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RERF conducts research and studies—for peaceful purposes—on the medical effects of radiation on humans with a view toward contributing to the maintenance of the health and welfare of atomic-bomb survivors and to the enhancement of the health of all mankind.

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Editorial Policy

Contributions to Update receive editorial review only and do not receive scientific peer review. The opinions expressed herein are those of the authors only and do not reflect RERF policies or positions.

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The 36th Meeting of the Board of Directors, Hiroshima

The 36th meeting of the Board of Directors was held on May 26–27, 2001 in the Auditorium of the Hiroshima Laboratory. Seventeen people were in attendance, including directors, supervisors, and observers from the Ministry of Health, Labour and Welfare (Japan), Department of Energy (U.S.), and National Academy of Sciences (U.S.). The Board normally meets once per year to consider important operational issues of RERF.

Following approval of the minutes of the 34th and 35th Board meetings, Dr. Shigenobu Nagataki, RERF Chairman, presented three reports: “Summary of the past four years,” “Present problems,” and “Future research plans.” With regard to the Health Effects Study on the Children of A-bomb Survivors, it was reported that the full-scale mail survey had been initiated based on the agreement with the All Japan Second Generation A-bomb Victims Liaison Council.

Dr. Nagataki further commented on three additional issues: “Role of the Operating Committee,” “Role of the Chief Scientist,” and “Future plans.” In terms of the future plans, he emphasized the need to promote collaboration with other research organizations.

Dr. Seymour Abrahamson, Vice Chairman and Chief of Research, explained in detail proposals for establishing liaisons with statistics programs at universities and promoting the recruitment of research scientists, based on the recommendations of the Multinational Peer Review of the Statistics Program. That was followed by deliberation on the past year’s research activities and audit reports, current year’s working budget, and coming year’s provisional budget plan, all of which were approved.

Finally, the Board elected Dr. Burton G. Bennett (former Secretary of the United Nations Scientific Committee on the Effects of Atomic Radiation [UNSCEAR]) as Chairman, Senjun Taira (RERF Permanent Director) as Vice Chairman, and Dr. Eiichi Tahara (professor emeritus of Hiroshima University School of Medicine) as Permanent Director. Their terms of office begin on 1 July 2001 and extend for a period of four years.

List of Participants

Permanent Directors:

Shigenobu Nagataki, Chairman
Seymour Abrahamson, Vice Chairman and Chief of Research

Senjun Taira, Permanent Director

Visiting Directors:

Patricia A. Buffler, Dean Emeritus and Professor of Epidemiology, School of Public Health, University of California, Berkeley

Jonathan M. Samet, Professor and Chairman, Department of Epidemiology, The School of Hygiene and Public Health, The Johns Hopkins University

Richard B. Setlow, Senior Biophysicist, Brookhaven National Laboratory, and Adjunct Professor of Biochemistry and Cell Biology, State University of New York at Stony Brook (submitted a letter of attorney)

Kazuaki Arichi, Councilor, The Japan Institute of International Affairs

Toshiyuki Kumatori, Consultant, Radiation Effects Association (submitted a letter of attorney)

Masumi Ohike, Former Chairman, Board of Directors, Japan Anti-Tuberculosis Association

Supervisors:

David Williams, Senior Financial Advisor, National Academy of Sciences

Shudo Yamazaki, Former Director-General, National Institute of Infectious Diseases

Scientific Councilor:

Tomio Hirohata, Professor Emeritus, Kyushu University Faculty of Medicine

Representatives of Allied Agencies:

Kazuhiro Kanayama, Chief, Medical Care Activities Unit, General Affairs Division, Health Service Bureau, Ministry of Health, Labour and Welfare

James H. Hall, Minister-Counselor (Science), Embassy of the United States of America

Giulia R. Bisconti, Energy Attaché, Director, U.S. Department of Energy Asia Office, Embassy of the United States of America

Evan Double, Director, Board on Radiation Effects Research, National Research Council, National Academy of Sciences

Catherine S. Berkley, Administrative Associate, Board on Radiation Effects Research, National Research Council, National Academy of Sciences

Secretariat:

Masaharu Yoshikawa, Chief of Secretariat

Richard D. Sperry, Administrative Advisor, Secretariat

The 28th Meeting of RERF Scientific Council

The 28th meeting of the Scientific Council was held in Hiroshima on April 9–11, 2001. The meeting was co-chaired by Dr. J. Martin Brown of Stanford University and Dr. Tomio Hirohata of Kyushu University. After introductory remarks by RERF Chairman, Dr. Shigenobu Nagataki, and Vice Chairman/Chief of Research, Dr. Seymour Abrahamson, presentations were made by each of the RERF departments: Clinical Studies, Epidemiology, Statistics, Radiobiology, and Genetics.

In addition to overview presentations, more specific reports of RERF projects were presented as follows:

- Breast cancer molecular analysis (Yuko Hirai, Radiobiology)
- Immunological homeostasis (Yoichiro Kusunoki, Radiobiology)
- In utero* cytogenetics (Yoshiaki Kodama, Genetics)
- Molecular analysis of induced mutations in mice (Junichi Asakawa, Genetics)
- Progress in DNA microarray technology (Norio Takahashi, Genetics)
- Plans for the F₁ clinical study (Saeko Fujiwara, Clinical Studies)
- Microbial infection in the Adult Health Study (Masayuki Hakoda, Clinical Studies)
- F₁ mail survey and mortality in the F₁ population (Akihiko Suyama, Epidemiology, Nagasaki)
- Radiation interactions in lung, breast, and liver cancer (Gerald B. Sharp, Epidemiology)
- DS86 dosimetry revision (Shoichiro Fujita and Harry M. Cullings, Statistics)
- Effect of radiation on menopause (Michiko Yamada, Clinical Studies)
- Natural menopause in Nagasaki women (Shizue Izumi, Statistics)
- Role of body mass index, serum cholesterol, systolic blood pressure, and menopause (Masazumi Akahoshi, Clinical Studies, Nagasaki)

The Scientific Council affirmed the emphasis given at RERF to the main core projects on the health effects of radiation. In addition to the epidemiology and clinical studies, the Council recognized that research to elucidate the etiology of diseases is important to keep RERF in the forefront of scientific research and to attract young scientists. The primary general recommendations were as follows, along with brief indication of RERF follow-up on them:

- Reiteration of the previous Scientific Council recommendation that there be small-group “brain-

storming” sessions where scientists discuss research goals. [*Such sessions have been initiated or resumed at the department level and are contributing to development of a Long Range Plan for RERF.*]

- The Scientific Council might henceforth conduct their formal review only on alternate years, with some less formal interaction in the non-review years. [*The Board of Directors requested that a decision on this be postponed. Discussions are continuing in regard to a possible restructuring of the review process.*]
- Careful consideration should be given to the possibility of individual RERF scientists obtaining their own research grants. [*The Ministry of Education, Culture, Sports, Science and Technology (MEXT) has recently recognized RERF as eligible for submitting grant applications, and proposals for Grants-in-Aid from MEXT have been made for 13 projects. Seven of these have now been awarded.*]
- Consideration should be given to longitudinal continuation of the new F₁ clinical study beyond the initial investigation, with biennial mail contact and repeat clinical visits each 10 years. [*Very serious consideration is being given to that, including clinical visits more frequently than each 10 years.*]

Extensive recommendations were then made for each research department, which are summarized below.

Radiobiology – Appointment of 2–3 new staff at the doctoral level should be made. The animal facilities should be upgraded. Additional tumor tissue, especially breast cancer tissue, should be obtained from the Adult Health Study (AHS) participants for use in studies utilizing modern genomic techniques such as profiling of genetic expression in tumors, e.g., in early and late onset breast cancers. The department should continue to develop expertise in this area and institute the necessary collaborations to perform these studies.

Genetics – The current direction of the department to develop expertise in new molecular-based technologies to identify genetic alterations is supported. The staff should be expanded with young, short-term investigators. Collaborations should be established with other scientists both within and outside RERF. Joint departmental retreats organized around a research theme may be helpful for problem solving, technology sharing, and generation of new research ideas. The department should be involved in the RERF F₁ study during the planning and implementation stages to be sure that appropriate data and specimen collection plans are included.

Clinical Studies – The F₁ study should be made into a longitudinal study using mail contact with participants every 2 years, and a repeat full clinical study should be performed every 10 years. Current studies that are inactive or making poor progress should be closed and a mechanism should be set up to limit approval of clinical research proposals to five years with resubmission with a progress report if it is necessary for the study to continue for a longer period. A clinical research review committee should be created, made up of clinicians and statisticians to review clinical research proposals for scientific validity. It should include outside experts as well as RERF personnel.

Epidemiology – The continued surveillance of the Life Span Study (LSS) sample, *in utero* cohort, and F₁ cohort is essential for the mission of RERF. Because of improved survival of cancer patients, analysis based on cancer incidence is becoming more important than mortality analysis. RERF should continue to be involved in the tumor registries in Hiroshima and Nagasaki cities in order to maintain the current high standards of the tumor registries, which enables RERF to make nearly complete assessment of incidence cancer cases among survivors. The maintenance of the current system to abstract cancer records of survivors at hospitals in both Hiroshima and Nagasaki cities is desirable. The information obtained during the surveillance of many years of the LSS cohort, such as several mail surveys should be explored. Cooperation between the Departments of Clinical Studies and Epidemiology is desirable to conduct “nested” case-control studies using stored serum. Careful consideration should be given to develop first-rate scientific hypotheses to be tested in such studies.

Statistics – The Council endorsed the recommendations of the recently completed peer review of the Department of Statistics. In response to these, the department will make further efforts to increase its visibility to the Japanese statistical community and to recruit Japanese as well as foreign statisticians to RERF. At least one statistician should be added to the Scientific Council in the future. Efforts to develop new statistical techniques applicable to RERF data

will be increased. These will include: improved dose response models for predicting health effects at low radiation exposures and continued exploration of mechanistic models for cancer induction. Analyses of data on the primary RERF cohorts will continue to be a major focus, and attention will be given to the committee’s specific suggestions in this regard. These include use of adjusted survivor doses taking into account random errors. More discussion within the department and throughout RERF will be necessary on confounding, adjustment, and interpretation issues. Also, further discussion will be needed to consider whether there should be an oversight group to consider design, analysis, access, and documentation of databases for current and future studies.

Members of the Scientific Council

Tomio Hirohata, Professor Emeritus, Kyushu University Faculty of Medicine/Professor, Nakamura Gakuen University

Yusuke Nakamura, Director of Human Genome Center, Laboratory of Molecular Medicine, Institute of Medical Science, The University of Tokyo (absent)

Masao Sasaki, Professor Emeritus, Kyoto University (absent)

Yasuhito Sasaki, Chairman, Board of Directors, National Institute of Radiological Sciences

Shinichiro Ushigome, Visiting Professor, Jikei University School of Medicine

J. Martin Brown, Professor and Division Chairman, Division of Radiation Biology, Department of Radiation Oncology, Stanford University School of Medicine

Joe W. Gray, Professor of Laboratory Medicine, Radiation Oncology, University of California, San Francisco (absent)

Gloria M. Petersen, Professor of Clinical Epidemiology, Mayo Medical School

Theodore L. Phillips, Professor and Chairman, Radiation Oncology, Cancer Center, School of Medicine, University of California, San Francisco

Susan Preston-Martin, Professor, Department of Preventive Medicine, Keck School of Medicine, University of Southern California

New Chairman and Chief of Research

At the Board of Directors meeting held in Hiroshima on 26–28 May 2001, Dr. Burton G. Bennett was appointed to succeed Dr. Shigenobu Nagataki as Chairman and Dr. Eiichi Tahara was appointed the new Chief of Research. The Chief of Research's position had been held by Dr. Seymour Abrahamson who also concurrently held the position of Vice Chairman. Dr. Senjun Taira was continued as a Director for a second term and was now appointed Vice Chairman.

This series of appointments was a complete break with past practice since the founding of RERF with Dr. Bennett being the first American Chairman, Dr. Taira being the first Japanese Vice Chairman, and Dr. Tahara becoming the first Japanese Chief of Research. The terms of all the Directors began 1 July 2001 and are for four years.

