Research Departments

Departments of Clinical Studies, Hiroshima and Nagasaki

The Adult Health Study (AHS) biennial examinations were initiated in 1958 and continue to this day. The purposes of these studies have been to determine (a) the types of diseases and (b) the physiological or biochemical abnormalities that may have occurred as a consequence of previous exposure to ionizing radiation, and to correlate this information with other life experiences and modes and patterns of death. The AHS clinical examination is the only point of regular direct contact with the survivors and functions as a principal source of biological materials for a wide variety of other special, invaluable studies.

The AHS has greatly increased in importance in recent years as a result of the accumulation of an enormous body of data from serial medical examinations, with and without the superimposed radiation aspects. Particularly noteworthy is the accumulating evidence of the radiation dose-related increase in non-cancer disease morbidity, such as cardiovascular disease, hyperparathyroidism, thyroid diseases, uterine myoma, chronic liver disease, and cataracts. These important, largely unexpected relationships could never be properly studied using death certificate data alone. Another unexpected finding made largely retrospectively is that radiation is associated with premature menopause, and this, in turn, may result in the earlier onset of other conditions, such as an increase in cholesterol levels and cardiovascular disease. Given the advancing age of the survivors, the time for such studies is limited, making it imperative that any/all research opportunities involving these individuals be considered in a timely manner.

The Departments of Clinical Studies in Hiroshima and Nagasaki follow exactly the same procedures for clinical examination and laboratory testing (history taking, physical examination, electrocardiography, chest X ray, abdominal ultrasonography, and hematological and biochemical examination) as well as computer data entry for AHS participants based on the AHS platform research protocol (RP 2-75), allowing analysis of combined AHS cohort data in the two cities. The subjects exposed at younger ages are an extremely important group in terms of risk assessment. We started to examine an augmented sample of up to 2,300 additional younger cohort subjects who were exposed at ages of 0–9 at the time of the bombings since October 2007 as a pilot study and November 2008 as a full-scale study (RP 3-07) and will examine them biennially from July 2010.

The F_1 Clinical Study (FOCS, RP 1-02) examinations began in January 2002 and were completed in September 2006. RERF conducted several transgenerational studies in the past, and is conducting the mortality follow-up study of A-bomb survivors' children and the genetic analyses of 1,000 family trios. The purpose of FOCS is to analyze the potential heritable effect(s) of A-bomb exposure by focusing on endpoints such as the prevalence of multifactorial diseases (e.g., diabetes, essential hypertension, coronary heart disease) or measurements reflecting the preclinical changes related to multifactorial diseases. However, owing to the young age of the F_1 group (mean age of 48), most of their disease experience is yet ahead, so we are converting the sample to a cohort to follow-up prospectively, and plans for the F_1 clinical follow-up study have almost been completed.

In addition to collaborating on the main study protocols, RP 2-75, RP 3-07, and RP 1-02, the two Clinical Studies Departments in Hiroshima and Nagasaki also actively collaborate, with departmental staff members serving as primary investigators in two additional Adult Health Studies (RP 7-09, RP-A3-09), 11 Special Clinical Studies (RPs 3-10, 2-10, 6-08, 3-05, 2-05, 1-05, 8-02, 3-00, 2-99, and 9-92, RP-A13-08), four Special Cancer Studies (RPs 1-09, 2-04, 1-04, and 6-02), and one Tumor Registry Study (RP 4-08). Further, Nagasaki Clinical Studies is working independently on four Special Clinical Studies (RP 5-00, RPs-A14-08, A10-08, and A8-08), while the Hiroshima department is working independently on four additional clinical studies (RPs 5-92, 3-89, and 4-85, RP-A1-10).

Two new studies related to cardiovascular disease, entitled "The association between chronic kidney disease and cardiovascular disease among atomic bomb survivors" (RP-A3-09) and "Study of arteriosclerosis in the Adult Health Study population (Part 1. Physiological indices of

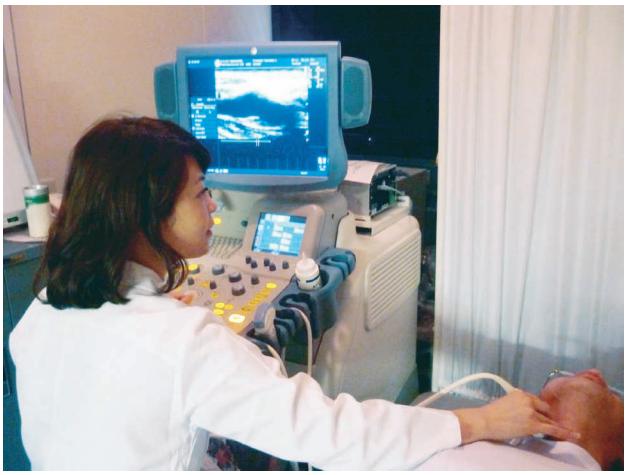
Research Scientists of Hiroshima Clinical Studies (First row from left) Michiko Yamada, Saeko Fujiwara, Kazuo Neriishi, (Second row from left) Ikuno Takahashi, Waka Ohishi, Yoshimi Tatsukawa

Research Scientists of Nagasaki Clinical Studies (First row from left) Ayumi Hida, Nobuko Sera, (Second row from left) Misa Imaizumi, Masazumi Akahoshi

arteriosclerosis)" (RP 7-09), have started. Three new RPs on radiation ocular effects, a follow-up of early-to-moderate cataracts to document cataract progression (RP 3-10), a study to evaluate retinal arteriolosclerosis as an early marker for cardiovascular disease risk and glaucoma (RP 2-10), and a study on ATM and cataract (RP-A1-10) have been approved by the RERF committees.

Sera have been collected and stored from the AHS participants during examinations since 1969. A Microsoft Access™-based user-friendly inventory of stored sera has been established in collaboration with the Department of Information Technology. A digital recording and alarm system for biological samples in deep freezers was established in Hiroshima in 2002. Four studies using stored sera are underway to evaluate interactions between radiation and infectious agents or hormones in the development of hepatocellular carcinoma (RPs 1-09 and 1-04), gastric cancer (RP 2-04), and breast cancer (RP 6-02).

A Radiation Cataractogenesis Workshop was held in March 2009 at RERF. The results from RERF, together with other data presented at the workshop, have provided strong evidence of a lower threshold for the radiation dose response than had been assumed previously. The workshop summary, including our current results, is in press in *Radiation Research*. Another review of the RERF and other epidemiologic cataract data is also in press in the same journal.



Carotid artery ultrasonography has started as part of our study on arteriosclerosis "Study of arteriosclerosis in the Adult Health Study population (Part 1. Physiological indices of arteriosclerosis)." This examination to investigate degree of arteriosclerosis by observing the walls of the carotid artery linking the neck and the brain can help predict cerebral infarction.

Department of Genetics

The Department of Genetics consists of two laboratories: Biochemical Genetics and Cytogenetics. The Biochemical Genetics group focuses on the detection of germline mutations within the offspring of radiation-exposed mammals (i.e., A-bomb survivors or experimental animals) and the development of the requisite technology for such detection. For that purpose, the group has established B-cell lines from nearly 1,300 mother-father-child trios, half of which include at least one parent proximally irradiated by the A-bomb. The Cytogenetics group focuses on the detection of chromosomal abnormalities in A-bomb survivors, as well as their offspring.

