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TITLE: Immune Response Genotypes and Risk of Young Adult Hodgkin Lymphoma

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14. ABSTRACT Hodgkin lymphoma (HL) is the first and second most common cancer among young women and men 20-39 years old, respectively. Our previous results from a small twin study suggested that an inherited imbalance in the immune response to infection could increase susceptibility to young adult HL. Here we will further test the hypothesis that the susceptible immunophenotype for HL is determined by a genetic tendency toward an exaggerated Th2 and/or inflammatory response and/or a depressed Th1 response, resulting from genotypes that regulate these responses. After 10 months of harmonizing the IRB materials between USC and the DOD, we have begun data collection. To date we have obtained and processed blood and saliva samples for DNA from 221 patients plus their parents to serve as controls. We will begin genotyping when we collect the projected 368 cases plus their parents or siblings (controls). With one more year in the field, we expect to reach our goal of 378 case-family sets. If we can identify the immune pathways responsible for this cancer, we may be able to design immunotherapy to prevent it, avoiding many lost years of productivity and loss of life that results from the treatment and its complications.					
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INTRODUCTION

BODY

The grant was awarded and funded late because this proposal was an alternate from 2005, and the funds only became available in September of 2006. We received the funding in October of 2006 so we were a month or so late in getting started. The study materials (questionnaires, informed consents, HIPAA) and database were developed. It took 10 months for the two IRBs (DOD and USC) to come to an agreement over the injury statement in the informed consent. Once that was finished, we received the case listing of newly diagnosed Hodgkin lymphoma patients in Los Angeles County. To date we have collected samples and extracted DNA from 221 cases and their family members (parents and/or siblings).

KEY RESEARCH FINDINGS

There are no key research findings to date from this study since we are still in the field recruiting patients and collecting samples.

CONCLUSIONS

There are no research conclusions based on this DOD supported proposal to date. Since there are 24 months left in the grant, we will continue recruiting patients this year and complete the genotyping and analysis of the samples in Year 4.

REFERENCES

APPENDICES

INTRODUCTION

There is strong evidence supporting the hypothesis that the young adult form of HL is caused by an aberrant immune response to a common childhood infection (as yet unknown), acquired relatively late (in adolescence or young adulthood)¹. There is additional evidence for a genetic contribution which may predispose to that aberrant immune response². We postulate that the genetic mechanism might involve a heritable propensity to produce higher levels of Th2/inflammatory cytokines, and lower levels of Th1 cytokines^{3,4}. This hypothesis is difficult to test directly since the disease alters the immune response. However, the production of these cytokines is governed in part by polymorphic genes, with functional variants responsible for some inter-individual variation^{5,6}. These genetic variants are immutable and can be studied at point in the disease process or at any phase of life.

Preliminary studies of twins with young adult HL support this hypothesis and suggest that genotypes associated with increased Th2 and inflammatory cytokines and decreased Th1 cytokines increase risk of young adult HL^{3,4}. Confirmation of these results in a population-based study is necessary. Cohort studies are impractical since cohorts would have to be designed to enroll individuals by adolescence in order to ensure that they were identified prior to peak occurrence of this cancer. In addition, the cancer is relatively rare and there are not enough cases within the appropriate age range in existing cohorts.

Case-control studies are also problematic. It is increasingly difficult to recruit a representative group of controls due to a decline in participation rates and an increase in barriers to recruitment (such as caller ID, use of cell phones, etc)⁷. Fortunately, a class of study designs based on the transmission disequilibrium test (TDT) eliminates some of these problems altogether and minimizes the others⁸. This approach uses a data structure of case-parent trios in which genotype-disease associations are measured by comparing genotypes in the case with those that could have been transmitted by his/her parents. Refinements in the method allow for enrollment of incomplete trios (e.g. case and one parent or case and one parent plus a sibling, etc.)⁹, although these other relative sets provide reduced power. The statistical and genetic properties of the resulting estimators have been extensively validated and shown to work well for diseases with sufficiently young age-at-onset to allow for enrollment of parents. We predict that this design will be appropriate to study genetic risk factors for this form of cancer since the targeted age group is relatively young (18-45 years old). Thus, we were awarded this grant proposal to test the following hypothesis and to achieve the following Technical Objectives in a 4-year study.

HYPOTHESES

- 1) To test the hypothesis that genotypes associated with increased secretion of T helper cell 2 cytokines and/or decreased secretion of T helper 1 and T regulatory cell cytokines are associated with an increased risk of young adult Hodgkin lymphoma [HL].
- 2) To test the hypothesis that variations in genotypes of other immune response elements (antigen processing proteins and other regulatory molecules) are associated with an increased risk of young adult Hodgkin lymphoma.
- 3) To determine if these associations vary by histologic subtype of Hodgkin lymphoma.
- 4) To determine if these associations vary by the presence or absence of Epstein-Barr virus (EBV) in the tumor.