Dr. Bennett comes to RERF after having been for many years Secretary of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). Since UNSCEAR serves as a primary organization utilizing the radiation and health research results from RERF, he is in a good position to provide leadership in maintaining and improving RERF contributions to the radiation protection community. Dr. Bennett had his basic education in physics with a M.S. degree from University of Washington and later a Ph.D. degree in environmental health sciences from New York University. Dr. & Mrs. Bennett lived in London for ten years where he worked on the United Nations Environment Programs and they later moved to Vienna for twelve years when he became Secretary of UNSCEAR. Most recently he had returned to the DOE Environmental Measurements Laboratory in New York City before coming to RERF.

When Dr. & Mrs. Bennett arrived in Hiroshima to take up the position of Chairman they were surprised as they got off the train to be unexpectedly met by a

television crew and reporter with questions. This was his introduction to the interest that has been shown by the media in the first American Chairman of RERF.

Dr. Tahara comes to RERF following a distinguished career as a pathologist at Hiroshima University, and is the first RERF Director to be appointed from the local community. In his work he has advanced the area of molecular methods in pathology, and is thus well suited to provide leadership to RERF in the new research areas stemming from the revolution in genomics. Dr. Tahara has most recently been a visiting professor at the University of California, San Diego after retiring from Hiroshima University in March 2000. He had a long career at Hiroshima University, beginning in 1968, with positions including Chief of the Pathology Department and Director of the Faculty of Medicine. He spent two years in Germany at Bonn University in the early part of his career. Dr. Tahara played a central role in establishing the Hiroshima Cancer Seminar Foundation in 1992.

Dr. Senjun Taira is the veteran of this group, now starting his second term as an RERF Director after a long career in the Ministry of Health, Labour and Welfare. He had assignments in many posts including the Japan International Cooperation Agency (JICA) before coming to RERF in 1997.

It is appropriate to mention here that the Chief Scientist, Dr. Charles Waldren, very ably complements these three directors in functions of the Executive Committee and other leadership of RERF research. Dr. Waldren spent most of his career at the University of Colorado Health Sciences Center, working in mutagenesis and DNA repair and their relationship to genetic disease. Before coming to RERF he was Professor of Radiological Sciences at the Colorado State University School of Veterinary Medicine.

Staff News

Drs. Hidetaka Eguchi and Kazuo Imai joined the Department of Epidemiology in July 2001 as Research Scientists. Both came from the Saitama Cancer Center Research Institute, to join Dr. Nakachi (Chief, Epidemiology), also from there, in the molecular epidemiology program at RERF. Dr. Eguchi received a Ph.D. in Life Chemistry at Tokyo Institute of Technology and Dr. Imai received a Ph.D. in Psychology at Tokyo Metropolitan University.

Dr. Masahiro Ito, Department of Genetics, retired

in December 2001. Dr. Ito graduated from Tokyo University of Agriculture in 1967, taking a research fellow position at University of Hokkaido. In the early days of the ABCC genetics program, Dr. Ito followed Dr. Awa from Hokkaido to ABCC in 1971, taking a position in Nagasaki (see Dr. Awa's article in this issue of *Update*). Dr. Ito played an important role for 20 years in Nagasaki, continuing in Hiroshima when the Nagasaki Radiobiology Department was merged with Hiroshima departments.

Multinational Peer Review: Clinical Studies

Performance highly evaluated, with recommended strengthening of the analysis of modifying factors related to onset of disease

The fifth Multinational Peer Review of RERF departments was held on November 28–30 at Hiroshima Laboratory Auditorium. Annual peer reviews have been held since 1997 in response to the Blue Ribbon Panel's recommendation of this practice. This review of Clinical Studies for both Hiroshima and Nagasaki completes the cycle of all departments, including also: Radiobiology, Epidemiology, Genetics, Statistics.

The meeting opened with an address by Dr. Burton G. Bennett, Chairman of RERF, and an RERF overview given by Chief of Research Dr. Eiichi Tahara. Following that, Dr. Gen Suzuki, Hiroshima Clinical Studies Department Chief reported on the major activities of his department and the Adult Health Study (AHS) program, and Dr. Masazumi Akahoshi, Nagasaki Clinical Studies Department Chief, summarized the activities of his department. Subsequently, the following presentations were made and active discussions held: *F₁ Health Study* (Saeko Fujiwara); *AHS longitudinal data analysis methodology* (Michiko Yamada); *Thyroid diseases* (Misa Imaizumi); *Inflammatory response and immune response to microbial infection* (Masayuki Hakoda); *Radiation exposure and diabetes mellitus* (Saeko Fujiwara); *Radiation exposure and senile cataract* (Kazuo Neriishi); *Cardiovascular disease* (Masazumi Akahoshi); *International Collaboration Studies (Ni-Hon-San comparative study on cardiovascular diseases, Ni-Hon-Sea comparative study on dementia)* (Michiko Yamada); *International and domestic collaboration study (Ni-Hon comparative study on osteoporosis)* (Saeko Fujiwara); *Future research plan—Sicca syndrome* (Ayumi Hida; read by Masazumi Akahoshi); *Future RERF cohort study* (Gen Suzuki).

As the RERF departments having direct contact with the A-bomb survivors and their children, Clinical Studies in Hiroshima and Nagasaki have contributed in many ways to the health and welfare of the A-bomb survivors and their children, including through the various research programs mentioned above. The departments also play a crucial role through the systematic collection of clinical data and biological samples that are important to research work of other RERF departments. The departments have placed special emphasis on research of the radiation effects on non-lethal diseases. The results are reported in twenty to thirty-plus scientific papers published each year in the major scientific journals in English and in Japanese.

The departments reported on future plans to conduct, in addition to the ongoing F₁ health examinations, research using stored biological samples and to perform analyses of newly found modifying factors and genetic traits related to the onset of diseases.

On the final day of the meeting, Dr. Theodore L. Phillips, panel chairman, provided a preliminary overview of the recommendations, and some other panel members added more specific comments. The detailed recommendations, received later, are summarized below. The review panel highly evaluated the research achievements of the clinical group and the papers they have published. It also recommended that the departments further strengthen the analytical program of modifying factors (confounders) related to the onset of diseases. As a conclusion, the panel encouraged the staff, saying that RERF will continue to be acknowledged as a globally important research institute if its future direction is established as utilizing RERF's long-accumulated data and samples for the research in elucidating the etiologies of diseases.

Summary of recommendations received in writing later is as follows:

- Initial review of new studies should be expanded to include outside reviewers when the RERF protocol committee does not have expertise in the area of study proposed.
- There should be a systematic strategy for prioritizing hypotheses and the use of blood products. Very strict policies on the use of preserved biological specimens and international review of any use of limited specimens should be instituted. Division of specimens into multiple tubes should only take place after such approval for their use, not on a routine basis.
- An annual review of open studies should be conducted by an RERF committee, to determine that progress is adequate to justify the investment of resources.
- The Departments of Clinical Studies should become more involved in studies of multifactorial influences on cancer incidence and outcome.
- The F₁ study should conduct the initial baseline examinations and contacts with the cohort as planned. During the initiation of this process the expected number of events in common diseases should be calculated in order to refine and reduce the number of clinical endpoints and allow

focus on high probability events in future re-examinations. High subject participation rates must be encouraged. Every effort should be made to maximize the power of the research to identify the hypothesized effects.

- In the AHS longitudinal study more attention should be paid to validation of endpoints such as death certificate data versus case-control studies.
- The classification systems for disease should be standardized.
- The thyroid disease study is a very nice piece of research. Consideration should be given to extending the study to Hiroshima and to initiating follow-up studies in subclinical hypothyroidism.
- The studies of infectious agents have shown that individual agents are not important but that the initiation of a chronic inflammatory process may be very important in cardiovascular disease and aging in general. Future studies should focus on mechanisms that trigger chronic inflammatory responses.
- The diabetes mellitus studies are very interesting but the discrepancy between results in Hiroshima and Nagasaki needs to be resolved by means of HLA typing.
- The cataract study may be very important in showing that radiation accelerates aging. It should be completed and consideration given to adding retinal photographs and fluorescein angiograms to the studies.
- The cardiac disease studies should concentrate on specific biochemical risk factors such as cholesterol levels etc. rather than on surrogate markers.
- The osteoporosis work is outstanding. Future studies may want to focus on environmental and

lifestyle factors influencing osteoporosis and on comparison of Japanese and Caucasians.

- The international studies are of high quality, benefit RERF, and should be continued and expanded if opportunities arise.
- Broader interaction of the clinical departments with the epidemiology departments is encouraged.

Peer Review Panel Members

Theodore L. Phillips, Professor and Chairman, Radiation Oncology, Cancer Center, School of Medicine, University of California, San Francisco (Panel Chair and RERF Scientific Councilor)

Yasuhito Sasaki, Chairman, Board of Directors, National Institute of Radiological Sciences (RERF Scientific Councilor)

John Danesh, Professor, Epidemiology and Medicine, Head of the Department of Public Health and Primary Care, University of Cambridge, Institute of Public Health

Donald R. Harkness, Professor Emeritus, University of Wisconsin Medical School

Yoshitomo Oka, Professor, Molecular Metabolism and Diabetes, Internal Medicine, Tohoku University School of Medicine

Hajime Orimo, Director, Metropolitan Geriatric Hospital

Kazuo Ueda, Professor, Kyushu University School of Health Sciences

Lon R. White, Principal Investigator, Honolulu-Asia Aging Study, Senior Neuroepidemiologist, Pacific Health Research Institute, Professor with joint appointments in the Schools of Nursing and Medicine, University of Hawaii at Manoa

Current Status of Dosimetry Revision

In the previous issue of RERF *Update* some information was given about reasons for revision of the DS86 radiation dose estimates for A-bomb survivors, and the progress at that time. Since then there has been substantial progress, including a meeting in Hiroshima in April 2002 of the Joint U.S.-Japanese Working Groups on the Reassessment of A-bomb Dosimetry. Below we will provide verbatim the press release following that meeting.

It is now expected that the fundamentals of a new dosimetry system may be completed by Fall 2002, with implementation of that system and preparation of a final report continuing for several months after that. Although there apparently will be smaller overall changes in dose estimates than anticipated, there will be some more specific improvements in accuracy. For example, there should be substantially improved shielding calculations for a large number of Nagasaki factory workers who received high doses, and for low-dose survivors in both cities who were at some distance from the bombs but shielded by terrain. Generally, the process of having very carefully considered every aspect of revision will lead to more confidence in the dosimetry system.

The April 4, 2002 press release is as follows:

Since the detonation of the A-bombs, the survivors of the bombings and the international community responsible for radiation protection standards have depended on the A-bomb radiation dosimetry systems for the determination of accurate radiation doses. As advances in technology for dose calculation from the bombs have been made, and the ability to check those calculations using activation measurements has improved, changes have been made in the dosimetry from T57D to T65D to the current DS86 system.

It is widely recognized that the DS86 system accurately calculates gamma rays, which constitute the majority of the radiation exposure to survivors. Since the implementation of DS86, thermal neutron activation measurements have been made that appear to differ from those calculated by the dosimetry system. Although neutrons are a small fraction of the total dose, it was thought to be important that this apparent discrepancy be addressed in order to give the survivors and the radiation risk assessment community confidence that the dosimetry system was accurate in all significant details.

In the 16 years since DS86 was implemented, remarkable advances have been made in computational capabilities and measurement technology.

These improvements now make it possible for scientists to measure and calculate trace amounts of activation from the bombs in ways and details not possible in the 1980's. Over the past 18 months, all of this new technical capability has been brought to bear in a further re-evaluation of the dosimetry system conducted by the Working Groups on the Reassessment of A-bomb Dosimetry in the U.S. and Japan.

The joint Japanese and U.S. Working Groups on the Reassessment of A-bomb Dosimetry met on April 3rd and 4th at the Radiation Effects Research Foundation in Hiroshima to review their work and discuss final preparations for a new dosimetry system, DS02, to replace DS86. The most detailed recalculation to date of the Hiroshima and Nagasaki bombs and an exhaustive evaluation of the data indicate the neutron discrepancy that gave rise to this reassessment will be resolved.

Changes in the nuclear data and calculation techniques of DS02, and extensive verification using nuclear test data, have refined the Nagasaki dosimetry. These refinements confirm the DS86 conclusion that 21 kilotons and height of 503 meters above the city are the most accurate parameters for the detonation. The degree to which the calculations for the Nagasaki detonation have been verified against nuclear tests now makes the Nagasaki calculation a benchmark for dose reconstruction. The refinements in the DS02 calculation for Nagasaki produce an increase of less than 10% in the dose from gamma rays while decreasing the neutron dose by 15 to 45% at distances of 1,000 to 2,000 meters from the bomb. Significant refinements in calculation of the shielding for workers in the torpedo factories reduce overall doses for these workers by 20 to 40%.

