

Active Research Protocols by Study Program

1 April 2013–31 March 2014

The 108 research protocols (RPs) including smaller-scale Type A protocols that were active during the fiscal year are listed below by study program with brief progress reports prepared by primary investigating departments, and listings of publications and oral presentations emanating from related studies follow. In addition, the title and researcher names for any smaller-scale, preliminary or pilot Type B and Type P protocols are listed at the end of each section of the Active Research Protocols by Study Program.

Investigating departments are identified by the following codes:

Clinical Studies, Hiroshima (CH)
 Clinical Studies, Nagasaki (CN)
 Epidemiology, Hiroshima (EH)
 Epidemiology, Nagasaki (EN)
 Genetics (G)
 Radiobiology/Molecular Epidemiology (R)
 Statistics (S)
 Information Technology (IT)
 RERF Director (D)
 RERF Associate Chief of Research (ACR)
 RERF Chief Scientist (CS)

Outside researchers are not listed with their affiliations here.

The following marks are used for publications and oral presentations based on RPs:

- ◆ Publications
- ⌘ Manuscripts in Press
- ❖ Oral Presentations

Protocols are presented by study program in reverse chronological order, and entries include the protocol title, investigators, and a brief description of the RP.

Each study program is followed by a listing of any publications that emanated from these studies as well as manuscripts accepted for publication during the fiscal year. These are presented in alphabetical order by first author. (Major publications are listed with an “Abstract” or “Summary Explanation.”)

Most of the outside authors are RERF consultants, expert advisors, or part-time professionals, and their listings with affiliations appear in a separate section of the annual report.

(Japanese) indicates that the original publication is in Japanese.

Oral presentations are included by study program after publications and manuscripts in press, and listed chronologically by meeting date.

Research Protocols 2-08, 1-75 (Platform Protocol), 2-61, A2-13, A1-13, A2-11, A1-11, A2-10, A1-09, A1-08 Life Span Study (LSS)

RP 2-08 Mail survey 2008 on epidemiological factors in the Extended Life Span Study population

Sakata R (EH), Nagano J, Grant EJ (EH), Sugiyama H (EH), Ohishi W (CH), Akahoshi M, Moriwaki H (EH), Mabuchi K, Ozasa K (EH), Kodama K (CS)

Objectives: To update the information on epidemiological factors that may confound or modify radiation effects among LSS subjects, and to obtain information on subsequent medical radiation exposures.

Background and Significance: Increased rates of malignant tumors and circulatory diseases indicate that important late effects of A-bomb radiation on health include common multifactorial diseases. The questionnaire information will permit more insights about the joint effects of or possible confounding by various environmental, lifestyle, and endogenous factors, upon the health effects of A-bomb radiation. ABCC-RERF has conducted several mail surveys for this purpose in the past. Since more than 15 years had passed since the last mail survey, another survey was conducted to update information on factors that might have changed longitudinally and to obtain new information on factors that need clarification from previous studies. For the first time, the survey collected information on exposures from radiotherapy and relatively high-dose diagnostic radiation procedures (e.g., CT scans).

Study Methods: Although the original targets of the survey were all 47,000 subjects who were alive as of 1 July 2007 in the Extended Life Span Study cohort (LSS-E85), the number was reduced to about 25,000 because of a new legal restriction regarding access to the resident registries, which limited our ability to obtain addresses. The content, validity, reproducibility, and feasibility of the questionnaire were evaluated through a pilot study (B45-06) as well as outside review. Multiple mailings were conducted for nonrespondents.

Study Progress: The survey was sent to 24,640 subjects. Data entry of questionnaire responses has been completed and data cleaning is underway. A summary report was written and sent to the subjects who returned a questionnaire. A supplemental interview for smoking and alcohol drinking for AHS subjects who did not respond to the mail survey is being conducted by the Department of Clinical Studies during clinic visits.

Results and Conclusions: The number of returned questionnaires with answers was 14,094. Information was also obtained from 332 AHS subjects who visited RERF for a health examination prior to November 30, 2013 who had not filled out a questionnaire. A preliminary examination of the medical irradiation data gave little indication that it was a confounder of the bombing radiation effect, though more detailed analyses are needed.

RP 1-75 Research plan for RERF study of life span of A bomb survivors, Hiroshima and Nagasaki

Ozasa K (EH), Shimizu Y, Grant EJ (EH), Sugiyama H (EH), Sakata R (EH), Sadakane A (EH), Soda M (EN), Cologne JB (S), Kodama K (CS)

Objectives: To ascertain the long-term health effects of exposure to ionizing radiation in the Life Span Study (LSS) cohort of A-bomb survivors.

Background and Significance: Because the LSS is a large cohort of all ages with a wide range of radiation doses and a long-term follow-up for mortality and cancer incidence, it provides key information for radiation risk assessment. Evaluation of the late health effects of radiation from the atomic bombs is the central goal of RERF research.

Study Methods: A cohort study. The LSS cohort of about 94,000 A-bomb survivors and 26,000 subjects who were not in city at the time of the bombing has been followed up since 1950 for their vital status and cause of death and since 1958 for cancer incidence. Individual A-bomb radiation doses have been estimated. Mail surveys have been conducted to obtain information on factors that might confound or modify the radiation effects.

Study Progress: An analysis of mortality of noncancer respiratory disease in relation to potential confounding variables and co-morbidities was published in *Radiation Research*, and an analysis of noncancer digestive disease is being conducted in RP-A1-11. The dose response for mortality of various heart disease subtypes over separate observation periods from 1950 to 2008 is being examined. Analyses of the effects of co-morbidity from other chronic diseases on the mortality of major noncancer diseases are being conducted in RP-A2-11. An analysis of the updated cancer incidence data (through 2007) is being conducted. Estimates of the LSS population who reside within the cancer registry catchment areas in Hiroshima and Nagasaki were updated in collaboration with the Department of Statistics.

Results and Conclusions: The risks of radiation apparently increased with radiation dose for some types of noncancer respiratory disease, but whether those associations might be spurious was investigated by examining disease misclassification (e.g., underlying malignancies) and possible confounding. An international symposium focusing on low-dose effects, especially in relation to the LSS, was held at RERF in December 2013. Since a majority of the subjects who were exposed when young are still alive and risk estimates for them are uncertain at this stage, a number of years of further follow-up will provide a much more informative characterization of radiation risks.

RP 2-61 Study of mortality and cancer incidence in people exposed *in utero*

Sugiyama H (EH), Shimizu Y, Preston DL, Suyama A, Cologne JB (S), Misumi M (S), Ozasa K (EH), Kodama K (CS)

Objectives: To investigate the nature of radiation effects on mortality and cancer incidence among people exposed *in utero* to atomic bomb radiation.

Background and Significance: Prenatal medical diagnostic x-ray exposure has been associated with increased risk of childhood cancer in several case-control studies. The

RERF *in utero* cohort, although small in size, has a large collective dose, with a subgroup with substantial radiation doses, and so can provide much information on the sensitivity of the embryo and fetus to late radiation effects. It is the only available cohort in the world with exclusively *in utero* radiation exposure and adulthood data on health risks.

Study Methods: Follow-up of mortality and cancer incidence in a cohort of 3,600 people who were *in utero* at the time of the bombings has now continued to age 62.

Study Progress: Analysis of the mortality data for 1950–2008 is underway with special emphases on the magnitudes and temporal patterns of radiation risks for cancer and noncancer deaths taking into account birth weight.

Results and Conclusions: Significant associations between radiation exposure and solid cancer mortality among females (ERR/Gy = 2.9; 95% CI: 0.56, 8.2), and noncancer disease mortality in both childhood and at ages of 40 years and older (ERR/Gy = 3.0; 95% CI: 0.45, 9.0, ERR/Gy = 1.9; 95% CI: 0.21, 5.3, respectively) were detected. The adult noncancer mortality risk was detected among *in utero* exposed individuals of low birth weight. The possibility that there is mediation between radiation and noncancer mortality by birth weight is being considered.

RP-A2-13 A proposal to join the biliary tract cancers pooling project

Grant EJ (EH), Hida A (CN), Ohishi W (CH), Ozasa K (EH)

Objectives: This is a proposal to join the Biliary Tract Cancers Pooling Project (BTCPP) being organized by Dr. Jill Koshiol of the U.S. National Cancer Institute (NCI). The BTCPP is a pooled analysis of individual-level data collected in cohort settings to study the etiology of biliary tract cancers, specifically studying possible associations with body mass index (BMI), smoking, and diabetes mellitus.

Background and Significance: Biliary tract cancers are rare (fewer than 10,000 total incident cases are expected in the U.S. in the current year) and little is known about their etiology. Due to rarity, few prospective cohort studies have sufficient power to study biliary tract cancer etiology; therefore a pooled study using prospective data is proposed.

Study Methods: Data will be pooled at the U.S. NCI and harmonized. Data will include height, weight, date of birth, age at baseline, BMI, smoking, diabetes, incident cancer (gallbladder) diagnosis date, and others if available (family history, NSAID drug use, physical activity, etc.).

Study Progress: This RP was approved in July 2013. Data have been prepared and sent to NCI. Current efforts are underway at NCI to harmonize the data.

Results and Conclusions: No results at this time. Study still in the data collection/harmonization phase.

RP-A1-13 A proposal to join the pooled analysis of radiation-related primary neoplasms of the brain and nervous system

Sakata R (EH), Sugiyama H (EH), Soda M (EN), Inskip P, Brenner A, Ozasa K (EH), Shore RE (D)

Objectives: To characterize in more detail the association between radiation and tumors of the central nervous system (CNS), particularly considering; 1) the shape of radiation dose-response curve, 2) the effect modification by age at irradiation and sex, 3) temporal patterns of radiation-related

risks, 4) the effect of dose fractionation, and 5) possible joint effects of radiotherapy and chemotherapy.

Background and Significance: It is well established that ionizing radiation can induce tumors of the CNS. Exposure during childhood appears to be more effective in tumor induction than adult exposure, but the data concerning adult exposure are sparse. Little is known about other factors that modify radiation-related risk. The association between radiation exposure and risk appears to be stronger for benign tumors (meningioma and schwannoma), than for malignant tumors (glioma).

Study Methods: Fifteen studies (11 cohort and 4 case-control studies) will join this pooled analysis. RERF will provide most of the data used in the previous study (Preston et al., JNCI 2002), but DS02 dose estimates will be used instead of the original DS86 doses. After the harmonizing of the pooled data, excess relative risks and excess absolute rate will be estimated at the National Cancer Institute (NCI). Using the best fitting dose-response model, multiplicative interactions of the ERR and EAR with sex, attained age, age at exposure, time since exposure, dose fractionation, and some study-specific characteristics (type of first cancer, chemotherapy, etc.) will be evaluated.

Study Progress: The research protocol at RERF was approved in June 2013. The project is currently under review at the National Institute of Health Office of Human Subjects Research Protections at NCI.

Results and Conclusions: Not yet. Results expected in 2015.

RP-A2-11 Mortality analysis of Life Span Study (LSS) cohort taking into account multiple causes of death indicated in death certificates

Takamori A, Kasagi F, Takahashi I (CH, EH), Ozasa K (EH), Yanagawa T

Objectives: To study the association between radiation and underlying/secondary causes of death that were identified from death certificates of LSS subjects.

Background and Significance: The LSS has mainly focused on relationships between underlying causes of death and radiation dose. However, diseases other than underlying causes of death (secondary causes of death) may modify the radiation risk estimates of death compared to the underlying cause of death and have rarely been studied.

Study Methods: The LSS follow-up period considered was 1950–2002. We are investigating whether the dose response determined only by underlying causes of death is different from that determined by both the underlying and secondary causes of death.

Study Progress: The dataset is being analyzed and a paper is being drafted.

Results and Conclusions: During all follow-up, a total of 49,603 deaths were identified among 86,611 LSS subjects. Circulatory disease was identified as the underlying cause of death in 18,705 deaths. For those cases, pneumonia was identified as one of the secondary causes of death in 1,331 deaths, renal disease in 498 deaths, diabetes mellitus in 401 deaths, and any cancer in 224 deaths.

RP-A1-11 Radiation exposure and the risk of mortality from noncancer diseases of the respiratory and digestive systems in the Life Span Study (LSS), 1950–2005

Pham TM, Sakata R (EH), Grant EJ (EH), Shimizu Y, Furukawa K (S), Takahashi I (CH, EH), Sugiyama H (EH), Soda M (EN), Ozasa K (EH)

Objectives: To examine the association between atomic-bomb radiation and the mortality risk of noncancer diseases of the respiratory and digestive systems in the LSS, 1950–2005. The goal is to examine the main specific conditions within those broad classifications and to determine the degree to which the apparent associations with radiation may be due to misdiagnoses of underlying cancer or cardiovascular disease.

Background and Significance: Findings regarding an association of radiation exposure and noncancer respiratory and digestive diseases have been sparse and non-conclusive. Previously examined radiation risks for those diseases in the LSS were derived from aggregate conditions.

Study Methods: The follow-up data from 1950 to 2005 for 86,611 subjects of the LSS cohort are being analyzed. The estimated risk will be adjusted for potential confounding variables (smoking, alcohol, body mass index, education, diabetes, occupation, and proximal/distal survivors) and for incidence/co-morbidity of cancer and co-morbidity of cardiovascular disease. Period-specific analyses will also be conducted since background disease structure in Japan has changed markedly in these several decades.

Study Progress: A paper on respiratory diseases was published in *Radiation Research*. For digestive diseases, a paper is being drafted.

Results and Conclusions: For nonmalignant respiratory disease risks, adjustments for indications of cancer and/or cardiovascular disease decreased the risk estimates for various subtypes of such disease, and only pneumonia/influenza remained statistically significant after adjustment. This shows that the association between noncancer respiratory diseases and radiation exposure in previous reports may, in part, be attributed to coincident cancer and/or cardiovascular diseases.

For digestive diseases, the linear excess relative risk estimates were small for the whole period. Liver disease accounted for approximately half of all digestive disease deaths. Some increased radiation risk was observed in the middle follow-up period, while the risks in the early as well as in the late periods tended to be small. Such difference in risks by period needs to be discussed from the viewpoint of biological mechanisms, confounding, and artifacts of misdiagnosis.

RP-A2-10 A semi-parametric survival extrapolation method: Model validation using RERF cohort

Fang C-T, Wang J-D, Hwang J-S, Furukawa K (S), Kasagi F, Soda M (EN), Suyama A, Ozasa K (EH), Cullings HM (S)

Objectives: To examine the robustness of a semi-parametric survival extrapolation method based on the constant excess hazard model (developed by Dr. Hwang) using LSS cohort data. The long-follow-up data in the LSS cohort provide a good opportunity to evaluate temporal extrapolation using the model.

Background and Significance: The knowledge of how long patients can expect to live after the diagnosis of a disease is essential for cost-effectiveness evaluation of medical interventions. Researchers need to look beyond the follow-up limit of clinical trials and take a lifetime perspective when measuring the impact of a new medical therapy. Because there are usually only very limited follow-up data for newer medical interventions, a robust statistical method for survival extrapolation is particularly important. Previously-used parametric survival models can make reasonably good short-term prediction. Problems may arise, however, when there is a high right-censoring rate or long-term projection.

Our group therefore conceived an innovative approach that borrows information from national vital statistics data, and incorporates this information in a semi-parametric survival extrapolation method based on the logit survival ratio between patients with a disease and an age- and gender-matched reference population (see details in the Method section), assuming a constant excess hazard. In that case, the logit survival ratio curve will converge to a straight line over time, which allows linear extrapolation.

Study Methods: We propose to examine whether the logit survival ratio curves between atomic bomb survivors who (1) are with or without radiation exposure, or (2) had a specific cancer vs. an age- and gender-matched reference population generated from Japanese vital statistics by a Monte Carlo method, will converge to a straight line over time. In addition, we will test different strategies for estimating the slope in logit survival ratio plots in order to find the best strategy for choosing the sample size. A software program has been developed by Prof. J-S Hwang (Academia Sinica, Taiwan) to facilitate these calculations.

Study Progress: Statistical analysis has been completed.

Results and Conclusions: For the period 1950–1998, the logit survival ratio between atomic bomb survivors who were exposed to the highest-dose radiation (HDR) (dose $\geq 1,000$ mGy) (2,375 persons) and an age- and gender-matched reference group selected from those without radiation exposure (17,830 persons), the slope eventually randomly fluctuated between +0.005 and -0.020 . For site-specific cancers (stomach, lung, liver, colon, breast, pancreas) the logit survival ratio between patients with cancer and the matched references, the slopes also show overt random variations over time as the sample size decreases, especially for cancers with a smaller sample size. To solve this problem, we derived two new mathematical theorems on the properties of the logit survival ratio, based on the constant excess hazard assumption. Using these, we developed a set of simple yet robust rules for selecting the best slope for extrapolating the survival curves. With the selected slope, we further evaluate the accuracy of long-term projection by comparing the difference between the predicted and actual survival curves. The semi-parametric model had excellent accuracy in extrapolation: the differences between the predicted and actual survival probabilities at the end of follow-up were all below 0.05 (in absolute value). We concluded that the semi-parametric method based on the constant excess hazard assumption is a robust statistical method for lifetime survival extrapolation.

RP-A1-09 Biologically based mechanistic modeling of leukemia in the Life Span Study

Dekkers F, Bijwaard H, Cullings HM (S), Soda M (EN), Sugiyama H (EH), Kasagi F, Suyama A

Objectives: Apply a biologically based two-mutation carcinogenesis model to individual leukemia incidence data in the LSS cohort.

Background and Significance: The Netherlands National Institute for Public Health and the Environment (RIVM) has a history of developing Moolgavkar-type two-mutation models for leukemia in laboratory animals and humans that attempt to incorporate biological concepts of the disease. The biologically-based leukemia model results can be compared with our empirical-model results, and may be informative as to how to transfer risk estimates to other radiation-exposed groups, such as western populations with chronic or low-dose exposures.

Study Methods: RIVM has developed a maximum-likelihood, Two Mutation Carcinogenesis (TMC) model that takes relevant radiobiological information into account more than most other two-stage models have done by estimating mutation rates of interest. Risks will be estimated for LSS data for three main radiogenic leukemia subtypes (acute myeloid leukemia, chronic myeloid leukemia, and acute lymphocytic leukemia).

Study Progress: An earlier analysis under the assumption of a fixed lag time led to problems. A minimum lag time between the creation of the first malignant cell and leukemia diagnosis provides better model fitting. The value for lag time obtained in statistical data fitting is largely determined by the earliest cases. Since the LSS does not contain information on cases that occurred in the first years after the bombings, using the LSS data alone results in implausibly large values. In the past year, the limited information available on early cases was used to obtain a more realistic value for the lag time. Data fitting to determine the sensitivity of the other parameters in the model to the uncertainty in the lag time are in progress.

Results and Conclusions: Preliminary TMC analyses indicated that different background mutation rates were found for men and women. All other parameters are identical for the two sexes. This suggests that risk estimates for populations with different baseline leukemia incidence can be obtained from the model. Similarly, risks can be derived for chronic exposures. This work was presented at the 14th International Congress of Radiation Research (ICRR), Warsaw, Poland, 2011.

RP-A1-08 Bayesian MCMC applied to individual cancer incidence data in atomic bomb survivors

Cullings HM (S), Little MP, Furukawa K (S), Nishi N, Soda M (EN), Suyama A, Sakata R (EH), Kasagi F, Molitor J

Objectives: The uncertainty in survivor dose estimates and the resulting effect on RERF studies is a longstanding concern at RERF. It is currently addressed by adjusting doses using correction factors based on regression calibration and assumptions about the probability distributions of the errors. This study intends to examine the effect of assumptions about the error distributions on important types of RERF risk estimation studies by using Bayesian models for individual data with fully specified likelihoods rather than the

standard approach of Poisson regression on grouped data. For a variety of radiosensitive cancer sites, estimates obtained using Bayesian Markov Chain Monte Carlo (MCMC) methods will be compared with those obtained using the regression calibration (substitution) method, where both methods are based on individual data.

Background and Significance: A major source of uncertainty concerns extrapolation of risks at high doses and high dose rates to low doses and low dose rates and the impact of both systematic and random dosimetric errors on those. It is well recognized that measurement error can alter substantially the shape of this relationship and hence the derived population risk estimates.

Pierce et al. (*Radiat Res* 1990; 123:275–84) carried out a dose adjustment prior to the model fitting to allow for random dosimetric errors by substituting the expectation of the “true dose” given the estimated dose. This leads to reasonable adjusted point estimates of the model parameters for linear dose-effect relationships but is only approximate for non-linear ones, and does not fully take account of all the variability induced by the measurement errors.

The proposed Bayesian approach to the measurement error problem links three basic sub-models: the disease model, the measurement model and the exposure model, and dosimetric uncertainty is reflected in the variability of the model parameters. In principle this allows for more of the uncertainties to be taken into account, and will produce a wider, but more realistic, uncertainty envelope. However, it is possible that in practice this may not happen to any great extent.

Study Methods: The posterior distribution of the risk parameters for the various data sets of interest will be obtained by samples from MCMC algorithms. Using individual data will allow a fully Bayesian analysis to be carried out, in particular one that embraces the full range of uncertainties, for example relating to the form of the error distribution. However, given the complexities and iterative nature of the model and the large sample size, the computational requirements are a hurdle.

Study Progress: In April 2009, Dr. Furukawa presented some initial results from simulation studies to compare a Bayesian approach for the dose error adjustment to regression calibration in normal and logistic regression. The work initiated by Dr. Li (Imperial College London) in 2008 (nested case-control data of leukemia and thyroid cancer) made little progress in the past year due to Dr. Li’s relocation.

The computational burden was still a major issue for Cox regression on individual data, the primary goal of this project. We held discussions with Dr. Pierce about computational feasibility issues during his visit in October 2010. As he suggested, we started exploring some alternative approaches to Bayesian MCMC that may be more computationally tractable, such as multiple imputation. Some related results from a collaboration with Dr. Doi were presented at the International Biometrics Society meeting in Kobe in August 2012. This work made no progress in 2013, primarily due to lack of time because of other priorities related to dosimetry and dose-error, particularly the work described under the dosimetry RP 18-59 and the dose-error RPs A4-10 and A5-11.

Results and Conclusions: None yet.

Effects of rain falling shortly after the atomic bombings

Ozasa K (EH), Grant EJ (EH), Sakata R (EH), Cullings HM (S)

Objectives: To clarify the association of exposure to rain falling shortly after the atomic bombings (fallout rain) in Hiroshima and Nagasaki and the development of acute radiation syndromes and long-term health outcomes.

Background and Significance: Although some information on exposure to fallout rain was collected in the early interview surveys in the 1950s–60s, it had not been used for the analysis of health effects because the data were crude and non-quantitative. However, as a result of the findings on the effects of “black rain” published by outside groups and the nuclear power plant accident in 2011, public concern about radioactive fallout has grown, and it was thought that an investigation of the association of exposure to fallout rain and health outcomes was needed in the light of such concerns.

Study Methods: Individual information on exposure status (yes/no/unknown) to fallout rain and development of acute symptoms was abstracted. Combined with follow-up information, the risk of exposure to fallout rain was estimated for overall death, cause of death, and cancer incidence. Cross-sectional analyses between rain exposure and acute symptoms also were conducted.

Study Progress: Papers on the risk of fallout rain exposure for long-term health effects and association of rain exposure and development of acute symptoms were drafted and are under review in coordination with the RERF Dosimetry Committee and the Department of Statistics.

Results and Conclusions: No substantial increases in risks of cancer or other diseases were observed in association with reported exposure to fallout rain. Some associations were observed between reported exposure to fallout rain and reported development of acute symptoms (possibly due to a combination of exceptional cases and recall bias). Therefore, we concluded that the reported fallout rain was not likely to be homogeneously radioactive at a considerable level, which accords with area measurements made soon after the bombings.

Life Span Study Publications

RERF Report (RR)

◆ Pham TM, Sakata R, Grant EJ, Shimizu Y, Furukawa K, Takahashi I, Sugiyama H, Kasagi F, Soda M, Suyama A, Shore RE, Ozasa K: Radiation exposure and the risk of mortality from noncancer respiratory diseases in the Life Span Study, 1950–2005. *Radiat Res* 2013 (November); 180(5):539–45. (RR 2-13) © 2013 by Radiation Research Society

[Study Rationale] The Life Span Study (LSS) has found that exposure to A-bomb radiation is associated with mortality from noncancer respiratory diseases (hereinafter, simply called respiratory diseases). However, this relationship may be a secondary association caused by the fact that respiratory diseases that can develop at the terminal stage of illnesses such as cancer and circulatory diseases, which have already been linked to radiation exposure, are designated as the cause of death. Considering that the biological mechanisms behind the

association between radiation exposure and respiratory diseases are unclear, further examination was necessary before reaching any conclusion about whether the association between radiation exposure and noncancer respiratory disease mortality was genuine or an artifact disease misclassification.

[Explanation] Objectives While the LSS has clarified the association between A-bomb radiation exposure and cancer, attention has also recently been paid to noncancer diseases. The two purposes of the study were (1) to examine the associations of radiation dose with the main subclasses of deaths coded as respiratory diseases on the death certificates; and (2) to determine the degree to which the associations between radiation exposure and respiratory diseases were genuine or were caused by biases due to lifestyle and sociodemographic factors or disease misclassification. **Methods** The subjects of the present study were 86,611 people with estimated lung doses from among the 120,321 members of the LSS, which was established on the basis of the Japan 1950 National Census. Based on the International Classification of Diseases, respiratory diseases recorded as causes of death of these subjects were classified into acute respiratory infection, pneumonia/influenza, chronic obstructive lung disease, bronchial asthma, and other respiratory diseases. The present study was based on a follow-up conducted from 1950 through 2005. To determine the degree of association of radiation exposure with mortality from these diseases, excess relative risks (ERR) were estimated using Cox regression analyses. **Results** (1) Association between radiation exposure and respiratory diseases: During the study period, 5,515 deaths due to respiratory diseases occurred. The ERR per Gy for all noncancer respiratory diseases was significantly elevated, being 0.17 (95% confidence interval [CI]: 0.08, 0.27). (The ERR of 0.17 here indicates a 17% increase in the risk at 1 Gy of exposure as compared with unexposed subjects, but with a range of uncertainty from 8% to 27%). The ERR for each of the respective diseases varied, being 0.20 (95% CI: 0.09, 0.34) for pneumonia/influenza, 0.08 (95% CI: -0.14, 0.37) for chronic obstructive lung disease, 0.16 (95% CI: -0.10, 0.52) for bronchial asthma, and -0.16 (95% CI: <0, 0.40) for acute respiratory infection. Adjustments for lifestyle and socioeconomic factors had virtually no impact on the risk estimates. When the study period was divided into shorter intervals (1950–1964, 1965–1979, and 1980–2005), the association between radiation exposure and respiratory diseases was observed to be stronger in the period 1980–2005. Until the 1970s, most respiratory disease deaths in Japan were from acute infections, but in the 1980s respiratory disease deaths started to be observed primarily as diseases accompanying the terminal stages of cancer and circulatory diseases among the elderly. It was thus plausible that the suggestive association between radiation exposure and respiratory diseases might reflect cancer and circulatory disease in this later period. (2) Effects of cause-of-death misclassification: Because in the present study information on cause of death was obtained from death certificates, it is important that the underlying cause of death recorded on death certificates be accurate in order to correctly estimate

risks. Many patients with cancer or circulatory disease die of respiratory diseases at the terminal stage of such illnesses. Thus, there is a concern that although the actual underlying cause of death is cancer or circulatory diseases, respiratory diseases may be mistakenly recorded as the cause of death. With this in mind, analysis was conducted after excluding subjects with a history of cancer or circulatory diseases from among deaths due to respiratory diseases. As a result, the ERRs decreased by about 35%, and except for pneumonia/influenza, no statistically significant association between radiation exposure and mortality from respiratory diseases was observed. Care should be taken in interpreting the results of the present study because the study could only partially correct for other causes of death. Some study subjects did not live in Hiroshima or Nagasaki prefectures where our tumor registries could have identified associated cancers, and death certificates often list only one “cause” of death, rather than identifying the important accompanying causes as well, such as cancer or cardiovascular disease. Thus, the apparent associations of radiation with respiratory diseases may have been reduced even more if we had complete ascertainment of the alternative underlying causes. However, the present study indicates that at least part of the apparent association of radiation exposure with noncancer respiratory disease deaths is likely due to reporting artifacts on the death certificates, but not due to sociodemographic or lifestyle biases.

Other Journal Publications

- ◆ Mettler FA Jr, Constine LS, Nosske D, Shore RE: Ninth annual Warren K. Sinclair keynote address: Effects of childhood radiation exposure: An issue from computed tomography scans to Fukushima. *Health Phys* 2013 (November); 105(5):424–9.
- ◆ Nakamura N: Radiation risks at low doses. *Igaku Butsuri [Jpn J Med Phys]* 2013 (May); 32(4):202–8. (Japanese)
- ◆ Ozasa K: Effects of radiation in atomic-bomb survivors who were young at the time of bombing. *Nihon Shoni Ketsueki/Gan Gakkai Zasshi [Jpn J Pediatr Hematol Oncol]* 2013; 50(1):15–7. (Japanese)
- ◆ Ozasa K: Health effects of radiation. Yasumura S, ed. *Public Health at Nuclear Disaster—Report from Fukushima*. Tokyo: Nanzando; 2014, pp 18–24. (Japanese)
- ◆ Preston RJ, Boice JD Jr, Brill AB, Chakraborty R, Conolly R, Hoffman FO, Hornung RW, Kocher DC, Land CE, Shore RE, Woloschak GE: Uncertainties in estimating health risks associated with exposure to ionising radiation. *J Radiol Prot* 2013 (September); 33(3):573–88. (related to *Atomic-bomb Dosimetry Studies*)
- ◆ Takahashi I, Ohishi W, Mettler FA Jr, Ozasa K, Jacob P, Bab N, Lipshultz SE, Stewart FA, Nabika T, Niwa Y, Takahashi N, Akahoshi M, Kodama K, Shore RE, the International Radiation and Cardiovascular Disease Workshop Participants: A report from the 2013 International Workshop: Radiation and Cardiovascular Disease, Hiroshima, Japan. *J Radiol Prot* 2013 (December); 33(4):869–80. (related to *Adult Health Study* and *Cell Biology Study*)
- ◆ United Nations Scientific Committee on Effects of Atomic Radiation (Kelly GN, Golikov V, Constine LS, Nagel HD, Nosske D, Shore RE): Sources, Effects and Risks of Ionizing

Radiation, Volume II. Scientific Annex B: Effects of radiation exposure of children. New York: United Nations; 2013, 269 p. (related to *Adult Health Study*)

Manuscript in Press

❖ Ozasa K: Late health effects of atomic bomb radiation. Tajima K et al., eds. *Advances and Future Directions of Cancer Epidemiology and Prevention—Extended Abstracts for the 44th International Symposium of the Princess Takamatsu Cancer Research Fund*, Tokyo, Japan, 13–15 November 2013. Tokyo: Princess Takamatsu Cancer Research Fund.