TECHNICAL OBJECTIVES

- 1) To identify, enroll and collect blood specimens from 368 adolescents and young adults 18 years of age or older at the time of participation, diagnosed with Hodgkin lymphoma at ages 15-45 years, and from their parents, in a case-parent-trio study.
- 2) To genotype 1,536 SNPs of genes encoding cytokines, their receptors, antigen processing genes, and other immune response factors using the Illumina genotyping platform.
- 3) To conduct a transmission disequilibrium test and conditional logistic statistical modeling to test the association between genotypes and young adult Hodgkin lymphoma risk.
- 4) To collect tumor tissue from cases to validate histological subtype of Hodgkin lymphoma.
- 5) To collect tumor tissue from cases to determine the presence or absence of EBV.
- 6) To analyze the statistical data resulting from the genotyping.

BODY

The grant was awarded and funded late because this proposal was an alternate from 2005, and the funds only became available in September of 2006. We received the funding in October of 2006 so we were a month or so late in getting started. The study materials (questionnaires, informed consents, HIPAA) and database were developed. It took 10 months for the two IRBs (DOD and USC) to come to an agreement over the injury statement in the informed consent. Once that was finished, we received the case listing of newly diagnosed Hodgkin lymphoma patients in Los Angeles County and commenced with the study. We have received blood and/or saliva samples from 221 patients and their family members (parents and/or siblings). Questionnaires have been entered for all of these subjects (patients and family members) and pathology reports have been reviewed and submitted to the Population Tissue Retrieval Program for collection of tumor slides from patients (for Epstein-Barr virus testing). We have collected tissue from 54 patients so far and have sent these to our consultant Dr. Lawrence Weiss at City of Hope to test for the Epstein-Barr virus. (Slides from 8 patients were positive and 46 were negative for the virus). A summary of the completed tasks from the proposal Statement of Work is given below.

Task 1: To prepare and submit IRB documents (protocol detail, physician, patient and parent letters, HIPAA forms, tumor block release forms and questionnaires). **COMPLETE**

Task 2: To develop a tracking database to log participants and non-participants, and keep track of interview and specimen collection dates. **COMPLETE**

Task 3: To identify patients 18-45 years old diagnosed with Hodgkin lymphoma from October 1, 2005 through March 31, 2009 while living in Los Angeles County, California within three months of diagnosis through rapid reporting by the USC Cancer Surveillance Program. **IN PROGRESS**

Task 4: To contact and recruit 368 of these patients for the study **IN PROGRESS**

Task 5: To contact and recruit parents of these patients for the study. **IN PROGRESS**

Task 6: To obtain blood specimens and questionnaires on 368 patients and their parents **IN PROGRESS**

Task 7: To request tumor tissue blocks of the 368 enrolled patients from hospitals through the USC Slide/Block Retrieval Program (PI directs the program). We estimate that we can obtain 80% of the tissue blocks requested. **IN PROGRESS**

Task 8: To section tumor tissue blocks and stain 1 of the 10 resulting slides with H & E and review the histology for verification of diagnosis. **IN PROGRESS**

Task 9: To determine the presence or absence of Epstein-Barr virus in the tumor cells using in situ hybridization. **IN PROGRESS**

Task 10: To process blood specimens from subjects including collection and storage of serum, isolation of buffy coat and extraction of DNA. **IN PROGRESS**

Task 11: To aliquot extracted DNA into 96-well plates to prepare for genetic analysis and perform genotyping of 1,536 single nucleotide polymorphisms from extracted DNA from all subjects using the Illumina assay platform. **IN PROGRESS**

Task 12: To perform statistical analysis of the data to determine which genotypes are associated with young adult Hodgkin lymphoma risk. **NOT YET BEGUN (ON TRACK)**

Task 13: To write a paper summarizing the findings. **NOT YET BEGUN (ON TRACK)**

Task 14: To produce annual reports summarizing the progress of the grant (depending on the requirements, months 12, 24, 36, 48). **12-and 24-MONTH REPORT SUBMITTED**

KEY RESEARCH ACCOMPLISHMENTS

There are no key research findings to date.

REPORTABLE OUTCOMES

There are none to date.

CONCLUSIONS

There are no research conclusions based on this DOD supported proposal to date. Although there initially was a delay due to late notification of the award and late funding we have caught up with recruitment and we are now on schedule. We will conduct the genotyping and analysis of the samples in Year 4 of the proposal.

