

■ **FY2014 Highlights in Research Progress**

■ **Clinical Studies**

Thyroid nodules among atomic bomb survivors exposed in childhood

Few studies have evaluated the association of thyroid nodules with radiation dose among adults exposed to radiation in childhood. In this study, between October 2007 and October 2011, we performed thyroid examinations in 3,087 of the Adult Health Study (AHS) participants who were younger than 10 years at exposure and evaluated radiation dose responses on the prevalence of thyroid nodules. Data from 2,668 participants with known atomic bomb thyroid radiation doses (mean age, 68.2 years; 1,213 men and 1,455 women; mean dose, 0.182 Gy; median dose, 0.018 Gy) were analyzed.

The prevalence of all thyroid nodules (≥ 10 mm diameter, 17.6%), solid nodules (16.0%), malignant tumors (1.8%), benign nodules (7.0%), and cysts (1.8%) were significantly associated with thyroid radiation dose. Dose effects for the prevalence of all nodules and solid nodules were significantly higher with earlier childhood exposure, while no interactions with dose were seen for sex, family history of thyroid disease, antithyroid antibodies, or seaweed intake. No dose-response relationships were observed for small (< 10 mm diameter) thyroid nodules. In conclusion, radiation effects on the prevalence of thyroid nodules exist in atomic bomb survivors 62 to 66 years after their exposure in childhood. However, radiation exposure is not associated with small thyroid nodules.

Exposure to atomic bomb radiation and age-related macular degeneration in later life

As they age, atomic bomb survivors may be at increased risk of age-related macular degeneration (AMD), but the relationship between AMD and radiation exposure has remained unclear. We therefore investigated the association between AMD prevalence and radiation exposure using logistic regression model with adjustment for relevant factors (e.g. age, sex, city, smoking habits, body mass index, diabetes, hypertension, hyperlipidemia, and white blood cell count), among 1,824 subjects who participated in Adult Health Study (AHS) ophthalmological examinations during 2006–2008 and were diagnosed as early/late AMD. The prevalence of early AMD was 10.5%, and that of late AMD was 0.3%, with relevant factors-adjusted odds ratio (95% confidence interval) per 1 Gy being 0.93 (0.75–1.15) for early AMD and 0.79 (0.21–2.94) for late AMD, respectively. The prevalence of small drusen deposits (diameter < 125 μm), one important manifestation of AMD, decreased with dose but was not statistically significant. In this study, no association was observed between AMD prevalence and radiation exposure. The issue of statistical power, however, cannot be denied completely because this was the first study based on fundus images, and very few participants had late AMD. Further follow-up on AMD development and progress will be needed.

■ **Genetics**

Radiation-induced somatic cell mutations identified using whole genome sequencing of clones derived from a human B cell line

To characterize and quantify somatic cell mutations induced by X-irradiation, we conducted whole genome sequencing of DNA obtained from clones established from a human B lymphocyte cell line following graded doses of X-rays (0–4 Gy). For these pilot studies we analyzed two clones from the 0 Gy control group, two from the 2 Gy group, and three from the 4 Gy group. Whole genome sequencing of DNA from these seven clones and DNA from the original cell line was carried out by the 90 bp paired-end method using an Illumina HiSeq 2000. After obtaining 90 Gb of raw sequence data per sample, we analyzed the data in collaboration with Dr. Akihiro Fujimoto (Riken). After the verification, the number of true point mutations and indels (small insertion and deletion less than 5 bp) in the controls was estimated to be 100–150 and 4–8, respectively. The frequency of such mutations increased as a function of dose. Inversion and translocation type mutations were also identified only in the irradiated groups.

