Research Departments

Departments of Clinical Studies, Hiroshima and Nagasaki

The Department of Clinical Studies conducts health-examination programs of the Adult Health Study (AHS) and the F_1 (children of the atomic-bomb survivors) Clinical Study that provide the opportunity for a number of specific investigations to be conducted into a variety of health outcomes.

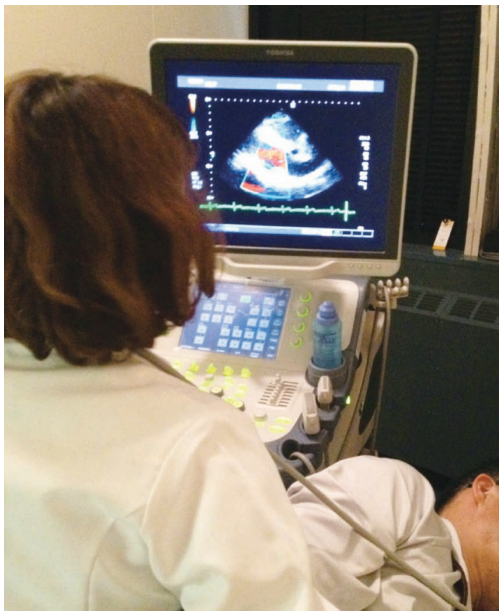
The AHS biennial health examinations were initiated in 1958 and continue today. These health examinations represent the only point of regular direct contact with the survivors and provide health benefits to that population through early disease detection. Such examinations function as the principal source of biological materials that make possible a wide variety of valuable studies by numerous RERF departments and outside investigators. Sera and blood cells have been collected from the AHS participants and stored since 1969 and 1990, respectively. The AHS program has greatly contributed to RERF's mission of 1) assessing noncancer disease risk from radiation, 2) determining radiation effects on physiological or biochemical abnormalities and correlating this information with other life experiences and modes and patterns of disease, and 3) epidemiologically elucidating mechanisms of radiation effects on cancer and noncancer diseases using stored biosamples and clinical, physiological, and epidemiological information that were obtained through the health examinations.

The AHS continually increases in importance as a result of the accumulation of an immense body of data from the total of 27 rounds of health examinations carried out to date. The data have provided the strongest available evidence of radiation dose-related increases in morbidity from noncancer disease, such as cardiovascular disease (CVD), stroke, hyperparathyroidism, thyroid disease, uterine myoma, chronic liver disease, and cataract, plus subclinical risk indicators and conditions such as circulatory inflammation.

More than a decade ago the Department of Clinical Studies began the program of F_1 Clinical Study examinations, which were conducted for about 12,000 individuals to analyze the potential heritable effect(s) of A-bomb exposure on polygenic, multifactorial diseases (e.g., diabetes, essential hypertension, coronary heart disease, and stroke) from 2002 to 2006. However, owing to the young age of the F_1 group (mean age of about 49 years at that time), most of their disease experience is still ahead, so we converted the sample to a cohort for prospective follow-up and are now partway through a second round of examinations.

Research Scientists of Hiroshima Clinical Studies (First row from left) Michiko Yamada, Waka Ohishi, Yoshimi Tatsukawa, (Second row from left) Ikuno Takahashi, Keiko Ueda, Eiji Katsurada

Research Scientists of Nagasaki Clinical Studies (From left) Misa Imaizumi, Masazumi Akahoshi, Ayumi Hida, Nobuko Sera



Echocardiography is one of the most commonly used non-invasive examinations, after the electrocardiogram, for the diagnosis and assessment of heart diseases. It provides information about cardiac size and morphology by processing data collected through ultrasound waves. This modality also makes it possible to quantify intracardiac blood flow velocity, pressure gradient, and movement of myocardial tissue.

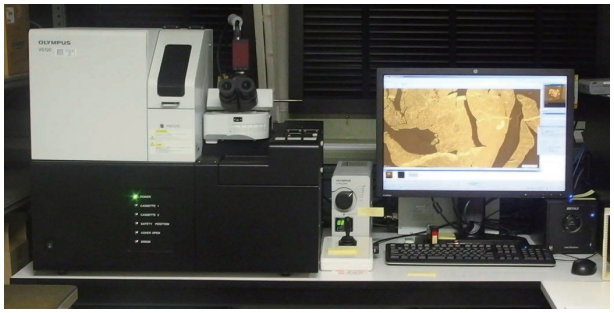
Department of Genetics

The Department of Genetics is divided into two laboratories: the Cytogenetics Laboratory and the Biochemical Genetics Laboratory. The main thrust of the Cytogenetics Laboratory is somatic mutations and biodosimetry, while the Biochemical Genetics Laboratory focuses on heritable mutations in the F_1 generation.

