Report on the Health Effects Study of the Children of A-bomb Survivors

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Scientific and Ethics Committees for the Health Effects Study of the Children of A-bomb Survivors

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Report of the Scientific and Ethics Committees for the Health Effects Study of the Children of A-bomb Survivors

Summary

Study overview

The Health Effects Study of the Children of A-bomb Survivors has been conducted for seven years since 2000 to investigate whether there exists any relationship between the prevalences of adult-onset multifactorial diseases (hypertension, diabetes mellitus, hypercholesterolemia, myocardial infarction, angina pectoris, and stroke) and parental radiation exposure.

It is considered that multifactorial diseases develop due to both genetic and lifestyle factors. Since the prevalences of multifactorial diseases increase in adulthood, it is meaningful to evaluate association of the diseases with the mechanisms of genetic effects of radiation. The Health Effects Study is the first to investigate such association in human subjects.

For the conduct of this study, starting in its planning stage, committees of outside experts – the Scientific Committee, the Ethics Committee and the Analysis Subcommittee – were established to investigate the appropriateness of study objectives and plans as well as the ethical issues involving study methods. Thus far, there have been five meetings of the Scientific Committee, four meetings of the Ethics Committee, six joint meetings of the Scientific and Ethics Committees, and four meetings of the Analysis Subcommittee.

Study subjects

From about 77,000 subjects of RERF's ongoing F1 Study, a total of 24,673 individuals were selected as the mail survey cohort for the Health Effects Study of the Children of A-bomb Survivors. The mail-survey subjects were individuals with permanent and present addresses either in Hiroshima or Nagasaki, or in the cities' adjacent areas, and with one or both parents exposed to 5 mGy of radiation or more, and gender-, city- and age-matched individuals with both parents exposed to less than 5 mGy or not exposed to radiation. The mail survey employed a self-administered questionnaire with questions regarding health status, smoking, alcohol intake, diet, physical activity, family size, occupation, educational history, and pregnancy/delivery. To solicit participation in the clinical health examinations, the primary objective of the Health Effects Study, an inquiry was made to confirm willingness to undergo health examinations, and those wishing to undergo the examinations were then enrolled in the clinical study. Those expressing their desire to undergo health examinations in the mail survey comprised 57.3% of the total, 84.5% of whom (11,951 individuals) actually underwent examinations. Lifestyle data obtained from responses to the questionnaires were incorporated into the analysis of multifactorial diseases observed through the health examinations.

Statistical analysis

When any of hypertension, diabetes mellitus, hypercholesterolemia, myocardial infarction, angina pectoris, or stroke was identified, the person was considered to have multifactorial disease. By using the presence or absence of multifactorial disease as the outcome variable, relationship with parental radiation dose (separately for father and mother)

was analyzed using a logistic regression analysis. Common risk factors for multifactorial diseases, including age, gender, BMI, drinking/smoking habits, menopause (females only), and parental history of multifactorial diseases, were included in the analysis. Furthermore, degree of effects of factors that might be considered to modify radiation effects, including gender, parental age at the time of bombing (ATB), and time from exposure to birth, were studied. To confirm possible effects of the study subject's health status and his/her parents' exposure status on the participation rate for the health examinations, those who participated and those who did not participate in the examinations were compared.

Analysis results

– In analysis of the data from the current study, when multifactorial diseases in children were combined, no evidence suggesting increased risk associated with parental radiation exposure was observed (with an odds ratio for paternal dose of 1 Gy being 0.91, and that for maternal dose of 1 Gy being 0.98; statistical significance was not achieved with either).

– In males, a negative association between paternal dose and prevalence of multifactorial diseases was suggested. However, careful interpretation of this finding is necessary.

Evaluation and recommendations

1. The Health Effects Study of the Children of A-bomb Survivors has been properly conducted with use of methods approved by the Scientific Committee, under conditions established by the Ethics Committee. In addition, it was confirmed that the collected data were analyzed in a proper manner with use of statistical methods approved by the Analysis Subcommittee.
2. The present study did not show a positive association between parental radiation exposure and health effects among offspring, although a negative association was suggested between paternal dose and the prevalence of multifactorial diseases among male children. Given that the average age of the study population, 48.6 years, is relatively young, careful interpretation of such findings is required by continuation of the study, including the items which did not reveal significant association.
3. The proportion of the mail survey cohort participating in the health examinations was 48.8%. Among the subject population, the number of males was about 19% higher than that of females, while the number of females was 10% higher than that of males among the health-examination participants. RERF needs to also take into account this matter when interpreting the results.
4. It was recommended that disease-specific analysis be performed if possible, in addition to our combined analysis of all multifactorial diseases.
5. In the future, as the study population ages, it is necessary to consider the possibility that RERF should embark on research into further health-related indices besides the study items examined up to this point. It is also hoped that the study is continued, given that, due to progress in gene analysis technologies, introduction of new perspectives, such as association between genetic disposition and disease onset, can be anticipated in the future.
6. It is recommended to continuously study the fixed F1 population in a prospective manner, by taking into account the above-mentioned points.
7. We, along with RERF, would also like to express our deep appreciation to the children of A-bomb survivors who have cooperated in this study

1. Background

With regard to genetic effects of radiation, various experimental studies conducted in the 1920s and later indicate the possibility that ionizing radiation induces mutations in germ cells and chromosomal abnormalities, which can cause genetic disorders.

A number of studies involving human subjects on genetic effects have been conducted at the Atomic Bomb Casualty Commission (ABCC) and the Radiation Effects Research Foundation (RERF). The early genetic program studied congenital abnormalities, stillbirth, and prenatal deaths. That research was followed by studies of chromosomal aberrations, protein alterations by one-dimensional electrophoresis, and changes in enzyme activity. Furthermore, long-term mortality and cancer incidence follow-up studies of a large cohort of children of A-bomb survivors are being continued. To date, the studies have produced no evidence of dose-related genetic effects in the children of A-bomb survivors.

Genetic diseases are broadly classified into single-gene diseases, which are caused by a single gene mutation, chromosomal disorders, and multifactorial diseases. Examples of such multifactorial diseases include congenital anomalies that are often observed at birth (e.g., neural tube defect, cardiovascular malformation, cleft lip and/or palate) and many common adult-onset diseases (e.g., coronary heart disease, diabetes, essential hypertension, cancer, etc.).