Radiation dose received in distant past can be estimated from cytogenetic data of blood lymphocytes

Chromosome aberration frequencies in blood lymphocytes have long been used for estimation of individual radiation doses received, although the approach cannot be used for dose calculation in A-bomb survivors. This is because the aberration yield depends on photon energy while A-bomb photon energy was distributed widely, making simulation experiments difficult to conduct. Further, since too many years had passed since exposure, immature lymphoid cells at the time of irradiation underwent differentiation and entered the lymphocyte pool, but the calibration curve for dose estimation is for mature lymphocytes and cannot be applied. In the present study, we measured both tooth doses (by means of electron spin resonance: ESR, which is nearly photon-energy independent) and cytogenetic doses on the same 100 survivors to obtain an empirical interrelationship. It was found that ESR doses agreed closely with cytogenetic doses under the assumptions that all the lymphocytes were exposed to mono-energetic cobalt-60 gamma rays and were mature at the time of radiation exposure. The results show that cytogenetic data in blood lymphocytes can now be used to estimate radiation dose exposure in the distant past without introduction of specific correction factors.

■ **Epidemiology**

Radiation health risk among atomic bomb survivors

The association of atomic bomb radiation and skin cancer was published based on the results of a pathological review of cases that occurred among members of the Life Span Study (LSS). With regard to basal cell cancer, the best description of the data was a linear-threshold model with a threshold dose of 0.6 Gy. The risk was estimated to increase by 74% for those exposed to 1 Gy of radiation at age of 30 compared to those non-exposed. The risk was estimated to increase by 11% with each one-year decrease in age at exposure. On the other hand, attained age did not significantly affect radiation risks.

Using a population of individuals responding to surveys from 1949 to 1961, we compared mortality and cancer incidence between those reporting rain exposure shortly after the atomic bombing and those reporting no such rain exposure. No increase in mortality or cancer incidence was observed in those reporting rain exposure.

In the area of dosimetry, Epidemiology Department members have contributed to revised individual dosimetry estimates based on a review of original questionnaire data and locating survivors' locations at the time of bombing using modern geographical information systems mapping software. The new estimates will be used in future manuscripts.

Radiobiology/Molecular Epidemiology

Age and radiation effects on the production of reactive oxygen species in blood cells of atomic bomb survivors

Reactive oxygen species (ROS) play an important role in cell-mediated immune responses, although overproduction and excessive accumulation of ROS may cause enhanced risks of inflammation-related diseases such as selected cancers and coronary heart disease. In this study, we measured ROS (H_2O_2 and O_2^-) levels in the blood cells of 2,789 atomic bomb survivors. Intracellular ROS (O_2^-) levels in lymphocytes and granulocytes increased with age and radiation dose. Specifically, O_2^- levels in memory CD8 T cells increased with age and radiation dose. These results suggest that ROS levels, specifically O_2^- levels in specific blood cells, are affected by aging and radiation exposure.

Induction of *ALK* rearrangements in human thyroid epithelial cells by *in vitro* X-ray irradiation

EML4-ALK fusion genes have been frequently observed in exposed papillary thyroid cancer cases showing solid/trabecular architectures in the cancerous regions among atomic bomb survivors. To clarify whether radiation exposure induces *ALK* rearrangements in human thyroid epithelial cells, we conducted X-ray irradiation experiments using an immortalized human thyroid epithelial cell line (Nthy-ori 3) and investigated the occurrence of *EML4-ALK* fusion genes in the irradiated cells. As a result, X-ray irradiation experiments with single doses of 0, 0.2, 1, or 5 Gy indicated that *EML4-ALK* fusion events in the Nthy-ori 3 cells increased with increased dose.

Statistics

Radiation risk assessment and dosimetry

Members of the Statistics Department published several first-author papers, including a paper on accounting for neutron exposure in studies of the atomic bomb survivors, a paper on handling missing smoking history data in survival analysis, a paper on spline methods in risk regression for the atomic bomb survivors, and a review paper on the impact of radiation on the health of the survivors, the last of which was based on a presentation to the U.S. National Council on Radiation Protection and Measurements. A member of the Statistics Department was a co-author on a statistical paper together with a student of an RERF Expert Advisor who spent time at RERF to do collaborative research using RERF data, and two members of the department were co-authors on a commentary from the National Academy of Sciences (NAS) on a critical review of the NAS BEIR VII Report and use of the linear no-threshold (LNT) hypothesis in radiation risk assessment. Members of the Statistics Department continued collaboration with external investigators in several areas related to new radiation risk models and related statistical methods, including multi-model inference for circulatory disease outcomes, a semi-parametric statistical method to correct for the effects of random errors in dose estimates on risk regressions, and implementation of a new version of a program already in use at RERF to adjust dose estimates for the effect of random errors. They also collaborated on research related to causal models, such as the mediating and moderating effects of serum sex hormones on radiation risk of breast cancer.