Life Span Study Oral Presentations

- ❖ Ozasa K. Basic methodologies of radiation epidemiology. 50th Radiation Science Meeting, 19 April 2013, Osaka
- ❖ Nakamura N. How to tell low dose risks to the public. 54th Late A-bomb Effects Research Meeting, 2 June 2013, Hiroshima
- ❖ Ozasa K, Grant EJ, Sakata R, Sugiyama H, Soda M, Sadakane A. Risk of radiation exposure for second primary cancer incidence. 36th Annual Meeting of the Japanese Society of Cancer Epidemiology, 20–21 June 2013, Gifu
- ❖ Sakata R, Sugiyama H, Soda M, Grant EJ, Sadakane A, Ozasa K. Difference by smoking status in effects of atomic-bomb radiation on histological type-specific stomach cancer. 36th Annual Meeting of the Japanese Society of Cancer Epidemiology, 20–21 June 2013, Gifu
- ❖ Nakamura N, Sakata R. Explanation on “no age-at-exposure effect on the relative risk of breast cancer in atomic-bomb survivors.” 38th Annual Meeting of the Chugoku Area Radiation Research Society, 26 July 2013, Hiroshima
- ❖ Sakata R, McGale P, Grant EJ, Ozasa K, Peto R, Darby SC. Smoking reduces life expectancy in Japanese the same as other populations. 10th Asia Pacific Conference on Tobacco or Health (APACT) Conference, 18–21 August 2013, Chiba
- ❖ Grant EJ, Cullings HM, Sakata R, Sugiyama H, Sadakane A, Ozasa K. Impact of missing doses in the Life Span Study of atomic bomb survivors. 40th Annual Meeting of the European Radiation Research Society, 1–5 September 2013, Dublin, Ireland
- ❖ Sakata R, Sugiyama H, Soda M, Grant EJ, Sadakane A, Ozasa K. Joint effects of smoking status and radiation on stomach cancer by histological type in the atomic bomb survivor cohort. 40th Annual Meeting of the European Radiation Research Society, 1–5 September 2013, Dublin, Ireland
- ❖ Nakashima E. Radiation dose response estimation with emphasis on low dose range: Application of restricted cubic spline models to all solid cancer mortality data in atomic-bomb survivors. FY2013 Japan Joint Statistical meeting, 8–11 September 2013, Osaka
- ❖ Ozasa K, Shimizu Y, Takahashi I, Sakata R, Sugiyama H, Sadakane A, Grant EJ, Kodama K. Dose response of mortality risk of cardiovascular disease in the atomic-bomb survivor’s life span study. 5th International MELODI (Multidisciplinary European Low Dose Initiative) Workshop, 7–10 October 2013, Brussels, Belgium
- ❖ Ozasa K, Shimizu Y, Takahashi I, Sakata R, Sugiyama H,

Sadakane A, Grant EJ. Dose-response of radiation risk of heart disease in the atomic-bomb survivors. 56th Annual Meeting of the Japan Radiation Research Society, 18–20 October 2013, Aomori

- ❖ Ozasa K. Evaluation and protection of health effects of radiation. 72nd Annual Meeting of the Japanese Society of Public Health, 23–25 October 2013, Tsu
- ❖ Ozasa K. Late health effects of atomic bomb radiation. 44th International Symposium of the Princess Takamatsu Cancer Research Fund, 13–15 November 2013, Tokyo
- ❖ Furukawa K. A semi-parametric approach to dose-response estimation in radiation risk analysis. 2013 SRA (Society for Risk Analysis) Annual Meeting, 8–11 December 2013, Baltimore, Maryland, USA
- ❖ Shore RE. Presentation to ICRP Task Group. 1st ICRP TG91, Kickoff Meeting, 10–11 December 2013, Munich, Germany
- ❖ Takahashi I. Impact of radiation on cardiovascular disease in the follow-up study of atomic-bomb survivors. 2013 Seminar of Radiation Epidemiology, 19 December 2013, Tokyo (related to *Adult Health Study*)
- ❖ Sugiyama H, Misumi M, Sakata R, Sadakane A, Grant EJ, Soda M, Ozasa K. Association of cancer and non-cancer mortality, radiation and birth weight among *in-utero* A-bomb survivors (1950–2008). 24th Annual Scientific Meeting of the Japan Epidemiological Association, 23–25 January 2014, Sendai
- ❖ Shore RE, Furukawa K, Imaizumi M. Thyroid disease among A-bomb survivors exposed in childhood. International Workshop on Radiation and Thyroid Cancer, 21–23 February 2014, Tokyo (related to *Adult Health Study*)
- ❖ Ozasa K, Grant EJ, Sugiyama H, Sakata R, Sadakane A, Takahashi I, Shimizu Y. Evaluation of effects of atomic-bomb radiation in epidemiological studies. 6th International Systems Radiation Biology Workshop, 5–7 March 2014, Chiba

**Research Protocols 2-11, 7-10, 7-09, 2-75 (Platform Protocol), A6-12, A3-09
Adult Health Study (AHS)**

RP 2-11 Study of arteriosclerosis in the Adult Health Study population (Part 2. Analysis of the cytokine network regulating differentiation of mesenchymal stem cells in artery)

Takahashi I (CH), Ohishi W (CH), Hayashi T (R), Cologne JB (S), Takahashi T, Kusunoki Y (R), Ozasa K (EH), Kihara Y, Matsumoto M, Fujiwara S

Objectives: To test the hypothesis that arteriosclerosis develops due to abnormalities in mesenchymal tissues because cell damage from radiation exposure triggers the cytokine network involved in repair of such tissue damage in arteries.

Background and Significance: AHS data have suggested a relationship between radiation exposure and the prevalence or incidence of stroke, ischemic heart disease, and aortic calcification. However, the pathogenetic mechanism(s) underlying arteriosclerosis due to radiation exposure is unclear. Some have hypothesized that arteriosclerosis is primarily an inflammatory disease, but it appears to have a complex pathology, of which inflammation is only one component. We hypothesize that atherosclerosis develops due to abnormalities in mesenchymal tissues because cell damage from radiation exposure triggers the cytokine network involved in repair of such tissue damage in arteries.

Study Methods: This is a cross-sectional study among 2,000 AHS subjects (including those exposed at young ages) in Hiroshima. We will measure several multi-functional cytokines involved in the “cytokine network” that are possibly related to radiation-induced atherogenesis and relate them to physiological measurements of atherosclerotic cardiovascular outcomes (based on RP 7-09). We will then examine if the “cytokine network” functions to either moderate or mediate the radiation effect upon atherosclerotic cardiovascular outcomes.

Study Progress: Data collection began in April, 2011. We are measuring blood cytokine levels related to CVD such as pentraxin-3, osteopontin, osteoprotegerin, high mobility group box (HMGB)-1, VEGF, and apolipoprotein (Apo)-J for about 2,100 AHS subjects.

Results and Conclusions: None yet. Results expected in 2015.

RP 7-10 Study of body composition of the Hiroshima Adult Health Study population

Tatsukawa Y (CH), Fujiwara S, Harris TB, Misumi M (S), Ohishi W (CH), Yamada M (CH), Kasagi F

Objectives: The major objectives in this research protocol are to test whether radiation exposure is related to an increased incidence of arteriosclerotic diseases and their risk factors through modifications in body composition.

Background and Significance: Recent studies in childhood cancer survivors have suggested that radiation exposure causes modifications in body composition (such as higher body fat mass and lower lean mass). These findings reflect high exposures from radiotherapy (e.g., Hodgkin disease treatment), but extrapolation to lower

doses is unknown. It is important to examine the association between radiation dose and body composition, and to determine whether radiation exposure is related to an increased incidence of arteriosclerotic diseases and their risk factors partly through modifications of body composition.

Study Methods: Study subjects total approximately 2,200 Hiroshima AHS participants who underwent whole-body composition examination by dual energy X-ray absorptiometry (DEXA) during the period 1994–96. With those data it is possible to evaluate the whole-body/regional (trunk, limb, etc.) fat mass and lean mass and to relate those to other AHS data on cardiovascular diseases.

Study Progress: We completed the analyses regarding the effects of radiation dose on modifications in body composition. The result shows A-bomb radiation dose was associated with modifications in body composition. The manuscript has been accepted by the *International Journal of Obesity*.

Results and Conclusions: A-bomb radiation dose was associated significantly and negatively with BMI in both sexes and appendicular lean mass (a surrogate marker for muscle mass). It was positively associated with trunk-to-limb fat ratio in women who were less than 15 years old at the time of exposure. In conclusion, significant associations of radiation dose with BMI and body composition were observed 50 years after A-bomb exposure.

RP 7-09 Study of arteriosclerosis in the Adult Health Study population (Part 1. Physiological indices of arteriosclerosis)

Takahashi I (CH), Hida A (CN), Kohata M, Yamada M (CH), Ohishi W (CH), Cologne JB (S), Misumi M (S), Takahashi T, Kihara Y, Matsumoto M, Fujiwara S

Objectives: The goal is to study one of the potential mechanisms by which radiation may promote cardiovascular disease. We will evaluate acceleration of arterial stiffness by radiation among AHS subjects (including the expanded group of younger survivors).

Background and Significance: Past studies have reported a significant association between radiation exposure and atherosclerotic disease mortality/morbidity among A-bomb survivors.

Atherosclerosis conceptually has two aspects: atherosclerosis (fatty degeneration) and sclerosis (arterial stiffness). While acceleration of arterial stiffness might be caused by radiation-induced structural changes in arterial walls, it has not been fully investigated. In this study, we will evaluate the associations of radiation and arterial stiffness taking into account correlations among stiffness indices and atheromatous disease indices/risk factors.

Study Methods: This is a cross-sectional study among all AHS subjects in Hiroshima and Nagasaki. The associations of radiation and the arterial stiffness indices (brachial-ankle pulse wave velocity [baPWV], augmentation index [AI]) will be analyzed with consideration of atheromatous disease indices (ankle-brachial blood pressure index [ABI], carotid intima-media wall thickness [CIMT], aortic calcification, and left ventricular hypertrophy) and atherosclerosis risk factors (Framingham risk scores).

Study Progress: Data collection began in April, 2010 and is underway. We have examined about 3,900 AHS subjects

since the beginning of the study. For the purpose of assessing the features of our measuring ABI among the elderly subjects, we conducted a comparative study between the different principle ABI measures. We found that ABI values obtained by our method may provide more accurate results than diagnoses based on the conventional method. A paper on these findings has been published (*Vasc Health Risk Manag* 2013; 9:89–94).

Results and Conclusions: None yet. Results expected in 2015.

RP 2-75 Research plan for RERF Adult Health Study (AHS), Hiroshima and Nagasaki

Ohishi W (CH), Yamada M (CH), Tatsukawa Y (CH), Takahashi I (CH), Ueda K (CH), Hida A (CN), Sera N (CN), Imaizumi M (CN), Haruta D (CN), Soda M (EN), Fujiwara S

Objectives: To evaluate in a systematic fashion the age and radiation exposure-dependent changes in the clinical status of long-term survivors (AHS cohort) of the atomic bombings, and to provide extensive biological specimens and information concerning lifestyle or other potential risk factors for many fields of study, which include cytology, genetics, immunology, radiobiology, and medical dosimetry.

Background and Significance: The AHS program of biennial comprehensive medical examinations is the only program in the world to provide long-term health screening of a representative irradiated population of all exposure-ages and a wide range of doses, and therefore can provide unique longitudinal information on subclinical and clinical disease risk, plus biosamples for numerous clinical and radiobiology studies. It began in 1958 with a targeted population of about 20,000 survivors and controls in the contact areas of Hiroshima and Nagasaki, was enriched in 1978 with about 2,400 additional higher-dose subjects and all available (~1,000) persons who were exposed *in utero*, and expanded again in 2008 with over 1,900 young exposed subjects (<10 years old at the bombings) to increase our ability to document radiation effects among those exposed when young.

Study Methods: The study examines differences in rates of a wide variety of diseases or pre-clinical disorders by level of radiation exposure. During the 27th cycle (July 2010–June 2012), 2,705 individuals among the original 1958–1978 AHS cohort were examined, representing approximately 65% of that cohort who were still living in the catchment area, and 1,728 individuals in the 2008 expanded AHS group.

Study Progress: Health examinations are continuing. The biological samples collected are used for clinical determinations and stored for ongoing and future studies. Evaluation of possible interactions has been assessed between radiation and various biological factors based on stored samples with respect to health outcomes; the factors include infectious agents, hormones, inflammatory markers, and a variety of other phenotypic and genetic factors. In addition to ongoing collaborative studies to evaluate possible interactions between radiation and infectious agents or hormones in the development of hepatocellular carcinoma (HCC), gastric cancer, and breast cancer, recently initiated collaborative studies include ones on: the association between thyroid function and various endpoints, as well as the association of progressive cardiac conduction defect and

weight fluctuation with cancer and cardiovascular disease. Because significant associations between radiation exposure and atherosclerotic disease mortality/morbidity have been reported in past studies, we are evaluating arterial stiffness and several multi-functional cytokines to study potential mechanisms by which radiation may promote cardiovascular disease. The informed consent form has been updated to obtain more specific permission for preservation and later use of blood and urine samples collected at current and previous examinations. Detailed data collection on alcohol consumption started in both Hiroshima and Nagasaki in 2013.

Results: Reports of new findings include: evaluating the associations of radiation exposure with body mass index and body composition, chronic kidney disease defined by using e-GFR criteria, and glaucoma prevalence, or the interaction of radiation with IL-6 on HCC risk.

RP-A6-12 Choice reaction time of middle-aged and elderly Japanese as a predictor of cardiovascular mortality: Radiation Effects Research Foundation Adult Health Study

Shimizu M, Misumi M (S), Yamada M (CH), Ohishi W (CH), Yamamoto H, Kihara Y

Objectives: The association between choice reaction time and subsequent coronary heart disease (CHD) and stroke mortality in middle-aged and elderly individuals based on a roughly 35-year follow-up of participants of the Adult Health Study (AHS) will be studied.

Background and Significance: Reaction time, certain biological indices that exhibit aging-related changes, and an index of cognitive function, were reported to predict life prognosis and vascular disease mortality. However, large-scale studies of general populations and cohort studies with a broad age range were limited, and there are few reports from studies on Asian cohorts.

Study Methods: In the AHS, reaction time was measured by Bogitch's flash reaction test for about 5,000 Hiroshima participants aged 35–74 years old, between 1970 and 1972. Vital status of the subjects was followed up from the time of baseline reaction time measurement until the end of 2007, with causes of death recorded in death certificates coded using the International Classification of Diseases (ICD). The association between reaction time and circulatory disease mortality will be investigated.

Study Progress: The analysis has been completed. The manuscript was submitted to the RERF review committee.

Results and Conclusions: None yet.

RP-A3-09 The association between chronic kidney disease and cardiovascular disease among atomic bomb survivors

Haruta D (CN), Tsuneto A, Takahashi I (CH), Hida A (CN), Sera N (CN), Imaizumi M (CN), Yamada M (CH), Neriishi K, Ohishi W (CH), Tatsukawa Y (CH), Nakashima E (S), Misumi M (S), Fujiwara S, Akahoshi M

Objectives: To clarify whether radiation is associated with chronic kidney disease (CKD) and whether CKD is involved in the mechanism(s) linking radiation exposure and cardiovascular disease (CVD).

Background and Significance: The association

between atomic-bomb radiation exposure and CVD has recently been drawing attention. CKD has recently been recognized as a risk factor for CVD. CKD and CVD share many common risk factors such as obesity, insulin resistance, impaired glucose tolerance, hypertension, dyslipidemia, and nephritis. This is the first RERF study to examine whether CKD serves as a CVD risk factor and the possible involvement of A-bomb radiation exposure in the disease process.

Study Methods: In this analysis, we will identify prevalent cases of CKD diagnosed during the four-year period between January 1988 and December 1991 (baseline period) and incident cases of CKD diagnosed between January 1992 and December 2006 (follow-up period) in the AHS cohort. We will also identify both prevalent and incident cases of CVD during the above-mentioned periods, respectively. CVD includes coronary heart disease (CHD) and stroke in this RP. Based on these data, we will determine whether the effects of A-bomb radiation exposure can be observed for several endpoints, with adjustment for other risk factors: (1) Associations of CKD prevalence or incidence with radiation dose and CKD risk factors. (2) Associations of CHD and stroke prevalence with radiation dose, CKD risk factors, and previous prevalent or incident CKD.

Study Progress: The data to analyze the association of prevalent cases of CKD with radiation dose and CKD risk factors and the incidence data on CKD have been collected but the incidence data on CVD need to be compiled and cleaned.

Results and Conclusions: None yet. Results expected in 2015.

Adult Health Study Publications

RERF Reports (RR)

◆ Takahashi Y, Misumi M, Yamada M, Masunari N, Oyama H, Nakanishi S, Fukunaga M, Fujiwara S: Alteration of body mass index and body composition in atomic bomb survivors. *Int J Obesity* 2013 (August); 37(8):1123–8. (RR 4-12) © 2013 Macmillan Publishers Limited. (This abstract was reprinted by permission of Nature Publishing Group.)

[Abstract] Objective: Obesity, underweight, sarcopenia, and excess accumulation of abdominal fat are associated with a risk of death and adverse health outcomes. Our aim was to determine whether body mass index (BMI) and body composition, assessed with dual-energy X-ray absorptiometry (DXA), are associated with radiation exposure among atomic bomb (A-bomb) survivors.

Design: This was a cross-sectional study conducted in the Adult Health Study of the Radiation Effects Research Foundation. **Subjects:** We examined 2686 subjects (834 men and 1852 women), aged 48–89 years (0–40 years at A-bomb exposure), for BMI analysis. Among them, 550 men and 1179 women underwent DXA in 1994–1996 and were eligible for a body composition study. **Results:** After being adjusted for age and other potential confounding factors, A-bomb radiation dose was associated significantly and negatively with BMI in both sexes ($P = 0.01$ in men, $P = 0.03$ in women) and appendicular lean mass ($P < 0.001$ in men, $P = 0.05$ in women). It was positively associated with trunk-to-limb fat ratio in women who were less than 15 years old at the time of exposure ($P = 0.03$).

Conclusions: This is the first study to report a significant dose response for BMI and body composition 50 years after A-bomb radiation exposure. We will need to conduct further studies to evaluate whether these alterations affect health status.

Other Journal Publications

◆ Takahashi I, Ohishi W, Mettler FA Jr, Ozasa K, Jacob P, Bab N, Lipshultz SE, Stewart FA, Nabika T, Niwa Y, Takahashi N, Akahoshi M, Kodama K, Shore RE, the International Radiation and Cardiovascular Disease Workshop Participants: A report from the 2013 International Workshop: Radiation and Cardiovascular Disease, Hiroshima, Japan. *J Radiol Prot* 2013 (December); 33(4):869–80. (related to *Life Span Study* and *Cell Biology Study*)

◆ United Nations Scientific Committee on Effects of Atomic Radiation (Kelly GN, Golikov V, Constine LS, Nagel HD, Nosske D, Shore RE): Sources, Effects and Risks of Ionizing Radiation, Volume II. Scientific Annex B: Effects of radiation exposure of children. New York: United Nations; 2013, 269 p. (related to *Life Span Study*)

Adult Health Study Oral Presentations

❖ Tatsukawa Y, Yamada M, Ohishi W, Fujiwara S, Nakanishi S. Effect of regional body fat distribution on development of diabetes. 56th Annual Scientific Meeting of the Japan Diabetes Society, 16–18 May 2013, Kumamoto (related to *Special Clinical Studies*)

❖ Takahashi I. Analyses of the association between arteriosclerosis in microvessels and microvascular damages. 13th Clinical Conference of Blood Pressure and Pulse Wave, 8 June 2013, Osaka (related to *Special Clinical Studies*)

❖ Tatsukawa Y, Yamada M, Fujiwara S, Ohishi W, Nakanishi S. Association between trunk and leg fat and incidence of diabetes. Endocrine Society's 95th Annual Meeting & Expo, 15–18 June 2013, San Francisco, California, USA

❖ Yamada M. Radiation effects on aging: Radiation Effects Research Foundation Adult Health Study. 20th International Association of Gerontology and Geriatrics World Congress Gerontology and Geriatrics, 23–27 June 2013, Seoul, South Korea

❖ Takahashi I, Matsumoto M. Epidemiological studies about hypertension and atherosclerosis—Adult Health Study in Hiroshima and Nagasaki. 9th Meeting for Clinical Studies of Hypertension, 6 July 2013, Sapporo

❖ Hayashi T, Hu Y, Furukawa K, Ohishi W, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Age, smoking, and radiation effects on the production of reactive oxygen species in blood cells of atomic-bomb survivors. 20th Annual Meeting of the Japanese Society of Immunotoxicology, 12–13 September 2013, Tokyo (related to *Immunology Studies*)

❖ Neriishi K, Nakashima E, Minamoto A. Radiation accelerates age of cataract surgery. 59th Annual Meeting of the Radiation Research Society, 15–19 September 2013, New Orleans, Louisiana, USA

❖ Takahashi I, Ohishi W, Kodama K, Matsumoto M. Lifetime risk of stroke and the association between radiation and stroke incidence among the Japanese atomic-bomb survivors. 6th Korea-Japan Joint Stroke Conference, 5–6 October 2013, Osaka

- ❖ Shore RE, Nakashima E, Hida A, Neriishi K. Cataract risk in A-bomb survivors and comparison studies. 5th International MELODI (Multidisciplinary European Low Dose Initiative) Workshop, 7–10 October 2013, Brussels, Belgium (related to *Special Clinical Studies*)
- ❖ Ohishi W, Ueda K, Tatsukawa Y, Nakashima E, Yamada M, Kohata M, Takahashi I, Tsuge M, Chayama K. Effects of lifestyle and insulin resistance on liver fibrosis in nonalcoholic fatty liver disease. 17th Annual Meeting of the Japan Society of Hepatology, 9–12 October 2013, Tokyo (related to *Special Clinical Studies*)
- ❖ Hayashi T, Hu Y, Furukawa K, Ohishi W, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Age and radiation effects on intracellular reactive oxygen species in the blood cells of atomic-bomb survivors and their association with immune-related biomarkers. 56th Annual Meeting of the Japan Radiation Research Society, 18–20 October 2013, Aomori (related to *Immunology Studies*)
- ❖ Neriishi K. Prevalence and incidence of cataract surgery in A-bomb survivors. 67th Annual Congress of Japan Clinical Ophthalmology, 31 October 2013, Yokohama
- ❖ Ohishi W, Ueda K, Tatsukawa Y, Nakashima E, Yamada M, Takahashi I, Tsuge M, Chayama K. Effects of lifestyle, serum adiponectin and radiation on prevalence and progression of nonalcoholic fatty liver disease (NAFLD). 64th Annual Meeting of the American Association for the Study of Liver Diseases, 1–5 November 2013, Washington DC, USA (related to *Special Clinical Studies*)
- ❖ Takahashi I, Yamada M, Tatsukawa Y, Ueda K, Hida A, Matsumoto M, Ohishi W. Impact of weight change following cessation of smoking on the incidence of cardiovascular disease incidence: A 30-year follow-up study of Japanese cohort. American Heart Association Scientific Sessions 2013, 16–20 November 2013, Dallas, Texas, USA
- ❖ Yanagi M, Kiuchi Y, Takahashi I, Misumi M, Neriishi K. Association between glaucoma subtypes and retinal vascular calibers. Hiroshima District Society of Ophthalmology, 1 December 2013, Hiroshima
- ❖ Hu Y, Ohishi W, Yoshida K, Kyoizumi S, Kusunoki Y, Hayashi T. Relationship of reactive oxygen species in blood cells with age, radiation and *IL-6R* gene polymorphisms among atomic-bomb survivors. 42nd Annual Meeting of the Japanese Society for Immunology, 11–13 December 2013, Chiba (related to *Immunology Studies*)
- ❖ Takahashi I. Impact of radiation on cardiovascular disease in the follow-up study of atomic-bomb survivors. 2013 Seminar of Radiation Epidemiology, 19 December 2013, Tokyo (related to *Life Span Study*)
- ❖ Shore RE, Furukawa K, Imaizumi M. Thyroid disease among A-bomb survivors exposed in childhood. International Workshop on Radiation and Thyroid Cancer, 21–23 February 2014, Tokyo (related to *Life Span Study*)

Research Protocols 4-10 (Platform Protocol), A3-12 F₁ Clinical Study

RP 4-10 Longitudinal clinical study of the F₁ offspring of A-bomb survivors

Ohishi W (CH), Fujiwara S, Tatsukawa Y (CH), Furukawa K (S), Hsu W-L, Takahashi N, Satoh Y (G), Kusunoki Y (R), Yamada M (CH), Takahashi I (CH), Ueda K (CH), Hida A (CN), Imaizumi M (CN), Sera N (CN), Haruta D (CN), Grant EJ (EH), Ozasa K (EH), Cologne JB (S), Cullings HM (S), Kodama Y (G), Katayama H (IT), Watanabe T (EH), Nakamura N

Objectives: The objective of this study is to elucidate the effects of parental exposure to A-bomb radiation on the development of polygenic, multifactorial diseases and subclinical conditions among the F₁ offspring.

Background and Significance: The previous cross-sectional F₁ clinical study from 2002 to 2006 provided no evidence for an increased prevalence of adult-onset multifactorial diseases due to parental radiation exposure, but the study subjects were still quite young. The rationale for this study is that definitive human data can be obtained only if a high-quality clinical study is continued until the subjects become elderly, when many multifactorial diseases develop. Self-selection bias also tends to be minimized when prospective longitudinal data are obtained, because such data allow estimates of disease incidence.

Study Methods: This prospective study will conduct quadrennial health examinations for up to 12,500 subjects who responded favorably by mail or telephone to participate in health examinations between May 2000 and November 2008. Multifactorial diseases detected via these examinations will be analyzed in relation to the radiation doses of their parents, taking into consideration confounding factors.

Study Progress: We have started the longitudinal part of the F₁ clinical study in November 2010. We sent brochures that provide an overview of the health examinations to 10,047 potential study subjects for the past year and have contacted them by telephone to request their participation in the health examination. Of those, 7,517 subjects have undergone health examinations, and 78 subjects are due to participate in health examinations (participation rate is 75.6%). Among those who were examined in 2002–06, 82.4% are participating again.

Results and Conclusions: The previous cross-sectional F₁ clinical study provided no evidence of a risk from parental radiation exposure for hypercholesterolemia, hypertension, diabetes, angina, myocardial infarction, or stroke when the various endpoints were examined individually (*J Radiol Prot* 2013; 33:281–93).

RP-A3-12 Continued preservation of frozen fresh thyroid samples obtained from children of A-bomb survivors

Imaizumi M (CN), Ohishi W (CH), Sera N (CN), Hida A (CN), Yamada M (CH), Hamatani K (R), Akahoshi M

Objectives: The purpose of the present study is continued preservation of the existing frozen fresh thyroid tissues from thyroid cancer cases detected among the subjects of the Health Effects Study of the Children of A-bomb Survivors in preparation for future molecular studies.

Background and Significance: Thyroid cancer is one of the cancers affected by radiation among A-bomb survivors. Even though evidence linking parental radiation exposure with solid cancer incidence among the offspring of A-bomb survivors has proved elusive, these offspring are just now entering their cancer-prone years. It is necessary to consider the possibility that future epidemiological research may suggest such effects. Furthermore, mechanisms behind thyroid cancer development are not yet fully understood. Under the present circumstances, preservation of as many frozen thyroid cancer samples as possible is indispensable for future molecular research of thyroid carcinogenesis. At RERF, thyroid ultrasound screening performed in the Health Effects Study of the Children of A-bomb Survivors during the period 2002–2006 detected dozens of cases of thyroid cancer, which were then referred for surgical removal. From among those cases, frozen fresh thyroid samples from 36 cases are currently stored at RERF. Continued preservation of the relevant frozen fresh thyroid samples will be valuable for future molecular research.

Study Methods: We continue to store the frozen fresh thyroid samples using liquid nitrogen. Sample data (research identification number, date of surgery, site [tumor or tumor-free], quantity of sample) have been stored in a database.

Study Progress: We are continuing to store the frozen fresh thyroid samples.

Results and Conclusions: None. This RP is only for continued preservation of frozen fresh samples from thyroid cancer cases detected among the F₁ population in preparation for future molecular research. A research protocol using the samples will be separately prepared in the future.

F₁ Clinical Study Publication

RERF Report (RR)

◆ Tatsukawa Y, Cologne JB, Hsu WL, Yamada M, Ohishi W, Hida A, Furukawa K, Takahashi N, Nakamura N, Suyama A, Ozasa K, Akahoshi M, Fujiwara S, Shore RE: Radiation risk of individual multifactorial diseases in offspring of the atomic-bomb survivors: A clinical health study. *J Radiol Prot* 2013 (June); 33(2):281–93. doi: 10.1088/0952-4746/33/2/281 (RR 28-11) © 2013 IOP Publishing Ltd. (*This abstract was reprinted by permission of IOP Publishing.*)

[Abstract] There is no convincing evidence regarding radiation-induced heritable risks of adult-onset multifactorial diseases in humans, although it is important from the standpoint of protection and management of populations exposed to radiation. The objective of the present study was to examine whether parental exposure to atomic-bomb (A-bomb) radiation led to an increased risk of common polygenic, multifactorial diseases—hypertension, hypercholesterolemia, diabetes mellitus, angina pectoris, myocardial infarction or stroke—in the first-generation (F₁) offspring of A-bomb survivors. A total of 11,951 F₁ offspring of survivors in Hiroshima or Nagasaki, conceived after the bombing, underwent health examinations to assess disease prevalence. We found no evidence that paternal or maternal A-bomb radiation dose, or the sum of their doses, was associated with an increased risk of any multifactorial diseases in either male or female offspring. None of the 18 radiation dose-response slopes, adjusted for other risk factors for the diseases, was

statistically significantly elevated. However, the study population is still in mid-life (mean age 48.6 years), and will express much of its multifactorial disease incidence in the future, so ongoing longitudinal follow-up will provide increasingly informative risk estimates regarding hereditary genetic effects for incidence of adult-onset multifactorial disease.

F₁ Clinical Studies Oral Presentation

❖ Tatsukawa Y, Cologne JB, Yamada M, Ohishi W, Hida A, Furukawa K, Takahashi N, Nakamura N, Ozasa K, Akahoshi M, Fujiwara S, Shore RE. Association between parental radiation exposure and prevalence of multifactorial disease: F₁ clinical study. 54th Late A-bomb Effects Research Meeting, 2 June 2013, Hiroshima

Research Protocols 5-09, 4-09, 3-09, 4-04 and 5-04, 1-03, 4-02, 2-97, 2-90, 7-87, B48-09 and P2-11, B44-06, P1-13

Immunology Studies

RP 5-09 Effects of radiation exposure and aging on hematopoietic stem cells (HSCs) and dendritic cells (DCs)—Analyses of numerical and functional changes

Kusunoki Y (R), Kyoizumi S (R), Kajimura J (R), Yoshida K (R), Hayashi T (R), Geyer SM, Misumi M (S), Ohishi W (CH), Ozasa K (EH), Hirabayashi Y, Iwama A, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng N-P, Murasko D, Seed TM, Nakachi K

Objectives: To delineate the long-term consequences of prior A-bomb irradiation and advancing age on homeostatic control of HSCs and DCs, we will analyze numerical and functional changes within the circulating HSC and DC pools among A-bomb survivors.

Background and Significance: There is accumulating evidence that suggests accelerated immunosenescence in A-bomb survivors. However, the mechanisms of radiation-associated immunosenescence are poorly understood. We hypothesize that radiation exposure induces premature aging of HSCs, resulting in reduced numbers and impaired self-renewal abilities that in turn accelerate loss of lymphoid potential. We also hypothesize that A-bomb irradiation affects innate and adaptive immunity, possibly by altering DC populations toward a T-cell suppressor phenotype. Animal models will also be explored for a better understanding of the reconstitution of hematopoietic and immune systems following radiation-induced damage.

Study Methods: Numerical and functional changes are analyzed in relation to radiation dose within the circulating HSC and DC pools among about 260 AHS subjects in Hiroshima. In order to confirm the results obtained in the A-bomb survivor studies, we have developed a series of *in vitro* assay systems and mouse experimental models to determine the functional and differentiation status of HSC and DC populations following exposure to ionizing irradiation.

Study Progress: Functional analyses of circulating HSCs and DCs among the AHS subjects (target numbers: 258 and 259 for HSC and DC studies, respectively) have nearly been completed. Based on results from HSC analyses among in-house volunteers, we submitted one manuscript on the linkage between DC and T-cell commitments in human circulating HSCs.

Results and Conclusions: In our HSC assay system, DC precursor frequencies (PFs) correlated with T-cell PFs but not with NK-cell PFs, suggesting a linkage between DC and T-cell potentials in circulating HSCs.

RP 4-09 Effects of ionizing radiation exposure and aging on vaccination responses

Hayashi T (R), Kusunoki Y (R), Yoshida K (R), Ito R (R), Ohishi W (CH), Ozasa K (EH), Geyer SM, Hirabayashi Y, Iwama A, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng N-P, Murasko D, Seed TM, Nakachi K

Objectives: It is important to examine whether the radiation-impaired immune system modifies health-related status, in this case the vaccine response, in aging A-bomb survivors. The purpose of this study is to evaluate the effects of prior A-bomb radiation exposure on the immunological capacity of aging individuals to respond to influenza vaccination.

Background and Significance: RERF's epidemiology and clinical studies have long indicated increased risks of age-related and immune/inflammation-related diseases among A-bomb survivors. Further, the noted radiation effects on the immune system are similar to those associated with aging.

Study Methods: On the basis of a pilot study in FY2010 with 50 AHS subjects, full-scale studies were conducted in FY2011 and FY2012 with a total of 300 additional AHS subjects, who were selected by stratified random sampling of dose and age groups and gender. The primary endpoint is change in anti-influenza virus antibody titer levels before and three weeks after influenza vaccination. Secondary endpoints include plasma levels of cytokines and inflammation-related proteins, lymphocyte subsets, and intracellular activation markers. The responses of peripheral blood mononuclear cells (PBMCs) to stimulation following 24 and 96 hours culture time were also determined by comparing cytokine concentrations in supernatants between stimulated and unstimulated cells.

Study Progress: We analyzed PBMC supernatant cytokine concentration data for the FY2011 full-scale study. This sample set was derived from 156 AHS subjects before and three weeks after flu immunization. The effects of age, gender, and radiation dose were evaluated relative to the noted cytokine responses to stimulation and/or vaccination.

