Active Research Protocols by Study Program

1 April 2011–31 March 2012

The 115 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.

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. (*RERF Reports* are listed with abstracts before other journal publications.)

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 3-08, 2-08, 1-75 (Platform Protocol), 2-61, A2-11, A1-11, A2-10, A1-09, A11-08, A7-08, A3-08, A1-08

Life Span Study (LSS)

RP 3-08 Mortality in relation to smoking and other lifestyle factors in a Japanese population

Sakata R (EH), McGale P, Darby S, Grant EJ (EH), Boreham J, Sugiyama H (EH), Soda M (EN), Shimizu Y (EH), Tatsukawa Y (CH), Yamada M (CH), Moriwaki H (EH), Kasagi F, Ozasa K (EH), Suyama A (EN), Kodama K (CS), Peto R

The question has been raised as to why the magnitude of effects from smoking cigarettes appears to be less in Japan than in western countries. An initial issue is to determine if the magnitude is, in fact, less once identical methods of data definition, stratification, and analysis are used in western and Japanese populations. The LSS provides a unique opportunity to examine this, because of the large cohort size, smoking data gathered repeatedly over >35 years, and complete mortality ascertainment. So a comparison with the extensive British doctors' smoking study (Doll et al., *British Medical Journal* 2004; 328:1519) is being conducted in collaboration with Oxford University. The main analyses focus on individuals for whom there is LSS information on cigarette smoking from the mail surveys. Mortality is being examined in relation to cigarette smoking status, categorized by the same rules used in the analysis in the British doctors' study.

Proportional increases in smoking-related mortality were greater in those who started smoking at a younger age, and in later birth cohorts. In current smokers born in 1920 or thereafter, all-cause mortality compared with never smokers was more than doubled (RRs males: 2.27 [95% CI 1.99–2.59], females: 3.03 [2.24–4.09]). Lower hazards reported in previous Japanese studies may have been due to earlier birth cohorts that had started smoking at later ages and smoked fewer cigarettes per day than more recent birth cohorts. A paper is currently being prepared.

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

Sakata R (EH), Nishi N, Nagano J, Grant EJ (EH), Sugiyama H (EH), Hsu WL (S), Fujiwara S (CH), Akahoshi M (CN), Moriwaki H (EH), Mabuchi K, Suyama A (EN), Ozasa K (EH), Kodama K (CS)

A mail survey has been started on all 47,000 subjects who are alive in the Extended Life Span Study cohort (LSS-E85) in order to update information on epidemiological factors such as lifestyles, history of diagnostic and therapeutic radiation exposure, height, weight, financial situation, disease history, menstruation, and psychosocial factors. Such information will be used to examine factors that may confound or modify the health effects of radiation. Due to the large size, long-term follow-up, and advanced age of the cohort, an effort was made to broaden the focus to

include overall aging markers. Additional targeted data include history of major diseases as well as mental and physical health status.

Questionnaires were mailed to 24,640 subjects who had responded to the previous mail survey in 1991 and thus had address information available at RERF. However, 23% of the mail was returned as "undeliverable," with an additional 2% of the recipients found to be deceased on the basis of information received from families. Seventy-seven percent of the subjects thought to have received the questionnaire responded. The responses were entered into computers and tabulated, with a leaflet showing the major findings and a letter of appreciation mailed to the questionnaire respondents. The responses will be entered into a database to make them available as basic data for various epidemiological studies.

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

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

This is the long-term follow-up of a fixed cohort of 93,000 atomic-bomb survivors and 27,000 unexposed individuals. The follow-up began in 1950 using the family registration system, the *koseki*, which assures virtually complete mortality ascertainment. Through tumor registries in Hiroshima and Nagasaki, it has also become possible to study cancer incidence in a large fraction of the LSS. It also serves as the sampling frame for the Adult Health Study clinical subcohort.

Results of analyses of cancer and noncancer mortality data through 2003 using the DS02 were published (Ozasa et al., *Radiation Research* 2012; 177:229–43). The risk of total solid cancers from radiation increases almost linearly over the full-dose range. The risk of cancers significantly increased for most major sites including esophagus, stomach, colon, liver, gall bladder, lung, female breast, ovary, and urinary tract. An increased risk of non-neoplastic diseases including circulatory, respiratory, and digestive system was observed at moderate dose or higher, but the degree to which these are causal relationships requires further investigation. Presently, detailed analyses of respiratory and digestive diseases are under way. There was no evidence of a radiation effect on infectious or external causes of death.

Elevated risk of radiation-associated early natural menopause was observed among female A-bomb survivors (Sakata et al., *Radiation Research* 2011; 176:787–95). Analysis of osteosarcoma based on cancer registry data between 1958 and 2001 showed that risk of osteosarcoma increased by ERR/Gy of 7.5 above the threshold of 0.85 Gy (Samartzis et al., *The Journal of Bone and Joint Surgery. American Volume* 2011; 93:1–8).

At the end of 2007, 37% of LSS subjects, including 84% of those exposed before age 10, were alive, so continued follow-up is essential. The LSS mortality data have demonstrated an excess risk of death from cancers, but the causal nature of associations between radiation exposure and circulatory, digestive, and respiratory diseases is less certain.

RP 2-61 Study of mortality in children exposed *in utero*

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

This is an ongoing long-term follow-up of a cohort of about 3,600 persons who were *in utero* at the time of the atomic bombings. The *in utero* cohort, although small in size, can provide much information on the health effects associated with radiation exposure occurring during embryonic and fetal development. It is the only available cohort in the world with exclusively *in utero* radiation exposure and adulthood data on health risks.

A recently published report (Preston et al., *Journal of the National Cancer Institute* 2008; 100:428–36) showed that the excess risk of adulthood cancer incidence following *in utero* exposure appears to be somewhat smaller than that seen in those exposed as children, and that the temporal pattern of the excess risks following *in utero* exposures also differs from that seen for childhood exposures. In particular, while the excess rate tends to increase markedly with age for those exposed as children, the excess rate does not increase with age for those exposed *in utero*. Relative risks in the earliest years of follow-up (13–20 years after exposure), are reasonably consistent with what one might expect based on other studies of childhood cancer following *in utero* exposure; however there is only weak evidence of an increase in cancer incidence rates for adult-onset cancers.

Analysis of the mortality data for 1950–2003 has almost been completed with a special emphasis on temporal patterns of radiation risks for cancer and noncancer deaths. The mortality data revealed that a decreasing temporal trend in the ERR with advancing age among the *in utero* cohort is more rapid than the trend among the childhood-exposure cohort. The difference in the EAR temporal pattern, relatively constant for the *in utero* cohort, but markedly increasing with age for the childhood exposure, was also observed in the cancer mortality data. In the *in utero* cohort, although there is no significant variation in solid cancer risk by trimester, higher risks were seen in the first and the third trimester than in the second trimester. Unlike cancer mortality, the *in utero* sex-averaged ERR of noncancer mortality at attained age 50 years is larger than that observed among those exposed in early childhood. In the *in utero* cohort noncancer mortality risk was seen especially for those exposed in the second trimester.

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), Suyama A (EN), Ozasa K (EH), Yanagawa T

The principal purpose of this study is to evaluate effects of radiation exposure on mortality among A-bomb survivors, taking into account secondary causes of death. This study is based on the Life Span Study (LSS) cohort. The proposed study will start with identification of secondary causes of death specific to major underlying causes of death that developed during the follow-up period. Next, we will examine whether or not mortality dose response with only the underlying causes of death considered as was done

conventionally is different from that with due consideration given to both the underlying and secondary causes of death. With regard to major underlying causes of death, we will also determine whether different combinations of the same underlying cause of death with secondary causes of death change dose response. With regard to two secondary causes of death specific to the same underlying cause of death, furthermore, we will investigate association (synergistic or antagonistic) among A-bomb survivors between dose-response curves with both of the two secondary causes of death and those with only one of the causes. The aforementioned work is anticipated to hint at effects of secondary causes of death on association between underlying causes of death and radiation exposure. This study is assumed to be important for understanding of association between deaths reflecting secondary causes and radiation exposure among the LSS cohort, specifically with regard to noncancer deaths.

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

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

Although associations of radiation exposures with cancer risk have been well documented, those with noncancer have been sparse or only reported in combined organs. Since diseases from those organs often share different risk factors and physiopathology, it is important to examine the effects of radiation with those diseases. In the present study, we will examine the risk of radiation exposure for mortality from diseases of respiratory and digestive systems. We will use the data from the atomic bomb survivors with estimated radiation doses in the Life Span Study cohort in Hiroshima and Nagasaki for the follow-up period from 1950 to 2005. We will estimate the risk of death from noncancer disease of respiratory system after radiation exposure for acute upper respiratory infections; acute bronchitis; pneumonia and influenza; chronic obstructive pulmonary disease; asthma; and other diseases of the respiratory system. Deaths due to noncancer diseases of the digestive system will also be assessed, including diseases of the esophagus, stomach, duodenum, liver (especially liver cirrhosis), and others. Individual doses of radiation exposure for study subjects will be used according to the Dosimetry System 2002 (DS02). Weighted lung dose will be applied for all diseases of the respiratory system. Weighted dose for target organs of digestive system will be used, but weighted colon dose for diseases of non-specific or combined organs of the digestive system. Information on potential confounding factors including socio-demographic and lifestyle factors of study subjects were obtained through the previous mail and clinical surveys. Cox proportional hazard regression models will be used to estimate the risks of mortality from these diseases according to radiation dose exposures, and to evaluate the variation of the dose-response effects with respect to potential confounding factors.

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

Fang CT, Wang JD, Hwang JS, Hsu WL (S), Furukawa K (S), Kasagi F, Soda M (EN), Suyama A (EN) , Ozasa K (EH), Cullings HM (S)

The knowledge that how long patients can expect to live after the diagnosis of a disease is essential for cost-effectiveness evaluation of medical interventions. Our research group has developed a semi-parametric survival extrapolation method based on logit survival ratio between patient cohort and a reference population. Our method is built on one assumption that the excess hazard associated with a particular type of disease remains constant over time. In mathematical terms, this means that the logit survival ratio curve will converge to a straight line over time and therefore allows linear extrapolation. The method has been proven to be accurate for short-term projection of Taiwanese HIV-infected patients. However, due to limited follow-up time, we had not yet been able to validate its accuracy in life-long projection. The Life Span Study (LSS) cohort of the atomic-bomb survivors from Hiroshima and Nagasaki is unique that maintains one of the longest follow-up data in the world and will allow us to empirically examine the robustness of the constant excess hazard assumption in life-long projection scenario. We propose to examine whether the logit survival ratio curve between atomic-bomb survivors with (1) with or without radiation exposure or (2) a specific cancer (e.g., leukemia, stomach cancer, lung cancer, liver cancer, colon cancer, breast cancer, and pancreatic cancer) and Japanese reference population will converge to a straight line over time. In addition, we would test different strategies of estimating the slope of the logit of survival ratio.

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

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

At RERF ample experience exists with modeling predominantly solid cancer in the Life Span Study (LSS) whereas the Dutch National Institute for Public Health and the Environment (RIVM) has a history of developing and applying a Moolgavkar-type two-mutation model to leukemia in both laboratory animals and humans. These complementary backgrounds provide a unique opportunity to model the partly radiation-induced leukemia incidence in the LSS in a collaborative effort. Such a biologically-based leukemia model would not only make the comparison of calculated risks for the A-bomb survivors with epidemiological estimates possible, but more importantly: it would provide a means to transfer risks, for instance, to chronic and low dose exposures and to other western populations. Such risk estimates are of great interest for radiation protection purposes. Collaborative and data sharing agreements were completed and statistical analysis is in progress. During a visit to RERF in November 2009 Dr. Dekkers of RIVM created data files that can be used as input for the two-mutation carcinogenesis (TMC) model from the LSS data on leukemia incidence. Preliminary analyses indicate that the data can be described using the TMC model. Dr. Dekkers has continued making progress in model fitting. In 2011, Dr. Dekkers presented her results in the poster

session at the 14th International Congress of Radiation Research held in Warsaw, Poland.

RP-A11-08 Relationship between radiation exposure and kidney disease among A-bomb survivors

Adams MJ, Grant EJ (EH), Kodama K (CS), Shimizu Y (EH), Kasagi F, Suyama A (EN), Sakata R (EH), Fujiwara S (CH), Akahoshi M (CN)

The purpose of this study is to evaluate whether increasing radiation dose is associated with kidney disease mortality in atomic-bomb survivors after adjusting for other known risk factors for kidney disease incidence that were collected among LSS cohort members.

Results indicated a significant quadratic dose relationship between radiation dose and possible chronic renal disease mortality, which was similar in shape to that observed between radiation and incidence of hypertension in this population. Our results suggest that renal dysfunction could be part of the mechanism causing increased CVD risk after whole body irradiation, a hypothesis that deserves further study (Adams et al., *Radiation Research* 2012; 177:220–8).

RP-A7-08 Risk estimates of bladder, ureter and renal pelvis cancers among atomic bomb survivors after adjustments for lifestyle factors

Grant EJ (EH), Kasagi F, Suyama A (EN), Shimizu Y (EH), Soda M (EN), Sugiyama H (EH), Sakata R (EH), Yamada M (CH), Hsu WL (S), Nagano J, De Roos A, Kopecky K, Davis S

The primary goal of this study is to re-evaluate the radiation risk of urothelial cancers (UC) after accounting for lifestyle factors also known to be associated with these cancers.

The first of three manuscripts from this project was published (Grant et al., *Radiation Research* 2012; 177:86–98). The paper used a full cohort design and the results indicated that radiation risk estimates were not modified by such lifestyle factors as smoking, drinking, vegetable and fruit consumption, and attained educational level, despite the strong dependence of UC on smoking.

The last lifestyle factor under consideration is occupational exposures to classes of chemicals that cause UC. Using a stratified case-cohort design, occupational exposures to aromatic amines and polycyclic hydrocarbons were determined via recoding survey data and using a job exposure matrix. Two manuscripts are in preparation. The first is a methodology paper on the occupational exposure assignments. The second manuscript reports the results of the stratified analysis.

RP-A3-08 Potential confounding or interaction of anthropometric factors with radiation on colon cancer incidence among atomic bomb survivors in the Life Span Study (LSS) cohort

Semmens E, Li CI, Sugiyama H (EH), Moriwaki H (EH), Sakata R (EH), Soda M (EN), Kasagi F, Yamada M (CH), Fujiwara S (CH), Akahoshi M (CN), Davis S, Kopecky KJ, Mabuchi K, Kodama K (CS)

The primary purpose of this project is to assess how anthropometric factors may confound or modify the relationship between radiation dose and risk of colon cancer incidence among atomic-bomb survivors in the Life Span Study (LSS) cohort. Anthropometric data obtained through questionnaires in 1965, 1969, 1978, and 1991 from members of the Life Span Study (LSS) cohort and from clinic records of members of the Adult Health Study (AHS) were utilized.

Results indicate that increasing BMI was significantly associated with increasing risks of colon cancer, however, radiation risk estimates were not modified by those associations.

This RP was developed under the auspices of the Radiation Research Partnership Program with the University of Washington and Kurume University. Ms. Semmens, a PhD student at the University of Washington, carried out the analyses and has submitted a paper to a scientific journal.

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

Little MP, Cullings HM (S), Furukawa K (S), Ozasa K (EH), Soda M (EN), Sakata R (EH)

The purpose of this protocol is to investigate the effects of errors in survivors' dose estimates on the estimation of radiation risk of cancer in the LSS, using Bayesian models with individual data under several models for assumed dose errors, and to compare the results to those expected from the theory associated with standard methods employed at RERF. It is well understood that measurement errors exist in DS02 dose estimates and that these affect risk estimates. Several researchers have investigated the problem with a view towards removing measurement-error bias in risk estimates. The method of dose adjustment currently employed at RERF is the so-called "regression calibration" method, which entails replacing the DS02 dose estimate with the expected value of the individual's true dose given their estimated dose. Although this approach leads to reasonable adjusted point estimates of risk parameters for linear dose-response models, it is an approximate method when used with non-linear dose-effect relationships and does not fully take account of the variability in risk estimates induced by the measurement errors.

Bayesian models can in principle incorporate a much wider variety of error models and can evaluate the resulting bias and imprecision of risk estimates using individual data rather than the grouped data typically used in the past at RERF. Because these models require evaluation of the likelihood by the computationally intensive method of Markov Chain Monte Carlo (MCMC) integration, computational requirements are an issue. Earlier, a student working under Dr. Little, Philip Li, found that computational requirements forced him to retreat from the full LSS data and work with a sub-sample using a nested case-control design, which he evaluated for a few key outcome measures such as leukemia and thyroid cancer incidence.

Dr. Furukawa published a paper on using multiple imputation of missing data on smoking for risk estimation of lung cancer, which is an example of an alternative to full-likelihood Bayesian MCMC calculations that is at present more computationally feasible. Dr. Cullings began working on an approach to a computationally feasible Bayesian analysis of the effect of dose error using a method for

grouped data set forth in a 2008 paper by Dr. Little and others.

Life Span Study Publications

RERF Reports (RR)

♦ Adams MJ, Grant EJ, Kodama K, Shimizu Y, Kasagi F, Suyama A, Sakata R, Akahoshi M: Radiation dose associated with renal failure mortality: A potential pathway to partially explain increased cardiovascular disease mortality observed after whole-body irradiation. Radiation Research 2012 (February); 177(2):220–8. **(RR 9-11)**

[Abstract] Whole-body and thoracic ionizing radiation exposure are associated with increased cardiovascular disease (CVD) risk. In atomic bomb survivors, radiation dose is also associated with increased hypertension incidence, suggesting that radiation dose may be associated with chronic renal failure (CRF), thus explaining part of the mechanism for increased CVD. Multivariate Poisson regression was used to evaluate the association of radiation dose with various definitions of chronic kidney disease (CKD) mortality in the Life Span Study (LSS) of atomic bomb survivors. A secondary analysis was performed using a subsample for whom self-reported information on hypertension and diabetes, the two biggest risk factors for CRF, had been collected. We found a significant association between radiation dose and only our broadest definition of CRF among the full cohort. A quadratic dose excess relative risk model (ERR/Gy^2 = 0.091 [95% CI: 0.05, 0.198]) fit minimally better than a linear model. Within the subsample, association was also observed only with the broadest CRF definition (ERR/Gy^2 = 0.15 [95% CI: 0.02, 0.32]). Adjustment for hypertension and diabetes improved model fit but did not substantially change the ERR/Gy^2 estimate, which was 0.17 (95% CI: 0.04, 0.35). We found a significant quadratic dose relationship between radiation dose and possible chronic renal disease mortality that is similar in shape to that observed between radiation and incidence of hypertension in this population. Our results suggest that renal dysfunction could be part of the mechanism causing increased CVD risk after whole-body irradiation, a hypothesis that deserves further study.

♦ Cologne JB, Grant EJ, Nakashima E, Chen Y, Funamoto S, Katayama H: Protecting privacy of shared epidemiologic data without compromising analysis potential. Journal of Environmental and Public Health 2012 (February); Volume 2012, Article ID 421989, 9 pages. doi:10.1155/2012/421989 **(RR 2-06)**

[Abstract] ***Objective:*** Ensuring privacy of research subjects when epidemiologic data are shared with outside collaborators involves masking (modifying) the data, but overmasking can compromise utility (analysis potential). Methods of statistical disclosure control for protecting privacy may be impractical for individual researchers involved in small-scale collaborations. ***Methods:*** We investigated a simple approach based on measures of disclosure risk and analytical utility that are straightforward for epidemiologic researchers to derive. The method is illustrated using data from the Japanese Atomic-bomb Survivor population. ***Results:*** Masking by modest rounding did not adequately enhance security but rounding to remove several digits of relative accuracy effectively reduced the risk of identification without substantially reducing utility. Grouping or adding random noise led to noticeable bias. ***Conclusions:*** When sharing epidemiologic data, it is recommended that masking be performed using rounding. Specific treatment should be determined separately in individual situations after consideration of the disclosure risks and analysis needs.

♦ Ozasa K, Shimizu Y, Suyama A, Kasagi F, Soda M, Grant EJ, Sakata R, Sugiyama H, Kodama K: Studies of the mortality of atomic bomb survivors, Report 14, 1950–2003: An overview of cancer and noncancer diseases. Radiation Research 2012 (March); 177(3):229–43. **(RR 4-11)**

[Abstract] This is the 14th report in a series of periodic general reports on mortality in the Life Span Study (LSS) cohort of atomic bomb survivors followed by the Radiation Effects Research Foundation to investigate the late health effects of the radiation from the atomic bombs. During the period 1950–2003, 58% of the 86,611 LSS cohort members with DS02 dose estimates have died. The 6 years of additional follow-up since the previous report provide substantially more information at longer periods after radiation exposure (17% more cancer deaths), especially among those under age 10 at exposure (58% more deaths). Poisson regression methods were used to investigate the magnitude of the radiation-associated risks, the shape of the dose response, and effect modification by gender, age at exposure, and attained age. The risk of all causes of death was positively associated with radiation dose. Importantly, for solid cancers the additive radiation risk (i.e., excess cancer cases per 10^4 person-years per Gy) continues to increase throughout life with a linear dose–response relationship. The sex-averaged excess relative risk per Gy was 0.42 (95% confidence interval [CI]: 0.32, 0.53) for all solid cancer at age 70 years after exposure at age 30 based on a linear model. The risk increased by about 29% per decade decrease in age at exposure (95% CI: 17%, 41%). The estimated lowest dose range with a significant ERR for all solid cancer was 0 to 0.20 Gy, and a formal dose-threshold analysis indicated no threshold; i.e., zero dose was the best estimate of the threshold. The risk of cancer mortality increased significantly for most major sites, including stomach, lung, liver, colon, breast, gallbladder, esophagus, bladder and ovary, whereas rectum, pancreas, uterus, prostate and kidney parenchyma did not have significantly increased risks. An increased risk of non-neoplastic diseases including the circulatory, respiratory and digestive systems was observed, but whether these are causal relationships requires further investigation. There was no evidence of a radiation effect for infectious or external causes of death.

♦ Sakata R, Shimizu Y, Soda M, Yamada M, Hsu WL, Hayashi M, Ozasa K: Effect of radiation on age at menopause among atomic bomb survivors. Radiation Research 2011 (December); 176(6):787–95. **(RR 7-11)**

[Abstract] Exposure to ionizing radiation has been thought

to induce ovarian failure and premature menopause. Proximally exposed female atomic bomb survivors were reported to experience menopause immediately after the exposure more often than those who were distally exposed. However, it remains unclear whether such effects were caused by physical injury and psychological trauma or by direct effects of radiation on the ovaries. The objective of this study was to see if there are any late health effects associated with the exposure to atomic bomb radiation in terms of age at menopause in a cohort of 21,259 Life Span Study female A-bomb survivors. Excess absolute rates (EAR) of natural and artificial menopause were estimated using Poisson regression. A linear threshold model with a knot at 0.40 Gy (95% confidence interval [CI]: 0.13, 0.62) was the best fit for a dose response of natural menopause (EAR at 1 Gy at age of 50 years = 19.4/1,000 person-years, 95% CI: 10.4, 30.8) and a linear threshold model with a knot at 0.22 Gy (95% CI: 0.14, 0.34) was the best fit for artificial menopause (EAR at 1 Gy at age of 50 years for females who were exposed at age of 20 years = 14.5/1,000 person-years, 95% CI: 10.2, 20.1). Effect modification by attained age indicated that EARs peaked around 50 years of age for both natural and artificial menopause. Although effect modification by age at exposure was not significant for natural menopause, the EAR for artificial menopause tended to be larger in females exposed at young ages. On the cumulative incidence curve of natural menopause, the median age at menopause was 0.3 years younger in females exposed to radiation of 1 Gy compared with unexposed females. The median age was 1 year younger for combined natural and artificial menopause in the same comparison. In conclusion, age at menopause was thought to decrease with increasing radiation dose for both natural and artificial menopause occurring at least 5 years after the exposure.

♦ Samartzis D, Nishi N, Hayashi M, Cologne JB, Cullings HM, Kodama K, Miles EF, Funamoto S, Suyama A, Soda M, Kasagi F: Exposure to ionizing radiation and development of bone sarcoma: New insights based on atomic-bomb survivors of Hiroshima and Nagasaki. Journal of Bone and Joint Surgery-American Volume 2011 (June); 93:1–8. **(RR 9-08)**

 (related to *Tumor and Tissue Registry*)

[Abstract] ***Background:*** Radiation-induced bone sarcoma has been associated with high doses of ionizing radiation from therapeutic or occupation-related exposures. However, the development of bone sarcoma following exposure to lower doses of ionizing radiation remains speculative. ***Methods:*** A cohort analysis based on the Life Span Study (n = 120,321) was performed to assess the development of bone sarcoma in atomic-bomb survivors of Hiroshima and Nagasaki followed from 1958 to 2001. The excess relative risk per gray of ionizing radiation absorbed by the bone marrow was estimated. Additional subject demographic, survival, and clinical factors were evaluated. ***Results:*** Nineteen cases of bone sarcoma (in eleven males and eight females) were identified among the 80,181 subjects who met the inclusion criteria, corresponding to an incidence of 0.9 per 100,000 person-years. The mean ages at the time of the bombing and at diagnosis were 32.4 and 61.6 years, respectively. The mean bone marrow dose was 0.43 Gy. Osteosarcoma was the most commonly identified bone sarcoma. The most common bone sarcoma site was the pelvis. The overall unadjusted five-year survival rate was 25%. A dose threshold was found at 0.85 Gy (95% confidence interval, 0.12 to 1.85 Gy), with a linear dose-response association above this threshold. The linear slope equaled an excess relative risk of 7.5 per Gy (95% confidence interval, 1.34 to 23.14 per Gy) in excess of 0.85 Gy. ***Conclusions:*** On the basis of what we believe is one of the longest and largest prospective studies assessing the development of bone sarcoma in individuals exposed to ionizing radiation, it appears that the development of radiation-induced bone sarcoma may be associated with exposure to much lower doses of ionizing radiation than have previously been reported. Such new insights may potentially improve bone sarcoma prevention measures and broaden our understanding of the role of ionizing radiation from various sources on the development of malignant tumors. This study stresses the need to become increasingly aware of the various health risks that may be attributable to even low levels of ionizing radiation exposure.

Other Journal Publications

♦ Kodama K, Ozasa K, Okubo T: Radiation and cancer risk in atomic-bomb survivors. Journal of Radiological Protection 2012 (March); 32(1):N51–4. (Special section: Selected articles from Proceedings of the International Expert Symposium in Fukushima: Radiation and Health Risks, 11–12 September 2011) (related to *Special Cancer Studies* and *Tumor and Tissue Registries*)

♦ Ozasa K: Cohort studies of the atomic bomb survivors at the Radiation Effects Research Foundation. Nippon Rinsho [Japanese Journal of Clinical Medicine] 2012 (March); 70(3):399–404. (Japanese)

♦ Ozasa K: Effects of low-dose radiation on the human body. Igaku Butsuri [Japanese Journal of Medical Physics] 2011; 31(Sup. 3):41–50. (Proceedings of the Medical Physics Training Session 2011) (Japanese)

♦ Ozasa K: Epidemiological studies of the atomic bomb survivors in Hiroshima and Nagasaki. Kyoto Furitsu Ikadaigaku Zasshi [Journal of Kyoto Prefectural University of Medicine] 2011 (December); 120(12):903–11. (Japanese)

♦ Ozasa K: Health effects of atomic-bomb radiation of Hiroshima and Nagasaki. Nogyo to Keizai [Agriculture & Economics] 2012 (January); 78(1):53–60. (Extra edition of January, 2012) (Japanese)

♦ Ozasa K, Shimizu Y, Sakata R, Sugiyama H, Grant EJ, Soda M, Kasagi F, Suyama A: Risk of cancer and non-cancer diseases in the atomic bomb survivors. Radiation Protection Dosimetry 2011 (April):1–4.

♦ Sakata R, Shimizu Y, Soda M, Grant EJ, Sugiyama H, Pham TM, Hsu WL, Suyama A, Ozasa K: Recent RERF epidemiological studies of an atomic bomb (A-bomb) survivor cohort: Early onset of menopause observed among A-bomb survivor females. Nakashima M, et al., eds. Global Strategic Center for Radiation Health Risk Control: A new challenge of radiation health risk management. Proceedings

of the 6th International Symposium of Nagasaki University Global COE Program "Global Strategic Center for Radiation Health Risk Control." Nagasaki: Nagasaki Newspaper Publish; 2012 March. pp 145–9.

♦ Shore RE, Ozasa K, Suyama A, Fujiwara S, Akahoshi M, Cullings HM, Kodama Y, Kodaira M, Hayashi T, Hamatani K: Recent findings by the radiation effects research foundation on the health of atomic bomb survivors and their offspring. Nakashima M, et al., eds. A New Challenge of Radiation Health Risk Management. Proceedings of the 6th International Symposium of Nagasaki University Global COE Program "Global Strategic Center for Radiation Health Risk Control." Nagasaki: Nagasaki Newspaper Publish; 2012(March). pp 27–33. (related to *Adult Health Study*)

Manuscripts in Press

⌘ Kodama K, Ozasa K, Katayama H, Shore RE, Okubo T: Radiation effects on cancer risks in the Life Span Study cohort. Radiation Protection Dosimetry.

