, C., Flores, R., Armengol, L., Vilella, E., Gabau, E., Guitart, M. and Guillamat, R. (2009) Association of common copy number variants at the glutathione S-transferase genes and rare novel genomic changes with schizophrenia. Mol Psychiatry, 15, 1023-1033.

Rotunno, M., Lam, T. K., Vogt, A., Bertazzi, P. A., Lubin, J. H., Caporaso, N. E. and Landi, M. T. (2012) GSTM1 and GSTT1 copy numbers and mRNA expression in lung cancer. Mol Carcinog.

Sal'nikova, L., Chumachenko, A., Lapteva, N. S., Vesnina, I., Kuznetsova, G. and Rubanovich, A. (2011) Allelic variants of polymorphic genes associated with a higher frequency of chromosome aberrations. Russian Journal of Genetics, 47, 1364-1371.

Samavat, H. and Mozdarani, H. (2004) Chromosomal aberrations in Iranian radiation workers due to chronic exposure of X-irradiation. International Journal of Low Radiation, 1, 216-222.

Sprenger, R., Schlagenhaufer, R., Kerb, R., Bruhn, C., Brockmöller, J., Roots, I. and Brinkmann, U. (2000) Characterization of the glutathione S-transferase GSTT1 deletion: discrimination of all genotypes by polymerase chain reaction indicates a trimodular genotype-phenotype correlation. Pharmacogenet Genom, 10, 557.

Sram, R. J., Rossner, P., Rubes, J., Beskid, O., Dusek, Z., Chvatalova, I., Schmuczerova, J., Milcova, A., Solansky, I. and Bavorova, H. (2006) Possible genetic damage in the Czech nuclear power plant workers. Mutat Res, 593, 50-63.

Straume, T., Amundson, S. A., Blakely, W. F., Burns, F. J., Chen, A., Dainiak, N., Franklin, S., Leary, J. A., Loftus, D. J. and Morgan, W. F. (2008) NASA Radiation Biomarker WorkshopSeptember 27-28, 2007. Radiat Res, 170, 393-405.

Tamer, L., Calikoglu, M., Ates, N. A., Yildirim, H., Ercan, B., Saritas, E., Unlu, A. and Atik, U. (2004) Glutathione-S-transferase gene polymorphisms (GSTT1, GSTM1, GSTP1) as increased risk factors for asthma. Respirology, 9, 493-498.

Timofeeva, M., Jäger, B., Rosenberger, A., Sauter, W., Wichmann, H. E., Bickeböller, H. and Risch, A. (2009) A multiplex real-time PCR method for detection of GSTM1 and GSTT1 copy numbers. Clin Biochem, 42, 500-509.

Tiwari, P., Kumar, A., Balakrishnan, S., Kushwaha, H. S. and Mishra, K. P. (2009) Radiation-induced micronucleus formation and DNA damage in human lymphocytes and their prevention by antioxidant thiols. Mutat Res, 676, 62-68.

Trachootham, D., Alexandre, J. and Huang, P. (2009) Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov, 8, 579-591.

Tsai, P. C., Huang, W., Lee, Y. C., Chan, S. H. and Guo, Y. L. (2006) Genetic polymorphisms in CYP1A1 and GSTM1 predispose humans to PCBs/PCDFs-induced skin lesions. Chemosphere, 63, 1410-1418.

Uhm, Y. K., Yoon, S. H., Kang, I. J., Chung, J. H., Yim, S. V. and Lee, M. H. (2007) Association of glutathione S-transferase gene polymorphisms (GSTM1 and GSTT1) of vitiligo in Korean population. Life Sci, 81, 223-227.

Vasil'eva, Z. Z., Besmirbaev, R., Bekmanov, B. and Vorobtsova, I. Association of genetic polymorphism in GSTM1 and GSTT1 with the frequency of chromosomal aberrations in the uranium workers]. Radiats Biol Radioecol 50, 148.

Vasil'eva, Z. Z., Besmirbaev, R., Bekmanov, B. and Vorobtsova, I. (2010) Association of genetic polymorphism in GSTM1 and GSTT1 with the frequency of chromosomal aberrations in the uranium workers. Radiats Biol Radioecol 50, 148.

Wan, J., Shi, J., Hui, L., Wu, D., Jin, X., Zhao, N., Huang, W., Xia, Z. and Hu, G. (2002) Association of genetic polymorphisms in CYP2E1, MPO, NQO1, GSTM1, and GSTT1 genes with benzene poisoning. Environ Health Perspect, 110, 1213.

Zakeri, F. and Hirobe, T. (2010) A cytogenetic approach to the effects of low levels of ionizing radiations on occupationally exposed individuals. Eur J Radiol, 73, 191-195.