In the Cytogenetics Laboratory, the frequency of stable-type chromosome aberrations (translocations) was examined previously by the solid Giemsa method and is currently being studied using fluorescence *in situ* hybridization (FISH). The results of this study showed that a wide scatter of individual translocation frequencies against physical dose was observed by FISH, as was seen in the previous Giemsa study. Another method of biodosimetry being utilized is electron spin resonance (ESR) using tooth enamel. We now know that both FISH and ESR data fit reasonably well to each other. We anticipate that such biodosimetric data will provide information on possible random and systematic dose uncertainties in individual doses calculated by DS02 and prove to be valuable for use in cancer risk estimation. Currently, the laboratory has expanded its scope of research and is also studying genetic factors in breast and skin cancers, conducting a cytogenetic study on *in utero* exposed mouse thyroid cells, developing a green fluorescent protein (GFP) mouse model for quantitative measurement of germ-cell mutations, and finding markers for unrepairable DNA radiation damage.

The Biochemical Genetics Laboratory has been collecting blood samples and establishing Epstein-Barr virus (EBV)-transformed cell lines from members of survivor families (mother, father, and offspring) for molecular studies. We also are developing competence in more advanced DNA-based assays. Several studies were conducted that screened mutations at hyper-variable mini- and micro-satellite loci—about 1,000 loci per person by two-dimensional gel electrophoresis and 2,500 loci per person by BAC (bacterial artificial chromosome) arrays. None of those studies indicated statistically significant genetic effects of parental exposure to radiation. Recently, high-density comparative genomic hybridization (CGH) microarray methods using over one million probes have been introduced to detect relatively large deletion/amplification mutations throughout the genome. This method is currently being used to estimate the transgenerational effects of radiation in the F_1 offspring of A-bomb survivors, with the offspring of irradiated male mice and female rats being used as experimental models of human exposure. We are also examining the possibility of conducting whole genome sequencing-based genetic studies using next-generation sequencer technology.

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



Olympus Virtual Slide VS120 system. This device is equipped in the laboratory for fully-automated scanning and digitalization of histopathological samples. It enables the scanning and analysis of 100 glass-slides, totaling up to 300 tissue slices, in a one-batch process, and now is utilized for the detection of radiation-induced somatic and germ cell mutations as green-fluorescent cells in a model mouse system newly developed at RERF.

Department of Radiobiology/Molecular Epidemiology

The Department of Radiobiology/Molecular Epidemiology focuses on two research themes: immunology and oncology. This department seeks to elucidate the mechanisms underlying the radiation-associated increases of various common cancers and other diseases. Immunologic effects are investigated with two approaches: immunobiology and immunogenome studies, which primarily examine immunological profiles and inter-individual genetic variations among A-bomb survivors, respectively. Oncology studies analyze early molecular events of radiation-associated cancers, seeking to find carcinogenic pathways that preferentially occur in these cancers.

The immunobiology studies hypothesize that radiation accelerates immune attenuation, partially resulting in enhanced risks of chronic diseases among A-bomb survivors. To understand the mechanisms of radiation-induced immune dysfunction, we are investigating radiation-related alterations of immune cells (such as lymphocytes, hematopoietic stem cells [HSC], and dendritic cells [DC]) and organs (such as the thymus) that are potentially involved. The effects of radiation on the influenza vaccine response are being evaluated as a direct health-related outcome. We also are developing an integrated scoring system for evaluating the immunological/inflammatory status of A-bomb survivors to provide estimates of the role of immunocompetence in radiation-disease associations.

The immunogenome studies evaluate the genetic basis for inter-individual differences in immune function and the impact of genetics on susceptibility to radiation-associated diseases. The results obtained from phenotype-genotype association analyses utilizing cumulative immunology data will have the potential to contribute to the individualized prevention of radiation-associated diseases in A-bomb survivors and other exposed populations.

The oncology studies aim to clarify mechanistic relationships between radiation exposure and cancer development among A-bomb survivors. Toward this end, we have been analyzing early molecular events in the development of thyroid, colorectal, and lung cancers in the Life Span Study (LSS) cohort. We also are beginning to evaluate epigenetic alterations (i.e., modification of genetic function by means other than through changing the DNA) in normal blood cell subsets from Adult Health Study (AHS) subjects, based on the hypothesis that radiation might cause epigenetic changes that lead to increased risks of selected diseases.