⌘ Ozasa K: Effects of atomic-bomb radiation on children. Child Health. (Japanese)

⌘ Samartzis D, Nishi N, Cologne JB, Hayashi M, Kodama K, Miles EF, Funamoto S, Suyama A, Soda M, Kasagi F: Ionizing radiation exposure and the development of soft tissue sarcomas in atomic-bomb survivors. Journal of Bone and Joint Surgery-American Volume. (related to *Tumor and Tissue Registries*)

Life Span Study Oral Presentations

❖ Shore RE. Low-dose epidemiology studies: Summary and issues to consider. NAS Study Around Nuclear Plants—Feasibility Assessment, 18–19 April 2011, Chicago, Illinois, USA

❖ Ozasa K. Epidemiological studies of the late health effects of atomic-bomb radiation in Hiroshima and Nagasaki. 18th International Congress of Dentomaxillofacial Radiology, 25–29 May 2011, Hiroshima

❖ Grant EJ, Kubo T, Sakata R, Cologne JB, Suyama A, Ozasa K. The urothelial carcinoma risks of radiation, smoking and occupational exposures: A case-cohort study with stratified sampling among A-bomb survivors. IEA World Congress of Epidemiology 2011, 7–11 August 2011, Edinburgh, Scotland

❖ Dekkers F, Bijwaard H, van Dillen T, Hsu WL, Cullings HM, Soda M, Sugiyama H. Leukemia in atomic bomb survivors: Towards a biophysical model. 14th International Congress of Radiation Research, 28 August–1 September 2011, Warsaw, Poland

❖ Kodama K, Ozasa K, Shore RE. Update on radiation risk estimates from A-bomb survivors. 14th International Congress of Radiation Research, 28 August–1 September 2011, Warsaw, Poland (related to *Adult Health Study*)

❖ Ozasa K, Shimizu Y, Suyama A, Soda M, Grant EJ, Sakata R, Sugiyama H, Kodama K. Radiation risk of cancer and noncancer mortality in atomic bomb survivors, 1950–2003. 14th International Congress of Radiation Research, 28 August–1 September 2011, Warsaw, Poland

❖ Sakata R, Shimizu Y, Grant EJ, Sugiyama H, Soda M, Suyama A, Ozasa K. Effect modification by smoking status on radiation effect for stomach cancer mortality among atomic bomb survivors. 14th International Congress of Radiation Research, 28 August–1 September 2011, Warsaw, Poland

❖ Kodama K, Ozasa K, Okubo T. Radiation and cancer risk in atomic bomb survivors. The International Experts Symposium on Radiation and Health Risks, 11–12 September 2011, Fukushima (related to *Adult Health Study*)

❖ Kodama K, Ozasa K, Suyama A, Grant EJ, Yamada M. Epidemiological studies to determine long-term health effects in atomic bomb survivors. 23rd General Assembly of Japanese Association of Medical Sciences, 17 September 2011, Tokyo (related to *Adult Health Study*)

❖ Shore RE, Ozasa K, Shimizu Y, Neriishi K. Applications of Hiroshima-Nagasaki A-bomb research to epidemiologic studies of nuclear accidents. Symposium on Radiation Epidemiology, 28 September 2011, Mainz, Germany (related to *Adult Health Study*)

❖ Ozasa K. Cohort studies of late health effects of radiation among the atomic-bomb survivors. 70th Annual Meeting of the Japanese Cancer Association, 3–5 October 2111, Nagoya

❖ Ozasa K. Epidemiological follow-up studies on the atomic-bomb survivors. 70th Annual Meeting of the Japanese Society of Public Health, 19–21 October 2011, Akita (related to *F_1 Studies* and *Tumor and Tissue Registries*)

❖ Sakata R, Shimizu Y, Soda M, Grant EJ, Sugiyama H, Pham TM, Hsu WL, Suyama A, Ozasa K. Recent epidemiological studies on RERF A-bomb survivor cohort: Early onset of menopause observed among A-bomb survivor females. 6th International Symposium of Nagasaki University Global COE Program, 20–22 October 2011, Nagasaki

❖ Shore RE, Ozasa K, Suyama A, Fujiwara S, Akahoshi M, Cullings HM, Kodama Y, Kodaira M, Hayashi T, Hamatani K. Recent findings by the Radiation Effects Research Foundation on the health of atomic bomb survivors and their offspring. International Symposium of Nagasaki University Global COE Program, 22 October 2011, Nagasaki (related to *Adult Health Study*, *Immunology Studies*, *Cytogenetics Studies*, and *F_1 Studies*)

❖ Ozasa K, Shimizu Y. Late health effects at low-dose levels among the atomic-bomb survivors. 54th Annual Meeting of the Japan Radiation Research Society, 17–19 November 2011, Kobe

❖ Ozasa K. Radiation effects on young people based on the atomic bomb survivor studies. 53rd Annual Meeting of the Japanese Society of Pediatric Hematology/Oncology, 25–27 November 2011, Maebashi

❖ Ozasa K. Radiation effects at low-dose levels and effects of age at bombing among the atomic-bomb survivors. Radiation Epidemiology Research Meeting 2011, 14 December 2011, Tokyo

❖ Grant EJ, Shimizu Y, Sugiyama H, Sakata R, Pham TM, Cologne JB, Suyama A, Ozasa K. The sex-specific urothelial carcinoma risks of radiation and smoking among A-bomb survivors. 22nd General Meeting of the Japan Epidemiological Association, 26–28 January 2012, Tokyo

❖ Pham TM, Grant EJ, Shimizu Y, Sakata R, Furukawa K, Takahashi I, Sugiyama H, Soda M, Suyama A, Ozasa K. Radiation risk for non-cancer respiratory and digestive diseases in the Life Span Study (LSS). 22nd General Meeting of the Japan Epidemiological Association, 26–28 January 2012, Tokyo

❖ Sakata R, Grant EJ, Sugiyama H, Shimizu Y, Soda M, Suyama A, Ozasa K. Results from the 2008 mail survey of

the Radiaation Effects Research Foundation Life Span Study cohort. 22nd General Meeting of the Japan Epidemiological Association, 26–28 January 2012, Tokyo (related to *Adult Health Study*)

❖ Sakata R. Radiation risk estimation for A-bomb survivors' cohort. Young Researcher's Association of Japan Health Physics Society Seminar, 3 March 2012, Tokyo

Research Protocols 2-11, 7-10, 7-09, 3-07, 2-75 (Platform Protocol), 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 (CH)

Background and purposes: Reports regarding mortality and incidence of arteriosclerotic diseases among A-bomb survivors have suggested that radiation induces arteriosclerosis, but the mechanism of such induction is unclear. It is difficult to explain the entire picture of the complex clinical condition of arteriosclerosis with the conventional hypothesis that arteriosclerosis is an inflammatory disease. In this study, we will consider arteriosclerosis based on the "inflammation-response-to-injury" hypothesis and evaluate the effects of radiation exposure based on the hypothesis that diseases related to "artery-bone metabolism-immunity" are abnormalities in differentiation and proliferation of arterial mesenchymal tissue. By verifying this new hypothesis, it may become possible to better understand arterial effects from radiation.

Study methods: For about 2,100 Adult Health Study (AHS) subjects in Hiroshima (including those exposed at young ages), we will measure several multi-functional cytokines including: pentraxin (PTX)-3; osteopontin (OPN); osteoprotegerin (OPG); receptor activator of nuclear factor (NF)-κB ligand, RANKL; vascular endothelial growth factor (VEGF)-A; high mobility group box (HMGB)-1; apolipoprotein (Apo)-J, also called clusterin; interleukin (IL)-17; and reactive oxygen species (ROS). Study period will be four years (two cycles) starting in 2010.

Study progress: Examinations are under way.

Results: Not yet reported.

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

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

Background: Some recent results from studies of atomic-bomb (A-bomb) survivors have shown a positive association between radiation dose and incidence of arteriosclerotic diseases such as hypertension and myocardial infarction (MI). The underlying mechanisms of radiation dose effects on arteriosclerotic diseases, however, remain elusive. In addition, there are limited numbers of reports on the presence or absence of racial differences in the health effects of body composition.

Objectives: The objectives of this prospective study include: 1) testing whether radiation exposure is related to increased incidence of arteriosclerotic diseases and their risk factors through modifications in body composition; 2) examining effects of body composition modifications, particularly aging-related loss of muscle mass (sarcopenia), on the health of the Japanese people, such as the prevalence and incidence of arteriosclerotic diseases, and associated

risk factors and mortality; and 3) comparing Hiroshima Adult Health Study (AHS) participants and U.S. Health, Aging, and Body Composition (ABC) Study participants, with respect to potential racial differences in health effects associated with body composition (international collaborative study).

Methods: Study subjects will total approximately 2,200 Hiroshima AHS participants who underwent whole-body composition examination by dual energy X-ray absorptiometry (DEXA) during the period 1994–1996. In the Hiroshima AHS, measurements of whole-body/regional (trunk, limb, etc.) fat mass (FM), lean mass (LM), and bone mineral content (BMC) were conducted by DEXA starting in 1994. Those data are already stored in a database and available for use. In this prospective study, we will examine relationships between DEXA-based body composition and radiation dose, and the prevalence and incidence of arteriosclerotic diseases plus associated risk factors and mortality. Mortality endpoints will include all causes, ischemic heart disease, stroke, and possibly other cardiovascular diseases, if feasible.

This study may be useful to elucidate mechanisms of the relationship of radiation dose to arteriosclerotic diseases and their risk factors. Furthermore, the international collaborative study should be meaningful for both the A-bomb survivors and the general Japanese population.

Study progress: Data cleaning has been completed and analysis initiated to determine the effects of radiation dose on body composition.

Results: Preliminary statistical analysis of possible radiation effects on body composition showed negative association between radiation dose and body mass index (BMI). Further analyses will be conducted.

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

Takahashi I (CH), Hida A (CN), Akahoshi M (CN), Kohata M, Yamada M (CH), Hsu WL (S), Misumi M (S), Takahashi T, Kihara Y, Matsumoto M, Fujiwara S (CH)

Purposes: We will evaluate acceleration of arterial stiffness by radiation among AHS subjects including the expanded group of younger survivors in order to study one of the potential mechanisms by which radiation may promote cardiovascular disease.

Background: Past studies have reported a significant association between radiation exposure and atherosclerotic disease mortality/morbidity among A-bomb survivors. Atherosclerosis conceptually has two aspects: atherosis (the 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 both in Hiroshima and Nagasaki.

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 over four years starting 2010 with consideration for atheromatous disease indices (ankle-brachial blood pressure index [ABI], intima-media wall thickness [IMT], aortic calcification, and left ventricular hypertrophy) and atherosclerosis risk factors (Framingham risk scores).

Study progress: Examinations are under way.

Results: Not yet reported.

RP 3-07 Clinical health study for expanded group of younger A-bomb survivors

Akahoshi M (CN), Yamada M (CH), Hida A (CN), Ohishi W (CH), Ozasa K (EH), Kasagi F, Suyama A (EN), Furukawa K (S), Cullings HM (S), Hayashi T (R), Nakachi K, Kodama Y (G), Katayama H (IT), Kodama K (CS), Nakamura N (CS), Fujiwara S (CH)

LSS data show that those exposed to A-bomb radiation at a young age have a greater cancer risk than those exposed when older. AHS data show similar results for benign thyroid tumors, hyperparathyroidism, hepatitis B virus (HBV) infection, and myocardial infarction. By expanding the cohort of younger survivors, we will enhance the statistical power and precision for estimating risks among those who were young at the time of the bombing.

The existing AHS cohort consisted of all identified heavily exposed individuals and a small fraction of those lightly or moderately exposed. Adding more of the latter to the AHS cohort will provide a much better assessment of low and moderate radiation dose effects in younger subjects and will increase the number of biological samples from younger survivors for molecular biological studies in the future.

Since subjects who received the lowest doses (<5 mGy) are already the largest group of AHS subjects, little statistical power would be gained by the addition of many more such subjects. We therefore plan to solicit 30% of the potentially eligible who were exposed to <5 mGy, 80% of those exposed to 5–20 mGy, and all exposed to 20–1,000 mGy which will add up to 2,300 subjects of whom the majority received 20–1,000 mGy. We will focus on noncancer disease (liver disease, thyroid disease, ophthalmologic disease, and cardiovascular disease) in addition to cancer.

In 2011, we added to the AHS cohort 1,941 younger survivors who agreed to cooperate in the longitudinal study. The added subjects will undergo biennial health examinations just like the AHS participants.

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

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

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 specimen and information concerning lifestyle or other potential risk factors for many fields of study, which include cytology, genetics, immunology, radiobiology, and medical dosimetry.

Background: The AHS program of biennial comprehensive medical examinations began in 1958 with a targeted population of about 20,000 survivors and controls in the contact areas of Hiroshima and Nagasaki. In 1978, the

sample was enriched with about 2,400 additional higher-dose subjects and all available (~1,000) persons who were exposed *in utero,* while about 5,000 not-in-city study subjects were dropped as being in different environment from other unexposed study subjects. We have added about 1,900 young exposed subjects (<10 years old at the bombings) to the study in 2008–2010.

Study methods: The study attempts to examine differences in diseases or pre-clinical disorders by radiation exposures. During the 26th cycle (July 2008–June 2010), 3,236 individuals were examined, representing approximately 65% of the AHS cohort still living in the contact areas of interest.

Study progress: Health examinations have been continued. The biological specimens collected are used for clinical determinations and stored for future studies. Evaluation of possible interactions between radiation and infectious agents or hormones and cancer risk, and phenotypic and genetic factors has been conducted using stored specimens. Recently initiated radiation-related studies include those on cytokines related to arteriosclerosis and assessment of neurocognitive function among subjects exposed when young.

Results: Reports of new findings include hepatocellular carcinoma risk with regard to radiation and hepatitis virus infection, radiation/hormone/growth factor levels in non-breast cancer subjects, lifetime risk of stroke, and incidence and prognostic value of abnormal electrocardiographic early repolarization.

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

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), Hsu WL (S), Misumi M (S), Fujiwara S (CH), Akahoshi M (CN)

The association between atomic-bomb radiation exposure and cardiovascular disease (CVD) has recently been drawing attention. A-bomb radiation exposure has been reported to be associated with a number of CVD risk factors. Chronic kidney disease (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. No study has been conducted at RERF thus far as to whether CKD is related to radiation, and whether CKD might serve as a mediating variable in the association of radiation with CVD.

In this analysis, we will identify prevalent cases of CKD diagnosed during the four-year baseline period (1988–91) and incident cases of CKD diagnosed during the 15-year follow-up period of 1992–2006 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. 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 prevalent cases of CKD with radiation dose and CKD risk factors.
(2) Associations of incident cases of CKD with radiation dose and CKD risk factors.
(3) Associations of prevalent cases of CHD and stroke with radiation dose, CKD risk factors, and prevalent cases of CKD.
(4) Associations of incident cases of CHD and stroke with radiation dose, CKD risk factors, and prevalent cases of CKD.

In November 2009, we started data collection to analyze association of prevalent CKD cases with radiation and CKD risk factors.

Adult Health Study Publications

RERF Reports (RR)

♦ Cologne JB, Grant EJ, Nakashima E, Chen Y, Funamoto S, Katayama H: Protecting privacy of shared epidemiologic data without compromising analysis potential. Journal of Environmental and Public Health 2012 (February); Volume 2012, Article ID 421989, 9 pages. doi:10.1155/2012/421989 **(RR 2–06)** (refer to abstract in *Life Span Study* Publications)

♦ Masunari N, Fujiwara S, Kasagi F, Takahashi I, Yamada M, Nakamura T: Height loss starting in middle age predicts increased mortality in the elderly. Journal of Bone and Mineral Research 2012 (January); 27(1):138–45. **(RR 2-11)** © American Society for Bone and Mineral Research (*This abstract was reprinted by permission of the Society.*) (related to *Special Clinical Studies*)

[Abstract] The purpose of this study was to determine the mortality risk among Japanese men and women with height loss starting in middle age, taking into account lifestyle and physical factors. A total of 2,498 subjects (755 men and 1,743 women) aged 47 to 91 years old underwent physical examinations during the period 1994 to 1995. Those individuals were followed for mortality status through 2003. Mortality risk was estimated using an age-stratified Cox proportional hazards model. In addition to sex, adjustment factors such as radiation dose, lifestyle, and physical factors measured at the baseline—including smoking status, alcohol intake, total cholesterol, blood pressure, and diagnosed diseases—were used for analysis of total mortality and mortality from each cause of death. There were a total of 302 all-cause deaths, 46 coronary heart disease and stroke deaths, 58 respiratory deaths including 45 pneumonia deaths, and 132 cancer deaths during the follow-up period. Participants were followed for 20,787 person-years after baseline. Prior history of vertebral deformity and hip fracture were not associated with mortality risk. However, more than 2 cm of height loss starting in middle age showed a significant association with all-cause mortality among the study participants (HR = 1.76, 95% CI 1.31 to 2.38, $p = 0.0002$), after adjustment was made for sex, attained age, atomic-bomb radiation exposure, and lifestyle and physical factors. Such height loss also was significantly associated with death due to coronary heart disease or stroke (HR = 3.35, 95% CI 1.63 to 6.86, $p = 0.0010$), as well as respiratory-disease death (HR = 2.52, 95% CI 1.25 to 5.22, $p = 0.0130$), but not cancer death. Continuous HL also was associated with all-cause mortality and CHD- or stroke-caused mortality. Association between height loss and mortality was still significant, even after excluding persons with vertebral deformity. Height loss of more than 2 cm starting in middle age was an independent risk factor for cardiovascular and

respiratory-disease mortality among the elderly, even after adjusting for potential risk factors.

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♦ Nakashima E, Fujii Y, Neriishi K, Minamoto A: Assessment of misclassification in a binary response: Recovering information on clinically significant cataract prevalence from cataract surgery data in atomic-bomb survivors. Journal of the Japan Statistical Society 2011 (June); 41(1):17–31. **(RR 14-07)**

[Abstract] Cataract surgery results when a patient decides to undergo lens surgery following a diagnosis of a clinically significant cataract (CSC). Because the presence of a CSC is generally latent and unobserved, a person might not receive cataract surgery even if the person has a CSC. This misclassification needs to be adjusted in the statistical analysis of CSC so as to reduce the bias in the parameter estimation. Following Magder and Hughes (1997) and using the cataract surgery data on atomic-bomb survivors at the Radiation Effects Research Foundation, we used this method for estimating the prevalence of CSC in a linear logistic dose response model taking account of the sensitivity and/or specificity of the decision for lens surgery. The estimated sensitivity was 0.385 (95% CI: 0.268, 0.517) and the estimated specificity was perfect. The odds ratio estimate for the radiation dose response changed from 1.39 (95% CI: 1.24, 1.55) to 1.58 (95% CI: 1.26, 1.98) when allowing for the imperfect sensitivity. A large sample simulation study with a continuous covariate was conducted, assuming either imperfect sensitivity or imperfect specificity, to investigate the performance of the method. Results indicated that the parameter estimates are almost correct. We calculated the asymptotic relative efficiency (ARE) for a simple logistic regression slope estimate and showed that the ARE depends only on the values of slope and intercept parameters.

♦ Takahashi I, Abbott RD, Ohshita T, Takahashi T, Ozasa K, Akahoshi M, Fujiwara S, Kodama K, Matsumoto M: A prospective follow-up study of the association of radiation exposure with fatal and non-fatal stroke among atomic bomb survivors in Hiroshima and Nagasaki (1980–2003). BMJ Open 2012 (February); 2(1):e000654. **(RR 13-11)**

[Abstract] ***Objective:*** Use of medical radiotherapy has increased markedly in recent decades. Whether the consequence includes an increased risk of cardiovascular disease remains to be determined. The purpose of this study was to examine the association between radiation exposure and the incidence of stroke among Japanese atomic bomb survivors. ***Design:*** A prospective follow-up study. ***Setting and participants:*** Radiation exposure from the atomic bombing was assessed in 9,515 subjects (34.8% men) with 24-year follow-up from 1980. Subjects were free of prevalent stroke when follow-up began. ***Outcome measures:*** Stroke events and the underlying cause of death were reviewed to confirm the first-ever stroke. Subtypes (ischaemic and haemorrhagic events) were categorised based on established criteria according to the definitions of typical/atypical stroke symptoms. ***Results:*** Overall mean radiation dose (±SD) in units of gray (Gy) was 0.38 ± 0.58 (range: 0–3.5). During the study period, 235 haemorrhagic and 607 ischaemic events were identified. For men, after adjusting for age and concomitant risk factors, the risk of haemorrhagic stroke rose consistently from 11.6 to 29.1 per 10,000 person-years as doses increased from <0.05 to ≥2 Gy (p = 0.009). Incidence also rose within the dose range <1 Gy (p = 0.004) with no dose threshold. In women, the risk of haemorrhagic stroke rose with increasing radiation exposure but not until doses reached a threshold of 1.3 Gy (95% CI 0.5 to 2.3). Among women, for doses <1.3 Gy, differences in stroke risk were modest (13.5 per 10,000 person-years), while it increased to 20.3 per 10,000 person-years for doses that ranged from 1.3 to <2.2 Gy and to 48.6 per 10,000 person-years for doses that were higher (p = 0.002). In both sexes, dose was unrelated to ischaemic stroke. ***Conclusion:*** While the risk of haemorrhagic stroke increases with rising radiation exposure for both sexes, effects in women are less apparent until doses exceed a threshold at 1.3 Gy.

This article has been accepted for publication in *BMJ Open* 2012;2:e000654 following peer review and can also be viewed on the journal's website at www.bmjopen.bmj.com.

♦ Takahashi I, Geyer SM, Nishi N, Ohshita T, Takahashi T, Akahoshi M, Fujiwara S, Kodama K, Matsumoto M: Lifetime risk of stroke and impact of hypertension: Estimates from the Adult Health Study in Hiroshima and Nagasaki. Hypertension Research 2011 (May); 34(5):649–54. doi:10.1038/hr.2011.7 **(RR 15-09)**

[Abstract] Very few reports have been published on lifetime risk (LTR) of stroke by blood pressure (BP) group. This study included participants in the Radiation Effects Research Foundation Adult Health Study who have been followed up by biennial health examinations since 1958. We calculated the LTR of stroke for various BP-based groups among 7,847 subjects who had not been diagnosed with stroke before the index age of 55 years using cumulative incidence analysis adjusting for competing risks. By 2003, 868 subjects had suffered stroke (512 [58.9%] were women and 542 [62.4%] experienced ischemic stroke). BP was a significant factor in determining risk of stroke for men and women, with distributions of cumulative risk for stroke significantly different across BP groups. The LTR of all-stroke for normotension (systolic BP/diastolic BP <120/80 mmHg), prehypertension (120–139/80–89 mmHg), stage 1 hypertension (140–159/90–99 mmHg) and stage 2 hypertension (>160/100 mmHg) were 13.8–16.9–25.8–25.8% in men and 16.0–19.9–24.0–30.5% in women, respectively ($p < 0.001$ among BP groups in both sexes). The estimates did not differ significantly ($p = 0.16$) between normotensive and prehypertensive subjects. One in five Japanese atomic bomb survivor subjects experienced stroke over their lifetime from the age of 55 years. Long-term stroke risks were elevated in

those with hypertension (≥140/90 mmHg) at any of the index ages of 45, 55, 65 and 75 years.

Other Journal Publication

♦ Shore RE, Ozasa K, Suyama A, Fujiwara S, Akahoshi M, Cullings HM, Kodama Y, Kodaira M, Hayashi T, Hamatani K: Recent findings by the radiation effects research foundation on the health of atomic bomb survivors and their offspring. Nakashima M, et al., eds. A New Challenge of Radiation Health Risk Management. Proceedings of the 6th International Symposium of Nagasaki University Global COE Program "Global Strategic Center for Radiation Health Risk Control." Nagasaki: Nagasaki Newspaper Publish; 2012(March). pp 27–33. (related to *Life Span Study*)

Manuscript in Press

⌘ Sera N, Hida A, Imaizumi M, Nakashima E, Akahoshi M: The association between chronic kidney disease and cardiovascular disease risk factors in atomic bomb survivors. Radiation Research.

Adult Health Study Oral Presentations

❖ Kodama K, Ozasa K, Shore RE. Update on radiation risk estimates from A-bomb survivors. 14th International Congress of Radiation Research, 28 August–1 September 2011, Warsaw, Poland (related to *Life Span Study*)

❖ Kodama K, Ozasa K, Okubo T. Radiation and cancer risk in atomic bomb survivors. The International Experts Symposium on Radiation and Health Risks, 11–12 September 2011, Fukushima (related to *Life Span Study*)

❖ Kodama K, Ozasa K, Suyama A, Grant EJ, Yamada M. Epidemiological studies to determine long-term health effects in atomic bomb survivors. 23rd General Assembly of Japanese Association of Medical Sciences, 17 September 2011, Tokyo (related to *Life Span Study*)

❖ Neriishi K. Current status of ophthalmologic study of A-bomb survivors. Exploratory Workshop on Radiation-Induced Lens Opacities Resulting from Low Dose Exposure, 20–23 September 2011, Bombon, France (related to *Special Clinical Studies*)

❖ Shore RE, Ozasa K, Shimizu Y, Neriishi K. Applications of Hiroshima-Nagasaki A-bomb research to epidemiologic studies of nuclear accidents. Symposium on Radiation Epidemiology, 28 September 2011, Mainz, Germany (related to *Life Span Study*)

❖ Takahashi I, Hida A, Akahoshi M, Fujiwara S, Takahashi T, Matsumoto M. The relationship between arteriosclerotic indexes and central blood pressure among the Adult Health Study (Part 1: Report of interim reports from the cross-sectional study). 34th Annual Scientific Meeting of the Japanese Society of Hypertension, 20–22 October 2011, Utsunomiya

❖ Shore RE, Ozasa K, Suyama A, Fujiwara S, Akahoshi M, Cullings HM, Kodama Y, Kodaira M, Hayashi T, Hamatani K. Recent findings by the Radiation Effects Research Foundation on the health of atomic bomb survivors and their offspring. International Symposium of Nagasaki University Global COE Program, 22 October 2011, Nagasaki (related to *Life Span Study*, *Immunology Studies*, *Cytogenetics Studies*, and *F_1 Studies*)

❖ Yamada M, Kasagi F, Sasaki H. Reaction time predicts mortality in a middle-aged and elderly Japanese population: Adult Health Study at the Radiation Effects Research Foundation. 9th Asia/Oceania Regional Congress of Gerontology and Geriatrics, 23–27 October 2011, Melbourne, Australia

❖ Sakata R, Grant EJ, Sugiyama H, Shimizu Y, Soda M, Suyama A, Ozasa K. Results from the 2008 mail survey of the Radiaation Effects Research Foundation Life Span Study cohort. 22nd General Meeting of the Japan Epidemiological Association, 26–28 January 2012, Tokyo (related to *Life Span Study*)

❖ Takahashi I, Abbott RD, Ohshita T, Takahashi T, Ozasa K, Akahoshi M, Fujiwara S, Kodama K, Matsumoto M. Radiation exposure and the incidence of hemorrhagic stroke among atomic bomb survivirs in Hiroshia and Nagasaki. 22nd General Meeting of the Japan Epidemiological Association, 26–28 January 2012, Tokyo

❖ Takahashi I, Abbott RD, Ohshita T, Takahashi T, Ozasa K, Akahoshi M, Fujiwara S, Kodama K, Matsumoto M. Relationship between hemorrhagic stroke incidence and radiation exposure among atomic bomb survivors in Hiroshima and Nagasaki. ACC (American College of Cardiology) Annual Scientific Session, 13–16 March 2012, San Diego, California, USA

Research Protocols 4-10 (Platform Protocol), 1-02 (Platform Protocol), A3-12
F_1 Clinical Study

RP 4-10 Longitudinal clinical study of the F_1 offspring of A-bomb survivors

Ohishi W (CH), Fujiwara S (CH), Akahoshi M (CN), Suyama A (EN), Furukawa K (S), Hsu WL (S), Takahashi N (R), Satoh Y (G), Kusunoki Y (R), Yamada M (CH), Tatsukawa Y (CH), Takahashi I (CH), Neriishi K, Hida A (CN), Imaizumi M (CN), Sera N (CN), Grant EJ (EH), Ozasa K (EH), Cologne JB (S), Cullings HM (S), Kodama Y (G), Katayama H (IT), Watanabe T (EH), Nakamura N (CS)

Objectives: To elucidate the effects of parental exposure to A-bomb radiation on the development of multifactorial diseases and subclinical conditions among the F_1 offspring of A-bomb survivors.

Background and significance: The initial cross-sectional clinical study of the F_1 offspring of A-bomb survivors (FOCS) provided no evidence for increased prevalence of adult-onset multifactorial diseases attributable to parental radiation exposure. The rationale for this study is that a high-quality clinical follow-up of participants through their later years, the period when people are prone to lifestyle diseases, will provide more reliable human data. In addition, prospective longitudinal data will allow analysis of incidence, enabling us to minimize self-selection bias.

Methods: In this prospective study, we will conduct periodic health examinations every four years of about 12,500 individuals who responded to our inquiry by mail or telephone between May 2000 and November 2008 indicating their willingness to undergo health examinations. The study will investigate association between multifactorial diseases identified through such examinations and parental radiation dose, taking into account any confounding factors.

Study progress: The longitudinal clinical study of the F_1 offspring of A-bomb survivors was initiated in November 2010. Over the past one-year period, we sent a brochure outlining the health examinations to 3,145 individuals and by telephone asked for their participation in the examinations. Of these individuals, 2,095 have already undergone the health examinations and 82 are expected to participate in the near future.