국 문 초 록

최근 원자력 발전소 종사자의 방사선피폭은 국제방사선보호위원회가 권고한 직무 선량한도를 초과하지 않는 범위 내에서 잘 관리되고 있다. 하지만 원전종사자에서의 저 선량 방서선의 직업적 또는 만성적 노출은 증가함에 따라, 이의 인체에 대한 영향에 관심이 계속해서 높아지고 있다.

본 연구는 매년 20 mSv를 초과하지 않았으며, 저 선량 방사선에 만성적으로 노출되어 온 180명의 원자력 발전소 종사자들과 45명의 대조군의 세포유전학적 변화에 대해 알아보고자 하였으며, 이를 위해 방사선 노출에 대한 생물학적 선량측정지표로 이용되며 이온화 방사선의 폭로유무 및 돌연변이 유발원과 발암성에 대한 유전적 장해 등을 평가할 수 있는 염색체이상(chromosome aberration)과 소핵(micronuclei) 분석법을 실시하였다. 또한 동일한 노출군과 대조군을 대상으로, DNA손상 및 항산화에 관여하는 Glutathione S-Transferase mu 1 (GSTM1) 과 theta 1 (GSTT1)의 유전자 복제수 변이와 염색체손상 빈도와의 관련성을 보고자 하였으며, 이를 위해 대규모 분자역학연구에서 적용하기 적합한 정량 실시간 PCR (quantitative real-time PCR) 분석법을 사용하였다.

본 연구에서 원자력 발전소 종사자들의 염색체 이상 빈도는 대조군보다 유의하게 높았으며 (Mann Whitney test; $p<.001$), 원자력 발전소 종사자들의 최근 1.5년의 개인선량계상 방사선 피폭 모니터링 측정치가 증가함에 따라 염색체형 이상(Chromosome-type aberration)의 빈도가 유의하게 증가하였다 (Pearson's correlation coefficient test; $r = 0.17$, $p = 0.02$). 또한 포아슨 회귀분석 분석결과, 방사선 피폭량과 염섹체이상 빈도와의 관계에 영향을 미칠 수 있는 연령, 흡연, 음주여부를 보정한 후에도 최근 1.5년과의 방사선 피폭 측청치와 염책체형이상 빈도와 유의한 상관관계를 확인할 수 있었다. 노출군을 최근 1.5년간의 방사선 피폭 모니터링 측정치에 따라 고노출군(≥7.5

mSv) 과 저노출군(＜7.5 mSv)으로 구분하여 분석한 결과 고노출군에서 염색분체형 이상 (Chromatid type aberration)의 빈도와 GSTM1, GSTT1의 유전자복제수와 음의 상관관계를 보였으며 (Kendall τ correlation coefficient; -0.17, p = 0.058; -0.17, p = 0.054), 연령, 흡연, 음주여부를 보정하였을 시 각각의 GSTM1 , GSTT1의 유전자 복제수가 0개인 경우에 비해 유전자 복제수가 2개 이상인 경우 염색분체형 이상의 빈도비(Frequency ratio)는 0.71 (95% CI 0.51 - 0.98, p = 0.04), 0.70 (95% CI 0.49 - 0.99, p = 0.05)로 낮게 나타났다. 또한 고노출군에서의 GSTM1과 GSTT1의 유전자 복제수의 합이 증가함에 따라 염색분체형 이상의 빈도가 유의하게 감소함을 관찰하였으며 (FR = 0.84, 95% CI 1.23 - 4.39, p = 0.009), 대조군과 저노출군에서의 GSTM1,GSTT1 유전자 복제수 합이 3개 이상인 경우에 비해 고노출군에서 GSTM1과 GSTT1의 유전자 복제수 합이 0개인 경우 염색분체형 이상의 빈도비는 1.36 (95% CI 1.03-1.81, p = 0.04)로 유의하게 낮게 나타났다.

이상의 결과를 토대로 염색체이상 빈도의 분석은 저선량 방사선의 노출 정도를 효과적으로 반영해 줄 수 있음을 확인하였으며, 저선량 방사선 노출의 생물학적 지표로 활용될 수 있을 것으로 판단된다. 또한 저선량 방사선 노출에 의한 염색체이상 빈도가 방사선 노출로 인한 DNA 손상복구 및 산화적 스트레스 (oxidative stress) 억제에 관여하는 GSTs 유전자의 복제수 변이와 유의한 상관관계를 있음을 확인함에 따라, 이러한 개인별 GSTM1,T1 유전자 복제수 변이가 방사선 노출에 대한 효과적인 감수성 지표로 활용될 수 있을것으로 판된된다.

주제어: 이온화 방사선, 생체지표, 염색체 이상, 소핵, 유전자 복제수 변이, Glutathione S-transferase mu-1 (GSTM1), glutathione S-transferase theta-1 (GSTT1).

학 번: 2009-30671