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GENE-RAD-RISK

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FINAL REPORT

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1 List of partners

International Agency for Research on Cancer (IARC), Lyon, France
Institut Gustave Roussy (IGR), France
Istituto G. Gaslini (IGG), Italy
Netherlands Cancer Institute (NKI), Netherlands
Imperial College of Science, Technology and Medicine (ICSTM), United Kingdom
Institute of Cancer Research, United Kingdom
The Chancellor, Masters and Scholars of the University of Cambridge, United Kingdom
University of Birmingham, United Kingdom
Centre René Huguenin, France

2 Coordination

Dr. Elisabeth Cardis – until 23 March 2008
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3 Introduction

Breast cancer is the most common cancer and a leading cause of death from cancer among women worldwide. Known risk factors for breast cancer among others include genetic susceptibility, and exposure to ionising radiation. The relative risks of breast cancer for women exposed to external radiation in childhood, adolescence and early reproductive years are among the highest known radiation related risks for any solid cancer type. A number of genes increase breast cancer susceptibility and are known to be involved in detection and repair of radiation-induced DNA damage. DNA repair mechanisms play an important role in the cellular response to radiation exposure and hence on the risk of radiation induced cancer. Mutations or polymorphisms in these genes may therefore render cells more sensitive to radiation-induced cancer.

4 Objectives

The overall objective of the GENE-RAD-RISK project was to conduct complementary multi-national nested case-control and cohort studies of breast cancer in two different populations – childhood cancer survivors and Hodgkin lymphoma patients chosen on the basis of high prevalence of radiation exposure from radiotherapy early in life, and BRCA1/2 mutation carriers with high prevalence of known mutations in susceptibility genes. The project aimed at examining the role of radiation exposure in the aetiology of breast cancer in young women and studying its possible interaction with genetic susceptibility in the *BRCA1/2* mutation carrier cohort. A supplementary objective was to evaluate the possible modifying effects of other risk factors for breast cancer, including cancer therapies other than radiation.
Overall, nearly 2,500 breast cancer patients and unaffected (breast cancer free) *BRCA1/2* mutation carriers from France, Netherlands and UK agreed to participate in the study. Among the childhood cancer and HD survivors, 391 breast cancer patients or proxies and 876 control patients from France, Italy, Netherlands and UK agreed to be interviewed.
The consortium brought together a powerful team with a wide range of expertise – epidemiologists, radiation oncologists and physicists, dosimetrists, molecular biologists and statisticians. The majority of the members of the consortium have known each other

professionally for many years and have been involved in successful multinational collaborations previously.
The project specifically addressed two very important issues: "to better characterize the risks associated with low and protracted exposures to ionising radiation of different forms" and the request for "epidemiological studies of exposed populations that offer the greatest potential to help resolve major public health concerns". The results of this project not only contribute to our understanding of the mechanisms of carcinogenesis, but also have important public health implications for the protection of patients treated with radiotherapy.

5 Description of the research performed

The objectives of this project were achieved by carrying out epidemiological studies of breast cancer in young women within cohorts of childhood cancer and Hodgkin lymphoma survivors and of known carriers of mutations in the *BRCA1* and *BRCA2* genes. This involved:
Workpackage 1 - Case-control studies in populations with medical radiation exposures were conducted to study the effects of medical radiation exposure received in childhood, adolescence and early adulthood and to set the stage for future analyses of gene-radiation interactions. Advantage has been made to group already existing national cohorts of childhood cancer survivors and Hodgkin lymphoma patients in France, Italy, Netherlands and UK. Because of particular interest for this project were radiation exposures that occurred in childhood, adolescence or early reproductive years, the age restrictions to patients who survived a first cancer diagnosed before the age of 35 were implied.
Workpackage 2 - Cohort study of populations of *BRCA1/BRCA2* mutation carriers. A study was conducted as a complement to the above cohorts of subjects exposed to medical radiation, who are not a priori carriers of genetic mutations. Analyses evaluated an impact of the interaction between genes and radiation from diagnostic medical procedures on breast cancer risk in this potentially susceptible population.