Results and conclusions: None yet.

RP 1-02 Health effects study of the children of A-bomb survivors: Clinical health study

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Objectives: To assess the possible genetic effects and associated long-term health consequences among children of the atomic-bomb survivors.

Background and significance: Animal experiments have shown that radiation exposure induces germ cell mutations and chromosome aberrations that may cause hereditary diseases. To date the studies have produced no evidence of dose-related genetic effects on the children of A-bomb survivors. In terms of human health, further evaluation and accumulation of evidence regarding radiation-induced genetic risk are crucial.

Study methods: Prevalence study from 2002 to 2006.

Study progress: A paper on the overall prevalence of the selected multi-factorial diseases in relation to radiation dose was published. Analyses of radiation-related genetic effects on individual multi-factorial diseases such as hypertension, hypercholesterolemia, and diabetes were completed with a manuscript submitted for internal review.

Results and conclusions: This cross-sectional study provided no evidence for an overall increased prevalence of adult-onset multi-factorial diseases in relation to parental radiation exposure among nearly 12,000 offspring of A-bomb survivors (Fujiwara et al., *Radiation Research* 2008; 170:451–7). Furthermore, analyses addressing individual diseases did not prove that parental radiation exposure increased risk of hypercholesterolemia, hypertension, diabetes, angina, myocardial infarction, or stroke among the offspring of A-bomb survivors.

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), Fujiwara S (CH), Akahoshi M (CN)

Thyroid cancer is one of the radiation-affected cancers 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, with such research ongoing. Furthermore, although there have been no reports examining effects of parental radiation exposure on thyroid cancer among the offspring, it is necessary to consider the possibility that future epidemiological research may suggest such effects. In recent years, genetic research into thyroid cancer has achieved significant progress mainly in terms of understanding *RET/PTC* rearrangements and *BRAF* mutations. However, mechanisms behind thyroid cancer development and radiation effects on such mechanisms 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 into effects of parental radiation exposure on thyroid cancers developing among the offspring of A-bomb survivors. Furthermore, it is believed that such thyroid cancer samples will be valuable for elucidation of molecular mechanisms behind 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 surgically removed. From among these cases, frozen fresh thyroid samples deriving from 36 cases are currently stored at RERF. The purpose of this RP is continued preservation of the relevant frozen fresh thyroid samples currently in custody of RERF in preparation for future molecular research.

Research Protocols 5-09, 4-09, 3-09, 4-04 and 5-04, 1-03, 4-02, 2-97, 1-93, 2-90, 7-87, 3-87

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), Fujiwara S (CH), Ozasa K (EH), Hirabayashi Y, Iwama A, Koyasu S, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng NP, Murasko D, Seed TM, Douple EB (ACR), Nakachi K

This study aims to delineate the long-term consequences of prior A-bomb irradiation and advancing age on homeostatic control of HSCs and DCs. Based on accumulating evidence for accelerated immunosenescence in A-bomb survivors, we initiated an international collaboration study on mechanisms of radiation-related immunosenescence, with support by funding from the U.S. National Institute of Allergy and Infectious Diseases (NIAID). In that study, we hypothesize that radiation exposure induces premature aging of HSCs, resulting in reduced numbers and impaired self-renewal ability, 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. Numerical and functional changes in relation to radiation dose will be analyzed within the circulating HSC and DC pools among several hundred individuals who are currently participating in the Hiroshima AHS. In order to confirm the results of the A-bomb survivor studies, we will develop a series of *in vitro* and *in vivo* assay systems to determine the functional and differentiation status of HSC and DC populations following ionizing irradiation.

We established assays for the numbers and functions of human peripheral blood HSC and DC populations at RERF and have begun studies of the AHS population in FY2011. At the collaborative research institutes outside RERF, experiments are being conducted to investigate the processes of hematopoietic and immune reconstitution following radiation-induced damage, using a number of mouse models. The mouse model experiments include studies on effects of radiation and aging on bone marrow stromal and thymic epithelial cells and investigations of radiation effects on *in vivo* human hematopoietic function using SCID-hu mice containing human blood cells.

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

Hayashi T (R), Kusunoki Y (R), Imai K (R), Yoshida K (R), Ito R (R), Ohishi W (CH), Fujiwara S (CH), Ozasa K (EH), Hirabayashi Y, Iwama A, Koyasu S, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng NP, Murasko D, Seed TM, Douple EB (ACR), Nakachi K

The RERF's epidemiology and clinical studies have long indicated increased risks of age-related and immune system/inflammation-related diseases among A-bomb survivors. Further, the noted radiation effects on the immune system are similar to those associated with aging. It is important to examine whether the radiation-impaired immune system modifies the response to vaccination. 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. We will recruit 50 AHS subjects for a pilot study and 300 AHS subjects for the full-scale study by stratified random sampling on dose, age, and gender. Collection of blood serum, plasma, and lymphocyte samples will be conducted immediately before and three weeks after the vaccination. The primary endpoint will be the change in anti-influenza virus antibody titer levels from before to three weeks after vaccination. Secondary endpoints to be analyzed include levels of cytokines and inflammation-related proteins, lymphocyte subsets, and intracellular activation markers (mRNA and protein). Those parameters will be analyzed in relation to age and dose of prior radiation exposure.

In a pilot vaccination effects study conducted in 2010, we collected blood samples immediately before and three weeks after the vaccination from 50 A-bomb survivors and 20 in-house volunteers and measured anti-influenza virus antibody titer levels using the hemagglutination inhibition test (HI test). The HI test results indicated that post-vaccination antibody titer levels against A/H1N1 and A/H3N2 antigens were significantly higher among most of the AHS subjects and young controls than their pre-vaccination levels. Since for the first cycle of the full-scale study in 2011 as many as 150 participants were expected, we called on the Hiroshima City Medical Association (HCMA) and other local medical associations (Asa District Medical Association, Aki District Medical Association, and Saeki District Medical Association) for cooperation in this study, with this cycle successfully completed on the basis of comprehensive cooperation with the aforementioned medical associations. As a result, we succeeded in obtaining cooperation from 157 AHS subjects who had a history of influenza vaccination within the previous five years and also planned to receive influenza vaccination from their attending physicians this coming season, 140 attending physicians, and 23 in-house RERF volunteers. Finally, we collected blood samples before and three weeks after the vaccination from 180 people consisting of these AHS subjects and in-house volunteers.

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

Hayashi T (R), Kusunoki Y (R), Imai K (R), Yoshida K (R), Ito R (R), Ohishi W (CH), Fujiwara S (CH), Ozasa K (EH), Furukawa K (S), Hirabayashi Y, Iwama A, Koyasu S, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng NP, Murasko D, Seed TM, Douple EB (ACR), Nakachi K

The immunology study unique to RERF constitutes repeated observations of various immunological parameters in A-bomb survivors with long-term follow-up, demonstrating significant radiation-related alterations in the immune system among survivors, found even today. The objective of this study is to develop an integrated scoring system for evaluating immunological and inflammatory status of individuals as a function of age and radiation dose. This

study consists of a cross-sectional analysis and a longitudinal analysis. The proposed cross-sectional analysis will include about 3,600 Hiroshima AHS subjects. Measurements will be made in plasma and blood of the immunological and inflammation-related markers. The proposed longitudinal analysis will include a random subset of 300 AHS subjects. Biomarkers will be measured on two sets of plasma samples collected from the 300 AHS subjects ten years apart, using a multiple assay system for the simultaneous quantitative determination of plasma cytokines. Telomere-length assays will also be conducted on DNA from the same 600 samples. The results will be utilized to construct an integrated scoring system that effectively will reflect overall immune-related health, and how that immune status differs across varying age and radiation-exposed groups.

We have thus far measured plasma levels of reactive oxygen species (ROS) in 1,520 Hiroshima AHS subjects, ROS (H_2O_2 and $O_2^{\cdot-}$) levels in blood cells from 1,600 people, plasma levels of 27 types of cytokine/chemokine-related biomarkers in 1,700 individuals, and lymphocyte subset frequencies in 2,300 people. A preliminary analysis indicated increased plasma ROS levels with increase in radiation dose. A preliminary analysis of intracellular ROS levels did not detect association between H_2O_2 levels in T cells and age and radiation dose but revealed age-dependent increase in intracellular $O_2^{\cdot-}$ levels for each of the T-cell subsets as well as radiation exposure-related increase in intracellular $O_2^{\cdot-}$ levels for CD3, CD4, CD8, and naïve CD8 T cells.

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), Morishita Y (R), Nagamura H (R), Maki M (R), Kusunoki Y (R), Yoshida K (R), Imai K (R), Cologne JB (S), Tahara E, Fujiwara S (CH), Akahoshi M (CN), Nakachi K

Epidemiological studies have clearly demonstrated long-lasting impacts of A-bomb radiation on human health, including dose-dependent increases in the incidence/mortality of inflammation-related cancers. Although enhanced inflammation has been consistently observed among A-bomb survivors, roles of inflammatory responses in radiation carcinogenesis are not understood. The purpose of this study is to assess whether genetic backgrounds of individuals affect their susceptibility to cancer, particularly as this may be a modifier of the radiation response. The study will focus on polymorphisms of genes encoding molecules that are possibly involved in immunological defenses against cancer development or in inflammatory responses that may modify cancer risk. A preliminary study showed that the risk of the intestinal-type gastric cancer was modulated primarily by *IL-10* haplotypes, while the risk of the diffuse-type gastric cancer was affected by both *IL-10* haplotypes and radiation exposure at a high radiation dose. We also found a synergistically increased risk of colon cancer for those with a certain *IL-18* genotype and high radiation exposures.

This fiscal year we have focused on NKG2D, a cell surface receptor expressed on NK and CD8 T cells, reviewing association between *NKG2D* haplotypes and persistent HCV infection as well as development of hepatocellular carcinoma (HCC). High risk of persistent HCV infection was previously observed among AHS subjects with certain *NKG2D* haplotypes. In this study we performed analysis of a combination of *NKG2D* haplotypes and high-dose radiation exposures, resulting in a finding of increased HCC risk among high-dose subjects with specific *NKG2D* haplotypes. The aforementioned results suggest that *NKG2D* haplotypes affect HCV clearance after HCV infection and course of persistent infection through their involvement as a genetic factor in individual differences in NK activity and cytotoxic T-lymphocyte functions, and also play a role in radiation-related HCC risk.

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

Hayashi T (R), Morishita Y (R), Nagamura H (R), Yoshida K (R), Kusunoki Y (R), Nakashima E (S), Tatsukawa Y (CH), Fujiwara S (CH), Akahoshi M (CN), Imai K (R), Nakachi K

Although early studies of A-bomb survivors did not show associations between radiation exposure and risk of diabetes, data on AHS subjects in 1992–1994 indicated a significant positive radiation-diabetes association in Hiroshima but not in Nagasaki (radiation dose-city interaction; $p < 0.001$), after adjusting for sex, 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 immune responses, and that the radiation-diabetes association is especially relevant to a subgroup of A-bomb survivors who have a specific *HLA* class II haplotype. That observation suggests that the effects of radiation on the development of diabetes may vary according to genetic background. The purpose of the study is to assess the effects of radiation and various genetic factors on risk of diabetes mellitus (DM) in terms of a case-control study within the AHS cohort.

We have identified *DQA1* and *DRB1* genotypes in 711 DM patients (483 and 228 in Hiroshima and Nagasaki, respectively) and 1,878 controls (966 and 912 in Hiroshima and Nagasaki, respectively), and newly examined *DQB1* genotype in 262 controls and *TP53Arg72Pro* gene polymorphism in 1,260 individuals (Hiroshima, 416 DM patients and 844 controls). Our preliminary results on *TP53Arg72Pro* gene polymorphism suggest that radiation exposure might significantly increase DM risk among *TP53Pro/Pro* gene polymorphism subjects.

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

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

We wish to examine the proposition that radiation exposure can seriously perturb one or more of the processes involved in T-cell homeostasis in humans. The type and extent of any substantial impairment (or in some situations, imbalance) of immunological defense systems may well be

an important element in excessive, and perhaps ongoing, cancer and non-cancer disease processes. Our basic strategy involves studying the blood leukocytes of about 1,000 A-bomb survivors who are current or recent participants in the AHS population. Those leukocyte evaluations will determine (1) numbers of T lymphocytes that contain T-cell receptor-rearrangement excision circles (TRECs; an indicator of production of new, naïve T cells), (2) average lengths of telomere repeats in T lymphocytes (an indicator of T-cell senescence), and (3) composition of lymphocyte subsets and other immunity-determining cell populations.

To date, we have found dose-dependent reductions in sizes of naïve T-cell populations, as well as increased percentages of memory T-cell populations, with radiation dose among study subjects. Preliminary analyses showed that the number of lymphocytes containing TRECs in the CD4 T-cell fraction tended to decrease with age as well as with radiation dose. We also observed a similar decreasing trend (i.e., age- or dose-associated decrease) in telomere lengths of CD8 T-cell populations among the survivors. In addition, we found a dose-dependent increase in the percentages of functionally weak memory subsets and regulatory T cells in the CD4 T-cell population of the survivors. Furthermore, the percentages of both T_H1 and T_H2 cells significantly increased with age and radiation dose in the CD4 T-cell populations of A-bomb survivors, and there was an inverse association between the percentage of T_H1 cells and temporal platelet count-change in individuals with hepatitis C virus persistent infection, suggesting the possibility that enhanced T_H1 cell-mediated immunity is involved in progression of viral hepatitis. Those results are consistent with the hypothesis that A-bomb radiation exposure may have perturbed T-cell homeostasis and thereby accelerated immunosenescence.

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

Hayashi T (R), Kusunoki Y (R), Yoshida K (R), Akahoshi M (CN), Fujiwara S (CH), Nakachi K

For studying late effects of radiation exposure in A-bomb survivors, analyses of gene alterations due to radiation are essential in investigations of genetic instability, genetic susceptibility, and molecular oncology. With new technologies, the investigations can be conducted using extremely small amounts of DNA. This RP was designed to supplement RP 2-90 by providing a means of storing DNA for multiple, small-scale molecular analyses that otherwise would be wasteful of stored DNA from stock sources (i.e., biospecimens from RP 2-90). Blood samples, destined for DNA extraction and subsequent molecular analyses, are lyophilized and stored for use in current and future studies.

Blood remaining after clinical examination is used in this study. As the Immunogenome Studies including SNP analyses have started with a requirement of large amounts of DNA, as many nucleated cells as possible are harvested and directly stored at –80°C. Since the erythrocyte sedimentation test conducted in the Department of Clinical Studies was terminated in April 2008, additional blood cells are available for storage. We have so far cryopreserved 18,272 blood samples from 3,446 AHS participants in Hiroshima and 11,811 from 2,538 AHS participants in Nagasaki. It was determined by PCR amplification that DNA extracted from laboratory control blood stored at –80°C for 14 years on paper was not significantly affected by storage. We have also collected 3,011 blood cell samples (2,017 in Hiroshima and 994 in Nagasaki) from the expanded group of young A-bomb survivors since December 2007.

RP 1-93 Repertoire of T-cell antigen receptors and activity of hematopoietic progenitor cells in peripheral blood of atomic-bomb survivors (Addendum to RPs 7-89 [terminated], 4-87 [terminated], and 3-87)

Hayashi T (R), Kusunoki Y (R), Ohishi W (CH), Fujiwara S (CH), Yoshida K (R), Imai K (R), Nakachi K

A follow-up of possible radiation-induced alterations in blood cell populations of the survivors has been carried out to further elucidate the late effects of A-bomb radiation on the lymphohematopoietic system and their relationship to selected diseases being observed in the A-bomb survivors. The purpose of this study is to examine radiation effects on the hematolymphoid system related to inflammation and genomic instability that might lead to the development of aging-associated diseases including cancer. We hypothesize that exposure to A-bomb radiation has induced significant abnormalities in T-cell function and changed the proportion of some T-cell subsets in A-bomb survivors, resulting in chronic inflammation that has been enhanced more than in non-exposed individuals.

This fiscal year, we measured intracellular ROS levels of 3,800 AHS subjects (2,300 AHS subjects and 1,500 expanded younger AHS subjects). We also measured serum biomarkers of lipid metabolism in 449 non-AHS control samples using the Bio-Plex Pro cytokine system. We found that cigarette smoking was involved in increased serum levels of plasminogen activator inhibitor-1 (PAI-1) and in decreased serum insulin levels.

RP 2-90 Cryopreservation of blood cells from Hiroshima and Nagasaki Adult Health Study participants (See also RP 2-97)

Hayashi T (R), Kusunoki Y (R), Yoshida K (R), Akahoshi M (CN), Fujiwara S (CH), Nakachi K

Since A-bomb survivors are now reaching advanced ages, we propose to cryopreserve live blood cells from all AHS participants to ensure a readily available source of materials for future studies. Lymphocytes of approximately 7,000 survivors have been preserved, and medical histories of these subjects have been recorded at the Department of Clinical Studies. About 1,500 of those subjects developed cancers before or after preservation of their lymphocytes. Biological materials from about 2,000 subjects have been used to assess the effects of A-bomb radiation on various endpoints, including a range of immune functions in addition to somatic mutations. Molecular epidemiological analysis of genetic susceptibility to cancer and other diseases is one of the most important research areas that we wish to pursue in the future. Blood samples collected from the same individuals at different times (e.g., approximately 10-year intervals) will permit the conduct of a unique longitudinal

study to assess age and joint age-by-radiation effects on various blood biomarkers. To facilitate such studies, we will collect as many blood samples as possible from current AHS participants and cryopreserve mononuclear cells isolated from these samples.

This fiscal year, we have cryopreserved blood cells from 2,049 AHS participants in total: 1,185 in Hiroshima including 508 young members from the newly expanded AHS group and 864 in Nagasaki including 285 from the expanded group. We confirmed that the viability of cryopreserved cells was more than 80% and that thawed lymphocytes expressed surface antigens and immunological functions as expected for live lymphocytes.

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 [terminated])

Hayashi T (R), Kusunoki Y (R), Yoshida K (R), Akahoshi M (CN), Fujiwara S (CH), Nakachi K

For this study, it was initially proposed to cryopreserve EBV-transformed B-cell lines from high-dose and control survivors for new cell biology studies, e.g., those dealing with radiosensitivity. However, it soon became obvious that the resulting B-cell lines would be of considerable use in a variety of other research fields, including and perhaps especially those involving alterations to immune functions as well as those in which the role of genetic background in disease development is being investigated. Cell lines from 807 Hiroshima AHS participants have been cryopreserved for future studies since this project began in 1987. Following a recommendation by the Multinational Peer Review Panel, we began to accelerate EBV transformation of lymphocytes from AHS participants in June 1998. The AHS subjects for this study are the high-dose (1 Gy or more) and control (less than 0.005 Gy) groups; these total roughly 3,500 in Hiroshima and Nagasaki. About 500 samples that overlap with the F_1 study have been immortalized and are being stored at the Department of Genetics. To date, the immortalization of lymphocytes from Hiroshima subjects is nearing completion. In the targeted subsets, 1,887 subjects in Hiroshima and 854 in Nagasaki have had lymphocytes immortalized. We began immortalizing lymphocytes from Nagasaki AHS participants in October 2000, and lymphocytes from 830 Nagasaki AHS participants have been successfully transformed. Following a recommendation by the Multinational Peer Review Panel, the Committee on Biological Samples requested that samples from each of the AHS participants be divided and stored both in Hiroshima and Nagasaki, with the aim of avoiding the loss of such valuable samples due to disasters and other problems. To this end, we have completed storage of at least four vials for each sample for a portion of the subject population. Since samples prepared from 921 Hiroshima participants and 207 Nagasaki participants totaled fewer than four vials, however, we are conducting the work of preparing at least four vials for each sample.

RP 3-87 Cellular immune function and its relationship to *in vitro* T-lymphocyte radiosensitivity and MN blood group locus mutation frequency in A-bomb survivors: Precursor frequency analysis of mitogen- and antigen-responsive blood lymphocytes **(See also RP 1-93)**

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

This study aims to evaluate T-cell functions and analyze their relationships to individual sensitivity among A-bomb survivors relative to radiation-induced genetic damage. We analyzed frequencies of T cells that can react to a given stimulus *in vitro* and measured percentages of various lymphocyte subsets in the peripheral blood. Radiation sensitivity was assessed among individuals, using *in vitro* radiosensitivity of T lymphocytes along with T-cell receptor (*TCR*), and glycophorin-A (*GPA*) mutation frequencies (Mf) *in vivo*. Our results indicate that A-bomb radiation has led to a decrease in CD4 T-cell fractions as well as a decrease in frequencies of the T cells that are capable of producing IL-2; conversely, B-cell percentages appear to have increased somewhat. We also showed that *TCR* mutation assay can be used as a sensitive indicator of recent radiation exposure but not as a radiosensitivity biomarker in A-bomb survivors. In contrast, erythrocyte *GPA* Mf increased with radiation dose in A-bomb survivors, and a follow-up study showed that the slope of the dose-response curve was significantly higher in the cancer group than in the cancer-free group among Hiroshima subjects, suggesting that the mutability of somatic genes of irradiated Hiroshima survivors might be associated with cancer susceptibility. On the basis of the assumption that this association in part involves possible difference(s) in genetic background, we initiated association analyses between genotypes of DNA repair genes and *GPA* Mf. Preliminary results suggest that there is an association between the dose-response curve of *GPA* Mf among survivors and p53-binding protein 1 (*P53BP1*) gene polymorphism, but not between the dose-response curve and *ATM* or *NBS1* gene polymorphisms. This RP was terminated in FY2011 and we will continue investigating relationships between polymorphisms of DNA repair-related genes and *in vitro* lymphocyte radiosensitivity or *in vivo* somatic mutation frequency among A-bomb survivors under RP 4-04 (immunogenome study).

Immunology Studies Publications

RERF Report (RR)

♦ Yoshida K, Ohishi W, Nakashima E, Fujiwara S, Akahoshi M, Kasagi F, Chayama K, Hakoda M, Kyoizumi S, Nakachi K, Hayashi T, Kusunoki Y: Lymphocyte subset characterization associated with persistent hepatitis C virus infection and subsequent progression of liver fibrosis. Human Immunology 2011 (October); 72(10):821–6. doi:10.1016/j.humimm.2011.05.029 http://www.sciencedirect.com/science/journal/01988859 **(RR 14-10)**

 (related to *Special Clinical Studies*)

[Abstract] This study aims to deepen the understanding of lymphocyte phenotypes related to the course of hepatitis C virus (HCV) infection and progression of liver fibrosis

in a cohort of atomic bomb survivors. The study subjects comprise 3 groups: 162 HCV persistently infected, 145 spontaneously cleared, and 3,511 uninfected individuals. We observed increased percentages of peripheral blood T_H1 and total CD8 T cells and decreased percentages of natural killer (NK) cells in the HCV persistence group compared with the other 2 groups after adjustment for age, gender, and radiation exposure dose. Subsequently, we determined that increased T_H1 cell percentages in the HCV persistence group were significantly associated with an accelerated time-course reduction in platelet counts—accelerated progression of liver fibrosis—whereas T_C1 and NK cell percentages were inversely associated with progression. This study suggests that T_H1 immunity is enhanced by persistent HCV infection and that percentages of peripheral T_H1, T_C1, and NK cells may help predict progression of liver fibrosis.

Manuscript in Press

⌘ Hayashi T, Morishita Y, Khattree R, Misumi M, Sasaki K, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Imai K, Kusunoki Y, Nakachi K: Evaluation of systemic markers of inflammation in atomic-bomb survivors with special reference to radiation and age effects. FASEB Journal.

Immunology Studies Oral Presentations

❖ Hayashi T, Imai K, Nakachi K. Molecular epidemiology of lifestyle-related diseases in relation to biological effects of cigarette smoking, using the data from a general population cohort study. FY2010 Research Meeting of Smoking Research Foundation, 22 July 2011, Tokyo

❖ Hayashi T, Kusunoki Y, Nakachi K. Radiation effects on immunogenetic susceptibility to cancer found in atomic-bomb survivors. 70th Annual Meeting of the Japanese Cancer Association, 3–5 October 2011, Nagoya

❖ Imai K, Nakachi K, Hayashi T, Kusunoki Y. The cancer risk-related *NKG2D* haplotypes are associated with the NKG2D protein expression on NK and CD8 T cells. 70th Annual Meeting of the Japanese Cancer Association, 3–5 October 2011, Nagoya

❖ Ohishi W, Yoshida K, Kusunoki Y, Nakashima E, Fujiwara S, Hayashi T, Nakachi K, Chayama K. Study of immunological characteristics associated with disease progression in chronic hepatitis C virus infection. 15th Annual Meeting of the Japan Society of Hepatology, 20–23 October 2011, Fukuoka (related to *Special Clinical Studies*)

❖ Shore RE, Ozasa K, Suyama A, Fujiwara S, Akahoshi M, Cullings HM, Kodama Y, Kodaira M, Hayashi T, Hamatani K. Recent findings by the Radiation Effects Research Foundation on the health of atomic bomb survivors and their offspring. International Symposium of Nagasaki University Global COE Program, 22 October 2011, Nagasaki (related to *Life Span Study*, *Adult Health Study*, *Cytogenetics Studies*, and *F_1 Studies*)

❖ Ohishi W, Yoshida K, Kusunoki Y, Nakashima E, Fujiwara S, Hayashi T, Nakachi K, Chayama K. Immunological features associated with disease progression in persistent hepatitis C virus infection. 62nd Annual Meeting of the American Association for the Study of Liver Diseases, 4–8 November 2011, San Francisco, California, USA (related to *Special Clinical Studies*)

❖ Kusunoki Y. Possible involvement of immunological alterations in radiation-associated non-cancer disease. 54th Annual Meeting of the Japan Radiation Research Society, 17–19 November 2011, Kobe

❖ Nakachi K. Radiation effects on immune system. Japan-Russia Open Symposium on Childhood Cancer, 18 November 2011, Chiba

Research Protocols 3-11, 3-10, 2-10, 6-08, 4-08, 3-05, 2-05, 1-05, 8-02, 5-00, 3-00, 2-99, 9-92, 5-92, 3-89, 4-85, A2-12, A1-12, A6-11, A4-11, A1-10, A5-09, A14-08, A13-08, A10-08, A8-08, A4-08

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), Akahoshi M (CN), Kasagi F, Abbott RD (S), Khattree R (S), Ohshita T, Miyachi T, Matsumoto M, Tsujino A, Mimori Y, Krull KR, Fujiwara S (CH)

Objectives: We will examine late-life neurocognitive function among the Adult Health Study (AHS) subjects who were exposed *in utero* or aged <12 years at the time of the atomic bombing of Hiroshima and Nagasaki. Objectives, are (1) to examine associations between radiation exposure and neurocognitive function as assessed by neuropsychological examination, (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 with advancing age and the occurrence of dementia.

Background: Reports from various studies of A-bomb radiation exposure and radiotherapy show that developing brains *in utero* and in infancy are susceptible to radiation's effects.

Study methods: Study participants will be those exposed *in utero* or aged ≤12 years at the time of the atomic bombings of Hiroshima and Nagasaki. In the period from 2011 through 2015, we plan to examine neurocognitive function in about 1,050 persons in Hiroshima and Nagasaki using the Cognitive Abilities Screening Instrument (CASI) and the Childhood Cancer Survivors Study (CCSS)-Neurocognitive Questionnaire (NCQ). CASI will be conducted by nurse interview and NCQ by mailing of self-recording questionnaire for participants to fill out.

Study progress: Assessment of neurocognitive function using CASI and NCQ has been initiated.

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 S, Minamoto A, Kiuchi Y, Kitaoka T, Nakashima E (S), Fujiwara S (CH), Akahoshi M (CN)

The ophthalmological study of A-bomb survivors conducted between 2000 and 2002 based on RP 3-00 showed statistically significant dose-response relationship for posterior subcapsular and cortical cataract. Furthermore, a lower dose threshold or no threshold is suggested. The objective of this addendum study is to further investigate the following unanswered questions of RP 3-00: (1) whether time-dependent progression is seen in radiation-induced cataract and (2) whether dose response relationship is observed when cataract cases are assessed using a radiation-specific classification method (Merriam-Focht method).

Study methods: (1) AHS participants who were 13 years old or younger at the time of atomic bombings will be studied. (2) Assessment will be done by ophthalmologists based on the Lens Opacities Classification System Version II (LOCS II) and Merriam-Focht method. (3) Various confounding factors will be taken into consideration at the time of analysis. (4) Digital images of the lens and retina will be stored.

In accordance with the above methods, the ophthalmological study started in Hiroshima and Nagasaki in August 2010. By March 2012, 295 subjects participated in the examinations by ophthalmologists in Hiroshima and 193 in Nagasaki.

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, Abbott RD (S), Itakura K, Kawasaki R, Nakashima E (S), Hsu WL (S), Yokoyama T, Takamatsu M, Kinoshita H, Tsuiki E, Uematsu M, Kumagami T, Kiuchi Y, Kitaoka T, Fujiwara S (CH), Hida A (CN), Akahoshi M (CN), Neriishi K

Purposes: We will investigate if retinal arteriolosclerosis is involved as an intermediate risk factor in radiation-associated glaucoma. We investigate the relationship between radiation and the prevalence of age-related macular degeneration using the same fundus images as well.