REFERENCES

- 1) Glaser S, Jarrett R. The epidemiology of Hodgkin's disease. London, UK: Saunders; 1996.
- 2) Mack T, Cozen W, Shibata D, et al. Concordance for Hodgkin's disease in identical twins suggesting gene
- 3) Cozen W, Gill P, Ingles S, et al. IL-6 levels and genotype are associated with risk of young adult Hodgkin's lymphoma. *Blood* 2004;103:3216-21.
- 4) Cozen W, Gill PS, Salam MT, et al. Interleukin-2, Interleukin-12 and Interferon- γ levels and risk of young adult Hodgkin lymphoma. *Blood* (accepted)
- 5) de Crean A, Posthuma D, Remarque E, van den Biggelaar A, Westendorp R. Heritability estimates of innate immunity: an extended twin study. *Genes Immun.* 2005; 6: 167-170.
- 6) Hohler T, Reuss E, Adams P, et al. A genetic basis for INF- γ production and T-bet expression in humans. *J Immunol*; 2005; 175: 5457-5462.
- 7) Shen M, Cozen W, Huang L, et al. Census and geographic differences between respondents and non-respondents in a case control study of non-Hodgkin lymphoma. *AJE*, in press.
- 8) Clayton D. A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. *American Journal of Human Genetics* 1999;65:1170-7.
- 9) Knapp M. The transmission/disequilibrium test and parental-genotype reconstruction: the reconstruction-combined transmission/ disequilibrium test. *American Journal of Human Genetics* 1999;64:861-70.

APPENDICES

None



University of
Southern California



Children's Hospital Los Angeles
International Leader in Pediatrics

Proposal #HS-07-00066

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TITLE OF PROPOSAL:

Immune Response Genotypes and Risk of Young Adult Hodgkin Lymphoma
Continuing Review: HS-07-00066-CR001 ([ContRev2008](#))

Action Date: **2/8/2008**

Action Taken: **Approve**

Committee: Institutional Review Board Vice
Chairman

Note: Your iStar Continuing Review Application received by the IRB on 1/30/08 was reviewed by Dr. Robert Larsen on 2/8/08. The Continuing Review qualifies for expedited review as category 2,3,7. The Continuing Review was APPROVED.

APPROVAL FOR YOUR STUDY IS VALID FROM 2/8/08 UNTIL 2/7/09.

As the Principal Investigator you are required to ensure that this research and the actions of all project personnel involved in conducting the study will conform with the research project and its modifications approved by the IRB; HHS regulations (45CFR46); FDA regulations (21CFR50,56); International Conference on Harmonization Good Clinical Practice Consolidated Guideline; IRB Policies and Procedures and applicable state laws. Failure to comply may result in suspension or termination of your research project, notification of appropriate governmental agencies by the IRB, and/or suspension of your freedom to present or publish results. Any proposed changes in the research project must be submitted, reviewed and approved by the IRB before the change can be implemented. The only exception is a change necessary to eliminate apparent immediate hazards to the research subjects. In such a case, the IRB should be promptly informed of the change following its implementation for IRB review. You must inform the IRB immediately if you become aware of any violations of HHS regulations (45CFR46), FDA regulations (21CFR50,56), applicable state laws or IRB Policies and Procedures for the protection of human subjects. You are required to notify the IRB office in the event of any action by the sponsor, funding agency or FDA, including warnings, suspension or termination of your participation in this trial. You must maintain all required research records and recognize the IRB is authorized to inspect these records.

APPROVAL FOR YOUR STUDY WILL EXPIRE AT THE END OF THE DAY (i.e., MIDNIGHT) ON 2/7/09.

IRB approval is valid for a maximum period of one year with continuing review by the IRB required at least annually in order to maintain approval status. You must not enter subjects on the study if IRB approval expires. In this case you must immediately submit an amendment to obtain permission to continue study treatments/interventions on currently enrolled subjects.

Adult case informed consent, Adult Siblings Informed consent, Parent of Cases, and Parent of Minors Informed consent all dated 4/10/07 were

APPROVED.

Informed consent must be obtained by the investigator or person authorized to obtain informed consent from all research subjects or their legally authorized representatives. You must ensure that all project personnel involved in the process of consent/assent are trained properly and are fully aware of their responsibilities relative to the obtainment of informed consent/assent according to the IRB guidelines and applicable federal regulations.

The IRB office has stamped the approved informed consent forms for use in this research project. It should be photocopied, as appropriate. You may not use these informed consent form documents to consent new subjects after their expiration date. The study subject must sign and date the informed consent documents. The person obtaining informed consent must also sign the study consent form at the time consent is obtained. One copy of the informed consent should be given to the study subject, one copy placed in the hospital medical record, and the investigator should retain one copy.

Informed consent is obtained in the research participant's language. If the participant speaks Spanish and the informed consent document has been translated into Spanish, you must utilize the Spanish informed consent document, the Spanish Experimental Subject's Bill of Rights, and the Spanish HIPAA Authorization form. For participants who speak other languages, you must have a translator verbally translate the English informed consent document into those languages for the participants. The English informed consent serves as a summary. The translator, the person obtaining informed consent and the witness sign the English informed consent document. The participant and witness sign the Short Form informed consent document, which must be in the participant's language. The IRB has translated the Short Form consent into multiple languages, which are available on the IRB website. In addition, the participant signs the Experimental Subject's Bill of Rights in the participant's language. The IRB has translated the Experimental Subject's Bill of Rights into multiple languages which are also available on the IRB website (<http://www.usc.edu/admin/provost/oprs/hsirb/forms>).

Attachments:

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