Research Scientists of Radiobiology/Molecular Epidemiology (First row from left) Kei Nakachi (RERF Consultant), Evan B. Douple (Associate Chief of Research), Yoichiro Kusunoki, Tomonori Hayashi, Kazue Imai, (Second row from left) Junko Kajimura, Reiko Ito, Kengo Yoshida, Seishi Kyoizumi (NIAID Project Research Scientist), Kiyohiro Hamatani, Masataka Taga, (Photo added later; at top from left) Norio Takahashi (Consultant, Vice Chairman's Office), Yasuharu Niwa

Pyrosequencer. Analysis of the methylation status of CpG sites through use of this apparatus is known as pyrosequencing. This method enables the quantitative analysis of methylation levels of multiple CpG sites, within the range of about 30 base pairs, from the primer-binding site.

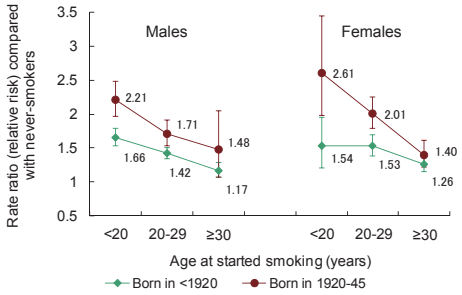
Department of Epidemiology, Hiroshima and Nagasaki

RERF aims to characterize and quantify the late health effects of radiation, based on data of the atomic-bomb survivors. The Department of Epidemiology's follow-up of the Life Span Study (LSS) of survivors and the *in utero* and F_1 cohorts is crucial to accomplish these purposes. Follow-up outcomes include deaths and causes of death wherever they may occur in Japan, and cancer incidence in Hiroshima and Nagasaki prefectures, where a large percentage of the survivors still dwell. Histological specimens of cancer cases are also collected by tissue registries in collaboration with community pathologists. Around 36% of the LSS cohort members were still alive at the end of 2008, including 84% of those who were less than 10 years old at the time of bombing (ATB). Moreover, 88% of the *in utero* and 90% of the F_1 cohorts are still alive. Therefore, continued follow-up of these young age groups for an additional 20 years or more is clearly essential. It is important to establish consistency between epidemiological evidence and biological mechanisms of radiation effects. It

Research Scientists of Hiroshima Epidemiology (First row from left) Ritsu Sakata, Kotaro Ozasa, Eric J. Grant, Yukiko Shimizu (Part-time Professional), (Second row from left) Atsuko Sadakane, Hiromi Sugiyama, Ikuno Takahashi (concurrent)

Research Scientists of Nagasaki Epidemiology (From left) Kotaro Ozasa (concurrent), Midori Soda

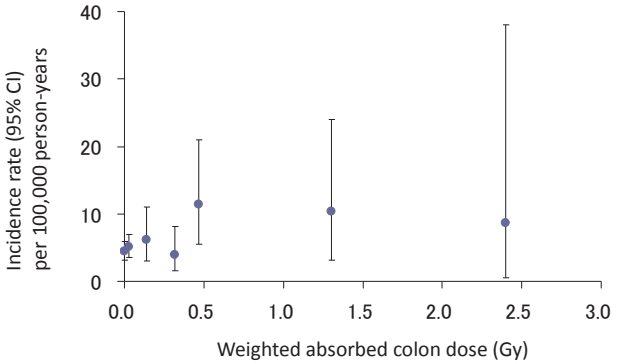
Relative risk of all-cause death among current smokers compared with never smokers by sex, year of birth, and age at starting smoking



Smokers born in 1920–45 had a higher risk of smoking for all-cause death compared with smokers born in 1920 or earlier for any age at starting smoking in both sexes. The difference was larger in smokers who started smoking at a young age. The risk decreased with increasing age at started smoking in both sexes and both birth cohorts.

(Sakata R, Grant EJ, et al., *BMJ* 2012; 345:e7093)

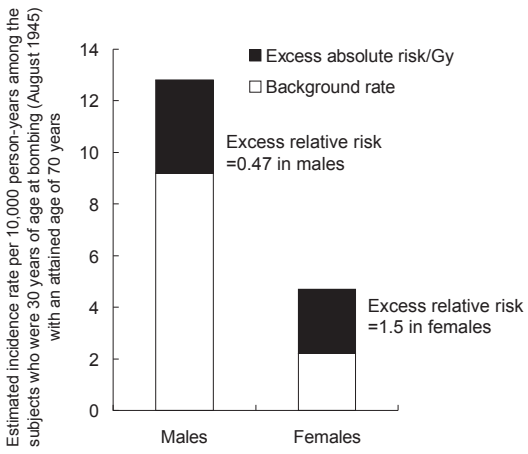
Soft-tissue sarcoma rates by colon dose adjusted for age and birth-year



Soft-tissue sarcoma incidence rates by weighted absorbed colon dose (Gy), which is the sum of the gamma radiation dose plus 10 times the neutron dose. The figure shows the incidence rates and 95% CIs for the person-year weighted mean values within dose categories (<0.005, 0.005–0.1, 0.1–0.2, 0.2–0.5, 0.5–1, 1–2, and >2).