Background: The preliminary analysis of glaucoma study (RP 1-05) indicated that normal tension glaucoma prevalence is significantly associated with A-bomb radiation, and it is indicated that retinal arteriolosclerosis may associate with normal tension glaucoma as a causal factor. Therefore, we planned an evaluation of radiation effects on standardized retinal measurement values of stored retina images. This study would provide evidence regarding a possible mechanism for radiation-associated glaucoma.

Study methods: Subjects are 2,722 persons who underwent screening examinations, including retinal images, for glaucoma during 2006–2008, according to glaucoma study (RP 1-05). Among them, those with complete data including known radiation dose are subjects for a causal pathway analysis concerning glaucoma. The ophthalmologic markers such as retinal vessel diameter and age-related macular degeneration level will be measured and assessed by the graders who were trained at the Melbourne center to maintain adequate intra- and inter-rater agreement, using a semi-automated computer program. Those fundus images are also available to diagnose age-related macular degeneration as well. We furthermore investigate the association between radiation and age-related macular degeneration, which had been never reported yet.

Study progress: Assessment of retinal vessel diameter and age-related macular degeneration level was completed and analyses are currently being conducted.

Results: Not yet reported.

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

Ohishi W (CH), Tatsukawa Y (CH), Fujiwara S (CH), Hsu

WL (S), Kohata M, Yamada M (CH), Ozasa K (EH), Tsuge M, Chayama K

Objectives: To determine whether A-bomb radiation exposure increased liver stiffness, which is a marker of liver fibrosis severity, and to examine whether liver fibrosis is involved in, through insulin resistance, the development of arteriosclerotic diseases, on the basis of the hypothesis that radiation exposure leads to progression of liver fibrosis regardless of hepatitis virus infection status.

Background and significance: Liver fibrosis accelerates progression of chronic hepatitis B or C and non-alcoholic fatty liver disease to liver cirrhosis and/or hepatocellular carcinoma. RERF's data have shown that risks of chronic liver disease and liver cirrhosis are related to radiation dose. In addition, radiation effects have been observed in incidence of such arteriosclerotic diseases as hypertension and cardiovascular disease in the Life Span Study and Adult Health Study (AHS) cohorts.

Study methods: Possible association between liver elasticity and radiation dose will be investigated to test the hypothesis that radiation exposure is involved in the increased risks of chronic hepatitis and liver cirrhosis. Furthermore, we will elucidate mechanisms of radiation effects underlying these diseases by examining whether increased liver fibrosis is involved in the development of arteriosclerotic diseases through insulin resistance.

Study progress: During the period November 2008–November 2011, we measured liver stiffness with an elastometer for about 2,500 survivors, with the measurement now completed for younger survivors in the AHS cohort (less than 10 years of age at the time of atomic bombings). We also measured blood cytokine levels by ELISA or multiplex bead array assay for about 2,450 survivors.

Results and conclusions: None yet.

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), Fujiwara S (CH), Akahoshi M (CN), 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

The goal of this project is to confirm the adequacy of a storage method for cataract tissues of AHS participants who undergo a cataract operation, and to collect and store the tissue for future analyses. Our recent study has indicated that the odds ratio at 1 Gy of prevalence of those who underwent cataract operation was 1.39 (95% confidence interval: 1.24, 1.55). AHS participants who were young at the time of the bombings are expected to reach the age of cataract operation within the next decades. The stored cataract tissues are expected to contribute significantly to future research on radiation-induced cataract. When enough numbers of tissues are collected, a new research protocol for biological studies will be prepared.

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 also conducted. Actual program of collection and storage of lens tissue started in December 2008 in Hiroshima and in November 2009 in Nagasaki. A total of 54 samples in Hiroshima and 17 samples in Nagasaki have been collected and stored as of March 2012. We performed histological staining of collected anterior lens capsule samples and extraction of RNA/DNA from phacoemulsified lens liquid samples on a trial basis, reviewing the availability of such materials for future research studies.

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

Tatsukawa Y (CH), Hsu WL (S), Neriishi K, Nakashima E (S), Little MP, Nishi N, Soda M (EN), Yamada M (CH), Fujiwara S (CH), Cologne JB (S), Akahoshi M (CN)

Background: Experimental and epidemiological studies report a relationship between inflammation and cancer. Studies of A-bomb survivors also report radiation dose-dependent increases of inflammatory biomarkers.

Objectives: To investigate the relationship between inflammatory biomarkers and cancer incidence and effects of radiation on cancer development via inflammation among 12,870 Adult Health Study (AHS) participants followed from 1965 to 1999.

Methods: Various inflammatory biomarkers have been measured in the AHS. Because associations among radiation, inflammatory biomarkers, and cancer are complex, various statistical methods will be used to assess these associations.

Results and study progress: Analysis of longitudinal trends in white blood cell (WBC) counts showed a significant increase of leukocyte counts among males exposed to A-bomb radiation before the age of 20 and among females regardless of age at the time of bombing in the heavily exposed group (>2 Gy). The results were published in *Journal of Radiation Research* (Hsu et al., 2010; 51:431–9). Subsequently, a joint model to estimate causal associations among radiation, inflammation, and solid cancer incidence based on WBC counts as inflammatory indices was specified and analysis conducted. The results showed that there is a significant causal association between radiation and long-term WBC count as well as solid cancer incidence. The proportion of the mediating effect from inflammation is about 7% of the total radiation effect on cancer. A manuscript on these results has been submitted to an international journal.

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

Fujiwara S (CH), Ohishi W (CH), Akahoshi M (CN), Cologne JB (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, and to determine if these factors also increased the risk of chronic inflammation and myocardial infarction.

Background and significance: The Life Span Study and the Adult Health Study (AHS) cohort members were selected from among survivors alive in 1950; it has since

been reported that a "healthy survivor effect" was suspected for noncancer mortality among survivors. The hypothesis is that genetic polymorphisms controlling responsiveness to external stresses can alter survival rates following acute radiation exposures but also increase myocardial infarction in later life.

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 will be conducted. We will analyze and compare single nucleotide polymorphisms (SNP) in *LTA* and *TLR2* genes between the cases and controls.

Study progress: The genotyping (*LTA* and *TRL2* genes) was completed, and a preliminary analysis was conducted for possible association between *LTA* and *TLR2* genotype frequencies and A-bomb radiation. Construction of datasets including clinical information was initiated.

Results and conclusions: None yet.

RP 1-05 Glaucoma study in atomic bomb survivors

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

Objectives: To investigate possible association between radiation dose and glaucoma prevalence.

Background and significance: It is well known clinically that acute, high radiation exposure can induce glaucoma. However, the relationship between glaucoma and radiation exposure within the general population and the Adult Health Study (AHS) cohort with moderate doses is unclear.

Study methods: A comprehensive study of glaucoma involving examination of intraocular pressure, retinal image, and visual field was conducted during 2006–2008, with more detailed examinations administered to potential glaucoma cases.

Study progress: Among 1,589 subjects with known radiation dose (mean age: 74.3), we detected 284 (17.9%) cases of all glaucoma types, most of which were normal tension glaucoma. Various aspects, including possible non-participation bias, were analyzed as proposed by the glaucoma study group. A manuscript is very close to completion.

Results and conclusions: A preliminary analysis using the generalized estimating equation method, with adjustment for sex, age, city, cataract surgery, and diabetes mellitus, revealed increased odds ratios in normal tension glaucoma cases. However, this finding should be interpreted with caution, taking into account the low participation rate.

RP 8-02 Ophthalmologic study of children of atomic bomb survivors (Addendum to RP 1-02)

Minamoto A, Yokoyama T, Mishima HK, Kitaoka T, Nakashima E (S), Hida A (CN), Tatsukawa Y (CH), Neriishi K, Fujiwara S (CH), Akahoshi M (CN)

Background: Cataract is a multifactorial disease that stems from a combination of environmental and genetic factors. Animal experiments have suggested the possibility of an increase in the incidence of congenital cataracts in relation to radiation exposure. Therefore, it is important to quantitatively examine lens opacity and retinal arteriolosclerosis in the offspring of A-bomb survivors.

Objectives: To investigate on a quantitative basis the opacity of eye lenses and retinal arteriolosclerosis of the offspring of A-bomb survivors, and to look into the relationship of these values with several risk factors.

Methods: Lens opacity and retinal arteriolosclerosis will be quantified using LOCS-II and/or Scheie classification systems. Data will be analyzed by regression analysis, taking into consideration various risk factors.

Study progress: In this study, a questionnaire survey related to history of ophthalmologic disease was conducted for about 10,000 offspring of A-bomb survivors. In addition, ophthalmologic examination was performed for 2,517 offspring. Data from the questionnaire are being cleaned. Further analyses will be conducted.

Results: The preliminary results indicate that there was no association between parental radiation exposure and any types of cataract in the offspring.

RP 5-00 The prevalence, incidence, and prognosis of the Brugada type electrocardiogram: A population-based study of four decades

Haruta D, Matsuo K, Akahoshi M (CN), Nakashima E (S), Suyama A (EN), Seto S

The objectives of this study are to clarify the incidence and prognosis of the Brugada type electrocardiogram (ECG) and examine the relationship between Brugada type ECG and sex hormones.

The incidence and prognosis of the Brugada syndrome, a new clinical entity causing sudden death due to ventricular fibrillation (VF), has not been fully elucidated. Although Brugada syndrome, which is linked to mutations of the *SCN5A* gene, is inherited with equal frequency by men and women, most of the reported cases have been adult men.

We identify the Brugada type ECG cases by reviewing all the ECG recordings from 1958 to 1999 in 4,788 Nagasaki AHS subjects who were under age 50 in 1958 to calculate the incidence. We ascertain the sudden death cases from all deceased subjects to evaluate the prognosis of Brugada type ECG cases. We also evaluate the association between Brugada type ECG and prostatic cancer, which is related to testosterone.

We found an association between the Brugada type ECG and prostatic cancer. In two surgical castration and one hormonal castration cases, the Brugada type ECG disappeared after castration; leading to a new therapeutic strategy to prevent sudden death.

The incidence of the Brugada type ECG was 31.4 persons/100,000 person-years in males, nine times higher than in females. Brugada type ECG cases had a higher risk of sudden death (RR = 52, 95% CI: 23–128) than controls. Brugada type ECG cases had a higher risk for prostate cancer (RR = 5, 95% CI: 2–15).

RP 3-00 Ophthalmologic study of atomic bomb survivors

Minamoto A, Hida A (CN), Tatsukawa Y (CH), Yokoyama T, Neriishi K, Mishima HK, Kitaoka T, Nakashima E (S), Fujiwara S (CH), Akahoshi M (CN)

The goal of this study is to evaluate the prevalence of radiation cataracts within two study subject groups, namely

(1) a select group of survivors who were relatively young (13 years old or younger) at the time of the atomic bombing, but had not been previously given ophthalmic examinations; and (2) a larger group that had been evaluated previously, but by older methods. For both groups, dose-response analyses were conducted for posterior subcapsular axial opacities and polychromatic changes and for peripheral opacities using a standard grading system, while adjusting for a variety of potential confounding factors. Further, all digital computer images of radiation cataracts were stored for future assessments. A total of 883 persons underwent ophthalmologic examinations in Hiroshima and Nagasaki, from which four papers were published (Minamoto et al., *International Journal of Radiation Biology* 2004; 80:339–45, Nakashima et al., *Health Physics* 2006; 90[2]:154–60, Nakashima et al., *Annals of the Institute of Statistical Mathematics* 2008; 60[3]:465–82, and Minamoto et al., *Journal of Photochemistry and Photobiology B: Biology* 2011; 103[2]:105–10). An analysis of the prevalence of severe cataract cases with surgical lens removal was also published (Neriishi et al., *Radiation Research* 2007; 168:404–8).

Stored lens images collected during 2000–2002 were used to conduct a re-evaluation with the Merriam-Focht cataract scoring method, a radiation-specific classification system used in numerous studies, such as among Chernobyl clean-up workers. The preliminary results of an opacity re-evaluation with the Merriam-Focht method indicate that the dose responses for the two major research cohorts, A-bomb survivors and Chernobyl clean-up workers, are almost identical.

The results regarding cataract surgery incidence from 1986 to 2005 showed a low radiation dose-effect threshold for cataracts, and a related paper has been submitted to *Radiology*.

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

Imaizumi M (CN), Usa T, Tominaga T, Akahoshi M (CN), Soda M (EN), Neriishi K, Fujiwara S (CH), Yamada M (CH), Kodama K (CS), Nakashima E (S), Shibata Y, Okubo M, Ashizawa K, Sera N (CN), Eguchi K

The objectives of the current thyroid disease study in Hiroshima and Nagasaki AHS cohort are to investigate whether there are positive associations between radiation dose and thyroid nodules, autoimmune thyroid diseases, and thyroid dysfunction, and further, to examine whether thyroid cancers frequently develop among subjects with thyroid nodules detected in an earlier study (1984–87).

We found that malignant thyroid tumor, benign nodules, and cysts were increased with radiation dose and the relationships were more significant in those exposed at younger ages. On the other hand, autoimmune hypothyroidism and Graves' disease were not associated with radiation dose (Imaizumi et al., *JAMA* 2006; 295[9]:1011–22). No significant dose-responses for thyroid diseases were observed among those exposed *in utero*, although the risk estimates were similar to those with juvenile exposure and the null results may reflect limited statistical power (Imaizumi et al., *Journal of Clinical Endocrinology and Metabolism* 2008; 93:1641–8). We more frequently detected cancer in subjects with solid thyroid nodules than in nodule-free controls (Imaizumi et al., *Journal of Clinical Endocrinology and Metabolism* 2005; 90:5009–14), suggesting that a thyroid nodule is a risk factor for subsequent thyroid cancer.

Based on RP 3-07, we are now conducting thyroid examinations in the newly expanded cohort of AHS subjects to study the effects of low dose radiation on thyroid diseases exposed at younger age, and the results of the examinations will be analyzed in the future.

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), Fujiwara S (CH), Cologne JB (S), Cullings HM (S), Nakashima E (S), Yoshida K (R), Kusunoki Y (R), Hayashi T (R), Akahoshi M (CN), Chayama K

Objectives: The hypothesis behind this study is that radiation may increase the incidence of hepatocellular carcinoma either by increasing the rates of hepatitis B virus (HBV) or hepatitis C virus (HCV) infection or by facilitating clinical progression after hepatitis virus infection. The purposes of this study are to investigate the associations of radiation dose with natural history of HBV or HCV infection in the Adult Health Study (AHS) cohort.

Background and significance: Our previous studies demonstrated that the prevalence of hepatitis B surface antigen (HBsAg) increased with radiation dose among the AHS population. The percentage of subjects unable to clear the virus increased significantly with radiation dose among those who had received blood transfusions. No association was found between radiation dose and prevalence of anti-HCV positivity, but 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) association between radiation dose and HBV activity by examining hepatitis B e-antigen (HBeAg), HBV DNA, and HBeAg and HBsAg seroconversion rates; (2) association between radiation dose and natural history of chronic hepatitis B and C; and (3) clinical pathological characteristics, immunogenetic background, and effects of radiation dose with regard to progression following hepatitis virus infection in the AHS cohort.

Study progress: A paper regarding lymphocyte subset characteristics relating to persistent infection and clinical progression following HCV infection was published. We have nearly completed longitudinal analysis of HCC incidence based on radiation-related HBV/HCV infection status in the AHS population.

Results and conclusions: Increased percentage of Th1 cells and decreased percentages of Tc1 cells and NK cells were significantly associated with progression of liver fibrosis (Yoshida et al., *Human Immunology* 2011; 72:821–6).

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

Yamada M (CH), Fujiwara S (CH), Mimori Y, Sasaki H, Akahoshi M (CN), Matsumoto M, Kasagi F, White LR

Objectives: In this study, we examined the effects of radiation exposure on cognitive function, the prevalence and incidence of dementia, and other age-related physiologic

variables such as reaction time at older ages among adult survivors in the AHS.

Background: This study investigates the hypothesis that the effects of ionizing radiation on the mature central nervous system may 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). Dementia prevalence was observed, and 2,286 dementia-free subjects were followed up to assess dementia incidence.

Study progress: We initiated assessment of cognitive function and other psychoneurological functions among those exposed to radiation between ages 0–12 and among *in utero* survivors. We developed a database of CASI screening conducted on a longitudinal basis.

Results: No association was found between previous radiation exposure and cognitive impairment and/or development of dementia among subjects exposed at ≥13 years of age. A new research on radiation exposure in children and *in utero* survivors and late-life neurocognitive function has begun.

RP 3-89 Osteoporosis in Hiroshima atomic-bomb survivors

Fujiwara S (CH), 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, even after adjusting for age, weight, and age at menopause. We are utilizing accumulated data on BMD and fracture for national and international collaborative studies.

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.

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 (Fujiwara et al., *Osteoporosis International* 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 4-85 Incidence and risk factors of coronary heart disease (CHD) in Japanese men living in Japan and Hawaii, 1966–78 (Addendum to Research Plan TR 12-71)

Yamada M (CH), Kodama K (CS), Tatsukawa Y (CH), Shimizu Y (EH), Sasaki H, Takahashi I (CH), Fujiwara S (CH), Curb JD, Rodriguez B, Yano K

Objectives: The objective of the study is to investigate the relationship between risk factors and the incidence of cardiovascular disease (CVD) among Japanese men living in Japan and in the United States (The NI-HON-SAN project). The epidemiologic methodology developed by this project has been applied for the entire AHS cohort.

Background: The epidemiologic methodology developed by this project has indicated a weak, but very consistent association between radiation dose and various endpoints of atherosclerosis, including myocardial infarction, thromboembolic stroke, hemorrhagic stroke, calcification of the aortic arch, retinal arteriosclerosis, isolated systolic hypertension, and abnormal pulse wave velocity.

Study methods: The cases of CHD (mainly acute myocardial infarction) and cerebrovascular disease are being ascertained through periodic examination, mortality surveillance, autopsy, etc. In order to obtain more detailed information, particularly on acute coronary events, a mail survey for morbidity surveillance has been conducted every six months in the AHS cohort since 1995. Data regarding atherosclerotic endpoints and risk factors have been collected.

Study progress: In 2010, measurements of central blood pressure, augmentation index (AI), brachial-ankle pulse wave velocity (baPWV), and ankle brachial index (ABI) were initiated and research to examine cytokines related to arteriosclerosis was begun.

Results: This study is helping the cardiovascular disease working group develop and confirm hypotheses regarding low-dose radiation effects on cardiovascular disease.

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), Suyama A (EN), Ozasa K (EH), Cologne JB (S), Araki Y, Hsu WL (S), Cullings HM (S), Fujiwara S (CH), Akahoshi M (CN)

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 pattern in terms of the frequency and magnitude of weight change. In this study, we first calculate a classical measure of weight fluctuation and, to address the above issue, 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 statistical methods compared with those used in previous studies.

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

Makita N, Akahoshi M (CN), Haruta D, Maemura K, Fujiwara S (CH), Nakashima E (S)

Progressive cardiac conduction defect (PCCD) is a hereditary lethal arrhythmia characterized by electrocardiographic findings of atrioventricular block and bundle branch block caused by progressive degenerative fibrosis of 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.

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

Makita N, Akahoshi M (CN), Haruta D, Maemura K, Fujiwara S (CH)

Congenital short QT syndrome 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 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 Radiation Effects Research Foundation (RERF) Adult Health Study (AHS).

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

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

It has been reported that atomic bomb radiation exposure increases the risk of cardiovascular disease (CVD). In addition, atomic bomb radiation is related to fatty liver, hypertension, abnormal lipid profiles, impaired glucose tolerance, and inflammation. The entire aforementioned are closely 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. We examined 1,366 Nagasaki Adult Health Study (AHS) subjects (521 male, 845 female) 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 mellitus (DM), hyperlipidemia, metabolic syndrome, angina pectoris, myocardial infarction, and stroke based on universally accepted diagnostic criteria after taking consideration of medication history. Using this data set, we plan to investigate the causal pathways and associations among atomic bomb radiation, visceral fat accumulation and its sequels (fatty liver, hypertension, hyperlipidemia, type 2 DM), and CVD. This will provide a better understanding of the mechanism(s) and the effects of atomic bomb radiation on CVD.

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

Purposes: This protocol aims to investigate an association between polymorphisms of *ATM* and other genes and the dose-dependent prevalence of cataract in A-bomb survivors.

Backgrounds: An association between ataxia telangiectasia mutated (*ATM*) gene and lens radiosensitivity has been well documented in experimental animals. Worgul et al. reported that atm heterozygous (+/–) mice are more sensitive to radiation-induced cataracts than are their wild-type counterparts. Kleiman et al. reported that Mrad9 and atm haploinsufficiency enhance spontaneous and X-ray-induced cataractogenesis in mice. A-bomb survivors demonstrated a significant dose-response relationship in prevalence of cataract with A-bomb radiation, and genotyping data on *ATM* and other genes are available from another study at RERF (RP 4-04).

Study methods: Subjects are 5,126 AHS participants who underwent medical examination during 2000–2001. Among them, those who have an information of polymorphism of *ATM* and other genes in the study of RP 4-04 and who agreed to use the information for the study were selected. In 5,126 AHS participants, there were 645 persons with cataract surgery. The dataset of polymorphisms of *ATM* and other genes will be obtained from the Department of Radiobiology/Molecular Epidemiology and merged with the cataract surgery data. The prevalence of cataract will be compared by radiation dose and polymorphisms of *ATM* and other genes. Two types of analysis are envisioned. One would be based on individual candidate SNPs and the other on haplotypes.

Study progress: Analyses are under way.

Results: Not yet reported.

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

Kakuma T, Araki Y, Hsu WL (S), Nakashima E (S), Neriishi K

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, i.e., a joint model in which the associations among radiation, inflammation, and incidence of cataract surgery are simultaneously modeled and estimated in the time-to-event analysis for the A-bomb survivors. The RP was approved in fall 2009. Dr. Hsu analyzed the data using Mplus, a specialized statistical package for latent variable models. The preliminary results from Mplus indicated significant causal association of radiation, inflammation, and cataract surgery. The proportion of indirect effect, i.e., radiation effect on cataract mediated by inflammation, was about 8%. Further analysis is in progress. Dr. Nakashima also started the model fitting using Mplus but used different model specifications. Drs. Kakuma and Araki at Kurume University are working on deriving the theoretical framework under the same causal hypothesis and plan to analyze the model in STATA.

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

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

The early repolarization pattern (ERP) has been considered to be benign, but one recent high-profile clinical study reported its potential arrhythmogenicity, suggesting the possibility that ERP is a cause of idiopathic ventricular fibrillation leading to sudden death.

Of 7,564 subjects (3,374 men and 4,190 women) followed biennially in Nagasaki since 1958, we will investigate all ECG records of 5,976 subjects who have been examined at least once in Nagasaki between 1958 and 2004. We found 650 prevalent ERP cases and 779 incident ERP cases. The incidence of ERP peaked during the fourth decade of life. ERP appeared intermittently on inferior and/or lateral leads. We found 27 and 42 sudden death cases in 1,429 ERP cases and 4,507 control subjects, respectively. The rates of sudden death in ERP cases and control subjects are 1.89% and 0.93%, leading to approximately two times higher rate of sudden death in ERP cases compared to that in control subjects. In this study, we found 7 sudden death cases (17.5%) among 40 Brugada type ECG cases. While the rate of sudden death in ERP cases is approximately one-tenth of that in Brugada type ECG cases, ERP is an important issue of public health, because approximately 32% of subjects exhibit ERP and 36% of sudden death are related to ERP. A related paper was published in *Circulation* 2011; 123:2931–77.

RP-A13-08 Prognostic significance of ventricular premature contractions (VPCs) in taking consideration of their origins

Haruta D, Nakashima E (S), Fujiwara S (CH), Akahoshi M (CN)

Ventricular premature contractions (VPCs) are common arrythmias in patients with and without structural heart diseases, and the presence of VPC in regular 12-lead ECG recordings is a significant and independent predictor for cardiovascular mortality.

We will extract VPC cases using the ECG database among AHS subjects (4,092 in Hiroshima and 2,642 in Nagasaki) who underwent a regular 12-lead ECG recording in Nagasaki and Hiroshima from January 1990 to December 1993 and classify VPC into three groups according to the morphology of VPC in the precordial lead; (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. Information of deceased cases and cause of death until December 2005 will be used to assess the prognostic significance for cardiovascular mortality between cases with and without VPC. We will characterize the certainty of the VPC diagnosis by whether it was observed at only one or more than one clinic visit. We will conduct Cox proportional hazards analyses to assess the prognostic significance of VPC, frequency of VPC diagnosis, and morphology of VPC for cardiovascular mortality after adjusting for age, sex, and underlying diseases.

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)

This is an international collaborative study involving 11 cohorts in Europe, U.S.A., Australia, Brazil, and Japan (Nagasaki AHS). The objective of this study is to assess the relationship between subclinical thyroid dysfunction and coronary heart disease and mortality.

No constant results have been obtained from prospective cohort studies regarding the association between subclinical hypothyroidism and cardiovascular outcomes. These conflicting results might reflect differences in participants' age, gender, thyroid-stimulating hormone (TSH) levels or preexisting cardiovascular disease.

We performed an individual participant data analysis of 55,287 participants (542,494 person-years) in 11 prospective cohort studies. We examined the risk of coronary heart disease (CHD) events and CHD mortality in all cohorts. With subclinical hypothyroidism, CHD risks did not significantly differ by age, gender, or preexisting cardiovascular disease. At the same time, subclinical hypothyroidism is associated with an increased risk of CHD events and CHD mortality in those with higher TSH levels, especially those with TSH ≥10 mIU/L (Rodondi et al., *JAMA* 2010; 304[12]:1365–74). Association between subclinical hypothyroidism and cardiovascular disease risk factors will be reviewed in the future.

RP-A8-08 Incidence and risk factors of fatty liver

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

Since fatty liver predicts ischemic heart disease, the incidence and predictors of fatty liver need examination. This study aims to determine fatty liver incidence and predictive variables.

Using abdominal ultrasonography, we followed biennially during 1990–2007 (mean follow-up, 11.6 ± 4.6 years) 1,635 Nagasaki atomic-bomb survivors (606 men) without fatty liver at baseline. We examined potential predictive variables with the Cox proportional hazards model and longitudinal trends with the Wilcoxon rank-sum test.

323 (124 males) new fatty liver cases were diagnosed. The incidence was 19.9/1,000 person-years (22.3 for men, 18.6 for women) and peaked in the sixth decade of life.

After controlling for age, sex, smoking, and drinking habits, obesity, low HDL-cholesterol, hypertriglyceridemia, glucose intolerance, and hypertension were predictive of fatty liver. In the multivariate analysis including all variables, obesity, hypertriglyceridemia, and hypertension remained predictive. In fatty liver cases, body mass index and serum triglycerides, but not systolic or diastolic blood pressure, increased significantly and steadily up to the time of the diagnosis. Radiation dose was not associated with fatty liver (RR = 0.92, 95% CI: 0.8–1.1). In summary, obesity, hypertriglyceridemia, and, to a lesser extent, hypertension might serve as predictive variables for fatty liver.

RP-A4-08 Study on the estimability of waist circumference and its application to risk analysis for metabolic syndrome

Nakamura T, Ichimaru S (CN), Ishida N, Soda M (EN), Akahoshi M (CN), Cullings HM (S), Nakashima E (S), Misumi M (S)

Metabolic Syndrome (MS) is a combination of medical risk factors for developing cardiovascular disease and diabetes. World-wide, many different sets of criteria have been proposed for the diagnosis of this syndrome. A common feature of these criteria is that they all utilize abdominal fat accumulation, as estimated by the waist circumference (WC) measured at the level of the umbilicus, as one criterion for the diagnosis of the syndrome. The objective of this research is to examine the projection of estimates of WC obtained from recently measured WC on RERF AHS subjects who came to the clinic in 2005–2006 to AHS subjects who had MS related deaths prior to 2005 when no WC measures were taken. Using the 2005–2006 data, we will obtain theoretically unbiased estimates of WC and their measurement error, for each subject ten years prior to 2005 (when WC was not measured) through an extrapolation procedure that incorporates an analysis of other correlated covariates from the accumulated health examination data at RERF. Then, a proportional hazards model adjusted for the measurement error will be developed. Finally, we will describe how to design a retrospective cohort study for MS using RERF data. Dr. Ishida completed an analysis for her Ph.D. thesis and wrote a paper about the risk of the MS-related causes of death that was published in the *Japanese Journal of Biometrics*. The results of risk estimation for death indicate that, for those of Semi-MS (those with at least two of dyslipidemia, hypertension, and impaired glucose tolerance), the larger the WC, the smaller the risk of MS-related death, contrary to expectations. Dr. Ichimaru of Nagasaki RERF prepared a manuscript in which deaths occurring within five years after study inception were omitted in order to minimize the effect of latent disorders, which resulted in WC's no longer being associated with MS-related deaths. This RP was terminated in June 2012 at the end of its term.