In the past, screening for chromosomal abnormalities and gene mutations in germ cells was carried out in the two laboratories by examining the children of survivors selected from the F_1 cohort. However, over the past decade, the cytogenetics program has focused on screening for somatic chromosome abnormalities in survivors and the assessment of these biological responses for the purpose of biodosimetry. Complementary studies involving electron spin resonance (ESR) of tooth enamel obtained from survivors continue to provide supporting data as a validation check on the A-bomb DS02 dosimetry.

The Department of Genetics is currently assuming the research lead for 13 active protocols. They include: Biochemical Genetics, RPs 1-10, 2-07, 1-07, 1-97, and 5-85; Cytogenetics, RPs 6-09, 1-08, 6-00, and 8-93, RP-A4-09; Atomic-bomb Dosimetry, RPs 3-04, 1-92, and 10-86.

Biochemical Genetics Laboratory

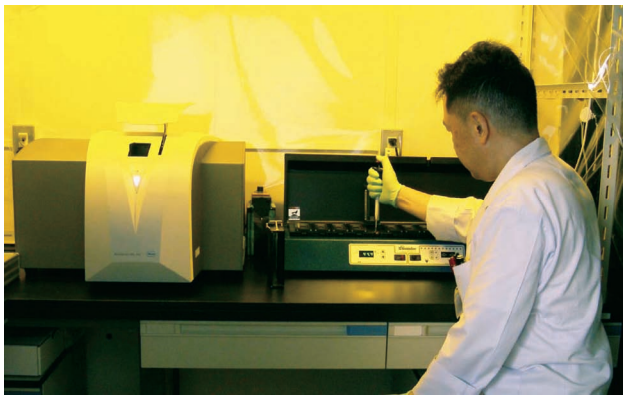
The Biochemical Genetics Laboratory has conducted studies to see whether or not a significant increase in mutation rate has occurred among the children of A-bomb survivors, but extensive studies in the past have yielded no clear and unambiguous evidence of such transgenerational effects.

We have been collecting blood samples and establishing EBV-transformed cell lines from members of survivor families (mother, father, and offspring) to establish DNA resources for molecular studies. Two pilot studies on minisatellite and microsatellite loci, which exhibit high spontaneous mutation rates in germ cells, were conducted

Research Scientists of Genetics (First row from left) Yuko Hirai, Asao Noda, Yoshiaki Kodama, Mieko Kodaira, Kanya Hamasaki, (Second row from left) Nori Nakamura (Chief Scientist), Jun-ichi Asakawa, Yasunari Satoh, Norio Takahashi

using DNA from cell lines of 100 families (50 coming from parents with the highest exposures and 50 from non-exposed, control groups). However they did not show any significant increase in the mutation rates by exposure to radiation (Kodaira et al., *Radiation Research* 2004; 162:350–6 and Kodaira et al., *Radiation Research* 2010; 173:205–13).

We have developed two techniques to detect mutations throughout the whole genome. One is a two-dimensional electrophoresis (2-DE) of DNA, coupled with a quantitative image analysis of ^{32}P-labeled DNA fragments. Based upon our mouse experiments as a model of human male exposure to radiation (Asakawa et al., *Radiation Research* 2004; 161:380–90 and Asakawa et al., *Radiation Research* 2007; 168:158–67), the mean mutation induction rate is considerably lower than the mean rate at seven loci obtained from past mega-mouse experiments that formerly served as a primary basis for estimates of genetic risk to humans. Another technique is a microarray-based comparative genomic hybridization (aCGH) method. We first conducted a pilot study to establish our aCGH system using a microarray consisting of 2,300 BAC (bacterial artificial chromosome)-clones. The results from the pilot study, including the molecular characteristics of the rare variants, were published (Takahashi et al., *Annals of Human Genetics* 2008; 72:193–204 and Takahashi et al., *Cytogenetic Genome Research* 2008; 123:224–33). Following the pilot study, a larger population study, where 225 offspring born to high-dose-exposed parents (≥1 Gy) were examined by the array having 2,500 BAC clones, identified three *de novo* mutations (one deletion and two duplications) in the gametes that originated from exposed fathers.



It is now possible to establish a high-density array with 2.1 million probes (60 bases selected from 2.1 million sites on the human and mouse genomes) attached to one glass slide. To conduct research with this high-density microarray, Roche NimbleGen's MS200 Microarray Scanner (left) and Hybridization System (right) were purchased. These devices are located in an ozone-free dark room.

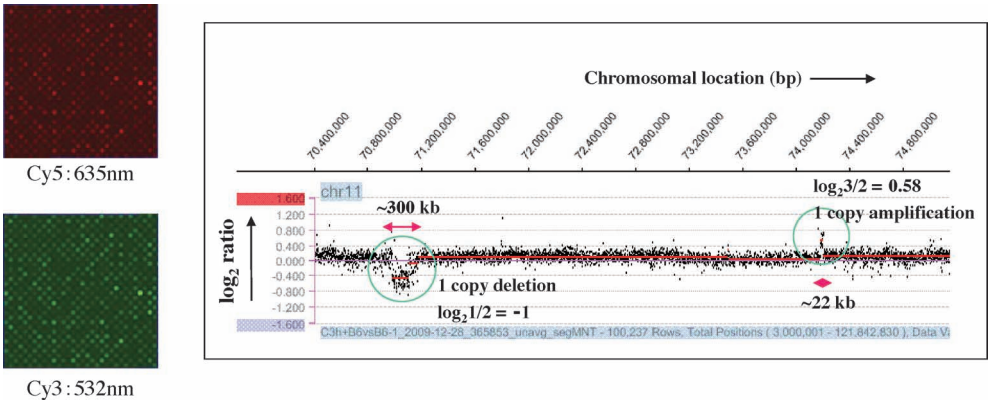
The laboratory is currently developing plans to conduct studies of radiation-associated germline mutations in both animal models and humans using high-density arrays (1–2 million probes) for detecting copy number variations.

Cytogenetics Laboratory

The Cytogenetics Laboratory's research activities span five areas. The first area is to collect survivors' cytogenetic information in the blood lymphocytes in order to either validate or demonstrate biases in the individual radiation dose estimates made by conventional physical measurements. The second area is to determine whether parental exposure to A-bomb radiation increases the number of offspring carrying chromosomal abnormalities. Third, studies are being undertaken to compare DS02 dose estimates and those made using ESR measurements of teeth. Fourth, studies of the genetics of radiation-associated breast cancer, skin cancer, and hypertension are being undertaken. Fifth, development of a novel animal model system for estimating genetic effects of radiation at low doses is also being conducted.

Cytogenetics studies of survivors

In the past, over 4,000 survivors were examined for chromosome translocation frequency (3,000 by the conventional Giemsa-staining method and 1,000 by the new fluorescence *in situ* hybridization [FISH]), and the individual frequencies varied extensively when plotted against DS86/DS02 doses. The frequencies were never used to calculate radiation doses, primarily because too many years had passed since radiation exposure and hence a substantial, but unknown and variable, fraction of currently observed lymphocytes are derived from irradiated bone marrow stem



For comparison purposes, one DNA sample is labeled with red fluorescent dye (Cy5) and the other with green fluorescent dye (Cy3). The same probes on the microarray are then arrayed. Gene copy number is estimated based on degree of fluorescence of each probe. Using this method, it is possible to accurately detect changes (deletion/amplification) in gene copy number, irrespective of the scale of such changes.

cells at various levels (but their radiosensitivities for induction of translocations are not fully understood). We anticipate that ESR measurements of tooth enamel will prove to be useful in addressing dosimetry issues because translocation frequency and ESR-estimated dose (calibrated by Co-60 gamma rays) fit rather closely, and ESR data are a better candidate to calculate gamma-ray doses from the A-bomb, provided that the energy dependency of gamma-ray photons can be taken into account (see *A-bomb Dosimetry Studies* to be described in a later section).