Until the mid 1980's, risk estimation for radiation-related genetic disorders drew primarily on laboratory and human data for single-gene diseases. However, the 1996 report from the International Commission on Radiological Protection (ICRP) Task Group on Risk Estimation for Multifactorial Disease assumed a statistical model to predict radiation-induced mutations in multifactorial diseases on the basis of laboratory data from animals. Since no previous study evaluated genetic risk for multifactorial diseases in the human population, however, the Blue Ribbon Panel, convened in 1996 to evaluate RERF research, recommended that "consideration be given to further investigation into the health of the offspring (F1 cohort) since it may well yield valuable information on genetic effects, especially when conducted together with research using the new molecular genetics techniques." In response to this recommendation, RERF planned and initiated a health study of the children of A-bomb survivors, consisting of a mail survey and a clinical study.

2. Mail survey

Objectives

Objectives of this health effects study's mail survey is creation of opportunity to directly contact children of A-bomb survivors, who are registered as part of RERF's study population but have rarely been contacted thus far, and inquiring about their willingness to participate in a clinical health examination program for studying genetic effects of parental radiation exposure on the onset of multifactorial diseases. Those who indicated desire to participate in the clinical study were incorporated into the clinical study cohort, and data on lifestyle factors were collected from clinical examination participants through use of another questionnaire. Such lifestyle data was also expected to serve as a useful information source for conduct of the ongoing cancer mortality and incidence studies on children of A-bomb survivors.

Subjects and methods

1) Study subjects

(1) Determination of the subject size necessary for the health effects study

RERF started determination of the subject size by taking into account the main objective of this health effects study, which was to examine effects of parental radiation exposure on multifactorial diseases.

In consideration of the aforementioned risk estimation for multifactorial diseases by the ICRP task group, RERF established a minimum goal of over 10,000 participants for the clinical examinations.

(2) Establishment of study population

The basic mail survey cohort was established by selecting, from among the F1 mortality follow-up cohort of 76, 814 people (Table 1), all known to be alive at the time of selection who were children of A-bomb survivors with parental dose ≥ 0.005 Gy for both or either of the parents, and gender-, city-, age-matched individuals born to parents with dose < 0.005 Gy or unexposed, totaling 33,786 participants (Table 2).

Table 1. City-, gender-specific composition of the F1 mortality follow-up cohort

	Hiroshima	Nagasaki	Total
Men	24,824	14,574	39,398
	51.7%	50.6%	51.3%
Women	23,190	14,226	37,416
	48.3%	49.4%	48.7%
Total	48,014	28,800	76,814
	100.0%	100.0%	100.0%

Table 2. City-, gender-specific composition of the basic mail survey cohort

	Hiroshima	Nagasaki	Total
Men	10,978	6,222	17,200
	50.6%	51.5%	50.9%
Women	10,716	5,870	16,586
	49.4%	48.5%	49.1%
Total	21,694	12,092	33,786
	100.0%	100.0%	100.0%

From among the basic mail survey cohort and individuals whose *koseki* attachments were already obtained, 17,698 people whose family registry was in the cities of Hiroshima or Nagasaki and whose current address is in the clinical study catchment area (i.e. in or near the cities) were extracted to create a mail survey cohort (Table 3). Such selection brought about imbalance in the gender ratio in the Hiroshima group, which was attributable to differences between Hiroshima and Nagasaki, including change of family registry due to marriage, and change of current address for occupational reasons. Since the selection for both cities was conducted based on the same criteria, however, RERF established this population as the original cohort.

Table 3. City-, gender-specific composition of the mail survey cohort

	Hiroshima	Nagasaki	Total
Men	7,040	3,050	10,090
	59.8%	51.4%	57.0%
Women	4,727	2,881	7,608
	40.2%	48.6%	43.0%
Total	11,767	5,931	17,698
	100.0%	100.0%	100.0%

A pilot mail survey was conducted on 300 subjects extracted from among the original mail survey cohort, with half of that total expressing willingness to participate in clinical examinations. The pilot survey thus suggested that the mail survey cohort should include at least 20,000 subjects for the purpose of attaining the aforementioned target of over 10,000 participants for the clinical examinations.

For that reason, the original cohort was augmented with the addition of 9,813 individuals who satisfied the conditions that one parent was exposed to 0.005-0.999 Gy of radiation according to DS86, and the other with no dose information or DS86 dose estimate not available. In addition, the target family registry areas were expanded to completely cover the catchment areas. With regard to the high dose group of ≥ 1 Gy, the target areas were further expanded to cover the entire country. RERF thereby collected *koseki* attachments and added to the cohort those individuals residing in either city or the cities' surrounding areas (catchment areas).

Ultimately, the mail survey cohort was composed of a total of 24,673 subjects, including 16,348 in Hiroshima (9,238 males and 7,110 females) and 8,325 in Nagasaki (4,151 males and 4,174 females) (Table 4), with the target cohort size attained.

Table 4. City-, gender-, age-specific mail survey subjects

		Age group					
		-29	30-39	40-49	50-	Total	%
Hiroshima	Men	426	1,540	3,755	3,517	9,238	56.5
	Women	470	1,274	2,692	2,674	7,110	43.5
	Subtotal	896	2,814	6,447	6,191	16,348	100.0
Nagasaki	Men	205	815	1,911	1,220	4,151	49.9
	Women	252	868	1,899	1,155	4,174	50.1
	Subtotal	457	1,683	3,810	2,375	8,325	100.0
Total	Men	631	2,355	5,666	4,737	13,389	54.3
	Women	722	2,142	4,591	3,829	11,284	45.7
	Subtotal	1,353	4,497	10,257	8,566	24,673	100.0

Table 5. Full-scale mail survey subjects and background of selection

		Maternal Dose (Gy)						
		No Info	Unexposed	<0.005	0.005-0.999	>=1.0	Unknown	計
Paternal dose (Gy)	No Info				1,821	140		1,961
	Unexposed		3,773	3,236	3,847	379		11,235
	<0.005		1,142	1,773	1,179	108		4,202
	0.005-0.999	2,012	1,309	1,043	1,346	50	266	6,026
	>=1.0	169	217	201	95	24	34	740
	Unknown				360	35	114	509
	Total	2,181	6,441	6,253	8,648	736	414	24,673

Dosimetry system: DS86

1) Mail survey subjects selected based on initial target dose range :
2) Mail survey subjects after expansion of target dose range :

2) Study methods

(1) Questionnaire and related materials

A self-administered questionnaire with a letter asking for cooperation and a brochure were sent to 24,673 mail survey subjects. The letter emphasized that those selected for the survey would include not only children of A-bomb survivors but also, for the purpose of comparison, children born to non-survivor parents, and it also explained the mail survey subject selection method. The brochure provided detailed explanation of the background, purpose, and justification of the study, outline of the clinical health examination program, and handling of personal information.