Similar refinements in the new DS02 calculation of the Hiroshima bomb produce better agreement between neutron calculations and measurements. New measurements of high-energy neutron activation at Hiroshima were particularly important in confirming the calculations. A measurement intercomparison is being carried out to confirm this agreement. The reassessment for DS02 confirms the DS86 conclusion that the bomb produced an explosion that was equivalent to 15 kilotons. A careful review of the data for the height of the detonation indicates that best agreement is achieved by increasing the burst height by approximately three percent to 600 meters. While this change produces better agreement between the calculation and the measurements, it produces almost no change in survivor

doses. Gamma-ray doses are unchanged within the first 2,000 meters of the bomb. Neutron doses are essentially unchanged for survivors out to approximately 1,500 meters, beyond which they are slightly decreased.

Thus, within the uncertainties of such difficult measurements and complex calculations, acceptable agreement has been achieved for both the neutron and gamma radiations. As a practical matter, while

the DS02 system will be more accurate than DS86, it will produce little change in the overall radiation doses for survivors. However, DS02 should produce a much more confident basis for the entire radiation dose to the survivors than was previously available.

Measurement results of exposed samples collected have been essential for dose reassessment. We would like to express our sincere appreciation to the citizens who have helped us collect the samples.

Ten Thousand Days Atop Hijiya

Akio Awa

Preamble

As of June 30, 1995, I retired from the Radiation Effects Research Foundation (RERF). I joined the Atomic Bomb Casualty Commission (ABCC) on January 16, 1967 and have spent 28 and half years with this organization. Alternatively put, I have spent 10,393 days at Hijiya—8 years and 3 months with the ABCC and the remaining 20 years and 3 months with the RERF. During this period, I was under the guidance of two ABCC Directors (Drs. George B. Darling and LeRoy R. Allen) and three RERF Chairmen (Drs. Hisao Yamashita, Masao Tamaki, and Itsuzo Shigematsu).

It was my pleasure to participate in research at the Cytogenetics Laboratory. A number of colleagues have gathered here and departed. At present, there is virtually no one who is familiar with the history of the laboratory. Before everything fades away and is forgotten, as a former permanent member of the laboratory, I feel obliged to prepare a message regarding its history. This document is intended to take the form of a personal account of the events I encountered over the past decades in the Cytogenetics Laboratory. Fortunately, Dr. Seymour Abrahamson, former Chief of Research and editor-in-chief of *RERF Update* kindly offered me an opportunity to publish this here. I am aware that this article will inevitably contain some errors and am solely responsible for them.

The document will consist of two parts, published separately. This part one details the genesis of Cytogenetics Laboratory, and a series of chromosome studies based on the Adult Health Study (AHS) population and a summary of this work. Part two will describe an outline of another important research mission, i.e., cytogenetic study of the children of atomic-bomb survivors, the so-called F₁ study. Included here also are my recollections of many of those unforgettable people to whom I am indebted in many ways, and who have—even now—guided me spiritually and scientifically.

Part 1. Cytogenetics Laboratory at ABCC/RERF—Past and Present

1. Prologue

In 1952, I entered the Hokkaido University undergraduate course in science, and completed the Biology course in the Faculty of Science in 1956. By coincidence, the period between 1952 and 1956 was an epoch-making era in the twentieth century de-

velopment of biology. In April of 1953, Watson and Crick first uncovered a famous double-helix structure of deoxyribose nucleic acid (DNA), and published a short but path-breaking paper in *Nature*.¹ A few years later, in early 1956, Tjio and Levan demonstrated unequivocally that the true number of human chromosomes is 46.² These important findings had a strong impact on me throughout my scientific career.

In the postgraduate course at Hokkaido University, my thesis work was to study human and mammalian chromosomes under the guidance of Professor Sajiro Makino (1906–1989). As will be fully described later, Dr. Makino has been internationally recognized as a human and mammalian cytogeneticist. He was also well known as an excellent leader for the education of his youngster colleagues.

I was a lazy postgraduate student with a short and capricious temper unsuitable for scientific research. Surprisingly, I was fond of microscopic work, and was able to accurately draw and analyze chromosomes directly under the microscope. This may be related to my hobby since childhood of drawing cartoons. This acquired habit became an indispensable tool for performing time-consuming work on detection of chromosome aberrations.

2. Human Cytogenetics — *çà et là* A. All about Chromosomes in Man*

Chromosomes are present in the cell nucleus. Their basic molecular component is DNA. As mentioned before, the exact number of human chromosomes is 46. They are small and slender, only visible by the use of a microscope; even the longest of the chromosomes is less than 1/100 mm. Each chromosome carries a constriction called “centromere,” which is an important landmark to characterize individual chromosomes.

Chromosomes can be observed only when cells are at a stage of cell division (mitosis) called “metaphase.” The period of metaphase is extremely short, so that in order to examine chromosomes, it is necessary to obtain tissue samples whose cells are actively proliferating. Until tissue culture technique was introduced to biological research, it was difficult to get the required fresh materials from healthy people. This was the reason why progress in human chromosome study had been greatly delayed.

In early 1950's, there occurred a remarkable wave of technical progress in biology. Tissue culture methods, as mentioned above, became in routine use for

medical and biological research. Particularly noteworthy was a new development of peripheral blood culture by Moorhead and his colleagues in 1960.³ Fresh human specimens for subsequent culture were easily obtained aseptically by vein puncture. For chromosome analysis, it requires only a few milliliters of peripheral blood. Moreover, it takes only two days of cultivation *in vitro* to yield sufficient number of mitotic cells.

Besides tissue culture techniques, other technological improvements have helped expand the knowledge of human chromosomes. These include (1) use of colchicines that arrest the mitotic cells at metaphase, and (2) an “air-dry” method⁴ that can spread metaphase chromosomes two-dimensionally on microscopic slides, and more importantly, overlapping of chromosomes is reduced considerably. Use of enlarged photographic prints of metaphases also helped facilitate the efficiency of chromosome analysis.

Such technical advances have made detailed chromosome analyses far easier, and have prompted the development of human clinical genetics. Certain genetic diseases are now known to be associated with chromosome anomalies due either to changes in number (chromosome aneuploidy) or in structure (structural rearrangement). For instance, the chromosome number of those with Down syndrome is 47, with an addition of an extra chromosome 21.

The introduction of lymphocyte culture method also has expanded the research area in radiation cytogenetics. A research group in Edinburgh University studied lymphocyte chromosomes in patients given X-ray treatment for ankylosing spondylitis.⁵ They reported that a variety of X-ray-induced chromosome damage was observed. Furthermore, they later confirmed not only their previous findings but also demonstrated that cells carrying aberrations could persist for years in patients’ circulating lymphocytes.⁶ These findings aroused the interest of scientists in radiation research, and need for investigation of radiation effects onto atomic-bomb survivors of Hiroshima and Nagasaki.

B. All about Radiation-induced Chromosome Damage

a. Formation of chromosome aberrations

Chromosomes are packed in a mass-like structure, called a nucleus, in the interphase cell—during an interval between two mitoses. When the nucleus is exposed either to ionizing radiation, or some other agents such as toxic chemicals and certain kind of viruses, chromosome threads are broken, and the number of induced breaks is proportional to the amount of dose administered. With the aid of repair enzyme, broken ends of affected chromosomes are largely restored to an original state. However, as the radiation dose increases, breaks are (1) left unrestored as chromosome

fragments, or (2) joined between wrong broken partners, thus newly producing chromosome aberrations—more precisely, exchange aberrations, or structural rearrangements of chromosomes. The evidence from experiments to date has shown that chromosome aberration frequency increases with increasing radiation dose, and that the frequency is influenced by radiation quality such as neutron, α -, β -, γ -, and X-rays.

b. Types of chromosome aberrations

As shown in Figure 1, an exchange between two breaks within a chromosome results in the formation of either a ring chromosome (plus a fragment without centromere, called acentric fragment) or an inversion of a chromosome. These categories of aberrations are termed “intra-chromosomal exchange.” Theoretically, both types of aberrations occur with an equal probability. Similarly, exchanges of breaks between different chromosomes result in a dicentric chromosome plus an acentric fragment, on the one hand, and on the other hand, two-translocated chromosomes, called “inter-chromosomal exchange.” “Dicentric” means a chromosome having two centromeres. Although there are many other types of chromosome aberrations, for convenience I will deal exclusively with the following four main types of aberrations, i.e., rings, dicentrics, inversions, and translocations.

Chromosome aberrations are classified in different ways, as seen in Figure 1. One group consists of rings and dicentrics, while the other with inversions and translocations. The former is called “asymmetric exchange,” and the latter “symmetric exchange.” Due to their morphological peculiarity, the former type of aberration is unequivocally detectable. On the contrary, symmetric exchange is difficult to identify with certainty, because the changes in shape and length of aberrant chromosomes often are so subtle that they are not discriminated as aberrant chromosomes. To score inversions and translocations with full efficiency requires long experience and expertise.

However, dicentrics and rings are known to cause mitotic disturbance because of their structural peculiarity. The cells carrying such aberrations are lost in the subsequent cell generations. Thus, their frequency decreases sharply with time, being a drawback in use of dicentrics and rings as a biological marker for those who were irradiated many years prior to chromosome examination. In contrast, there is no such disadvantage for translocations and inversions at mitosis, and the level of such frequency is maintained constantly for decades after exposure to radiation. For this reason, asymmetric exchanges are also called unstable aberrations (Cu type), and symmetric ones as stable aberrations (Cs type). I shall use this terminology exclusively throughout this article.

Figure 1. Diagrammatic representation of four major types of chromosome aberrations following exposure to ionizing radiation

EXCHANGES		REMARKS
Intra-chromosomal	Inter-chromosomal	
<p>RING PLUS FRAGMENT</p> <p>INVERSION</p>	<p>DICENTRIC PLUS FRAGMENT</p> <p>RECIPROCAL TRANSLOCATION</p>	<p>Easily detected but unstable over time</p> <p>Difficult to detect but stable over time</p>

The types of chromosome aberrations and the way to detect them have been fully described in the RERF Home Page (www.rerf.jp/Gene/eng/giensa.htm).

3. Birth of Cytogenetics Laboratory

The first ABCC genetics program was the extensive search for untoward pregnancy outcomes (1948–1954) initiated by Dr. James V. Neel and colleagues. In that era chromosome analysis was rather primitive, e.g. even the number of chromosomes was not established until the work of Tjio and Levan in 1956.² It was only in the 1960's that the ABCC cytogenetic studies began to flourish. See Dr. William J. Schull's book⁷ for further historical perspective.

During the period between 1963 and 1964, both Drs. Michael A. Bender and Ernest H.Y. Chu, cytogenetic experts in Oak Ridge National Laboratory, came to ABCC for a site visit to seek the possibility of initiating cytogenetic programs there. Based on their recommendation, Dr. Richard E. Slavin and colleagues in ABCC Pathology Department started the cytogenetic project. One of their results on the cytogenetic screening of Down syndrome cases in Hiroshima was published in 1967.⁸

Dr. Arthur D. Bloom began a full study in 1965. Prior to his assignment with the ABCC, he received training for human chromosomes under Dr. J.H. Tjio. The Cytogenetics Laboratory was transferred from the Department of Pathology to the Clinical Laboratory headed by Dr. Howard B. Hamilton.

In collaboration with Dr. Nanao Kamada (Hiroshima University, former director of Research Institute of Radiation Biology and Medicine) and Dr. Tetsuya Iseki (Nagasaki University, current president of Nagasaki Prefectural Medical Association), both Drs. Bloom and Shotaro Neriishi (Nagasaki Branch Laboratory) started the cytogenetic survey on the

Adult Health Study (AHS) population. They also studied some survivors who experienced prenatal exposure to A-bomb radiation (*in utero* survivors).

Their initial AHS study consisted of 174 proximally exposed survivors, whose estimated radiation dose (T65D system) was more than 200 rad,** and the 181 controls (0 rad group). They reported a significant elevation in the frequency of gross chromosome damage (such as dicentrics, rings, acentric fragments, and abnormal marker chromosomes of exchange type).^{9,10} In this study, however, they did not analyze the data for demonstration of a dose-response relationship.