Special Clinical Studies Publications

RERF Reports (RR)

♦ Fujiwara S, Hamaya E, Goto W, Masunari N, Furukawa K, Fukunaga M, Nakamura T, Miyauchi A, Chen P: Vertebral fracture status and the World Health Organization risk factors for predicting osteoporotic fracture risk in Japan. Bone 2011 (June); 49(3):520–5. doi:10.1016/j.bone.2011.05.021 http://www.sciencedirect.com/science/article/pii/S8756328211010106 **(RR 4-10)**

[Abstract] ***Introduction:*** Vertebral fractures are the most common osteoporotic fracture and the prevalence of vertebral fracture is commonly assessed in clinical practice in Japan. The objective of this study was to evaluate potential risk factors for osteoporotic fractures, including morphometric spine fracture status and the WHO risk factors for predicting 4-year fracture risk. ***Methods:*** A population-based community cohort, the Adult Health Study, consisting of 2,613 men and women with mean age of 65 enrolled in Hiroshima was followed prospectively for 4 years. The prevalence and incidence of spine fractures were identified from lateral and posterior–anterior spine radiographs using a semiquantitative method. Information on incident nonvertebral fragility fractures (hip, proximal humeral, and forearm) was collected at interviews by trained nurses and physicians during biennial health examinations. ***Results:*** A model, including spine fracture status in addition to the WHO risk factors, appeared to provide greater prognostic information regarding future fracture risk (gradient of risk/standard deviation: GR/SD = 2.73) than a model with the WHO risk factors alone (GR/SD = 2.54). In univariate analyses, age, bone mineral density (BMD), prior clinical fracture, and spine fracture status had the highest gradient of risk. The presence of multiple prevalent spine or non-spine fractures significantly increased fracture risk, but, their contributions to the gradient of risk were similar to those when fracture status was categorized as a binary variable. A model considering those four risk factors yielded GR/SD = 2.67, indicating that it could capture most of the predictive information provided by the model with spine fracture status plus the WHO risk factors. ***Conclusions:*** The use of age, BMD, prior clinical fracture and spine fracture predicted future fracture risk with greater simplicity and higher prognostic accuracy than consideration of the risk factors included in the WHO tool.

♦ Haruta D, Matsuo K, Tsuneto A, Ichimaru S, Hida A, Sera N, Imaizumi M, Nakashima E, Maemura K, Akahoshi M: Incidence and prognostic value of early repolarization pattern in the 12-lead electrocardiogram. Circulation 2011 (June); 123(25):2931–7. **(RR 8-10)**

[Abstract] ***Background:*** Early repolarization pattern is a common ECG finding characterized by J-point elevation and QRS notching or slurring in the inferior and/or lateral leads, yet little is known about its incidence and long-term prognosis in Asian populations. ***Methods and Results:*** We reviewed all the ECG records of the 5,976 atomic-bomb survivors who were examined at least once during our biennial health examination in Nagasaki, Japan, between July 1958 and December 2004. We defined early repolarization pattern as ≥0.1-mV elevation of the J point or ST segment, with notching or slurring in at least 2 inferior and/or lateral leads. We assessed unexpected, cardiac, and all-cause death risk by Cox analysis. We identified 1,429 early repolarization pattern cases (779

incident cases) during follow-up, yielding a positive rate of 23.9% and an incidence rate of 715 per 100,000 person-years. Early repolarization pattern had an elevated risk of unexpected death (hazard ratio, 1.83; 95% confidence interval, 1.12 to 2.97; $P = 0.02$) and a decreased risk of cardiac (hazard ratio, 0.75; 95% confidence interval, 0.60 to 0.93; $P < 0.01$) and all-cause (hazard ratio, 0.85; 95% confidence interval, 0.78 to 0.93; $P < 0.01$) death. In addition, both slurring and notching were related to higher risk of unexpected death (hazard ratio, 2.09; 95% confidence interval, 1.06 to 4.12; $P = 0.03$), as was early repolarization pattern manifestation in both inferior and lateral leads (hazard ratio, 2.50; 95% confidence interval, 1.29 to 4.83; $P < 0.01$). ***Conclusions:*** Early repolarization pattern is associated with an elevated risk of unexpected death and a decreased risk of cardiac and all-cause death. Specific early repolarization pattern morphologies and location are associated with an adverse prognosis.

♦ Imaizumi M, Sera N, Ueki I, Horie I, Ando T, Usa T, Ichimaru S, Nakashima E, Hida A, Soda M, Tominaga T, Ashizawa K, Maeda R, Nagataki S, Akahoshi M: Risk for progression to overt hypothyroidism in an elderly Japanese population with subclinical hypothyroidism. Thyroid 2011 (November); 21(11):1177–82. **(RR 10-10)**

[Abstract] ***Background:*** Few population-based studies report the changes with time in thyroid function tests in patients with subclinical hypothyroidism. We compared the risk for developing overt hypothyroidism in patients with subclinical hypothyroidism and euthyroid controls from the same population of elderly Japanese. We also sought associations of selected parameters with the development of overt hypothyroidism in the subclinical hypothyroid and euthyroid groups. ***Methods:*** We measured thyrotropin (TSH) and free thyroxine (T4) levels at baseline examinations performed from 2000 to 2003 in the cohort of Japanese atomic-bomb survivors and identified 71 patients with spontaneous subclinical hypothyroidism (normal free T4 and TSH >4.5 mIU/L without a history of thyroid treatment, mean age 70 year) and 562 euthyroid controls. We re-examined TSH and free T4 levels an average of 4.2 years later (range, 1.9–6.9). ***Results:*** The risk for progression to overt hypothyroidism was significantly increased in subclinical hypothyroid patients (7.0%) compared with control subjects (1.6%) after adjusting for age and sex (odds ratio, 4.56; $p = 0.009$). Higher baseline TSH levels were associated with progression from subclinical to overt hypothyroidism ($p = 0.02$) in the multivariate analysis, including age, sex, antithyroid peroxidase antibody, and ultrasonography (US) findings. The analysis using binary TSH data suggested that a TSH level >8 mIU/L was a predictive value for development of overt hypothyroidism ($p = 0.005$). On the other hand, serum TSH levels spontaneously normalized in 38 (53.5%) of the patients with subclinical hypothyroidism. In the multivariate analysis, normalization of TSH levels was associated with lower baseline TSH levels ($p = 0.004$) and normal and homogenous thyroid US findings ($p = 0.04$). Atomic-bomb radiation dose was not associated with subclinical hypothyroidism or its course. ***Conclusions:*** Subclinical hypothyroidism was four times more likely to be associated with development of overt hypothyroidism than euthyroid controls in the sample population of Japanese elderly. TSH levels in half of the patients normalized spontaneously when assessed after an average follow-up period of 4.2 years. Baseline TSH level and thyroid US findings are potential predictors of future thyroid function in subclinical hypothyroidism.

♦ Masunari N, Fujiwara S, Kasagi F, Takahashi I, Yamada M, Nakamura T: Height loss starting in middle age predicts increased mortality in the elderly. Journal of Bone and Mineral Research 2012 (January); 27(1):138–45. **(RR 2-11)** (refer to abstract in *Adult Health Study* Publications)

♦ Minamoto A, Neriishi K, Nakashima E: UV radiation may explain intercity difference for cataract in A-bomb survivors. Journal of Photochemistry and Photobiology B: Biology 2011 (May); 103(2):105–10. **(RR 5-10)**

[Abstract] Accurate assessment of risk factors is important for the evaluation of radiation-induced ocular lens damage. Our previous study identified a significant city difference between Hiroshima and Nagasaki atomic-bomb survivors in terms of cataract prevalence, prompting further analysis. This study analyzed the sites of lens opacities and used model fitting that incorporated the variable impact of UV on the eye, based on the hypothesis that the city difference in the prevalence of cataract was due to differences in UV radiation between the two cities. The results suggested that cataracts among Nagasaki residents were more frequently located at the inferior nasal portion of the lens compared to cataracts in Hiroshima residents, with no ionizing radiation-specific localization observed. Based on the angles of incidence, UV was suggested as a possible cause of the city difference. We therefore analyzed models of city differences in terms of UVA and UVB levels. The UVB model provided a better fit than the UVA model, suggesting that UVB might account for the city difference. The current study implicated the geographic location of the subject, the investigation period, and outdoor activities as potentially important surrogate factors for UVB influence in radiation-induced cataract. In addition, the superior temporal portion of the lens seemed the most suitable for evaluating the effects of ionizing radiation because of the lesser amount of UVB interference at that site.

♦ Yoshida K, Ohishi W, Nakashima E, Fujiwara S, Akahoshi M, Kasagi F, Chayama K, Hakoda M, Kyoizumi S, Nakachi K, Hayashi T, Kusunoki Y: Lymphocyte subset characterization associated with persistent hepatitis C virus infection and subsequent progression of liver fibrosis. Human Immunology 2011 (October); 72(10):821–6. doi:10.1016/j.humimm.2011.05.029 http://www.sciencedirect.com/science/journal/01988859 **(RR 14-10)** (refer to abstract in *Immunology Studies* Publications)

Other Journal Publication

♦ Neriishi K: Cataract: based on A-bomb survivor studies. Nippon Rinsho [Japanese Journal of Clinical Medicine] 2012 (March); 70(3):441–5. (Japanese)

Manuscript in Press

⌘ Yamada M: Epidemiological study on dementia—Follow up study among atomic bomb survivors. Ronenki Ninchisho Kenkyukai Shi [Proceedings of the Annual Meeting of the Japanese Research Group on Senile Dementia]. (Japanese)

Special Clinical Studies Oral Presentations

❖ Imaizumi M. Subclinical hypothyroidism and coronary heart disease among the elderly. 84th Annual Meeting of the Japan Endocrine Society, 21–23 April 2011, Kobe

❖ Yanagi M, Neriishi K, Kawasaki R, Nakashima E, Fujiwara S, Akahoshi M, Takahashi I, Wang JJ, Wong TY, Kiuchi Y. Relationship between estimated radiation dose and retinal vascular caliber in atomic bomb survivors. ARVO 2011 Annual Meeting, 1–5 May 2011, Fort Lauderdale, Florida, USA

❖ Fujiwara S. Application of FRAX(R) to a Japanese cohort—Criteria for timing of initiation of treatment. 84th Annual Congress of Japanese Orthopaedic Association, 12–15 May 2011, Yokohama

❖ Tatsukawa Y, Yamada M, Yamane K, Fujiwara S. Effects of diabetes mellitus and metabolic syndrome on incidence of peripheral artery disease. 54th Annual Scientific Meeting of the Japan Diabetes Society, 19–21 May 2011, Sapporo

❖ Fujiwara S. An approach to vertebral fracture assessment: Epidemiology of vertebral fracture among Japanese. 31st Annual Meeting of the Japanese Society for Bone Morphometry, 20–22 May 2011, Gifu

❖ Yamada M, Kasagi F, Mimori Y, Miyachi T, Ohshita T, Sasaki H. Midlife and elderly biologic function and dementia: Radiation Effects Research Foundation Adult Health Study. Alzheimer's Association International Conference on Alzheimer's Disease 2011, 16–20 July 2011, Paris, France

❖ Haruta D, Matsuo K, Tsuneto A, Ichimaru S, Hida A, Sera N, Imaizumi M, Nakashima E, Maemura K, Akahoshi M. Prevalence, incidence and prognostic value of early repolarization pattern. 75th Annual Meeting of the Japanese Circulation Society, 3–4 August 2011, Yokohama

❖ Nakashima E, Neriishi K, Minamoto A. An analysis of cortical cataract localization data from A-bomb survivors, 2000–2002. 2011 Joint Statistics Meeting, 4–7 September 2011, Fukuoka

❖ Mimori Y, Yamada M. Epidemiology of dementia and related risk factors: Radiation Effects Research Foundataion Adult Health Study. 1st Annual Meeting of Japan Society for Dementia Prevention, 9–11 September 2011, Yonago

❖ Sasaki H, Yamada M, Kasagi F. Exercise epidemiologic studies in Adult Health Study. 14th Scientific meeting of Japanese Association of Exercise Epidemiology, 15 September 2011, Shimonoseki

❖ Fujiwara S, Masunari N, Kasagi F, Nakamura T. Height loss starting in middle age predicts increased mortality in elderly men and women. 33rd Annual Meeting of the American Society of Bone and Mineral Research, 16–20 September 2011, San Diego, California, USA

❖ Neriishi K. Current status of ophthalmologic study of A-bomb survivors. Exploratory Workshop on Radiation-Induced Lens Opacities Resulting from Low Dose Exposure, 20–23 September 2011, Bombon, France (related to *Adult Health Study*)

❖ Ohishi W, Yoshida K, Kusunoki Y, Nakashima E, Fujiwara S, Hayashi T, Nakachi K, Chayama K. Study of immunological characteristics associated with disease progression in chronic hepatitis C virus infection. 15th Annual Meeting of the Japan Society of Hepatology, 20–23 October 2011, Fukuoka (related to *Immunology Studies*)

❖ Fujiwara S. Epidemiology of vertebral fracture among Japanese. 13th Annual Meeting of the Japan Osteoporosis Society, 3–5 November 2011, Kobe

❖ Fujiwara S. Fracture prediction with bone markers (examination using T score). 13th Annual Meeting of the Japan Osteoporosis Society, 3–5 November 2011, Kobe

❖ Masunari N, Fujiwara S. Study on screening of individuals at high risk of osteoporosis bone fracture based on quantitative ultrasound (QUS) and WHO fracture risk assessment tool (FRAX). 13th Annual Meeting of the Japan Osteoporosis Society, 3–5 November 2011, Kobe

❖ Fujiwara S. Application of FRAX and limitation in year 2011: Japanese perspectives. Joint Symposium, Korean Osteoporosis Society and Japan Osteoporosis Society, 3–5 November 2011, Kobe

❖ Ohishi W, Yoshida K, Kusunoki Y, Nakashima E, Fujiwara S, Hayashi T, Nakachi K, Chayama K. Immunological features associated with disease progression in persistent hepatitis C virus infection. 62nd Annual Meeting of the American Association for the Study of Liver Diseases, 4–8 November 2011, San Francisco, California, USA (related to *Immunology Studies*)

❖ Takahashi N, Sasaki K, Shimoichi Y, Sugita K, Katayama H, Satoh Y. The segmental copy-number variations (CNVs) observed in Japanese by BAC-array-CGH—Summary of relatively rare CNVs. 56th Annual Meeting of the Japan Society of Human Genetics, 10–13 November 2011, Chiba (related to *Biochemical Genetics Studies*)

❖ Ichimaru S, Hida A, Imaizumi M, Onishi N, Nakamura T, Akahoshi M. Non significance of waist circumference in the diagnosis of metabolic syndrome: An epidemiological perspective. Health System Reform in Asia Conference, 9–12 December 2011, Hong Kong, China

❖ Imaizumi M. Radiation Effects on Thyroid: What have been learned from the atomic bomb radiation and Chernobyl accident. Radiation Epidemiology Research Meeting 2011, 14 December 2011, Tokyo

❖ Imaizumi M. Radiation effects on thyroid: What have been learned from the atomic bomb radiation and Chernobyl accident. Meeting: Concerns after the accident in the nuclear power plant of Fukushima, 15 March 2012, Tokyo

❖ Fujiwara S, Masunari N, Harada A. Prediction of osteoporotic fracture risk based on daily activity and health status of the elderly in Hiroshima cohort. IOF European Congress on Osteoporosis and Osteoarthritis, 21–24 March 2012, Bordeaux, France

❖ Tatsukawa Y, Yamada M, Nakanishi S, Fujiwara S. Effects of diabetes mellitus and metabolic syndrome on incidence of peripheral artery disease. 16th International Symposium on Atherosclerosis, 25–29 March 2012, Sydney, Australia

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), Suyama A (EN)

The latest paper regarding number of solid cancer cases in the Life Span Study (LSS) cohort between 1958 and 1998 reported it as 17,448, of which the majority of cases requiring surgical procedures are believed to have undergone surgery at major hospitals in Hiroshima and Nagasaki. Such surgical cancer samples (in paraffin blocks) are maintained independently at such hospitals. Some hospitals, however, are forced to consider discarding the samples due to the lengthy storage they have to commit to and the difficulty they have in securing storage space. In order to carry out pathological studies, such as in-depth molecular biological research on radiation carcinogenesis in the LSS cohort, long-term storage of such samples needs to be assured. The objective of this study is to develop an archival system for surgical cancer samples from atomic-bomb survivors in collaboration with pathologists of the major hospitals in Hiroshima and Nagasaki. For development of this system, surgical samples from the LSS cohort members stored at each hospital will be identified and reviewed for their storage status and usability in future studies. Upon identification of such samples, each hospital will be requested to store them over an extended period of time. When it becomes difficult for a hospital to continue maintaining the samples, they will be transferred to and stored at RERF. In addition to this research protocol (RP)'s collaborating investigators and their organizations, other major hospitals and their pathologists in Hiroshima and Nagasaki will be requested to participate in this RP. RERF will create and manage the database about all stored samples. This RP determines the procedures of collaborating research projects in which the storage samples will be used and the guidelines for the utilization, and the participants in this RP should respect them.

RP 5-89 Pathology studies in Hiroshima and Nagasaki. Revised research plan

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

The pathology program started at the inception of ABCC has undergone several major revisions through the years. Autopsy rates peaked at 40–45% in the early 1960s but subsequently declined and the autopsy program was terminated in 1988. In 1987, the pathology program was redesigned to conduct surgical pathoepidemiological follow-up studies of atomic-bomb survivors in order to verify diagnoses and to detect specific histologic, cytologic, or other tissue changes directly or indirectly attributable to irradiation (RP 9-88).

A large number of LSS autopsy (about 7,500 cases) and surgical tissue samples (about 13,000 cases) have been collected through the ABCC-RERF pathology program over the years. These archived tissues have been useful for molecular studies as well as usual pathological investigation. Many of the recent tissue samples are stored in the outside hospitals at which subjects in the LSS were diagnosed but are obtained under agreements signed between the hospitals and RERF. A new project for storage of surgically removed histopathological specimens (tissue blocks) has been initiated (RP 1-12) among Hiroshima University and hospitals in the Hiroshima area, Nagasaki University and hospitals in the Nagasaki area, and RERF. It is chaired by Dr. Okubo and funded by the Ministry of Health, Labour and Welfare.

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

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

The development of multiple primary cancers (MPC) may be related to both exposure to carcinogenic factors and high cancer susceptibility. Thus, the relationship between A-bomb irradiation and the development of MPC among A-bomb survivors is an important issue in elucidating the influence of the bombings on carcinogenesis as a late health effect. In identifying MPC cases, it is essential to determine whether the second cancer is primary or metastatic.

The purpose of this protocol is to identify more accurately true MPC diagnoses vs. metastatic disease by modern histopathological methods as an initial step for evaluating the relationship between MPC and radiation exposure in A-bomb survivors. We will identify MPC cases histopathologically with immunohistochemical methods to differentiate primary and metastatic cases, using as subjects A-bomb survivors in Nagasaki in the Life Span Study (LSS) population. This study will contribute to accurate identification of cases in order to obtain further information that can be used for examining the effects of radiation exposure on cancer risks.

There were 648 patients who had two or more cancers which had been histologically diagnosed in a total of 6,305 primary-cancer patients bearing cancer among 38,107 in the Nagasaki LSS cohort between 1958 and 2003. After reviewing HE-stained tissue specimens and immunohistochemistry findings of the 648 cases, we found 595 cases were MPC, 41 cases (7%) were not MPC cases, 10 cases were undetermined and 2 cases were uncollectible.

Research Protocols 1-11, 5-10, 5-02, 1-97 and 2-01
Cell Biology Studies

RP 1-11 Study of radiation-induced circulatory diseases using animal models

Takahashi N (R), Niwa Y (R), Ohishi W (CH), Hayashi T (R), Murakami H (R), Hsu WL (S), Kokubo T, Inaba T, Oghiso Y, Tanaka S, Kusunoki Y (R)

The Life Span Study (LSS) data indicate radiation-associated risk for hypertensive heart disease and stroke, and the Adult Health Study (AHS) data suggest radiation-associated risk for hypertension. We hypothesize that radiation may result in higher risk for circulatory diseases (CD). However, there are a limited number of animal model studies that have examined the relationship between radiation and CD at doses under 4 Gy, although some papers report that various biomarkers are affected by radiation. In this study, we propose the use of spontaneous hypertensive rat-stroke prone (SHRSP) rats as rat CD models. Radiation doses from a brief single exposure were given to the rats at 4, 2, and 1 Gy, with non-exposed (0 Gy) rats used as controls. For each dose group, four rats were used. The study was conducted according to the following two approaches: 1) the rats were raised until their natural deaths to observe life span; or 2) blood samples were obtained and autopsies and pathology search conducted immediately after observation of stroke-like symptoms to obtain fresh samples for histopathology search and blood biomarker measurement. The Research Institute for Radiation Biology and Medicine (Hiroshima University) irradiated and raised the rats, while the Institute for Environmental Sciences conducted the pathology search. 【Results】1) Statistically significant shortening of lifespan was observed in the irradiated groups compared with the non-irradiated groups; 2) the histopathology search led to the observation that the extent and severity of vascular legions were significantly more marked in the irradiated groups than the non-irradiated groups. Moreover, we will measure candidate serum markers that have shown radiation dose effects based on previous AHS studies and 23 biomarkers in plasma samples by immunoassay. In the present study, significant dose effects were observed even among the rats exposed at 1 Gy. Because of this result, we are now preparing an RP based on which we plan to study rats exposed to low-dose radiation of less than 1 Gy. This study should provide mechanistic information on the association between radiation exposure and development of CD.

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

Certain solid cancers show a significant and relatively high excess relative risk (ERR) from radiation exposure in the Life Span Study (LSS) of atomic-bomb (A-bomb) survivors. Those victims who had A-bomb exposure at a young age (0–19 years old) show a higher ERR of solid cancers, even 60 years after exposure, than do those exposed at over 20 years of age. Pathological studies have in the past provided significant information about the histological characteristics of various solid cancers that developed among A-bomb survivors, some of which seem to differ from those in the non-exposed population.

Although little has been proved about the molecular characteristics of solid cancers among A-bomb survivors, preliminary results obtained from our pilot study on colon and rectal cancers among A-bomb survivors implied that prior atomic radiation exposure might influence microsatellite instability (MSI) status and result in an increase in the relative frequency of MSI-high (MSI-H) colon cancer. In addition, MSI-related molecular events, typically genetic and epigenetic alterations of the *MLH1* and Ras-signaling-related genes, might also be influenced by radiation exposure.

Determination of colon and rectal cancers with MSI or chromosomal instability (CIN) is an important issue that forms the basis of this study. MSI status will be examined by DNA fragment analysis using six different microsatellite markers. In addition, CIN status will be examined by analysis of gain or loss of CIN-related chromosome loci with real-time polymerase chain reaction (PCR) or the PCR-restriction fragment length polymorphism (PCR-RFLP) method using single nucleotide polymorphism (SNP) loci, respectively.

Based on findings obtained from the pilot study, we propose the following hypothesis: The carcinogenic pathway with MSI may preferentially occur in colon cancer, but not in rectal cancer, among A-bomb survivors. To test this hypothesis, the following questions will be clarified by examination of about 140 cases with colon or rectal cancers including 20 subjects exposed to high radiation dose: 1) Whether radiation exposure has a stronger relationship with MSI-H colon cancer than with microsatellite stable/MSI-low (MSS/MSI-L) colon cancer or overall rectal cancer; 2) If this is the case, is radiation exposure associated with the occurrence of genetic and epigenetic alterations, specifically methylation of MSI-related genes (DNA repair genes, Ras-signaling-related genes, CpG island methylator phenotype [CIMP]-related genes) in colon cancer?; 3) Whether radiation exposure is associated with CIN-positive colon and rectal cancers; 4) If answer to question 3) is yes, whether radiation exposure influences the most important initial event (i.e., *adenomatous polyposis coli* [*APC*] gene alteration) or subsequent events; and 5) Are there any differences in pathological parameters (e.g., differentiation, development, progression, and host immune reaction to cancer) between non-exposed and exposed MSI- or CIN-positive colon and rectal cancers?

In this study, we will examine those questions in the order of descending priorities indicated by the item numbers 1) to 5). DNA extraction from cancer tissue and surrounding noncancer tissue was completed and analysis of MSI status has been initiated.

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), Eguchi H, Taga M (R), Ito R (R), Imai K (R), Soda M (EN), Katayama H (IT), Cologne JB (S), Hayashi Y, Nakachi K, Kusunoki Y (R)

Thyroid cancer is one of the malignancies most closely associated with radiation in humans. We have previously found that rearrangement of *RET* proto-oncogene could be induced in human thyroid cells by *in vitro* and *in vivo* X-ray irradiation. *RET* rearrangements occurred in 60–80% of thyroid cancers in post-Chernobyl children and also in patients with histories of radiation therapy. On the other hand, $BRAF^{V600E}$ gene mutation is also an early event in adult-onset thyroid carcinogenesis. We hypothesize that the high incidence of papillary thyroid cancer (PTC) in adult survivors who were exposed to A-bomb radiation at young ages is in part a result of *RET/PTC* rearrangements and other chromosomal rearrangements induced by radiation. To examine that hypothesis, we are analyzing *RET/PTC* rearrangements, $BRAF^{V600E}$ mutation, and other alterations in cancer tissues of adult-onset PTC patients from the LSS cohort.

We examined point mutations of *BRAF, K-RAS*, *N-RAS,* and *H-RAS* genes as well as rearrangements of *RET*, *NTRK1,* and *BRAF* genes, in 105 adult-onset PTC patients (including 26 patients not exposed to radiation) from A-bomb survivors. The frequency of PTC cases with chromosomal rearrangements, mainly *RET/PTC* rearrangements, significantly increased with radiation dose, while point mutations including $BRAF^{V600E}$ significantly decreased with radiation dose. We recently found anaplastic lymphoma kinase (*ALK*) gene rearrangements for the first time in PTC among 10 of 25 PTC cases with non-detected gene alterations (i.e., no alterations in the *RET*, *NTRK1*, *BRAF*, and *RAS* genes).

We identified partner genes of *ALK* rearrangements by 5′ RACE method or inverse PCR method in all 10 cases. In nine of the cases, fusion genes of exon 13 in echinoderm microtubule-associated protein-like 4 (*EML4*) and exon 20 in *ALK* were identified, and the remaining case had a fusion gene of *EML4* exon 20 and *ALK* exon 20. Moreover, we investigated *ALK* gene rearrangements in 45 of 80 PTC cases with alterations in *RET*, *NTRK1*, *BRAF*, or *RAS* gene, and failed to find rearrangements in 43 of the cases. However, the two remaining cases could have *ALK* rearrangements, regarding which verification work is ongoing. We also are examining *ALK* rearrangements in the remaining 35 cases.

RP 1-97 Pilot study of the genetic background of the Adult Health Study (AHS) population: Identification of markers in potential candidate genes associated with hypertension

RP 2-01 The acquisition of signed informed consent forms from the donors (or their proxies) for genomic studies conducted either at RERF or at other research institutes as collaborative study using previously collected blood samples (Addendum to RP 1-97)

Takahashi N (R), Murakami H (R), Yamada M (CH), Kasagi F, Kodama K (CS)

The aim of this study is to test the hypothesis that differences in genetic background in the survivors modify the radiation risk for non-cancer diseases. For this purpose, we established a small-scale case-control study in the AHS and initially sought to identify key polygenic elements that may be predisposing to hypertension. A nationwide project started in 2000 served to complement our effort by initiating a large-scale search for hypertension-related genes. From that effort, it was found that 38 polymorphisms of single nucleotide polymorphisms (SNPs) were significantly associated with hypertension (about 2,300 study subjects consisting of 1,100 cases and 1,200 controls). A summary paper was published (Kohara et al., *Hypertension Research* 2008; 31:203–12).

The consortium recommends that many SNPs accumulated during the study would be applied to new field and used for validation of new results obtained by studies.

Since one group of the consortium demonstrated that A subtype of trimeric intracellular cation-specific channels (*TRIC-A*) knockout mice developed hypertension, this gene is expected to be one candidate gene of hypertension. The studies were conducted to examine whether the SNPs around the gene (*TRIC-A*) are associated with hypertension or not. The study revealed that SNPs were associated with essential hypertension (Yamazaki et al., *Cell Metabolism* 2011; 14[2]:231–41). To date, association studies for the candidate genes are still ongoing. After the SNPs showing strong association with hypertension are accumulated, they will be applied to the AHS population for the study of radiation-associated cardiovascular diseases of A-bomb survivors.

Cell Biology Studies Publication

Manuscript in Press

⌘ Hamatani K, Mukai M, Takahashi K, Hayashi Y, Nakachi K, Kusunoki Y: Rearranged anaplastic lymphoma kinase (*ALK*) gene in adult-onset papillary thyroid cancer among atomic bomb survivors. Thyroid.