Members of the Statistics Department continued intramural work and extramural collaboration in dosimetry, including preparation of a major new manuscript on RERF's efforts at improvement of survivor location estimates and terrain-shielding calculations, preparation of a statistical manuscript on the "simulation-extrapolation" (SIMEX) method for evaluating the effect of random errors in dose on risk regressions, and contribution by two members of the department to a new paper by a member of the Epidemiology Department on the long-term effects in the atomic bomb survivors of reported exposure to "fallout rain." Members of the Statistics Department also continued to support the Genetics Department's analyses of ESR measurements in donated teeth, and organized a small international meeting jointly with the Epidemiology Department on dosimetry issues for RERF cohorts.

Statistical methodology for other RERF studies

Members of the Statistics Department consulted at the inception of numerous studies, and contributed to the extensive new analyses of cancer incidence under a contract with the U.S. National Cancer Institute, as well as several analyses of immunological data in the final phase of the U.S. National Institute of Allergy and Infectious Diseases (NIAID) contract, and a member of the Statistics Department prepared a statistical manuscript on power calculations for nested case-control studies relevant to many studies at RERF. The Statistics Department provided analytical support for a large number of RERF studies during the year, including at least 15 published or submitted manuscripts and numerous presentations at scientific meetings.

Information Technology

To cope with the termination of support for Windows XP, the Information Technology Department replaced XP on staff PCs with Windows 7 in an orderly manner. The department also moved forward with the virtualization of servers, continuing work from the previous year. As a result, 17 virtual server hosts and 56 virtual machines are currently in operation. With regard to core systems, the SAS system was updated to the latest version. Mail server software was updated, and the mailing list system was replaced with a multifunctional version.

As a security measure, an asset-management server was newly introduced and is currently in operation. The asset-management server has enabled further detailed usage monitoring of individual PCs and enhanced security of the RERF network. Furthermore, the existing security camera system was replaced with high-definition equipment, and another four security cameras were added. A total of nine security cameras are currently monitoring the facilities, including the parking lots.

The Library and Archives Section is working on retrospective data entry of its archived materials, and completion of this project is in sight. It has also endeavored to improve staff services, including extension of the collection of electronic materials beyond electronic journals. Furthermore, the Library and Archives Section has made great progress in computerizing materials in the custody of the Archives Office; digitization will keep this material from deteriorating and simplify access. Work has been completed for photographs and newspaper/magazine articles and is currently underway for documents. On October 10, 2014, the Historical Material Management Committee hosted the Third ABCC-RERF History Forum at the Hiroshima Laboratory and posted a report on the external website.

■ Research Progress by Project

■ Cardiovascular Disease Study

The question of whether A-bomb radiation exposure causes cardiovascular diseases (CVD) has not yet been resolved, continuing to be one of the critical themes in the field of radiation health effects research.

To address this issue comprehensively, based on research in the areas of epidemiology as well as clinical and basic medicine, RERF established the Cardiovascular Disease Study Working Group, consisting of the RERF vice chairman, chief scientist, department chiefs, and research scientists. The group started working as a 'project team' in 2008. Thus far, the team has: (1) summarized the results of all the studies on CVD conducted at RERF; (2) streamlined specific research hypotheses to be verified; and (3) considered new studies to be conducted in the future, such as a study on arteriosclerosis and animal experiments. Based on this work, the team has planned and conducted new research, including studies of stroke, chronic kidney disease, and arteriosclerosis indices, as well as biomarkers related to these diseases and immunological functions, as part of the Adult Health Study (AHS). Detailed additional analyses are also being undertaken in the Life Span Study (LSS), and animal experiments using spontaneous hypertensive rats are being conducted.