In the 1960's Japanese scientists outside ABCC published reports on chromosome aberrations in atomic-bomb survivors. In 1968 Sasaki and Miyata examined 52 Hiroshima atomic-bomb survivors and published an excellent paper in *Nature*.¹¹ They scored mainly dicentrics, rings, and acentric fragments out of an average of more than 1,000



Front row (from left to right): Dr. Shotaro Neriishi, Dr. Sajiro Makino, and Dr. Howard B. Hamilton. Back row: Dr. Michihiro C. Yoshida, Dr. Takeo Honda, and Dr. Toshio Sofuni [1967]

metaphases per person, and made radiation dose estimations for individual survivors on the basis of their bio-dosimetric formula, called the Q_{dr} method. Their results showed a close relationship between biological and physical dose estimates, the latter of which was derived from both the distance from the hypocenter and the types of shielding materials between A-bomb burst point and individual survivors.

4. Expanded Cytogenetic Study: 1967–1975

In early December of 1966, Professor Makino in Hokkaido University was invited to ABCC to explore collaboration between Hokkaido University and ABCC. I accompanied Dr. Makino to ABCC, and negotiated with the representatives of ABCC, i.e., Drs. Hamilton, Bloom, and Hiroshi Maki (ABCC Associate Director). Dr. Darling, ABCC Director, did not attend this meeting since he was out town. After a lengthy discussion, we reached to the agreement that Hokkaido University would support ABCC cytogenetic projects by participation of Dr. Makino's research staff.

Only a month later, in January of 1967, Dr. Takeo Honda and I were assigned to ABCC as permanent research associates. In the same year, two more members also came to ABCC, Dr. Michihiro Yoshida (1967–1969) to Nagasaki and Dr. Toshio Sofuni (1967–1979) to Hiroshima. In the subsequent years, others from Hokkaido joined the ABCC: Messrs. Hachiro Shimba and Kazuo Ohtaki in Hiroshima, and Mr. Masahiro Itoh in Nagasaki.

The addition of research staff continued. Dr. Mimako Nakano from Hiroshima University joined us in 1977. Mr. Yoshiaki Kodama came to RERF in 1980 as a replacement for Dr. Sofuni, who was assigned to National Institute of Hygienic Science in Tokyo. Finally in 1994, Dr. Nori Nakamura, currently Chief of Genetics Department, was transferred from the Radiobiology Department. Dr. Sadayuki Ban was also part of the Cytogenetics Laboratory from 1985 to 1994.

Dr. Bloom returned to the United States after three



Dr. Howard B. Hamilton
[1981]



Dr. Arthur D. Bloom
[1983]

years with the ABCC and joined to Dr. Neel's group at University of Michigan Medical School in Ann Arbor. Indeed, he did a good job and his name should long be remembered as a founder of ABCC Cytogenetics Laboratory. Mr. Shozo Iida, then chief technician of the laboratory, also left for the U.S. to help Dr. Bloom set up a new cytogenetic laboratory in Michigan.

After Dr. Bloom's departure to the U.S., I was asked to take over the management of the laboratory. I carefully looked at the ABCC organization chart and tried to remember the names of key staff members in other departments. I also went around in ABCC to confirm what I had remembered. All of this helped me a great deal when we had to establish an inter-departmental management system for Dr. Neel's new project on biochemical screening of the F_1 children of survivors.

Prior to the onset of laboratory management, I faced with two urgent issues. Firstly, we all felt the study should be oriented to the determination of the dose-response relationship in A-bomb survivors. To do this, however, we needed to expand the sample size to cover survivors in all dose ranges. Sample selection from among the AHS participants was made in collaboration with a statistician in charge of cytogenetic project, Mr. Takashi Matsui, now professor in Statistics at Dokkyo University. Indeed, he was our best partner. In those days, only statisticians could deal with radiation dose data (T65D). Any members other than statisticians were not allowed to obtain individual dose of survivors, to avoid any observers' bias prior to or in the course of survey.

Secondly, the level of techniques both for lymphocyte culture and preparation of microscopic slides was rather poor in our laboratory. At that time, it was difficult for us to routinely observe more than 100 cells per person. In addition, it was already more than 20 years since A-bomb explosions in 1945. We had thus anticipated that most of the cells carrying unstable aberrations might be eliminated from circulating blood of survivors. Consequently, even for heavily exposed survivors, we might fail to detect any unstable aberrations within the ranges of 100 analyzable metaphases per person. We empirically knew that we could find on an average about 5% of cells bearing stable aberrations at 100 rad.

We thus decided as a routine procedure to score all types of stable and unstable aberrations per 100 metaphases per survivors. Our decision was quite contrary to the general strategy of scoring exclusively unstable aberrations, an easy and well established biosimetry employed in most of cytogenetic laboratories in the world. We knew that those researchers who analyze stable aberrations were in the minority group. We were convinced that stable aberration scoring could equally be efficient for the

analysis of dose-response relationship, provided that the pooled data were analyzed by breakdown of survivors into 100 rad-interval dose groups.

In the course of the routine microscopic work, what worried us was finding too many metaphases with false negative aberrations, i.e., metaphases judged as normal even when true aberrations were present. Our data would have lost credibility if aberrations were overlooked by misjudgment of the observers, though it is impossible to eliminate all mistakes. In order to minimize the number of false negative aberrations, all of the cells in which definite and suspected structural aberrations were detected by direct microscopy were then photographed and subjected to karyotype analysis. Where possible, we vigorously pursued the origin of chromosomes involved in the exchanges of aberrations by karyotyping. Our obligatory scoring procedure was (1) to record on the laboratory score sheets such items as case ID number, slide number, cell number, and the X-Y axis location of the cell on the microscopic stage, and (2) to take photographs for all metaphases suspected by examiners of having any abnormality. Considering the cost of a frame of 35 mm negative films, this procedure seemed to be the most reliable and the cheapest. By this system, ability of observers to detect aberrations became very much improved and consistent. It is worth noting that nearly 400,000 frames of metaphase pictures in 35 mm negative films have been kept to date in the large film cabinets of the laboratory.

The results of expanded AHS surveys have been intermittently reported elsewhere.^{12,13,14} Major findings of the study are as follows: (1) The frequency of aberrant cells increased with increasing radiation dose, and was generally higher in Hiroshima than in Nagasaki. (2) The mode of dose-response relationship was linear in Hiroshima, while it was dose-squared fashion in Nagasaki. The observed difference might reflect the difference in a mixed ratio of neutron and gamma rays between the two cities. This view, however, was re-evaluated upon adopting a new dosimetry system of DS86, in which neutron dose was drastically reduced from Hiroshima radiation spectrum. The observed inter-city difference in the neutron-gamma rays thus diminished. Even now, however, inter-city difference in the frequency of chromosome aberrations still exists, though lesser in degree, when data are reanalyzed using DS86 system. (3) Frequency of stable aberrations (translocations and inversions) is predominantly higher than that of unstable aberrations (dicentrics and rings). The former type of aberration is the major contributor for the dose-response relationship. The dose-response for unstable aberrations was also demonstrated. (4) There was evidence of *in vivo* clones of cells with cytogenetically identical aberrations in high dose survivors. (5) There was a small fraction of survivors either with high dose and low aberra-

tion frequency or with low dose and high aberration frequency. These survivors were regarded as overdispersion cases outlying the normal range of the dose response relationship. We termed them "cytogenetic outliers." Our interpretation was that the outlying values stemmed from errors in dose estimate rather than due to biological individual difference. Obviously, we needed further validation of cytogenetically outlying cases.

In February 1975, before the re-organization of ABCC to RERF, a special scientific committee meeting was held at ABCC, chaired by Dr. James F. Crow (professor in Genetics, University of Wisconsin), called the "Crow Committee." The charge to the committee was to carefully review each item of the ABCC scientific programs as to which of the items should be taken over by the new foundation. When the recommendation of the committee¹⁵ was reported, we cytogenetic members were very happy to know that the report included the issue of "cytogenetic outliers," and recommended pursuing this even more vigorously. Part of the Crow Committee recommendation is directly cited as follows.

"It would be especially valuable to study persons whose cytogenetic findings are grossly discrepant with regard to estimated dose. On the one hand, such a study may lead to improved dosimetry; on the other, it might reveal possible human phenotypes with extreme radiation resistance or susceptibility. Much concern has been expressed for persons who may be especially susceptible to chemicals; this might well be a concern also with radiation."

After the re-organization of ABCC to RERF, except for the F₁ cytogenetic work our AHS study was concentrated to conduct repeat examinations of survivors who were cytogenetically categorized in different groups. In one of the categories, we randomly chose an appropriate number of survivors, for whom cytogenetic survey had already conducted, in all dose ranges, to see if aberration frequency noted previously would be consistently maintained. Alternatively put, had our ability to detect aberration been maintained constantly over time? Important inclusions of survivors into repeat examination were the



Dr. James F. Crow (left) and the author [1997]

outliers, both high-dose with low aberration frequency and low-dose with high aberration. Also included were survivors carrying a clone of cells with cytogenetically identical aberration.

The results of repeated examinations reconfirmed our previous findings. Aberration frequency for each of survivors had been maintained consistently for long period of time. The same held true for both outliers and clone-carriers. As for controversial cases of outliers, it was proved that the discrepancy of aberration frequency with regard to the dose was found to stem from errors in dosimetry rather than from difference in radiosensitivity or radioresistance of survivors. Interestingly, clones of aberrant cells with a constant frequency still continued to persist for many years in the body of clone carriers.

In 1982, the T65D A-bomb radiation dose estimate system was criticized as having many problems. Thus a team of scientists from the U.S. and Japan was formed, and started a re-evaluation work that took them several years to complete. In 1986, a new system "Dosimetry System 1986," called DS86, was finally established. One of the major changes from the previous system was a substantial reduction of neutron dose and increase in gamma dose in Hiroshima. Thus the ratio of neutron to gamma estimates differed less between Hiroshima and Nagasaki than before.

When a new system was available, our cytogenetic data were reanalyzed using DS86 values. The results indicated that the inter-city difference in aberration frequency per unit dose became smaller than that of the previous result. The degree of difference in the shape of dose-response curves also became smaller, but there still existed some inter-city difference; Hiroshima frequency is still higher than in Nagasaki.¹⁶

We had a difficult time between 1982 and 1986 when dosimetric work was still underway. All of our studies were directly related to A-bomb radiation dose. Doing studies without dose was actually impossible. Thus we spent most of the time carrying out a detailed analysis of the types and frequencies of stable aberrations, data being derived from both G-band and conventional staining analyses. The data revealed that translocations were found to be about two-thirds of the total stable aberrations, and they were a primary contributor to the dose-response relationship.¹⁷ It was also demonstrated that, provided that G-band method can detect all types of aberrations with a full efficiency, conventional analysis could detect aberrations of about 70% of the G-band efficiency.

The year 1988 was a dawn of a new molecular study at the Cytogenetics Laboratory. In close collaboration with Lawrence Livermore National



Microscopy Room in Cytogenetics Laboratory (Hiroshima) [1988]

Laboratory (LLNL) in California, a new technique called a chromosome painting method, or more precisely the FISH technique, an abbreviated form of fluorescence *in situ* hybridization, was introduced to our laboratory. By this technique translocations can be detected easily, rapidly, and accurately, so that the technique seemed to be promising for identification of stable aberrations in A-bomb survivors, and it would be eventually employed as our routine cytogenetic procedure. Since the procedure of technique is too complicated and highly technical, I do not describe it here in detail. In principle, composite probes from chromosomes 1, 2, and 4 are hybridized to the same chromosomes in the microscopic slide. Thus the chromosome of interest is selectively painted, while the rest of non-target chromosomes are left unstained (or stained with other dye). Any translocations involving chromosomes 1, 2, and 4 are visualized as a bicolor chromosome(s) so that, even for lay people, they are easily distinguishable from other elements in the metaphase. In 1989, a new joint research program between LLNL and RERF was approved.¹⁸ This research protocol was to examine the feasibility of the FISH technique as a routine screening procedure for A-bomb survivors (www.rerf.jp/Gene/eng/fish.htm).