(Samartzis D et al., *J Bone Joint Surg Am* 2013; 95:222–9)

Excess relative risk and excess absolute risk of urothelial carcinoma incidence (1958–2001)



(Grant EJ et al., Radiat Res 2012; 178:86–98)

is also essential to epidemiologically evaluate other risk factors for confounding or modification of radiation risks, and to more precisely determine the magnitude of risk for radiosensitive subgroups such as those who were in early childhood or *in utero* at the time of exposure. International risk assessment groups use the results from these cohorts as the primary basis for radiation-risk estimation. The LSS mortality and incidence data have been periodically analyzed, and those results were heavily relied upon for the creation of numerous radiation-risk reports, including by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (2006), the International Commission on Radiological Protection (ICRP) (2007), and the National Academy of Sciences Committee on the Biological Effects of Ionizing Radiation (BEIR) VII (2005).

High-quality cancer-incidence data are essential to evaluate the radiation risks for cancers occurring in the above cohorts, given that only a fraction of cancers are lethal. For this, RERF has vigorously managed the Hiroshima and Nagasaki tumor/tissue registries. The data from these registries have long been included in the "Cancer Incidence in Five Continents (CI5)" (by the International Agency for Research on Cancer [IARC]/International Association of Cancer Registries [IACR]), a compilation of worldwide cancer incidence data, and are given the highest rating by that consortium. The data for 2003–2007 were used in the latest 10th edition (CI5-10). The data on childhood cancer in 1990–2007 are scheduled to be published in the "International Incidence of Childhood Cancer, Volume 3 (IICC-3)" (by IARC/IACR). A preservation program of survivors' tissues resected during surgery is under construction in collaboration with hospitals in Hiroshima and Nagasaki. These materials, which are utilized by all RERF departments, are important for investigations of cancer pathogenesis associated with radiation exposure.

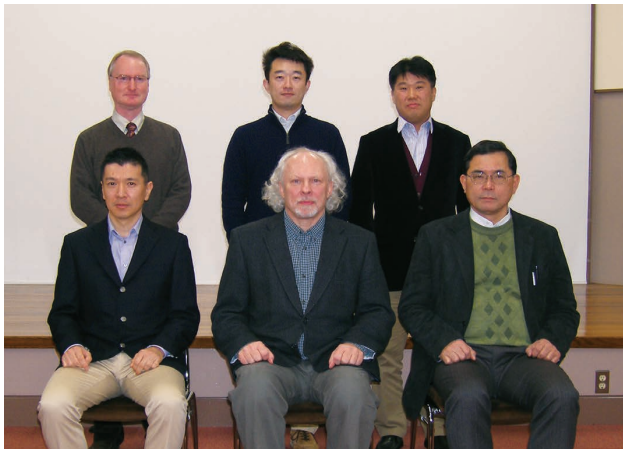
Department of Statistics

The Department of Statistics has historically led in the development of analytical methods for major aspects of the RERF research program of risk estimation. This includes the development of tools and methods to handle various problem areas in the longitudinal data of major RERF cohorts, such as dose uncertainty and undocumented out-migration from cancer tumor registry catchment areas, among many other examples.

We have evaluated or developed special methods of sub-cohort sampling to increase efficiency and address special issues of effect-modifiers or -mediators. We also develop many special methods to meet the requirements of RERF's basic-science research in genetics, immunology, radiation biology, and molecular epidemiology. Finally, the Department of Statistics implements RERF dosimetry systems and maintains a database of survivor dose estimates, presently using the DS02 system provided by a combined external and internal scientific working group, and provides key statistical and dosimetric support to RERF projects in biodosimetry.