Workpackage 3 - Dose reconstruction encompassed activities related to the calculation of organ-specific doses for each study member of the medically-exposed cohorts in WP1, and the uncertainties in these estimates. Radiation doses from radiotherapy received to the site of the breast cancer for cases (and to the same site for matched controls) were individually estimated. Retrospective dose estimations will be performed using the Dos_EG software package which was developed by the GRR study partner - IGR and validated in a series of inter-comparison exercises with the method used in the past by M. Stowall team for the Hodgkin lymphoma patients included in the NKI cohort from the Netherlands.
Estimation of radiation dose from diagnostic exposures for the WP 2 subjects involve compilation of a table of average radiation dose (and typical range) for each diagnostic procedure of interest and type of equipment, by country and time period. Estimation of dose to individuals was then based on the use of relevant information from the questionnaire coupled with the information from the table.
Workpackage 4 - Integration: statistical analyses - risk estimates and consequences for radiation protection included analyses of the separate groupings of studies (WP1 and WP2) using individual dose estimates to the breast (WP1) or a cumulative breast dose score as an approximation of organ dose (WP2) from WP 3. Conclusions concerning the identification of susceptible groups were drafted; however, more precise recommendations for radiation protection require mutation screening of coding sequences of candidate genes in the DNA collected from study subjects, which was not included in the current grant.
Workpackage 5 - Consortium management. This workpackage was specifically devoted to the overall management of the project and required substantial coordination efforts between different complementary work packages to ensure overall progress of the study.

6 Main achievements

- Development of study documents: core protocols for case-control studies nested within cohorts of childhood cancer survivors and HD patients, and for cohort studies of *BRC1/BRC2* mutation carriers; development a GENE-RAD-RISK questionnaire in order to establish a core set of questions related to potential risk factors for breast cancer that were integrated into existing questionnaires or used independently; establishment of country-specific procedures for implementation of study protocol; development of standard forms to abstract information from medical records concerning the diagnosis of the first tumour (WP1), radiotherapy and chemotherapy for the first tumour (WP1), the diagnosis of breast cancer (WP1 and WP2), information on diagnostic radiation for subjects who did not receive radiotherapy (WP1 and WP2), and information on risk factors for breast cancer for subjects who were deceased (WP1 and WP2);
- Creation of the databases to enter information from study questionnaire and medical records, particularly concerning radiotherapy and chemotherapy for first cancer (WP1), also related to the study follow-up;
- Identification of cases and controls within WP1. Overall number of cases ascertained in the study exceeded the numbers projected at the start of the project (669 cases instead of 553) for both sub-populations – HD patients (464 instead of 419) and childhood cancer survivors (205 instead of 144);
- Identification of eligible cases of breast cancer diagnosed within the *BRCA1/BRCA2* cohorts (WP2) (total 1,431);
- Collection of information on medical radiation exposure and potential confounding factors by administrating the study questionnaire to the subjects who gave their consent. Overall, 391 (58%) eligible cases or their proxies and 876 (64%) controls were interviewed within WP1 and 1,011 (71%) breast cancer cases and 1,487 (79%) non-affected carriers within WP2 were interviewed. Medical records were located an information extracted on radiotherapy, chemotherapy and breast cancer diagnosis for both – alive and deceased patients. Collection of the data required for dose reconstruction was essential for the successful completion of the project. Major efforts were invested in the participating centres to locate radiotherapy charts and films. Radiotherapy records were photocopied, anonymised, and scanned before being sent to Institut Gustave Roussy for radiation dose construction. Information on cytotoxic drug (drug name, dose, route, cycle, start and end date etc) was abstracted from the records and double data entered on a database that was specifically developed for this purpose. Software to calculate the cumulative dose of cytotoxic drug exposure in mg/m2 was specifically developed
- Collection of biological samples within WP1, quality control, DNA extraction and whole genome amplification in preparation for gene analyses for which separate funding will be sought in the future. Overall, a blood or saliva sample was obtained from 46% of the cases and 49% of the controls;
- Direct individual radiotherapy dose calculations for WP1 were performed using the Dos_EG software package which was developed by IGR and validated in a series of inter-comparison exercises with the method previously used for the HD patients included in the NKI cohort. For each patient, a simple mathematical phantom anatomy was generated according to patient dimensions. With this voxelized phantom the dose was obtained in the whole breast and in any selected region of the breast to represent the dose to a breast cancer site for the cases (and the same site for their respective controls). For each patient and for each breast, the following dose estimations were performed: the mean whole breast

dose; the dose at the level of the nipples, the dose to each quadrant. Uncertainty related to the data on radiotherapy was evaluated and taken into account;