Cell Biology Studies Oral Presentations

❖ Ito R, Eguchi H, Hamatani K, Taga M, Takahashi K, Oue N, Yasui W, Nakachi K, Kusunoki Y. Analysis of CpG island methylator phenotype (CIMP) pathway in colorectal cancer among atomic-bomb (A-bomb) survivors. 100th Annual Meeting of the Japanese Society of Pathology, 28–30 April 2011, Yokohama

❖ Ito R, Eguchi H, Hamatani K, Taga M, Oue N, Yasui W, Nakachi K, Kusunoki Y. Genetic and epigenetic alterations in colorectal cancer among atomic-bomb survivors. 2nd AACR International Conference on Frontiers in Basic Cancer Research, 14–18 September 2011, San Francisco, California, USA

❖ Hamatani K, Takahashi K, Ito R, Taga M, Niwa Y, Nakachi K, Kusunoki Y. Characteristics of adult-onset papillary thyroid cancer with rearranged *ALK* gene developed from atomic bomb survivors. 70th Annual Meeting of the Japanese Cancer Association, 3–5 October 2011, Nagoya

Research Protocols 4-11, 1-10, 2-07, 1-07, 5-85 and 1-01

Biochemical Genetics Studies

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 (CS), Asakawa J (G)

The genetic effects of atomic-bomb (A-bomb) radiation (trans-generational effect) have not been fully elucidated due to low rates of both spontaneous mutation and radiation-induced mutation in germ cells. Since radiation-induced mutations are primarily deletions initiated with DNA double-strand breaks, we propose a comparative genome hybridization (CGH) study using high-density microarrays. In the proposed study, we will analyze a total of 688 DNA samples from both parents of 184 families (high-dose exposure is limited to either parent) and 320 offspring (160 from paternally and 160 from maternally exposed families). We plan to use 3 × 1.4 M high-density microarray that contains three identical sets of 1.4 million probes (average interval 2.2 kb) on a single slide. The multiplex format of the microarray permits us to analyze three DNA samples at a time on a single slide. We will determine the parental origin of mutations to be detected based on the single nucleotide polymorphism (SNP) information existing in the genome region involved in the mutational events. The analyses of 320 offspring will enable us to test 7.36×10^6 loci that were exposed and unexposed, respectively, assuming that our genome consists of 23,000 genes. If we use the currently available mutation rates obtained through irradiation of spermatogonia of male mice, we would expect 16.2 mutations (95% confidence interval [CI]: 9–25) among the exposed paternal alleles and 3.7 mutations (95% CI: 0–8) among the unexposed alleles (the estimated statistical power to detect radiation effect is more than 80%). On the other hand, we do not expect enough mutations in 160 offspring of the maternally exposed families to detect an elevated rate because the mutation rate obtained in irradiated rat immature oocytes seems to be much lower than that in mouse spermatogonia. Nonetheless, this group is indispensable because the paternal alleles are not exposed to radiation and hence serve as the control of the above-mentioned paternally exposed families. The analyses will provide far more information than is currently available on the risk associated with either maternal or paternal radiation exposure.

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

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

The purpose of the study is to estimate the mutation induction rate following 4 Gy of gamma irradiation of mouse spermatogonia, and to molecularly characterize the mutations as an animal model of human male exposure. Based on recent advances in the comparative genomic hybridization (CGH) microarray technology, the RERF Scientific Council recommended that RERF conduct a pilot study using HD-array CGH with mouse and rat DNA samples that were used for DNA 2-DE studies. This pilot 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) from the offspring of Hiroshima and Nagasaki survivors whose radiation doses are much smaller than those used in animal studies. We will estimate the mutation induction rate by examining DNA samples of 80 F_1 mice derived from the spermatogonia of male mice irradiated with 4 Gy of γ-rays and 80 F_1 mice in the control group by a HD-array CGH technique. To detect small deletions harboring only 2–3 probes accurately, we have improved the experimental and analytical methods for the CGH platform. We finished the CGH experiment on the DNA samples of 80 exposed and 80 control mice. We have been analyzing the data to identify mutation candidates and will confirm the putative mutations to be detected molecularly.

RP 2-07 Study on genetic effects of radiation by array comparative genome hybridization (array-CGH) method—validation of ability of the methodology for the genetic study

Takahashi N (R), Satoh Y (G), Kodaira M (G), Katayama H (IT), Kodama Y (G), Cologne JB (S)

For the large population of F_1 offspring of A-bomb survivors, the bacterial artificial chromosome DNA-microarray-based comparative genome hybridization (BAC-aCGH) method was selected and refined in order to effectively identify "radiation-induced *de novo* mutations." The results of a pilot study revealed that our BAC-aCGH system could detect changes in copy number variation (CNV) with good sensitivity (Takahashi et al., *Annals of Human Genetics* 2008; 72:193–204 and Takahashi et al., *Cytogenetic Genome Research* 2008; 123:224–33).

In this RP, the genomic DNA from 225 offspring born to parents, at least one of whom was exposed to high doses (≥1.0 Gy) of radiation, had been screened using our BAC-aCGH system that consists of approximately 2,500 BAC clones.

We finished a population survey and found various types of CNVs. In FY2010, we summarized the data by focusing on highly polymorphic CNVs (in ≥5% of the individuals). We found a total of 680 CNVs at 16 different BAC-regions in the genome. These results were published in *Journal of Biomedicine and Biotechnology* (Takahashi et al., Volume 2011, Article ID 820472).

Relatively rare variations have recently received attention from scientists who conjecture a hypothesis called "common disease and rare variants." Therefore, we have summarized the data by focusing on relatively less frequent CNVs (in less than 5.0% of the individuals). We found a total of 126 relevant CNVs at 52 different BAC-regions in the genome. Thirty-two CNVs observed in this study were novel and had not been previously reported, indicating that there may be diversity between the Japanese and other populations. Some of the CNVs contained genes that might be useful markers for investigation of association between phenotypic heterogeneity and genotype among individuals. These CNVs are expected to contribute to future CNV-disease association studies. The results were published in *Journal of Biomedicine*

and Biotechnology (Satoh et al., Vol. 2012, Article ID 789024).

RP 1-07 Estimation of genetic risk of radiation on immature oocytes of rats by using two-dimensional DNA analysis: An animal model for human female exposure

Asakawa J (G), Kamiguchi Y, Nakamura N (CS), Katayama H (IT), Cullings HM (S)

Up till now, there has been no suitable animal model to study the genetic effects of female gonadal exposure. Mouse immature oocytes are highly sensitive to ionizing radiation and readily die by apoptosis at rather modest doses. As we recognized the powerful nature of the 2-DE method for screening mutations in any mammals whose genomic information is unknown, we searched for the most appropriate laboratory animal model of radiation mutagenesis in human female gametes. We considered several key requirements: (a) the intrinsic radiosensitivity of immature oocytes, (b) availability of several inbred strains bearing variation in base sequences so that the parental origin of mutations in the F_1 can be assessed, and (c) ease in raising pups. After evaluating the suitability of various rodent species, we reached a tentative conclusion that a rat model may be the best animal model currently available, especially since rat immature oocytes are less sensitive to ionizing radiation than mouse oocytes.

Dr. Kamiguchi (Asahikawa Medical College) prepared F_1 rats after mating 2.5 Gy-exposed females (strain SD) with unirradiated males (strain BN) for our future screening of mutations using the 2-DE method. We prepared two types of gels, 1–5-kb fragments and 5–10-kb fragments, per animal. We analyzed a total of 3,000 gels prepared from 1,500 F_1 rats. In other word, we analyzed about 2.2 million spots (loci), each derived from maternal SD or paternal BN strains. A total of 24 mutations, 13 from the control and 11 from the 2.5-Gy exposed group, were confirmed as newly arisen germline mutations by the results on DNA from three different tissues (spleen, kidney, and liver) and by family studies. We also cloned and sequenced 21 normal DNA fragments where mutations were detected (three DNA fragments are not yet cloned). Four mutations, two each from the control and exposed groups, were deletions, but none of them occurred in the exposed maternal alleles. The majority of the mutations (11/13 mutations in the control and 9/11 in the exposed group) seemed to have occurred at microsatellite sequences (i.e., alterations in the number of repeats and probably spontaneous in origin). In summary, we have observed no indication for the transgenerational effects of radiation following exposure of immature oocytes of rats to 2.5 Gy of γ rays.

RP 5-85 Culture of permanent lymphocyte 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)

Takahashi N (R), Satoh Y (G), Murakami H (R), Katayama H (IT), Fujiwara S (CH), Akahoshi M (CN)

The purpose of this RP is to establish lymphoblastoid cell lines by transformation of B-cells from members (parents and children) of about 1,300 families as biological resources for current and future genetic studies, e.g., studies of mini- and microsatellite markers and DNA microarrays that can be used to address the issue of transgenerational radiation effects. In addition, collection of blood samples and establishment of permanent cell lines from those individuals omitted from the original cohort, but later added, are also being done.

No written informed consent was obtained when this RP (RP 5-85) was originally approved in 1985. In order to avoid possible ethical issues concerning work in the future, signed consent forms are now being collected from all participants (RP 1-01).

We are continuing the effort to collect blood samples from as many family members as possible. Although we have been successful in obtaining informed consent from the majority of blood donors, we need to make a continuing effort to obtain written consent from additional donors. The cumulative numbers of children from whom cell lines were established and from whom signed informed consent forms were obtained are shown in the Table.

In addition to this effort, we started to collect untreated blood samples for future genetics studies with new technologies, such as a next generation sequencer. We collected 149 new blood samples from F_1 Clinical Study participants (as a part of RP 4-10).

We are making efforts to safely maintain our archival specimens. Moreover, whenever the stocks of the frozen EB-transformed samples are utilized for any study, we replenish them and return them to the archive.

Table. The cumulative numbers of children with established cell lines and signed informed consent forms

Father's dose (Gy)	Mother's dose (Gy)				
	≥1	0< <1	0	Unknown	Total
≥1	2	24	146	2	174
0< <1	5	63	201	9	278
0	109	284	610	46	1,049
Unknown	0	7	30	0	37
Total	116	378	987	57	1,538

Biochemical Genetics Studies Publication

RERF Report (RR)

♦ Satoh Y, Sasaki K, Shimoichi Y, Sugita K, Katayama H, Takahashi N: Copy-number variations observed in a Japanese population by BAC array CGH: Summary of relatively rare CNVs. Journal of Biomedicine and Biotechnology 2012 (January); Volume 2012, Article ID 789024, 10 pages. **(RR 8-11)**

[Abstract] Copy-number variations (CNVs) may contribute to genetic variation in humans. Reports regarding existence and characteristics of CNVs in a large apparently healthy Japanese cohort are quite limited. We report the data from a screening of 213 unrelated Japanese individuals using comparative genomic hybridization based on a bacterial artificial chromosome microarray (BAC aCGH). In a previous paper, we summarized the data by focusing

on highly polymorphic CNVs (in ≥5.0% of the individuals). However, rare variations have recently received attention from scientists who espouse a hypothesis called "common disease and rare variants." Here, we report CNVs identified in fewer than 10 individuals in our study population. We found a total of 126 CNVs at 52 different BAC regions in the genome. The CNVs observed at 27 of the 52 BAC-regions were found in only one unrelated individual. The majority of CNVs found in this study were not identified in the Japanese who were examined in the other studies. Family studies were conducted, and the results demonstrated that the CNVs were inherited from one parent in the families.

Biochemical Genetics Studies Oral Presentations

❖ Takahashi N, Sasaki K, Shimoichi Y, Sugita K, Katayama H, Satoh Y. The segmental copy-number variations (CNVs) observed in Japanese by BAC-array-CGH—Summary of relatively rare CNVs. 56th Annual Meeting of the Japan Society of Human Genetics, 10–13 November 2011, Chiba (related to *Special Clinical Studies*)

❖ Nakamura N. May human germline mutagens be identified in the future? 40th Environmental Mutagen Society Annual Meeting, 21–23 November 2011, Tokyo

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 (CS), Hsu WL (S), Kodama Y (G)

Through epidemiologic studies on childhood cancer conducted since the 1950s, it has been suggested that fetuses are highly sensitive to radiation for induction of cancers. However, whether the third-trimester exposure to diagnostic X rays has induced childhood cancers or not is still under debate. At RERF, we found that peripheral blood lymphocytes of A-bomb survivors who were exposed *in utero* did not record chromosome damage when examined at the age of about 40 years, while the lymphocytes of the mothers showed a clear dose-related increase of translocation frequencies. A similar lack of response was obtained in mouse lympho-hematopoietic cells (peripheral blood T cells, spleen T cells, and bone marrow cells) following fetal irradiation and examination in adult mice. However, our latest results in rat mammary epithelial cells showed that the radiation effect (chromosome aberrations) was clearly maintained in adult rats that were irradiated as fetuses, and the frequency was nearly the same as that of the irradiated mothers. Therefore, it is suggested that chromosome aberration frequency in adults following fetal irradiation may vary among tissues. In the present study, we propose to examine thyroid epithelial cells in mice to see whether or not the radiation effect may be recorded like mammary epithelial cells following fetal irradiation. The thyroid cells were chosen since the thyroid gland is known to be one of the organs more susceptible to radiation-induced carcinogenesis as is the mammary gland. The results may help to explain the cancer risks of A-bomb survivors who were exposed *in utero*.

RP6-09 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 (CS), Noda A (G), Cullings HM (S), Ozasa K (EH), Tokuoka S, Yonehara S, Fujihara M, Moriwaki S, Nishigori C, Mabuchi K, Kraemer KH, Land CE, Kodama Y (G)

The frequency of patients with cancer-prone recessive hereditary disorders, such as xeroderma pigmentosum (XP), is usually low, but carriers (heterozygotes) are not rare. However, there is little data regarding cancer risk in the carriers of the heterozygotes, as they are generally difficult to identify. This study will focus on a founder mutation allele of *XPA* gene, which is an inactive mutation allele known to cause severe disease phenotypes under homozygous conditions. The mutation heterozygotes were found in about 1% of the general population (9/1,020 in our previous study; Hirai et al., *Mutation Research* 2006; 41:231–7), which is unique to Japanese. The condition provides a unique advantage in effective screening of such carriers.

The purpose of the study is to elucidate the frequency of carriers of an inactive, founder mutation allele of the *XPA* gene among nonmelanoma skin cancer patients, and compare the frequency to the frequency in a general population.

In this fiscal year, we examined 350 nonmelanoma skin cancers and found five XPA heterozygotes.

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

Noda A (G), Hirai Y (G), Kodama Y (G), Cullings HM (S), Nakamura N (CS)

Last year we reported the generation of HPRT-dupGFP mice. These genetically-modified mice have partial duplication in the *HPRT* locus, and living somatic and germ cells fluoresce green when reversion from this partial duplication occurs. This technique has enabled detection and measurement of mutations in all *in vivo* tissues and cells without destroying tissue structures. Using these mice, we expect to detect how radiation-induced somatic and germline mutations occur *in vivo*, how such mutations and subsequent proliferation affect the mice, and how such effects are passed on to the offspring. This year, we examined whether mutations in the liver, pancreas, small intestine, and spleen lymphoid cells are induced by radiation exposure. There is a possibility that we can study low-dose radiation effects if our efforts to automate a system to measure somatic mutations at the tissue level succeed. Aiming to study *in vivo* mutations, we held an international workshop titled "Radiation Effects on Mutation in Somatic and Germline Stem Cells" and discussed the kinds of studies that can be conducted using these model mice.

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

Hirai Y (G), Tokuoka S, Cologne JB (S), Mabuchi K, Land CE, Noda A (G), Kodama Y (G), Nakamura N (CS)

The purpose of the study is 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. So far, frequent founder mutations among Japanese did not explain the observed high risk.

We examine the feasibility of specific single nucleotide polymorphisms (SNPs) that were suggested as contributing to the early-onset breast cancers. We examined MDM2 SNP309(G/T) among about 550 breast cancer cases in Hiroshima. A significant difference in the frequency of GG genotype was observed in the group A (A-bomb survivor, early onset) compared to other groups.

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), Takahashi N (R), Kusunoki Y (R), Shimizu Y (EH), Nakashima E (S), Cullings HM (S), Misumi M (S), Nakamura N (CS)

Biodosimetric study using FISH

We examined 69 survivors (39 from Hiroshima and 30 from Nagasaki) by FISH. So far, a total of 1,100 survivors in Hiroshima and 672 survivors in Nagasaki have been examined with FISH. Confirmations of clonal chromosome aberrations among the translocations detected, and corrections of them are necessary for accurate dose estimation, and were conducted following routine FISH analysis. To date, numbers of such "clean" FISH data are for about 1,600 survivors (1,000 from Hiroshima and 600 from Nagasaki).

Puzzling cytogenetic results for survivors exposed *in utero*—animal experiments

We have previously found that survivors who were exposed *in utero* showed almost no dose response in the translocation frequency in blood lymphocytes when examined at age 40, which was confirmed by subsequent mouse experiments. In order to see whether the above finding is unique in hematopoietic cells, we examined chromosome aberration frequencies in rat mammary epithelial cells following fetal irradiation. The results indicated that irradiated fetal mammary tissue recorded radiation damage like their mothers when examined at 6–45 weeks after irradiation, whereas lymphocytes did not record the damage. Thus, it is quite clear that the lack of translocation dose response following fetal exposure is tissue-dependent. A paper describing the results is under preparation.

Nation-wide cytogenetic study network in Japan

In 2002, the National Institute of Radiological Science (NIRS) in Chiba organized a "Chromosome Network Committee," which includes seven laboratories in Japan (Y. Kodama represents the RERF laboratory). The committee's objective is to create a nation-wide biodosimetry network system for radiological accidents. On March 11, 2011, nuclear accident occurred in Fukushima No.1 Nuclear Power Plant (NPP) following the earthquake and tsunami. Soon after the accident, Chromosome Network Committee members were asked to standing by for possible biodosimetric work in large number of cases. To date, chromosome tests were done in 12 NPP workers at NIRS. Fortunately, their estimated doses were relatively low. Now we (Chromosome Network Committee) have a plan to examine the chromosomes of some 100 NPP workers whose estimated doses are more than 100 mSv. NIRS is currently negotiating about the task with Tokyo Electric Power Company who has a responsibility for Fukushima NPP.

RP-A4-09 Detection of unrepairable DNA damages (DNA double strand breaks) in the past-irradiated cells and tissues

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

A long time after exposure to ionizing radiation (more than one year) we are able to successfully detect unrepairable chromosomal damage (DSB-foci) in a dose dependent manner in normal human fibroblasts. DSB-foci that are believed to have occurred due to radiation exposure were observed in the tissues of model animals. However, we were unable to strictly distinguish between repairable and unrepairable damage. Biochemical characterization of

unrepairable damage is thus necessary. On the other hand, it was revealed that cells with unrepairable DSB-foci exhibit premature aging. This finding suggests the possibility that functional aging is accelerated in exposed tissues. A manuscript incorporating the findings obtained thus far was accepted by the *Journal of Cell Science*.

RP-A2-09 Comprehensive analysis of radiation-induced genetic-damage regions 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)

This collaborative study was planned in response to the request by Dr. Homma, Japan National Institute of Health Sciences (NIHS). The request is based upon a background of information showing that ionizing radiation induces various types of structural alterations in the genome, but that little is known about how large chromosomal regions are altered and what types of genetic alterations preferentially remain in a normal cell following a given dose of ionizing irradiation. To comprehensively analyze damaged genome regions following radiation exposure, DNA from human peripheral blood T-cell populations clonally propagated after *in vitro* X-irradiation will be assessed by a comparative genomic hybridization (CGH) method with DNA from unirradiated blood mononuclear cells. Mutated genome regions that will be identified by this CGH assay will further be assessed for their relevance to chromosomal aberrations by G-banding and multi-color fluorescence *in situ* hybridization (mFISH) methods. Since each clonal population from irradiated blood of an individual will be analyzed in comparisons with unirradiated blood cells of the same individual, radiation-induced genome alterations can extensively be evaluated at a single-cell level. Because normal, untransformed cells will be analyzed, genome alterations observed in this study will mostly be specific to radiation exposure and may partly be involved in molecular events in the process of radiation carcinogenesis.

Peripheral blood mononuclear cells from one healthy volunteer were irradiated with either 0 Gy or 1 Gy of X-rays *in vitro*, and about 30 T-cell colonies of single-cell origin each in the 0-Gy and 1-Gy groups were collected. Cells from those T-cell colonies were sent to NIHS. Regarding T-cell colonies with genome alterations detected by CGH analyses based on the DNA extracted from the relevant cells, chromosome analysis and base sequence analysis of such alteration sites are under way.

Cytogenetics Studies Publications

RERF Report (RR)

♦ Nakamura N, Hirai Y, Kodama Y: Gamma-ray and neutron dosimetry by EPR and AMS, using tooth enamel from atomic-bomb survivors: A mini review. Radiation Protection Dosimetry 2012 (January); 149(1):79–83. **(RR 26-11)**

 (related to *Atomic-bomb Dosimetry Studies*)

[Abstract] The electron paramagnetic resonance (EPR, or electron spin resonance) method was used to measure CO_2^-. radicals recorded in tooth enamel by exposure to atomic-bomb gamma rays. The EPR-estimated doses (i.e., ^{60}Co gamma-ray equivalent dose) were generally in good correlation with cytogenetic data of the same survivors, whereas plots of EPR-estimated dose or cytogenetically estimated dose against DS02 doses turned out to scatter more widely. Because those survivors whose EPR doses were higher (or lower) than DS02 doses tended to show also higher (or lower) responses for cytogenetic responses, the apparent variation appears primarily due to problems in individual DS02 doses rather than the measurement errors associated with the EPR or cytogenetic technique. A part of the enamel samples were also used for evaluation of neutron doses by measuring ^{41}Ca/^{40}Ca ratios using the accelerator mass spectrometry technique. The results for the measured ratios were on average ~85% of the calculated ratios by DS02 (but within the 95% confidence bounds of the simulated results), which lends support to DS02-derived neutron doses to the survivors.

Other Journal Publication

♦ Noda A, Nakamura N: Revisiting mutation rate calculations. Hoshasen Seibutsu Kenkyu [Radiation Biology Research Communications] 2012 (March); 47(1):1–7.

Manuscript in Press

⌘ Kodama Y, Noda A, Booth C, Breault D, Suda T, Hendry J, Shinohara T, Rube C, Nishimura EK, Mitani H, Nakamura N, Niwa O: International workshop: Radiation effects on mutation in somatic and germline stem cells. International Journal of Radiation Biology.

Cytogenetics Studies Oral Presentations

❖ Nakamura N, Nakano M, Shimada Y, Kodama Y. Why chromosome aberration in lymphocytes did not persist following fetal exposure? 18th International Congress of Dentomaxillofacial Radiology, 25–29 May 2011, Hiroshima

❖ Hamasaki K, Nakano M, Ohtaki K, Shimada Y, Nishimura M, Yoshida MA, Nakata A, Nakamura N, Kodama Y. Chromosome aberration frequency following fetal exposure to ionizing radiation differs by the tissue. 14th International Congress of Radiation Research, 28 August–1 September 2011, Warsaw, Poland

❖ Shore RE, Ozasa K, Suyama A, Fujiwara S, Akahoshi M, Cullings HM, Kodama Y, Kodaira M, Hayashi T, Hamatani K. Recent findings by the Radiation Effects Research Foundation on the health of atomic bomb survivors and their offspring. International Symposium of Nagasaki University Global COE Program, 22 October 2011, Nagasaki (related to *Life Span Study*, *Adult Health Study*, *Immunology Studies*, and *F_1 Studies*)

❖ Kodama Y. Cytogenetic studies of atomic bomb survivors. 62nd Annual Meeting of the Chromosome Research Society, 11–13 November 2011, Hiratsuka

❖ Noda A, Suemori H, Hirai Y, Kodama Y, Nakamura N. New approaches to develop *in vivo* model systems that detect somatic and germ line mutations (report III). 54th Annual Meeting of the Japan Radiation Research Society, 17–19 November 2011, Kobe

❖ Noda A, Hirai Y, Suemori H, Kodama Y, Nakamura N. A genetically altered mouse strain which contains a partial,

tandem duplication at the endogenous HPRT gene and gives rise to GFP-positive mutant cells following reversion of the *HPRT* gene. 40th Annual Meeting of the Japanese Environmental Mutagen Society, 21–23 November 2011, Tokyo

❖ Noda A, Hirai Y, Nakamura N, Kodama Y. Role of unrepairable DNA double strand breaks generated by ionizing radiation in determining. 50th International Symposium of Research Institute for Radiation Biology and Medicine, Hiroshima University, 20–21 February 2012, Hiroshima

Research Protocols 3-02, 4-75 (Platform Protocol)
F_1 Studies—Genetic Effects of Atomic Radiation on Children Born to Bombing Survivors

RP 3-02 Health effects study of the children of A-bomb survivors: Mail survey

Suyama A (EN), Grant EJ (EH), Furukawa K (S), Sakata R (EH), Ozasa K (EH), Kodama K (CS), Watanabe T (EH), Fujiwara S (CH), Cologne JB (S)

Studies of genetic effects have been a primary focus of the ABCC and RERF research program since its inception more than 50 years ago. Among the surviving members of the F_1 mortality cohort, a mail survey cohort was selected that included 24,673 members whose *koseki* and current address were in the catchment areas of the clinical Adult Health Study plus a small number of children of high-dose parents whose *koseki* was outside the catchment area and whose current address was in the catchment area. This survey was designed primarily to ascertain baseline epidemiological data on the F_1 subjects, and secondarily to identify F_1 cohort members willing to participate in clinical health examinations to investigate possible relationships of parental radiation doses to adult-onset noncancer diseases in the F_1 cohort.

The mail survey cohort consisted of 13,389 males and 11,284 females. The survey was carried out between 2000 and 2006. At the end of the survey, 16,756 (68%) subjects responded, 7,584 (31%) did not respond, and the rest were outside of the contact area, address unknown, or deceased. A total of 14,145 (57%) subjects agreed to participate in the health examination and 11,951 (71% of questionnaire respondents) actually came to RERF for a clinical examination. We made a final report brochure that summarized parts of the questionnaire and the F_1 clinical study and sent it to the survey respondents as a thank-you for their participation.

We published the results of the F_1 clinical study analysis, which included covariates from the mail survey data, investigating possible associations of parental radiation dose to F_1 multifactorial diseases (Fujiwara, Suyama, et al., *Radiation Research* 2008; 170:451–7). We will conduct the descriptive analysis of the full mail survey data and prepare a report for publication.

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

Suyama A (EN), Grant EJ (EH), Furukawa K (S), Sakata R (EH), Cullings HM (S), Shimizu Y (EH), Ozasa K (EH), Kodama K (CS), Cologne JB (S)

Since somatic and germinal mutations are thought to promote cancer and non-cancer disease development through numerous mechanisms, one might infer that possible radiation-induced, germinal mutations among A-bomb survivors would increase risk of cancer and non-cancer diseases in the F_1 generation. Although several experimental studies have found fairly large effects of radiation on mutation rates in the F_1 generation, others have reported such induced mutations are very rare. So an

inquiry into the genetic effects of parental exposure in humans on the mortality and cancer incidence of their children is an important and timely undertaking.

The F_1 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. About 41,000 F_1 subjects could be included in the mortality and cancer incidence parental dose-response analyses after appropriate exclusions due to missing parental dose information, etc.

The follow-up period of the current analyses was May 1946 to December 2003. During this period, 1,745 persons died and we have confirmed 418 cases of solid tumor, 57 cases of hematopoietic tumor, and 1,270 cases of noncancer diseases (infectious diseases 260, respiratory diseases 164, digestive diseases 230, circulatory diseases 285, and other diseases 331) in the F_1 cohort. Disease mortality rates were examined in relation to individual paternal and maternal gonadal doses using a Poisson regression model adjusted by city, age, birth-year, parental age at exposure, and parental age at child's birth. To date, neither non-cancer nor cancer mortality is significantly associated with paternal or maternal dose, but the F_1 cohort is still young (mean age <50) and has not yet expressed most of their eventual disease risk.

We will submit a draft paper of updated F_1 mortality and incidence followed-up through 2007 and will begin analyses of cancer incidence follow-up through 2005 by estimating the population for the F_1 cancer incidence study in the same way as for the LSS cancer incidence analysis.

F_1 Studies Oral Presentations

❖ Ozasa K. Epidemiological follow-up studies on the atomic-bomb survivors. 70th Annual Meeting of the Japanese Society of Public Health, 19–21 October 2011, Akita (related to *Life Span Study* and *Tumor and Tissue Registries*)

❖ Shore RE, Ozasa K, Suyama A, Fujiwara S, Akahoshi M, Cullings HM, Kodama Y, Kodaira M, Hayashi T, Hamatani K. Recent findings by the Radiation Effects Research Foundation on the health of atomic bomb survivors and their offspring. International Symposium of Nagasaki University Global COE Program, 22 October 2011, Nagasaki (related to *Life Span Study*, *Adult Health Study*, *Immunology Studies*, and *Cytogenetics Studies*)

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, 2-92, 6-91 and 5-11, 9-88, 2-86, 29-60, 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), Suyama A (EN), Ozasa K (EH), Fujiwara S (CH), Akahoshi M (CN)

Thyroid cancer is one of the cancers most affected by radiation and shows increased incidence with increase of radiation dose among A-bomb survivors. In recent years, genetic studies of thyroid cancer have achieved significant progress mainly in terms of *RET/PTC* rearrangements and *BRAF* mutations. However, it is not the case that mechanisms behind thyroid cancer development and radiation effects on such mechanisms are fully understood. For elucidation of molecular mechanisms of thyroid cancer development among A-bomb survivors, collection and storage of thyroid tumor samples are indispensable. With introduction of thyroid ultrasound screening to health examinations conducted by RERF's Departments of Clinical Studies, a large number of thyroid tumors are now being detected. It is believed that future collection of as many thyroid tumor samples as possible, without regard to radiation dose or distinction between benign and malignant tumors, would contribute to mechanistic elucidation of thyroid cancer development and radiation carcinogenesis.

In accordance with research protocol (RP) 2-86 "Collection of surgically removed cancer tissues from A-bomb survivors: Special reference to thyroid and breast cancers," RERF has worked on collection and cryopreservation of fresh thyroid cancer tissues from A-bomb survivors and non-exposed controls, with an eye on their use for future molecular biological studies. The present RP, an addendum to the aforementioned RP 2-86, aims at preservation of fresh thyroid tumor tissues surgically removed from the Adult Health Study (AHS) subjects for future studies including gene analyses.