The questionnaire elicited information on (a) subject name, gender, date of birth, and address, (b) present and past health status, (c) personal habits such as smoking, drinking, diet, and exercise, (d) socioeconomic status including occupation and education level, (e) obstetric and gynecologic history (women only), and (f) willingness to participate in the clinical health examination program.

(2) Mailing

The questionnaire was sent to all 24,673 mail survey subjects using addresses identified from *koseki* attachments, which were acquired for academic purposes. The pilot mail survey was conducted in 2000, and the full-scale survey was carried out over a four-year period, with questionnaires sent to 24,373 members, excluding the pilot mail survey subjects, four times, in 2001, 2003, 2004, and 2005. These four sub-cohorts were divided randomly by gender, age, current address, and parental exposure.

RERF sent up to two reminders with copies of the questionnaire to non-responders. Supplementary mailing or telephone interviews were conducted if returned questionnaires contained incomplete answers to key questions.

Questionnaires returned to RERF due to unknown address indicated the possibility that the relevant subject migrated after RERF had identified the current address with use of *koseki* attachments. In such cases, RERF obtained the new *koseki* attachments, identified the current address, and resent the questionnaire.

In 2006, RERF sent an interim study report to all those who had refused to cooperate in the study through 2005, explained that health examinations were available through the end of September 2006, and encouraged such individuals to participate in the clinical study. With regard to those who did not return the questionnaire, RERF also prepared and enclosed a different questionnaire for the purpose of politely requesting opinions about the study.

For handling a variety of inquires about the study, RERF introduced a toll-free telephone service. In addition, a 500-yen gift certificate and a letter of appreciation were sent to those who returned the questionnaire.

Results

The mail survey resulted in 16,368 subjects (66.3% of the total) returning the questionnaire, with 388 (1.6%) responding by telephone and other means, bringing the subtotal to 16,756 (67.9%). (Table 6)

Table 6. Outline of mail survey and health examination

	No. of subjects		Contact		Health Exam		Health Exam	
					Willing	Not willing	Participated	Did not participate
Responded by questionnaire	16,368	66.3%	Yes	16,756 (67.9%)	2,611 (10.6%)	14,145 (57.3%)	11,951 (48.4%)	4,805 (19.5%)
Direct contact by telephone, etc.	388	1.6%						
Deceased, address unknown, outside prefecture	718	2.9%	No	7,917	7,917	0	0	7,917
Did not respond	7,199	29.2%		(32.1%)	(32.1%)	(0.0%)	(0.0%)	(32.1%)
Total	24,673	100.0%		24,673	10,528	14,145	11,951	12,722

% indicates proportion of total subjects (24,674).

RERF defined the target current address area as the catchment areas (in and around the cities). A total of 718 individuals (2.9%) comprised those who had migrated out of the target areas during the survey period, those whose questionnaires mailed to the identified current address failed to reach the addressee and was returned to RERF, and those who were alive at the start of survey but died during the survey period.

RERF encouraged the non-responders to cooperate in the study a maximum of three times, including the aforementioned 2006 attempt, but, in the end, a total of 7,199 subjects (29.2%) failed to return the questionnaire.

From among 16,756 responders, 14,145 subjects (57.3% of the original total) expressed willingness to participate in the health examinations. Of this number, 11,951 (48.4%) actually underwent the exams. The city- and gender-specific composition of these 11,951 people is shown in Table 7 below.

Table 7. City-, gender-specific composition of FOCS

	Hiroshima	Nagasaki	Total
Men	4,018	1,684	5,702
	50.4%	42.3%	47.7%
Women	3,955	2,294	6,249
	49.6%	57.7%	52.3%
Total	7,973	3,978	11,951
	100.0%	100.0%	100.0%

Consequently, over 10,000 mail survey subjects underwent health examinations, attaining the goal established by the mail survey for creation of the FOCS subject cohort.

With regard to gender ratio, the ratio of the F1 mortality follow-up cohort was nearly evenly divided (Table 1). By taking into account the criteria of family registry and current address, however, the male-female ratio of the 24,673 mail survey subjects (13,389 men and 11,284 women) was 54% to 46%, 19% higher for men than women. The ratio of the 11,951 FOCS subjects (5,702 men and 6,249 women) was 48% to 52%, 10% higher for women than men.

Such imbalance in the male-female ratio of the mail survey subjects is likely attributable to the criteria of family registry and current address.

Data management and personal information protection

With regard to lifestyle-related data obtained from the questionnaires, data entry was performed in accordance with a newly prepared manual for data entry definitions, by way of double entry, and through automatic collation and checking on computers. In the final phase, RERF prepared a manual for data cleaning definitions, thereby checking for distribution and logical inconsistencies, and then created the mail survey database, data from which were incorporated into analysis on multifactorial diseases in the FOCS.

Items other than subject name, address, and telephone number were anonymized in a linkable manner and then stored in the database. Access to personal identifiers was limited to authorized staff members, and the returned questionnaires and other materials concerned

were preserved in a locked cabinet in a room equipped with a reinforced locking system. Any other personal information was strictly safeguarded in compliance with the Ethical Guidelines for Epidemiological Research.

Ethical considerations

The mail survey was the first direct contact with RERF for these health effects study subjects, a sub-cohort of the F1 mortality follow-up cohort, and hence ethical consideration was necessary because some of those contacted may not have known that their parents had been exposed to A-bomb radiation or may have been trying to conceal such parental exposure.

(1) Ethical consideration of research design and methodology

As mentioned in the Background section, RERF gave careful consideration to research design and methodology, especially to the method used for identifying current address.

As already mentioned, the letter enclosed along with the questionnaire explained that the study subjects were to include children of A-bomb survivors and, for the purpose of comparison, children born around the same time whose parents were not A-bomb survivors. In addition, the enclosed brochure explained how the subject population was selected from among the F1 mortality follow-up cohort and that current address was identified with use of *koseki* attachments, as well as detailed the handling of personal information.

In compliance with the Ethical Guidelines for Epidemiological Research as well as in light of human rights protection, the mail survey questionnaire noted that responses to such socioeconomic questions concerning education level and such female-specific items as obstetric/gynecological history were not obligatory for those not wishing to respond, in an attempt to carefully explain the human rights issues. In addition, based on deliberations by the Ethics Committee, the mail survey was conducted by taking into account subject opinions expressed by mail or with use of a toll-free telephone number even after initiation of the study.

