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14. ABSTRACT

The purpose of the proposed project is to identify common genetic variations (i.e., single nucleotide polymorphisms and haplotypes) in cytokine genes, as well as psychological characteristics that are significantly associated with the severity of pain and co-occurring symptoms (i.e., fatigue, sleep disturbance, and depressive symptoms) in a sample of women receiving chemotherapy for breast cancer. Using cluster analysis, our team has previously and consistently found two extreme patient subgroups: patients who report symptoms uniformly low (i.e., low scores on pain, fatigue, sleep disturbance, and depressive symptom inventories), and patients who report symptoms as more severe (i.e., high scores on all four symptom inventories). By the end of Year 1, 236 breast cancer patients were enrolled across the three research sites (UCSF, Alta Bates Summit Medical Center, and El Camino Hospital), and data collection forms were scanned and output to statistical analysis software. Moreover, the DNA samples of the participants were sent to the UCSF Genome Core for genotyping, and we expect that genotyping will be complete within the next two months.

15. SUBJECT TERMS

breast cancer, pain, chemotherapy, cytokine gene variation, fatigue, sleep disturbance, depressive symptoms, cluster analysis

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Introduction

The purpose of the proposed project is to identify common genetic variations (i.e., single nucleotide polymorphisms and haplotypes) in cytokine genes, as well as psychological characteristics that are significantly associated with the severity of pain and co-occurring symptoms (i.e., fatigue, sleep disturbance, and depressive symptoms) in a sample of women receiving chemotherapy for breast cancer. Substantial inter-individual variability exists in the experience of these cancer-related symptoms, and applying the use of cluster analysis in order to identify those patients who experience symptoms similarly, may be a powerful method for the discovery of underlying mechanisms of these distressing symptoms. Previously, our group identified, in two independent samples, two extreme distinct subgroups of patients: those who scored high (ALL HIGH) and those who scored low (ALL LOW) on measures of pain, fatigue, sleep disturbance and depression.[1] Previous candidate gene studies by our group found that variation in cytokine candidate genes were associated with the severity of fatigue,[2,3] sleep disturbance,[3] and depression[4] in patients undergoing radiation therapy and their family caregivers. Moreover, the symptom cluster of interest (i.e., pain, fatigue, sleep disturbance, depression) closely resembles components of cytokine-induced sickness behavior observed in animal models[5,6], suggesting that the cytokine signaling pathway may plan an important role in mediating these symptoms.

Body

Participant Recruitment, Enrollment, and Data Collection

Recently, UCSF incorporated to an electronic medical record system, APeX, which has made it easier to identify eligible patients as well as pertinent clinical information. I received the appropriate module training, and have used the system successfully to refine the search for eligible patients, as well as to collect pertinent clinical and demographic information after obtaining written informed consent. We (PI and research assistants) are screening pharmacy charts at the Infusion Center on a daily basis to identify eligible patients to approach the following day. Precise screening procedures vary by site, and typically, at El Camino and Alta Bates, nurses or physicians are required to verify participant suitability before the patient is approached to participate in the study.

Participant enrollment numbers were higher than projected at the end of Year 1. In total, 412 women were approached, 141 (34%) refused to participate, mainly due to feeling overwhelmed or being too busy, and 35 (8.5%) withdrew from the study after enrollment. Complete data was collected for 236 breast cancer patients, recruited from UCSF (N=174), Alta Bates (N=37), and El Camino Hospital (N=25). On average, women were 53.5 (± 10.7) years old (range: 21 – 86). For details regarding ethnicity and race, see Table 1 below.

Table 1. Breakdown of participants by ethnic and racial categories.