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), Hsu WL (S), Hida A (CN), Yamada M (CH), Katayama H (IT), Shimada Y, Fujiwara S (CH), Akahoshi M (CN), Suyama A (EN), Kasagi F, Ozasa K (EH)

Studies of cancer risks among A-bomb survivors have mainly focused on the relationship with A-bomb radiation. However, exposure to medical radiation, such as diagnostic X-ray exposure and radiotherapy, is on the increase globally. Under these circumstances, it is important to study the effects of medical radiation exposure in the LSS population. Those who underwent cancer radiotherapy after exposure to A-bomb radiation constitute a unique population, because they have been exposed to multiple radiation insults at different times (A-bomb and medical radiation). However, the combined effects of a prior exposure followed many years later by a subsequent exposure to ionizing radiation

have not been well addressed either by experimental or epidemiological studies. By determining the magnitude of cancer risks among those who were exposed to both atomic bomb and therapeutic radiation using a population-based epidemiological follow-up study, new light will be shed on how atomic-bomb radiation exposure modifies the risks of subsequent radiation exposure or vice versa.

In this study, we will follow up the (secondary) cancer incidence after radiotherapy and mortality from cancer and non-cancer diseases among 1,501 LSS subjects who were confirmed to have undergone radiotherapy based on three surveys during the 1960s to early 1980s. This study evaluates not only the effects of A-bomb radiation but also the effects of medical radiation and the combined effects of the two kinds of radiation.

A database has been created containing information on primary diseases for which therapy was conducted (malignant tumor and other diseases; kind of cancer in case of malignant tumor), age and date of radiotherapy, site of radiotherapy, and estimated radiation dose to major tissues and organs from radiotherapy, and analyses conducted.

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), Fujiwara S (CH), Cologne JB (S), Akahoshi M (CN), Niwa Y (R), Ozasa K (EH), Tsuge M, Chayama K

Objectives: To examine the contribution of insulin resistance to hepatocellular carcinoma (HCC) risk on the basis of the hypothesis that chronic inflammation due to radiation exposure may be involved in the development of HCC through insulin resistance, 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: Using stored sera collected before the diagnosis of HCC from the subjects of the nested case-control study based on RP 1-04, levels of blood cytokines related to chronic inflammation and insulin resistance, which are considered to be important factors contributing to acceleration of progression to HCC, will be measured to examine the contribution of such cytokines to HCC risk.

Study progress: Levels of each marker were stratified by tertile for the controls, and possible associations between cytokine levels and HCC risk were examined after adjusting for such confounders as HBV and HCV infection, lifestyle factors, and radiation dose.

Results and conclusions: Elevated IL-6 levels were significantly associated with HCC risk (P for trend = 0.019). This trend was observed even in the analysis without adjustment for confounders. These results suggest that IL-6 level is associated with elevated HCC risk independently of HBV and HCV infection, lifestyle factors, and radiation dose.

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), Suyama A (EN), Sekine I, Tokuoka S, Mabuchi K, Preston DL, Kodama K (CS), Ozasa (EH)

Breast cancer is one of major cancers in which a significant excess risk has been observed in relation to radiation, but no difference in histological distribution was observed in the past between exposed and control cases. Histological reviews according to the new classification system will permit a re-evaluation. The procedures of case collection were based on the guidelines of special cancer studies (RP 9-88). Of 2,116 possible breast cancer cases identified in the LSS cohort during 1950–2005, 1,732 cases were identified for histopathological review, and sample collection and pathologists' review were initiated. An investigation of "intrinsic subtypes" (defined by estrogen and progesterone receptors, and human epidermal growth factor receptor-2 [HER-2] status) to be determined by immunochemical staining will be conducted for applicable cases (RP 6-10). After completing the histological reviews, the risk of breast cancer for radiation will be evaluated within histological classes. More detailed analyses of the major histological classes will consider, as the numbers of cases permit, the shape of the dose-response relationship and effect modification by age at exposure, attained age, menopausal status, etc. Histological characteristic of breast cancer cases that were highly exposed to radiation will be summarized by the pathologists compared with those with low/no exposure. Histopathological review of about 800 cases has been completed, and preparation for immunochemical staining is currently under way.

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, Tokuoka S, Soda M (EN), Suyama A (EN), Kodama K (CS), Mabuchi K, Preston DL, Ozasa K (EH)

The excess risk of sarcomas of the soft tissues and bones associated with high therapeutic doses of radiation has been known for some time, but epidemiological data on the risk associated with radiation exposure at relatively low doses are very limited. The latest analysis of solid cancer incidence data of the LSS cohort provided, for the first time in this cohort, evidence of a significant dose response for broadly-classified sarcomas using the tumor registry-based incidence data. We are conducting a detailed, standardized pathology review of sarcomas in order to investigate the association between estimated radiation dose (DS02) and risk of sarcomas by histological types and sub-types. Soft-tissue and bone sarcomas occurring between 1957 and 2003 in the LSS cohort will be identified based on the guidelines of special cancer studies (RP 9-88). A panel of pathologists will review the specimens using the WHO's Histological Classification of Tumors of Soft Tissue and Bone (2002). Analyses will be performed to assess the radiation-related risk of sarcomas and to evaluate modifying effects, if any, of age, gender, and other factors. Among 4,318 possible cases,

160 were judged by pathologists to be in need of histological review. Of these, pathological specimens were collected for 127 cases, 88 of which were ascertained as malignant tumors of soft tissue and bone. Presently, clinical pictures are undergoing pathologic review and radiation risk is being assessed.

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

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

Among the LSS cohort, cases of cancer of the uterus will be ascertained based on the guidelines of special cancer studies (RP 9-88). Reported cases will be reviewed by a panel of pathologists for histological diagnoses according to the WHO Histological Classification of Female Genital Organs (2003). It has been decided that the case collection will start for corpus cancer. The procedures for cervical cancer are suspended because less relationship with radiation was shown in the incidence study and investigations on HPV infection require a heavier load for collecting specimens from collaborating hospitals.

Investigations on atypical hyperplastic endometrial lesions and cervical dysplastic lesions are also suspended because case collection through the passive surveillance mechanisms such as cancer registries cannot gather those cases without biases. Those early-stage lesions are thought be detected through cancer screening or incidental findings when patients visit clinics. Hence, the incidence rates of those lesions would depend on patients' medical service-seeking behaviors, which might differ by radiation dose because the frequency of other health conditions is associated with radiation exposure. Active surveillance such as uterine cancer screening among all LSS subjects would be required to avoid such biases, but there are no such systems. Therefore, in this study, the association between estimated radiation doses (DS02) and histologically confirmed corpus cancer and its sub-types will be investigated. Based on cancer registry information and other data, records of about 1,600 cases of possible uterine corpus cancer were reviewed. Of this total, about 400 cases were identified as requiring pathological review. Preparations for material collection are being made.

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

Fujiwara S (CH), Cullings HM (S), Ohishi W (CH), Hayashi T (R), Tahara E, Akahoshi M (CN)

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 factors—environmental factors (diet, smoking), host factors (age, *H. pylori* infection), and genetic factors—jointly affect the genesis of gastric cancer. We will investigate interactions between radiation exposure and these risk factors in developing gastric cancer.

Study methods: A nested case-control study was performed in the longitudinal cohort of atomic-bomb survivors using stored sera before diagnosis. Enrollees included about 300 cancer cases and 3 controls per case selected from cohort members matched on age, gender, city, time and type of serum storage, and radiation dose.

Study progress: A new research protocol is being developed for a collaborative study with Prof. Gerhard in Germany to investigate bacterial antigen using an immunoblotting that enables better identification of *H. pylori* than our method.

Results and conclusions: *H. pylori* infection, chronic gastritis, and smoking are all independent predictors of gastric cancer. Higher relative risks were noted with the diffuse type of gastric cancers, whereas much lower risks were noted with intestinal type of gastric cancers, after adjusting for these risk factors (Suzuki et al., *Cancer Epidemiology, Biomarkers and Prevention* 2007; 16:1224–8). The *LTA 252* genotype is associated with noncardiac gastric cancer of the diffuse type in Japan, and the genotype was an effect modifier for radiation dose (Suzuki et al., *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 (CH), Cologne JB (S), Akahoshi M (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: Reports based on mortality studies and tumor registries have shown that liver cancer occurrence increased with increase in radiation dose among A-bomb survivors, although such research failed to take into account hepatitis virus infection. Furthermore, previous studies conducted at RERF suggest possible existence of interaction between radiation dose and hepatitis virus infection. (Highly synergistic interaction between radiation exposure and persistent hepatitis C virus [HCV] infection was reported in HCC cases not accompanied by cirrhosis.) These issues may help explain differences in degree of HCC risk and radiation risk between Japanese populations and Western populations, in which HCV infection is uncommon, and hence are considered particularly crucial.

Study methods: Study subjects total 224 HCC cases and 644 controls, who were selected per case matched on sex, age, city, and time/methodology of sera storage and counter-matched on radiation exposure.

Study progress: A paper on HCC risk in relation to radiation and hepatitis virus infection was published.

Results and conclusions: After adjusting for alcohol consumption, smoking, and body mass index (BMI), the relative risk (RR) at 1 Gy of radiation exposure for HCC was 1.67 (95% CI: 1.22–2.35), while the RRs for HBV and HCV infection were 63 (20–241) and 83 (36–231), respectively. These estimates changed little when radiation and viral

effects were fit jointly. The RR at 1 Gy of radiation exposure for non-B, non-C HCC was 2.74 (95% CI: 1.26–7.04) with adjustment made for alcohol consumption, smoking, and BMI. These results indicated that radiation exposure is associated independently with increased risk of HCC, and that radiation exposure is a significant risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption, smoking, and BMI (Ohishi et al., *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), Sharp GB, Eguchi H, Nakachi K, Nakashima E (S), Izumi S, Fujiwara S (CH), Akahoshi M (CN), Key TJ, Stevens RG, Berrington A

Objectives: The purpose of this study is to characterize the joint effects of radiation and serum markers of hormonal status, oxidative stress, and phytoestrogen consumption on the development of breast and endometrial cancer.

Background and significance: Multiple breast cancer risk factors are known, but to what extent these risk factors play direct/indirect roles in radiation-induced cancer development remains unclear. Assessment of joint effects between radiation and other risk factors may contribute to elucidation of the mechanisms behind radiation-induced breast and endometrial cancer etiology.

Study methods: Serum measurements were conducted in the period 2007–2008 for 243 breast cancer cases with blood samples collected up to 30 years before breast cancer diagnosis and their 486 age-matched controls, who were selected by counter-matching on radiation dose. Statistical analysis and preparation of a manuscript are currently under way.

Study progress: Two papers regarding breast cancer risk in relation to radiation exposure and hormone or ferritin levels were published. Preparation of a manuscript on radiation risk for breast cancer with adjustment made for hormone levels is under way. We are currently preparing data to be shared with the Breast Cancer Association Consortium.

Results and conclusions: The results revealed that serum levels of estradiol and testosterone among postmenopausal women without cancer increased with increase of radiation dose (Grant et al., *Radiation Research* 2011; 176:678–87). The relative risks for breast cancer accompanying 1-log increase in ferritin level with and without adjustment made for radiation dose were 1.3 (95% CI: 1.0–1.7) and 1.4 (1.1–1.8), respectively. The joint effect of ferritin and radiation on postmenopausal breast cancer risk could not be assessed (Stevens et al., *Cancer Science* 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)

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

It has been reported that skin cancer incidence, especially nonmelanoma skin cancer, is increased by radiation among patients with radiotherapy and atomic-bomb survivors, and also reported that the effects of radiation upon skin cancer have a long latency period among atomic-bomb survivors. In the LSS cohort, there were a total of 700 skin cancer cases that developed skin cancer between 1958 and 1996 and were identified in accordance with the guidelines for special cancer studies (RP 9-88). Of the 700, 336 were first primary skin cancer cases, a designation that included malignant melanoma (n = 10), basal cell carcinoma (n = 123), squamous cell carcinoma (n = 144), Bowen's disease (n = 64), Paget's disease (n = 10), and other skin cancer cases (n = 15). ERRs were estimated assuming a linear dose response, and only basal cell carcinoma had a statistically significant positive dose response (ERR/Gy = 2.1, 95% confidence interval [CI] = 1.2–3.5, $P < 0.01$). The best fit dose-response model by Akaike information criteria (AIC) was a linear threshold model with an estimated dose threshold of 0.63 Gy (95% CI = 0.34–0.89). This model predicted an ERR/Gy and ERR at 1 Gy of 2.0 (95% CI = 0.69–4.3) and 0.74 (95% CI = 0.26–1.6), respectively. In conclusion, epidermal basal cells are sensitive to ionizing radiation, especially for people who were young at exposure. The threshold for a radiation dose response of basal cell carcinoma was estimated to be 0.63 Gy, lower than the 1.0 Gy reported in the previous study. A manuscript is in preparation and will be submitted in 2012.

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

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

The relationship between lymphoid malignancies and radiation exposure is complicated because of variability of diagnostic categories and uncertainty about the potential for radiation carcinogenesis for certain important diagnostic categories. On the other hand, advances in immunological studies of malignant lymphoma (ML) made it possible to use surface markers on tumor cells to distinguish B and T cells involved in malignant lymphomas, and a WHO classification scheme based on this method was introduced (2001).

The purpose of this study is to characterize the LSS sample in terms of risk for the broad spectrum of lymphoid malignancies by cell type in relation to radiation dose from the atomic bombings and other factors. In accordance with the procedures of case collection based on the guidelines of special cancer studies (RP 9-88), lymphoma cases are to be classified according to the WHO classification (published in 2001) using immunohistochemical studies into T-ML, B-ML, Hodgkin's lymphoma, or others. Further subtypes of B-ML will also be coded. The nested classification system by InterLymph will be applied to the confirmed cases.

Histopathological reviews identified 476 cases of ML, including 306 cases of B-cell origin (140 cases of diffuse large B-cell lymphoma), 130 cases of T-cell origin, and 13 cases of Hodgkin's lymphoma. Presently, characteristics of cases by individual dose are being reviewed and radiation risk assessed, with a paper currently being prepared.

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

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

Lung cancer is a well established late effect of radiation exposure in various irradiated populations, including the atomic-bomb survivors. The incidence analysis of the RERF tumor registry data for the period 1958–98 also observed a radiation-related increase in lung cancer risk. The ERR for radiation effects tends to increase with increasing age at exposure, while attained-age specific EAR estimates vary little with age at exposure. These patterns are the opposite of those seen for solid cancers as a group. However, several specific issues and questions remain, including the specificity of various cell types involved in radiation- versus smoking-related cancers, confounding effects and interaction of smoking and radiation exposure, and delineation of temporal trends with allowance made for age at exposure and attained-age effects. This RP was developed to address those questions and issues. In this study, definitive diagnoses of histological types will be conducted on the basis of the guidelines of special cancer studies (RP 9-88) regarding lung cancer incidence cases in the Life Span Study (LSS) for the period 1958–99. Radiation risks of lung cancer and its histological type-specific risks as well as smoking effects on such risks will be clarified through examination of smoking habits indicated in the LSS and AHS questionnaires conducted from 1965 through 1991.

Since a positive interaction between smoking and radiation risks was observed in 1,803 first primary lung cancer cases identified during the study period (Furukawa et al., *Radiation Research* 2010; 174:72–82), analysis of interaction between radiation risks and smoking in terms of each histological type was conducted and the results were accepted for publication by *Radiation Research*. Namely, both smoking and radiation exposure significantly increased the risk of each major lung cancer histological type. Smoking-associated excess relative risks were significantly larger for small-cell and squamous-cell carcinomas than for adenocarcinoma. The gender-averaged excess relative risks per 1 Gy of radiation (for never-smokers at age 70 after exposure at 30) were estimated as 1.49 (95% confidence interval: 0.1–4.6) for small-cell carcinoma, 0.75 (0.3–1.3) for adenocarcinoma, and 0.27 (0–1.5) for squamous-cell carcinoma. Under a model allowing radiation effects to vary with levels of smoking, the nature of the joint effect of smoking and radiation showed a similar pattern for different histological types, in which the radiation-associated excess relative risk tended to be larger for moderate-smokers than for heavy-smokers.

RP 2-92 Studies of ovarian tumor incidence among the RERF Extended Life Span Study cohort, 1950–87

Tokuoka S, Kawai K, Inai K, Shimizu Y (EH), Nakashima E (S), Tokunaga M, Soda M (EN), Mabuchi K, Kodama K (CS)

An increased risk of ovarian cancer among atomic-bomb survivors has been reported from an earlier site-specific study of ovarian cancer as well as from analyses of LSS mortality and incidence. No clear evidence exists for any specific histologic type being particularly associated with radiation exposure. The aim of this study is to examine and quantify the relationship between the development of malignant and benign ovarian tumors and exposure to atomic-bomb radiation in the LSS based on histologically confirmed cases. The present study extends the previous ovarian cancer series by seven years. A total of 601 ovarian tumors (182 malignant, 419 benign tumors) were histologically confirmed. Frequent histological types were "serous epithelial tumor" (48% for malignant and 37% for benign tumors), "mucinous epithelial tumors" (22% and 19%, respectively), "sex-cord stromal tumors" (7% and 12%, respectively), and "germ cell tumors" (3% and 28%, respectively), which was similar to other studies. There was a suggestion of variation in histological types of ovarian tumors in relation to radiation dose within the case series. The mucinous type seems to be less radiogenic than the other types. There was a significant apparent survival advantage for the mucinous type compared with the serous type. We confirmed this suggestion in the population-based incidence study. Within tumor types, there were no consistent differences in survival by radiation dose.

RP 6-91 Studies of 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)

Tokuoka S, Hayashi Y, Tsuda N, Tokunaga M, Yonehara S, Ito M, Sekine I, Mabuchi K, Furukawa K (S), Imaizumi M (CN), Ozasa K (EH), Kodama K (CS)

Thyroid cancer was one of the earliest solid cancers found to be increased in atomic-bomb survivors, and several thyroid cancer studies have been conducted among survivors over the last 40 years. This study includes pathological ascertainment for both benign and malignant thyroid tumor cases in the LSS cohort in accordance with the guidelines of special cancer studies (RP 9-88) for the period 1958–1995, as well as for malignant tumor cases in the local cancer registry and the tissue registry for the extension period through 2005. A total of 1,074 thyroid tumor cases were identified for the period through 1995. Histological verification was conducted for 1,036 (96%) of these cases, 697 with malignant tumors and 339 with benign tumors. Most of the malignant thyroid carcinomas were of the papillary type (95%), followed by the follicular type (2%), malignant lymphoma (1%), medullary (<1%), and undifferentiated carcinoma (1%). The 663 papillary carcinomas included 325 micro-carcinomas detected primarily at autopsy. A total of 160 thyroid cancer cases were extracted, and pathologically definitive diagnosis was conducted for 136 of these cases for the extension period through 2005.

During the follow-up period of 1958 to 2005, 371 thyroid cancer cases (excluding those with microcarcinoma with a diameter <10 mm) were identified as a first primary among the eligible subjects. Using a linear dose-response model, 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. About 36% of the thyroid cancer cases among those exposed prior to age 20 were estimated to be attributable to radiation exposure. While the magnitude of the excess risk has decreased with increasing attained age or time since exposure, the excess thyroid cancer risk associated with childhood exposure has persisted for more than 50 years after exposure. These results were submitted to a scientific journal.

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

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

These guidelines are intended to simplify the preparation of research plans for site-specific cancer incidence studies and provide uniformity in basic study design and operation. The guidelines state that case ascertainment would be undertaken through the Hiroshima and Nagasaki tumor registries supplemented by an extended case-finding search. A panel of collaborating pathologists specializing in pertinent areas would achieve agreement on histopathological classification and verification. Tumor types are to be classified using internationally accepted tumor classification systems. RERF and/or other participating epidemiologists and statisticians should perform data analyses. Under these guidelines, the following site-specific cancer incidence studies are currently being undertaken: Skin cancer (RPs 2-91 and 2-02), thyroid tumor (RPs 6-91 and 5-11), breast cancer (RPs 5-08 and 6-10), ovary cancer (RP 2-92), uterine cancer (both cervical and endometrial) (RP 1-06), lung cancer (RP 1-94), soft tissue and bone tumors (RP 4-07), and lymphoid malignancies (RP 3-94). The majority of the current site-specific studies are expected to be completed in the next three to four years.

RP 2-86 Collection of surgically removed cancer tissues from A-bomb survivors: Special reference to thyroid and breast cancers

Hamatani K (R), Taga M (R), Imai K (R), Nakachi K, Kusunoki Y (R)

Based on this protocol that is concerned with the collection and cryopreservation in liquid nitrogen of possibly radiation-induced fresh thyroid and breast cancer tissues to be used as a resource for future molecular oncology studies, we have collected tissue specimens thus far. However, this protocol is dated. Since a new addendum to this RP submitted by the Department of Clinical Studies was approved, the collection of thyroid cancer tissue specimens will be conducted by the Department of Clinical Studies based on the new addendum RP (RP 7-11). This RP 2-86 was terminated in FY2011.

RP 29-60 Detection of leukemia and related disorders

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

This case-finding program, known as the leukemia registry, was started in 1948, with the collaboration of hematologists and physicians involved in the diagnosis and treatment of leukemia patients in Hiroshima and Nagasaki. With the recent improvement in the Hiroshima and Nagasaki tumor registries, leukemia case ascertainment currently relies primarily on the tumor registries' activities. In the mid-1980s, more than 60% of leukemia cases in the leukemia registry were reclassified using modern diagnostic criteria and nomenclature, most notably the French-American-British (FAB) classification schema for the acute leukemias.

The risk estimation of myelodysplastic syndrome (MDS) conducted in Nagasaki showed a significant linear dose response. The excess relative risk (ERR) at 1 Gy was 4.27 (95% CI, 1.63–9.48) (Iwanaga et al., *Journal of Clinical Oncology* 2011; 29:428–34).

Comprehensive analyses based on the totality of data between 1950 and 2001 were conducted in collaboration with the Department of Statistics, and a manuscript was submitted to a scientific journal. Leukemias excluding chronic lymphocytic leukemia and adult T-cell leukemia showed nonlinear dose response, with considerable differences in risk by time since exposure and age at exposure. Radiation effects differed between men and women for non-Hodgkin's lymphomas, with Hodgkin's lymphoma and multiple myeloma showing no evidence of radiation risk.

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 Asian populations, Project 4: Diet and mortality among Asians

Grant EJ (EH), Ozasa K (EH), Suyama A (EN), Fujiwara S (CH), Akahoshi M (CN), Shore RE (D)

RERF has successfully begun collaboration with the Asia Cohort Consortium (ACC) under RP-A3-10. Analyses for the first project on lifestyles and cancers of the small intestine are well underway and a manuscript is expected in 2011. The ACC has approved three new projects and invites RERF to participate. The previous RP stipulated that participation in additional ACC projects requires RERF's explicit approval. In accordance with those terms, this research proposal seeks approval for three ACC projects of high scientific merit. Conclusions generated from these projects will be beneficial to the research community and may be beneficial to Asians around the world.

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

Cologne JB (S), Furukawa K (S), Langholz B, Izumi S, Fujii Y, Hsu WL (S), Grant EJ (EH), Kopecky KJ, Ohishi W (CH), Neriishi K, Fujiwara S (CH), Nakachi K, Cullings HM (S)

Objectives: To develop and evaluate methods for analyzing complex causal models—including mediation (effects of intermediate or intervening variables)—in counter-matched (exposure-based control selection) nested case-control data.

Background: Analyses of radiation risk and transportation of risk estimates to populations other than the atomic-bomb

survivors require consideration of risk factors other than radiation. Some of these factors are radiation related—so-called intermediate risk factors, or mediators—and as such require special methods of analysis. Methods for modeling intermediate risk factors with cohort data, such as structural equations models, are not immediately applicable to data from nested case-control studies. More traditional methods—such as path analysis—may be applied, but do not yield valid estimates of risk.

Design and methods: We will attempt to implement several approaches: path analysis, a regression substitution method, and a full likelihood method. After developing and successfully implementing the methods, we will compare results and assess statistical efficiency via simulation. The first step in evaluation of the methods will be to compare them to standard methods of analyzing complex causal models with cohort data, where the nested case-control sampling design is not a factor.

Subjects and data: The subjects of this research are those already defined under existing RERF RPs. The proposed research involves no new data collection. In terms of personal information protection, RERF procedures for data sharing (collaborative study involving the use of available data) and data-masking procedures will be adhered to.

Time line and expected publications: The research is expected to require about two years. The first year will be devoted to development of the methods and comparison among methods using cohort data. The second year will be devoted to focusing on the likelihood approach and comparing the methods using nested case-control data. Publications produced during the first year should include at least one methodological paper as well as finalization of the AHS liver-cancer cohort study analysis. Publications produced during the second year should include at least one methodological paper, one applied paper on methodological comparisons, and at least two papers based on analyses of joint effects of radiation and intermediate risk factors in the AHS nested case-control studies of breast cancer and liver 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 (EN), Fujiwara S (CH), Akahoshi M (CN), Shore RE (D)

This proposal outlines the framework by which RERF would participate in the Asian Cohort Consortium (ACC). Additionally, a proposal for the first collaborative project with the ACC is described.

The ACC is a collaborative cohort created in 2008 by researchers in Asia and the U.S.A. The ACC combines Asian cohorts from many institutions to study the relationships between environmental exposures and the etiology of disease (among other goals) with high statistical power. As RERF manages one of the largest and longest-running cohort studies in the world, RERF data would be a valuable addition to the ACC. RERF would also be able to propose ACC research projects that utilize the statistical power of the combined cohort.

RERF would supply lifestyle information collected from AHS and LSS questionnaires along with cancer incidence data for the current project. Additional projects would require an addendum to the initial RP and may require some additional data (e.g., death due to a particular disease). Data sent to the ACC would be individual data, suitably anonymized to ensure privacy. To avoid biasing results due to ionizing exposure, data would be restricted to cohort members with less than 100 mGy kerma exposures. Released data would be sent to the ACC data coordinating center wherein it would be analyzed by Center statisticians in response to written requests. Data cannot be released to other researchers for analyses nor can any project occur with RERF data without RERF's explicit authorization. An RERF representative would be expected to attend ACC meetings twice per year.

Since the ACC invited RERF to participate in a proposed project entitled "Tobacco smoking, alcohol drinking, body mass index, and risk of rare cancers," this research protocol was prepared to provide the group with materials for the project. This study's analyses were completed and a paper was accepted by the *Annals of Oncology*.

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

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

The purpose of this project is to improve our understanding of radiogenic thyroid cancer by conducting an updated and expanded pooled analysis of 16 studies that have a reasonable number of thyroid cancers in exposed individuals and individual thyroid dose estimates. While the results from the previous pooling study of thyroid cancer and radiation risk remain the most important summary of epidemiologic information on thyroid cancer risks from exposure to external radiation to date, questions remain regarding the risk associated with adult exposure, the shape of the dose-response curve at low and high doses, the effect of fractionated dose, the risk associated with specific thyroid cancer histological types, the effect of gender, and the relationship with attained age and time since exposure. Since the prior pooling study (Ron et al., *Radiation Research* 1995; 141:259–77), a considerable amount of new data has been generated, so a new pooled analysis will significantly add to what is known about radiation-related thyroid cancer.

The general approach of this collaborative study is to examine how the age-specific thyroid tumor risks depend on variables of interest. To carry out these analyses, data are cross-classified by age (or age of exposure), calendar period, study population, dose, and other variables. For each cell, the number of events and accumulated person years are recorded and the person-year weighted mean radiation doses calculated. The disease rates are then analyzed using Poisson regression.

This RP was approved in October 2008. Data have been sent to the U.S. National Cancer Institute where the pooled data are being analyzed. The first step of this collaborative study—analysis of thyroid cancer after radiotherapy for childhood cancer—has been completed, and a dataset including RERF's data for the analysis in the next step is being prepared.

RP-A5-08 Esophageal and gastric cancers: Patterns and predictors of risk in Hiroshima and Nagasaki, Japan

Kennedy BS, Mabuchi K, Chow WH, Kasagi F, Suyama A (EN), Shimizu Y (EH), Sugiyama H (EH), Soda M (EN), Sakata R (EH), Grant EJ (EH), Cologne JB (S), Cullings HM (S), Yamada M (CH)

The patterns and predictors of esophageal and gastric cancer risk are being examined within the Hiroshima and Nagasaki populations using several approaches. First, the temporal trends in age-adjusted incidence rates of esophageal and gastric cancers among males and females are being investigated using the tumor registries for Hiroshima and Nagasaki. Trends will be assessed by anatomic location as well as by histological cell type. Second, the effects of ionizing radiation on the risk of esophageal and gastric cancers among atomic-bomb survivors are being studied using the extended Life Span Study (LSS) population. Similar to the temporal trend analyses, this component of the study will examine the association of radiation dose with esophageal and gastric cancer risk by anatomic location and histological cell type, while controlling for potential confounders (e.g., age of exposure, attained age, time since exposure). Third, using supplemental information gathered on the atomic-bomb survivors, the predictors of esophageal and gastric cancers are being investigated, with an emphasis on potentially modifiable risk factors (e.g., tobacco smoking, alcohol consumption, body mass index, dietary patterns). Further, the population attributable risk (PAR) for these factors will be estimated in order to quantify their public health impact.