Cytogenetics studies of children of the atomic-bomb survivors

An extensive cytogenetic survey using conventional chromosome staining methods was conducted previously, and involved nearly 16,000 persons (8,000 born to exposed parent(s) and 8,000 to unexposed). Results of that survey failed to reveal a significant transgenerational genetic effect; only one *de novo* structural chromosome rearrangement in each group was noted. However, a cautionary note is warranted in that not all parents of aberration-carrying individuals were examined cytogenetically. Nevertheless, to date, there is no conclusive evidence of a radiation effect on germ cells of the A-bomb survivors.

Radioisotope Facility

The Radioisotope (RI) Facility is a laboratory where the radiation effects for either germ cells or somatic cells are examined by using liquid radioisotopes. Use of seven types of radioisotopes, including ^{32}P, ^{3}H, ^{51}Cr, and ^{125}I, is permitted. Recently, ^{32}P has been most frequently used for DNA analyses.

All of RERF's research departments share the RI facility. Eleven persons from the Department of Genetics and seven from the Department of Radiobiology/Molecular Epidemiology hold the appropriate registration for authorized use of the RI facility, and these registered staff receive education and training once a year and heath examinations twice a year. Three persons are facility administrators responsible for safe conduct of experiments.

Legal regulations concerning the facility's safe management are strictly followed. The Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT), the country's official supervisory authority for such laboratories, monitors operations and issues advisories when violations occur in such institutions to preclude recurrences. Upon receipt of such notices, we inspect our facility for any inadequacies and make efforts to improve our operations accordingly.

RI exhaust system. All the air released from the RI facilities passes through filters. Air is purified by the filter unit in the right foreground and emitted outside as exhaust by the fan in the background. Radiation level in the exhaust air is constantly measured by the gas monitor located on the left.

Department of Radiobiology/Molecular Epidemiology

To address the question of how A-bomb radiation affects human health and causes diseases, this department seeks to clarify the immunological and molecular biological mechanisms linking exposure and various disease outcomes among the A-bomb survivors, as well as to determine genetic factors involved in individual susceptibility to such radiation-associated diseases. On the basis of this mechanistic understanding, we can build a strong foundation for more robust risk assessments of radiation-associated diseases and for prevention and treatment of these diseases.

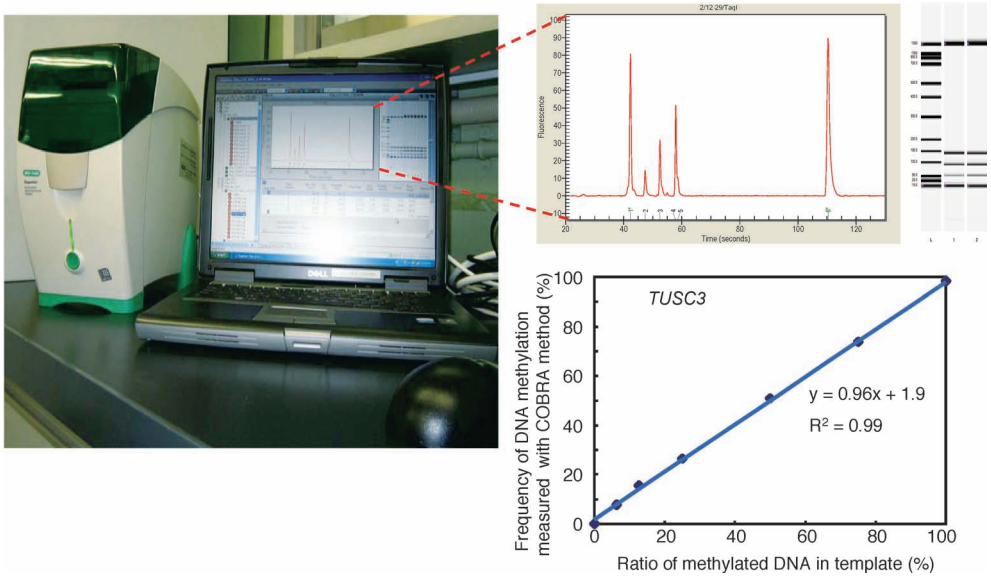
To understand mechanisms behind radiation-associated carcinogenesis, the Cell Biology Laboratory has been investigating molecular characteristics in selected cancers among A-bomb survivors. Those investigations have included *RET/PTC* rearrangements and a recently-found rearranged anaplastic lymphoma kinase (*ALK*) gene in thyroid cancer (RP 5-02), mutations of *p53*, *EGFR*, and *ALK* genes, global DNA hypomethylation in lung cancer (Pilot Study B37-04), and microsatellite instability-related alterations of *MLH1*, *MSH2*, and *RAS* signaling-related genes in colorectal cancer (B38-04).

Research Scientists of Radiobiology/Molecular Epidemiology (First row from left) Tomonori Hayashi, Kei Nakachi (RERF Consultant), Yoichiro Kusunoki, Evan B. Douple (Associate Chief of Research), Kiyohiro Hamatani, (Second row from left) Kengo Yoshida, Yasuharu Niwa, Reiko Ito, Kazue Imai, Masataka Taga

To assess radiation effects on normal cells, the Cell Biology Laboratory plans to conduct preliminary studies to assess epigenetic status in archival autopsied "non-cancer" tissues of A-bomb survivors and blood cell subsets of AHS subjects.

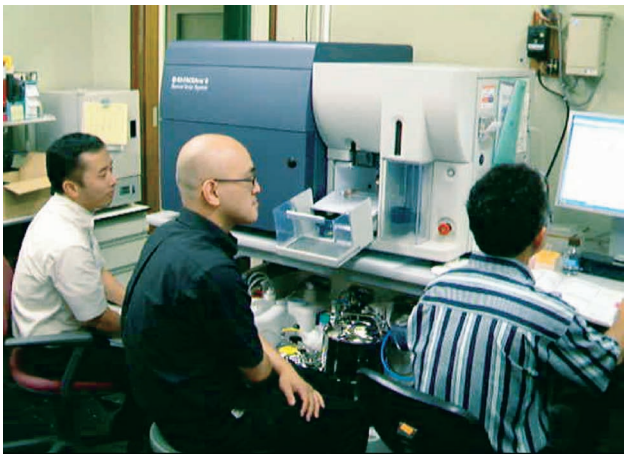
The Immunology Laboratory tests the hypothesis that ionizing radiation accelerates immunosenescence, resulting in enhanced risks of aging-related diseases among A-bomb survivors. Evidence supporting this hypothesis has been accumulated and is illustrated by altered immunity found among A-bomb survivors, such as (i) T-cell repertoire deviation and clonal expansion in memory T cells, (ii) decreased T-cell function and enhanced inflammation, (iii) decreases in naïve T-cell pools, (iv) increased T-cell populations with a compromised/senescent phenotype, and (v) genetic damage in the hematopoietic system. With support by funding from the U.S. National Institute of Allergy and Infectious Diseases (NIAID), this laboratory started an international collaboration to study mechanisms of radiation-related immunosenescence, influenza vaccine response, and development of an integrated scoring system for evaluating immunological and inflammatory status among A-bomb survivors.