(2) Ethical consideration of reminder for improving the response rate

As mentioned above, RERF sent an interim report in 2006 to all subjects other than those who refused to cooperate in the study, encouraging participation. In this regard, the Ethics Committees held deliberations with the aim of complying with the explanations regarding methodology made in advance to the subjects.

(3) Ethical consideration of use of sibling information for analysis

Since family clustering (among siblings) is observed for multifactorial diseases, information about subjects' siblings had to be incorporated into analysis. By considering the methods used to create the previous mortality follow-up cohort and the governmental approval already obtained, the Ethics Committee concluded that there was no ethical problem regarding use of sibling information.

3. Clinical Health Study on the Children of A-Bomb Survivors (FOCS)

Objectives

The objectives of the clinical health study on the children of A-bomb survivors (F_1 Clinical Study [FOCS]) were 1) to elucidate epidemiologically the effects of parental exposure to A-bomb radiation on the development of multifactorial diseases among the children of A-bomb survivors, 2) to preserve blood samples for future molecular biological studies, and 3) to contribute to the health and welfare of the F_1 population via health examinations and health guidance.

Subjects and methods

1) Study subjects

The individuals who responded to the mail survey questionnaire and did not refuse to participate in the clinical study were contacted by telephone and solicited to participate in FOCS. Of the recipients of the questionnaire, 57.3% expressed a willingness to participate in FOCS, of whom 84.5% , or 11,951 persons, actually underwent health examinations (Table 8). The participation rate in the mail survey cohort was 48.4% (43.0% for males and 55.0% for females; 48.8% for Hiroshima and 47.8% for Nagasaki) (Tables 6 and 9).

Table 8. Number of FOCS participants

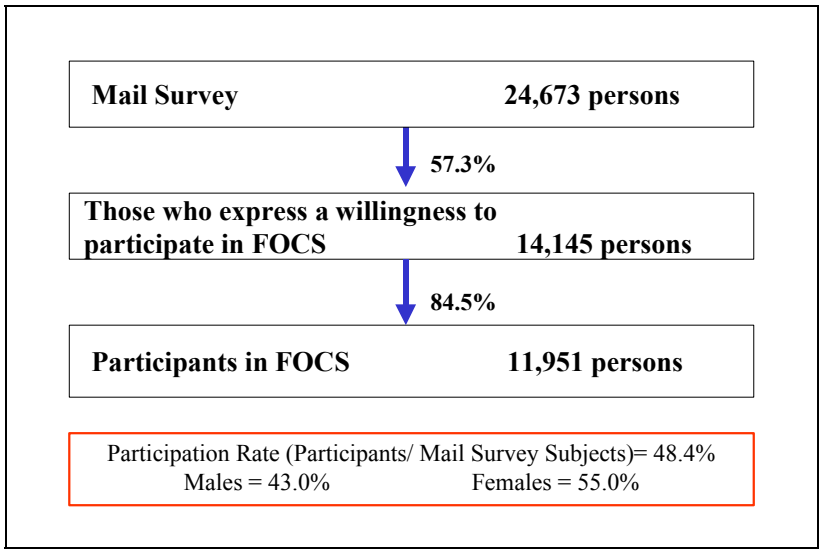


Table 9. Numbers of FOCS prospective and actual participants (by city)

	Mail survey (1)	Those expressing willingness to participate (2)	Participants (3)	Participation (%)	
				(3)/(2)	(3)/(1)
Hiroshima	16,348	9,495	7,973	84.0	48.8
Nagasaki	8,325	4,650	3,978	85.5	47.8
Total	24,673	14,145	11,951	84.5	48.4

2) Study methods

The Scientific and Ethics Committees provided scientific and ethical consideration by deliberating on such issues as procedures used for the mail survey and clinical study, health examination items, questionnaires, and informed consent, at several meetings. Consequently, FOCS was conducted as follows:

A pilot clinical study was conducted on about 500 people in Hiroshima and Nagasaki between January and June 2002 (Table 10). A full-scale clinical study was initiated in July 2002 after revisions were made to the above study items on the basis of the results of the pilot study, and was continued through September 2006. Night clinics and Saturday clinics were provided for the convenience of participants.

Table 10. F_1 health effects study

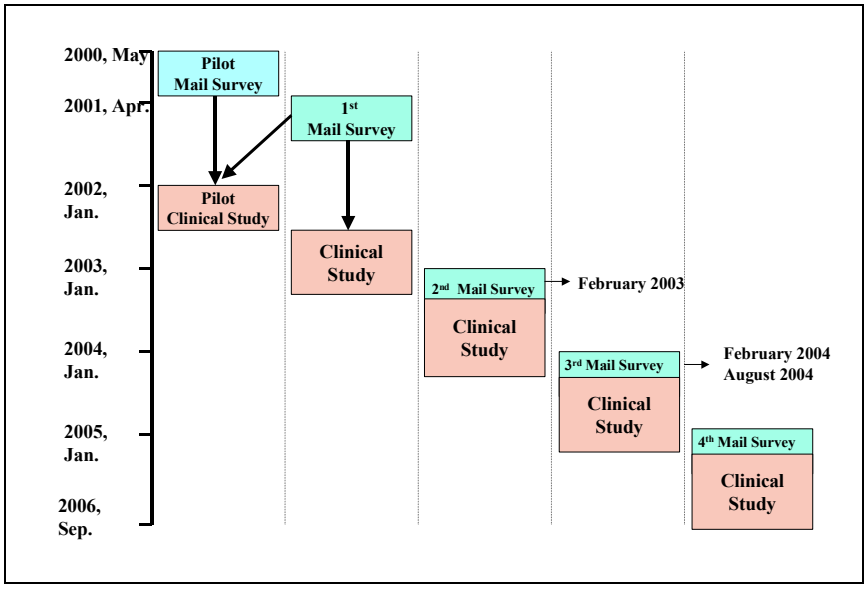


Table 11 shows details of the health examinations. Medical histories were ascertained by trained nurses through personal interviews.

Routine tests included measurement of height, weight, and blood pressure, urinalysis, examination of stool occult blood reaction, peripheral blood cell count, blood biochemistry, glucose metabolism, hepatitis virus, inflammation, and chest X-ray tests, stomach cancer screening (i.e., breath test for gastric *Helicobacter pylori* infection and serum pepsinogen I and II measurement), electrocardiography, abdominal and thyroid ultrasonography, breast cancer screening (palpation, and breast ultrasonography when necessary), sputum cytology (for heavy smokers), and osteoporosis test.