When I visited LLNL in the fall of 1988, I was strongly convinced that the technique seemed highly feasible, as far as I could see from observing all procedures with my own eyes. With a classical morphological analysis, I used to engage in analyzing stable aberrations in A-bomb survivors in a time-consuming process, but now it was possible to detect translocations more objectively and accurately than with the classic conventional technique I had employed. When those irradiated people who were exposed to ionizing radiation decades before examination are studied, it is my belief that stable aberrations are the most reliable cytogenetic marker. I thought that the dreams could come true, and we may be able to get rid of the minority.

A preliminary FISH study was conducted in combination with G-band analysis on 22 cases of

Hiroshima A-bomb survivors. The results satisfactorily showed that, as had been anticipated, there was good agreement of the data between FISH and G-band measurements. The FISH method was thus validated, and further indicated the utility of translocation frequency analysis for assessment of the level of acute exposure to radiation.¹⁹ Based on the result of the preliminary FISH study, a new screening project was proposed to examine AHS participants to further analyze the relationship between genomic translocation frequency and DS86 radiation dose for Hiroshima and Nagasaki survivors.²⁰

Now this document regarding the AHS cytogenetic study is close to the end. Before closing this chapter, I have some questions addressed to myself. Where do we stand now with a large body of cytogenetic data? How have the results of our data been evaluated now by others? Finally, have we done things in the right direction? It is still premature and thus difficult to evaluate our work by ourselves. Part of the answers may be in the evidence described below.

Our data suggests that a better-fitted dose-response relationship can be obtained when restricting to those survivors who were in individual (Japanese-style) houses at the time of explosion. It is these survivors whose estimated doses seem to be the most reliable. Furthermore, the difference in the shape of dose-response curves between Hiroshima and Nagasaki is thereby reduced to a certain extent.

Recently, Nakamura *et al* collected teeth obtained from Hiroshima survivors for whom cytogenetic analysis had already been completed, and estimated gamma-ray doses received by 69 Hiroshima survivors using electron spin resonance (ESR) of tooth enamel. The resulting measurement was compared with the corresponding stable aberration data. For 40 donors examined by both methods, there was the same pattern of the dose-response between ESR-estimated gamma dose and aberration data.²¹

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Evidence accumulated to date has shown a possible future study for survivors who were in the factories at the time of bombing in Nagasaki. They were relatively proximally exposed, but owing to complex shielding situations their physically estimated dose is either unavailable or inaccurate even if an estimate was attempted. Since they were exposed to appreciable radiation, and cancer risk estimation is limited by the numbers exposed at such levels, further study of the Nagasaki factory workers is urgently needed.

At present, no one knows the role and function of cytogenetically abnormal lymphocytes in the human body. It seems to me that the presence of lymphocytes with chromosome aberrations may have no direct bearing on the health of survivors. One of the potential future objectives would be to elucidate the clonal development of impaired lymphocytes at the site of the lymphoid stem cells, and determine their genetic and immunological implications.

– End of Part One –

Notes to the readers:

- * The following is the definition of the terms that are used frequently in this article. The terms “chromosome study” and “cytogenetic study” have been used synonymously and indiscriminately among the cytogeneticists. The study on the structure, function, and role of the chromosomes is called “cytogenetics.” The term “karyotype” is applied to “a systematized array of the chromosomes of a single cell prepared either by drawing or by photography, with extension in meaning that the chromosomes of a single cell can typify the chromosomes of an individual or even a species.”²²
- ** The term “rad” is an old radiation unit. A new unit is “gray (Gy)” (1 Gy = 100 rad).

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Radiation Risk, Longevity, and the Impact of the Comparison Group on Low-Dose Risk Estimates

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Note: This article is based on several recent RERF publications, including: “Longevity of atomic-bomb survivors” (Cologne and Preston [2000]¹), a modified version of this paper published in Japanese (Cologne *et al* [2001]²), and “Impact of comparison group on cohort dose response regression: An example using risk estimation in atomic-bomb survivors” (Cologne and Preston [2001]³).

In recent years articles appearing in the popular newspapers and magazines, such as *The Washington Post*, *The New York Times*, and *Time* magazine, have contained claims that atomic-bomb survivors are outliving their unexposed peers. The statements in these articles were based on some reports such as those of Mine *et al* (1990)⁴ that report increased life expectancy for male survivors in Nagasaki who received doses between 0.5 and 1.5 Gy, and Hayakawa *et al* (1989)⁵ who report that all-cause death mortality rates for survivors who were more than 1 km from the hypocenter were lower for than those for non-exposed residents of Hiroshima Prefecture. Kondo (1993)⁶ presents a summary of these and other findings (including data from RERF reports) to support his argument for reduced mortality (increased life expectancy) at low doses. On the other hand, RERF’s analyses of mortality in the Life Span Study provide clear evidence for dose-related increases in mortality rates from both cancer, Pierce *et al* (1996),⁷ and non-cancer diseases, Shimizu *et al* (1999).⁸ In view of these contrasting results—apparent radiation-associated increases in both cancer and non-cancer mortality and reports of greater longevity in some dose groups—we undertook to analyze recent Life Span Study mortality data (follow-up through 1994) in order to estimate longevity and to investigate the impact of the choice of a zero-dose comparison group on estimates of risk and longevity.

Increased mortality and life shortening caused by radiation

Table 1 presents our estimates of the risk for total mortality and longevity for the Life Span Study (LSS) cohort members in various dose groups. Risks for total mortality are measured relative to that for cohort members with a total shielded kerma estimate of 0 Gy. Longevity, or median life expectancy, is de-

finied as the age at which half of the population has died. The life-expectancy estimates are conditional on being alive at the start of follow-up. Since the estimates depend on city, gender, and age at the start of follow-up, the values in this table are standardized to represent averages over sex and city and for a person who was 34 years of age at the start of follow-up (the average for the LSS cohort). Median age at death

Table 1. Life expectancy by radiation dose^a

Dose range (Gy)	Mean dose (Gy)	No. of people	No. of deaths	Relative risk	Median age at death
0 (<0.005) ^b	0.0	34,064	16,775	1.000	81.082
0.005–	0.06	40,403	19,641	1.002	81.025
0.250–	0.36	4,899	2,548	1.031	80.435
0.500–	0.61	2,427	1,296	1.085	80.068
0.750–	0.86	1,360	693	1.120	80.312
1.000–	1.22	1,527	802	1.138	79.769
1.500–	1.90	1,160	619	1.259	77.994
2.500+	3.04	732	411	1.580	75.860
Unknown	—	7,097	3,151	1.031	80.945

^aSee also Figure 2.

^bThe dosimetry system assigns a dose estimate of zero to all persons whose calculated dose estimate would be less than 0.005 Gy free-in-air kerma.

for the LSS zero dose group was virtually identical in the two cities (81 years, 56 days in Hiroshima, 81 years, 15 days in Nagasaki). Females lived an average 7 years, 141 days longer than males. Figure 1 presents the estimated standardized survival curves for selected dose groups.

The death rates are increased and lifespan decreased in each non-zero dose group considered here. For survivors with dose estimates between 0.005 and 0.25 Gy (mean 0.055 Gy), the estimated decrease in life expectancy was three weeks, while that for survivors with dose estimates greater than 1 Gy (mean dose 2.25 Gy) was 2.6 years. There is no evidence of significant heterogeneity in the radiation-associated risks or longevity changes within the lowest non-zero dose category ($P > 0.5$). The estimated life expectancy for LSS survivors with estimated doses of at

least 0.005 Gy (mean dose 0.27 Gy) was a little more than four months less than that for cohort members in the zero dose group. Because the LSS cohort was constructed to over-represent survivors who were within 2.5 km of the hypocenters, the average loss of life among the larger population of all atomic-bomb-radiation-exposed individuals who survived acute causes of death would be less than four months. The results suggest that life shortening in acutely exposed humans is about 1–2% per Gy. As illustrated in Figure 2, life expectancy exhibits a nearly linear dependence on dose over the 0 to 1 Gy range with median loss-of-life estimates of about 0.12 years at 0.1 Gy and 1.3 years at 1 Gy. At 1 Gy about 60% of the total life lost can be attributed to solid cancer, 30% to non-cancer, and 10% to leukemia. Because of non-linearity in the leukemia mortality dose response and possible non-linearity in the non-cancer

Figure1. Survival curves for selected dose groups. Survival was estimated with adjustment for city, gender, and year of birth—all centered at their mean values—using Cox regression.

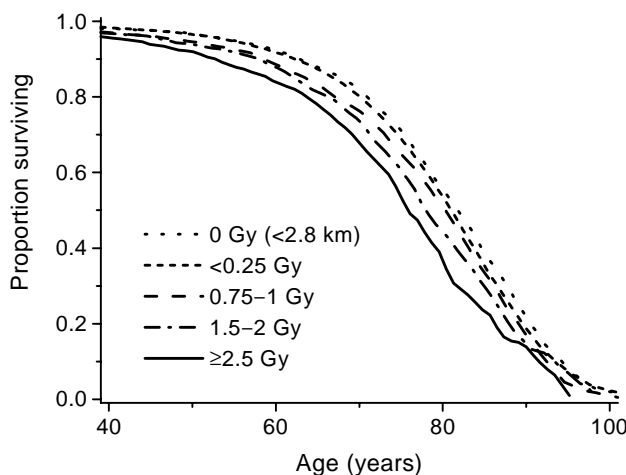
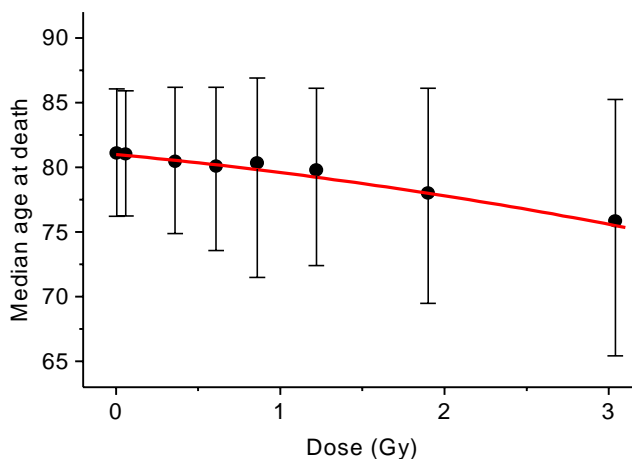


Figure2. Longevity as a function of radiation dose. The curve was estimated using a weighted least squares regression fit to the estimated median life expectancies of Table 1, using within-stratum mean doses as the independent variable and numbers of persons as the weights. The line represents the equation: median age at death = $81 - 1.2 \times \text{dose} - 0.2 \times \text{dose}^2$



mortality dose response, solid cancers are likely to contribute a greater proportion of the total life lost at lower doses. Thus, cancer is the major cause, but not the sole cause, of radiation related life shortening in the LSS. While the LSS results in Table 1 clearly indicate radiation-associated increases in risk (and decreases in lifespan) at doses in excess of 0.25 Gy they also indicate that mortality risk and longevity changes associated with lower doses are small. These results do not support claims that radiation-exposed atomic-bomb survivors are living longer than their peers.

Risk estimates and the choice of comparison group

Because the changes in risk associated with low-dose exposures are small and estimation of them requires the use of a comparison group, it is difficult to develop accurate estimates of these risks. The ideal comparison group should be comparable to the exposed cohort in every way except for the radiation exposure. However, detection of small changes in risks is complicated by the fact that, for reasons other than the radiation exposure, rates in seemingly similar populations can easily differ by as much or more than the likely low-dose radiation effects. It may be possible to measure and make statistical adjustments for some of the factors that cause these differences, but even when some such adjustments are feasible (as is the case, for example, with age and sex) they cannot completely eliminate differences between the exposed and unexposed populations. If the unexposed group is not comparable to the exposed group, risk estimates, particularly those at low doses, will be distorted (biased).

The LSS includes almost 94,000 survivors who were within 10 km of the hypocenter at the time of the bomb and a group of a little more than 26,000 people who were temporarily away from the cities at the time of the bombs. This latter group is referred to as the not-in-city (NIC) group. In order to reduce the likelihood of bias, the cohort was constructed to insure that the age and sex distribution of distal survivors and the NIC group is similar to that for

survivors in the cohort who received significant radiation exposures (i.e., more than 5 mGy). As has been noted in many RERF reports, despite this matching, cancer and non-cancer death rates for the NIC portion of the cohort are lower than those for survivors whose estimated dose is less than 5 mGy. Because of this little-understood difference in rates, the NIC group has not been used in most recent analyses of LSS mortality and cancer incidence. It is also useful to consider the degree of variability in death rates seen for survivors in the LSS cohort who received little or no radiation exposure from the bomb. This “zero” dose group includes the 25,532 LSS cohort members who were between 3 and 10 km from the hypocenter at the time of the bombs and 8,532 of the 68,137 cohort members who were within 3 km of the hypocenters.