Results and Conclusions: Age group (below or above 75) had a significant effect on post-vaccination IL-1RA, RANTES, and IL-5. Gender significantly affected IL-2 in pre-vaccination and MIP-1b in post-vaccination. Regarding the changes in cytokine concentrations before and after vaccination, age group exerted significant effects on the changes of MIP-1b, IL-5, IL-15, and IL-7 when the ratios (post-vaccination/pre-vaccination) were used. On the other hand, gender exerted significant effects on the changes of MIP-1b, IP-10, IL-6, IL-1RA, IL-8 when the differences (post-vaccination – pre-vaccination) were used. Radiation dose significantly affected the changes (ratios) of GM-CSF and IL-4.

RP 3-09 Development of an integrated scoring system for human immune competence as it relates to age and ionizing radiation

Hayashi T (R), Hu Y (R), Kusunoki Y (R), Yoshida K (R), Ito R (R), Ohishi W (CH), Ozasa K (EH), Furukawa K (S), Geyer SM, Hirabayashi Y, Iwama A, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng N-P, Murasko D, Seed TM, Nakachi K

Objectives: The objective of this study is to develop an integrated scoring system for evaluating the immunological and inflammatory status of individuals as a function of age and radiation dose, and predicting the effects of radiation on the immune system and somatic mutation in exposed subjects.

Background and Significance: This immunology study, unique to RERF, consists of repeated observations of various immunological parameters in A-bomb survivors with long-term follow-up, which may demonstrate significant radiation-related alterations in the immune system among the survivors, even 65 years after the atomic bombings.

Study Methods: A cross-sectional analysis includes about 3,600 Hiroshima AHS subjects. Measurements of numerous immunological and inflammation-related markers such as blood inflammatory cytokines and lymphocyte subsets are underway. A longitudinal analysis also is being conducted on a subset of 300 selected AHS subjects. Plasma biomarkers are measured using cytokine multiplex assays on two sets of samples collected from the 300 AHS subjects 10 years apart. Telomere length assays are also underway using two sets of DNA samples similarly collected from the same AHS subjects. The results will be utilized to construct an integrated scoring system.

Study Progress: In the cross-sectional analysis, we investigated the effects of age and radiation exposure on reactive oxygen species (ROS) levels in the blood cells obtained from 2,789 Japanese A-bomb survivors. H_2O_2 levels in monocyte and granulocyte fractions increased with age ($P < 0.001$) but not with radiation dose. No effects of age and radiation dose on H_2O_2 levels were observed for T-cell subsets. On the other hand, O_2^- levels in both lymphocyte and granulocyte fractions increased with age and radiation dose ($P < 0.05$). In addition, O_2^- levels in T cells, especially in $CD8^+$ T cells, increased with age and radiation dose ($P < 0.05$).

Results and Conclusions: ROS levels in selected types of immune cells are likely affected by age and radiation. Intracellular ROS may be a useful marker to estimate risk of inflammation-related disease development.

RP 4-04 Relationship between cancer development and genetic polymorphisms among A-bomb survivors, focusing on immune-related genes
RP 5-04 Identification of cancer-related gene polymorphisms and immunological markers (Addendum to RP 4-04)

Hayashi T (R), Hu Y (R), Cologne JB (S), Ito R (R), Ohishi W (CH), Kusunoki Y (R), Yoshida K (R), Hida A (CN), Nakachi K

Objectives: This study aims to assess whether immunogenetic backgrounds of A-bomb survivors affect susceptibility to cancer.

Background and Significance: Epidemiological studies have demonstrated long-lasting impacts of A-bomb radiation on the incidence/mortality of inflammation-related cancers such as gastrointestinal cancer. Although enhanced inflammation has been consistently observed among A-bomb survivors, roles of inflammatory responses in radiation carcinogenesis are not understood. Therefore, we are investigating relationships among risks of radiation-associated cancers, individuals' genetic backgrounds, and radiation exposure. Since we observed that the slope of the radiation dose response curve of *glycophorin A* (GPA) mutation frequency was significantly higher in the cancer group than in the cancer-free group, relationships between somatic mutability and individual genetic backgrounds are

being also examined.

Study Methods: Using DNA from 4,690 A-bomb survivors including 1,359 cancer cases, we have been conducting a series of case-cohort and case-control studies of various radiation-associated cancers in terms of polymorphisms of immune/inflammation-related genes and other cancer-associated genes such as DNA repair genes and drug-metabolizing enzyme genes, which are also known to be involved in inflammatory responses. In this study, risks of cancers are evaluated as functions of both radiation dose and genotypes.

Study Progress: We examined the association between risk of gastric cancer (93 intestinal-type and 96 diffuse-type) and radiation as a function of *IL-10* haplotypes (comprising major haplotype allele *IL10-ATTA* and minor haplotype allele *IL10-GGCG*) to investigate inter-individual differences in gastric cancer susceptibility associated with radiation exposure. The relative risk (RR) of intestinal-type gastric cancer among A-bomb survivors harboring the minor homozygote was significantly high, (2.2, 95% CI: 1.1–4.3), compared to those harboring the major homozygote. However, no significant association was observed between radiation exposure and intestinal-type gastric cancer. The *IL-10* minor haplotype allele was not a risk factor for diffuse-type gastric cancer. The excess relative risk (ERR) per Gy of radiation exposure by haplotype showed statistical significance only for the major homozygote (0.46/Gy, 95% CI: 0.02–1.4). On the other hand, the ERR per Gy for the minor homozygote was close to 0, with no statistical significance. Therefore, the *IL-10* minor haplotype allele might act to suppress the radiation-associated risk elevation of diffuse-type gastric cancer. There were distinguishable dose-response curves of GPA mutant frequencies by *p53-binding protein 1* haplotypes.

Results and Conclusions: *IL-10* haplotypes may be associated with the development of radiation-related diffuse-type gastric cancer. Immune/inflammation-related gene polymorphisms may potentially be involved in inter-individual differences in radiation-related cancer risks. Somatic mutability following radiation exposure may be partly dependent on individual genetic backgrounds, specifically related to DNA double-strand break repair.

RP 1-03 A study of gene polymorphisms and their possible role in the development of diabetes mellitus in the Adult Health Study population

Hayashi T (R), Hu Y (R), Nakashima E (S), Tatsukawa Y (CH), Ohishi W (CH), Yoshida K (R), Kusunoki Y (R), Hida A (CN), Nakachi K

Objectives: This study aims to assess the effect of radiation and various genetic factors on the development of diabetes mellitus (DM) in the AHS cohort, and determine whether differences in frequencies of any particular genotypes between Hiroshima and Nagasaki survivors may account for why a significant association between risk of DM and radiation dose is observed in A-bomb survivors in Hiroshima but not Nagasaki.

Background and Significance: Although early studies did not show associations between radiation exposure and DM, data on AHS subjects in 1992–1994 indicated a significant positive radiation-DM association in Hiroshima

but not in Nagasaki, after adjusting for gender, age, and body mass index. This somewhat puzzling finding may reflect genetic differences between the Hiroshima and Nagasaki populations. Our preliminary results suggest that radiation may persistently impair the immune system and that the radiation-DM association may be especially relevant to a sub-group of A-bomb survivors who have a specific *HLA* class II haplotype.

Study Methods: We investigated the relationships between *HLA*-related genetic factors, risk of DM, and radiation dose in the AHS subjects in a case-control study. A total of 3,242 AHS subjects, comprising DM cases (569 in Hiroshima and 307 in Nagasaki) and controls (1,138 in Hiroshima and 1,228 in Nagasaki), were selected by the Department of Statistics and genotyped for *HLA* and *HLA*-related gene polymorphisms by the Department of Radiobiology/Molecular Epidemiology. **Study Progress:** We have determined *DRB1/DQB1/DQA1, A/B/C*, and *MICA/MICB* genotypes for 864, 795, and 790 DM cases and 2,187, 2,127, and 2,152 control subjects, respectively.

Results and Conclusions: Data analysis and genotyping are underway. Results will be obtained in 2015.

RP 4-02 Perturbation of T-cell homeostasis in atomic-bomb survivors

Yoshida K (R), Kajimura J (R), Hayashi T (R), Ohishi W (CH), Nakashima E (S), Kodama Y (G), Nakachi K, Kusunoki Y (R)

Objectives: In this study we are testing the hypothesis that radiation exposure may perturb T-cell homeostasis, consequently elevating risks of various diseases.

Background and Significance: Perturbed homeostasis of the T-cell immune system is believed to be a primary cause of increased morbidity from selected infectious and inflammation-related diseases, which include both many acute and chronic diseases. So far, we have found four dose-dependent changes in T-cell profiling of A-bomb survivors: 1) reduced sizes of both naïve CD4 and naïve CD8 T-cell populations; 2) decreased repertoire of T-cell receptors in memory CD4 T cells; 3) increased percentages of functionally weak memory CD4 T cells; and 4) inverse associations between the relative frequency of naïve CD4 T cells and plasma levels of inflammatory cytokines. These results are consistent with the above hypothesis. We will further examine relationships between changes in T-cell profiles and subsequent disease development. We anticipate that this study will contribute to determining how, and to what degree, such T-cell immunity perturbations are involved in disease development in A-bomb survivors.

Study Methods: Among the AHS subjects, we are analyzing 1) the percentages of peripheral blood lymphocyte subsets by flow cytometry, 2) the numbers of T-cell receptor excision circles (TRECs) in CD4 and CD8 T-cell fractions by real-time PCR, 3) the average telomere lengths of both naïve and memory T-cell populations by flow fluorescent *in situ* hybridization (FISH), and 4) associations between the T-cell parameters and clinical outcome such as disease development.

Study Progress: TREC-bearing T-cell numbers, an indicator of individually differing capacity to produce new T cells, were measured among about 1,000 A-bomb survivors

in recent blood samples. The average telomere lengths of T lymphocytes in individual survivors have also been measured. Statistical analysis of radiation effects on TREC numbers was performed, and a manuscript has been submitted.

Results and Conclusions: TREC-bearing T-cell numbers clearly decreased with age, positively correlating with naïve T-cell numbers. We, however, found that TREC numbers were not associated with radiation dose, but were inversely associated with obesity indicators and obesity-related diseases such as diabetes mellitus and fatty liver, suggesting a notable relationship of obesity with T-cell homeostasis.

RP 2-97 Lyophilization of blood samples to be obtained from Adult Health Study subjects in Hiroshima and Nagasaki for later DNA extraction (Addendum to RP 2-90)

Hayashi T (R), Hu Y (R), Yoshida K (R), Kusunoki Y (R), Ohishi W (CH), Hida A (CN), Nakachi K

Objectives: The purpose of this study is to supplement RP 2-90 by providing a means of storing DNA from stock sources that otherwise would be wasted so that DNA would be available for multiple small-scale molecular analyses (i.e., biospecimens from RP 2-90).

Background and Significance: In studying the late effects of radiation exposure in A-bomb survivors, analyses of gene alterations due to radiation are essential in investigations of genomic instability, genetic susceptibility, and molecular oncology. With new technologies, investigations can now be conducted with extremely small amounts of DNA.

Study Methods: Blood samples, destined for DNA extraction and subsequent molecular analyses, are lyophilized and stored for use in current and future studies.

Study Progress: We have thus far cryopreserved 23,332 and 14,402 blood sample vials from AHS participants in Hiroshima and Nagasaki, respectively, including the expanded AHS participants who were exposed to radiation at the age of nine or younger. The PCR amplification of DNA extracted from laboratory control blood stored at -80°C for 16 years on paper showed no recognizable degradation of DNA after the long-term storage.

Results and Conclusions: This project provides a valuable resource for future molecular and genetic studies of radiation effects.

RP 2-90 Cryopreservation of blood cells from Hiroshima and Nagasaki Adult Health Study participants

Hayashi T (R), Hu Y (R), Kusunoki Y (R), Yoshida K (R), Hida A (CN), Ohishi W (CH), Nakachi K

Objectives: The purpose of this study is to cryopreserve viable blood mononuclear cells from all AHS participants to ensure that appropriate materials will be available for ongoing studies of the late effects of human exposure to A-bomb radiation as well as future analyses using advanced techniques.

Background and Significance: Various aspects of the medical effects of human exposure to A-bomb radiation have been studied at ABCC-RERF. It is reasonable to expect that technological improvements in analytical methodologies will

continue and that studies of effects that were previously not practical will become feasible in the future.

Study Methods: Peripheral blood mononuclear cells (PBMCs) are separated from 4 ml of heparinized blood using the Ficol/Hypaque density gradient centrifugation technique. PBMCs are preserved in a tank of liquid nitrogen.

Study Progress: We have cryopreserved blood cells from Hiroshima and Nagasaki AHS participants. We have also collected lymphocyte samples from the expanded group of A-bomb survivors since October 2008 (those who were exposed to radiation at the age of nine years or younger).

Results and Conclusions: In FY2013, we have cryopreserved blood cells from 1,049 AHS participants from Hiroshima and 807 from Nagasaki, including the expanded group members who were exposed to radiation at the age of nine years or younger. We confirmed that the viability of cryopreserved cells was more than 80% and that thawed lymphocytes expressed normal surface antigens and immunological functions.

RP 7-87 X-ray radiosensitivity of lymphocytes *in vitro* from A-bomb survivors. Part 3: Transformation of B cells by Epstein-Barr virus and their cryopreservation (Addendum to RP 3-86)

Hayashi T (R), Hu Y (R), Kusunoki Y (R), Yoshida K (R), Hida A (CN), Ohishi W (CH), Nakachi K

Objectives: The initial purpose of this project was to cryopreserve Epstein-Barr virus (EBV)-transformed B-cell lines from high-dose and control survivors for new cell biology studies, e.g., those dealing with radiosensitivity. The resulting B-cell lines are also of considerable use in studying radiation effects related to immune functions and the role of genetic backgrounds underlying disease development.

Background and Significance: According to the recommendation of the Multinational Peer Review Panel for the radiobiology program (1998), we have been cryopreserving EBV-transformed B-cell lines from high-dose (≥ 1 Gy) and control A-bomb survivors for molecular epidemiological, immunological, and other genomic and proteomic studies. About 500 samples that overlapped with the F₁ study have been immortalized and are being stored in the Department of Genetics.

Study Methods: The AHS subjects for this study are the high dose (≥ 1 Gy) and control (< 0.005 Gy) groups. The total number of participants in those groups is about 3,500 in Hiroshima and Nagasaki. Peripheral blood lymphocytes from A-bomb survivors are being transformed by EBV and cryopreserved in liquid nitrogen.

Study Progress: We have completed EBV transformation of mononuclear cells from 2,743 AHS subjects.

Results and Conclusions: The immortalization of lymphocytes from Hiroshima AHS subjects is nearing completion (1,887 subjects) and lymphocytes from 856 Nagasaki AHS participants have also been successfully transformed. About 2,500 samples have been distributed to both Hiroshima and Nagasaki storage facilities.

RP-B48-09 Evaluation of the existence and utility of the thymus autopsy specimens available in the RERF tissue archives for future analyses of thymus architecture and function in response to aging and exposure to ionizing radiation

RP-P2-11 Evaluation of the existence and utility of the thymus autopsy specimens that are available from LSS subjects who died at 70 or more years old in the RERF tissue archives and that are available from LSS subjects in Hiroshima University Hospital (Addendum to RP-B48-09)

Yoshida K (R), Kusunoki Y (R), Ozasa K (EH), Ito R (R), Kajimura J (R), Kyoizumi S (R), Hayashi T (R), Misumi M (S), Arihiro K, Geyer SM, Sempowski GD, Manley NR, van den Brink MRM, Double EB, Nakachi K

RP-B44-06 Establishment of new assay systems to facilitate evaluation of genetic instability in human hematolymphoid cells by flow cytometry

Kusunoki Y (R), Kajimura J (R), Hamasaki K (G), Yoshida K (R), Hayashi T (R), Imai K, Furukawa K (S), Cologne JB (S), Nakachi K

RP-P1-13 Establishment of methods for analyzing transcriptome in human blood cells using high-throughput sequencing

Yoshida K (R), Kyoizumi S (R), Hayashi T (R), Kajimura J (R), Misumi M (S), Matsui H, Kanai A, Inaba T, Ikee K, Gojobori T, Nakachi K, Kusunoki Y (R)

Immunology Studies Publications

RERF Reports (RR)

◆ Hayashi T, Ito R, Cologne JB, Maki M, Morishita Y, Nagamura H, Sasaki K, Hayashi I, Imai K, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Ohishi W, Fujiwara S, Akahoshi M, Nakachi K: Effects of *IL-10* haplotype and atomic bomb radiation exposure on gastric cancer risk. *Radiat Res* 2013 (July); 180(1):60–9. (RR 11-12) © 2013 by Radiation Research Society

[Abstract] Gastric cancer (GC) is one of the cancers that reveal increased risk of mortality and incidence in atomic bomb survivors. The incidence of gastric cancer in the Life Span Study cohort of the Radiation Effects Research Foundation (RERF) increased with radiation dose (gender-averaged excess relative risk per Gy = 0.28) and remains high more than 65 years after exposure. To assess a possible role of gene-environment interaction, we examined the dose response for gastric cancer incidence based on immunosuppression-related *IL-10* genotype, in a cohort study with 200 cancer cases (93 intestinal, 96 diffuse, and 11 other types) among 4,690 atomic bomb survivors participating in an immunological substudy. Using a single haplotype block composed of four haplotype-tagging SNPs (comprising the major haplotype allele *IL-10-ATTA* and the minor haplotype allele *IL-10-GGCG*, which are categorized by *IL-10* polymorphisms at -819A>G and -592T>G, +1177T>C and +1589A>G), multiplicative and additive models for joint effects of radiation and this *IL-10* haplotyping were examined. The *IL-10* minor haplotype allele(s) was a risk factor for intestinal type gastric cancer but not for diffuse type

gastric cancer. Radiation was not associated with intestinal type gastric cancer. In diffuse type gastric cancer, the haplotype-specific excess relative risk (ERR) for radiation was statistically significant only in the major homozygote category of *IL-10* (ERR = 0.46/Gy, $P = 0.037$), whereas estimated ERR for radiation with the minor *IL-10* homozygotes was close to 0 and nonsignificant. Thus, the minor *IL-10* haplotype might act to reduce the radiation related risk of diffuse-type gastric cancer. The results suggest that this *IL-10* haplotyping might be involved in development of radiation-associated gastric cancer of the diffuse type, and that *IL-10* haplotypes may explain individual differences in the radiation-related risk of gastric cancer.

◆ Kyoizumi S, Kubo Y, Kajimura J, Yoshida K, Imai K, Hayashi T, Nakachi K, Young LF, Moore MA, van den Brink MRM, Kusunoki Y: Age-associated changes in the differentiation potentials of human circulating hematopoietic progenitors to T- or NK-lineage cells. *J Immunol* 2013 (June); 190(12):6164–72. (RR 13-12) © The Journal of Immunology (This abstract was reprinted by permission of The Journal of Immunology.)

[Abstract] Age-associated changes of T and NK cell (T/NK) potential of human hematopoietic stem cells are unknown. In this study, we enumerate and characterize T/NK precursors among CD34⁺Lin⁻ cell populations circulating in normal human adult peripheral blood (PB) by a limiting-dilution assay using coculture with OP9-DL1 stroma cells expressing Notch 1 ligand, Delta-like 1. The frequency of T cell precursors in CD34⁺Lin⁻ cells was found to decrease with donor age, whereas the ratio of NK to T cell precursor frequency (NK/T ratio) increased with age, suggesting that lymphoid differentiation potential of PB progenitors shifts from T to NK cell lineage with aging. Clonal analyses of CD34⁺Lin⁻ cells showed that differences in the NK/T ratio were attributable to different distributions of single- and dual-lineage T/NK precursor clones. Because nearly all of the clones retained monocyte and/or granulocyte differentiation potentials in coculture with OP9-DL1 cells, T/NK precursors in PB are considered to be contained in the pool of T/NK/myeloid multipotent progenitors. The age-associated increase in NK over T cell commitment might occur in precursor cells with T/NK/myeloid potential.

◆ Yoshida K, Kusunoki Y, Cologne JB, Kyoizumi S, Maki M, Nakachi K, Hayashi T: Radiation-dose response of *glycophorin A* somatic mutation in erythrocytes associated with gene polymorphisms of *p53 binding protein 1*. *Mutat Res-Gen Tox En* 2013 (July); 755(1):49–54. (RR 1-13) © 2013 Elsevier B.V. (This abstract was reprinted by permission of Elsevier.)

[Abstract] Information on individual variations in response to ionizing radiation is still quite limited. Previous studies of atomic-bomb survivors revealed that somatic mutations at the *glycophorin A* (*GPA*) gene locus in erythrocytes were significantly elevated with radiation exposure dose, and that the dose response was significantly higher in survivors with subsequent cancer development compared to those without cancer development. Noteworthy in these studies were great inter-individual differences in *GPA* mutant fraction even in persons with similar radiation

doses. It is hypothesized that persistent *GPA* mutations in erythrocytes of atomic-bomb survivors are derived from those in long-lived hematopoietic stem cell (HSC) populations, and that individual genetic backgrounds, specifically related to DNA double-strand break repair, contribute to individual differences in HSC mutability following radiation exposure. Thus, we examined the relationship between radiation exposure, *GPA* mutant fraction in erythrocytes, and single nucleotide polymorphisms (SNPs) of the key gene involved in DNA double-strand break repair, *p53 binding protein 1* (*53BP1*). *53BP1* SNPs and inferred haplotypes demonstrated a significant interaction with radiation dose, suggesting that radiation-dose response of *GPA* somatic mutation is partly dependent on *53BP1* genotype. It is also possible that *53BP1* plays a significant role in DNA double-strand break repair in HSCs following radiation exposure.

◆ Yoshida K, Nakashima E, Kubo Y, Yamaoka M, Kajimura J, Kyoizumi S, Hayashi T, Ohishi W, Kusunoki Y: Inverse associations between obesity indicators and thymic T-cell production levels in aging atomic-bomb survivors. *PLoS ONE* 2014 (March); 9(3):e91985. (RR 8-13) © Yoshida et al.

[Study Findings] T-cell receptor excision circles, or TRECs, are circular molecules of DNA excised from chromosomes when T cells are produced in the thymus. They indicate new production of T cells, and their numbers, which decrease with aging, represent an immunological marker of thymic capacity to produce naïve T cells. In this study, low TREC numbers were found to be associated with diseases related to high body mass index (BMI) and obesity, such as diabetes and fatty liver. These results suggest the possibility that reduction of immunological competence associated with aging may be further accelerated by obesity.

[Explanation] The production of T cells, which are blood cells with immunological functions, starts in the bone marrow and then continues in the thymus. In previous immunological studies at the Radiation Effects Research Foundation (RERF), the number of naïve T cells in peripheral blood was found to decrease after radiation exposure, leading to a hypothesis describing the mechanism behind this decline as the effect of radiation exposure on the ability of the thymus to produce naïve T cells. Based on animal model studies conducted in recent years, obesity has been linked to the functional decline of the T-cell immune system associated with aging. However, findings based on studies of human populations were extremely limited. We therefore conducted a study of RERF's Adult Health Study (AHS) participants, whose health has been followed for many years, in order to determine the effects of age, radiation dose, and obesity on indicators of thymic capacity to produce naïve T cells (TREC number). **Objectives** The objectives of the study were to examine the relationship between radiation exposure and TREC number, an indicator of thymus capacity to produce naïve T cells, and to determine the association of TREC number to obesity indicators and diseases associated with obesity, after adjusting for the effects of age, sex, radiation exposure, alcohol consumption, and smoking. **Methods** Among 1,073 A-bomb survivors who participated in the AHS from 2003 to 2009, we measured TREC number per 10,000 CD4

T cells (helper T cells, which control immune response) and per 10,000 CD8 T cells (killer T cells, which detect and destroy infected or transformed cells) in peripheral blood, utilizing polymerase chain reaction. We used information obtained from the AHS on BMI as an indicator of obesity, total cholesterol level, HbA1c level (which represents blood glucose levels over the previous 2–3 months), and CRP level (an indicator of inflammation), as well as diseases related to obesity (type 2 diabetes, fatty liver, and hypertension), alcohol consumption, and smoking. We conducted linear regression statistical analysis, with adjustment made for age, sex, radiation dose, amount of alcohol consumed, and number of cigarettes smoked.

Results (1) Relationship between TREC number and age: Subject ages ranged from 58 to 109 years. Even in this elderly population, TREC number in CD4 and CD8 T cells decreased with age ($p < 0.001$ in terms of significance). (2) Relationship between TREC number and radiation dose: Radiation exposure had no effect on TREC number in either CD4 or CD8 T cells. We also conducted a preliminary analysis by selecting, from among the 1,073 subjects, a high-dose group (radiation dose of at least 1 Gy) and a control group (radiation dose of less than 5 mGy), matched by such factors as age and sex, and then comparing the two groups. No difference in TREC number was observed in relation to radiation dose. (3) Association between TREC number and obesity indicators: TREC number in CD4 and CD8 T cells significantly decreased with an increase of HbA1c and CRP levels ($p < 0.05$). TREC number also tended to be inversely associated with BMI. Further, TREC number was small in cases of diabetes and fatty liver ($p < 0.05$). In this study, no association between TREC number and radiation exposure was observed, but TREC number was found to decrease with an increase in the levels of obesity indicators in a human population. This result provides evidence to suggest that obesity may accelerate immunological aging in humans. Because obesity is known to increase the risk of several age-related diseases, attenuated immune competence due to the reduction in naïve T-cell production may be one of the developmental mechanisms of diseases associated with obesity.

Other Journal Publication

◆ Ohishi W, Fujiwara S, Chayama K: Study of viral hepatitis in a longitudinal cohort of A-bomb survivors. Elucidation of viral hepatitis based on a cohort study. Tokyo: Medical Review; 2013, pp 86–95. (Japanese) (related to *Special Clinical Studies* and *Special Cancer Studies*)

Manuscript in Press

⌘ Kyoizumi S, Kubo Y, Kajimura J, Yoshida K, Hayashi T, Nakachi K, Young LF, Moore MA, van den Brink MRM, Kusunoki Y: Linkage between dendritic and T cell commitments in human circulating hematopoietic progenitors. *J Immunol*.

Immunology Studies Oral Presentations

❖ Yoshida K, Kubo Y, Yamaoka M, Hayashi T, Ohishi W, Kusunoki Y. Decreased recent thymic emigrant number and shortened telomere length in obese A-bomb survivors. 42nd

Annual American Aging Association Meeting, 31 May–3 June 2013, Baltimore, Maryland, USA

❖ Hayashi T, Cologne JB, Yoshida K, Kyoizumi S, Kajimura J, Kusunoki Y, Nakachi K. Genetic susceptibility to radiation-associated colon and rectum cancers among atomic-bomb survivors. 39th Annual Meeting of Korean Cancer Association, 13–14 June 2013, Seoul, South Korea

❖ Hayashi T. Molecular epidemiology study of cigarette smoking effects on immunity- and inflammation-related biomarkers and lifestyle-related disease development. FY2012 Research Meeting of Smoking Research Foundation, 23 July 2013, Tokyo

❖ Hayashi T, Ohishi W, Imai K, Yoshida K, Hayashi I, Hu Y, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Immunogenetic factors of chronic hepatitis C and hepatocellular carcinoma development observed in A-bomb survivors. 15th International Congress of Immunology, 22–27 August 2013, Milan, Italy

❖ Hayashi T, Hu Y, Furukawa K, Ohishi W, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Age, smoking, and radiation effects on the production of reactive oxygen species in blood cells of atomic-bomb survivors. 20th Annual Meeting of the Japanese Society of Immunotoxicology, 12–13 September 2013, Tokyo (related to *Adult Health Study*)

❖ Hayashi T, Cologne JB, Yoshida K, Kyoizumi S, Kajimura J, Kusunoki Y, Nakachi K. Effects of *CD14* and *IL18* gene polymorphisms on radiation-associated colon and rectum cancer risks among atomic-bomb survivors. 22nd Annual Meeting of the Japanese Society for Histocompatibility and Immunogenetics, 14–16 September 2013, Fukushima

❖ Hayashi T, Kyoizumi S, Kusunoki Y, Nakachi K. Effects of aging and radiation exposure on intracellular ROS levels in atomic-bomb survivors. 72nd Annual Meeting of the Japanese Cancer Association, 3–5 October 2013, Yokohama

❖ Hayashi T, Hu Y, Furukawa K, Ohishi W, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Age and radiation effects on intracellular reactive oxygen species in the blood cells of atomic-bomb survivors and their association with immune-related biomarkers. 56th Annual Meeting of the Japan Radiation Research Society, 18–20 October 2013, Aomori (related to *Adult Health Study*)

❖ Hu Y, Ohishi W, Yoshida K, Kyoizumi S, Kusunoki Y, Hayashi T. Relationship of reactive oxygen species in blood cells with age, radiation and *IL-6R* gene polymorphisms among atomic-bomb survivors. 42nd Annual Meeting of the Japanese Society for Immunology, 11–13 December 2013, Chiba (related to *Adult Health Study*)

❖ Kyoizumi S, Yoshida K, Hayashi T, van den Brink MRM, Kusunoki Y. Linkage between dendritic-cell commitment and bifurcation into T- or NK-cell lineage in human circulating hematopoietic progenitors. 42nd Annual Meeting of the Japanese Society for Immunology, 11–13 December 2013, Chiba

Research Protocols 3-11, 3-10, 2-10, 6-08, 4-08, 3-05, 2-05, 1-05, 2-99, 9-92, 5-92, 3-89, A1-14, A5-13, A4-13, A3-13, A7-12, A4-12, A2-12, A1-12, A6-11, A4-11, A1-10, A5-09, A14-08, A13-08, A10-08

Special Clinical Studies

RP 3-11 Radiation exposure in children and *in utero* survivors of the Atomic Bombing of Hiroshima and Nagasaki and late-life neurocognitive function

Yamada M (CH), Hida A (CN), Kasagi F, Cologne JB (S), Landes R (S), Nagano Y, Matsumoto M, Tsujino A, Mimori Y, Sasaki H, Nakamura S, Krull KR

Objectives: Objectives, are (1) to examine associations between radiation exposure at ages 0–12 or *in utero* and neurocognitive function in late life as assessed by neuropsychological examinations, (2) to investigate the effects of sex, age, attained education, lifestyle, and comorbidity on neurocognitive function as risk factors or modifiers of radiation effects, and (3) to collect baseline data on cognitive function for the investigation of longitudinal progression in cognitive decline and the occurrence of dementia with advancing age.

Background and Significance: The atomic bomb study and various studies of childhood radiotherapy have shown that the brain is susceptible to radiation damage *in utero* and in early childhood. The proposed study is a unique opportunity to examine the prenatal and early childhood effects of radiation exposure on cognitive function as it appears more than 65 years later.

Study Methods: Study subjects are survivors exposed at ages 0–12 or *in utero*. We will evaluate neurocognitive function for about 1,050 subjects in Hiroshima and Nagasaki using the Cognitive Abilities Screening Instrument (CASI) and the Childhood Cancer Survivors Study (CCSS) Neurocognitive Questionnaire (NCQ) during the period 2011–2015. For the CASI, assessment is based on face-to-face interviews by trained nurse technicians during routine health examinations in the AHS. The self-administered NCQ will be conducted by mail survey.

Study Progress: Evaluation of neurocognitive function using CASI and NCQ was started in 2011. About 1,000 subjects have been investigated using CASI. About 1,300 subjects have answered the NCQ.

Results and Conclusions: None yet.

RP 3-10 Ophthalmologic follow-up study in Atomic-bomb survivors (Addendum to RP 3-00)

Hida A (CN), Tatsukawa Y (CH), Neriishi K, Yokoyama T, Takamatsu M, Yanagi M, Kumagami T, Uematsu M, Tsuiki E, Fukazawa Y, Minamoto A, Kiuchi Y, Kitaoka T, Nakashima E (S), Ohishi W (CH), Akahoshi M

Objectives: This addendum study proposes to investigate unanswered questions in the previous ophthalmologic study based on RP 3-00, (1) whether or not radiation-induced cataracts progress with time and (2) whether or not there is a dose response when cataracts are assessed by a radiation-specific classification system (the Merriam-Focht system).

Background and Significance: The ophthalmologic study conducted from 2000 to 2002 based on RP 3-00 had revealed a statistically significant dose response with

posterior subcapsular and cortical cataracts. The study also suggested a low or no dose threshold. However, important research questions still remain unanswered.

Study Methods: (1) Subjects will be those who were age 13 or less at the time of the bombs. (2) The Lens Opacity Classification System II and the Merriam-Focht system will be used for grading by the ophthalmologists. (3) A variety of potential confounding factors will be incorporated in the analysis. (4) Digital computer images of the lens and retina will be stored.