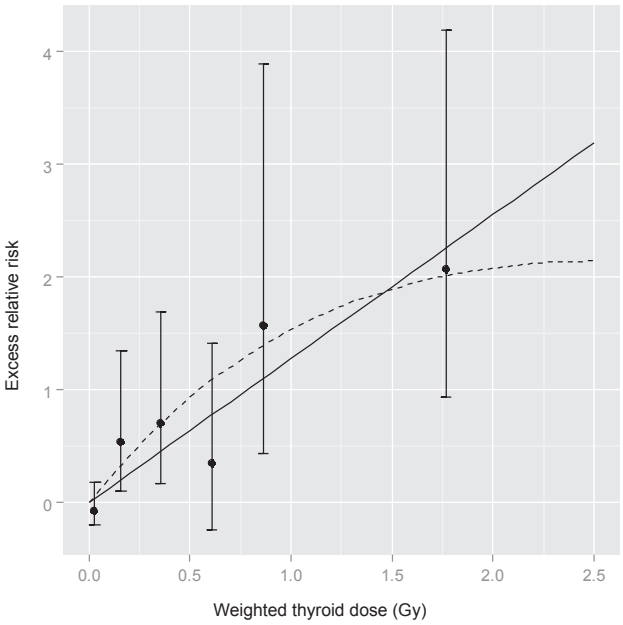
Unlike other departments, we engage in both consulting and research. Given the crucial importance of sound statistical advice in the design, analysis, and conduct of studies, the Department of Statistics seeks to adopt a proactive approach to consulting, beginning with early involvement in study design, and devotes a majority of its staff's efforts to this consulting role.

Work in dosimetry, including investigation into dose uncertainty, is by definition central to the RERF mission of evaluating the effects of ionizing radiation on human health, as such efforts provide denominator information for measures of dose response. The Department of Statistics does not undertake dosimetry-related research for its own purposes unless it stands to measurably improve the RERF dosimetry. In other areas, regarding both consulting and research, we provide information on study designs and the statistical power of potential studies. This is critical information in evaluating how effectively a given project will



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

be able to address its proposed scientific question, which in turn impacts its value to the RERF mission.



Fitted dose response functions for thyroid cancer incidence in the LSS cohort. The solid line is the fitted linear excess relative risk (ERR) dose response, and the dashed curve is the fitted ERR based on linear-exponential dose response model. The points are non-parametric estimates of the ERR in dose categories with 95% CIs. The line and points are all gender-averaged estimates at age 60 after exposure at age 10.

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, both of which are engaged in support activities for RERF's research.

The Systems Technology Section engages in the maintenance of network and hardware environments, including personal computers, the construction of a variety of databases for analysis (epidemiological research database, resource management database, Adult Health Study [AHS] database, etc.), and the development of relevant application software. The databases are managed in a technologically advanced manner with the aim of allowing RERF's researchers to effectively and rapidly access essential archives for research and administrative purposes. With the aim of helping research scientists understand those databases with their complex structure, the section has also been involved in such research-support activities as the creation of a data dictionary and a document-management database. Among the section's recent focuses are the prevention of illegal attacks on the RERF network and infections with computer viruses, and the addition of new functions for personal-information management at RERF in line with Japan's Personal Information Protection Law.

The Library and Archives Section is composed of the Library Unit and the Archives Unit, with the former handling the procedures for purchase and storage of scientific journals, management and preservation of books, and handling requests from RERF's research scientists for copies of papers. The demand for such services continues to grow rapidly. The Archives Unit is responsible for the storage, indexing, and digitizing of historical archival material, the processing of associated invoices, and the distribution of RERF publications. Although the number of orders for ABCC-RERF historical materials from outside RERF is on the rise, only two employees (one permanent staff and one temporary staff) are dealing with the entirety of the library and archives work. This shortage of personnel represents a major concern in terms of conducting the archival work.

ITD participates in a variety of collaborative projects with outside research organizations. For example, the department is providing technical expertise to the Osaka Medical Center for Cancer and Cardiovascular Diseases in constructing a comprehensive cancer control system in Osaka prefecture. Among ITD's research and collaborative activities are: participation in the World Health Organization (WHO) Radiation Emergency Medical Preparedness and Network (REMPAN); cooperative technical expertise for Hiroshima University as one of western Japan's tertiary

medical institutes for the radiation exposed; creation of an epidemiological research database devoted to the Ministry of Education, Culture, Sports, Science and Technology (MEXT) Grant-in-Aid for Scientific Research project on low-dose radiation effects from nuclear tests conducted in Semipalatinsk, Republic of Kazakhstan by the former Soviet Union; and participation as a member of the external advisory board in the SEMI-NUC project (feasibility assessment for a prospective cohort study of residents near the Semipalatinsk nuclear test site) conducted by the International Agency for Research on Cancer (IARC).