Ethnic category	Number (%) of subjects enrolled
Hispanic or Latino	12 (5.2)
Not Hispanic or Latino	217 (94.8)
Unreported	7 (3.0)
TOTAL:	**236 (100.0)**
Racial category	**Number (%) of subjects enrolled**
American/Indian or Alaska Native	2 (0.8)
Asian	33 (14.0)
Native Hawaiian or Other Pacific Islander	4 (1.7)
Black or African American	15 (6.4)
White	165 (69.9)
Mixed Ethnic Background	9 (3.8)
Other	7 (3.0)
Unreported	1 (0.4)
TOTAL:	**236 (100.0)**

Of note, this number includes only those participants who gave blood for genomic analysis and who completed demographic and clinical questionnaires, as well pain, fatigue, sleep disturbance, and depressive symptom inventories, that provides sufficient data for statistical analyses. Screening and data collection are tracked through a secure study log, maintained by our research coordinator, Ann Murai. Data are scanned and exported to a statistical software package (i.e., SPSS), using Optical Mark Recognition (OMR) technology by research assistants. In months 7 through 9 of year 1, I received training in how to design and troubleshoot OMR templates to ensure accurate scanning of paper data collection forms. Our research team has met monthly to discuss progress with regards to recruitment, enrollment, and data collection.

Training in Genetics

I completed the series of Human Population Genetics workshops, led by Dr. Aouizerat, in which I gained valuable knowledge in how to score genotype array data, using an existing data set. This training experience contributed to subsequent analyses of the existing array data, which I actually participated in, and from which a number of manuscripts were prepared for peer-review publication (on which I served as co-author, plus one in preparation as first author). This series provided me with the opportunity to learn basic

conceptual issues in human genetics, as well practical skills in haplotype construction, and how to use essential online resources to better understand specific polymorphisms and their potential functions. I met regularly with my co-mentor, Dr. Aouizerat, in order to ensure successful progress of the research project and various training/writing/analysis opportunities, as well as understanding of key conceptual issues. As a result of this training, and my involvement in the analysis and writing of various manuscripts (using the existing array data), I was able to help prepare the "Statistical analysis for genetic data" methods section for our series of manuscripts.

Construct and Perform Custom Genotyping Array

In the initial protocol, I reported five candidate genes to be interrogated with respect to their association with symptom severity, due to the established involvement of cytokines in mediating fatigue and sleep disturbance severity, compounded by physiological evidence of the involvement of particular pro- and anti-inflammatory cytokine genes in the primary symptom of interest (i.e., pain)[7,8]: TNFA (tumor necrosis factor-a), IL1B (interleukin 1B), IL6 (interleukin 6), IL10 (interleukin 10), IL13 (interleukin 13). I identified a number of specific polymorphisms (i.e., SNPs) for the array, based largely on reports in the literature of function or association with symptoms or disease. However, due to the needs of the parent project, as well as the associated benefit of economy of scale, a larger and more representative sample of SNPs will be assessed than proposed (approximately 4x the number of SNPs) for the current project. In addition, a number of ancestry informative markers (AIMS; SNPs known to vary by ethnicity) were included in the parent custom array. I will use the AIMS in my analyses in order to control for population substructure.

The custom array was designed in collaboration with my mentors, and was submitted to the UCSF Genome Core Facility. Given the timing and advantage of batching my array with that of the parent project, I chose not to submit my array after the first cohort of 100 patients was enrolled. Rather, I will run the array experiment in two, rather than three batches. Given the successful rate of enrollment, I will have a larger sample than projected for the genetic analysis. In addition, I will evaluate a larger number of cytokine candidate genes than those originally included in my application. The funds budgeted for genotyping in Year 1 contributed to the custom array designed for the parent project.

We expect to receive the genotypic data from the Genome Core within two months. At that time, I will begin genotype scoring, quality control analyses, and analyses for ancestry informative markers.