The immunogenome project in the Immunology Laboratory seeks to elucidate genetic factors involved in radiation sensitivity and disease susceptibility with use of stored lymphocyte DNA, and with a focus on gene-targeting analyses based on phenotype-genotype associations as well as genome-wide analyses. The study will analyze numerous candidate gene polymorphisms related to immunity, inflammation, DNA repair, cell cycle, and xenobiotic metabolism. We found that genetic polymorphisms of *IL-10*, *IL-18*, and *EGFR* modulated radiation dose-dependent risks of selected cancers among individual A-bomb survivors.



Application of the automated electrophoresis system to DNA methylation analysis (COBRA method). Electrophoresis using LabChip enables us to quantitatively measure the frequency of DNA methylation with COBRA method as shown in a lower right panel.

Current studies in the Department of Radiobiology/ Molecular Epidemiology are being conducted under 15 active protocols: 12 Immunology Studies (RPs 5-09, 4-09, 3-09, 5-04, 4-04, 1-03, 4-02, 2-97, 1-93, 2-90, 7-87, and 3-87), one Cell Biology Study (RP 5-02), one Special Cancer Study (RP 2-86), and one Cytogenetics Study (RP-A2-09). We collaborate with other departments at RERF and outside researchers on the projects.



A high-performance cell sorter, FACSAria II, has been introduced to the Immunology Laboratory (collaborative purchase with the Department of Genetics). Using this cell sorter, we are analyzing a variety of cell types, including hematopoietic stem cells, and performing chromosome sorting in collaboration with the Department of Genetics.

Departments of Epidemiology, Hiroshima and Nagasaki

The Department of Epidemiology plays a central role in the conduct of the long-term follow-up of the Life Span Study (LSS), *in utero*, and F_1 cohorts. The follow-up of these cohorts has relied on mortality surveillance through the use of the *koseki*, the nationwide family registration system, and on cancer morbidity data from tumor registries in Hiroshima and Nagasaki. Analyses of updated cancer and non-cancer mortality data (through 2003) in the LSS using the DS02 dosimetry system have been completed. The risk of total death significantly increased, largely because of an approximately linear dose-dependent increase in cancer, especially among subjects who were young at the time of the bombing. The risk of stroke and heart diseases also increases at moderate-to-high doses (Shimizu et al., *British Medical Journal* 2010; 340:b5349). In the *in utero* cohort, although solid cancer mortality risk appears to be lower than for those exposed in early childhood as similar as those seen in solid cancer incidence data, the risk of non-cancer diseases was suggestively higher in the *in utero*

Research Scientists of Hiroshima Epidemiology (First row from left) Shoji Tokuoka (RERF Consultant), Kotaro Ozasa, Fumiyoshi Kasagi, (Second row from left) Eric J. Grant, Ritsu Sakata, Hiromi Sugiyama

Research Scientists of Nagasaki Epidemiology (From left) Midori Soda, Akihiko Suyama

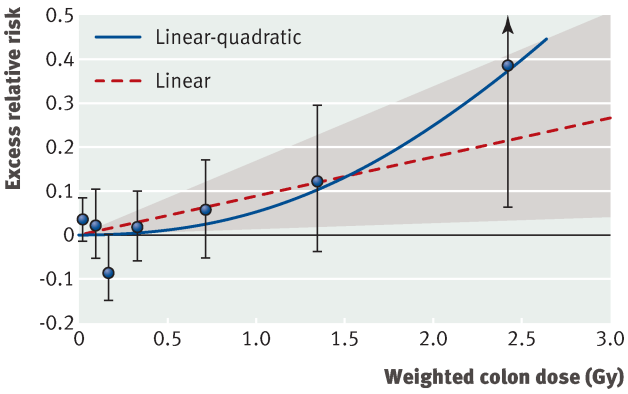


Figure 1. Radiation dose-response relation (excess relative risk per Gy) for death from stroke, showing linear and linear-quadratic functions. Shaded area is 95% confidence region for fitted linear line. Vertical lines are 95% confidence intervals for specific dose category risks. Point estimates of risk for each dose category are indicated by circles. *(Shimizu et al., BMJ 2010; 340:b5349)*

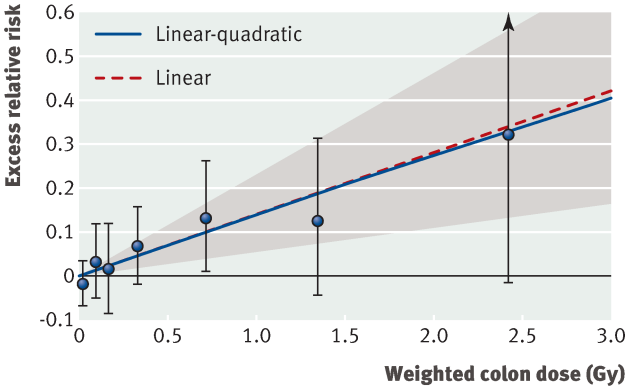


Figure 2. Radiation dose-response relation (excess relative risk per Gy) for death from heart disease, showing linear and linear-quadratic functions. Shaded area is 95% confidence region for fitted linear line. Vertical lines are 95% confidence intervals for specific dose category risks. Point estimates of risk for each dose category are indicated by circles. *(Shimizu et al., BMJ 2010; 340:b5349)*

cohort. In the F_1 cohort study, recent results corroborate previous analyses of earlier follow-up experience showing no association between cancer or non-cancer mortality, or cancer incidence in the children of A-bomb survivors and their paternal or maternal doses.

Continued follow-up of these cohorts is essential to clarify the temporal patterns of cancer risk as young subjects reach ages when background cancer risk increases. Due to the age distribution of the LSS, observed cancer deaths are estimated to increase yearly until they peak in about 2015 and then slowly decline (Furukawa et al., *Risk Analysis* 2009; 29:885–99). It is also important to evaluate non-cancer risks, such as for cardiovascular diseases and other diseases, which seem to increase with radiation exposure and aging. Although the total percentage of survivors is about 40% of the LSS subjects at the end of 2005, 86% of the LSS who were younger than 10 years old at the time of the bombing were still alive. Similarly, about 90% of the *in utero* cohort and the F_1 cohort are alive.

Detailed site-specific cancer studies that include histopathological reviews for various organ sites have been conducted among cancer cases within the LSS cohort for a long period. These are collaborative studies with pathologists in Hiroshima and Nagasaki, and the U.S. National Cancer Institute. Studies on skin, thyroid, ovary, and lung cancers are in their final stages of data analysis and/or have manuscripts in preparation and in press (Furukawa et al., *Radiation Research,* in press). Histopathological reviews for malignant lymphoma cases have been almost completed. We are currently collecting materials for soft tissue and bone tumors, and breast and uterine cancers.

Starting in the 1960s, mail surveys were conducted in the LSS for collecting information on non-radiation risk factors such as lifestyle and others that would be utilized for evaluation of confounding or modification of radiation risk. Several studies to evaluate confounding and modification of radiation risk by other non-radiation factors, for example, by menarche and first birth for breast cancer, anthropometric factors for colon cancer, and smoking and occupations for bladder cancer are underway or recently finished. Those studies are mainly conducted as part of a collaborative program called the Radiation Research Partnership with the University of Washington and Kurume University. Another project utilizing previous mail survey data is an evaluation of smoking effects on mortality and major causes of death in Japan, compared to the smoking risks observed in British studies. This study is being conducted in collaboration with Oxford University and is in the final stages. The department is planning to participate in the Asian Cohort Consortium (ACC), which includes more than one million subjects derived from 18 Asian cohorts and intends to investigate the association of lifestyle factors with relatively rare cancers.