Information on medication was obtained by asking participants to bring to RERF any medications they take (i.e., medicine prescribed by doctors [ethical pharmaceuticals], non-prescription drugs [over-the-counter drugs], and health foods). The pharmaceutical codes of such medicine were then checked by RERF pharmacists, who then inquired about dosage, and start and finish dates of the medication.

Participants were referred to hospitals for such special tests as endoscopy, computer tomography (CT), and magnetic resonance imaging (MRI), when requested by participants or

deemed necessary by physicians.

Diagnostic criteria standardized between Hiroshima and Nagasaki were used when making diagnoses (Table 12). The diseases under treatment were confirmed using the medicine brought to RERF by participants. When the participants were referred to hospitals, the information on final diagnoses was obtained from the hospitals to which they had been referred.

Table 11. Examination details

1.	**Consultation**
2.	**History taking**
3.	**Self-administered questionnaires** **Dietary habits, exercise, family history, obstetric and gynecological history**
4.	**Tests given to all participants who give consent** **Blood pressure, urinalysis, fecal occult blood, peripheral blood, blood biochemistry, ECG, chest X-ray, abdominal ultrasonography, glucose metabolism, hepatitis virus, etc.**
5.	**Tests given when requested by participants or deemed necessary by physicians** **1. Gynecological examination** **2. Diet guidance** **3. Special tests (referral to hospitals)**

Table 12. Diagnostic criteria

Diabetes mellitus	**Diagnosis based on glucose tolerance test, or medical history of DM under treatment, or HbA1c ≥ 6.5 or (glucose ≥ 126 mg/dL and meal time ≥ 10 hrs) or (glucose ≥ 200 mg/dL and meal time < 10 hrs)**
Hypercholesterolemia	**Medical history of hypercholesterolemia under treatment, or T-cho ≥ 220 mg/dL**
Hypertension	**Medical history of hypertension under treatment, or （1st Systolic BP ≥ 140 or 1st Diastolic BP ≥ 90 ） and （2nd Systolic BP ≥ 140 or 2nd Diastolic BP ≥ 90 ）**
Myocardial infarction	**Medical history of myocardial infarction and satisfy the conditions such as PTCA and stent insertion, or coronary artery bypass surgery, or coronary angiography, etc.**
Angina pectoris	**Medical history of angina pectoris and satisfy the conditions such as PTCA and stent insertion, or coronary artery bypass surgery, or coronary angiography, etc.**
Stroke	**Medical history of stroke and satisfy the conditions such as CT or MRI findings, or hemiplegia, etc.**

Ethical considerations

Informed consent was obtained in the following sequence: 1) Three informed consent forms (for the health examination items, the preservation of serum, plasma, and urine to use in the future studies excluding genetic study, and the preservation of blood to use in the future genetic studies) were sent to participants before the health examinations; and then 2) trained nurses explained the intent of the informed consent to the participants at the time of the health examinations. Such mailing of the informed consent forms allowed participants time to think beforehand. Explanation was provided to those who gave consent to the preservation

and use of donated samples that they were free to withdraw their consent.

The informed consent form for the preservation of blood to use in the future genetic studies was formulated in accordance with the "Ethical Guidelines for Human Genome/Gene Research," jointly developed by the Japanese Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labour and Welfare, and Ministry of Economy, Trade and Industry and enforced on April 1, 2001. For one of the items to which participants were asked to give consent, "the usage of the donated samples for other studies at RERF in the future," the following three options were given to the participants from which to choose, in line with the recommendation of the Ethics Committee: 1) "I agree. I wish to be given an explanation again and sign another consent form;" 2) "I agree. I do not need to be given an explanation again or sign another consent form;" and 3) "I don't agree."

Table 13 shows the percentages of participants who gave informed consent for preservation and use of donated samples in the future studies: 97.1% and 96.7% of them agreed with preservation and use of serum and plasma and of urine, respectively, for future studies other than genetic studies, but about 20% gave consent on the condition that they be given an explanation again when the samples that they donated are to be used for other studies. Four participants who gave consent at the time of the health examination withdrew their consent later.

The health examination data were stored in RERF's database tables after participant Master File numbers were replaced with linkable anonymous numbers. Examination results and findings were entered into medical charts, which are kept in a locked room.

For the purpose of genetic studies in the future, the donated blood samples were stored after donor master file numbers were replaced with linkable anonymous numbers and then with specific anonymous numbers. Files to link the Master File numbers with the specific anonymous numbers will only be used by an ID information administrator for linkage. Those linkage files will be stored in an offline storage device. This method of anonymity conforms to the aforementioned guidelines for ethics.

When publishing the results, only tabulated results will be reported. No information that can be used for identifying individuals will be disclosed.

Table 13. Acquisition status of informed consent for preservation and use of donated samples

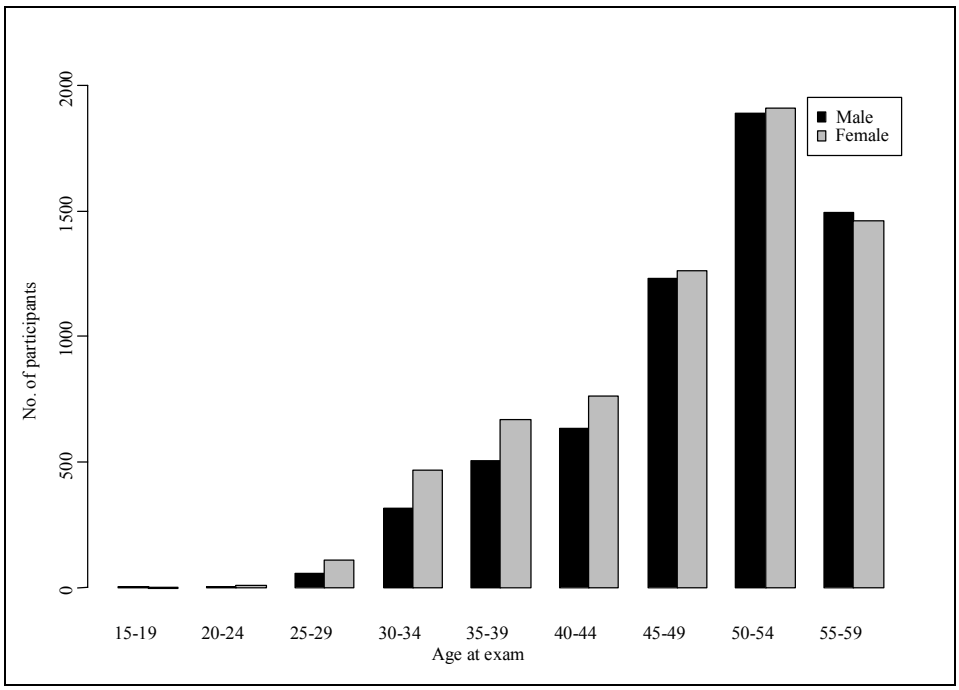
		No. of participants	Consented (%)		
			Study excluding genetic study		Genetic Study
			Serum • Plasma	Urine	Blood
Hiroshima	Male	4,020	98.5	98.4	98.3
	Female	3,967	97.1	96.2	96.8
	Total	7,987	97.8	97.3	97.6
Nagasaki	Male	1,682	98.1	97.9	97.7
	Female	2,282	94.2	93.4	94.4
	Total	3,964	95.9	95.3	95.8
Total	Male	5,702	98.4	98.2	98.1
	Female	6,249	96.0	95.2	95.9
	Total	11,951	97.1	96.7	97.0