Dr. Kennedy visited RERF as a Beebe fellow between January and June of 2008. At the end of his visit, Dr. Kennedy presented preliminary results of his research. He tracked the gastric cancer and esophageal trends in the Hiroshima population-based registry and estimated the radiation risks of specific sub-types of esophageal cancers. Additional analyses and a manuscript are in preparation. A manuscript is due in 2012.

Special Cancer Studies Publications

RERF Reports (RR)

♦ Grant EJ, Neriishi K, Cologne JB, Eguchi H, Hayashi T, Geyer SM, Izumi S, Nishi N, Land CE, Stevens RG, Sharp GB, Nakachi K: Associations of ionizing radiation and breast cancer-related serum hormone and growth factor levels in cancer-free female A-bomb survivors. Radiation Research 2011 (June); 176(5):678–87. **(RR 6-10)**

[Abstract] Levels of exposure to ionizing radiation are increasing for women worldwide due to the widespread use of CT and other radiologic diagnostic modalities. Exposure to ionizing radiation as well as increased levels of estradiol and other sex hormones are acknowledged breast cancer risk factors, but the effects of whole-body radiation on serum hormone levels in cancer-free women are unknown. This study examined whether ionizing radiation exposure is associated with levels of serum hormones and other markers that may mediate radiation-associated breast cancer risk. Serum samples were measured from cancer-free women who attended biennial health examinations with a wide range of past radiation exposure levels (N = 412, ages 26–79). The women were selected as controls for separate case-control studies from a cohort of A-bomb survivors. Outcome measures included serum levels of total estradiol, bioavailable estradiol, testosterone, progesterone, prolactin, insulin-like growth factor-1 (IGF1), insulin-like growth factor-binding protein 3 (IGFBP-3), and ferritin. Relationships were assessed using repeated-measures regression models fitted with generalized estimating equations. Geometric mean serum levels of total estradiol and bioavailable estradiol increased with 1 Gy of radiation dose among samples collected from postmenopausal women ($17\%_{1Gy}$, 95% CI: 1%–36% and $21\%_{1Gy}$, 95% CI: 4%–40%, respectively), while they decreased in samples collected from premenopausal women ($-11\%_{1Gy}$, 95% CI: –20%–1% and $-12\%_{1Gy}$, 95% CI: –20%– –2%, respectively). Interactions by menopausal status were significant (P = 0.003 and $P < 0.001$, respectively). Testosterone levels increased with radiation dose in postmenopausal samples ($30.0\%_{1Gy}$, 95% CI: 13%–49%) while they marginally decreased in premenopausal samples ($-10\%_{1Gy}$, 95% CI: –19%–0%) and the interaction by menopausal status was significant ($P < 0.001$). Serum levels of IGF1 increased linearly with radiation dose ($11\%_{1Gy}$, 95% CI: 2%–18%) and there was a significant interaction by menopausal status (P = 0.014). Radiation-associated changes in serum levels of estradiol, bioavailable estradiol, testosterone and IGF1 were modified by menopausal status at the time of collection. No associations with radiation were observed in serum levels of progesterone, prolactin, IGFBP-3 or ferritin.

♦ Ohishi W, Fujiwara S, Cologne JB, Suzuki G, Akahoshi M, Nishi N, Tsuge M, Chayama K: Impact of radiation and hepatitis virus infection on risk of hepatocellular carcinoma. Hepatology 2011 (April); 53(4):1237–45. **(RR 7-10)**

[Abstract] In cohort studies of atomic bomb survivors and Mayak nuclear facility workers, radiation-associated increases in liver cancer risk were observed, but hepatitis B virus (HBV) and hepatitis C virus (HCV) infections were not taken strictly into account. We identified 359 hepatocellular carcinoma (HCC) cases between 1970 and 2002 in the cohort of atomic bomb survivors and estimated cumulative incidence of HCC by radiation dose. To investigate contributions of radiation exposure and hepatitis virus infection to HCC risk, we conducted a nested case-control study using sera stored before HCC diagnosis in the longitudinal cohort of atomic bomb survivors. The study included 224 HCC cases and 644 controls that were matched to the cases on gender, age, city, and time and method of serum storage, and countermatched on radiation dose. The cumulative incidence of HCC by follow-up time and age increased significantly with radiation dose. The relative risk (RR) of HCC for radiation at 1 Gy was 1.67 (95% confidence interval: 1.22–2.35) with adjustment for alcohol consumption, body mass index (BMI), and smoking habit, whereas the RRs for HBV or HCV infection alone were 63 (20–241) and 83 (36–231) with such adjustment, respectively. Those

estimates changed little when radiation and hepatitis virus infection were fit simultaneously. The RR of non-B, non-C HCC at 1 Gy was 1.90 (1.02–3.92) without adjustment for alcohol consumption, BMI, or smoking habit and 2.74 (1.26–7.04) with such adjustment. ***Conclusion:*** These results indicate that radiation exposure and HBV and HCV infection are associated independently with increased HCC risk. In particular, radiation exposure was a significant risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption, BMI, or smoking habit.

♦ Stevens RG, Cologne JB, Nakachi K, Grant EJ, Neriishi K: Body iron stores and breast cancer risk in female atomic bomb survivors. Cancer Science 2011 (December); 102(12):2236–40. **(RR 5-11)**

[Abstract] Iron can be a potent pro-oxidant and, on this basis, elevated body iron may increase the risk of cancer. Although epidemiological evidence is mixed, there is overall support for this possibility. In addition, because of this same oxidative capacity, body iron levels may alter radiation sensitivity. In the present study, a nested case-control study of breast cancer was conducted in Japanese atomic bomb survivors. Stored serum samples from the Adult Health Study cohort were assayed for ferritin levels and joint statistical analyses were conducted of ferritin and radiation dose on the risk of breast cancer. Serum ferritin is the best feasible indicator of body iron levels in otherwise healthy people. A total of 107 cases and 212 controls were available for analysis. The relative risk (RR) of breast cancer for a 1 log unit increase in ferritin was 1.4 (95% confidence interval 1.1–1.8). This translates to an RR of 1.64 comparing high and low values of the interquartile range among controls (58 and 13.2 ng/mL, respectively). The results support the hypothesis that elevated body iron stores increase the risk of breast cancer. However, the study was inconclusive regarding the question of whether body iron alters radiation-induced breast cancer risk.

Other Journal Publications

♦ Kodama K, Ozasa K, Okubo T: Radiation and cancer risk in atomic-bomb survivors. Journal of Radiological Protection 2012 (March); 32(1):N51–4. (Special section: Selected articles from Proceedings of the International Expert Symposium in Fukushima: Radiation and Health Risks, 11–12 September 2011) (related to *Life Span Study* and *Tumor and Tissue Registry*)

♦ Ohishi W, Fujiwara S, Cologne JB, Chayama K: Effects of radiation exposure and lifestyle on risk of non-B, non-C hepatocellular carcinoma. Shokaki Naika [Japanese Journal of Gastroenterology] 2011 (October); 53(4):453–8. (Japanese)

Manuscripts in Press

⌘ Cologne JB: Commentary on "Development of a prediction model for 10-year risk of hepatocellular carcinoma: The Japan Public Health Center-based Prospective Study Cohort II" by Michikawa et al. Preventive Medicine.

⌘ Kodama K, Ozasa K, Katayama H, Shore RE, Okubo T: Radiation effects on cancer risks in the Life Span Study cohort. Radiation Protection Dosimetry.

Special Cancer Studies Oral Presentations

❖ Ohishi W, Fujiwara S, Cologne JB, Akahoshi M, Tsuge M, Chayama K. Risk factors of non-B, non-C hepatocellular carcinoma: A nested case-control study. 47th Annual Meeting of the Japan Society of Hepatology, 2–3 June 2011, Tokyo

❖ Cologne JB, Langholz BM, Imai K, Misumi M, Yoshida K, Hayashi T, Nakachi K. Multiphase analysis of case-cohort data in the RERF cancer and immunogenome study. FY2011 Annual Meeting of the Japanese Biometric Society, 2–3 June 2011, Osaka

❖ Stevens RG, Cologne JB, Nakachi K, Grant EJ, Neriishi K, Suyama A, Ozasa K, Akahoshi M, Fujiwara A. Body iron stores and breast cancer risk in female atomic bomb survivors. Scientific Meeting for Cancer Prevention 2011 Sapporo, 20–21 June 2011, Kyoto

❖ Furukawa K. Radiation, smoking and lung cancer: Epidemiological findings in the Life Span Study. The Joint NASA-NCI Workshop on Lung Cancer Risk Resulting from Space and Terrestrial Radiation, 27–28 June 2011, Bethesda, Maryland, USA

❖ Hsu WL, Prentice RL, Soda M, Ozasa K. The incidence of leukemia among atomic bomb survivors: 1950–2001. 14th International Congress of Radiation Research, 28 August–1 September 2011, Warsaw, Poland

❖ Furukawa K, Funamoto S. Multiple imputation for incomplete time-dependent variables: an application to analysis for smoking histories and lung cancer incidence among atomic-bomb survivors. 22nd General Meeting of the Japan Epidemiological Association, 26–28 January 2012, Tokyo

❖ Sugiyama H, Misumi M, Tokuoka S, Grant EJ, Sakata R, Shimizu Y, Ozasa K. Skin cancer incidence among atomic bomb survivors in Hiroshima and Nagasaki between 1958 and 1996. 22nd General Meeting of the Japan Epidemiological Association, 26–28 January 2012, Tokyo

❖ Grant EJ, Cologne JB, Sharp GB, Nakashima E, Nakachi K, Neriishi K. Radiation risk of breast cancer after adjustment for serum hormone levels among atomic bomb survivors. International Symposium on Natural Radiation Exposure and Low Dose Radiation Epidemiology Studies, 29 February–3 March 2012, Hirosaki

Research Protocol 18-61
Tumor and Tissue Registries, Hiroshima and Nagasaki

Note that RERF studies related to the tumor and tissue registries include RPs 7-11, 5-11, 6-10, 2-09, 1-09, 5-08, 4-07, 1-06, 2-04, 1-04, 6-02, 2-02, 3-94, 1-94, 2-92, 6-91, 2-91, 9-88, 2-86, 29-60, A3-11, A5-10, A3-10, A12-08, and A5-08 (all discussed under Special Cancer Studies*).*

RP 18-61 Tumor and tissue registry study in Hiroshima and Nagasaki

Soda M (EN), Ozasa K (EH), Sugiyama H (EH), Grant EJ (EH), Suyama A (EN), Kasagi F, Shimizu Y (EH), Katayama H (IT), Kodama K (CS)

This research protocol constitutes the framework for the operation of the Hiroshima and Nagasaki tumor registries, which provide for the systematic collection and management of tumor data in the populations of the cities and prefectures of Hiroshima and Nagasaki. These registries had been undertaken under the auspices of each city's medical association with technical and managerial support provided by RERF in Hiroshima since 1957 and in Nagasaki since 1958. Case ascertainment and data collection are based on the abstraction of medical records conducted by trained personnel at regularly scheduled hospital visits, producing high-quality incidence data. In addition, tissue registries to collect and archive tumor tissue samples were started in 1973 under the auspices of the Hiroshima Prefectural Medical Association and in 1974 by the Nagasaki City Medical Association (this registry presently is conducted in the southern part of the prefecture by the Nagasaki Prefectural Medical Association). Pathology slides and pathology reports are collected for each tumor, malignant or benign. Having a large number of pathology slides collected and stored at one location greatly facilitates the conduct of our many site-specific studies involving pathology reviews of cases diagnosed over many years. In 1984, the Nagasaki prefectural government took over the local cancer registry from the city of Nagasaki, and in 2002 the Hiroshima prefectural government established a local cancer registry. Both governments currently run their own respective registries. In 2005, the abstraction-based local cancer registry in the city of Hiroshima and its surroundings was placed under the auspices of the Hiroshima city government. The databases of the Hiroshima prefectural and city cancer registries were consolidated in accordance with rules for sharing of materials. The registry information is linked with the master list of the members of RERF's major cohort samples (LSS, *in utero*, and F_1) with the permission of each registry and serves as the source of RERF cancer incidence data.

Case collection by notifications and death certificates is almost completed through 2010 in both Hiroshima and Nagasaki. On-site record abstraction is nearly complete through 2009 in both Hiroshima and Nagasaki. However, due to the spread of hospital cancer registration, abstraction at major hospitals has been halted after abstraction of cases through 2005. As for tissue registry, case collection and registration have been completed through 2008 in the prefectures of Hiroshima and Nagasaki. The linkage between tumor and tissue registry information and RERF subjects has been completed through 2005.

The Hiroshima and Nagasaki tumor registry data, complemented by tissue registries both qualitatively and quantitatively, have regularly been included in the publications and website "Cancer Incidence in Five Continents" compiled by the International Association of Cancer Registries (IACR) and the International Agency for Research on Cancer (IARC) in Lyon, France, as the data satisfy their accuracy standards. This year, data between 2003 and 2007 were provided for use in the latest version of the reference work, Volume X. Data on childhood cancer between 1990 and 2007 were also provided for use in the "International Incidence of Childhood Cancer, Volume 3." In addition, data are provided every year to the National Cancer Center of Japan for compilation of the publication "Monitoring of Cancer Incidence in Japan." The population-based tumor registries in both Hiroshima and Nagasaki will continue to provide up-to-date cancer incidence and other information for the local communities.

Tumor and Tissue Registries Publications

RERF Report (RR)

♦ Samartzis D, Nishi N, Hayashi M, Cologne JB, Cullings HM, Kodama K, Miles EF, Funamoto S, Suyama A, Soda M, Kasagi F: Exposure to ionizing radiation and development of bone sarcoma: New insights based on atomic-bomb survivors of Hiroshima and Nagasaki. Journal of Bone and Joint Surgery-American Volume 2011 (June); 93:1–8. **(RR 9-08)** (refer to abstract in *Life Span Study* Publications)

Other Journal Publication

♦ Kodama K, Ozasa K, Okubo T: Radiation and cancer risk in atomic-bomb survivors. Journal of Radiological Protection 2012 (March); 32(1):N51–4. (Special section: Selected articles from Proceedings of the International Expert Symposium in Fukushima: Radiation and Health Risks, 11–12 September 2011) (related to *Life Span Study* and *Special Cancer Studies*)

Manuscripts in Press

⌘ Kodama K, Ozasa K, Katayama H, Shore RE, Okubo T: Radiation effects on cancer risks in the Life Span Study cohort. Radiation Protection Dosimetry.

⌘ Samartzis D, Nishi N, Cologne JB, Hayashi M, Kodama K, Miles EF, Funamoto S, Suyama A, Soda M, Kasagi F: Ionizing radiation exposure and the development of soft tissue sarcomas in atomic-bomb survivors. Journal of Bone and Joint Surgery-American Volume. (related to *Life Span Study*)

Tumor and Tissue Registries Oral Presentations

❖ Soda M. Possibility of secondary prevention (cancer screening) from viewpoint of clinical epidemiologist/cancer registry specialist. 20th Annual Meeting of the Japan Preventive Nephrology and Urology Meeting, 7–8 July 2011, Maebashi

❖ Sugiyama H, Ozasa K, Tsumura H, Arita K, Yasui W, Kajihara H. Effects of differences in methods of collecting local cancer registry data on completeness and information registered for respective items. 20th Annual Meeting on

Japanese Association of Cancer Registries, 14–15 September 2011, Chiba

❖ Ozasa K. Epidemiological follow-up studies on the atomic-bomb survivors. 70th Annual Meeting of the Japanese Society of Public Health, 19–21 October 2011, Akita (related to *Life Span Study* and *F_1 Studies*)

❖ Nagayoshi A, Yoshida M, Inada Y, Hayama S, Yamakawa S, Yamada T, Soda M, Suyama A. Ascertainment of vital status by acquisition of residence certificates. 49th Annual Meeting of the Nagasaki Public Health Association, 2 March 2012, Nagasaki

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 (CS), Kodama Y (G), Tomonaga M, Iijima Y, Mine M, Okumura Y, Kodama K (CS), Cullings HM (S), Akahoshi M (CN)

The purpose of the study is to investigate the possibility that Nagasaki survivors who were exposed in factories have overestimated doses. Twenty nine tooth samples donated from 23 survivors in Nagasaki were measured to estimate individual doses. ESR-estimated dose and the DS02 estimated dose (eye dose) were generally in agreement except for one survivor: he was exposed to radiation in a factory, and his ESR estimate was nearly one half of that estimated from DS02. Because the number of tooth samples examined was limited, it was not possible to derive a conclusion about a possible dose bias in DS02 estimates.

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 (CS), Kodama Y (G), Wada T, Rühm W, Wallner A

The purpose of the study is to estimate individual doses using tooth enamel by the ESR technique and to compare the results with DS02 doses, and with chromosome aberration frequencies in lymphocytes, from the same donors. We measured 5 molars in this fiscal year. Thus far, a total of 249 molars donated by 194 Hiroshima AHS participants were measured. Of them, 112 participants had FISH and/or Giemsa data. The individual ESR doses will compare the results with DS02 dose, and with chromosome dose from the same donors.

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 (CS), Fujiwara S (CH), Akahoshi M (CN)

The collection of teeth from A-bomb survivors is the first part of the project to estimate the radiation doses of A-bomb survivors using the ESR method. We started

Table. Cumulative number of teeth

DS02 kerma dose (total cGy)	Total cases collected	
	Hiroshima	Nagasaki
0	324 (19)*	16 (1)
1–30	382 (16)	2 (2)
31–100	317 (0)	5 (0)
101 and over	270 (0)	14 (0)
Not available	257 (10)	21 (5)
	Total 1,550 (45)	Total 58 (8)

* Numbers in parentheses represent cases collected during April 2011–March 2012.

collecting teeth from Hiroshima AHS participants in April 1987, and in November 2004 from Nagasaki AHS participants. As of March 2011, we have collected 1,550 tooth samples from Hiroshima AHS participants and 58 from Nagasaki AHS participants (Table). Nearly 20% of the collected samples are suitable for ESR measurement.

RP 18-59 Shielding survey and dosimetry study

Cullings HM (S), Grant EJ (EH), Watanabe T (EH), Funamoto S (S), Sakata R (EH)

Since well-characterized survivor dose estimates are essential to RERF research, the purpose of this protocol is to refine estimates of tissue kerma and organ-specific absorbed doses for Hiroshima and Nagasaki atomic-bomb survivors and to characterize the uncertainties in these estimates. Workers in the Department of Epidemiology, under the aegis of the RERF Dosimetry Committee, completed the process of improving estimated map coordinates for survivors with shielding histories by locating their neighborhood drawings on mosaics of geometrically corrected pre-bombing aerial photographs of both cities, and the Statistics Department began work on using digital terrain elevation data to provide elevation and terrain shielding data for the new locations to complete a set of revised dose calculations.

Regarding doses from residual radioactivity, Dr. Cullings continued to work on estimation of individual survivor doses from external exposure to gamma rays in 1) known areas of radioactive fallout and 2) worst-case scenarios of early entry on specific days into areas near the hypocenter affected by soil activation. He gave a related presentation at the annual meeting of the Health Physics Society in Sacramento, California. In response to assertions from outside investigators, the Department of Epidemiology prepared a release of information on individual survivors' data on reported exposure to "fallout rain." Dr. Sakata began analyses of those data in regard to health outcomes and Dr. Cullings began spatial analyses of the data to support that work, including analyses of any patterns in the locations of survivors with missing data. Dr. Ozasa also prepared a detailed critique of an early report from 1972 by an investigator from Oak Ridge National Laboratories and a member of the ABCC Field Investigations Section that alleged a connection between reported exposure to "fallout rain" and symptoms of acute radiation exposure such as epilation.

Dr. Cullings began collaborative work with Dr. D.A. Pierce (Oregon, U.S.A.) on dose-dependent weighting for neutron doses and prepared a related manuscript. Several collaborations related to estimating and correcting for dose uncertainty continued with external investigators, including work by Dr. Pierce on evaluating the effects of simulated dose errors on risk estimation, and a first paper by Dr. C.Y. Wang (Fred Hutchinson Cancer Research Center, Washington State, U.S.A.) under his NIH (National Institute of Health) grant to develop functional methods for adjusting for dose errors.

RERF held a special international workshop in March, 2011, on the potential for improving the organ dose calculations of dosimetry system DS02, and the proceedings were published in a special issue of the journal *Radiation Protection Dosimetry* that included fourteen full papers on related topics, including four by RERF authors.

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

Carter R, Miller A, Tekwe C, Cullings HM (S), Neriishi K, Kodama Y (G), Kusunoki Y (R), Nakamura N (CS), Ozasa K (EH), Imaizumi M (CN), Cologne JB (S), Nakashima E (S), Misumi M (S), Funamoto S (S), Stram DO, Douple EB (ACR)

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 assumption is incorrect, then the method leads to biased estimates of dose-response relationships. Previous applications of this substitution method have not made use of information on bio-dosimeters measured in a number of AHS subjects, which could be used to avoid 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 incorporate the information on bio-dosimeters and is the focus of this work. The need to assume a known parameter is obviated by the availability of observations on one or more instrumental variables. The data will consist of the existing AHS laboratory and clinical measurements and DS02 dose estimates, and diagnostic/disease incidence data, supplemented by various bio-dosimeters ascertained in the Departments of Genetics and Radiobiology/Molecular Epidemiology.

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 are under RERF review.

Results: 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.

Conclusions: 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 certain assumptions related to the parametric form of the error model.

RP-A4-10 Semi-parametric methods using radiation exposure and chromosome aberration data in atomic-bomb survivor studies

Wang CY, Cullings HM (S), Song X, Ozasa K (EH), Soda M (EN), Kodama Y (G), Davis S, Kopecky KJ

This research will develop semi-parametric statistical methods to adjust for the effects of radiation dose measurement error on the estimation of radiation dose responses for health effects in survivors of the atomic bombings of Hiroshima and Nagasaki. Although some measurement error methods have been applied to adjust for radiation measurement error in RERF data, further development of semi-parametric or non-parametric 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. An important aspect of this work is that the measurement error standard deviation will not have an assumed value, but rather will be estimated from the data, even though the data do not include replicate measurements or estimates of radiation doses. A first paper under this RP is in preparation.

Atomic-bomb Dosimetry Studies Publications

RERF Reports (RR)

♦ Chen J, Kerr GD, Cullings HM: A comparison of organ doses between mathematical and voxel phantoms with the DS02 photon fluences. Radiation Protection Dosimetry 2012 (January); 149(1):49–55. **(RR 22-11)**

[Abstract] The purpose of this study is to quantify dosimetric differences if modern sophisticated voxel phantoms were used in the dosimetry system DS02 rather than the mathematical phantoms. The mathematical models (ADAM and EVA) and voxel phantoms (REX and REGINA) developed in Germany allow a useful comparison as they are very close in body weight, body height and organ masses. In this study, organ doses are calculated with published fluence-to-absorbed-dose conversion coefficients derived from those two model sets for unidirectional plane beam irradiation geometries, with DS02 photon energy spectra at various distances from the hypocentre in Hiroshima. Results showed that organ doses from mathematical models generally agree well with those from voxel phantoms except for a few organs at lateral irradiation geometries and eye lenses at antero-posterior irradiation, even though there were significant differences between the two phantom sets and various uncertainties in dose calculations.

♦ Cullings HM: Recommended improvements to the DS02 dosimetry system's calculation of organ doses and their potential advantages for the Radiation Effects Research Foundation. Radiation Protection Dosimetry 2012 (January); 149(1):2–14. **(RR 24-11)**

[Abstract] The Radiation Effects Research Foundation (RERF) uses a dosimetry system to calculate radiation doses received by the Japanese atomic bomb survivors based on their reported location and shielding at the time of exposure. The current system, DS02, completed in 2003, calculates detailed doses to 15 particular organs of the body from neutrons and gamma rays, using new source terms and transport calculations as well as some other improvements in the calculation of terrain and structural shielding, but continues to use methods from an older system, DS86, to account for body self-shielding. Although recent developments in models of the human body from medical imaging, along with contemporary computer speed and software, allow for improvement of the calculated organ doses, before undertaking changes to the organ dose calculations, it is important to evaluate the improvements that can be made and their potential contribution to RERF's research. The analysis provided here suggests that the most important improvements can be made by providing calculations for more organs or tissues and by providing a larger series of age- and sex-specific models of the human body from birth to adulthood, as well as fetal models.

♦ Cullings HM, Kawamura H, Chen J: Body and organ dimensions of the 1945 Japanese population used in dosimetry system DS86 and data available for an expanded series of phantoms. Radiation Protection Dosimetry 2012 (January); 149(1):35–42. **(RR 23-11)**

[Abstract] The computational phantoms used in dosimetry system DS86 and re-used in DS02 were derived from models and methods developed at Oak Ridge National Laboratories (ORNL) in the US, but referred to Japanese anthropometric data for the Japanese population of 1945, from studies conducted at the Japanese National Institute of Radiological Sciences and other sources. The phantoms developed for DS86 were limited to three hermaphroditic models: infant, child and adult. After comparing data from Japanese and Western populations, phantoms were adapted from the pre-existing ORNL series, adjusting some organs in the adult phantom to reflect differences between Japanese and Western data, but not in the infant and child phantoms. To develop a new and larger series of more age- and sex-specific models, it appears necessary to rely on the original Japanese data and values derived from them, which can directly provide population-average body dimensions for various ages. Those data were re-analysed in conjunction with other Asian data for an Asian Reference Man model, providing a rather complete table of organ weights that could be used to scale organs for growth during childhood and adolescence. Although the resulting organ volumes might have some inaccuracies in relation to true population-average values, this is a minor concern because in the DS02 context organ size *per se* is less important than the correct body size and correct placement of the organ in the body.

♦ Hirai Y, Kodama Y, Cullings HM, Miyazawa C, Nakamura N: Electron spin resonance analysis of tooth enamel does not indicate exposures to large radiation doses in a large proportion of distally-exposed a-bomb survivors. Journal of Radiation Research 2011 (September); 52(5):600–8. http://www.oxfordjournals.org/our_journals/jrr/ **(RR 13-10)**

[Abstract] The atomic bombs in Hiroshima and Nagasaki led to two different types of radiation exposure; one was direct and brief and the other was indirect and persistent. The latter (so-called exposure to residual radiation) resulted from the presence of neutron activation products in the soil, or from fission products present in the fallout. Compared with the doses from direct exposures, estimations of individual doses from residual radiation have been much more complicated, and estimates vary widely among researchers. The present report bases its conclusions on radiation doses recorded in tooth enamel from survivors in Hiroshima. Those survivors were present at distances of about 3 km or greater from the hypocenter at the time of the explosion, and have DS02 estimated doses (direct exposure doses) of less than 5 mGy (and are regarded as control subjects). Individual doses were estimated by measuring CO_2^- radicals in tooth enamel with the electron spin resonance (ESR; or electron paramagnetic resonance, EPR) method. The results from 56 molars donated by 49 survivors provided estimated doses which vary from –200 mGy to 500 mGy, and the median dose was 17 mGy (25% and 75% quartiles are –54 mGy and 137 mGy, respectively) for the buccal parts and 13 mGy (25% and 75% quartiles: –49 mGy and 87 mGy, respectively) for the lingual parts of the molars. Three molars had ESR-estimated doses of 300 to 400 mGy for both the buccal and lingual parts, which indicates possible exposures to excess doses of penetrating radiation, although the origin of such radiation remains to be determined. The results did not support claims that a large fraction of distally-exposed survivors received large doses (e.g., 1 Gy) of external penetrating radiation resulting from residual radiation.

♦ Nakamura N, Hirai Y, Kodama Y: Gamma-ray and neutron dosimetry by EPR and AMS, using tooth enamel from atomic-bomb survivors: A mini review. Radiation Protection Dosimetry 2012 (January); 149(1):79–83. **(RR 26-11)** (refer to abstract in *Cytogenetics Studies* Publications)

Other Journal Publication

♦ Fattibene P, Wieser A, Adolfsson E, Benevides LA, Brai M, Callens F, Chumak V, Ciesielski B, Della Monaca S, Emerich K, Gustafsson H, Hirai Y, et al: The 4th international comparison on EPR dosimetry with tooth enamel. Part 1: Report on the results. Radiation Measurements 2011 (May); 46:765–71.

Atomic-bomb Dosimetry Studies Oral Presentations

❖ Hirai Y, Kodama Y, Tomonaga M, Iijima Y, Mine M, Cullings HM, Wada T, Nakamura N. Evaluating individual radiation doses with electron spin resonance analysis of tooth enamel from Nagasaki atomic bomb survivors. 36th Annual Meeting of the Chugoku Area Radiation Research Society, 28 July 2011, Hiroshima

❖ Cullings HM. Plans for a new series of phantoms for the calculation of organ doses of the Japanese atomic bomb survivors by dosimetry system DS02. 3rd International Workshop on Computational Phantoms for Radiation Protection, Imaging and Radiotherapy, 7–9 August 2011, Beijing, China

❖ Cullings HM. Geospatial analysis of ^{137}Cs measured in soil from the "black rain" area of Hiroshima. 14th International Congress of Radiation Research, 28 August–1 September 2011, Warsaw, Poland

❖ Cullings HM. Reported occurrence of severe epilation vs. location among the Life Span Study cohort of atomic bomb survivors. 17th Hiroshima International Symposium, 25–26 January 2012, Hiroshima