A new mail survey in the LSS cohort has been started, 17 years after the last full-scale survey. New information will be collected in this survey including diagnostic and therapeutic medical radiation exposures, as it is important to evaluate radiation from other sources in addition to the A-bomb. The first phase of the study targets 5,100 persons eligible for recruitment into the Adult Health Study and has almost finished. Next, a pilot study will be conducted to test the feasibility of doing saliva collection for extracting subjects' DNA from the balance of the LSS cohort.

The Department of Epidemiology should and will play a key role in the design and conduct of various interdepartmental research activities by providing information on cause of death and cancer incidence of the subjects derived from LSS, *in utero*, and F_1 cohorts to them. The Department of Epidemiology is engaged in the working groups involving all departments within RERF such as the F_1 clinical study, a program of studies evaluating radiation risks and mechanisms of cardiovascular diseases, and revision of individual doses. Also the department has many individual projects with other departments at RERF. A project for the storage of histopathological specimens from the subjects of our cohorts and atomic-bomb survivors is being planned including universities and hospitals in Hiroshima and Nagasaki, which is a collaborative project chaired by Dr. Okubo, and funded by the Ministry of Health, Labour and Welfare.

The epidemiology research activities in Hiroshima and Nagasaki Laboratories are carried out following the same

research protocols and procedures by five epidemiologists in Hiroshima and two in Nagasaki. Two part-time pathologists are also highly involved in the research. The Epidemiology staff works closely with the Statistics Department staff in study design, data analysis, and major report preparation; they also work with the Department of Information Technology in database design and development.

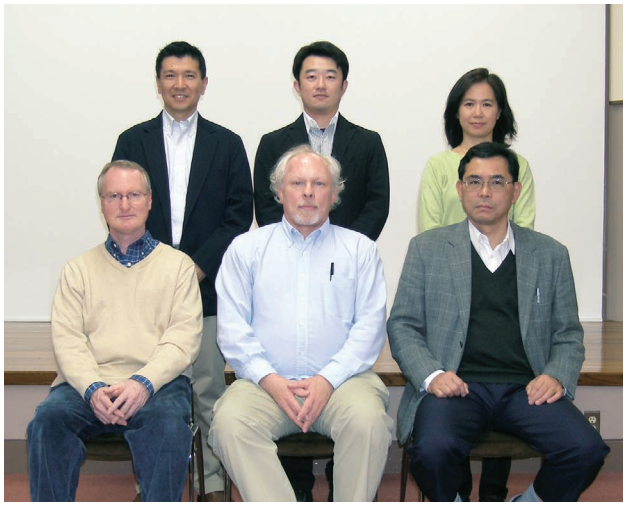
This year the Hiroshima and Nagasaki Departments of Epidemiology have worked as primary investigators on 31 active research protocols including 9 smaller type-A protocols, spanning five major research areas: LSS (RPs 3-08, 2-08, 2-06, 1-75, and 2-61, RPs-A11-08, A9-08, A7-08, A6-08, A3-08, and A1-07); F_1 Study (RPs 3-02 and 4-75); Tumor Registry (RP 18-61 and tissue registry study with no RP number); Histopathology (RP 5-89, RP-A2-08); and Special Cancer Studies (RPs 2-09, 5-08, 4-07, 1-06, 2-02, 3-94, 1-94, 2-92, 6-91, 2-91, 9-88, and 29-60, RPs-A12-08 and A5-08). A1-07 and A6-08 were successfully terminated during 2009. We were collaborating investigators in many other RP investigations as well.

Department of Statistics

The Department of Statistics fulfills three broad missions listed in order of priority: (1) provide statistical consultation to and collaborate with researchers in other RERF departments; (2) conduct research pertaining to radiation risk estimation (including implementation and characterization of A-bomb dosimetry as well as assessment of risk modification by age, time, and other factors); (3) perform basic methodological research to implement and extend biostatistical methods relevant to RERF studies.

During FY2009, the Department of Statistics hosted a number of visiting scientists and collaborators. The research staff worked on numerous statistical consulting projects in support of RERF research protocols as well as statistical methods research directed at improving radiation effects risk assessment. The statistical research is summarized briefly here, and both research and consulting work are addressed in following sections. Three key areas of research are development of 1) methods for individual as opposed to grouped data in major risk assessment studies, 2) new types of generalized models for joint effects of multiple risk factors, and 3) methods for quantifying and correcting for uncertainty in survivors' dose estimates in risk assessment.

A related area involves new methods for using geospatial information about survivors' locations and possibly their residential histories in risk assessment, beyond simply using distance to calculate dose. Other important work involves special nested designs for efficient and unbiased sub-samples for studies requiring, e.g., lab analysis of biosamples. Another area involves consideration of causal relationships involving intermediate variables, and some of our newest work combines both sub-sample designs and causal relationships. Research in dosimetry made considerable progress during the last year, in areas such as use of Geographical Information System technology to improve dose estimates and quantitative assessment of potential doses from residual radiation, i.e., local radioactive fallout and neutron-activated soil. We are also actively working on methods to improve utilization of clinical laboratory data, mechanistic modeling of cancer, and other areas.



Research Scientists of Statistics (First row from left) John B. Cologne, Harry M. Cullings, Eiji Nakashima, (Second row from left) Kyoji Furukawa, Munechika Misumi, Wan Ling Hsu

Overview of Department Staff Research Activities in FY2009

Radiation effects research and risk assessment

Dr. Hsu continued to finalize analyses of the incidence of leukemia, lymphoma, and multiple myeloma and also collaborated with Dr. Masako Iwanaga at Nagasaki University on investigating myelodysplastic syndromes (MDS) in Nagasaki A-bomb survivors. She also published a paper on longitudinal trends in white blood cell counts in the Adult Health Study (AHS) in relation to radiation dose. A summary of dose response in men is shown in Figure 1. Dr. Cullings submitted a manuscript on the application of geospatial hotspotting to cancer incidence data to *Environmental and Ecological Statistics* and gave presentations related to geospatial hotspotting at three international meetings. Dr. Cologne and others in the Department of Statistics wrote a paper on "Attributable risk for radiation in the presence of other risk factors," which was accepted for publication in *Health Physics*. Figure 2 shows the differing risk relationships of multiplicative vs. additive models for radiation in the presence of another risk factor.

Dosimetry

Dr. Cullings began analyzing data obtained by Dr. Nakamura, RERF Chief Scientist, and evaluated by Dr. Albrecht Wieser of the Helmholtz Institute of Munich, Germany, on measurements of atomic-bomb gamma ray dose to tooth enamel by electron spin resonance (ESR), for a manuscript on results in unexposed controls. Two papers on the measurement of thermal neutron activation in teeth by measuring ^{41}Ca levels, in collaboration with investigators at the Helmholtz Institute Munich, are in press at *Radiation Research*; Dr. Cullings contributed related analyses and is a co-author on the second paper. Ongoing work in other aspects of dosimetry is described under RP 18-59 in "Active Research Protocols by Study Program."

Methodological research

We are engaged in a number of methodological research activities focused on improving the estimation of radiation risk and related issues. These fall into four major areas: risk analysis and dosimetry, complex modeling, study design, and methods related to specific RERF studies.

There is a continuing need for research on statistical methods for adjusting risk estimates for bias and imprecision caused by errors in dose estimation. This encompasses both efforts to better characterize errors in dose estimates and to develop ways of correcting risk estimates for those errors. We are examining a number of different approaches, which are discussed further below under "*Collaboration with outside organizations*."