Study Progress: The ophthalmologic examinations of 468 participants in Hiroshima and 235 participants in Nagasaki were completed by March 2013. It was learned that the digital images of the lens were inadequate for grading lens opacity changes over time because of limited standardized examination methodology, image quality, and the fact that retro-illumination photos were not taken using a retro-illumination type camera. Those factors also limited the comparison of the two opacity scoring systems.

Results and Conclusions: None yet.

RP 2-10 Evaluation of retinal arteriolosclerosis and age-related macular degeneration using stored retina images with standardized measurements in relation to glaucoma development in atomic bomb survivors and to association with aortic arteriosclerosis (Addendum to RP 1-05)

Takahashi I (CH), Yanagi M, Misumi M (S), Itakura K, Kawasaki R, Nakashima E (S), Yokoyama T, Takamatsu M, Kinoshita H, Tsuiki E, Uematsu M, Kumagami T, Kiuchi Y, Kitaoka T, Fujiwara S, Hida A (CN), Akahoshi M, Neriishi K

Objectives: To investigate if retinal arteriolosclerosis is associated with radiation dose and is involved as an intermediate risk factor in radiation-associated glaucoma.

Background: Retinal vessel caliber is a well-established, non-invasive marker of microvascular disorders, which may contribute to the initiation and/or progression of radiation-induced cardiovascular diseases. Furthermore, alterations of ocular perfusion could cause ischemia and poor perfusion of the optic disc with subsequent glaucomatous damage. Preliminary analyses of the glaucoma study during 2006–2008 suggested a relationship between radiation and an increased prevalence of normal-tension glaucoma among A-bomb survivors. To investigate the pathological background of glaucoma, we planned to calculate retinal vessel diameters, which are possibly involved in glaucoma genesis through eye-circulation impediment. In addition, we will be able to investigate the relationship between radiation and age-related macular degeneration (AMD), which will be assessed using the same retinal images.

Study Methods: This is a cross-sectional study of AHS subjects for whom retinal photography was performed during 2006–2008. Vessel calibers and AMD were assessed from the digitized retinal images at the University of Melbourne using a computer-assisted program.

Study Progress: Well-trained graders at the Melbourne (Australia) ophthalmology center have calculated the width of retinal arterial and venous vessels and independently graded macular degeneration using a semi-automated computer program. All grading was performed blinded with respect to radiation dose. First, we analyzed smoking effects

on retinal vessel calibers and published the findings (*Invest Ophthalmol Vis Sci* 2014; 55(1):405–11). Retinal vessel calibers are also being analyzed in relation to radiation dose. The macular degeneration prevalence data are being analyzed and a manuscript is being drafted.

Results: In women, central retinal vein equivalent (CRVE) caliber showed a consistent increase with number of cigarettes smoked/day, consistent with previous findings by others. However, females who quit smoking ≥ 10 years ago, had similar CRVE calibers to never smokers, a new finding in the literature. There was no association of smoking and arterial caliber. No significant association of radiation dose was found for either early or late AMD.

RP 6-08 Liver stiffness study using elastometer in Hiroshima atomic-bomb survivors

Ohishi W (CH), Tatsukawa Y (CH), Ueda K (CH), Fujiwara S, Nakashima E (S), Kohata M, Yamada M (CH), Ozasa K (EH), Tsuge M, Chayama K

Objectives: The hypothesis behind this study is that radiation exposure may accelerate the severity of liver fibrosis irrespective of hepatitis virus infection. The objective is to determine whether A-bomb radiation exposure has increased liver stiffness, which serves as a marker of liver fibrosis severity, and to investigate the possibility that liver fibrosis is involved in the development of atherosclerotic diseases by inducing insulin resistance.

Background and Significance: Liver fibrosis sometimes progresses into liver cirrhosis and hepatocellular carcinoma (HCC), among chronic type B or C liver disease and nonalcoholic steatohepatitis cases. The RERF data have shown that both chronic liver disease and liver cirrhosis are related to radiation dose. In the LSS and AHS populations, radiation effects on the incidence of atherosclerotic diseases, such as hypertension and cardiovascular disease, have also been observed.

Study Methods: We will examine the relationship between liver stiffness and radiation dose, in order to determine whether this is a plausible pathway by which radiation exposure is involved in increased chronic hepatitis and liver cirrhosis. We will also examine whether an increase in liver fibrosis is involved, through insulin resistance, in the development of atherosclerotic diseases, in order to elucidate mechanisms of radiation effects underlying these diseases.

Study Progress: We completed measurement of liver stiffness with the elastometer and blood cytokine levels such as TNF- α , IL-6, PAI-1, adiponectin, resistin, and IGF-1 for 2,911 Hiroshima AHS participants. The data cleaning of the data set, which includes levels of liver stiffness, blood cytokine, and other clinical examination and information data, is underway. Early descriptive statistics and preliminary analyses for younger A-bomb survivors exposed before age 10 were conducted.

Results and Conclusions: None yet. Results expected in 2014.

RP 4-08 Investigation of storage conditions for cataract tissue of A-bomb survivors, and its collection and storage program

Hida A (CN), Tatsukawa Y (CH), Neriishi K, Blakely EA, Chang P, Nakashima E (S), Ohishi W (CH), Akahoshi M,

Hayashi T (R), Ito R (R), Nakachi K, Minamoto A, Yokoyama T, Toda S, Uematsu M, Tsuiki E, Kiuchi Y, Kitaoka T, Shirai A, Cucinotta FA, Chylack LT

Objectives: Little is known about the mechanisms by which radiation causes cataracts. Samples of cataract tissue from radiation-exposed study subjects will provide an opportunity to study mechanisms and pathways in more detail. The goal of this preliminary project is to confirm the adequacy of the storage method for cataract tissues of the AHS participants who undergo a cataract operation, and to collect and store the tissue for future analyses.

Background and Significance: Our recent study found a radiation effect with an odds ratio at 1 Gy of 1.39 (95% confidence interval 1.24, 1.55) for the prevalence of cataract operations. Cataract extractions become more common as the AHS participants age. The potential to evaluate the molecular biology from stored lens tissues will add significantly to our knowledge about radiation-induced cataract.

Study Methods: Meetings with specialists were held to establish the adequacy of the storage method for cataract tissues of the AHS participants. Training of staff for collection and storage of the tissue for future analyses was conducted.

Study Progress: The program of collection and storage of lens tissue was completed. Fifty-four samples in Hiroshima and 20 samples in Nagasaki have been collected and stored as of December 2013. Among these, opacities in lenses had previously been detected at RERF for 13 cases. We could extract RNA and DNA from the lens epithelial cells attached to the anterior capsule.

Results and Conclusions: None yet.

RP 3-05 Inflammation and cancer incidence in atomic bomb survivors

Tatsukawa Y (CH), Nakashima E (S), Little MP, Ozasa K (EH), Soda M (EN), Yamada M (CH), Cologne JB (S)

Objectives: To explore effects of inflammation levels on radiation risk for cancer development.

Background and Significance: Experimental and epidemiological studies report a relationship between inflammation and cancer. Because A-bomb survivors have radiation dose-dependent increases in inflammatory biomarkers, we are investigating the relationship between selected biomarkers and cancer incidence among 12,870 AHS participants followed from 1965 to 1999 and their possible role in radiation risk for cancer.

Study Methods: A number of inflammation biomarkers have been measured in the AHS cohort. Since associations between radiation, biomarkers, and cancer outcomes are complex, we are using several statistical analysis approaches to characterize them.

Study Progress: Initial analyses of the joint effects of white blood cell (WBC) and radiation or smoking on all solid cancer incidence using a causal model were completed and a manuscript has been approved after internal review. Following unsuccessful journal submission, the manuscript is currently under revision.

Results and Conclusions: Longitudinal trends in WBC counts over time are elevated among subjects exposed to ≥ 2 Gy radiation (result published in the *Journal of Radiation Research* in 2010). There is a significant mediation effect by the longitudinal WBC count upon the radiation risk for all

solid cancer, with the proportion of total radiation effect attributed to mediation by WBC being about 7%. Whether there is mediation by WBC of the radiation risk for specific cancer sites could be determined statistically for only certain common cancers; the mediation proportion for lung cancer was much higher (27%) than for all solid cancers. A manuscript has been internally approved and is currently in the journal review process.

RP 2-05 Could genetic factors cause population bias among proximal A-bomb survivors?—A test of whether the same genetic factors are risk factors for high inflammatory status and myocardial infarction among A-bomb survivors 40–50 years later

Ohishi W (CH), Takahashi I (CH), Cologne JB (S), Hida A (CN), Fujiwara S

Objectives: To evaluate a set of underlying genetic factors associated with survival among atomic-bomb survivors who probably suffered from radiation injuries, burns, and early infections. Specifically, it is hypothesized that if there was survival bias due to the early sequelae of the bombing, then genetic profiles which are associated with the degree of inflammatory responses to biologic stressors would likely differ among high-dose survivors vs. high-dose non-survivors, which could cause the survivors to differ from (virtually) unexposed controls in either the frequency of relevant genes or in the genotype Hardy-Weinberg Equilibrium (HWE). Secondly, since the targeted inflammatory genes may also be implicated in cardiovascular disease, we will also examine whether they and radiation dose are jointly related to increased risk of cardiovascular disease.

Background and Significance: Since the Life Span Study (LSS) and Adult Health Study (AHS) cohort members had to have survived until 1950 and 1958, respectively, a “healthy survivor effect” may have been operative with respect to noncancer mortality among the high-dose survivors. The hypothesis is that genetic polymorphisms controlling responsiveness to external stresses can alter survival rates following acute radiation exposures and also increase cardiovascular disease in later life. Specifically, candidate genetic polymorphisms in the *LTA* and *TLR2* genes were identified as important stress-responsiveness and chronic inflammation genes.

Study Methods: A case-control study among younger survivors who participated in the first health examination in 1958–59 and were exposed to at least 1 Gy of radiation, and their sex-, age-, and city-matched controls exposed distally to less than 5 mGy will be conducted. We will analyze selected *LTA* and *TLR2* genetic polymorphisms to determine if there is dose-related evidence for differences in their frequency or in HWE. We will also assess the joint association of radiation and the *LTA* and *TLR2* genotypes to see if the radiation effect is modified by genotype.

Study Progress: The relationship between the *LTA* and *TLR2* genotype frequency and A-bomb radiation exposure is being examined. The preliminary results showed that frequencies of *LTA* and *TLR2* genotypes, which are advantageous to infection, are not significantly different between proximal and distal survivors. We also preliminarily examined the relationship between *LTA* or *TLR2* genotype

and CRP levels among subgroups of proximal and distal survivors based on previous research findings that the *LTA252G* allele is associated with high CRP levels (*Atherosclerosis* 2004; 176:91–4). A significant relationship between *LTA* or *TLR2* and CRP levels was not observed in either proximal or distal survivors.

Results and Conclusions: None yet. Results expected in 2014.

RP 1-05 Glaucoma study in atomic bomb survivors

Kiuchi Y, Yokoyama T, Uematsu M, Tsuiki E, Kitaoka T, Nakashima E (S), Khattree R, Neriishi K, Hida A (CN), Fujiwara S, Akahoshi M

Objectives: To determine the relationship between radiation dose and prevalence of glaucoma.

Background and Significance: Although it is well known clinically that acute, high radiation exposure can induce glaucoma, the relationship between glaucoma and radiation exposure within the general population at moderate doses, such as in the AHS cohort, is unclear.

Study Methods: We conducted comprehensive ophthalmologic examinations, including intraocular pressure measurements, retinal images, and a visual field examination, with further detailed examinations for potential cases during 2006–2008.

Study Progress: Among 1,589 subjects with known radiation dose (mean age 74.3) we detected 284 (17.9%) cases of glaucoma overall, most of whom presented with normal tension glaucoma. Based on suggestions of a Glaucoma Study Group, various aspects of the study, including possible non-participation bias, were analyzed and a manuscript was published in *Radiation Research*.

Results and Conclusions: The analyses using the generalized estimating equation method, with adjustment for sex, age, city, cataract surgery, and diabetes mellitus, revealed an elevated odds ratio in the case of normal tension glaucoma, but the findings need to be interpreted cautiously given possible biases associated with the high, non-random non-participation rates (*Radiat Res* 2013; 180(4):422–30).

RP 2-99 Thyroid diseases in Hiroshima and Nagasaki atomic-bomb survivors

Imaizumi M (CN), Ohishi W (CH), Usa T, Akahoshi M, Neriishi K, Sera N (CN), Yamada M (CH), Nakashima E (S), Sugino K, Hida A (CN)

Objectives: To investigate whether there are positive associations between radiation dose and thyroid diseases in the AHS cohort, and to examine how frequently thyroid cancer has developed among subjects with thyroid nodules detected in an earlier study (1984–87).

Background and Significance: An earlier study of thyroid disease in the Nagasaki AHS cohort in 1984–87 showed an approximately linear association between thyroid radiation dose and the prevalence of thyroid nodules and suggested an association between autoimmune hypothyroidism and radiation dose (*JAMA* 1994; 272:364). The current thyroid study is conducted in both Hiroshima and Nagasaki AHS cohort members to clarify the radiation dose response for thyroid diseases. A second objective is to examine how frequently thyroid cancer develops among

irradiated subjects with thyroid nodules detected in the previous study (1984–87). If the frequency is high, this would impact guidelines for clinicians regarding follow-up of nodules and incidental tumors.

Study Methods: Thyroid examinations are performed (blinded as to radiation dose) in AHS cohort members and dose responses for thyroid disorders are analyzed. For the second objective, incident thyroid cancer is ascertained among those with thyroid nodules in 1984–87.

Study Progress: We performed thyroid examinations in AHS cohort members between 2000 and 2003. We are continuing the follow-up of AHS subjects for detecting the development of thyroid cancers. We conducted thyroid examinations in AHS subjects exposed at younger ages (<10 years old at exposure) between 2008 and 2011 (RP 3-07) for further study of radiation effects on thyroid diseases and are conducting analyses.

Results and Conclusions: We found that both malignant thyroid tumors and benign nodules were increased with radiation dose and the relationships were significantly higher in those exposed at younger ages. On the other hand, autoimmune hypothyroidism and Graves' disease were not associated with radiation dose (*JAMA* 2006; 295(9):1011–22). No significant dose responses for thyroid diseases were observed among those exposed *in utero* (*J Clin Endocrinol Metab* 2008; 93:1641–8), though that may reflect lack of statistical power to detect effects. We more frequently detected cancer in subjects with solid thyroid nodules than in nodule-free controls (*J Clin Endocrinol Metab* 2005; 90:5009–14). Significant linear dose-response relationships were observed for the prevalence of thyroid nodules among AHS subjects exposed at younger ages (<10 years old at exposure).

RP 9-92 Study of liver diseases in the Adult Health Study sample: Relationship between radiation dose and infection by hepatitis B and C viruses

Ohishi W (CH), Cologne JB (S), Cullings HM (S), Nakashima E (S), Yoshida K (R), Kusunoki Y (R), Hayashi T (R), Ueda K (CH), Hida A (CN), Fujiwara S, Chayama K

Objectives: The hypothesis behind this study is that radiation may increase the incidence of hepatocellular carcinoma (HCC) either by increasing the rates of chronic hepatitis B (HBV) and C (HCV) virus infection, or by accelerating the disease progression after hepatitis virus infection. The objective of the study is to investigate the relationship between radiation dose and the natural history of HBV or HCV infection among AHS cohort.

Background and Significance: Our previous studies demonstrated that the prevalence of hepatitis HBsAg increased with radiation dose among the AHS. The percent among subjects who were unable to clear the virus increased significantly with radiation dose among those receiving blood transfusions. No relationship was found between radiation dose and the prevalence of anti-HCV, but the radiation-dose response for chronic liver disease among anti-HCV-positive subjects was suggestively greater than that among anti-HCV-negative subjects.

Study Methods: We will examine (1) the relation between radiation dose and HBV activity ascertained by measuring

hepatitis B e-antigen (HBeAg) and HBV DNA, and the HBeAg or HBsAg seroconversion rates, (2) the relation between radiation dose and the natural history of chronic type B or C liver diseases, and (3) the effects of clinicopathological features, immunogenetic background, and radiation dose on the course following hepatitis virus infection among the AHS cohort.

Study Progress: We have finished debugging the program to create the analysis data set using the latest cancer follow-up data, AHS HBV/HCV data, and other risk factor information.

Results and Conclusions: Subjects with persistent HCV infection showed Th1-dominant immunological features. Increased Th1 cell percentages were significantly associated with accelerated progression of liver fibrosis, while Tc1 and NK cell percentages were inversely associated with fibrosis progression (*Hum Immunol* 2011; 72:821–6).

RP 5-92 Study of senile dementia among the Adult Health Study subjects

Yamada M (CH), Mimori Y, Cologne JB (S), Landes R (S), Sasaki H, Matsumoto M, White LR

Objectives: In this study, we are examining the effects of radiation exposure on cognitive function, the prevalence and incidence of dementia, and other age-related physiologic variables such as reaction time in older age among adult survivors in the AHS.

Background and Significance: This study investigates the hypothesis that the effects of ionizing radiation on the mature central nervous system could possibly be manifested as accelerated neurological aging. In the late 1980s, a collaborative study of dementia using standardized procedures to compare Japanese-Americans living in Seattle and Honolulu with the AHS cohort was initiated to identify whether the prevalence, incidence, and causes of dementia were the same across cultures (the NI-HON-SEA study).

Study Methods: Study subjects were survivors exposed at ≥13 years of age. We evaluated cognitive performance for about 3,113 subjects in Hiroshima and Nagasaki with the Cognitive Abilities Screening Instrument (CASI) during the period 1992–1998. The prevalence of dementia and its subtypes was assessed among 2,648 Hiroshima AHS subjects aged 60 years or older at baseline examination (1992–1996). A total of 2,286 dementia-free subjects at baseline examination have been followed up to assess dementia incidence and results were published.

Study Progress: The database of the CASI longitudinal examinations had been restructured. Longitudinal analyses regarding trajectories of cognitive function and effects of demographic factors have been initiated in cooperation with the Department of Statistics.

Results and Conclusions: No association was found between previous radiation exposure and cognitive impairment and/or development of dementia among subjects exposed at ≥13 years of age. Additional longitudinal analyses regarding radiation effects on cognitive decline have been prepared.

RP 3-89 Osteoporosis in Hiroshima atomic-bomb survivors

Fujiwara S, Takahashi I (CH), Ohishi W (CH), Masunari N,

Furukawa K (S), Nakamura T, Yoshimura N, Fukunaga M, Orimo H

Objectives: To determine the relationship between ionizing radiation and the prevalence and severity of osteoporosis as a potential, long-term health consequence of prior radiation exposure.

Background and Significance: Our working hypothesis is that acute ionizing radiation might accelerate the aging process as manifested by increased osteoporosis. To date, preliminary analyses of bone mineral density (BMD) in long-term atomic-bomb survivors do not suggest radiation exposure-related changes in BMD after adjusting for age, weight, and age at menopause. We are utilizing accumulated data on BMD and fracture for national and international collaborative studies that provide new insights regarding BMD and guidelines for health maintenance of atomic bomb survivors and others.

Study Methods: Longitudinal follow-up study of BMD as a part of routine health examinations.

Study Progress: We are conducting international and national collaboration studies using accumulated data and information related to this RP and have published several papers.

Results and Conclusions: As a collaborative study with the WHO working group, we have published a Japanese version of the WHO fracture risk assessment tool including age, sex, BMD, prior fracture, smoking, alcohol drinking, and so on as risk factors (*Osteoporos Int* 2008; 19:429–35). Our papers from the AHS have contributed to development of a WHO fracture risk assessment tool, Japanese guidelines for prevention and treatment, guidelines for treatment of steroid-induced osteoporosis, and so on.

RP-A1-14 Association of chronic kidney disease and albuminuria with cardiovascular diseases among A-bomb survivors

Sera N (CN), Hida A (CN), Haruta D (CN), Imaizumi M (CN), Takahashi I (CH), Yamada M (CH), Tatsukawa Y (CH), Nakashima E (S), Ohishi W (CH), Akahoshi M, Kawakami A

Previous studies have clarified the association between radiation exposure and cardiovascular diseases (CVD) among A-bomb survivors.

Reports indicate that exposure to A-bomb radiation is also associated with cardiovascular risk factors such as fatty liver, hypertension, dyslipidemia, and inflammation markers. The involvement of such cardiovascular risk factors in the increase of CVD among A-bomb survivors has also been suggested. Chronic kidney disease (CKD) has been recently recognized as a risk factor for CVD. Kidney disorders due to high-dose radiation exposure have been reported previously; and the association between renal failure mortality and radiation dose among A-bomb survivors has also recently been observed. Studies on the Adult Health Study (AHS) cohort in Nagasaki have also indicated the association of severe renal dysfunction (estimated glomerular filtration rate [eGFR] <30 ml/min/1.73 m²) and CKD (diagnosed based on eGFR <60 ml/min/1.73 m²) with radiation dose, newly suggesting that CKD is potentially involved in CVD increase among A-bomb survivors.

We previously used only eGFR <60 ml/min/1.73 m² to

diagnose CKD at RERF, but in 2008 we began measuring urinary albumin (urine albumin-to-creatinine ratio [UACR]) for AHS participants to use the results for CKD diagnosis. Unlike eGFR, albuminuria is less likely to be affected by age and is considered as a marker of glomerular capillary endothelial damage. Albuminuria, a risk factor for CVD independent of eGFR, is believed to increase CVD risks even when eGFR is still normal. It has been suggested that albuminuria and decreased eGFR elevate CVD risks through different mechanisms.

Using both UACR and eGFR, we will make diagnoses and detailed categorizations of CKD for AHS participants in Hiroshima and Nagasaki to review their association with CVD prevalence and radiation dose for each of the CKD categories.

RP-A5-13 The association between thyroid status and the progression of renal function over time

den Elzen W, Meuwese CL, Gussekloo J, Rodondi N, Imaizumi M (CN), Ohishi W (CH)

Objectives: The study hypothesis is that thyroid function is associated with the progression of renal dysfunction over time. The objectives of the present analysis are: 1) to study the association between thyroid function and renal function cross-sectionally, 2) to study the association between thyroid function and changes in renal function and the development of chronic kidney disease (CKD) over time, and 3) to study age as an effect-modifier in the previous associations.

Background and Significance: The prevalence of CKD increases with age. Throughout all age categories CKD associates with an increased risk of adverse cardiovascular outcomes. Like CKD, the prevalence of overt and subclinical hypothyroidism increases with age. In the general population, overt hypothyroidism and subclinical hypothyroidism are both associated with an increased cardiovascular risk, which could be attributed to various cardiovascular effects of thyroid hormones. End-stage renal disease is frequently accompanied by thyroid hormone alterations in the absence of primary hypothalamic-pituitary-thyroidal dysfunction. Conversely, small observational studies have indicated deterioration in renal function due to the effects of overt and subclinical hypothyroidism. By using combined data from the Thyroid Studies Collaboration, it will be possible to study the effects of a low thyroid function on renal function.

Study Methods: At this point, 15 large international cohorts including the Hiroshima and Nagasaki AHS have renal function data. We will perform a pooled analysis of individual participants in the studies to determine whether there is an association between low thyroid function and renal function.

Study Progress: We have started compiling the data of the cohorts.

Results and Conclusions: None yet. Results expected in 2015.

RP-A4-13 Subclinical thyroid dysfunction and adverse bone outcomes (Addendum to RP-A10-08)

Blum MR, da Costa B, Imaizumi M (CN), Rodondi N

Objectives: To study: (1) the association between

subclinical thyroid dysfunction and subsequent fracture risk (hip, non-spine), (2) the association between subclinical thyroid dysfunction and a greater bone loss as assessed by serial measurements of hip and spine bone mineral density (BMD), (3) the association between thyroid stimulating hormone (TSH) levels within the normal range and/or thyroxine therapy and increased fracture risk (hip, non-spine) and/or lower BMD, and (4) if fracture risk in subclinical thyroid dysfunction is mediated by its effects on BMD.

Background and Significance: Overt hyperthyroidism is known to be associated with osteoporosis and fractures, and overt hypothyroidism has been shown to decrease bone turnover. Excess thyroid hormone (hyperthyroidism) affects osteoclasts and osteoblasts and leads to bone loss, clinically resulting in osteoporosis and increased fracture risk. Excess TSH (hypothyroidism) also has direct effects on bone remodeling by directly or indirectly acting on osteoclast and osteoblast activity in experimental studies. Subclinical hyperthyroidism (SHyper) is characterized by low TSH and normal to high-normal free T4 levels, which could induce loss of BMD and increased fracture risk. Conversely, subclinical hypothyroidism (SHypo) could cause adverse bone outcomes by direct effects of TSH on bone turnover with subsequent change of bone quality. However, studies on the association of SHyper or SHypo with BMD and fractures are limited and controversial. By using combined data from the Thyroid Studies Collaboration, we want to study the associations of serum TSH levels and the risk of bone fracture or BMD.

Study Methods: We will perform an individual participant pooled data analysis from all available prospective cohorts of adults with measurement of baseline thyroid function and fracture outcomes and BMD.

Study Progress: In 13 prospective cohort studies including the Nagasaki AHS with 69,795 participants during 757,219 person-years of follow-up, we analyzed the association between subclinical thyroid dysfunction and subsequent fracture risk.

Results and Conclusions: In the pooled analyses, subclinical hyperthyroidism is associated with an increased risk of hip and other fractures. On the other hand, subclinical hypothyroidism was not associated with fracture risk.

RP-A3-13 Serum TSH levels and the risk of stroke (Addendum to RP-A10-08)

van Dijk B, Chaker L, Peeters RP, Franco O, Imaizumi M (CN)

Objectives: (1) To study the association between suppressed or elevated thyroid stimulating hormone (TSH) levels and stroke, (2) to study the associations between low-normal or high-normal TSH levels and stroke, and (3) to study if the relationship between a suppressed TSH and stroke is mediated via atrial fibrillation, and if the relationship between an elevated TSH and stroke is driven via atherosclerosis (using carotid intima-media thickness as an index).

Background and Significance: Subclinical thyroid disease has been associated with a higher risk of cardiovascular disease in various studies. The underlying hypothesis is that subclinical hypothyroidism may increase the risk of

atherosclerosis, whereas subclinical hyperthyroidism is related to an increased risk of atrial fibrillation. Both atherosclerosis and atrial fibrillation are known risk factors for the development of stroke, suggesting that subclinical thyroid disease may also be associated with an increased risk of stroke. However, only very few epidemiological studies evaluated subclinical thyroid disease as a risk factor for stroke. At this point, 14 cohorts with stroke data have joined the Thyroid Studies Collaboration, providing considerable statistical power to assess the association between both high and low TSH levels and the risk of stroke.

Study Methods: We will perform a pooled analysis of individual participants in large international cohort studies to determine whether there are the associations of serum TSH levels and the risk of stroke.

Study Progress: We are performing a pooled analysis to determine the association between elevated TSH levels (subclinical hypothyroidism) and stroke. The 14 prospective studies including the Nagasaki AHS included 41,777 participants with 427,608 person-years of follow-up.

Results and Conclusions: None yet. Results expected in 2014.

RP-A7-12 Serum TSH within the reference range and the risk of coronary heart disease (Addendum to RP-A10-08)

Åsvold BO, Imaizumi M (CN)

Objectives: To study the associations of serum thyrotropin (TSH) concentrations within the reference range with 1) the risk of a first-time coronary heart disease event and specifically, the risk of first-time myocardial infarction, 2) survival after myocardial infarction, 3) the risk of death from coronary heart disease.

Background and Significance: We previously reported that subclinical hypothyroidism, defined as elevated serum TSH levels with normal thyroxine concentrations, was associated with an increased risk of coronary heart disease events and mortality based on the pooled data from the Thyroid Studies Collaboration, a multicenter, international collaboration which includes RERF. Recently, it has been noted that some people with TSH in the upper part of the normal reference range have an early stage of hypothyroidism. However, an association between TSH within the reference range and coronary heart disease has not been studied. By using combined data from the Thyroid Studies Collaboration, we will study the association of TSH within the reference range with the risk of coronary heart disease, the risk of myocardial infarction, survival from myocardial infarction, and the risk of death from coronary heart disease.

Study Methods: For all study cohorts participating in the Thyroid Studies Collaboration we will analyze individuals with TSH within the reference range. For each cohort, we will use Cox regression analysis to estimate hazard ratios for a first-time coronary heart disease event, first-time myocardial infarction, and death from coronary heart disease by categories of TSH within the reference range, using the lower part of the TSH reference range (0.45–1.49 mU/L) as a reference group. We will use random-effects meta-analysis to obtain pooled estimates, analogous to the method used in two prior papers from the Thyroid Studies Collaboration (*JAMA* 2010; 304:1365 and *Arch Intern Med*

2012; 172:799).

Study Progress: Analyses are underway.

Results and Conclusions: Not yet. Results expected in 2014.

RP-A4-12 MRI-based study of importance of diagnosis of fatty infiltration of the salivary glands among patients with xerostomia

Takagi Y, Hida A (CN), Nakamura H, Sumi M, Akahoshi M, Nakamura T

Objectives: Using MRI imaging, we evaluated the salivary glands of patients with xerostomia to study the status and prevalence of “fatty salivary glands” and the condition’s causal relationship with glandular dysfunction.

Background and Significance: Fatty degeneration and fatty deposition are known to occur in salivary gland tissue of patients with Sjögren’s syndrome (SS) and dyslipidemia (DL), a situation that can be confirmed by MRI. Although the association between “fatty salivary glands” and glandular dysfunction has been suggested, the former’s clinical significance and mechanism are not fully understood.

Study Methods: Among 235 subjects who were examined and underwent MRI examination at the Xerostomia Clinic of the Nagasaki University Hospital, 155 subjects were referred from RERF between 2003 and 2005 based on a pilot study of AHS sicca syndrome. The subjects comprised 80 patients with SS, 70 patients with DL, and 85 non-SS and non-DL subjects. Salivary gland MRI imaging was evaluated retrospectively to confirm status and prevalence of fatty salivary glands and to examine the condition’s causal relationship with glandular dysfunction.

Study Progress: The analysis of MRI imaging of 235 subjects was finished and a paper has been written.

Results and Conclusions: The salivary flow rates of SS patients were significantly correlated with the percentages of fat area in the glands. The salivary flow rates of non-SS patients were not correlated with the percentages of fatty area in the glands. Further, salivary flow rates of DL and non-DL patients were similar, irrespective of the presence or absence of fat infiltration in these patients.

RP-A2-12 Weight fluctuation and cancer and cardiovascular disease incidence and mortality in Japanese

Nanri A, Mizoue T, Sera N (CN), Takahashi I (CH), Soda M (EN), Ozasa K (EH), Cologne JB (S), Araki Y, Cullings HM (S), Ohishi W (CH), Hida A (CN), Akahoshi M

Objectives: We identify patterns of weight fluctuation and examine their association with subsequent morbidity and mortality of cancer and cardiovascular disease in the AHS cohort.

Background and Significance: Obesity and underweight at a single point in time, and weight gain and loss, have been reported to be associated with increased mortality. Some studies have also found an association between weight fluctuation and mortality. However, measures of weight fluctuation in previous studies have had limited ability to capture weight fluctuation patterns in terms of the frequency and magnitude of weight change.

Study Methods: In this study, we first calculate a classical measure of weight fluctuation and, to address weight

fluctuation patterns, we will further develop innovative measures that facilitate examining the relationship of longitudinal patterns in weight change to morbidity and mortality. We will prospectively examine the association of the new indicators of weight fluctuation with morbidity and mortality from all causes, cancer, and cardiovascular disease using Cox regression. Analyses will be based on 5,790 subjects in the Adult Health Study (AHS) cohort who were aged 20 to 49 years old at baseline (1958) and had health examination seven times or more between baseline and start of follow-up 20 years later (1978). The analysis should provide improved estimates of the risk of weight fluctuation based on enhanced conceptual and statistical approaches compared with those used in previous studies.

Study Progress: We have conducted analyses since 2013.

Results and Conclusions: None yet. Results expected in 2016.

RP-A1-12 Study for the epidemiological and genetic basis of progressive cardiac conduction defect

Makita N, Akahoshi M, Haruta D (CN), Maemura K, Ohishi W (CH), Hida A (CN), Nakashima E (S)

Objectives: We will focus on progressive cardiac conduction defect (PCCD), which is a hereditary lethal arrhythmia.

Background and Significance: PCCD is a hereditary lethal arrhythmia characterized by electrocardiographic findings of atrioventricular block and bundle branch block caused by progressive degenerative fibrosis of the cardiac conduction system. Sudden death and pacemaker implantation are outcomes of PCCD and three genes have been reported as responsible for PCCD. We will determine the possible PCCD cases who progressed to sudden death or pacemaker implantation from bundle branch block in AHS and conduct gene analysis in these possible PCCD cases.