In order to investigate the degree of heterogeneity in the risks for the zero dose group we divided this group into four parts based on distance from the hypocenter and estimated the standardized mortality ratios (SMR) for these subgroups relative to mortality rates of for all zero-dose in-city survivors with adjustment for city, gender, age, and birth cohort. The results are shown in Table 2; an SMR of 1 means that the rates do not differ from the average rate for all zero-dose cohort members who were within 10 km of the hypocenter at the time of the bombs.

These estimates suggest that background death rates vary by about 10% within the zero-dose survivor group used for most RERF analyses. They also suggest that the SMR tends to increase with distance from the hypocenter. (A test for this trend indicates that it is statistically significant with $P < 0.001$.) Variation in mortality rates with distance in the zero-dose survivor group could be due to geographic differences in lifestyle, socioeconomic status, regional differences in health care, and/or occupation. In 1945, areas that were more than 3 km from the hypocenter were more rural than the urban central city areas on which the bombs were dropped and residents of those areas were generally poorer than city residents. The

Table 2. Standardized mortality ratio (SMR) for geographically distinct zero-dose groups in the Life Span Study

Group	People	SMR	
		Estimate	95% confidence interval
Survivors (within 10 km of the hypocenter ATB ^a)			
within 3 km	8,532	0.95	0.92, 0.98
3–5 km	18,352	1.01	0.99, 1.03
5–7 km	5,188	1.03	0.995, 1.07
7–10 km	1,992	1.07	1.00, 1.13
Local residents absent from city ATB			
Not in city	26,531	0.95	0.93, 0.97

^aAt the time of the bombs

low rates for proximal zero-dose survivors (which we will take as survivors who were within 3 km of the hypocenter at the time of the bombs) may also partially reflect a healthy-survivor selection effect. Although more work on selection is necessary, results from analyses of LSS non-cancer mortality by Shimizu *et al* (1999),⁸ suggest that there is evidence for such selection for noncancer deaths, but that impact of this selection had largely disappeared by the late 1960's.

It is also noteworthy that the SMR for the NIC group (for whom no selection effects would be expected) is significantly less than 1 and quite similar to that seen for the proximal zero-dose survivors. The reasons for this are unclear. However, it is useful to consider how the NIC group was selected. The primary selection was based on data obtained during special daytime censuses of people in Hiroshima or Nagasaki cities on October 1, 1950 and, for Hiroshima, supplementary censuses carried out on October 1 of 1951 and 1952. The unexposed group was defined to include people identified in these surveys whose family registry (*honseki*) was in Hiroshima or Nagasaki but who were not in or near the cities at the time of the bombs. These surveys were more likely to identify people who lived in or fairly near the city centers than people who lived in the more rural outlying areas. Thus, one might expect members of the NIC group to be more like the proximal-exposed survivors than some of the distally-exposed survivors. The LSS mail survey data provide some evidence to support such a view.

It is of interest to consider the impact of variability in the death rates among the zero-dose survivors on radiation risk estimates obtained from the LSS. To do this we examined how the choice of the zero-dose comparison group affects both the linear dose-response slope estimate and the evidence for dose-response non-linearity. Table 3 summarizes our findings (presented in more detail in Cologne and Preston [2001]³). For the analyses summarized in the first row of this table, all information about baseline risks is derived from survivors with non-zero (positive) dose estimates, using a dose-response model in which the baseline risk is estimated by the model parameters with dose equal

to zero. In the other analyses the 0–3 km zero-dose group, the 3–10 km zero-dose group, or all zero-dose survivors were used to determine the level of the baseline risks.

These results indicate that the choice of the comparison group has a relatively small effect on the estimate of, or inference about, the slope in a linear dose response model. Neither did we find that the choice of comparison group affected inferences on gender effects or age-time patterns in the excess risks. However, the choice of the comparison group has a marked impact on inference about the shape of the dose response and, hence, low-dose risk estimates for total mortality. The results presented in Pierce and Preston (2000)⁹ also suggest that choice of the comparison group has some impact on inference about low dose risks and the shape of the dose response. Analyses based solely on proximal survivors (positive dose only or all proximal survivors) provide no suggestion of significant non-linearity in the dose response, while in analyses in which the more distal survivors were allowed to contribute to the determination of the baseline rate level, there were indications of a lack of linearity, specifically significant upward curvature in the dose response as can be seen in Figure 3.

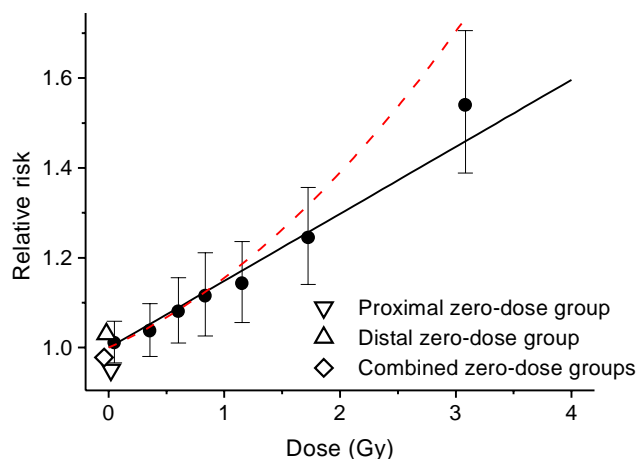
The present work reveals that small biases in the risk estimate can result from the choice of zero-dose comparison groups in analyzing atomic-bomb survivor data, but demonstrates that primary results from the LSS do not depend to a large extent on the traditional comparison group. However, because of the variability in death rates seen for various groups of zero-dose survivors in the LSS, it is questionable whether it is appropriate to include persons beyond 3 km in the analyses, when one is concerned with the nature of the dose response relationship over the low dose range (e.g., 5 to 200 mGy). Because of the large number of cohort members with very low doses (e.g., 5 to 10 mGy), background mortality rates can be precisely estimated from the data for proximal survivors or even from survivors with positive dose estimates. Our results suggest that detailed analyses of low-dose effects should focus on the proximally-exposed (within 3 km) individuals only.

Table 3. Effect of comparison group on estimated excess relative risk (ERR) of mortality

Comparison group	ERR per Gy	Deviation from positive-dose-only ERR (%)	P-value for non-linearity
Positive dose only ^a	0.212	—	0.11
0–3 km	0.224	5.7	0.22
3–10 km	0.196	–7.5	0.025
0–10 km	0.206	–2.8	0.059

^aThe intercept was estimated by the dose-response regression fit to the non-zero-dose persons; the zero-dose groups were fit by separate parameters.

Figure 3. Relative risk of death by radiation dose using internal standardization. The positive-dose points and regression line are from the internally standardized analysis of only those with significant radiation doses (5 mGy or more). Relative to the estimated background rate from that analysis, the SMR's for zero-dose proximal and distal survivors are shown separately (triangles), and as a single zero-dose group (diamond). The dashed curve is the linear-quadratic dose response when the distal, but not proximal, zero-dose persons are included in the analysis as is sometimes done by others. This is probably misleading because the nonlinearity of that curve is primarily due to the nature of the dose response at zero and very low doses, reflecting difficulties with the choice of comparison group.



Conclusions

The LSS cohort is well-suited for addressing issues of relative risk, loss of life, and low-dose radiation effects for several reasons. It is a well-defined cohort with virtually complete follow-up, doses are well characterized, and there are substantial numbers of people exposed to low doses. Because of these features, internal standardization of the risk regression estimates is possible, so that an external estimate of background rates is not required. Furthermore, follow-up began five years after exposure, which would eliminate mortality due to acute radiation effects and other bomb-related trauma but not most delayed radiation effects on mortality (except for a small number of early leukemia deaths).

Variation with dose in the relative risk of death from all causes and changes in lifespan are two ways of describing and quantifying the same phenomenon—the effect of exposure on the population age-specific rate of death. An increase in one measure implies a decrease in the other; when the relative risk increases, the age-specific mortality rate becomes higher than the background rate, and so some people will die at a younger age as a result of their exposure. Reasons for favoring the use of relative risks or excess rates to summarize radiation risk include their direct relationship to impact of radiation exposure on health and the relative ease with which they can be computed and adjusted for confounding or modifying factors. However, relative risks and excess rates cannot really be understood without rather extensive knowledge and understanding of background mor-

tality rates. Loss-of-life is a seemingly simple, relatively intuitive, general summary that has some meaning to people outside of the fields of statistics and epidemiology. Despite its intuitive appeal, loss of life is not an adequate summary of radiation effects in a population since, among other things, it does not provide any information on the proportion of the population that are actually affected by a given exposure, it fails to indicate when these exposure-associated cases might be expected to occur, and it is not very useful when one needs to focus on the impact of an exposure on specific outcomes (e.g., solid cancer).

Using total mortality and avoiding the use of any particular dose-response model by using dose groups, we found compelling evidence that radiation exposure has increased total mortality rates (and hence shortened lives) for atomic-bomb survivors with doses in excess of 0.25 Gy and some indications, albeit not statistically significant, that there is slight life shortening at lower doses. There is no evidence of increased longevity in any dose range in the LSS. We suspect that others' claims of greater longevity in certain dose groups may partly reflect biases resulting from the choice of comparison group. At least for total mortality in the LSS, the zero-dose group is not necessary for inference about either radiation risk or effect modification by sex and age at exposure.

We do not recommend the use of total mortality or dose groups for describing radiation risks. This approach was taken because of our interest in com-

paring the LSS cohort to other studies. However, the message seems clear. Studies that compare radiation-exposed survivors of the bombings to the distal (zero-dose) survivors or to the general population can produce biased results, with the bias especially pronounced at low doses. Furthermore our results suggest

that the Life Span Study of atomic-bomb survivors contains enough proximal, low-dose survivors to support useful inferences about radiation risks, particularly low-dose risks based only on survivors who were within 3 km of the hypocenters.

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Multifactorial Diseases in the Post-Genomic-Sequencing Era and Our Current Position

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Department of Genetics

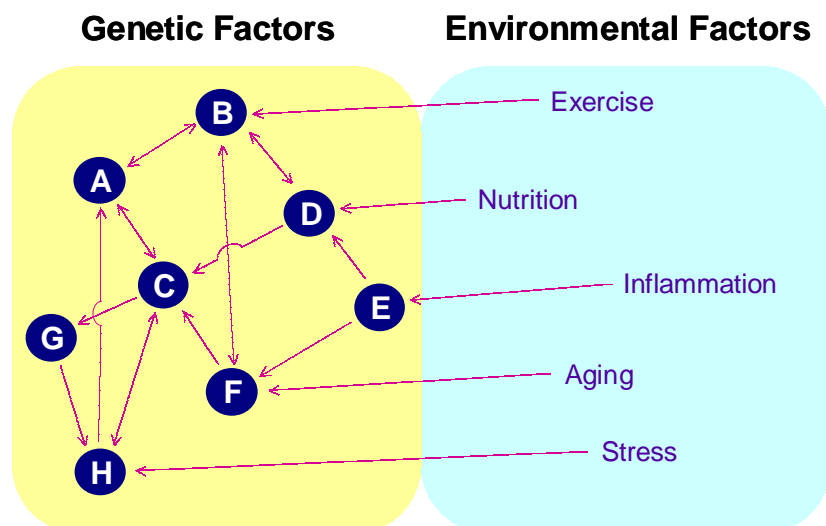
In the field of human genome research, nearly complete human chromosome sequences of number 22 and number 21 were published in December 1999 and May 2000, respectively. Subsequently, two different groups have independently published draft sequences of the entire human genome in the March 2001 issues of *Nature* and *Science*, respectively. Nearly complete information on the base sequences of chromosome 20 was reported in December 2001. The International Consortium announced that nearly complete sequences of the entire human genome would be produced by 2003. Now we are entering the so-called “post-sequence” era, in which the sequence information enables us to analyze functions of all possible gene products or proteins.