- Calculation of a cumulative breast dose score as an approximation of organ dose from radiodiagnostic procedures for patients within WP2. The procedures considered included: fluoroscopy, conventional X-rays, mammograms, radionuclide scans and CT-scans. The estimates of the breast dose were derived from a literature review of published studies and 1. institutional reports assessing radiation dose delivered to the breast from radiological examinations and expert judgment;
- Methods for analyzing epidemiological data which may include both categorical and continuous variables and are capable of dealing with many variables were developed and can be applied for future analyses of gene-radiation interactions once the DNA samples collected within the current project are analysed;
- Analyses of the relation between radiation dose and breast cancer risk in the three sub-populations, namely, childhood cancer survivors, Hodgkin lymphoma patients and carriers of *BRCA1* and *BRCA2* mutations, using individual dose estimates and individual cumulative dose scores were conducted. The risk of breast cancer in relation to radiation dose among childhood cancer survivors treated with radiotherapy at very young ages is largely unknown since few studies quantified the risks of breast cancer by the radiation dose received to the breast. Moreover, the proportion of very young children include in our study is higher compared to most of the studies conducted until now- 31% of cases had their first cancer below the age of 10 and 62% - between 10 and 14 years. In addition, majority of the cases (68%) had other cancers than Hodgkin's lymphoma; this resulted into lower doses, on average, to the breast site from radiotherapy because of the first cancer. The findings of this study indicating that survivors treated with any radiotherapy were at a 2-fold significantly increased risk of breast cancer compared to survivors never treated with radiotherapy are very important for predicting long-term risk of radiation induced breast cancer after radiotherapy in childhood. A significant linear dose-response relationship was observed with increasing cumulative radiation dose received to the breast tumour site. In this study, exposure to any chemotherapeutic agent did not increase the radiation induced risk of breast cancer significantly. In the subpopulation of Hodgkin lymphoma patients, the preliminary analyses showed that odds ratio (OR) of developing breast cancer increased linearly with increasing radiation dose. Analyses of ORs of breast cancer by treatment modality indicate that Hodgkin lymphoma survivors treated with chemotherapy had lower risk compared to those treated with radiotherapy only. In the combined cohort of *BRC1/BRCA2* mutation carriers, exposure to diagnostic radiation before age 30 increased breast cancer risk. Risks were observed at dose levels considerably lower than at which increase were found in other studies and indicate that the medical need for diagnostic procedures using ionising radiation must be balanced against the potential radiation risk in this sensitive population.

7 Exploitation and dissemination of the results

All the results were discussed in the frame of the GENE-RAD-RISK project and detailed in the technical reports and manuscripts. These are still preliminary because either they have been submitted to peer-review literature and are undergoing revisions following the reviewers comments received or some further analyses are still ongoing and final results will be published later.

Preliminary study results were presented in several scientific conferences. The list of publications already submitted is presented below:

1. RC Reulen, I Diallo, F de Vathaire, M Terenziani, FE van Leeuwen, R Haupt, C Magnani, V Tenet, E Cardis, A Kesminiene, MM Hawkins. *Breast Cancer after Childhood Cancer: an international collaborative study.*
2. A Pijpe, N Andrieu, DF. Easton, A Kesminiene, E Cardis, C Noguès, M Gauthier-Villars, , P Manders, ChJ. van Asperen, M Ausems, H Meijers-Heijboer, I Thierry-Chef, M Hauptmann, D Goldgar, MA Rookus, and FE. van Leeuwen. *Diagnostic radiation exposure and breast cancer risk in BRCA1/2 mutation carriers in the GENE-RAD-RISK study*.
3. Morris D, Sovio U, Little MP, A *Bayesian approach to fitting latent factor models for epidemiological data.*
4. Morris D, Sovio U, Jarvelin M-R, Little MP, *Use of the partial least squares algorithm adapted to non-linear regression*.
5. I. Thierry-Chef, Steven Simon, Robert Weinstock, Martha Linet. *Reconstruction of Radiation Doses to the Female Breast from Mammography from 1960 to the Present*. Accepted for publication in *Radiation Research 2011*.