Study Methods: Among 16,170 individuals who underwent health examinations in the Adult Health Study in Nagasaki and Hiroshima between 1967 and 2010, we will extract those who were diagnosed with right bundle branch block (RBBB; 828 cases). They will be categorized into two groups: a group (non-PCCD) that did not show progression of the bundle branch block, and a case group (PCCD) that progressed into sick sinus syndrome (SSS) or complete atrioventricular block (AVB). The latter group includes individuals who have undergone pacemaker implantation. Endpoints of the study are pacemaker implantation (32 cases).

At Nagasaki University, genomic DNA will be extracted from peripheral lymphocytes of subjects in the case group, and the genetic screening will be conducted on the following genes: connexin genes, which modulate the electrical connection between cardiac cells (connexin 40, 43, 45), Na channels (*SCN10A*, *SCN4B*), which have been raised as candidate genes affecting cardiac conduction in genome-wide association analysis, and the cytoskeletal protein lamin, which plays a role in maintenance of structure of cellular nucleus and modulation of transcription. Exons of the respective genes will be amplified by the PCR method, and the base sequences will be analyzed with an ABI 3130 sequencer.

Study Progress: We detected 520 incident RBBB cases and selected 1,038 age- and sex-matched controls among

16,170 AHS subjects. We found 14 and 6 pacemaker implanted cases due to SSS or AVB in incident RBBB cases and age- and sex-matched controls, respectively.

Results and Conclusions: RBBB cases progress to AVB and SSS that requires pacemaker implantation. The hazard ratio for the risk of pacemaker implantation due to all causes was 4.54 (95% confidential interval 1.74–11.88).

RP-A6-11 Study on the genetic background of short QT

Makita N, Akahoshi M, Haruta D (CN), Maemura K, Ohishi W (CH), Hida A (CN)

Objectives: Congenital short QT syndrome (SQT) is a genetic disease characterized by sudden death due to ventricular arrhythmia, and five cardiac ion channel genes have been reported as responsible genes. Because only several dozen patients with short QT have been reported, we will conduct gene analysis in two subjects with short QT (prevalence was 0.01%) who were identified by Dr. Moriya in the RERF AHS.

Background and Significance: SQT is a genetic arrhythmia characterized by shortened electrocardiographic QT interval (QTc <350 msec) and sudden death. In 2007, Moriya et al. at the Nagasaki Laboratory of RERF examined electrocardiograms of 19,153 subjects in a follow-up program, and observed short QT (QTc <350 msec) in two of them. This study is aimed at determining whether there are SQT gene abnormalities in the two subjects who exhibited SQT in the RERF follow-up program.

Study Methods: Blood samples are available from both SQT cases. The gene analysis will be conducted at the Department of Molecular Physiology of Nagasaki University. The genes to be analyzed are those for three K channels (*KCNH2*, *KCNQ1*, and *KCNJ2*) and two Ca channels (*CACNA1C* and *CACNB2*) that have been reported as SQT genes. We will amplify the exons of the respective genes by PCR technique and read the DNA sequences using an ABI 3130 capillary sequencer. When gene abnormalities are observed, we will prepare mutant cDNA and transfect this DNA into CHO cell culture in order to analyze electrophysiological characteristics of mutant channels by the whole-cell patch clamp method to determine the functional abnormalities that tend to shorten the action potential of cardiomyocytes.

Study Progress: One case is still alive and the other case was deceased. We obtained blood from the living case in 2013.

Results and Conclusions: None yet. Results expected in 2015.

RP-A4-11 Role of visceral fat and its effects in the association between radiation dose and cardiovascular disease

Sera N (CN), Nakashima E (S), Carter RL, Hida A (CN), Imaizumi M (CN), Cullings HM (S), Akahoshi M

Objectives: We plan to investigate the causal pathways and associations among atomic bomb (A-bomb) radiation, visceral fat accumulation and its sequelae (fatty liver, hypertension, hyperlipidemia, type 2 diabetes mellitus), and cardiovascular disease (CVD).

Background and Significance: It has been reported

that A-bomb radiation exposure increases the risk of CVD. In addition, A-bomb radiation is related to fatty liver, hypertension, abnormal lipid profiles, impaired glucose tolerance, and inflammation, which are all related to visceral fat accumulation. Free fatty acids (FFAs) and several adipokines (inflammatory cytokines, adiponectin, angiotensinogen, and plasminogen activator inhibitor-1 [PAI-1]) are secreted from visceral fat (adipocytes and macrophages in fat tissue). FFAs and adipokines, along with visceral fat accumulation, may contribute to the mechanism(s) of radiation-induced CVD. Thus visceral fat accumulation and metabolic and/or inflammatory parameters relating to FFAs, and adipokines may be part of the causal pathway that explains the association between A-bomb radiation and CVD.

Study Methods: We examined 1,366 Nagasaki Adult Health Study (AHS) subjects (521 males, 845 females) from 2004 through 2007 and collected (1) the data of surrogate markers of visceral fat accumulation and atherosclerosis, and (2) metabolic or inflammatory data relating to FFAs and adipokines. We also identified the cases of hypertension, type 2 diabetes, hyperlipidemia, metabolic syndrome, angina pectoris, myocardial infarction, and stroke, based on standard diagnostic criteria, after taking consideration of medication history. Using this dataset, we plan to investigate the potential causal pathways and associations among A-bomb radiation, visceral fat accumulation and its sequelae (fatty liver, hypertension, hyperlipidemia, type 2 diabetes), and CVD. The mediating effects of visceral fat accumulation on the radiation-CVD association will be investigated using the conceptual model: radiation + visceral fat accumulation → inflammation → metabolic function → atherosclerosis → CVD.

Study Progress: We have conducted preliminary demographic analyses and found positive associations of ischemic heart disease with hypertension ($p = 0.0019$) and type 2 diabetes mellitus ($p = 0.0065$).

Results and Conclusions: None yet. Results expected in 2014.

RP A1-10 Radiosensitivity difference of cataract surgery in A-bomb survivors by polymorphisms of ATM and other genes

Takahashi I (CH), Hayashi T (R), Misumi M (S), Nakachi K, Nakashima E (S), Neriishi K

Objectives: This study aim is to investigate an association between *ATM* polymorphisms and radiosensitivity differences with respect to cataract surgery prevalence among A-bomb survivors.

Background and Significance: The mechanisms of the relationship between radiation and cataract in A-bomb survivors remain unclear. We are investigating if *ATM* gene polymorphisms, which have been shown to enhance X-ray-induced cataractogenesis in animal experiments, are related to radiation-induced cataract surgery prevalence among A-bomb survivors.

Study Methods: The study is examining the prevalence of surgically removed cataracts in the AHS in relation to radiation dose and the presence of selected polymorphisms in *ATM* and other DNA repair genes. The examination included 3,744 subjects during 2000 and 2001.

Study Progress: We reviewed the utility of cataract

surgery as a measure for this genetic study and the appropriate analyses for radiation by gene interaction.

Results and Conclusions: None yet.

RP-A5-09 Application of causal modeling on radiation, inflammation, and cataract surgery among Adult Health Study population

Kakuma T, Araki Y, Hsu W-L, Nakashima E (S), Neriishi K

Objectives: To apply a joint model to investigate how radiation and other risk factors, such as smoking, result in elevated inflammation levels and a higher risk of cataract surgery.

Background and Significance: An RERF study has indicated a significant dose response for the prevalence of cataract surgery in A-bomb survivors. It is hypothesized that the effect of radiation exposure on cataract risk may be mediated through an inflammation process. This study proposes to apply a causal model to address the complex associations of radiation, inflammation, and cataract incidence.

Study Methods: This study proposes to apply a joint model in which the associations among radiation, inflammation, and incidence of cataract surgery, allowing inflammation to be a mediating variable, are estimated in a time-to-event analysis for the A-bomb survivors.

Study Progress: Analyses have been delayed due to a problem with statistical methods.

Results and Conclusions: The data obtained from 3,942 Adult Health Study participants indicate that radiation exposure is significantly associated with cataract ($P < 0.001$) and elevated inflammation ($P = 0.016$), and inflammation is a significant risk factor for cataract ($P = 0.008$). The mediating proportion via inflammation is roughly 7.2% of the total radiation effect at 1 Gy. The joint model supports the hypothesized causal pathway of radiation exposure, chronic inflammation, and cataract, although inflammation accounts for only a small fraction of the radiation effect. However, in this preliminary analysis, inflammatory diseases were not taken into account.

There is a methodological question as to whether the analysis should use longitudinal data or cross-sectional data, as the Kurume group reported that the Cox model did not work well with the data set. The prevalence analysis using M-plus software can be made for the inflammatory measurements measured during the period from 1988 to 1992 with cataract surgery data during 1986 to 2005, and a latent variable that reflects the inflammation that will be produced by factor analysis using M-plus. The indirect effect of eye radiation dose via inflammation on cataract surgery and the direct effect of eye radiation dose will be jointly estimated by M-plus, including the effect of inflammatory diseases. A formal analysis should be made before writing a manuscript.

RP-A14-08 The incidence and prognostic value of the early repolarization electrocardiogram pattern

Haruta D (CN), Tsuneto A, Nakashima E (S), Akahoshi M

Objectives: To examine the incidence of the early repolarization pattern (ERP) and its prognostic value in terms of mortality from unexpected death, cardiac disease mortality,

and total mortality among Nagasaki AHS subjects.

Background and Significance: The majority of sudden cardiac deaths are caused by ventricular tachyarrhythmias which occur in persons with a structurally normal heart. The ERP has been considered benign, but one experimental study reported its potential arrhythmogenicity (Gussak I. et al., *J Electrocardiol* 2000; 33:299–309), suggesting the possibility that ERP is a cause of idiopathic ventricular fibrillation leading to sudden death.

Study Methods: We reviewed all the ECG records of 5,976 AHS subjects who were examined at least once between 1958 and 2004. The Brugada-type ECG cases were excluded. We identified those who showed ERP and calculated the incidence during the follow-up period after excluding prevalent ERP cases. We assessed the cause of death using death certificates and calculated the risk of: 1) unexpected deaths including sudden death or unexplained accidental death; 2) cardiac disease mortality; and 3) total mortality in ERP cases by means of Cox proportional hazards analysis with adjustment for age, sex and underlying cardiovascular diseases.

Study Progress: We have finished analyses of the associations of ERP with unexpected death, cardiac death and total mortality.

Results and Conclusions: ERP incidence was 715 per 100,000 person-years and ERP was observed in 35.5% of unexpected death cases. ERP had an elevated risk of unexpected death (HR 1.83, 95% CI 1.12–2.97), and a decreased risk of cardiac (HR 0.75, 95% CI 0.60–0.93) and all-cause (HR 0.83, 95% CI 0.78–0.93) death. ERP has significant public health implications to prevent unexpected death.

RP-A13-08 Prognostic significance of VPCs in taking consideration of their origins

Haruta D (CN), Nakashima E (S), Ohishi W (CH), Hida A (CN), Akahoshi M

Objectives: Ventricular premature contractions (VPC) in regular 12-lead electrocardiogram (ECG) recordings have again gained attention as a predictive variable for cardiovascular mortality in clinical populations. Thus, we would like to evaluate the significance of VPC in regular 12-lead ECG recordings as a risk factor for cardiovascular mortality in our representative, general population.

Background and Significance: Since the CAST trials found no evidence for the theory that VPC suppression reduces risk, VPC has generally been ignored on a routine ECG examination. But, some recent reports have indicated that the presence of VPC in regular 12-lead ECG recordings is a significant and independent predictor of cardiovascular mortality.

Study Methods: We will identify VPC cases from AHS subjects (4,092 in Hiroshima and 2,642 in Nagasaki) who underwent regular 12-lead ECG recording from 1990 to December 1993. We will identify subjects with VPC, and classify into three groups according to VPC morphology: (1) left bundle branch block (LBBB) type that originates from the right ventricle; (2) right bundle branch block (RBBB) type that originates from the left ventricle; and (3) unidentified type. We will compare the basic characteristics at VPC diagnosis, and the underlying diseases between

those with and without VPC and also among VPC cases with LBBB type, RBBB type, and unidentified type. Information on mortality and cause of death until December 2005 will be used to assess the prognostic significance for cardiovascular mortality between those with and without VPC. We will conduct a Cox proportional hazards analysis to assess the prognostic significance of VPC, frequency of cardiovascular mortality according to VPC diagnosis, and morphology of VPC with adjustment for age, sex, and underlying diseases.

Study Progress: We have reviewed the ECGs of all the VPC cases and divided them into three types (LBBB type, RBBB type, unidentified type). We ascertained the underlying diseases and cause of death.

Results and Conclusions: None yet. Results expected in 2015.

RP-A10-08 The association between subclinical thyroid dysfunction and cardiovascular disease and mortality: An individual participant pooled analysis of large international cohort studies

Rodondi N, Gussekloo J, Imaizumi M (CN)

Objectives: 1) To assess the relationship between subclinical thyroid dysfunction and coronary heart disease (CHD) and mortality, 2) to assess whether these relationships persist after adjusting for important potential confounders, and 3) to determine whether these relationships differ according to: age and gender, race, TSH levels, and presence or absence of prevalent cardiovascular disease.

Background and Significance: Subclinical hypothyroidism has been reported to be associated with elevated cholesterol and increased risk for atherosclerosis. We previously reported on risk for ischemic heart disease and all-cause mortality in subclinical hypothyroidism (*J Clin Endocrinol Metab* 2004; 89:3365). In this study, we found a significant association between the prevalence of ischemic heart disease and subclinical hypothyroidism and a possible increased mortality in men with subclinical hypothyroidism by using the data of 2,856 Nagasaki AHS subjects examined between 1984 and 1987. However, several reports from other cohorts on the relationship between subclinical hypothyroidism and CHD events and mortality are conflicting. Only a pooled analysis of the large cohort studies with individual participant data might confirm these associations, explore the potential differences, and clarify the conflicting data.

Study Methods: We are performing a pooled analysis of individual participants in large international cohort studies to determine whether there is an association between subclinical thyroid dysfunction and cardiovascular disease and mortality.

Study Progress: The 11 prospective studies including the Nagasaki AHS included 55,287 participants with 542,494 person-years of follow-up. The analyses led to a high-profile publication (Rodondi et al. [including Imaizumi M], *JAMA* 2010; 304(12):1365–74). We are evaluating the association between subclinical hypothyroidism and cardiovascular risk factors according to age based on the small-scale RP related to this RP. Other related studies (RP-A7-12, A3-13, and A4-13) have started as addendums to this RP.

Results and Conclusions: Subclinical hypothyroidism is associated with an increased risk of CHD events and CHD mortality in those with higher TSH levels, particularly in

those with a TSH concentration of 10 mIU/L or greater.

Special Clinical Studies Publications

RERF Reports (RR)

◆ Kiuchi Y, Yokoyama T, Takamatsu M, Tsuiki E, Uematsu M, Kinoshita H, Kumagami T, Kitaoka T, Minamoto A, Neriishi K, Nakashima E, Khattree R, Hida A, Fujiwara S, Akahoshi M: Glaucoma in atomic bomb survivors. *Radiat Res* 2013 (October); 180(4):422–30. (RR 8-12) © 2013 by Radiation Research Society

[Study Findings] A previous paper reported that glaucoma incidence in the Adult Health Study (AHS), based on medical history information, decreased with increasing atomic-bomb radiation dose. This finding prompted the conduct of a cross-sectional glaucoma study based on systematic ophthalmologic examinations. The study's results suggested that the prevalence of normal-tension glaucoma, but not glaucoma with elevated eye pressure, may have increased with A-bomb radiation dose. The estimated odds ratio at 1 Gy was 1.31 (95% confidence interval: 1.11–1.53, $P = 0.001$) for normal tension glaucoma. However, uncertainties exist in the results because of the study's low participation rate and the resulting possibility of selection biases. The findings therefore should be interpreted cautiously until confirmed by other studies.

[Explanation] While glaucoma has been found in 7–11% of patients as a complication of high radiation dose (e.g., radiotherapy), it is not understood whether radiation at low-to-moderate doses gives rise to glaucoma. Systematic ophthalmologic examinations were conducted to evaluate the possibility of excess glaucoma cases in relation to A-bomb radiation exposure. **Study purpose** To elucidate the association between glaucoma prevalence and A-bomb radiation, taking other potential risk factors into consideration. **Subjects and methods** Of the 2,699 people who underwent health examinations in the AHS clinical program between October 2006 and September 2008, the prevalence of glaucoma in relation to estimated A-bomb doses was analyzed among the 1,589 people who participated in the glaucoma study. However, the low participation rate (59%) introduced more uncertainty into the results and the potential for selection biases. Upon conducting medical interviews and ophthalmologic examinations, individuals with an indication of ocular disease, including glaucoma, were referred to local medical institutions for more comprehensive evaluations. Glaucoma was diagnosed by glaucoma specialists on the basis of specific changes in the optic nerve head, visual field test results, and other related ocular findings. **Results** Of the 1,589 people, a total of 284 (17.9%) individuals were diagnosed as having glaucoma in one or both eyes: 36 (2.3%) primary open-angle glaucoma cases (intraocular pressure ≥ 21 mmHg), 226 (14.2%) normal-tension glaucoma cases, and 25 (1.6%) primary closed-angle glaucoma cases. Logistic regression analysis with adjustment for gender, age, city, cataract surgery, and diabetes mellitus, revealed an estimated odds ratio at 1 Gy of 1.31 (95% confidence interval: 1.11–1.53, $P = 0.001$) for normal-tension glaucoma, but no association with radiation dose was observed for the other types of glaucoma. **Conclusion** This study's findings suggest that

the prevalence of normal-tension glaucoma may increase with A-bomb radiation dose. However, the low participation rate in the study and possible potential for selection biases introduced as a result warrant a cautious interpretation of these results. **Significance of this study and tasks ahead** This study, based on diagnoses made by ophthalmologists, suggests that normal tension glaucoma may increase with A-bomb radiation dose. However, this finding must be confirmed by other studies, and an epidemiological study that minimizes uncertainties and potential selection biases needs to be conducted. If the association is confirmed, then studies could be pursued of possible underlying biological mechanisms.

◆ Yanagi M, Misumi M, Kawasaki R, Takahashi I, Itakura K, Fujiwara S, Akahoshi M, Neriishi K, Wong TY, Kiuchi Y: Is the association between smoking and the retinal venular diameter reversible following smoking cessation? *Invest Ophthalm Vis Sci* 2014 (January); 55(1):405–11. (RR 17-12) © 2014 The Association for Research in Vision and Ophthalmology, Inc.

[Study Findings] After adjustment for such related factors as age, sex, blood pressure, body mass index (BMI), lipid profile, and radiation dose, this analysis of female A-bomb survivors showed that (1) the caliber of veins in the retina increases with the number of cigarettes smoked, and that (2) vein (venular) caliber differences between former smokers and those who never smoked diminished, until 10 years after quitting smoking their calibers were nearly identical.

[Explanation] Smoking has long been considered a risk factor for circulatory diseases (hypertension, myocardial infarction, and stroke), and it may have harmful effects on the structure of blood vessels. Retinal vessel caliber, measured from digital images of the fundus, is considered a good indicator of blood vessel structure, and such changes have been reported to be associated with smoking in various epidemiological studies. However, there has been little or no data regarding whether the association increases with the number of cigarettes smoked, and whether there also are changes in retinal vessel caliber among people who have stopped smoking. This epidemiological study addresses those gaps in our knowledge about smoking and blood vessel caliber.

Objectives To elucidate the association between habitual smoking or smoking cessation and retinal vessel caliber changes among A-bomb survivors in Hiroshima and Nagasaki who have been followed by the Radiation Effects Research Foundation through biennial health examinations (Adult Health Study: AHS). **Methods** Digital images of the fundus/retina were made for 1,664 participants in AHS ophthalmological examinations from 2006 to 2008. To observe retinal vessel caliber, standard indices, called the central retinal artery and vein equivalents (CRAE and CRVE), were calculated using image analysis software. To assess smoking habits, current smoking status was confirmed based on AHS medical history records, and the average number of cigarettes smoked per day was calculated. Information about smoking cessation was confirmed based on past AHS medical history records and mail survey information, and the number of years of smoking cessation was calculated. Allowing for related

factors such as age, sex, blood pressure, BMI, lipid profile, diabetes, C-reactive protein, white blood cell count, and radiation dose, the association between habitual smoking or smoking cessation and changes in retinal vessel (artery/vein) caliber was evaluated. **Results** (1) CRAE: No significant association was observed between habitual smoking or the number of years of smoking cessation and retinal artery caliber in either males or females. (2) CRVE: Retinal venular caliber was positively associated with the number of cigarettes smoked per day among female smokers. Compared with non-smokers, CRVE was increased by about 5% on average for those who smoked 10 or more cigarettes per day (trend $p = 0.001$). Among females, while the retinal venular caliber of those who had stopped smoking for less than 10 years was significantly larger than that of non-smokers, the venular caliber for never smokers and those who had stopped smoking for 10 or more years was indistinguishable ($p = 0.99$). No significant associations of either retinal artery or venular calibers were observed among males for smoking frequency or the number of years of smoking cessation.

Discussion (1) The results of this study, which find that retinal venular caliber is significantly associated with habitual smoking and the number of years of smoking cessation, are similar to those of several other epidemiological studies, such as the Rotterdam Study and the Singapore/Malay Study. Increased venular caliber is generally believed to be associated with circulatory diseases (e.g., cerebral ischemia). Smoking is known to accelerate both inflammation and vascular endothelial dysfunction. Yet in this study an analysis adjusting for the effects of typical inflammation markers (white blood cell count and C-reactive protein) still showed an association between smoking and retinal venular caliber, which suggests there may be non-inflammation-mediated effects on blood vessels as a result of smoking. (2) Generally, aging effects on the blood vessels appear earlier in males than in females. In this study, smoking effects were not observed in males. A possible reason for this is that men tend to experience accelerated effects of aging on the blood vessels, such as hardening of vessel walls, which may have masked smoking effects on the vessels among our study cohort at the time of examination (average age: 73.8). (3) The results of this study suggest that among Japanese females smoking cessation for a sufficient period of time may reverse the effects of smoking on retinal venular caliber. These results also shed some mechanistic light on the various reports that 10 or more years of smoking cessation decrease the risk of mortality from myocardial infarction and cerebral infarction.

Other Journal Publications

◆ Neriishi K: Cataract in A-bomb survivors. *Nihon Hakunaisho Gakkaishi [J Jpn Soc Cataract Res]* 2013; 25:75–9. (Japanese)

◆ Ohishi W, Fujiwara S, Chayama K: Study of viral hepatitis in a longitudinal cohort of A-bomb survivors. Elucidation of viral hepatitis based on a cohort study. Tokyo: Medical Review; 2013, pp 86–95. (Japanese) (related to *Immunology Studies* and *Special Cancer Studies*)

Special Clinical Studies Oral Presentations

- ❖ Tatsukawa Y, Yamada M, Ohishi W, Fujiwara S, Nakanishi S. Effect of regional body fat distribution on development of diabetes. 56th Annual Scientific Meeting of the Japan Diabetes Society, 16–18 May 2013, Kumamoto (related to *Adult Health Study*)
- ❖ Fujiwara S. Cohort study of osteoporosis. 86th Annual Congress of Japanese Orthopaedic Association, 23–26 May 2013, Hiroshima
- ❖ Imaizumi M, Furukawa K. Thyroid diseases in Hiroshima and Nagasaki A-bomb survivors. 54th Late A-bomb Effects Research Meeting, 2 June 2013, Hiroshima
- ❖ Takahashi I. Analyses of the association between arteriosclerosis in microvessels and microvascular damages. 13th Clinical Conference of Blood Pressure and Pulse Wave, 8 June 2013, Osaka (related to *Adult Health Study*)
- ❖ Kusumoto S, Kawano H, Koide Y, Ikeda S, Takeno M, Eguchi M, Yonekura T, Akahoshi M, Maemura K. Clinical course of incidence right bundle branch block in relation to pacemaker implantation due to high-degree atrioventricular block and sick sinus syndrome. 114th Meeting of Kyushu Branch of the Japanese Circulation Society, 29 June 2013, Fukuoka
- ❖ Shore RE, Yamada M, Hida A, Neriishi K. Cataract and CNS effects in the Japanese atomic bomb survivors. 59th Annual Meeting of the Radiation Research Society, 15–19 September 2013, New Orleans, Louisiana, USA (related to *Special Cancer Studies*)
- ❖ Shore RE, Nakashima E, Hida A, Neriishi K. Cataract risk in A-bomb survivors and comparison studies. 5th International MELODI (Multidisciplinary European Low Dose Initiative) Workshop, 7–10 October 2013, Brussels, Belgium (related to *Adult Health Study*)
- ❖ Ohishi W, Ueda K, Tatsukawa Y, Nakashima E, Yamada M, Kohata M, Takahashi I, Tsuge M, Chayama K. Effects of lifestyle and insulin resistance on liver fibrosis in nonalcoholic fatty liver disease. 17th Annual Meeting of the Japan Society of Hepatology, 9–12 October 2013, Tokyo (related to *Adult Health Study*)
- ❖ Yamada M. Trajectories of cognitive function among Japanese women: Radiation Effects Research Foundation Adult Health Study. 5th Scientific Meeting of the Asia Pacific Menopause Federation, 18–20 October 2013, Tokyo
- ❖ Minamoto A. Association between the evaluation of lens opacity by slitlamp biomicroscopy and radiation dose. 67th Annual Congress of Japan Clinical Ophthalmology, 31 October 2013, Yokohama
- ❖ Ohishi W, Ueda K, Tatsukawa Y, Nakashima E, Yamada M, Takahashi I, Tsuge M, Chayama K. Effects of lifestyle, serum adiponectin and radiation on prevalence and progression of nonalcoholic fatty liver disease (NAFLD). 64th Annual Meeting of the American Association for the Study of Liver Diseases, 1–5 November 2013, Washington DC, USA (related to *Adult Health Study*)

Research Protocols 1-12, 5-89 (Platform Protocol), A2-08 Histopathology Studies

RP 1-12 Development of an archival system for surgical cancer samples from atomic-bomb survivors

Ozasa K (EH), Sugiyama H (EH), Soda M (EN), Yasui W, Arihiro K, Fujihara M, Arita K, Nishisaka T, Matsuura H, Nakashima M, Shigematsu K, Takahara O, Kusunoki Y (R), Katayama H (IT)

Objectives: To develop an archival system for surgical cancer samples from the atomic-bomb survivors in collaboration with pathologists of the major hospitals in Hiroshima and Nagasaki.

Background and Significance: To clarify the mechanisms of site-specific differences in cancer risks, the shapes of dose-response curves, and the effects of age at the time of bombing and attained age or time since exposure, pathological studies and potentially molecular biological studies of carcinogenesis mechanisms are conducted. These studies should contribute to improved health care for the atomic-bomb survivors and all those suffering from radiation damage in the future.

Study Methods: The major hospitals in Hiroshima and Nagasaki will be collaborators and they will keep the surgical samples derived from LSS cohort members in accordance with common procedures that are defined in the RP. The guidelines to use the stored samples for research are also defined in the RP.

Study Progress: Preparation of an operations protocol for the procedures between individual hospitals and RERF is being planned.

Results and Conclusions: None yet.

RP 5-89 Pathology studies in Hiroshima and Nagasaki, revised research plan (Formerly RP 3-75)

Ozasa K (EH), Yonehara S, Fujihara M, Soda M (EN), Kodama K (CS)

Objectives: This RP is a revised platform protocol for the conduct of pathology studies at RERF. Pathologists in community and university hospitals are involved in the site-specific cancer incidence studies.

Background and Significance: The RERF autopsy and surgical programs (ABCC TR 4-61 and RERF RP 3-75) were terminated and this RP is the replacement for them.

Study Methods: Intensive efforts are made to seek the cooperation of pathologists at other medical institution in providing pathological materials. We are collecting microscopic slides for those whose autopsies were performed at local hospitals in Hiroshima and Nagasaki since 1989 after terminating the autopsy program at RERF.

Study Progress: A series of site-specific studies has been conducted. We are taking an inventory of formalin-fixed paraffin-embedded tissues that are stored in RERF. Those specimens are from around 7,000 autopsies or surgical operations. We collected two autopsy cases in Nagasaki this year.

Results and Conclusions: Essential methods of collecting and storing pathological materials were established.

RP-A2-08 Histopathological identification of multiple primary cancers occurring in Nagasaki atomic-bomb survivors

Nakashima M, Soda M (EN), Furukawa K (S), Sekine I, Yamashita S, Shibata Y, Kodama K (CS)

Objectives: The purpose of this protocol is to identify true multiple primary cancers (MPC) diagnoses by modern histopathological methods as an initial step in evaluating the relationship between MPC and radiation exposure in A-bomb survivors.

Background and Significance: A recent analysis at the Atomic Bomb Disease Institute of the Nagasaki University Graduate School of Biomedical Sciences, using similar histopathological methods, found a strong association between MPC rates and distance from the hypocenter, especially for exposure at young ages. The present study is being conducted within the LSS so that actual dose estimates can be applied to the risk estimation.

Study Methods: Based on immunohistochemistry for cytokeratin-7 (CK7) and CK20 (cytoskeleton proteins), thyroid transcription factor-1 and prostatic specific antigen (tissue-specific markers), lung surfactant PE-10, and ovarian cancer marker CA125, MPC of various sites can be distinguished from metastatic tumors. Pathological review by the principal investigator intends to discriminate metastases from second primary cancers by determining the cell type or tissue of origin.

Study Progress: Among the Nagasaki LSS cohort (N = 38,107), there were a total of 6,305 primary-cancer patients between 1958 and 2003. After reviewing the HE-stained tissue specimens and immunohistochemistry findings among 648 patients who had two or more cancers, 595 cases were identified as MPC, but 41 cases (7%) were not. Pathological specimens of another four cases were inadequate for immunohistochemical staining and specimens of eight cases were not available.

Results and Conclusions: Not yet.

Research Protocols 1-14, 1-13, 1-11 and 2-12, 5-10, 5-02, B49-10, B34-03 and B35-04, P1-11

Cell Biology Studies

RP 1-14 A study of the biological significance of the *EML4-ALK* fusion gene in radiation-associated thyroid carcinogenesis using conditional transgenic mice

Hamatani K (R), Ito R (R), Taga M (R), Niwa Y (R), KimY-M (S), Hayashi Y, Eguchi H, Kusunoki Y (R)

Rearranged anaplastic lymphoma kinase (*ALK*) gene (echinoderm microtubule-associated protein-like 4 [*EML4*]-*ALK* fusion gene), which we found for the first time in papillary thyroid cancer (PTC), are likely to be highly correlated with atomic-bomb (A-bomb) radiation exposure and to occur in a fundamentally exclusive manner in *RET*, neurotrophic tyrosine kinase receptor 1 (*NTRK1*), *BRAF*, and *RAS* gene alterations. Interestingly, just as lung adenocarcinoma cases with the *EML4-ALK* fusion gene show histological characteristics significantly different from those without the *EML4-ALK* fusion gene, this fusion gene-positive PTC has characteristic solid/trabecular architectures at a high frequency, suggesting that the *EML4-ALK* fusion gene plays a key role in architectural alterations related to the histopathological characteristics of the cancer tissue that concerns our oncology study. We thus hypothesize that the *EML4-ALK* fusion gene plays an important role in causing PTC and is a result of radiation. Function of the fusion gene may be different from *RET/PTC* rearrangements in terms of their pathological consequences. With use of conditional transgenic mice harboring the *EML4-ALK* gene, we will test the hypotheses from the following viewpoints: One is to evaluate evidence for generation of PTC from the transgenic mice with this fusion gene. The second is to demonstrate effects of radiation in tumorigenesis in these transgenic mice, i.e., the shortened latency and/or the enhanced aggressiveness of the tumors.

RP 1-13 Analyses of molecular characteristics of lung cancer among atomic-bomb survivors

Taga M (R), Hamatani K (R), Ito R (R), Niwa Y (R), Grant EJ (E), Ozasa K (E), Katayama H (IT), Misumi M (S), Sui H, Harris CC, Yasui W, Kusunoki Y (R)

Objectives: The aim of this study is to clarify molecular oncological characteristics of lung cancer among A-bomb survivors.

Background and Significance: Molecular mechanisms of how radiation exposure is associated with the development of lung cancer are unclear. Based on results from a previous pilot study (RP-B37-04), we hypothesize that radiation exposure may affect the profile of genetic and/or epigenetic alterations associated with lung carcinogenesis, working together with cigarette smoking.