In FY2014, we completed data collection for the aforementioned AHS study of arteriosclerosis indices and disease-related biomarkers. We also embarked on a study using echocardiography as part of AHS. In addition, a new research protocol on atrial fibrillation among the AHS population has been approved for implementation.

■ Collaborating Immunology Study

To understand mechanisms underlying radiation-induced immunosenescence and this phenomenon's implications in the various diseases experienced by A-bomb survivors, RERF researchers started in 2009 a five-year collaborative study with four Japanese and five U.S. institutions, based on funding provided by the U.S. National Institute of Allergy and Infectious Diseases (NIAID); now, this study is being continued with a one-year extension, until September 2015. In the study, RERF researchers focus on five projects that analyze radiation and aging effects on 1) hematopoietic stem cells, 2) dendritic cells, 3) immune responsiveness to influenza vaccination, 4) development of an integrated scoring system of immunity, and 5) thymus architecture and function. Measurements of immune parameters using biosamples donated by A-bomb survivors are nearly completed, with cooperation from AHS participants and their attending physicians, medical associations, and the RERF Departments of Radiobiology/Molecular Epidemiology, Clinical Studies, Information Technology, Epidemiology, and Statistics, and data analyses and manuscript preparations are in progress.

■ Collaborative Cancer Studies

RERF-National Cancer Institute collaborative cancer pathology studies

A five-year research contract with the U.S. National Cancer Institute (NCI) was renewed in 2014. The contract includes comprehensive cancer incidence analyses, analyses of site-specific cancers based on standardized reviews by pathology panels, and optional studies of genome sequencing of selected cancers. Currently, a comprehensive analysis of cancer incidence for 1958–2009 is being conducted. In the pathological review-based studies, analyses of malignant lymphoma cases that occurred between 1950 and 1995 and bone and soft-tissue tumors for cases between 1958 and 2003 are being conducted. Histological review and classification of "intrinsic subtypes" of breast cancer based on intracellular hormone receptors are continuing for the cases that occurred between 1958 and 2005.

Effects of *IL6R* genotype on colorectal cancer risk among atomic bomb survivors

This study investigated the association between radiation exposure and colorectal cancer (CRC) risk based on *IL6R* genotypes in the immunogenome (IMG) study cohort (a total of 4,673 individuals, including 222 CRC cases). As a result, when the IMG cohort was divided into two groups by *IL6R* genotype and three groups by radiation dose (non-exposed, middle-dose exposed, and high-dose exposed), the highest relative risk of CRC was observed for a combination of the highest exposure dose with certain *IL6R* genotype, as compared to the control group (non-exposed individuals) with other *IL6R* genotypes. These results suggest the possibility that *IL6R* genotypes may be associated with individual variance in radiation-related CRC risk among A-bomb survivors.

■ F1 Clinical Study

A clinical study of the F₁ offspring of A-bomb survivors was conducted from 2002 through 2006 to examine the effects of parental radiation exposure on prevalence of adult-onset multifactorial diseases among F₁ subjects. In this prevalence study (first round of health examinations), the results of that study's combined or individual analysis of multifactorial diseases in children showed no evidence of increased risk related to parental radiation exposure. In that study, however, the average age of the F₁ subjects who underwent health examinations was young, at around 49 years, just at the beginning of the age range in which diseases frequently occur. At the same time, there tended to be a bias related to decision-making among the participants concerning whether or not to undergo health examinations. Thus, the need for continuation of the study was recommended, and a longitudinal study for the nearly 12,000 subjects was initiated on November 24, 2010.

About 10,200 subjects have taken part in the longitudinal study during the four years since its inception (i.e., the second examination cycle). With a participation rate of 77.2%, the study has come close to achieving its initial goal of 80% participation. The study has now moved into its third examination cycle and is progressing smoothly. Preliminary tabulations have been conducted on the prevalence and incidence of multifactorial diseases among participants during the first three years of the four-year second cycle, and these data will be used to determine a plan for any future analysis.