We are developing methods for risk estimation using individual data with Bayesian models evaluated by Markov Chain Monte Carlo (MCMC) integration. This can do many

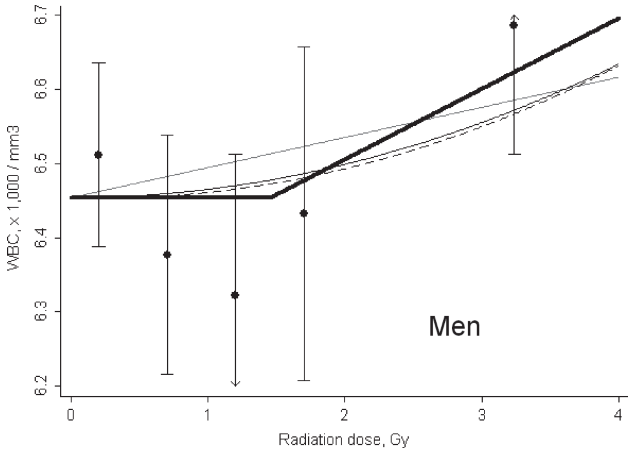


Figure 1. White blood cell count vs. bone marrow dose in LSS men, adjusted to age = 30 and BMI = 21. Several models (L, Q, LQ, threshold) are shown.

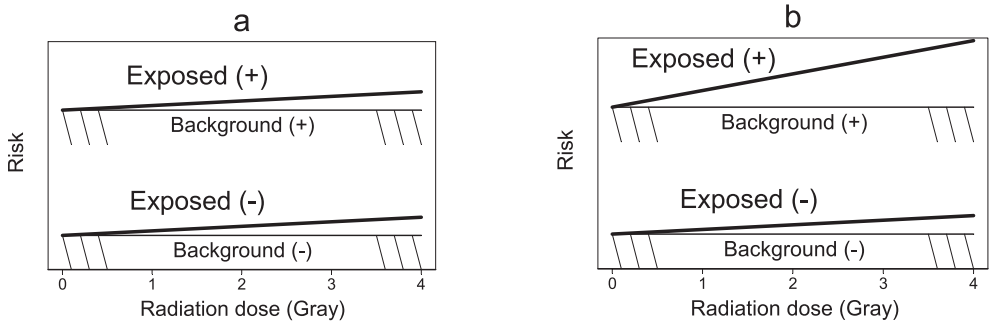


Figure 2. Risk for exposure to radiation in the presence (+) or absence (–) of another factor, depending on whether the joint effect is additive (a) or multiplicative (b).

things that traditional Poisson regression cannot, such as incorporating mechanistic hypotheses and family relationships (possible clustering in terms of radiation sensitivity); modeling missing-data mechanisms (e.g., for mail-survey data); developing flexible methods of inference about the shape of the dose response (especially low-dose), and utilizing more flexible and detailed error models for uncertainties in dose estimates. Preliminary results of such analyses were used to evaluate the effect of dose error under several error models.

We are collaborating with several groups of external investigators on mechanistic models of carcinogenesis and on models for complex causal mechanisms including intermediate risk factors, which are discussed further below under "*Collaboration with outside organizations*." We are also developing methods to analyze complex causal models involving intermediate risk factors in the nested case-control study design, and have drafted a new RP: "Methods for assessing joint effects of radiation and intermediate risk factors in nested case-control studies," which is a high priority because several nested case-control studies under analysis in the AHS involve intermediate risk factors that themselves are associated with radiation dose.

Several RERF studies have been designed as nested case-control studies with risk set-based subject selection and counter-matching (stratified sampling) on radiation dose. We are developing methods for handling problems such as 1) optimum definition of stratification, and 2) re-utilization of controls originally matched to cases that later had to be dropped.

In the area of methods of analysis for individual RERF studies, we are doing work to assess effects of misclassification using data on cataract surgery as a surrogate for cataract prevalence. Another area involving clinical data is extending mixture distributions to left-censored data (data with a lower limit of detection) to analyze antibody titers associated with *H. pylori* and gastric cancer, including two-dimensional analysis of the joint distributions of related titers. Work is also ongoing in comparing various approaches to the design (whether or not to stratify on radiation) and analysis (which of several competing methods of weighting cases not in the case-cohort sub-cohort) of the case-cohort study of radiation, immunogenes, and cancer in the Department of Radiobiology/Molecular Epidemiology; this is in collaboration with Dr. Bryan Langholz of the University of Southern California.

Collaboration with other RERF departments

Dr. Nakashima's ongoing collaborative projects with the Department of Clinical Studies include a continuation of cataract and glaucoma studies, a metabolic risk factor study relating sub-clinical hypothyroidism and lipid infiltration in the parotid gland to visceral fat and coronary heart disease, and the continuing F_1 ophthalmology study.

Dr. Cullings did consulting and collaboration with several investigators in the Department of Genetics: Dr. Asakawa (binomial statistics of rare mutational events in mouse and rat germline mutation assays, including preparation of a new RP using high density oligonucleotide microarrays to detect mutations as copy number variation), Dr. Noda (relation of mutational frequency in flow cytometry to mutation rate), and Dr. Nakamura (ESR and ^{41}Ca dosimetry measurements of tooth enamel). Dr. Cullings is also engaged in extensive collaborations with investigators in Epidemiology and other RERF departments under the aegis of the RERF Dosimetry Committee (RP 18-59, above).

A large grant was awarded by the U.S. National Institute of Allergy and Infectious Diseases (NIAID) to the Department of Radiobiology/Molecular Epidemiology. It includes three RPs focused on aging and immunosenescence and the impact of radiation on immunological and inflammation markers, including studies on the effect of radiation on dendritic cells and hematopoietic stem cells, response to influenza vaccine in aging AHS subjects, and developing a scoring system to assess immunological aging. Dr. Geyer developed study designs and analysis plans for these projects. Mr. Misumi has been working on these projects in consultation with Dr. Geyer, who is now in the U.S. The project relating to response to influenza vaccine has begun as a pilot study.

Dr. Cologne collaborated with Dr. Yoshida and others in the Department of Radiobiology/Molecular Epidemiology on the analysis of interaction between radiation and an *EGFR* gene repeat polymorphism in lung cancer, which was published in *Carcinogenesis*. He also collaborates with Dr. Hayashi in the Department of Radiobiology/Molecular Epidemiology in various studies of immunogenes and cancer focusing on gene-environment interaction with radiation (RP 4-04; currently *HLA-DRB1* and *IL-10* gene polymorphisms and risk of radiation-associated gastric cancer). He has the lead statistical role in the nested case-control study of breast/endometrial cancer (RP 6-02, described in this report), which is led by Dr. Neriishi in the Department of Clinical Studies. He has been extending analyses of the F_1 Clinical Study (FOCS; RP 1-02) cross-sectional data by investigating individual multifactorial outcomes jointly using multivariate-outcome models to assess potential differential effects of parental radiation exposure. Dr. Cologne also collaborates with Drs. Takahashi and Satoh of the Department of Genetics (Biochemical Genetics) on studies of trans-generational effects of radiation assessed using array comparative genome hybridization (array-CGH) methods (RP 2-07) and is involved in their new proposal making use of single nucleotide polymorphisms (SNP) arrays. He continues collaborating with Dr. Ohishi of the Department of Clinical Studies regarding the nested case-control study of viral hepatitis and liver cancer, for which a complex analysis involving hepatitis B virus (HBV) infection as an intermediate, radiation-related risk factor has been conducted with a manuscript in preparation. He has an ongoing collaboration with the Department of Epidemiology, in particular involving former Beebe Fellow Dr. Samartzis, on analyses of radiation effects on bone sarcomas and soft tissue sarcomas.