Study Methods: We use 1) LSS surgical lung cancer tissue specimens collected by a local pathologist network in Hiroshima (RP-B35-04) and 2) LSS autopsy lung cancer tissue specimens stored at RERF. Using biological materials 1) and 2), we analyze genetic and epigenetic alterations associated with lung carcinogenesis in relation to patho-epidemiological factors including radiation dose and cigarette smoking history. For instance, we examine loss of

heterozygosity (LOH) of *p53*, *p16*, and *RASSF1A*, mutations of *p53*, *EGFR*, and *K-ras*, and methylation of *p16*, *RASSF1A*, and *LINE1* and Alu retrotransposones (a marker of global methylation in genomic DNA). In addition, we examine selected gene rearrangements (e.g., rearranged *ALK*) in LSS lung adenocarcinoma specimens that have been reported in sporadic lung adenocarcinoma.

Study Progress: 1) Thus far from LSS surgical non-small cell lung cancer (NSCLC) tissue specimens we have collected 20 radiation-exposed (dose > 0) and 18 non-exposed (dose = 0) specimens. We found that *p53* mutation frequency and *RASSF1A* methylation levels in exposed NSCLC cases compared to non-exposed NSCLC were suggestively higher and lower, respectively, although not statistically significant. Gene rearrangements were also examined in lung adenocarcinoma cases. No *RET* rearrangements were found, and *ALK* rearrangements were detected in two adenocarcinoma cases among those thus far examined; although the former and the latter rearrangements have been observed in about 1% and 5%, respectively, of lung adenocarcinoma cases among the general Japanese population. Preparation of tissue sections from the autopsy lung cancer cases stored at RERF has recently started. Analyses of genetic and epigenetic alterations in LSS NSCLC cases will be completed in 2018.

Results and Conclusions: Analyses of *ALK* and *RET* rearrangements feasible in LSS lung adenocarcinoma and will be completed in 2014.

RP 1-11 Study of Radiation-induced Circulatory Diseases Using Animal Models

RP 2-12 A Study of Circulatory Diseases Using Animal Models Irradiated with Lower Doses (Addendum to RP 1-11)

Takahashi N (RC), Niwa Y (R), Murakami H. (R), Ohishi W (CH), Misumi M (S), Kusunoki Y (R), Inaba T, Nagamachi A, Kokubo T, Oghiso Y, Tanaka I, Tanaka S

Objectives: The purpose of this study is to assess the relationship between radiation and circulatory diseases (CD) at doses of 4 Gy or less. Data from pathological analysis and measurement of blood biomarkers may provide mechanistic information on the relationship between radiation exposure and development of CD.

Background and Significance: Based on epidemiological and clinical observations from the LSS and AHS, we hypothesize that radiation exposure may cause a high risk of CD. For assessing this hypothesis, we are conducting animal studies where the spontaneous hypertensive rat-stroke prone (SHRSP) rats and SHR rats irradiated with moderate doses are used. Most of the data available from animal studies of radiation and CD have used doses of 2 Gy or more. Through this study, we can also obtain information on the mechanisms of radiation-related CD.

Study Methods: In the study for SHRSP, male rats with age of five weeks are irradiated by gamma ray with 0.25, 0.5, 0.75 and 1 Gy, as well as a 0 Gy control. A parallel preliminary study had used doses of 1, 2, and 4 Gy. The study of SHRSP is conducted in two ways. 1) Whether there is an acceleration in time to show symptoms related to stroke. 2) For another subset of rats, we collect blood from the rats and sacrifice at eight weeks after irradiation to obtain fresh samples for

pathological analyses and measurement of blood biomarkers. In the study of SHR rats (who become hypertensive but are resistant to stroke), blood pressure is measured weekly until 25 weeks after irradiation. At that time the rats are euthanized to obtain fresh samples for measurement of biomarkers and pathological analyses.

Study Progress: Our preliminary study indicated that SHRSP irradiated with 1 to 4 Gy had shortened lifespans compared with unirradiated SHRSP rats. The severity of perivascular changes in such organs as the brain, heart, and kidney were more advanced in the irradiated than the unirradiated rats. To evaluate effects of lower doses, we are now conducting analyses of the SHRSP rats irradiated with 0, 0.25, 0.5, 0.75, and 1.0 Gy, with 10 in each dose group. Stroke onset experiments with a preliminary statistical analysis have been completed, and biomarker measurements and pathological analyses are in progress. To plan a full-scale SHR study, a preliminary experiment was initiated with four rats irradiated with 4 Gy and four unirradiated controls. We also gave an invited presentation of the study at the 2013 MELODI Workshop in Brussels, Belgium that generated considerable interest.

Results and Conclusions: Preliminary statistical analysis demonstrated that the symptoms of stroke in irradiated SHRSP were found significantly earlier than in controls. The preliminary SHR study suggested that systolic blood pressure levels in the rats irradiated with 4 Gy were higher than those in controls. The conclusions for our entire studies will be obtained in 2015.

RP 5-10 Analyses of molecular characteristics of colorectal cancer among atomic-bomb survivors

Ito R (R), Hamatani K (R), Taga M (R), Imai K, Ozasa K (EH), Katayama H (IT), Cologne JB (S), Misumi M (S), Izumi S, Oue N, Yasui W, Nakachi K, Kusunoki Y (R)

Objectives: The aim of this study is to clarify molecular oncological characteristics of colon and rectal cancers among A-bomb survivors.

Background and Significance: Radiation exposure is associated with an increased risk of colon cancer. In colorectal cancer, two major phenotypes, chromosomal instability (CIN) and microsatellite instability (MSI), characterize different carcinogenic pathways. We have focused on molecular events related to those phenotypes and are analyzing the molecular oncological characteristics of colorectal cancer among A-bomb survivors. Our hypothesis based on results from a pilot study in 33 cases is that the MSI pathway may preferentially occur in colorectal cancer among A-bomb survivors, since the five MSI-high (MSI-H) cases showed a significantly higher radiation dose than the other 28 cases. Since MSI-H cases have been more frequently observed in proximal colon cancers than distal colon or rectum cancers, the different radiation response for colon vs. rectal cancer may be explained if we can strengthen this hypothesis. However, MSI and CIN are not necessarily exclusive, and whether or not radiation exposure is associated with CIN-positive colon and rectal cancers is yet to be addressed. This study will help elucidate mechanisms to explain the observed increased risk of colon cancer, but not rectal cancer, among A-bomb survivors.

Study Methods: We use LSS archival surgical and autop-

sied colorectal cancer tissue specimens that are preserved at RERF as well as archival specimens from the Department of Molecular Pathology, Hiroshima University Graduate School of Biomedical Sciences, based on protocols B34-03, B35-04. Using DNA extracted from the micro-dissected cells, we examine MSI and CIN status and their related gene alterations in relation to patho-epidemiological factors including radiation dose.

Study Progress: We have analyzed 77 LSS colorectal cancer cases (including 33 cases previously analyzed in pilot study B38-04), of which 49 and 28 cases were collected from Hiroshima City Hospital and RERF's archives, respectively. Of them, a total of 14 MSI-H cases were found showing no significant association with radiation exposure status. In addition, no association between *MLH1* alterations and radiation exposure status was found. Since latency period (years elapsed since A-bombing till incidence) differed substantially between the cases stored at RERF (<20 years) and those at Hiroshima City Hospital (>35 years), we need to further analyze the potential influence of latency period with an increased number of cases, specifically those with longer latency period. Upon completion of analyses of MSI-related alterations, on which we are currently focusing, we will initiate analyses of CIN-related alterations.

Results and Conclusions: We will submit a manuscript about the characteristics of exposed cases with MSI-H status in FY2014.

RP 5-02 Papillary thyroid carcinomas in residents of Hiroshima and Nagasaki who were exposed to A-bomb radiation as children: A study of *RET* gene rearrangements and other DNA changes potentially responsible for the origins and/or development of these tumors

Hamatani K (R), Taga M (R), Ito R (R), Cologne JB (S), Soda M (EN), Imai K, Nakachi K, Kusunoki Y (R)

Objective: To elucidate the mechanisms of adult-onset papillary thyroid carcinogenesis among A-bomb survivors, we will clarify characteristics of gene alterations that occur at an early stage of thyroid carcinogenesis. We will use archival cancer tissue specimens from the LSS subjects.

Background and Significance: Rearrangements of the *RET* gene are induced in human thyroid cells by *in vitro* and *in vivo* x-ray irradiation. *RET* rearrangements occur at a low frequency of about 5–10% of sporadic adult-onset papillary thyroid cancer (PTC) cases. On the other hand, we observed that the relative frequency of PTC cases with *RET* rearrangements significantly increased with radiation dose, while the frequency of PTC with point mutations, typically *BRAF*^{V600E}, significantly decreased with dose.

Study Methods: Using DNA and RNA from archival tissue specimens of PTC from the LSS cohort, we are examining various gene alterations including *RET/PTC* and *ALK* rearrangements and the *BRAF*^{V600E} point mutation.

Study Progress: In addition to the PTC cases carrying *RET/PTC* and *NTRK1* rearrangements, we have thus far found *EMLA-ALK* fusion genes in 10 exposed PTC cases. This fusion was fundamentally exclusive of any *RET*, *NTRK1*, *BRAF*, or *RAS* gene alterations. To further assess gene alterations in exposed PTC cases carrying no known gene alterations, we examined *FGFR* or *PDGFR* rearrangements

in 15 PTC cases with no known gene alterations, but these 15 PTC cases showed no rearrangements of the *FGFR* or *PDGFR* gene. We have recently started analysis of new type rearrangements, i.e., acylglycerol kinase (*AGK*)/*BRAF* and the transcription factor ETS variant 6 (*ETV6*)/*NTRK3* rearrangements, which were recently reported in post-Chernobyl childhood thyroid tumors. We established real-time PCR conditions for use of archival formalin-fixed paraffin-embedded (FFPE) PTC specimens for future new thyroid study to analyze mRNA expression of such genes that were reported to show significantly different levels in surrounding noncancer tissues of post-Chernobyl PTC from those of sporadic PTC.

Results and Conclusions: Chromosomal rearrangements, such as *RET*, *NTRK1*, and *ALK* rearrangements, likely play an important role in radiation-associated adult-onset thyroid carcinogenesis. These rearrangements are mutually exclusive in our study cases as an early molecular event in carcinogenesis.

RP-B49-10 Establishment of methods for transfection of expression vectors into primary cultured epithelial cells of mouse thyroid tissues

Hamatani K (R), Niwa Y (R), Kusunoki Y (R)

RP-B34-03 A pilot study for systematic collection of tissue specimens of newly diagnosed cancer cases among members of the LSS cohort in Hiroshima

RP-B35-04 A pilot study for systematic collection of archival tissue specimens of cancer cases among the members of LSS cohort in Hiroshima (Addendum to RP-B34-03)

Kusunoki Y (R), Nakachi K, Kodama K (CS), Katayama H (IT), Fujiwara S, Taniyama K, Fukuhara T, Matsuura H, Hayashi Y, Fujihara M, Takeshima Y, Arihiro K, Yasui W

RP-P1-11 Aging effects on epigenetic status in blood cell subsets

Niwa Y (R), Hamatani K (R), Taga M (R), Ito R (R), Yoshida K (R), Kusunoki Y (R)

Cell Biology Studies Publication

Journal Publication

- ◆ Hamatani K: Fusion genes in radiation-associated solid cancer. Saibou [The Cell] 2013 (June); 45(6):6–9. (Japanese)
- ◆ Takahashi I, Ohishi W, Mettler FA Jr, Ozasa K, Jacob P, Bab N, Lipshultz SE, Stewart FA, Nabika T, Niwa Y, Takahashi N, Akahoshi M, Kodama K, Shore RE, the International Radiation and Cardiovascular Disease Workshop Participants: A report from the 2013 International Workshop: Radiation and Cardiovascular Disease, Hiroshima, Japan. J Radiol Prot 2013 (December); 33(4):869–80. (related to *Life Span Study* and *Adult Health Study*)

Cell Biology Studies Oral Presentations

- ❖ Ito R, Hamatani K, Yano S, Shinohara T, Takahashi K, Yasui W, Nakachi K, Kusunoki Y. Microsatellite instability (MSI) and MLH1 alteration in colorectal cancer among A-bomb survivors. 54th Late A-bomb Effects Research Meeting, 2 June 2013, Hiroshima
- ❖ Ito R, Hamatani K, Yano S, Shinohara T, Takahashi K, Oue

N, Yasui W, Nakachi K, Kusunoki Y. Microsatellite instability (MSI) and related gene alterations in colorectal cancer among A-bomb survivors. 102nd Annual Meeting of the Japanese Society of Pathology, 6–8 June 2013, Sapporo

❖ Hamatani K, Takahashi K, Nakachi K, Kusunoki Y. Molecular oncology study of papillary thyroid cancer among atomic-bomb survivors with a focus on early molecular events. 72nd Annual Meeting of the Japanese Cancer Association, 3–5 October 2013, Yokohama

❖ Niwa Y, Murakami H, Kusunoki Y, Ohishi W, Misumi M, Nagamachi A, Inaba T, Oghiso Y, Tanaka S, Takahashi N. Study of radiation related cardiovascular diseases (CVD) using animal models. 5th International MELODI (Multidisciplinary European Low Dose Initiative) Workshop, 7–10 October 2013, Brussels, Belgium

❖ Takahashi N, Murakami H, Ohishi W, Misumi M, Kusunoki Y, Nagamachi A, Inaba T, Oghiso Y, Tanaka S, Niwa Y. Study of radiation related circulatory diseases using a model animal; preliminary studies about introduction of spontaneous hypertensive rat (SHR). 56th Annual Meeting of the Japan Radiation Research Society, 18–20 October 2013, Aomori

Research Protocols 2-13, 4-11, 1-10, 5-85 and 1-01 Biochemical Genetics Studies

RP 2-13 Estimation of genetic risk of radiation on mature oocytes of mice by using next generation sequencer

Satoh Y (G), Furukawa K (S), Cullings HM (S), Nakamura N, Nishimura M, Shimada Y, Asakawa J (G)

Objectives: The purpose of this study is to examine the mutation induction rate following 4-Gy gamma irradiation in mouse mature oocytes by whole genome sequencing.

Background and Significance: Whole genome sequencing provides more detailed information on the genome compared to other previous techniques used in the Department of Genetics. In this study, we will conduct whole-genome sequencing of mouse parents and their offspring born to 4-Gy irradiated mature oocytes using a next generation sequencer. We will clarify the mutation frequency and spectrum for small deletions/insertions that are not detectable by previous studies. We also evaluate the feasibility of human studies using this method.

Study Methods: Followed by obtaining the control F₁ mice, we irradiated the same female mouse C57BL/6 and then immediately mated with the same unirradiated male mouse C3H. Genomic DNA from F₁ mice born before and after irradiation of the mother and the parents were sequenced with a next generation sequencer.

Study Progress: We completed whole genome sequencing of eight mouse samples and obtained their raw sequence data.

Results and Conclusions: Not yet obtained.

RP 4-11 Genetic study of atomic bomb radiation by using HD-microarray CGH analysis

Kodaira M (G), Satoh Y (G), Furukawa K (S), Nakamura N, Asakawa J (G)

Objectives: The purpose of the proposed study is to examine if the parental exposure to A-bomb radiation has induced deletion/amplification mutations in the genomes of the children.

Background and Significance: The heritable genetic effects of A-bomb radiation (trans-generational effect) have not been fully elucidated due to low rates of both spontaneous mutation and radiation-induced mutation. We have established the comparative genome hybridization (CGH) approach as a reliable mutation screening method by improving the repeatability, resolution, accuracy, and efficiency. Our improved CGH approach can now detect from small (3–5 kb) to large (~10 Mb) deletions with high accuracy. Since radiation-induced mutations are primarily deletions initiated with DNA double-strand breaks, we are conducting a CGH study using high-density microarrays.

Study Methods: We are analyzing 688 DNA samples from both parents of 184 families (exposure is restricted to either parent) and their 320 offspring (160 from paternally and 160 from maternally exposed families) using high-density microarrays with 1.4 million probes.

Study Progress: We established a database of polymorphic copy number variants among 50 Japanese. We expanded the CGH examination to a total of 639 DNA samples, 169 mother-father-child trios and 132 children. We finished the genetic

Table. The total number of children with informed consents and cell lines

Father's dose (Gy)	Mother's dose (Gy)							Total
	>2.00 ^a	1.50–1.99	1.00–1.49	0.50–0.99	0.01–0.49	0&Null ^c	Unknown ^d	
>2.00	2(2 ^b)	3		1	4(2)	67(19)	1	78(23)
1.50–1.99					3	44(14)	2	49(14)
1.00–1.49		1	7	5	17(6)	96(28)	3	129(34)
0.50–0.99		2(1)	1	13(4)	8	132(34)	4	160(39)
0.01–0.49	4	2	2	11	41(10)	114(41)	5(2)	179(53)
0&Null ^c	27(5)	44(8)	117(29)	292(69)	137(43)	655(110)	51	1,323(264)
Unknown ^d	2			7(2)	4(1)	39(1)		52(4)
Total	35(7)	52(9)	127(29)	329(75)	214(62)	1,147(247)	66(2)	1,970(431)

^a The doses shown in the table are rounded at the second decimal place.

^b The numbers in parentheses are the number of children whose blood samples have been re-collected.

^c The “null” individuals were spouses of the atomic-bomb survivors who were not in city or more than 10,000 m from the bomb and were not included in LSS cohort.

^d Distances from the hypocenter were known, but shielded conditions were unknown for individuals in this column.

analyses of the CGH data for 125 trios. We have identified about 30 mutation candidates and have conducted molecular validation for ~20 cases.

Results and Conclusions: Not yet obtained.

RP 1-10 Estimation of genetic effects of radiation in male germ cells of mice: Study for assessment of a high-density microarray CGH platform

Asakawa J (G), Kodaira M (G), Shimada Y, Cullings HM (S), Nakamura N

Objectives: The purpose of the study is, as an animal model of human male exposure, to estimate the mutation induction rate following 4 Gy of gamma irradiation of mouse spermatogonia, and to molecularly characterize the mutations.

Background and Significance: This study will provide crucial information necessary for planning future genetic studies. Interpretation of the results will be important in order to determine the feasibility of RERF examining the DNA obtained (e.g., whether or not duplications occur as frequently as deletions and whether both are of equal importance) from the offspring of Hiroshima and Nagasaki survivors whose radiation doses are much smaller than those used in animal studies.

Study Methods: We estimated the mutation induction rate by examining DNA samples of 100 F₁ mice derived from the spermatogonia of male mice irradiated with 4 Gy of γ rays and 100 F₁ mice in the control group by an HD-array CGH technique.

Study Progress: A total of 22 mutations, 10 in the exposed group (6 deletions in 6 mice, 4 amplifications in 4 mice) and 12 mutations in the control (7 deletions in 7 mice, 5 amplifications in 3 mice, i.e., 1 mouse had 3 amplifications) were detected. We molecularly characterized most of the mutations, including junction sequencing and identification of parental origin by SNP analyses.

Results and Conclusions: The results imply that the frequency of large deletions induced by radiation exposure may be considerably lower than currently estimated.

RP 5-85 Culture of lymphoblastoid cell lines as sources of biological samples for investigation of genetic effects of radiation on children of atomic bomb survivors

RP 1-01 The acquisition of signed informed consent forms from the donors (or their proxies) for whom permanent cell lines have been established (Addendum to RP 5-85)

Satoh Y (G), Takahashi N, Ohishi W (CH), Katayama H (IT), Hida A (CN)

Objectives: To archive untreated cells and to establish lymphoblastoid cell lines by transformation of B cells from members (parents and children) of families as biological resources for current and future genetic studies, and to obtain written informed consents from them.

Background and Significance: Blood cells have been obtained from 908 parent-child “trios” (including 1,500 children) and lymphoblastoid cell lines have been established. Moreover, as the new F₁ clinical follow-up study has started from November 2010, we have started to re-collect the blood samples from the participants in order to increase the numbers of untreated cells, which will be useful for analyses by newly developed technologies in the future.

Study Methods: An aliquot of lymphocytes separated from the donated blood was transformed by Epstein-Barr virus to establish cell lines while the remaining lymphocytes and poly-nuclear cells were cryopreserved without culturing. In the new F₁ clinical study, additional uncultured lymphocytes and poly-nuclear cells will be cryopreserved.

Study Progress: We collected new blood samples from 130 individuals this year.

Results and Conclusions: In the course of this project, we have established cell lines from 4,374 individuals with informed consents. The total number of children who gave us informed consents and whose cell lines were established is 1,970, as summarized below (Table) in relation to parental dose. And we have re-collected 431 specimens in the new F₁ clinical study. The numbers of re-collected blood samples are summarized inside the parentheses of the Table.

Biochemical Genetics Studies Oral Presentations

- ❖ Satoh Y. Analysis of the genetic effects of radiation on the mouse mature oocytes by the whole genome sequencing method. Expanded Group Meeting, 2013, Scientific Support Programs for Genome Science, 28–29 August 2013, Kobe
- ❖ Kodaira M, Miura A, Imanaka M, Tsuji T, Nakamoto Y, Nishimura M, Shimada Y, Asakawa J. Can we distinguish radiation-associated mutations from spontaneous mutations? 56th Annual Meeting of the Japan Radiation Research Society, 18–20 October 2013, Aomori

Research Protocols 6-11, 6-09, 1-08, 6-00, 8-93, A4-09, A2-09

Cytogenetics Studies**RP 6-11 A study of chromosome aberration frequency in thyroid cells following fetal exposure to ionizing radiation in mice**

Hamasaki K (G), Noda A (G), Nakamura N, Hsu W-L, Kodama Y (G)

Objectives: Aims of this study are to examine whether chromosome aberrations induced by *in utero* exposure to radiation persist in mouse thyroid cells and to better understand the tissue-specific characteristics of radiation-induced chromosome aberrations following fetal irradiation.

Background and Significance: Studying the biological effects of radiation to fetuses is essential for understanding the cancer risk to the fetus. We have previously reported that chromosome aberration frequency following fetal exposure to radiation may differ among different types of tissue; namely, it is low in lymphoid cells while remaining high in mammary epithelial cells in rats. To understand the mechanisms of the tissue dependency, we propose to examine thyroid cells of mice following fetal irradiation. The results obtained in this study may help to explain the cancer risks of A-bomb survivors who were exposed *in utero*.

Study Methods: To test the hypothesis that chromosome aberration frequency following fetal irradiation may differ by tissue, we will examine translocation frequency in mouse thyroid cells in three groups of mice; that is, 1) mice irradiated as fetuses, 2) mothers irradiated while pregnant, and 3) non irradiated mice. Using chromosome slides prepared following primary culture of thyroid epithelial cells, translocation frequencies will be determined and evaluated by a FISH (fluorescence *in situ* hybridization) method that labels chromosome 1 (green) and 3 (red). More than 500 metaphase cells will be needed for analysis in each group.

Study Progress: We successfully analyzed more than 1,000 metaphase cells in each of three groups by FISH. Current data indicate that there were 30 translocations in 1,155 metaphase cells in the fetus and 39 translocations in 1,149 cells in their irradiated mother, while no aberrations were observed in control mice (0/1,007).

Results and Conclusions: Chromosome damage in mouse thyroid cells following fetal exposure to radiation seems to persist, as does damage in rat mammary epithelial cells. These results suggest that chromosome aberration induction by radiation might be different between blood cells and non-blood cells, such as mammary and thyroid cells.

RP 6-09 Evaluation of the nonmelanoma skin cancer risk among heterozygotes bearing a founder mutation allele unique to a Japanese population at xeroderma pigmentosum group A (XPA) gene

Hirai Y (G), Nakamura N, Noda A (G), Cullings HM (S), Ozasa K (EH), Yonehara S, Fujihara M, Moriwaki S, Nishigori C, Mabuchi K, Kraemer KH, Land CE, Kodama Y (G)

Objectives: To evaluate the relative risk of developing skin cancers among carriers of an inactive allele of the XPA genes.

Background and Significance: The frequency of patients with cancer-prone recessive hereditary disorders, such as xeroderma pigmentosum (XP), is very rare, but carriers (heterozygotes) are not rare. However, there are few data regarding cancer risk in heterozygote carriers, as they are generally difficult to identify. This study will focus on a founder mutation allele of the *XPA* gene, which is an inactive allele known to cause severe disease phenotypes under homozygous conditions and the mutation heterozygotes are found in about 1% of the Japanese population, which is unique to the Japanese. Those conditions provide a unique advantage in effective screening of such carriers.

Study Methods: We will screen about 1,000 non-melanoma skin cancer specimens and an additional 500 chromosome slides as the control to estimate the frequency of the *XPA* heterozygotes using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Study Progress: We screened DNA from additional 380 non-melanoma skin cancers, and found five additional *XPA* heterozygotes among them.

Results and Conclusions: Thus far, we found five *XPA* heterozygotes among 682 chromosome slides, and 12 *XPA* heterozygotes among 857 non-melanoma skin cancers.

RP 1-08 Establishment of a recombinant mouse model for assessment of genetic effects of radiation at low doses

Noda A (G), Hidaka M (G), Sekihara K (G), Hirai Y (G), Kodama Y (G), Cullings HM (S), Nakamura N

Objectives: To make novel animal model systems which will allow us to estimate the genetic risk (mutagenic risk) of radiation at low doses. Risks of somatic mutations and genomic instability in various tissues will also be evaluated in irradiated individuals as well as F₁ populations born to irradiated parents.

Background and Significance: Since the genetic risk of radiation is difficult to assess at low doses, we plan to generate new mouse *in vivo* systems to detect and measure radiation-induced mutant frequencies occurring in germ cells, as well as in various somatic cells, at low doses. In these genetically engineered mice, mutant cells that are naturally occurring, or radiation-induced, become fluorescent in living tissues. That is a breakthrough of classical genetics which had to analyze millions of F₁ mice. With regards to somatic tissue, mutagenic risk assessment for target organs of radiation carcinogenesis will be validated *in situ*, without destroying tissue architecture.

Study Methods: By applying mouse embryonic stem (ES) cell knock-in techniques, recombinant mice will be generated. We plan to create two kinds of mouse strains. One is a gene reversion system (first generation knock-in): mice carrying a partial duplication of the *HPRT* gene in conjunction with the *GFP* gene at the 3' end of the duplicate (*HPRT^{dup}GFP* mice). In this case, reversion from the duplicate produces *HPRT-GFP* fusion proteins, thereby making the mutant cells fluorescent. The other is a gene forward mutation system (second generation knock-in); activating mutations of oncogenes, or inactivating mutations of tumor suppressor gene, make the cells GFP positive. In

the case of the second-generation knock-in mice, radiation-induced mutations will directly lead to the development of tumors. We plan to make such systems by using the *ras* or *p53* gene.

Study Progress: We have successfully made *HPRT^{dup}GFP* mice, the first generation knock-ins. In these mice, spontaneously arising and radiation-induced mutant cells (revertants) in the body could be detected clearly by observation of dissected tissues with a fluorescent microscope. Irradiation experiments were performed to evaluate radiation effects on various organs including the testis. An F₁ population born to irradiated fathers was also generated. Studies for fetus and neonate irradiation are also planned. For the second generation knock-ins, several strategies to make *p53-GFP* knock-ins were planned. This year, two new researchers, Drs. Hidaka and Sekihara, joined this project.

Results and Conclusions: We evaluated the mutagenic effects of radiation on cells that constitute various tissues, such as the liver, pancreas, spleen, small intestine, as well as testis. The paper is now in preparation.

RP 6-00 Molecular alterations in early-onset breast and ovarian cancers among atomic bomb survivors

Hirai Y (G), Nakamura N, Cologne JB (S), Mabuchi K, Land CE

Objectives: To test the hypothesis that the high incidence of early-onset breast cancers among A-bomb survivors may be due to the damaging effect of A-bomb radiation on the normal allele of a breast cancer susceptibility gene in women who are heterozygous carriers of germline mutations of the gene.

Background and Significance: Breast cancer is one of the most radiogenic tumors among A-bomb survivors. Further, the risk is particularly high among those who were exposed at ages below 20 and developed breast cancer before age 35 (early onset). We hypothesized that the high risk is due to the heterozygous inheritance of mutated breast cancer susceptibility genes and that radiation exposure had caused loss of the function of the remaining wild-type allele.

Study Methods: We are examining the plausibility of specific single nucleotide polymorphisms (SNPs) that have been suggested as contributing to early-onset breast cancers.

Study Progress: We reexamined TP53 codon72 polymorphism among 45 cases, because we could not obtain clearly defined results from them at the first examination.

Results and Conclusions: None yet.

RP 8-93 Cytogenetic study in the Adult Health Study population by fluorescence *in situ* hybridization (FISH)

Kodama Y (G), Hamasaki K (G), Noda A (G), Kodaira M (G), Kusunoki Y (R), Shimizu Y (EH), Cullings HM (S), Misumi M (S), Nakamura N

Objectives: The purpose of this study is to examine quantitatively radiation-induced chromosome aberrations (mainly translocations) in blood lymphocytes of atomic-bomb (A-bomb) survivors in the Adult Health Study (AHS) cohort using the fluorescence *in situ* hybridization (FISH) technique.

Background and Significance: A cytogenetic

investigation following ionizing radiation exposure is regarded as the most reliable tool for biological dosimetry. Blood lymphocytes can be considered as a natural, *in situ* dosimeter for the exposed individual. A further advantage is that the chromosome test is free from any recall bias while physical dose reconstruction requires precise information on location and shielding conditions for individual dose estimation that are critical but are often unavailable or subject to recall biases.

Study Methods: We have employed 2-color-FISH for detection of translocations involving chromosomes 1, 2, and 4. As a routine procedure, we score 500 FISH-stained metaphases per sample. All blood samples are coded so that the chromosome tests are being performed without any knowledge of individual physical doses.

Study Progress: In FY2013, blood samples were examined from 47 survivors (42 from Hiroshima and 5 from Nagasaki). To date, 1,142 survivors in Hiroshima and 681 survivors in Nagasaki have been examined with FISH.

Results and Conclusions: The dose response from current FISH data showed a wide scattering of individual translocation frequencies as we observed in the previous solid Giemsa staining study. However, the two sets of data (solid Giemsa and FISH) for the same individuals agreed well with each other.

RP-A4-09 Detection of unrepairable DNA damage (DNA double strand breaks) in cells and tissues post-irradiation

Noda A (G), Hirai Y (G), Nakamura N, Kodama Y (G)

Objectives: We hypothesized that radiation-induced unrepairable DNA double strand breaks (DSB) are permanently retained in non-apoptotic, non-dividing quiescent cells. We will find sensitive methods to detect such unrepairable DSB *in vitro* and *in vivo*, and apply them to archival tissues of A-bomb survivors so that retrospective dose estimation can be made.

Background and Significance: Following irradiation of cells *in vitro*, unrepairable DSBs form large repair foci consisting of repair and stress-signal proteins in the nuclei, possibly remaining for years post-irradiation. If we are able to distinctly detect such foci in the tissues at long times after irradiation (we guess they are retained permanently if the cells are non-dividing and have a very long life span, e.g., neuronal or pancreatic cells), it will help us to estimate radiation doses, as well as to elucidate causative mechanisms of the late effects of radiation in irradiated tissues.

Study Methods: (1) Biochemical identification of the specific components of the repair foci, (2) expression array screenings of the genes specifically expressed in cells bearing unrepairable DSBs, (3) isolation of monoclonal antibodies that distinctly react with unrepairable DSBs, were carried out.

Study Progress: At long times after exposure to ionizing radiation (up to one year) we are able to successfully detect unrepairable DSB-foci in a dose dependent manner in normal human fibroblasts that were kept under quiescent conditions. We also detected such damage in irradiated mouse tissues. Microscopic observation and chemical inhibitor treatment indicated that the damages affected nuclear membrane architecture, making deformed nuclei in

cells bearing unrepairable DSB-foci. Enforced expression of human telomerase ameliorated this effect and rescued the cells from damage-induced senescence. Genome-wide screening of expressed genes and comprehensive protein screenings revealed several candidate genes (proteins) that might be specific in the cells. Trials to generate mouse monoclonal antibodies against such proteins have been performed.

Results and Conclusions: Our results indicate that the nuclear membrane is involved in DSB repair, especially for such damage that is difficult to be subjected to normal repair. We submitted a paper. Comprehensive screenings to identify the genes (proteins) distinctly localized in unrepairable DSBs are still needed.

RP-A2-09 Comprehensive analysis of radiation-induced genetic damage in human peripheral blood T cells using comparative genomic hybridization (CGH) and cytogenetic techniques

Honma M, Ukai A, Hamasaki K (G), Kodama Y (G), Kusunoki Y (R)

Objectives: Comprehensive analysis of damaged genome regions following radiation exposure in human peripheral blood T-cell populations.