One of the most important aims in studying human genome/genes functions is to identify etiology of diseases. Post-sequence research that would be directly associated with genome information includes systematic and genome-wide gene expression analyses by utilizing **DNA chips and microarrays**, as well as approaches involving genetic epidemiology with the use of genetic polymorphisms as markers (terms in bold face are clarified in a Glossary at the end). Throughout the world, owing to such developments, it will become a trend to identify disease etiologies and apply individual information for medical treat-

ment. In the year 2000, the Japanese Government (led by the late Prime Minister Keizo Obuchi) initiated the “Millennium Project”; where one of the goals is a so-called “conquest of multifactorial diseases (lifestyle diseases).” RERF is on the verge of initiating a clinical health study for children of A-bomb survivors, primarily focusing on multifactorial diseases. It seems therefore timely to explain multifactorial diseases and strategies to be used in our future study. It would be my pleasure if this report can help you in understanding current research directions of multifactorial diseases as a whole.

Multifactorial diseases develop as a result of complicated interactions between environmental and genetic factors. In contrast to single-gene disorders (**Mendelian diseases**), occurrence of multifactorial diseases does not generally follow Mendelian patterns of inheritance. This is because many, diverse genes, such as A to H shown in Figure 1, are involved, and each gene has only a minute effect. These genes seem to be talking to each other through a complicated network in which the interaction of gene products varies among individuals both quantitatively and qualitatively. Moreover, expression profiles of genes (i.e., spectrum of genes regarding the amount of proteins produced from each gene) are affected by not only genetic but also non-genetic factors such as

Figure 1.



physical exercise, nutrition, inflammation, aging, and stress. These latter factors may further interact with each other. It is therefore obvious that identifying genes implicated in human multifactorial diseases is difficult.

Despite such difficulties, it is socially important to identify responsible genes because the prevalence of multifactorial diseases is so high in developed countries. As shown in Figure 2, if the mechanism for the development of multifactorial diseases could be elucidated, (1) new therapies and diagnostic methods and (2) individualized treatment would become possible. This is because even among patients showing similar symptoms or diagnoses, the etiologies may be different due to different genetic background or mechanisms involved. Further, if **polymorphic markers** associated with susceptibility in developing the multifactorial disease could be found, it would become possible to predict (3) individual susceptibility that will facilitate prevention and early detec-

tion/treatment of the diseases, and (4) individual drug responses including side-effects provided that further relevant individual markers continue to be recognized.

Figure 3 shows the strategy for identifying genes related to disease etiologies. Shown on the left is the knowledge-based method (also called functional cloning). This is a classical method to determine candidate genes through etiological, biochemical, or pathological knowledge. Many genes responsible for congenital metabolic disorders were identified by this method, while only proteins whose functions are well characterized can be used. Human Proteome Project (HUPO), which has been launched by a group of top-level proteomics researchers, aims at understanding interactions of all relevant proteins for normal functions of tissues. Theoretically, this approach will enable us to dissect any diseases at the molecular level.

However, it will certainly take many years to fully

Figure 2.

Why etiologies of multifactorial diseases should be elucidated so rapidly

Prevalence of such diseases is about 70% in general population

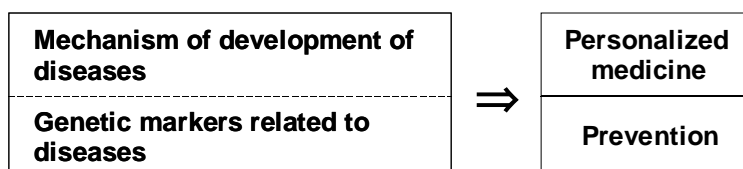
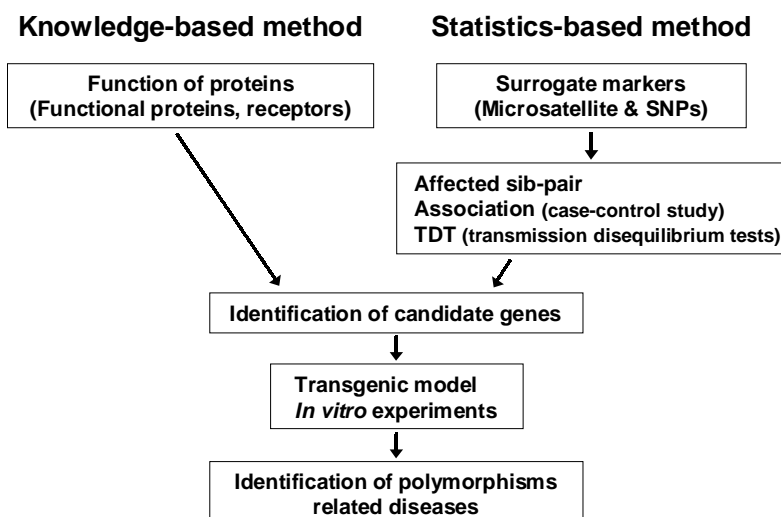


Figure 3.



understand the molecular mechanisms of every disease as the proteomics study has just begun. Therefore, a temporal and alternative option is to find candidate genes from patients' polymorphism information characteristic to the disease (right panel of Figure 3). Namely, this is to trace regions in the human genome that are highly associated with the phenotypes of concern. This method is commonly used in ongoing studies (including the Millennium Project), although there are some differences among methods in the details of applications (i.e., pedigree analysis, affected sib-pair analysis, association study, transmission disequilibrium tests, etc.).

In either approach, the candidate genes need to be confirmed by creating transgenic or knock-out models in animals or cells. Thereafter, typical polymorphisms affecting enzyme activities or enzyme stabilities, for example, will be used for clinical applications (Figure 2).

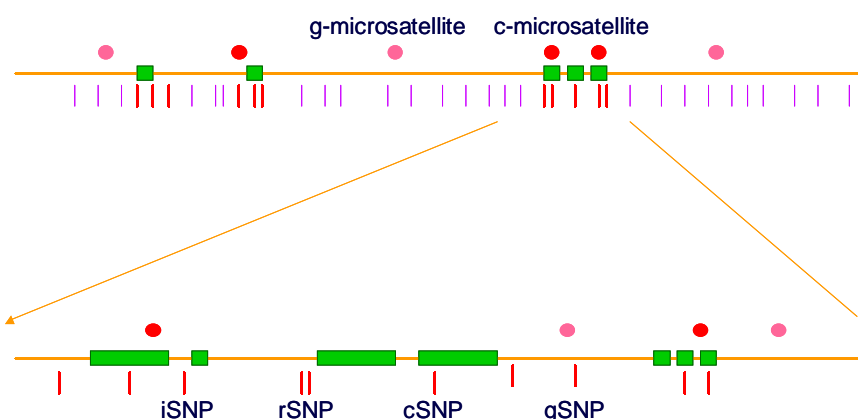
Figure 4 shows a typical strategy to identify candidate genes. This method has been widely adopted by researchers involved in the Japanese Millennium Project because it is believed to be the most effective and efficient method. The strategy uses a combination of microsatellites and SNPs (single nucleotide polymorphisms) as markers. Microsatellites represent base sequences comprising tandem repeats of short core sequences (2 to 9 bases) and the number of repeat units (n) varies among individuals (i.e., large number of **alleles** are present; $n = 1, 2, 3, 4, \dots$ etc). Selected sites with **heterozygosity indices** of usually ≥ 0.7 are to be used. However, microsatellites are not suitable for fine mapping of candidate genes because polymorphisms exist on the average in every 11 K base pairs (bp). This points to

the role of SNP because they are more abundant and hence more effective in fine mapping; existing in every 300–1000 bp. However, it is necessary to examine many markers because the heterozygosity index of a SNP is only about 0.3 because of its bi-allelic nature (i.e., there are only two alternative states at one site, either 0 or 1). Depending on the location of either microsatellite or SNPs in the genome, they are classified into several groups; **c** (exists on mature mRNA or its copy, cDNA), **i** (on intron), **r** (on regulatory sequence which controls RNA production of the gene), and **g** (on genome). In Japan, the predominant view is that we should focus on **c**, **i**, and **r** but not **g**, as the latter is considered to have little relevance to protein structure or its expression. In contrast, in the U.S., it is believed that all the markers should be probed exhaustively, because no one knows yet if **g** is really irrelevant or not.

The basic plan in the Millennium Project is to use 30,000 microsatellites uniformly distributed throughout the genome, i.e., one in every 100 Kb, for brief mapping of relevant genes. A statistical approach will clarify significant association of these markers with various phenotypes; presence or absence of a symptom, physiological measurement values such as blood pressure, thickness of a blood vessel wall, blood glucose level, blood concentration of stimulatory substances involved in physiological responses, etc. This first screening will use a relatively small number of subjects (100–200 people) because it aims at detecting the best candidate regions in the genome. Subsequently, a large number of subjects will be tested to narrow down the regions using SNP information and to confirm that the genes are indeed relevant to the disease.

Figure 4.

Strategy for genome-wide mapping of disease related genes



Courtesy of Professor Tetsuro Miki, Ehime University, School of Medicine

On the other hand, some critics argue that the microsatellite markers will not work well for mapping the disease-related genes, since these markers are not always associated with the genes. Thus, they assert that SNPs should be used from the beginning, even though SNPs typing is still very laborious.

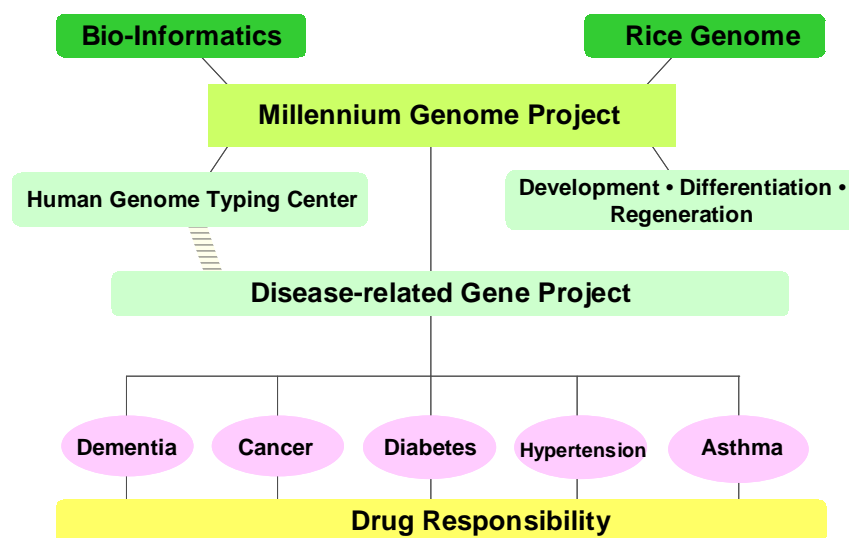
In FY2000, five Japanese Ministries established the Millennium Project consisting of 11 major projects among which is included the "Genome Project." Figure 5 shows an outline of the "Genome Project" which can be divided into four large fields. One of the key study projects, named "Disease-related Gene," is expected, within FY2000 to 2005, to identify the relevant genes to common diseases (such as dementia, cancer, diabetes, hypertension, asthma) and drug response following treatment of patients suffering from these five diseases. For the "Millennium Genome Project," tens of billions of yen a year is being invested in finding polymorphic markers, developing detection methods of SNPs (including a cohort study), and developing statistical methods to handle the data to be obtained. The project of the "Disease-related Gene" is intent on collecting samples from patients of five target diseases and appropriate control subjects. This group closely collaborates with the "Human Genome Typing Center" to which the members send specimens (DNA in many cases). The Center will undergo the most extensive typing systematically apart from small-scale typing effort in individual laboratories. Currently, the Center is making headway, having obtained over 20,000 SNPs, a number that will certainly continue to increase. The "Microsatellites Typing Center," under the direction of Professor

Inoko at Tokai University, has obtained 30,000 informative markers. The results from these centers will be analyzed statistically in collaboration with the "Bio-Informatics" group.

After completion of the Project, it is expected that many SNPs associated with the five main multifactorial diseases mentioned earlier, and that the drug responses will be found. Turning our eyes to the world, TSC (the SNP Consortium consisting of ten pharmaceutical companies and five academic organizations in USA and Europe) has already mapped more than 300,000 SNPs. Celera Genomics announced that they could provide more than 3.5 million SNP data identified by themselves, in addition to public data bases such as the Human Gene Mutation Database and the data base of SNPs (dbSNP) of National Center for Biological Information (NCBI).