Dr. Furukawa played a role as the primary statistician and investigator in collaborations with the Department of Epidemiology and the U.S. National Cancer Institute for analyses of site-specific Life Span Study (LSS) cancers (lung and thyroid cancers). A plot showing important results related to the joint effect of smoking and radiation on lung cancer is shown in Figure 3. Dr. Furukawa also conducted analyses for a study of second primary tumors in the LSS,

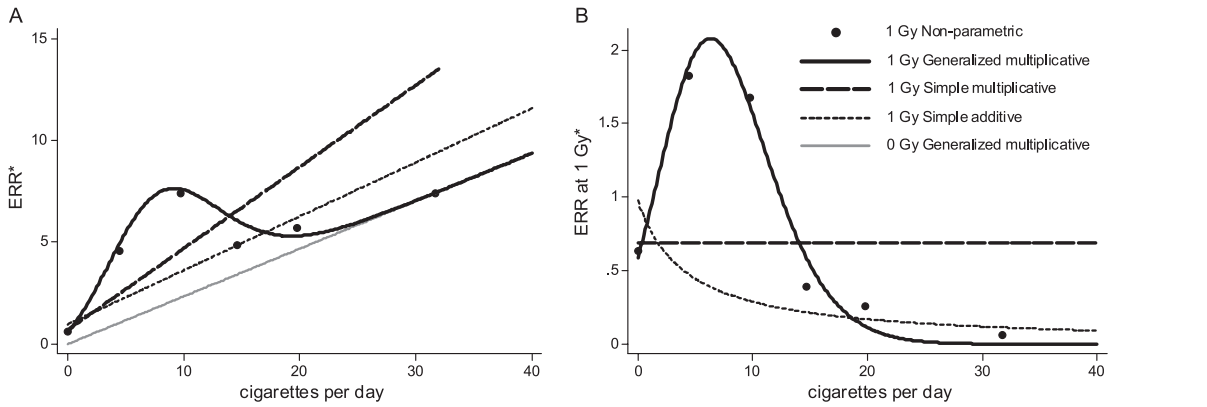


Figure 3. Variation of the excess relative risk (ERR) with smoking intensity. The gender-averaged risk estimates at age 70 following radiation exposure at age 30. Smoking was assumed to start at age 20 so that smoking duration was fixed at 50 years in this figure. Panel A describes the joint effect of radiation and smoking relative to the baseline rate for a non-exposed non-smoker. The thin long dashed line is the fitted ERR for a person with no radiation exposure. The solid line is the fitted ERR following exposure to 1 Gy under the generalized multiplicative model, the thick dashed line is the fitted risk under a simple multiplicative model, and the short-dashed line is the fitted ERR under a simple additive joint effect model. The points are based on a generalized multiplicative model in which smoking intensity categories replaced the linear-quadratic function of log intensity used in the generalized multiplicative model. Panel B presents radiation-associated excess risks for an exposure of 1 Gy relative to the risk of an unexposed person with the same smoking history.

which was led by Dr. Nishi of the Department of Epidemiology. Dr. Furukawa and Dr. Kamada (a former research assistant of the Department of Statistics) provided statistical support to a study of the genetic effects of parental radiation exposure on microsatellite mutations with the Department of Genetics.

Dr. Hsu continued collaborating with the Department of Epidemiology in analyses of leukemia, lymphoma, and multiple myeloma incidence for the LSS cohort. She also analyzed the risk of MDS among Nagasaki members of the LSS cohort for a collaborative pooled study with Dr. Iwanaga of Nagasaki University and the manuscript has been submitted. She collaborated with Dr. Takahashi of the Department of Clinical Studies in preparing for a research protocol "Study of arteriosclerosis in the Adult Health Study population (Part 1. Physiological indices of arteriosclerosis)" (RP 7-09). Drs. Hsu, Furukawa, and Cullings continued to be part of the FOCS statistical working group, under the lead of Dr. Cologne, to examine the effect of radiation on individual multifactorial disease outcomes.

Mr. Misumi consulted for the Department of Radiobiology/Molecular Epidemiology in the drafting of research proposals for analyses of molecular characteristics of colorectal and lung cancers among atomic-bomb survivors, and he performed the subject selection for the pilot phase of their NIAID vaccination study. Mr. Misumi also provided statistical support in the drafting of a research proposal on the study of radiation effects on body composition with the Department of Clinical Studies.

Collaboration with outside organizations

Under the RERF-Kurume University-University of Washington partnership program, Dr. Hsu is collaborating with Dr. Tatsuyuki Kakuma at Kurume University to apply joint modeling in examining the causal association among radiation, inflammatory markers, and postoperative cataract incidence for AHS subjects. A model was adopted for adjusting the intermediate inflammation factor in a time-to-event (survival model) analysis. Preliminary results showed a significant causal pathway by which inflammation mediates the effect of radiation in producing cataracts. Further investigation is ongoing.

Dr. Daniel Stram (University of Southern California) and collaborators at RERF have prepared a research proposal for major new work on biodosimetry and dose uncertainty, including the simultaneous use of biological dosimetry data with physical (DS02) dose estimates, data on acute signs of radiation injury such as epilation, and development of cancer. A separate proposal under RERF review incorporates proposed research on instrumental variables methods for evaluating and correcting for dose error, by a group at the State University of New York at Buffalo led by Dr. Randy Carter. In addition, Dr. C.Y. Wang of the Fred Hutchinson Cancer Research Center, under the auspices of the RERF-Kurume University-University of Washington partnership, has an RP under review and has obtained a review expected to result in a funding award from the National Institutes of Health (NIH) for a functional approach to handling dose error that requires few assumptions about the error distributions. Collaboration continued with Dr. Donald Pierce on work related to simulation of dose errors, during his visit to RERF in 2009. Dr. Cullings and Dr. Cologne, along with Dr. Shimizu of the Department of Epidemiology, continued their collaboration with Dr. Takesaburo Mori of Yokohama City University on analysis of data from the Japanese Thorotrast patient cohort, with comparison to a demographically similar subset of the LSS, with a manuscript presently in preparation.

Dr. Cullings and Dr. Hsu have been working with external collaborators on two-stage clonal expansion (TSCE) modeling approaches. Dr. Hsu has been working with Drs. Fieke Dekkers and Harmen Bijwaard at the National

Institute for Public Health and Environment in the Netherlands, where the research protocol to study their carcinogenesis modeling approach using RERF leukemia data is underway. Dr. Cullings is working with Dr. Jan Christian Kaiser from the German Helmholtz Institute on proposed carcinogenesis models using solid tumor data and completed a second paper, on use of TSCE models for individual data on cancer incidence, with Dr. Wolfgang Heidenreich of the same institute, which was published in *Radiation Research*.

Dr. Cologne and Dr. Cullings have been working with Dr. Bryan Langholz of the University of Southern California in ongoing work on nested case-control study design, in particular considering issues of bias and efficiency in counter matching. One important aspect relates to the nested case-control study of breast and endometrial cancer, where a large number of cases were unusable, freeing up for potential use with other matched sets many control specimens that had already been molecularly analyzed.