4. Analysis

Analytical methods

The data obtained from the 11,951 participants who underwent health examinations by the end of September 2006 were analyzed for association between parental exposure and the development of multifactorial diseases among the children of A-bomb survivors, using logistic regression. Table 14 shows the age distribution of the participants at the time of examination.

Table 14. Distribution of participant age at the time of examination



1) Outcome variable

When any of diabetes, hypertension, hypercholesterolemia, myocardial infarction, angina pectoris, or stroke was identified, the person was considered to have multifactorial disease. Presence or absence of multifactorial disease in each participant was used as the outcome variable. Table 15 shows the number of cases and prevalence of each disease. Prevalence of hypertension and hypercholesterolemia is relatively high, while the numbers of patients with myocardial infarction, angina pectoris, and stroke were small.

Table 15. Number of cases and prevalence of multifactorial diseases

	All	Male	Female
Diabetes	768 (6.4%)	553 (9.7%)	215 (3.4%)
Hypercholesterolemia	4,622 (38.7%)	2,096 (36.8%)	2,526 (40.4%)
Hypertension	3,152 (26.4%)	1,931 (33.9%)	1,221 (19.5%)
Angina pectoris	91 (0.8%)	61 (1.1%)	30 (0.5%)
Myocardial infarction	46 (0.4%)	44 (0.8%)	2 (0.0%)
Stroke	81 (0.7%)	50 (0.9%)	31 (0.5%)
Multifactorial diseases	6,530 (54.6%)	3,410 (59.8%)	3,120 (49.9%)

2) Parental radiation dose

For analysis of parental radiation dose, one of the main variables of interest in risk analysis, paternal and maternal doses were separately included in the models. For those without dose information (i.e., those in the "No information" or "Dose unknown" categories), dummy variables were used. Because there is no correlation between paternal and maternal doses (correlation coefficient = 0.02), these two variables are considered to be independent from each other, and thus inclusion of dose of one parent is unlikely to cause biases in the estimation of dose response for the other parent.

3) Background model

In addition to parental dose, the effects of the following confounding factors, which are generally known to be common risk factors for multifactorial diseases, were taken into consideration in building the background model.

* Age, gender, and city
* BMI, drinking, and smoking
* Menopausal status, age at menopause, and hormone replacement therapy (HRT) (women only)
* Parents' history of multifactorial diseases and parents' ages at birth
* Family size, occupational status, and living situation

Because the primary purpose of the analysis is to estimate the effects of parental exposure on the development of multifactorial diseases, particularly careful attention was paid to determine if factors other than radiation are confounders for radiation dose (confounders are factors related to both radiation dose and occurrence of multifactorial disease, the outcome variable). To avoid possible biases in risk estimation caused by inappropriate parametric assumptions, these non-radiation confounders are basically included as categorical variables.

The factors to be included in the background model were selected using stepwise selection methods based on the likelihood ratio test. In stepwise selection, a dose term was added to each of the two interaction terms, age*gender and city*gender, to build the basic model. The model for adjustment was chosen using stepwise inclusion of variables based on the likelihood ratio test; the effect with the lowest p value (if less than 0.1) was included at each step. When the interaction of a factor with gender was deemed significant, the interaction term in question was included in the model.

4) Effect modifier

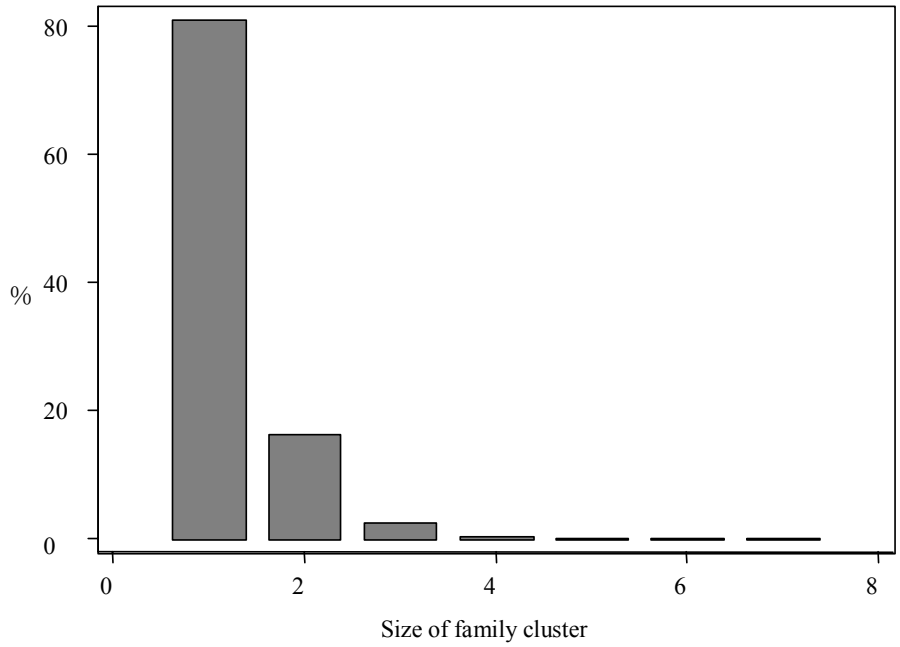
After the background model was built, effect modification was investigated with respect to gender, parental age at exposure, elapsed time from parental exposure to conception (birth), and parental history of multifactorial diseases, and was assessed by using likelihood ratio test with a p value of 0.05. Expertise and information available in genetics and radiobiology were used in identifying and categorizing such candidate effect modifiers.