Lastly, I would like to express my personal view how to use the massive data sets to be produced for RERF studies. If individual susceptibility could become predictable for development of multifactorial diseases, then, there will be little doubt that prevention of development of their diseases by introduction of appropriate change of their life style and early detection/treatment of their diseases would become possible to the participants of the on-going F_1 clinical study. The huge amount of polymorphism data (SNPs and microsatellites) would provide us good hint for understanding why incidence of some "non-cancer" diseases are higher in high dose survivors than non-exposed individuals. We should keep further discussions on the possible applications of the technique.

Figure 5.



From the Homepage of Ministry of Education, Culture, Sports, Science and Technology

Glossary

Allele; A gene that differs from the wild-type gene is generally called a mutant gene. Usually, the mutant gene has its function altered. However, if the difference is subtle and the mutant gene exists frequently in a population, it is difficult to describe which form is normal or mutant. Under such circumstances, different forms of genes are called alleles. In theory, any gene may have a number of alleles including functionally normal and abnormal ones.

DNA chip or microarray; Slide glasses containing multiple spots of DNA at a high density are generally termed DNA chips or microarrays. Each spot consists of multiple copies of a cloned DNA fragment so that a test DNA sample or a cDNA sample (artificial copy of mRNA) may hybridize quantitatively on the spot of homologous base sequences. The test DNA is tagged with fluorescent dye(s) before hybridization so that scanning of the slide with a narrow laser beam and measuring the emitted fluorescent light intensity will tell us the spots that showed scarce to intensive hybridization of the test DNA.

Heterozygosity index; This generally indicates the frequency of individuals bearing two different alleles in a population examined.

Mendelian inheritance or Mendelian diseases; Most of the human hereditary diseases that are not seriously affected by acquired conditions follow either dominant or recessive forms of inheritance that

were originally discovered by Mendel. Hemophilia is the well-known autosomal recessive disease among European Royal families. If the mutation happened in a gene located on the X chromosome, the affected individuals are mostly and characteristically males as females have two X chromosomes while males have only one. Color blindness is one of the well-known examples of X-chromosomal mutation.

Multifactorial diseases; In contrast to Mendelian diseases, multifactorial diseases are unique in that no single dominant mutation usually affects the disease onset but multiple genes provide the disease susceptibility. Because contribution of each gene is rather small, improved life style may affect the onset of the diseases. Diabetes mellitus and high-blood pressure are the examples.

Polymorphism; The traits that differ among apparently normal individuals are called polymorphism, such as ABO blood type. In the case of DNA polymorphism, base sequences differ among individuals whereas most of them have no effect on the phenotype.

SNP (pronounced as [snip]); Abbreviation of Single Nucleotide Polymorphism, which exists frequently in our genome. In most of the cases, a single base, say Guanine, is altered as another base, say Cytosine, in a fraction of population.

Facts and Figures

Department of Statistics

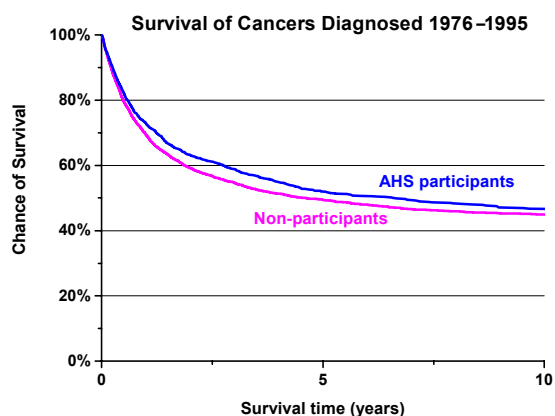
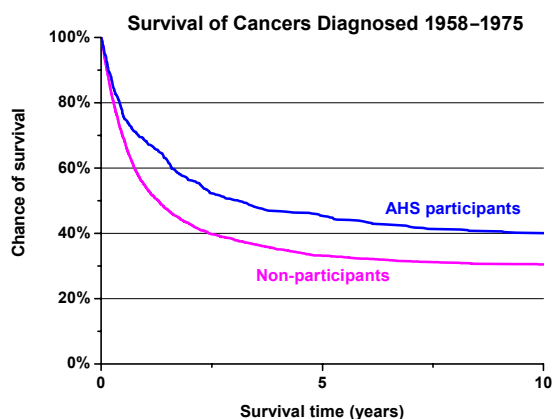
Effect of AHS Participation on Cancer Survival

We recently addressed the question of whether in the Life Span Study (LSS) there are differences in survival times between radiation-related and spontaneous cancers. Dr. Nori Nakamura of the Department of Genetics, in particular, raised the issue. Although our investigation, which will be reported elsewhere, found no evidence of association between survival time and atomic bomb radiation dose, it is noteworthy that we did find it necessary to adjust for participation in the RERF Adult Health Study (AHS). As we indicate and discuss here, participants in the AHS have tended to have longer survival times than non-participants, particularly during the program's earlier years. Because a larger proportion of high-dose than low-dose LSS cohort members were invited to participate in the AHS, it is essential that analyses of survival after cancer diagnosis be adjusted for AHS participation. Without this adjustment, there is a spurious indication that survival times are longer for those at higher radiation doses.

The figures here compare AHS participants (for at least one cycle) and other LSS members, showing the proportions who had not died of the originally-diagnosed cancer as a function of time since diagnosis. Survival times depend on age at diagnosis

and are here standardized to age 70. The curves tend to level off after about 10 years, at levels that can be considered as cure rates. Survival times, cure rates, and the contrast between AHS participants and others, all differ between the earlier and later parts of the AHS program.

Particularly during the earlier part of the AHS program, participants had both longer survival times and higher cure rates than non-participants. This is probably because participation leads to referrals based on suspicious clinical findings, which in turn leads to earlier diagnosis of cancer. It is for stomach cancer and female genital cancers that the distinction is greatest. Since earlier detection would in itself lead rather unremarkably to longer times between diagnosis and death, the difference in cure rates with participation is the more important aspect of the results seen. The smaller distinction seen during the latter part of the AHS program is probably due to introduction of alternative screening programs in the community and generally improved health care. Note that the cure rate for both groups in the latter period is somewhat higher than even that for AHS participants in the earlier period, probably reflecting a generally improved effectiveness of cancer therapy.



Historical Vignette 1955: Hiroshima Diary and The Hiroshima Maidens

Robert W. Miller

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In July 2000, the Mayor of Hiroshima asked several alumni of the Atomic Bomb Casualty Commission (ABCC) to send him Peace Messages for the 55th anniversary of the explosion on August 6. To my knowledge, these have not been published. My message was a historical vignette concerning two monumental ventures that gained international attention in 1955. They concerned collaborations between the U.S. physicians and Japanese with regard to the effects of the bomb. I witnessed the unfolding of these events when I was Chief of Pediatrics at the Hiroshima ABCC. In one, an American surgeon was key to the literary success of a Japanese physician. In the other, a daring venture sponsored by an American medical group owed its success largely to a Japanese-American woman who had returned to Japan to marry before the war.

In 1951, Dr. Warner Wells, a surgeon from the University of North Carolina, was at the ABCC (name changed in 1975 to the Radiation Effects Research Foundation), where survivors of the bomb are examined by the Japanese and American staff for late effects of radiation exposure. There was little need for a surgeon there, so he visited medical clinics in Hiroshima, where he lectured on surgical diseases and pursued his interest in overgrown scar tissue (keloids). He learned that Dr. Michihiko Hachiya, Director of the Communications Hospital, had written a diary during his 56-day hospitalization for wounds from the bomb. Dr. Wells sought him out and received permission to have the diary translated for publication in English. It had been published serially in the *Journal of the Communications Bureau*, where it circulated only among the medical staffs who provided care nationally for postal, telephone, and telegraph workers. Dr. Wells brought the publication to ABCC, where it was translated into imperfect English with an attractive Japanese "accent." It was inevitable that this feature would be lost in editing, carefully done by Dr. Wells.

It was very exciting to read the book reviews of *Hiroshima Diary (1)* in 1955. They were prominently featured by *The New York Times*, *Saturday Review*, *Newsweek*, and *Atlantic Monthly*, among others, and translated into 14 languages. The royalties, which must have been substantial, were declined for personal use by Dr. Hachiya and Dr. Wells. Dr. Hachiya requested \$10,000 for a fund to pay for the education of children who were made orphans by the bomb. When they repaid the loans, the funds were recycled.

Also in 1955, Norman Cousins, editor of the *Saturday Review*, arranged for 25 young Hiroshima women with severe scar contractures to be treated at Mt. Sinai Hospital in New York. At the last minute, while at ABCC, the New York group realized they needed as a chaperone a mature friend, guide and interpreter who knew both cultures. The visitors discussed the problem as they stood beside the desk of Helen Yokoyama, a Nisei interpreter who had majored in psychology at UCLA. Her travel documents were in order, and she was immediately available. She left with the maidens and was with them as they adjusted to the homes of Quaker families, where they stayed. She was with them at the hospital when they underwent surgery. Without her, the 18-month mission during which 138 operations were performed, would have had a rough course and may well have collapsed. The story is told in books by Rodney Barker [*The Hiroshima Maidens (2)*] and Anne Chisholm [*Faces of Hiroshima (3)*], which unfortunately are out of print. Helen Yokoyama repeatedly turned aside appeals to record the history of her experience.

Thus did working together in medicine help to heal the animosity from a war that had ended 10 years earlier.

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In Memoriam: Robert M. Heyssel

Dr. Heyssel served as Chief of the ABCC Department of Medicine during 1956–1958. Later, during a distinguished career at Johns Hopkins University, he became a member of the National Academy of Sciences Institute of Medicine. He received his B.S. in 1951 from the University of Missouri and his M.D. in 1953 from St. Louis University. In 1956, he joined the U.S. Public Health Service as a Senior Assistant Surgeon and was stationed at the ABCC in Hiroshima and Nagasaki for two years. After returning to the U.S., he did a fellowship in hematology at Washington University in St. Louis, then spent 10 years at the Vanderbilt University School of Medicine. Heyssel began his tenure at Johns Hopkins in 1968 as an Associate Dean of the School of Medicine and as an Administrator of the Johns Hopkins Hospital. He retired as President of the Johns Hopkins Health Care System in 1992, widely recognized as the chief architect of the Institution's highly regarded model of diversified modern health care delivery enterprise. He continued

to serve in many capacities until his death at age 72 on June 13, 2001 in Seaford, Delaware. His wife Maria, who kindly provided his picture for *Update*, survives him.



Research Protocols Approved 2001

RP 1-01 Culture of Permanent Lymphocyte Cell Lines as Sources of Biological Samples for Investigation of Genetic Effects of Radiation on Children of Atomic Bomb Survivors

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, Murakami H, Fujiwara S, Akahoshi M, Nakamura N

Based on RP 5-85, since 1985, blood samples have been collected from about 1,000 families consisting of both parents (at least one of whom is a survivor) and their children, and their permanent lymphocyte cell lines have been established. At that time the explanation for this project was carried out by the letter which was initially sent, and orally later when the subjects visited RERF for blood donation. However, this procedure for getting an informed consent does not necessarily seem to be suited to current conditions. Therefore, in this addendum we propose that a written informed consent be obtained from those who have previously donated blood, and their agreement be confirmed again.

RP 2-01 Pilot Study of Genetic Background of AHS Population: Identification of Markers in Potential Candidate Genes Associated with Hypertension

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, Murakami H, Yamada M, Kasagi F, Suzuki G

Based on RP 1-97, we examined the association between hypertension and polymorphic markers of hypertension-related candidate genes in the renin-angiotensin system among approximately 300 Adult Health Study (AHS) participants. However, only oral consent was obtained from the participants. We now wish to obtain signed consent forms, necessary for continued work on hypertension at RERF, from the AHS participants who donated their blood for DNA analysis. In addition, we propose to conduct a collaborative study with outside research organizations, by collaborating in the study "Elucidation of Hypertension-related Gene Groups," if informed consent for this study is independently obtained. This study is being conducted by Professor Akira Hata of the Department of Public Health, Asahikawa Medical College, as part of a nationwide study known as the Millennium Project, seeking to identify the candidate genes associated with a number of leading

diseases (hypertension, atherosclerosis, diabetes, asthma, osteoporosis, rheumatoid arthritis, etc.) in Japan. Informed consent is also requested of the donors if they so desire for use in these other studies. All of these studies will follow the requirements of the ethical guidelines developed by the Ministry of Health and Welfare.

Recent Publications

(Japanese): the original article is in Japanese; (JTr): a Japanese translation is available.

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