Background and Significance: Ionizing radiation induces various types of large structural alterations in the genome, and some genetic alterations may lead to cell transformation. However, little is known about how large chromosomal regions are altered and what types of genetic alterations preferentially remain in a normal cell following ionizing irradiation with a given dose. This approach may provide a novel way to seek possible mechanisms of somatic gene mutations leading to radiation-related cancers.

Study Methods: To determine structural alterations in the genome following radiation exposure, we have evaluated damaged genome regions in human peripheral blood T-cell populations that were clonally propagated after *in vitro* X-irradiation. By comparing DNA extracted from each irradiated T-cell clone with reference DNA also extracted from unirradiated peripheral blood mononuclear cells (PBMCs), damaged genome regions were identified by a comparative genomic hybridization (CGH) assay using a 244K human genome array that allows comprehensive analysis over the entire genome; the CGH was conducted using DNA extracted from each irradiated T-cell clone and reference DNA also extracted from unirradiated PBMCs but differently labeled with fluorescent dyes. G-banding and multi-color chromosome analyses have also been conducted for T-cell clones identified with gene deletions to confirm damaged genome regions at the chromosomal level.

Study Progress: All assays have been completed. A manuscript is in preparation.

Results and Conclusions: Among 33 T-cell clones from PBMCs irradiated with 1 Gy of X rays *in vitro*, 11 (33%) showed structural changes including deletions (14 kb–130 Mb) or amplification (28 kb) at least in one chromosome, and one clone showed trisomy. Molecular and cytogenetic analyses demonstrated that the majority of deletions were simple interstitial ones, and that one clone has a large terminal deletion (130 Mb) accompanying an unbalanced translocation. Fourteen non-irradiated T-cell clones were

similarly analyzed: One showed trisomy and the other 13 showed no structural changes. These results indicate that genome damage induced by X rays in normal human blood cells are usually repaired by non-homologous end-joining, resulting in simple interstitial deletions. Effects of radiation dose and dose rate as well as other DNA-damaging agents can be evaluated using a similar assay system.

Cytogenetics Studies Publication

RERF Report (RR)

◆ Nakano M, Nishimura M, Hamasaki K, Mishima S, Yoshida M, Nakata A, Shimada Y, Noda A, Nakamura N, Kodama Y: Fetal irradiation of rats induces persistent translocations in mammary epithelial cells similar to the level after adult irradiation, but not in hematology cells. *Radiat Res* 2014 (February); 181(2):172–6. (RR 3-13) © 2014 by Radiation Research Society

[Study Findings] Examination of rat chromosomes following fetal irradiation showed radiation-induced chromosome aberrations in mammary epithelial cells similar to those found in the mothers, but very few aberrations were observed in lymphocytes. This result demonstrates the presence of tissue specificity with regard to chromosome aberrations following fetal radiation exposure.

[Explanation] Previous studies on humans and mice have shown that fetal exposure to radiation fails to induce persistent chromosome aberrations in blood lymphocytes. Such a low level of response to radiation exposure is counterintuitive in view of the generally accepted belief that fetuses are highly sensitive to radiation. In this study, we examined chromosomes of mammary tissues in rats irradiated *in utero* to determine whether or not the same result is observed in other tissues. **Objectives** With the aim of determining whether or not the frequencies of chromosome aberration attributable to fetal radiation exposure differ by tissue type, this study examined mammary epithelial cells in rats. In addition, chromosomes of spleen lymphocytes were examined in a subgroup of rats to compare the frequencies of aberration between blood cells and epithelial cells. **Methods** Pregnant rats were irradiated at a dose of 2 Gy on day 17.5 of gestation. Mammary tissues were collected from the mothers and their offspring six, nine, and 45 weeks after irradiation. After mammary epithelial cells were cultured, chromosome samples were prepared by a conventional method. To detect translocations, we adopted the fluorescent *in situ* hybridization (FISH) technique to stain chromosomes 2 and 4 green and red, respectively, and 800 cells were examined for each rat. The same procedure was followed to examine the chromosomes of cultured spleen lymphocytes. **Results** (1) The mean translocation frequency in mammary epithelial cells of rats irradiated *in utero* with a dose of 2 Gy was 3.7% (n = 23). This figure is similar to the mean frequency of 2.9% observed in the mothers (n = 5). (2) The translocation frequency in spleen lymphocytes was 0.0–0.6% among rats irradiated *in utero* (mean frequency: 0.4%, n = 13), which was significantly lower than the mean translocation frequency of 3.5% observed in the mothers (n = 3). (3) Translocation frequencies were similar at six, nine, and 45 weeks after

irradiation. As noted above, this study showed that the frequency of chromosome aberrations in the mammary cells of rats irradiated *in utero*, and examined at six to 45 weeks of age, was as high as that in the mothers. In contrast, the translocation frequency in lymphocytes was high in the mothers but considerably lower among rats irradiated *in utero* (about 10% of that of the mothers), supporting the findings in previous studies on mice and humans. These results suggest tissue specificity in the chromosome aberration pattern in fetal exposure. If the translocation frequency is assumed to be an index of the carcinogenic effect of radiation, this study suggests that fetal radiation exposure may induce persistent carcinogenic damage in mammary stem cells and progenitor cells. However, the results of another report showed that fetal exposure in rats did not increase breast cancer risk. No clear reason has been identified that can explain this discrepancy, highlighting the need for further study.

Cytogenetics Studies Oral Presentations

❖ Hamasaki K, Noda A, Nakamura N, Kodama Y. Chromosome aberration frequency induced by fetal irradiation does not show the same pattern among the tissues. 59th Annual Meeting of the Radiation Research Society, 15–19 September 2013, New Orleans, Louisiana, USA

❖ Noda A, Mishima S, Hirai Y, Hamasaki K, Mitani H, Haga K, Kiyono T, Nakamura N, Kodama Y. Progerin, the causal protein for the accelerated premature aging in humans, enhances levels of unrepaired DNA damages following genotoxic stress insult. 44th Annual Meeting of Environmental Mutagenesis and Genomics Society, 21–25 September 2013, Monterey, California, USA

❖ Noda A, Hirai Y. New approaches toward identifying novel proteins that distinctly localize at radiation-induced unrepairable DSBs. 56th Annual Meeting of the Japan Radiation Research Society, 18–20 October 2013, Aomori

❖ Hamasaki K, Noda A, Nakamura N, Kodama Y. A study on chromosome aberrations induced in mouse thyroid cells following fetal X-ray irradiation. 64th Annual Meeting of the Chromosome Research Society, 8–10 November 2013, Toyama

**Research Protocols 4-75 (Platform Protocol)
F₁ Studies—Genetic Effects of Atomic
Radiation on Children Born to Bombing
Survivors**

RP 4-75 Research plan for RERF studies of the potential genetic effects of atomic radiation; Hiroshima and Nagasaki. Part 1. Mortality and cancer incidence study of children born to atomic-bomb survivors

Grant EJ (EH), Furukawa K (S), Sakata R (EH), Cullings H (S), Shimizu Y, Ozasa K (EH), Kodama K (CS), Cologne JB (S)

Objectives: To determine whether parental radiation exposure affects mortality and/or cancer incidence rates in their offspring.

Background and Significance: Animal studies have reported effects of radiation on mutation rates in the F₁ generation in accordance with the genetic hypothesis. This population provides one of the very few opportunities in the world to study the heritable genetic risks in humans into adulthood from exposure to ionizing radiation. As population exposures to medical radiation procedures increase before and during childbearing years, the public health implications of the results from this study continue to be timely and important.

Study Methods: The F₁ mortality cohort target sample consisted of 76,814 subjects. They were selected from the children born from May 1946 through December 1984 to parents with a variety of A-bomb radiation exposures, ranging from those known not to be in the city at the time of the bombing to those who were heavily exposed to acute radiation. The average age of the cohort as of 2008 was 52 years; 47% of the cohort was older than 55 years.

Study Progress: Analyses of updated cancer and noncancer mortality (through 2008) have been completed. A manuscript has been written and is currently under internal review.

Results and Conclusions: To date, we have found no significantly increased rates of noncancer or cancer mortality, or cancer incidence associated with paternal or maternal dose.

**Research Protocols 7-11, 2-09, 1-09, 5-08 and 6-10,
4-07, 1-06, 2-04, 1-04, 6-02, 2-91 and 2-02, 3-94, 1-94,
6-91 and 5-11, 9-88, 29-60, A5-12, A3-11, A5-10,
A3-10, A12-08, A5-08**

Special Cancer Studies

RP 7-11 Preservation of fresh thyroid samples obtained from Adult Health Study participants (Addendum to RP 2-86)

Imaizumi M (CN), Ohishi W (CH), Sera N (CN), Hida A (CN), Yamada M (CH), Hamatani K (R), Ozasa K (EH), Akahoshi M

Objectives: To preserve fresh thyroid samples removed from thyroid tumor cases detected among AHS subjects for future genetic and mechanistic studies on thyroid radiation carcinogenesis.

Background and Significance: Thyroid cancer shows an increased incidence with radiation dose among A-bomb survivors. However, the mechanisms of radiation induction of thyroid cancer are not fully understood. Collection of as many thyroid cancer samples as possible, could contribute to the mechanistic elucidation of thyroid cancer development and radiation carcinogenesis. Furthermore, since significant dose response is observed even for benign thyroid tumors, it is also important to compare cancer and benign tumors at the molecular level for elucidation of carcinogenic mechanisms. In accordance with an earlier research protocol RP 2-86, RERF has worked on the collection and cryopreservation of fresh thyroid cancer tissues from A-bomb survivors and non-exposed controls. Thyroid ultrasound examination has been performed in AHS health examinations since 2000, thereby leading to the detection of a large number of thyroid nodules. On account of the rapid decrease in the number of A-bomb survivors, it is important to collect systematically as many thyroid samples as possible, regardless of benign or malignant thyroid tumors, from AHS subjects. The present RP, an addendum to the aforementioned RP 2-86, aims at the systematic collection of fresh tissue from both benign and malignant thyroid tumors removed from AHS subjects.

Study Methods: When we are notified that thyroid surgery is to be performed in an AHS subject, we obtain advance consent from the donor subject and visit the hospital to obtain the fresh sample. One to two pieces of the sample will be rapidly frozen in liquid nitrogen.

Study Progress: We are continuing sample collection. We have obtained fresh samples from a total of 30 thyroid tumor cases (including samples collected based on RP 2-86).

Results and Conclusions: None. This RP is only for collection of frozen fresh samples from thyroid tumor cases detected among AHS subjects in preparation for future molecular research. Research protocols using the samples will be prepared separately in the future.

RP 2-09 Study on secondary cancer risks after radiotherapy among A-bomb survivors

Yoshinaga S, Soda M (EN), Akahane K, Doi K, Moriwaki H (EH), Hida A (CN), Yamada M (CH), Katayama H (IT), Shimada Y, Fujiwara S, Kasagi F, Ozasa K (EH)

Objectives: To evaluate the combined effects of A-bomb radiation and subsequent therapeutic radiation.

Background and Significance: Very limited information is available from epidemiological studies on the effects of exposure to multiple radiation sources. The RP focuses on secondary cancer risk of a subset from the LSS who had undergone radiotherapy subsequent to A-bomb radiation.

Study Methods: The subjects include 1,501 A-bomb survivors who were confirmed to have undergone radiotherapy and whose organ or tissue doses from that radiotherapy will be approximated. Secondary cancer risks from radiotherapy doses will be calculated adjusting for A-bomb radiation dose and also modifying A-bomb radiation effects.

Study Progress: Analysis is ongoing at the National Institute of Radiological Sciences (NIRS).

Results and Conclusions: None yet.

RP 1-09 A nested case-control study of factors contributing to acceleration of the development of hepatocellular carcinoma using stored sera (Addendum to RP 1-04)

Ohishi W (CH), Cologne JB (S), Fujiwara S, Ueda K (CH), Hida A (CN), Niwa Y (R), Ozasa K (EH), Tsuge M, Chayama K

Objectives: The hypothesis behind this study is that chronic inflammation and/or insulin resistance may be involved in increased risk of radiation-related hepatocellular carcinoma (HCC). The objective of this study is to examine the contribution of inflammation and/or insulin resistance to HCC risk, taking into account radiation exposure, hepatitis virus infection, lifestyle-related factors, and severity of liver fibrosis.

Background and Significance: This research protocol is an addendum to RP 1-04. In the previous study, we demonstrated that hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, obesity, and alcohol consumption are independent risk factors for HCC. The combination of HCV infection and increased body mass index (BMI) exerted a synergistic effect on risk of HCC.

Study Methods: We have measured blood cytokine levels related to chronic inflammation and/or insulin resistance that are considered important factors contributing to acceleration of the development of HCC using stored sera before HCC diagnosis among subjects of this nested case-control study (RP 1-04), as well as examined their contribution to HCC risk.

Study Progress: We have estimated the relationship between inflammatory marker levels and HCC risk based on the tertile distribution of each marker in the controls, after adjusting for confounding factors such as HBV and HCV infection, lifestyle-related factors, and radiation dose. A paper on serum inflammatory markers associated with HCC risk was published in the *International Journal of Cancer*.

Results and Conclusions: Higher serum levels of IL-6 were associated with increased HCC risk, independently of hepatitis virus infection, lifestyle-related factors, and radiation exposure. Interaction of obesity with IL-6 levels in relation to HCC risk was significant, but that of radiation with IL-6 levels was not observed (*Int J Cancer* 2014; 134:154–63).

RP 5-08 Breast cancer incidence among atomic-bomb survivors, 1950–2005

RP 6-10 Intrinsic subtypes of breast cancer among atomic-bomb survivors (Addendum to RP 5-08)

Yonehara S, Nishisaka T, Nakashima M, Furukawa K (S), Soda M (EN), Sekine I, Mabuchi K, Preston DL, Sadakane A (EH), Ozasa K (EH), Kodama K (CS)

Objectives: To clarify the relationship between radiation and breast cancer incidence based on histologically reviewed diagnoses according to current WHO classification criteria, and to clarify the immunohistochemical (IHC) characteristics of radiation-induced breast cancer. This study updates the past study by adding cases diagnosed from 1991–2005 and will provide more precise estimates of radiation effects.

Background and Significance: Breast cancer shows one of the highest associations with radiation, but no differences in the histological distributions were observed in the past between exposed and control cases. Histological reviews according to a new classification system along with IHC subtyping will contribute to characterization of radiation-induced breast cancer.

Study Methods: Potential breast cancer cases diagnosed during 1950–2005 collected from cancer registries and other available sources will be histologically reviewed by pathologists (RP 5-08). Among histologically confirmed breast cancer cases, suitable samples will undergo IHC staining to determine intrinsic subtypes of breast cancer according to presence or absence of estrogen and progesterone receptors (ER/PR) and Her2 (RP 6-10).

Study Progress: As of December 10, 2013, histopathological review has been completed by two pathologists for all 1,732 probable cases. The IHC staining has been completed for 90% of 1,396 suitable cases among the histologically confirmed breast cancer cases. Evaluation of intrinsic subtype has been made for approximately 700 cases.

Results and Conclusions: None yet.

RP 4-07 Pathology study of malignant tumors of soft tissue and bone among A-bomb survivors, 1957–2003

Yonehara S, Hayashi T, Daimaru Y, Sekine I, Soda M (EN), Neta G, Brenner A, Berrington de Gonzalez A, Mabuchi K, Preston DL, Ozasa K (EH), Kodama K (CS)

Objectives: To clarify the relationship between radiation and the incidence of soft tissue and bone tumors based on histologically reviewed diagnoses, and to clarify the histological characteristics of radiation-induced soft tissue and bone tumors.

Background and Significance: Sarcomas of the soft tissues and bones are known to be associated with high therapeutic doses of radiation. The broad classification of “sarcoma” showed a suggestive association with radiation in the recent LSS tumor incidence report. This study will provide more definitive evidence regarding the effects of radiation on histologically reviewed soft tissues and bone tumors.

Study Methods: Potential cases of soft tissue and bone tumors collected from cancer registries and other available sources will be histologically reviewed by pathologists.

Study Progress: A total of 91 cases with malignant tumor

of soft tissue and bone were histologically confirmed among 130 potential cases collected from collaborating hospitals. They were derived from 165 cases that were accepted by screening information sheets of 4,381 possible cases. Data analysis is being conducted in collaboration with U.S. NCI.

RP 1-06 Study on cancer of the uterus among A-bomb survivors, 1950–2003 (Addendum to RP 8-85)

Fujihara M, Matsuo T, Nishisaka T, Nakajima H, Hirai Y (G), Soda M (EN), Sekine I, Preston DL, Mabuchi K, Ozasa K (EH), Kodama K (CS)

Objectives: To clarify the relationship between radiation and uterine cancer incidence based on histologically reviewed diagnoses, and to clarify the histological characteristics of uterine cervix and corpus cancer associated with radiation.

Background and Significance: In the recent cancer incidence analyses, there was some suggestion that radiation may be associated with uterine corpus cancer, especially in subjects exposed at young ages, but no association was seen for cervical cancer. Other factors in the pathogenesis of cervical and corpus cancer include HPV infection and estrogens, respectively.

Study Methods: Uterine corpus cancer is given priority in this study. Potential corpus cancer cases collected from cancer registries and other available sources will be histologically reviewed by pathologists.

Study Progress: The first screening of case information sheets for a total of 1,592 possible cases of uterine corpus cancer has been completed and 381 cases were accepted for histological review. Collection of histological specimens for pathological review is being scheduled.

Results and Conclusions: None yet.

RP 2-04 A case-control study of atrophic gastritis and gastric cancer using frozen sera and genomic DNA: Identification of new biomarkers for chronic gastritis associated with gastric cancer

Ohishi W (CH), Ueda K (CH), Fujiwara S, Cullings HM (S), Hayashi T (R), Hida A (CN), Ozasa K (EH), Tahara E

Objectives: To determine whether the radiation exposure-dependent gastric cancer risk seen in the atomic bomb survivors is related to chronic tissue inflammation associated with *H. pylori* infection.

Background and Significance: Three major classes of factors—environmental (diet, smoking), host (age, *H. pylori* infection), and genetic—jointly affect the genesis of gastric cancer. We are investigating interactions between radiation exposure and these risk factors in developing gastric cancer to gain new insights on radiation risk susceptibility.

Study Methods: A nested case-control study was performed in the longitudinal cohort of atomic bomb survivors using stored sera obtained before diagnosis. Enrollees included about 300 gastric cancer cases and three controls per case selected from cohort members matched on age, gender, city, time of serum storage, and type of serum storage, and counter-matched on radiation dose to increase the statistical power.

Study Progress: A draft manuscript on the joint effects of

radiation and chronic atrophic gastritis upon gastric cancer risk is currently in the final stages of revision.

Results and Conclusions: *H. pylori* infection, chronic gastritis, and smoking are all independent predictors of gastric cancer. Higher radiation relative risks were noted with the diffuse type of gastric cancers, whereas much lower risks were noted with the intestinal type of gastric cancer, after adjusting for these risk factors (*Cancer Epidemiol Biomarkers Prev* 2007; 16:1224–8). The *LTA 252* genotype is associated with noncardiac gastric cancer of the diffuse type in Japan, and this genotype was an effect modifier for radiation dose (*Helicobacter* 2009; 14:571–9). Radiation risk was significant only for people without chronic gastritis in developing diffuse type noncardiac gastric cancers.

RP 1-04 A nested case-control study of hepatocellular carcinoma among atomic-bomb survivors using stored sera

Ohishi W (CH), Fujiwara S, Cologne JB (S), Ueda K (CH), Hida A (CN), Ozasa K (EH), Chayama K

Objectives: The primary objective of this study is to investigate the relationship between radiation exposure and the risk of hepatocellular carcinoma (HCC) among A-bomb survivors after taking into account hepatitis virus infection.

Background and Significance: An increased risk of liver cancer with radiation dose has been reported based on mortality studies and tumor registries among A-bomb survivors, but hepatitis virus infection status was not taken into account. Other previous studies at RERF provided suggestive evidence of a possible interaction, i.e., they reported supermultiplicative effects between radiation exposure and chronic HCV infection in the etiology of HCC without cirrhosis. These questions are especially important because they may help explain differences in the magnitude of radiation-HCC risk seen in this population vs. in western populations where HCV infection is uncommon.

Study Methods: The study included 224 HCC cases and 644 controls that were matched to the cases on gender, age, city, time and method of serum storage, and counter-matched on radiation dose.

Study Progress: Analyses of the joint effects of radiation and intermediate risk factors such as hepatitis virus infection is underway, along with the development of statistical methods.

Results and Conclusions: After adjusting for alcohol consumption, smoking habit, and body mass index (BMI), the RR (95% CI) at 1 Gy of radiation exposure for HCC was 1.67 (1.22–2.35), while the RRs for HBV or HCV infection were 63 (20–241) and 83 (36–231), respectively. Those estimates changed little when radiation and viral effects were fit simultaneously. The ERR (95% CI) at 1 Gy for non-B, non-C HCC was 2.74 (1.26–7.04) with adjustment for alcohol consumption, smoking habit, and BMI. These results indicated that radiation exposure is associated independently with increased HCC risk, and that radiation exposure was a significant risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption, smoking habit or BMI (*Hepatology* 2011; 53:1237–45).

RP 6-02 A nested case-control study of breast and endometrial cancer in the cohort of Japanese atomic bomb survivors

Ohishi W (CH), Neriishi K, Grant EJ (EH), Cologne JB (S), Ozasa K (EH), Sharp GB, Eguchi H, Nakachi K, Nakashima E (S), Izumi S, Key TJ, Stevens RG, Berrington de Gonzalez A
Objectives: To characterize the joint effects of radiation and serum-based indicators of hormonal status, oxidative stress, and phytoestrogen consumption in breast and endometrial cancer development.

Background and Significance: It is not known to what extent known breast-cancer risk factors are directly or indirectly involved in radiation-induced cancer. Assessing the joint effects of radiation and other factors using complex causal models may shed light on the mechanisms of radiogenic breast and endometrial cancer etiology.

Study Methods: Serum measurement from the 243 breast cancer cases whose blood samples were collected up to 30 years before cancer diagnosis and 486 age-matched controls, selected with counter-matching on radiation dose, were conducted in 2007–2008. Statistical analyses and manuscript preparations are under way.

Study Progress: Manuscripts were published on breast cancer risk related to radiation exposure and ferritin levels and on hormone levels in relation to radiation dose. A manuscript on radiation risk for breast cancer with hormone levels as an effect-modifier or mediating variable is under preparation. Data have been released for use by an international breast cancer consortium.

Results and Conclusions: Serum levels of estradiol and testosterone increased with radiation dose in cancer-free postmenopausal women (*Radiat Res* 2011; 176:678–87). The radiation-unadjusted and -adjusted relative risks (95% CI) of breast cancer for a 1 log unit increase in ferritin were 1.4 (1.1–1.8) and 1.3 (1.0–1.7), respectively. The mechanism of the joint effects of ferritin and radiation on postmenopausal breast cancer risk could not be assessed (*Cancer Sci* 2011; 102:2236–40).

RP 2-91 Studies on skin cancer incidence among the RERF Extended Life Span Study cohort, Hiroshima and Nagasaki, 1950–87

RP 2-02 Studies on skin cancer incidence among the RERF Life Span Study cohort, Hiroshima and Nagasaki (Addendum to RP 2-91)

Ozasa K (EH), Kishikawa M, Iseki M, Yonehara S, Soda M (EN), Mabuchi K, Preston DL, Sugiyama H (EH), Misumi M (S), Suyama A, Kodama K (CS)

Objectives: To clarify the relationship between radiation and skin cancer incidence based on histologically reviewed diagnosis, and to clarify the histological characteristics of radiation-induced skin cancer in the Life Span Study (LSS) cohort.

Background and Significance: It has been reported that skin cancer incidence, especially nonmelanoma skin cancer, increased with radiation exposure among patients with radiotherapy and atomic bomb survivors. The effects of radiation upon skin cancer have been reported to have a long latency period among atomic bomb survivors. RP 2-02 study is a 10-year extension of RP 2-91, which showed an excess of basal cell carcinoma (n = 80) during the period

from 1950 to 1987.

Study Methods: Potential skin tumor cases collected from cancer registries and other available sources were histologically reviewed by pathologists.

Study Progress: We confirmed a total of 336 cases with first primary skin cancer (123 basal cell carcinoma, 178 squamous cell carcinoma including *in situ* cancer, and others), adding 128 new cases to the previous 1958–1986 series. A paper was submitted to an international journal and is being revised.

Results and Conclusion: ERRs were estimated assuming a linear dose response, and only basal cell carcinoma had a statistically significant positive dose response. However, the basal cell carcinoma data fit a threshold model better than a linear model, and the dose threshold was estimated to be 0.63 Gy (95% CI: 0.30, 0.90).

RP 3-94 Incidence of lymphoid malignancies among the atomic-bomb survivors, 1950–90

Fujihara M, Namba K, Tokunaga M, Takahara O, Soda M (EN), Dohy H, Kamada N, Tomonaga M, Preston DL, Mabuchi K, Sakata R (EH), Ozasa K (EH), Kodama K (CS)

Objectives: To clarify the relationship between radiation and lymphoid malignancy incidence based on histologically reviewed diagnoses, and to clarify the histological characteristics of radiation-induced lymphoid malignancies.

Background and Significance: There have been controversial findings on the effects of radiation on lymphoid malignancies. It is necessary to thoroughly analyze the data on lymphoid malignancies in the A-bomb survivors through histological reviews based on current standardized diagnostic criteria.

Study Methods: Potential lymphoid malignancies collected from various available sources including cancer registries and others are histologically reviewed by pathologists.

Study Progress: A total of 476 definite and probable cases of malignant lymphomas (ML) from the period of 1950–1995 were identified for analyses. A paper on analysis of diagnosed cases is being drafted. An analysis of radiation risks by histological type is being conducted.

Results and Conclusions: Among T-cell lymphomas, mean age at exposure, age at disease onset, and latent period (defined as years from exposure to onset) appeared to be younger or shorter in the cases exposed to high doses of radiation although there were no such tendencies in B-cell lymphoma.

RP 1-94 Studies on lung cancer incidence among the atomic-bomb survivors, 1950–90

Ozasa K (EH), Egawa H, Matsuo T, Yonehara S, Nakashima E (S), Soda M (EN), Tokunaga M, Furukawa K (S), Mabuchi K, Preston DL, Kodama K (CS)

Objectives: To quantify the risk of lung cancer and the variation in histological subtypes with special interest in possible interactions with smoking.

Background and Significance: Radiation-related risks of lung cancer were observed in the latest tumor registry-based incidence and mortality reports. However, several specific questions are being addressed, including the specificity of various cell types involved in radiation- versus smoking-related cancers, confounding and joint effects of

smoking and radiation exposure.

Study Methods: A pathologist panel histologically confirmed 2,368 lung cancers among the 5,711 potential cases ascertained from cancer registries and other available sources between 1958 and 1999. The data on smoking habits were collected from a series of questionnaire surveys on the LSS and AHS conducted during 1965–1991.

Study Progress: The second paper on the joint effects of smoking and radiation on lung cancer by specific histological type was published (Egawa H et al., *Radiat Res* 2012; 178(3):191–201).

Results and Conclusions: Both smoking and radiation exposure appeared to significantly increase the risk of each of the major lung cancer histologic types. While the main effects of smoking and radiation varied in magnitude among the types, there appeared to be the possibility of a common pattern of the interaction between smoking and radiation exposure; the smoking-adjusted radiation effect among smokers tended to be larger for moderate smokers than for heavier smokers. This suggests, but does not prove, that heavy smoking may dominate over the radiation effect.

RP 6-91 Studies on thyroid tumor incidence among the RERF Extended Life Span Study cohort, 1950–87

RP 5-11 Extension of study period (to 2005) and amendment of review procedures of the “Studies on thyroid tumor incidence among the RERF Extended Life Span Study cohort, 1950–87” (Addendum to RP 6-91)

Ozasa K (EH), Hayashi Y, Tsuda N, Tokunaga M, Yonehara S, Ito M, Sekine I, Neta G, Brenner A, Mabuchi K, Preston DL, Furukawa K (S), Imaizumi M (CN), Kodama K (CS)

Objectives: To clarify the shape of the radiation dose-response relationships for malignant and benign tumors, variations in risk by modifiers, and the relative importance of different histological subtypes to radiation exposure based on histopathologically verified thyroid tumor cases.

Background and Significance: Thyroid cancer was one of the earliest solid cancers found to be increased among atomic-bomb survivors and other irradiated cohorts. This study includes the 1958–2005 period of case ascertainment for both benign and malignant thyroid tumor cases.

Study Methods: Potential thyroid tumor cases collected from cancer registries and other available sources were histologically reviewed by pathologists.

Study Progress: A total of 371 cases of thyroid cancer were histologically confirmed by the pathologist for the period of 1958–2005. A paper was published in collaboration with the Department of Statistics (Furukawa K et al., *Int J Cancer* 2013; 132:1222–6). A detailed analysis using information on lifestyle factors is being conducted in collaboration with the U.S. National Cancer Institute (NCI).

Results and Conclusions: A linear dose-response model fit the data well. The excess relative risk of thyroid cancer at 1 Gy of radiation exposure was estimated as 1.28 (95% confidence interval: 0.59–2.70) at age 60 after acute exposure at age 10. The risk decreased sharply with increasing age at exposure and there was little evidence of increased thyroid cancer rates for those exposed after age 20. Among those exposed as children or adolescents, the attributable fraction

decreased with attained age, but remained elevated (16%, 95% CI: 6–24; for people with >0.005 Gy weighted thyroid dose) during the latest follow-up period of 1996–2005.

RP 9-88 Guidelines for the conduct of site-specific cancer incidence studies among A-bomb survivors, Hiroshima and Nagasaki

Ozasa K (EH), Sekine I, Soda M (EN), Tokunaga M, Mabuchi K, Cullings HM (S), Kodama K (CS)

Objectives: To establish guidelines for pathological studies on the incidence of specific cancers in the LSS population. Guidelines include specifying basic study designs, methodology, and procedures to maintain uniformity. These guidelines are intended to simplify the preparation of subsequent research plans for site-specific cancer incidence studies and simultaneously provide for uniformity in basic design and operation of the studies.

Background and Significance: Some uncertainties have been detected in the accuracy of diagnoses based on death certificates or tumor and tissue registries in the LSS cohort studies. Standardized pathological reviews are therefore valuable. These guidelines are intended to simplify the preparation of subsequent research plans for site-specific cancer incidence studies and to provide uniformity in the basic design and conduct of studies.

Study Methods: The framework for the site-specific cancer studies includes three major areas: case ascertainment, pathological review, and data analysis.

Study Progress: Research protocols have been written for cancers and/or tumors of the liver (RP 5-90), salivary glands (RP 1-91), skin (RP 2-91, updated to RP 2-02), ovary (RP 2-92), thyroid (RP 6-91), central nervous system (RP 4-92), breast (RP 6-93, updated to RP 5-08, and RP 6-10 for intrinsic subtypes), lung (RP 1-94), lymphoid system (RP 3-94), uterus (RP 1-06), and soft tissue and bone (RP 4-07). Among them, studies of the liver (RP 5-90), salivary gland (RP 1-91), central nervous system (RP 4-92), and ovary (RP 2-92) have been completed and others are underway.

Results and Conclusions: Essential methods for site-specific cancer incidence studies with histology reviews were established.

RP 29-60 Detection of leukemia and related disorders

Ozasa K (EH), Soda M (EN), Sugiyama H (EH), Kodama K (CS), Tomonaga M, Kimura A, Kamada N, Dohy H, Miyazaki Y, Cologne JB (S)

Objectives: The purpose of the study is to determine the incidence and risks of leukemia and related hematological disorders in the radiation-exposed persons of Hiroshima and Nagasaki. Primary questions are those concerning the leukemogenic effects of ionizing radiation.

Background and Significance: A significant excess risk of radiation-induced leukemia was observed within five years after the bombings, and the latest data suggest the possibility of a small, continued excess of leukemia and an increased risk of myelodysplastic syndrome (MDS). Leukemia was specifically surveyed in the Leukemia Registry (TR 5-65) by ABCC from 1946 to the early 1990s and cases have been reclassified using a modern leukemia classification system. Therefore, leukemia has been more

completely surveyed than other malignancies. Currently leukemia cases are collected through usual population-based cancer registries.

Study Methods: Incident cases of leukemia and related hematological disorders have been collected through the Leukemia Registries and the population-based cancer registries in both cities.

Study Progress: A paper on incidence of leukemia, lymphoma, and multiple myeloma during 1950–2001 was published in collaboration with the Statistics Department in *Radiation Research*.