5) Family clusters

The distribution of sizes of family clusters in the clinically examined cohort is shown in Table 16. About 80% of the clusters had only one sibling in the clinically examined cohort;

in other words, about 66% of the clinical study participants had no siblings in the cohort. Very few clusters had three or more siblings in the cohort; however, the largest cluster had seven siblings in the cohort. Because of possible correlation in disease occurrence among siblings, usual methods of analysis treating all individuals as independent would not be correct in a study with two or more siblings in its cohort. Over-estimation of the precision of risk estimates, in particular, would have been likely in the present analysis. Therefore, risks were estimated, using generalized estimating equations (GEE) to adjust for sibling relationships (family clustering).

Table 16. Distribution of family cluster size in FOCS cohort



6) Assessment of self-selection bias

Individuals' decisions to participate or not to participate can potentially bias risk estimates if participation depends jointly on health status (presence/absence of multifactorial disease) and parental exposure (self-selection), and other such conditions, and there are two stages at which such decisions were made. The first is response/non-response to the mail survey, and the second is participation/non-participation in the clinical examination. To assess the potential for self-selection bias, those who underwent health examinations and those who did not were compared. Data that can be used as surrogate markers of outcome to assess self-selection bias would include information on cancer diagnosed in tumor registries among the entire mail survey sample and self-reported history of multifactorial diseases in the mail survey questionnaire among mail survey respondents. In addition, individuals who participated in the clinical study after multiple contacts could be used as surrogates for non-participants or non-responders to determine if there is any difference in outcome variables (presence/absence of multifactorial disease).

(1) Assessment of self-selection bias among mail survey sample

Possible self-selection bias was assessed using cancer registry data through 2001. The results suggested positive interaction between prior cancer diagnosis and whether or not the

mother was exposed to radiation (dichotomous value) in terms of rate of participation in the clinical examination.

Furthermore, dose response for cancer prevalence at the time of FOCS in the mail-survey sample with mother's dose differed significantly between participating females and non-participating females (odds ratio per Gy was 1.44 [95% CI: 1.01 to 2.06] among participating females and 0.88 [95% CI: 0.51 to 1.52] among non-participating females. No such effects were observed for males or father's dose.

(2) Assessment of self-selection bias among mail survey respondents

Self-reported conditions and clinical diagnoses were compared for each of the multifactorial diseases. Hypertension, myocardial infarction, diabetes, and stroke showed moderate to good agreement with Kappa index, ranging from 52% to 72%, while only fair agreement was observed for hyperlipidemia (as compared with hypercholesterolemia) and angina pectoris (Kappa index = 23% and 38%, respectively).

Analysis of relationship between self-reported health status and participation showed that there was 2% higher participation among persons with self-reported hyperlipidemia. Analysis also showed that there was association between participation and maternal dose: participation was 6% higher among persons whose parents had non-zero dose estimates, but among persons with self-reported hyperlipidemia whose mothers' doses were non-zero, participation was 10% higher. Hyperlipidemia is closely related with hypercholesterolemia, and, given that hypercholesterolemia is the predominant component of the combined multifactorial disease outcome (about 70%), this result could be suggesting that self-selection biases exist. However, it should be recalled that there was not very good agreement between self-reported hyperlipidemia and diagnosed hypercholesterolemia, and it therefore remains unknown to what extent self-selection based on self-reported hyperlipidemia may be used as a surrogate for indication of possible non-participation bias.

Individuals who had not responded to the questionnaire received up to four mails (i.e., questionnaires, reminders, or letters of request for participation), and about one-third of the clinical study participants were persons who responded only after repeated mailing. Investigation for possible relationship between the number of mailings and disease showed no clear correlation.

Results of analysis

1) Results of estimation

The final model included parental dose (separately for father's and mother's dose) as well as age, city, gender, age*gender interaction, city*gender interaction, body mass index, parental history of multifactorial disease for each parent, female menopause status, smoking status, drinking status and drinking*gender interaction, and occupation category.

Table 17 shows estimates of parental exposure effects on multifactorial disease occurrence among their offspring per Gy obtained from the final model by father's and mother's doses and for sum of both doses. For all clinical study participants, odds ratio for father's and mother's exposure per Gy was 0.91 and 0.98, respectively (left column in Table 17), which was not significant. When analysis was made for males and females separately, a

negative association was observed between father's dose and multifactorial diseases prevalence in male participants (OR: 0.76, 95% CI: 0.65 to 0.89) (middle column in Table 17).

Finally, the adequacy of the logistic regression model fit using linear dose terms was carefully examined by computing odds ratios for radiation dose categories. The results for the dose categories were consistent with those from the logistic regression. In addition, fractional polynomials and locally weighted regression techniques, two forms of smoothing that can detect departures from the assumed logistic regression linear model, especially in the low-dose range, were applied. No evidence of lack of model fit was observed.

Table 17 Prevalence odds ratio for multifactorial disease among children of atomic-bomb survivors in relation to parental radiation dose

	Relative odds per Gy 95% CI		
	All participants	Male offspring	Female offspring
Father's dose	0.91 0.81-1.01	0.76 0.65 - 0.89	1.04 0.90 - 1.21
Mother's dose	0.98 0.86-1.10	0.97 0.81 - 1.17	0.98 0.83 - 1.16
Sum of both doses	0.94 0.86-1.02	0.85 0.75 - 0.96	1.02 0.91 - 1.13

2) Adjustment for confounders

Table 18 shows the parental radiation exposure effect estimates at various stages of model building.

Table 18 Parental radiation exposure effect estimates (odds ratios) during model building—95% lower CI, OR estimate, 95% upper CI

Model	Paternal dose	Maternal dose
Dose only	0.72, 0.79, 0.88	0.80, 0.90, 1.00
Age only	0.79, 0.88, 0.97	0.85, 0.95, 1.07
Age*gender	0.80, 0.89, 0.99	0.85, 0.96, 1.08
Age*gender + city*gender	0.80, 0.89, 0.99	0.86, 0.97, 1.09
+ BMI	0.80, 0.90, 1.00	0.87, 0.99, 1.12
+ family history	0.81, 0.90, 1.01	0.87, 0.98, 1.11
+ female menopause	0.81, 0.91, 1.01	0.87, 0.98, 1.10
+ smoking	0.81, 0.90, 1.01	0.86, 0.98, 1.10
+ drinking*gender	0.81, 0.91, 1.01	0.86, 0.98, 1.10
+ job category	0.81, 0.91, 1.01	0.86, 0.98, 1.10

As indicated in the above table, except for age, no variables appeared to act as confounders, as the impact on radiation risk (odds ratio) was less than 2%, and the odds ratio changed by less than 2% overall between adjustment for age only and adjustment for all variables in the final model.