Department of Information Technology

Hiroaki Katayama, Research Scientist and Department Chief, ITD

The Department of Information Technology (ITD) consists of the Systems Technology Section and the Library and Archives Section. ITD serves as a center of information for RERF's varied research activities, and is staffed by a total of 15 full-time employees and one half-time employee. There are three major areas of focus of ITD. The first area is the management of the computer environment in RERF, with such tasks as network construction, the maintenance and development of hardware and software, and providing user support. ITD also manages various databases such as the Life Span Study (LSS), Adult Health Study (AHS), and bio-sample databases, and provides tools so that researchers can easily choose appropriate databases by obtaining information about them and ABCC-RERF historical data. The second area is the management of information by the Library and Archives Section. This includes the arrangement and preservation of ABCC-RERF archives, the handling of RERF publications and reports, and the management of the RERF Library. The third area is providing technical assistance to RERF in its many national and international collaborations. One example is software and documentation for a standard system for regional cancer registries that we have developed and has been adopted by many prefectures in Japan.

Systems Technology Section

Database activities

During the past year, we have implemented a number of essential structural changes to primary databases, and have designed and developed several new database systems. Those research database changes included:

(a) Division of the database, in which we separated the main database into two smaller databases with specific purposes, namely the resource database and research database. By separating identifiable information from research results, we can keep information secure. The change of the structure of the database does not adversely affect the clinical activities, data acquisition, or the research activities.

(b) Establishment of improved data backup methods and protocols, in which research data are kept on the hard disk and copied onto another hard disk, and then stored nightly onto magnetic tapes. Two magnetic tapes are produced for the data, with one being kept in the fire-resistant safe at the Hiroshima Laboratory, while the other is forwarded to the Nagasaki Laboratory for temporary storage; after one year, the backup tape in the Nagasaki Laboratory is sent back to Hiroshima, and then one of the backup tapes is stored permanently in an underground, fire-resistant

safe in the main branch of Hiroshima Bank.

(c) Development and implementation of a Data Dictionary System for users of the database: Since a new researcher has no knowledge about the database of RERF and the history of its data, we developed the Data Dictionary System to enable researchers to have access to the appropriate databases; the system includes historical descriptions, which can be very important for analysis.

(d) Improvement of the AHS Clinical Database System and related applications: This includes work on a blood-storage management system, an X-ray image digital filing system, a graphical tool used in the clinical examination rooms, a master file database system, and the cancer registry database system. We also store many digital image data such as retinal/lens images on a backup storage system.

(e) Designing and development of accounting and personnel databases for managerial purposes and other needs identified by RERF's Secretariat.

(f) Improvement of the Tumor Registry Database System and related applications: This includes work on both Hiroshima Prefectural Cancer Registry and Hiroshima City Cancer Registry management system. The database system is now handling not only cancer registry data but tissue registry data.

Network security activities

As the Internet connection and Intranet are absolutely essential for daily, ongoing RERF activities, we have constructed the RERF network so that its services cannot be cut off unexpectedly. In case of an electric power outage, an auxiliary emergency generator is in place and will provide an immediate, and if necessary sustained, source of electrical power for the network.

We have also set up various systems to secure the RERF network. These include; (a) a firewall system to prevent illegal access from outside, (b) a SPAM-blocking system, (c) a virus identifying and checking system to prevent viruses from incoming emails, (d) a web access check system to monitor the use of the website, (e) a Windows automatic update system to fix problems in Microsoft's Windows software, (f) a network traffic surveillance system to check the network load balance, (g) an auto-lock system using an IC-card recognition system for access to areas with sensitive identifiable information, (h) an IP camera system to record entrance to the gateways and the Radioisotope Facility, (i) an encryption system which encrypts personal information and records user activities on their PCs to prevent the leakage of information, and (j) a mail archiving system which can restore all incoming and outgoing emails at RERF.

Major work effort of the Systems Technology Section over the past year

We set up the IC-card security lock system at the Master File Section and the Tumor and Tissue Registry Office in the Department of Epidemiology, and the Clinical Administration Section in the Department of Clinical Studies, in both Hiroshima and Nagasaki. The system records when and who enters or leaves the room.

Since the mail server became too old and the load balance really high, we upgraded the mail server to provide improved performance and capacity. The new web-based interface through HTTP allows users to check their emails from outside and the new mail system also allows users to check their Emails using their mobile phone.

We also improved the bio-sample database. The Departments of Clinical Studies, Genetics, and Radiobiology/Molecular Epidemiology have their own stored bio-samples. The databases for bio-samples had been developed separately and not been integrated. During this year, with members of the Committee on Biological Samples, we discussed what kinds of information are needed in the database. Finally, we integrated the separate bio-sample databases and can now show information from the various databases on a single screen.

Library and Archives Section

Library Unit

The Library Unit is staffed with two full-time employee and one half-time employee serving the library needs of the RERF research staff. As a cost-saving measure, RERF purchased foreign journals through the U.S. National Academy of Sciences since 2003. This purchasing arrangement proved difficult at times; therefore we changed

Development and management of databases by Department of Information Technology

Department	Database	Department	Database
All departments	Research Management Information System	Genetics	DNA Microarray Database System
	Archives Search System		PLC (permanent lymphocyte cell lines) Storage Database
Clinical Studies	AHS Database System (including FOCS)	Radiobiology/Molecular Epidemiology	CS (cell storage) Database
	X-ray Digital Filing System		
	Blood Sample Database System		
Epidemiology	Index Database	Secretariat	
	Master File Database	Personnel Section	Personal Information Database System
	Tumor & Tissue Registry Database		Payroll Database System
	Tumor & Tissue Registry Form Digital Filing System	Accounting Section	Accounting Database System
			Budget Management System
	Death Information Digital Filing System	Supply Section	Equipments Management System
			Purchasing Management System

back to purchasing journals directly from a subscription service in 2008. We also reexamined the decision regarding the purchase of hard copies compared to subscribing to journals online. Finally, we decided to subscribe to 47 international journals including both hard-copy and online subscriptions and to others only online. We can now access most radiation-related journals, and a variety of other scientific journals, online.

Archives Unit

RERF historical documents are important and require proper cataloging and storage. Despite the enormity of the job and limited funding and personnel, the Archives Unit is making an effort to sort, digitize, and electronically store as many documents and photographs as possible. Because of personnel limitations, new approaches to this problem are being considered, including partnering with major historical/archival organizations.

Major work of the Library and Archives Section over the past year

Remarkable progress has been made in the classification of archival materials. The staff carefully checks each document, reads its contents and selects relevant codes for future search functions, then puts these codes into the database with other information. Finally, they scan the document and store the original document in a special container in the archives storage room. The project, started in August 2007, has so far scanned about 18,000 photos and 5,000 other documents. We have made an application program which will allow RERF users to view these archives from their PCs.

International and Domestic Collaborations

In the past, ITD's work was limited solely to RERF, but more recently, the Library and Archives Section has responded to many inquiries about RERF's scientific publications from both Japan and abroad, and has received increased requests for official documents and photographs from the past.

The Systems Technology Section has been involved in the third "Ten-Year Strategy for Cancer Control" project conducted by the Ministry of Health, Labour and Welfare. For this project, we have constructed a nationwide standardized model for a local tumor registry database system and are introducing it throughout Japan. It has now been adopted by 18 prefectures and 6 prefectures will introduce this system during 2010. In addition, as a collaborative study with the Hiroshima University Research Institute for Radiation Biology and Medicine, we have constructed a database in Semipalatinsk, Republic of Kazakhstan, to facilitate research on the effects of low-dose radiation exposure caused by nuclear tests conducted there by the former USSR over a period of many years. The latter example reflects ITD's active support to the Japanese government and various academic institutions nationwide; we also try to honor the requests of foreign institutions and their researchers when those requests are approved through proper channels at RERF.