Results and Conclusion: There was a non-linear dose response for leukemias (excluding chronic lymphocytic leukemia and adult T-cell leukemia) which varied markedly with time and age at exposure, with much of the evidence for this non-linearity arising from the acute myeloid leukemia risks. Although the leukemia excess risks generally declined with attained age or time since exposure, there was evidence that the radiation-associated excess leukemia risks, especially for acute myeloid leukemia, had persisted throughout the follow-up period out to 55 years after the bombings. As in earlier analyses, there was a weak suggestion of a radiation dose response for non-Hodgkin lymphoma among men with no indication of such an effect among women. There was no evidence of radiation-associated excess risks for either Hodgkin lymphoma or multiple myeloma.

RP-A5-12 A proposal to join the diet and bladder cancer pooling project

Grant EJ (EH), Ozasa K (EH), Ohishi W (CH), Hida A (CN)

Objectives: The Diet and Bladder Cancer Pooling Project (DBCPP) is a collaborative pooling project designed to investigate the association of diet and bladder cancer using data from bladder cancer studies that have been conducted around the world. The Principal Investigator of the project is Dr. Maurice Zeegers of Maastricht University (formerly of Cambridge University).

Background and Significance: Bladder cancer is the most expensive malignancy to treat from diagnosis until death, ranging from 96,000–187,000 U.S. dollars per patient. With 400,000 new patients occurring yearly worldwide, it is the 7th most common cancer. As is the case for many tumors, the development of bladder cancer is likely to be influenced by diet. However, the role of diet in bladder cancer could be more pronounced, as the bladder is an excretion organ. Previous research has suggested that 30% of all bladder cancers could have been prevented by dietary modifications. However, it is not yet clear which specific foods or nutrients are involved and therefore which dietary modifications should be recommended to prevent this disease. Efforts towards the prevention of this disease by dietary recommendations could directly lead to a substantial reduction of morbidity, mortality and healthcare costs. For example, a 30% decrease in bladder cancer in the USA would equal a reduction of 1.2 billion U.S. dollars of total medical care expenditures per year.

Study Methods: Pooled data from many different studies. Lifestyle data include smoking, alcohol consumption, education, and ingestion frequencies. Data will be harmonized as a first step. Later, pooled analyses will be performed.

Study Progress: As of 2011, the DBCPP had recruited 18 studies from the United States of America, Belgium, the Netherlands, Sweden, Italy, Germany, France, United Kingdom, Hungary, Romania, Slovakia, Spain, Singapore, and China representing more than 30,000 controls and 10,000 bladder cancer cases. RERF data were sent to Dr. Zeegers in October 2012. Currently, harmonizing procedures are taking place.

Results and Conclusions: None at this early point in the project.

RP-A3-11 A proposal to join the Asia Cohort Consortium. Project 2: BMI and total mortality (Phase II), Project 3: Body mass index and risk of intermediate and low rate cancers in Asia populations, Project 4: Diet and mortality among Asians

Grant EJ (EH), Ozasa K (EH), Ohishi W (CH), Hida A (CN), Shore RE (CR)

Objectives: This project builds upon the successful introduction of RP-A3-10, which established the collaboration between RERF and the Asia Cohort Consortium (ACC). This RP expands the data shared (additional cancer types and additional lifestyle data). New projects include another BMI and mortality study, a BMI and cancer incidence study, and a diet study.

Background and Significance: The ACC seeks to understand the relationship between environmental exposures and the etiology of disease through a newly established cohort of over one million healthy Asians who are followed for various disease endpoints, including cancer. Investigators from China, India, Japan, Korea, Malaysia, Singapore, Taiwan, and Bangladesh are included.

Study Methods: The Fred Hutchinson Cancer Research Center in Seattle, Washington, USA acts as the data coordinator for this multi-cohort study. Analyses use pooled data analyses for cohort data to derive risk estimates of various exposures.

Study Progress: This RP was approved in 2011. Additional data to support this RP have been uploaded to the project's coordinating system and harmonized with the other data. A paper on meat intake and mortality was published.

Results and Conclusions: Both ecological and individual analyses were performed. The ecological data showed that red meat consumption was lower in Asian countries compared to the USA. The pooled analysis (using individual records) found no association between total meat and risks of any cause of death among roughly 300,000 individuals representing eight prospective cohorts. Concerns of meat consumption being a surrogate for higher economic status may confound the results.

RP-A5-10 Methods for assessing joint effects of radiation and intermediate risk factors in nested case-control studies

Cologne J (S), Furukawa K (S), Grant E (EH), Ohishi W (CH), Cullings H (S)

Objectives: Extend regression methods for assessing mediation to nested case-control data that allow valid estimation of radiation risk and estimation of the amount of risk attributable to individual causal paths (especially mediation of radiation risk).

Background and Significance: Mediation of radiation risk by disease risk factors causally affected by radiation (e.g., radiation → HBV infection susceptibility → hepatocellular carcinoma; radiation → inflammation → heart disease) is now being explored in the atomic-bomb survivor studies. Regression models for assessing complex causal mechanisms are not immediately applicable to the nested case-control study design used in the RERF studies of liver cancer (RP 1-04), gastric cancer (RP 2-04), and breast cancer (RP 6-02), or to the case-cohort study design used in the RERF cancer and immunogenome study (RP 4-04).

Study Methods: We are applying a combination of statistical theory, computer simulation, and comparative analyses using actual data to assess various approaches to fitting causal models to nested case-control data. Methods are being evaluated using cohort data from the AHS follow-up study of hepatitis virus and liver disease (RP 9-92) and by computer simulation.

Study Progress: A manuscript has been completed describing joint effects of radiation and serum sex hormones using path analysis in the nested case-control study of breast cancer. A novel permutation test applied to path-analysis results was implemented for that paper to allow inference about mediation. Analysis of AHS liver cancer follow-up data is underway.

Results and Conclusions: Although the theoretical requirements for mediation are satisfied with certain serum sex hormones and growth factors in the case of breast cancer and with HBV in the case of liver cancer, there is no evidence of practical mediation so far in either study. Owing to natural variation and differences in magnitude of risk, potential mediators that are strong risk factors may not evidence a great deal of mediation of radiation risk despite highly significant dose responses. Further investigation is needed to determine how other causes of mediating risk factors compare with radiation in terms of overall risk of cancer.

RP-A3-10 A proposal to join the Asia Cohort Consortium. Project 1: Tobacco smoking, alcohol drinking, body mass index, and risk of rare cancers

Grant EJ (EH), Ozasa K (EH), Suyama A, Ohishi W (CH), Hida A (CN), Shore RE (D)

Objectives: This study creates a framework by which RERF becomes a member of the Asia Cohort Consortium (ACC), a multi-centered project with a combined cohort of over one million Asians with lifestyle, cancer incidence, and mortality data. In addition, this RP initiated RERF's first collaborative project with the ACC entitled: *Tobacco smoking, alcohol drinking, body mass index and risk of rare cancers*.

Background and Significance: Current knowledge on the causes of rare neoplasms (i.e., neoplasms with age-adjusted incidence rate below 1.0/100,000 in most human populations) is hampered by the difficulty to assemble a large enough set of cases to study even potent carcinogens with a high prevalence of exposure, such as tobacco smoking. Investigators from China, India, Japan, Korea, Malaysia, Singapore, Taiwan, and other countries have combined their cohorts to create a pooled analysis cohort of over 1,000,000 persons to address these and other

questions relating to lifestyle and the etiology of disease among Asians.

Study Methods: The project will require the integration of data on exposure of interest, covariates, and outcome among the participating cohorts. The coordinating center is the Fred Hutchinson Cancer Research Center in Seattle, Washington, USA. RERF data have been restricted to persons with less than 100 mGy shielded kerma exposure and to persons who participated in at least one lifestyle survey. Approximately 53,000 persons are included in the data contributed by RERF. Pooled methods for cohort data will be employed in the analyses.

Study Progress: RERF researchers have attended meetings, weekly telephone conferences and participated in manuscript preparation. Data have been harmonized and several papers have been published. This RP provides a foundation for additional research questions asked by the ACC and another RP has been written to expand the scope of the collaboration.

Results and Conclusions: A recently submitted paper (PLOS-Medicine) focuses on the burden of smoking in Asia using 21 cohorts and over one million people in a pooled analysis. The cohorts are representative of 71% of Asia's total population. We conclude that (among men ≥45 years) 11.4%, 30.5%, and 19.8% of deaths due to cardiovascular diseases, cancer, and respiratory diseases, respectively, were attributable to tobacco smoking. Applying these risks, we estimate that approximately 1.6 million deaths of adults ≥45 years in 2004 were attributable to smoking in the seven countries/regions covered in this analysis.

RP-A12-08 Second collaborative analysis of radiation-associated thyroid cancers

Sakata R (EH), Veiga L, Lubin J, Sugiyama H (EH), Shore RE (D)

Objectives: To improve our understanding of radiogenic thyroid cancer and probe additional questions that remain regarding risk of radiation-related thyroid cancer associated with juvenile or adult exposure.

Background and Significance: A causal relationship between ionizing radiation and thyroid neoplasia has been well established. The association for thyroid cancer was first identified among A-bomb survivors in 1963 (Socolow et al., *N Engl J Med* 1963). However, data have been limited with regard to several important radiation risk issues: the slope of the dose-response curve, dose fractionation, age at exposure, length of time since exposure, host susceptibility and histologic cell type. The original pooled analysis (Ron E et al., *Radiat Res* 1995; 141:259–77) of the risk of developing thyroid cancer after external radiation exposure was based on seven major epidemiologic studies. A new, pooled analysis based on considerably more data will significantly add to what is known about radiation-related thyroid cancer and its modifying factors.

Study Methods: An updated and expanded pooled analysis of 16 studies of radiation exposure and thyroid cancer risk will be carried out. To carry out the Poisson regression analyses, data were cross-classified by attained age, age at exposure, calendar period, study population, dose, and other variables of interest.

Study Progress: A paper on thyroid cancer incidence following radiotherapy for childhood cancer was published

in *Radiation Research*, and an analysis including the A-bomb study and other exposed populations is underway.

Results and Conclusions: The following results were obtained from the analysis of thyroid cancer following radiotherapy for childhood cancer. Dose-related relative risks increased approximately linearly up to about 10 Gy, then leveled off. The fitted RR at 10 Gy was 13.7 (95% confidence interval: 8.0–24.0). Dose-related excess RRs increased with decreasing age at exposure ($p < 0.01$), but did not vary with attained age or time since exposure, remaining elevated 25+ years after exposure.

RP-A5-08 Esophageal and gastric cancers: Patterns and predictors of risk in Hiroshima and Nagasaki, Japan

Kennedy BS, Mabuchi K, Chow WH, Shimizu Y (EH), Sugiyama H (EH), Soda M (EN), Sakata R (EH), Grant EJ (EH), Cologne JB (S), Cullings HM (S), Yamada M (CH)

Objectives: To determine the population-based temporal trends in esophageal and gastric cancer incidence in Hiroshima and Nagasaki by histopathology and anatomic site. To assess the radiation effects on esophageal and gastric cancer by histopathology and anatomic site. To identify which modifiable risk factors have the greatest public health impact on esophageal and gastric cancers in the LSS.

Background and Significance: Over the last several decades, incidence rates for esophageal cancer have varied, while incidence rates for stomach cancer have generally decreased in many countries. Prior work has suggested that these trends likely reflect patterns in certain cancer subcategories. Knowing the temporal patterns in Asian populations could provide important insights into the etiology of esophageal and gastric cancers. The LSS data have shown that the risks for esophageal and gastric cancers are significantly increased by radiation (Preston et al., *Radiat Res* 2007; 168:1–64). However, associations by anatomic location and histological cell type have not been specifically examined. Esophageal and gastric cancers, like other aerodigestive malignancies, have several risk factors in common: smoking, drinking alcohol, consuming low levels of fruits and vegetables, gastroesophageal reflux disease, and being overweight. However, among Asian populations, including those in Japan, it is not clear what proportion of esophageal and gastric cancers are due to these modifiable lifestyle factors. Differences in risk factor distributions may lead to differences in the population attributable risks (PAR) and, consequently, the public health impact of these factors.

Study Methods: For the temporal trend analysis, data from the population-based tumor registries of Hiroshima and Nagasaki were used. For the radiation effects and risk factor analyses, the LSS cohort data were used, analyzing sex, histopathology and location, age at exposure, attained age, and time since exposure. In the risk factor analysis, the main predictors were tobacco smoking, alcohol consumption, body mass index (BMI), and dietary patterns. The dietary data analysis emphasized the consumption of fruits and vegetables, which has been shown to lower the risk for esophageal/gastric cancer in prior studies. To assess the potential public health impact of several modifiable risk

factors, PARs for tobacco, alcohol, BMI, and dietary patterns will be estimated, using a method that allows for the determination of partial PARs for the modifiable risk factors, while controlling for the non-modifiable risk factors such as age and sex.

Study Progress: Two manuscripts have been written and are currently under internal review.

Results and Conclusions: For the population-based study: esophageal cancer increased for males and remained stable for females. Gastric cancer decreased for both males and females, mainly due to decreases in intestinal-type cancers. Gastric cancer early diagnosis increased in parallel with decreases in the death certificate only (DCO) fraction, especially for males; which may suggest lower screening rates for females. For the LSS-based radiation study: the lower anatomic segment showed the highest dose response (HR = 2.5) for esophageal cancer. For gastric cancer, diffuse cases showed the highest dose response (HR = 1.9). Smoking was shown to be the most important modifiable risk factor. Temporal trends in esophageal and gastric cancer rates were characterized. High radiation risks were observed in certain subtypes of these cancers. The results indicate that population risk reduction can be effected by avoiding smoking, by drinking alcohol and consuming hot/salty food only in moderation, and by increasing consumption of chicken, tofu, and fruit.

Special Cancer Studies Publications

RERF Report (RR)

◆ Ohishi W, Cologne JB, Fujiwara S, Suzuki G, Hayashi T, Niwa Y, Akahoshi M, Ueda K, Tsuge M, Chayama K: Serum interleukin-6 associated with hepatocellular carcinoma risk: A nested case-control study. *Int J Cancer* 2014 (January); 134(1):154–63. (RR 14-12) © 2013 UICC

[Abstract] Inflammatory markers have been associated with increased risk of several cancers, including colon, lung, breast and liver, but the evidence is inconsistent. We conducted a nested case-control study in the longitudinal cohort of atomic-bomb survivors. The study included 224 hepatocellular carcinoma (HCC) cases and 644 controls individually matched to cases on gender, age, city, and time and method of serum storage, and counter-matched on radiation dose. We measured C-reactive protein (CRP) and interleukin (IL)-6 using stored sera obtained within 6 years before HCC diagnosis from 188 HCC cases and 605 controls with adequate volumes of donated blood. Analyses with adjustment for hepatitis virus infection, alcohol consumption, smoking habit, body mass index (BMI), and radiation dose showed that relative risk (RR) of HCC [95% confidence interval (CI)] in the highest tertile of CRP levels was 1.94 (0.72–5.51) compared to the lowest tertile ($p = 0.20$). RR of HCC (95% CI) in the highest tertile of IL-6 levels was 5.12 (1.54–20.1) compared to the lowest tertile ($p = 0.007$). Among subjects with BMI >25.0 kg/m², a stronger association was found between a 1-standard deviation (SD) increase in log IL-6 and HCC risk compared to subjects in the middle quintile of BMI (21.3–22.9 kg/m²), resulting in adjusted RR (95% CI) of 3.09 (1.78–5.81; $p = 0.015$). The results indicate that higher serum levels of IL-6 are associated with increased HCC risk, independently of hepatitis virus infection, lifestyle-

related factors and radiation exposure. The association is especially pronounced among subjects with obesity.

Other Journal Publications

- ◆ Chen Y et al. (RERF: Grant EJ, Ozasa K, Ohishi W): Association between body mass index and cardiovascular disease mortality in east Asians and south Asians: pooled analysis of prospective data from the Asia Cohort Consortium. *BMJ* 2013 (October); 347:f5446.
- ◆ Lee JE et al. (RERF: Grant EJ, Ozasa K, Ohishi W): Meat intake and cause-specific mortality: A pooled analysis of Asian prospective cohort studies. *Am J Clin Nutr* 2013 (October); 98(4):1032–41.
- ◆ Lin Y et al. (RERF: Grant E, Ozasa K, Ohishi W): Association of body mass index and risk of death from pancreas cancer in Asians: findings from the Asia Cohort Consortium. *Eur J Cancer Prevent* 2013 (May); 22(3):244–50.
- ◆ Ohishi W, Fujiwara S, Chayama K: Study of viral hepatitis in a longitudinal cohort of A-bomb survivors. Elucidation of viral hepatitis based on a cohort study. Tokyo: Medical Review; 2013, pp 86–95. (Japanese) (related to *Immunology Studies* and *Special Clinical Studies*)

Manuscript in Press

⌘ Sugiyama H, Misumi M, Kishikawa M, Iseki M, Yonehara S, Hayashi T, Soda M, Tokuoka S, Shimizu Y, Sakata R, Grant EJ, Kasagi F, Mabuchi K, Suyama A, Ozasa K: Skin cancer incidence among atomic bomb survivors from 1958 to 1996. *Radiat Res*.

Special Cancer Studies Oral Presentations

- ❖ Ohishi W, Cologne JB, Ueda K, Hayashi T, Niwa Y, Fujiwara S, Tsuge M, Chayama K. Elevated IL-6 levels are associated with increased risks of hepatocellular carcinoma. 49th Annual Meeting of the Japan Society of Hepatology, 6–7 June 2013, Tokyo
- ❖ Shore RE, Yamada M, Hida A, Neriishi K. Cataract and CNS effects in the Japanese atomic bomb survivors. 59th Annual Meeting of the Radiation Research Society, 15–19 September 2013, New Orleans, Louisiana, USA (related to *Special Clinical Studies*)

Research Protocol 18-61

Tumor and Tissue Registries, Hiroshima and Nagasaki

RP 18-61 Tumor and tissue registry study in Hiroshima and Nagasaki

Soda M (EN), Ozasa K (EH), Sugiyama H (EH), Grant EJ (EH), Shimizu Y, Katayama H (IT), Kodama K (CS)

Objectives: The purpose of the study is to determine the incidence and risks of all types of neoplasms in the exposed persons of Hiroshima and Nagasaki.

Background and Significance: Population-based tumor registries have been active in Hiroshima since 1957 and in Nagasaki since 1958. Tissue registry systems were established in Hiroshima in 1973 and in Nagasaki in 1974. ABCC-RERF has assisted in the collection of cases and the management and maintenance of those databases. The information and materials are available in the Epidemiology Department for incidence studies, special cancer studies, case-control studies, etc.

Study Methods: Information on tumor diagnosis is collected through: notifications from hospitals and other medical facilities, on-site abstraction of medical records by RERF personnel, and death certificates. Information on pathological diagnosis of tumor and materials is collected from hospitals and local pathology laboratories for the tissue registries. Every year the Epidemiology Department retrieves the information on RERF study subjects from the registries with permission of the registry authorities.

Study Progress: Case collection by notifications and death certificates has been nearly completed through 2011 in both Hiroshima and Nagasaki prefectures. On-site record abstraction is complete through 2011 in Hiroshima city and 2012 in Nagasaki prefecture. Tissue diagnoses and samples are being collected through 2011 in Hiroshima prefecture and 2010 in Nagasaki prefecture. The Department of Epidemiology is linking registry information with RERF subjects, this is a laborious process and is currently being performed through 2009.

Results and Conclusion: Annual reports on incidence data for 2009 and 2010 in both Hiroshima and Nagasaki prefectures were published. Both registries expedited the publication successfully one year faster than the previous reports. The cancer incidence information through 2007 has been cross-checked with the database of the LSS, *in utero*, and F₁, and summarized. The new project to report the radiation-related ERR and EAR for solid cancer incidence among LSS has been initiated and data have been distributed to collaborating authors for analysis.

Tumor and Tissue Registries Publication

Journal Publication

- ◆ Sugiyama H: Effects of differences in methods of collecting local cancer registry data on completeness and information registered for respective items. *JACR Monograph* 2013 (December); No.19:116–20. (Japanese)

Tumor and Tissue Registries Oral Presentations

- ❖ Sugiyama H. Effects of differences in methods of collecting local cancer registry data on completeness and information registered for respective items. 22nd Annual

Meeting on Japanese Association of Cancer Registries, 13–14 June 2013, Akita

❖ Yamada T, Yoshida M, Inada Y, Hayama S, Nagayoshi A, Yamaguchi T, Soda M. Actual status of cancer care in Nagasaki prefecture and cancer registry promotion project. 22nd Annual Meeting on Japanese Association of Cancer Registries, 13–14 June 2013, Akita

Research Protocols 3-04, 1-92, 10-86, 18-59, A5-11, A4-10

Atomic-bomb Dosimetry Studies

RP 3-04 ESR measurements of tooth samples from Nagasaki survivors (Addendum to RP 1-92)

Hirai Y (G), Nakamura N, Kodama Y (G), Cullings HM (S), Hida A (CN), Tomonaga M, Iijima Y, Mine M

Objectives: To investigate the possibility that Nagasaki survivors who were exposed in factories have overestimated doses.

Background and Significance: It is difficult to estimate doses for Nagasaki survivors who were exposed in factories because of possible partial shielding by nearby machinery. Biodosimetric estimates can be used to validate or adjust the physical dose estimates. Tooth enamel from Nagasaki survivors, especially factory workers when available, will be measured by electron spin resonance (ESR) to evaluate radiation dose. Chromosome aberration frequency will be measured for the same donors. Based on the results, we will examine the validity of the factory worker DS02 dose estimates.

Study Methods: We use extracted teeth to measure the absorbed gamma-ray dose by ESR (^{60}Co gamma-ray equivalent dose). Measurements of chromosome aberration frequencies in blood lymphocytes will also be made from the same donors to compare the results.

Study Progress: We measured two molars in this fiscal year.

Results and Conclusions: Tooth collection is slow. Thus far, 31 tooth samples were measured with ESR. Because the number of tooth samples examined was limited, it was not possible to derive a conclusion about a possible dose bias. However, a close correlation was found between the ESR dose estimate and the cytogenetic dose estimate on the same survivors. Thus, these results may encourage the use of cytogenetic data, which is much more abundant than tooth data, for a better understanding of uncertainties associated with individual DS02 doses.

RP 1-92 Radiation dose estimates using tooth samples. Part 2: Use of electron spin resonance on tooth enamel from Hiroshima atomic bomb survivors

Hirai Y (G), Nakamura N, Kodama Y (G), Wada T, Cullings HM (S), Ohishi W (CH), Wieser A

Objectives: To estimate the individual doses using teeth by the electron spin resonance (ESR) technique and to compare the results with DS02 dose, and with chromosome aberration frequencies in lymphocytes, from the same donors.

Background and Significance: This study plan is to measure CO_2^- radicals induced by gamma-ray exposure in tooth enamel by means of the ESR technique (Ikeya et al., *Jpn J Appl Phy* 1984; 23:L697). For this purpose, we collected teeth according to RP 10-86. Since the ESR signal intensity is linearly related to gamma-ray dose, this technique enables us to estimate the gamma-ray dose of the survivors. ESR has proved to be a good choice in that the ESR-estimated doses agreed well with the cytogenetically estimated doses and jointly provide a good comparison basis to evaluate the

physical dose estimates (DS02).

Study Methods: We use tooth enamel to measure the absorbed gamma-ray dose by ESR (^{60}Co gamma-ray equivalent dose). Measurements of chromosome aberration frequencies in blood lymphocytes are made from the same donors and the results are compared.

Study Progress: We measured nine molars in this fiscal year. Thus far, a total of 283 molars donated by 220 Hiroshima AHS participants were measured.

Results and Conclusions: None yet.

RP 10-86 Radiation dose estimates using tooth samples. Part 1: Collection of tooth samples from A-bomb exposed people in Hiroshima and Nagasaki

Hirai Y (G), Nakamura N, Ohishi W (CH), Hida A (CN)

Objectives: To collect teeth from Adult Health Study (AHS) subjects who were exposed to the bomb within 2 km from the hypocenter and controls (estimated dose <5 mGy).

Background and Significance: Electron spin resonance (ESR; also called electron paramagnetic resonance, EPR) was first used to measure the cumulative radiation dose to the enamel of teeth from A-bomb survivors by Okajima's group at Nagasaki University. As the ESR signal intensity is linearly related to gamma-ray dose, this technique enables us to estimate individual doses. The collection of teeth from A-bomb survivors is the first part of the project to estimate the radiation dose of A-bomb survivors.

Study Methods: The Department of Clinical Studies sends letters to AHS participants twice a year for health monitoring. A sentence in the letters expresses our continued interest in the collection of teeth.

Study Progress: A total of 46 teeth were received from Hiroshima AHS participants and 16 from Nagasaki AHS participants during FY2013.

Results and Conclusions: Thus far, we have collected 1,632 tooth samples from Hiroshima AHS participants during the past 26 years and 89 from Nagasaki AHS participants during the past nine years. On average, nearly 10% of the collected samples are suitable for ESR measurement (i.e., molars that are not seriously decayed).

RP 18-59 Shielding survey and dosimetry study

Cullings HM (S), Grant EJ (EH), Watanabe T (EH), Funamoto S (S), Matsumoto N (S), Cologne JB (S)

Objectives: The purpose of this protocol is to refine dose estimates for Hiroshima and Nagasaki atomic-bomb survivors and to characterize the uncertainties in these estimates.

Background and Significance: Accurate and detailed dose estimates are essential to characterizing the radiation dose response of any health effect under study. Three successive systems for calculating survivor doses based on location and shielding have been developed and implemented: T65D, DS86, and DS02.

Study Methods: DS02 is based on calculations of the nuclear explosions and the generation, transport, and alteration by shielding of the neutrons and gamma rays that directly irradiated survivors. Methods have been developed at RERF to improve input data on survivor locations and shielding, including terrain shielding, to extend the systems

to longer distances and less detailed shielding data than those for which the core system performs calculations, and to characterize uncertainty in individual dose estimates and reduce the impact of uncertainties on radiation risk estimates.

Study Progress: Pre-2013 – In 2007 a new committee was initiated by the Chairman, Dr. Okubo, to examine a number of issues (residual radiation, improvement of input data, factory worker doses, etc.). Dr. Okubo and others in the Dosimetry Committee devised methods to make new estimates of map coordinates for individual survivors using archival RERF data with current technology. Also beginning in 2007, four new statistical collaborations on dose error and biodosimetry were begun with groups of external investigators.

2013 – The Epidemiology and Statistics Departments have continued to make progress in various areas.

The several years' work of re-estimating survivors' map coordinates and related terrain shielding has been completed and revised doses will be made available for the LSS in the RERF internal database in March 2014.

Improved input data for individual survivors' terrain shielding calculations were estimated for all survivors from new digital terrain elevation data, which were available on a horizontal grid with ~10 m spacing. This resulted in considerable improvement over the previous input data, which were either taken from work done in the 1960s (Nagasaki "globe terrain" cases) or were estimated for survivors behind mountains denoted as "distal terrain" in the implementation of DS02 using older digital elevation data on a ~50 m grid. This work, which also reflects changes in survivors' estimated locations based on the recent map work at RERF, required considerable time and effort during 2013, partly because systematic discrepancies were observed in relation to the old input data for the Nagasaki "globe terrain" cases (n = 315). These discrepancies were thoroughly investigated and the new terrain input data were thoroughly checked in a more general way. It was concluded that the original terrain input data for "globe terrain cases," which were estimated circa 1966 by manual methods using contours on the U.S. Army maps, were poorly estimated. Dr. Cullings made a presentation about the new location and terrain input data at the annual meeting of the Health Physics Society in July 2013. The entire process of the re-estimation of those data will be described in a journal paper and additional details will be documented in a RERF Report. We continued collaborative research with several external researchers to develop and compare statistical methods to estimate dose uncertainties and minimize their effects on risk estimation, including use of biodosimetry data. A new manuscript was submitted by a collaborator team.

We continued work on residual radiation, and Dr. Cullings and Dr. Grant collaborated with Dr. Sakata of Epidemiology on a manuscript currently in preparation related to the survivor data on exposure to "fallout rain."

Results and Conclusions: Work in progress confirms the suggestion of DS86 that the population dose increment in person-gray from known sources of residual radioactivity is a small fraction of direct doses calculated by DS02. Preliminary geospatial analyses of various biological

endpoints have not shown any patterns consistent with undocumented doses from residual radiation or other deficiencies in the dosimetry. Work on more sophisticated geospatial analyses and other aspects mentioned above is ongoing.

RP-A5-11 Bio-dosimeters as instrumental variables for assessing and dealing with uncertainty in atomic-bomb survivor dose estimates: Preliminary evaluation in the AHS sub-cohort

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Objectives: To develop and evaluate methods for dealing with dosimetry error in radiation risk estimation using ancillary information contained in various bio-dosimeters and biomarkers measured in a subset of the AHS cohort. The goal will include publications in substantive journals in addition to statistical journals.

Background and Significance: Inaccuracies and unknown quantities in the estimation of atomic-bomb survivor radiation doses can lead to bias and greater uncertainty in risk estimation. To date, methods for dealing with dosimetry errors have focused on substitution of expected doses based on an assumed model for the dose measurement-error distribution including assumed parameter values for the variance. If the assumed model is incorrect, then the method leads to biased estimates of dose-response relationships. Incorporating information on bio-dosimetric measurements could be used to address this issue.

Study Methods: The intent of this work is to develop approaches that incorporate information from biodosimeters or biomarkers to estimate measurement error variances. A method that treats biodosimetric data as so-called “instrumental variables” (i.e., variables that are correlated with true dose but uncorrelated with measurement error) can obviate the need to assume a known error-variance parameter. The data will consist of DS02 measurements and potential bio-dosimetric data from chromosome aberration, clinical/laboratory measurements and disease incidence.

Study Progress: Two graduate students, Austin Miller and Carmen Tekwe, have completed Ph.D. dissertations on relevant methods. Tekwe produced two manuscripts related to her dissertation, which have been submitted to journals.

Results and Conclusions: Analyses by the two graduate students have produced estimates that were reasonably consistent with the assumed values of error variance currently used in the methods employed by RERF for calculating “adjusted” survivor doses.

Methods based on instrumental variables are useful in obtaining estimates of the size of overall measurement error in RERF dosimetry (e.g., DS02) data, subject to the assumptions related to the parametric form of the error model for the instrumental variable(s) analysis, i.e., the probability distribution of errors in the survivor’s calculated DS02 doses.

RP-A4-10 Semiparametric methods using radiation exposure and chromosome aberration data in atomic-bomb survivors studies

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Objectives: Use semiparametric statistical methods to adjust for the effects of radiation dose measurement error on the estimation of radiation dose responses, incorporating information from biodosimetric data available on a part of the cohort.

Background and Significance: Although some measurement error methods have been applied to adjust for radiation measurement error in RERF data, further development of semiparametric or nonparametric methods is important to understand the radiation effect to cancer or other outcome variables. Dosimetry data may be considered as a surrogate variable for the unobserved underlying radiation exposure. Biomarkers such as percentage of stable chromosome aberrations can be treated as a type of instrumental variable for the unobserved radiation dose.

Study Methods: The AHS subcohort of about 4,000 who have DS02 radiation dose estimates, plus stable chromosome aberration data, and outcome data for diseases such as cardiovascular heart disease, stomach cancer, lung cancer, or breast cancer, will comprise the calibration sample. By using data from the calibration sample, we can estimate radiation dose responses for the entire LSS cohort, with an appropriate adjustment for the uncertainty in DS02 dose estimates. An important result here is that the measurement error standard deviation will not have an assumed value, but rather will be estimated from the data using an innovative method, even though the data do not include replicate measurements/estimates of radiation doses. We will consider logistic, Cox, and additive hazard regression models for the radiation dose response, with adjustment for smoking information, age at exposure, gender, education, and city.

Study Progress: A U.S. NIH grant was obtained (C-Y Wang, PI) to support the work. Statistical analysis is in progress. One related paper has been published and two others have been submitted by the non-RERF collaborators; these develop the method but do not use RERF data.

Results and Conclusions: Several methods have been developed that utilize different methods and different assumptions, for different situations in terms of available surrogate and instrumental variables and types of regression analysis.

Atomic-bomb Dosimetry Studies Publication

Journal Publication

◆ Preston RJ, Boice JD Jr, Brill AB, Chakraborty R, Conolly R, Hoffman FO, Hornung RW, Kocher DC, Land CE, Shore RE, Woloschak GE: Uncertainties in estimating health risks associated with exposure to ionising radiation. *J Radiol Prot* 2013 (September); 33(3):573–88. (related to *Life Span Study*)

Atomic-bomb Dosimetry Studies Oral Presentations

❖ Cullings HM, Grant EJ, Watanabe T, Oda T, Funamoto S, Ozasa K, Kodama K. Dose estimates resulting from improved location and terrain shielding data for the Japanese atomic bomb survivors. 58th Annual Meeting of the Health Physics Society, 7–11 July 2013, Madison, Wisconsin, USA