3) Result of effect modification inspection

Among the possible effect modifiers mentioned earlier, only gender was statistically

significant, in the case of father's dose (p = 0.0056) but not mother's dose (p = 0.99). For fathers' doses, the gender interaction odds ratio estimate was 1.35 (95% CI: 1.09 to 1.67). For mothers' doses, the gender interaction odds ratio estimate was 1.00 (95% CI: 0.79 to 1.27).

4) Result of analysis with family clusters taken into account

Analyses with and without taking family clusters into account and analysis by randomly selecting one sibling per family (average of 200 repetitions) were compared. Table 19 shows the results for the father's radiation exposure effect estimate and gender interaction. It is apparent that the presence of family cluster neither biased the estimated radiation effect parameter or gender modification, nor had a noticeable impact on the precision of these parameters.

Table 19 Impact of family clusters (siblings) on results of risk analysis

Approach	Father's dose (males)		Gender interaction (females)	
	OR [95% CI]	SE of log (OR)	OR [95% CI]	SE of log (OR)
Not accounting for clusters (N=11,951)	0.769 [0.66, 0.90]	0.081	1.35 [1.09, 1.67]	0.109
Accounting for clusters (N=11,951)	0.769 [0.65, 0.91]	0.083	1.35 [1.09, 1.68]	0.109
Randomly selecting one sib per family (200 repetitions; N=9,779)	0.768 [0.64, 0.92]	0.091	1.30 [1.01, 1.66]	0.126

Conclusions

In the present analysis, there was no statistically significant evidence for increased risk of multifactorial disease from parental radiation exposure (odds ratios for father's and mother's doses per Gy were 0.91 and 0.98, respectively, and neither was statistically significant).

The apparent decreased risk with paternal exposure in male children requires careful interpretation, as it is difficult to determine whether the decrease is biologically consistent or due to self-selection bias. When epidemiologic studies are expected to result in small to modest levels of risk, careful consideration of potential sources of bias is crucial

There was no significant impact of siblings on either the magnitude or precision of the estimated radiation odds ratios.

5. Evaluation and proposals

The Health Effects Study of the Children of A-bomb Survivors has been conducted for seven years since 2000 to investigate whether there exists any relationship between the prevalence rates of adult-onset multifactorial diseases (hypertension, diabetes mellitus, hypercholesterolemia, myocardial infarction, angina pectoris, and stroke) and parental radiation exposure. The study has been properly conducted with use of methods approved by the Scientific Committee, under conditions established by the Ethics Committee. In addition, it was confirmed that the collected data were analyzed in a proper manner with use of statistical methods approved by the Analysis Subcommittee.

Although large-scale intensive studies on the health effects of parental exposure to A-bomb radiation have been conducted, previous studies on gender ratio and some gene aberrations have shown no statistically significant difference. However, theoretically, this could be attributable to limitations in terms of the size of the study cohorts or radiation dose. The present study was significant in the sense that the outcomes of interest were health indicators commonly observed among the elderly and effects of the parental exposure on such health outcomes cannot be estimated based on theories or animal experiments.

In analysis of the data from the current study, when multifactorial diseases in children were combined, no evidence suggesting increased risk associated with parental radiation exposure was observed. Although negative association of paternal dose and prevalence rate of multifactorial diseases was suggested in males, other items revealed no significant difference, which might be attributable to the average age of the study population being relatively young, at 48.6 years. Yet, the present study was extremely significant as it provides baseline data for the effects yet to appear. In the future, as the study population ages, the cohort might begin to exhibit health effects, and due to progress in gene analysis technologies, introduction of new perspectives might be necessary. Both the Scientific and Ethics Committees for the Health Effects Study of the Children of A-bomb Survivors, which evaluated the study, recommend again that the study be continued and careful review be made.

We would also like to express our deep appreciation to the children of A-bomb survivors who have cooperated in this study.

March 30, 2007

Tadao Shimao, Chairman, Scientific Committee
Hiraku Takebe, Chairman, Ethics Committee
Committees for the Health Effects Study of the Children of A-bomb Survivors

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Meetings of Committees for Health Effects Study of the Children of A-bomb Survivors

	Date	Place	Committee
1	Dec. 13, 1999	Hiroshima	First meeting of Scientific Committee
2	Jan. 24, 2000	Hiroshima	First meeting of Ethics Committee
3	Sep. 19, 2000	Hiroshima	Second meeting of Scientific Committee
4	Oct. 10, 2000	Hiroshima	Second meeting of Ethics Committee
5	Apr. 24, 2001	Hiroshima	First joint meeting of Scientific and Ethics Committees (Third for each committee)
6	Jul. 6, 2001	Hiroshima	Fourth meeting of Ethics Committee
7	Jul. 10, 2001	Hiroshima	Fourth meeting of Scientific Committee
8	Dec. 6, 2001	Hiroshima	Fifth meeting of Scientific Committee
9	May 28, 2002	Hiroshima	Sixth meeting of Scientific Committee
10	Jun. 3, 2002	Hiroshima	Fifth meeting of Ethics Committee
11	Feb. 18, 2003	Hiroshima	Second joint meeting of Scientific and Ethics Committees
12	Feb. 19, 2004	Hiroshima	Third joint meeting of Scientific and Ethics Committees
13	Feb. 4, 2005	Hiroshima	Fourth joint meeting of Scientific and Ethics Committees
14	Apr. 20, 2005	Hiroshima	First meeting of Analysis Subcommittee
15	Feb. 3, 2006	Hiroshima	Second meeting of Analysis Subcommittee
16	Feb. 21, 2006	Hiroshima	Fifth joint meeting of Scientific and Ethics Committees
17	Dec. 8, 2006	Hiroshima	Third meeting of Analysis Subcommittee
18	Feb. 27, 2007	Hiroshima	Fourth meeting of Analysis Subcommittee
19	Feb. 27, 2007	Hiroshima	Sixth joint meeting of Scientific and Ethics Committees