Other Relevant Training

One-on-one meetings with mentor: Throughout Year 1, I met with my co-mentor, Dr. Miaskowski, on a bi-weekly basis in order to identify opportunities for secondary analyses and manuscript preparation for existing datasets, to ensure the progress of the proposed research, and to discuss career plans. As a result of these meetings, I have prepared a manuscript that describes life stressors amongst the first 105 oncology patients (including

46 women with breast cancer recruited for the proposed project). This manuscript is in revision to be resubmitted to the journal, *Psycho-Oncology.*

One-on-one meetings with biostatisticians: I met regularly with biostatisticians, Dr. Stephen Paul and Dr. Bruce Cooper, in order to gain valuable expertise in sophisticated statistical analyses. As a result, I have prepared the results section for a study using hierarchical linear modeling to identify predictors of the trajectory of fear of cancer recurrence in a sample of breast cancer patients. I have performed mixed effects linear modeling to determine how symptoms related to pain change over time in women with distinct pain severity trajectories following breast cancer surgery (I will complete and submit these manuscripts in Year 2). Finally, with the assistance of Dr. Cooper, I am learning how to conduct growth mixture modeling, with the goal of identifying subgroups (i.e., latent classes) of breast cancer patients with distinct trajectories of neuropathic pain following breast cancer surgery (I will complete this analysis and submit the manuscript in Year 2). I will meet with Dr. Cooper regularly in Year 2 in order to learn and successfully apply cluster analysis to the current dataset.

Oncology Symptom Management Research Group (OSMRG) Meetings: Our OSMRG meets biweekly to discuss the progress of ongoing analyses and manuscript preparation, particularly related to genetic analysis. These meetings increased my knowledge and skills in the analysis of genetic data, as well as the interpretation and communication (written and verbal) of genetic findings. As a result of my work with this team, I have served as co-author on a number of published papers (see Key Research Accomplishments) during Year 1.

Training in Clinical Research Summer Workshop: From July 31 – September 13, I participated in UCSF's Training in Clinical Research Summer Workshop. This workshop is a precursor to the Advanced Training in Clinical Research (ATCR) year-long certificate program. The workshop provided training in biostatistics, database management, and protocol development, presentation, and review. An additional part of the training was devoted to building a successful career in clinical research. These classes were both inspiring and truly transformative. The knowledge I acquired, and am currently acquiring in the ATCR program (which began September 18, 2012), in addition to superlative mentorship by Drs. Miaskowski and Aouizerat, will be great preparation for my pursuit of a successful career as an independent clinical research scientist.

Key Research Accomplishments

- Enrolled 236 participants in Year 1 (total projected for the duration of the fellowship: 300)
- Compiled data on relevant demographic questionnaires and symptom inventories
- Submitted custom genotyping array to UCSF Genome Core
- Submitted "Cumulative Life Stress in Oncology Patients Receiving Chemotherapy" as first author, based on a sample of 105 patients from the parent study (including 46 breast cancer patients from current sample), describing life stressors endorsed by the patients, how these differed with respect to patient demographic and clinical characteristics, and the association of life stress with important cancer-related

psychological outcomes (cancer-related post-traumatic stress, depressive symptoms, anxiety)
- o Currently in revision for resubmission to *Psycho-Oncology*
- Presented poster describing association of potassium channel genes in preoperative breast pain in sample of breast cancer patients; received Junior Investigator Poster Award by the Genetics and Pain Special Interest Group (manuscript in preparation for submission to *Pain* as first-author)
- Manuscript in preparation for submission "Trajectories of Fear of Cancer Recurrence in a Sample of Breast Cancer Patients Following Breast Cancer Surgery" as co-author
- o I prepared the results section of this manuscript (including tables, figures, figure legends, and text).
- o This questionnaire was administered to participants in the current study, and may be of interest as fear of recurrence may be a significant psychological factor associated with pain severity in women with breast cancer

Reportable Outcomes

Publications:

1. Miaskowski C, Cooper BA, Dhruva A, Dunn LB, Langford DJ, Cataldo JK, Baggott CR, Merriman JD, Dodd M, Lee K, West C, Paul SM, Aouizerat BE. Evidence of Associations between Cytokine Genes and Subjective Reports of Sleep Disturbance in Oncology Patients and Their Family Caregivers. PLoS One. 2012; 7(7):e40560.

2. McCann B, Miaskowski C, Koetters T, Baggott C, West C, Levine JD, Elboim C, Abrams G, Hamolsky D, Dunn L, Rugo H, Dodd M, Paul SM, Neuhaus J, Cooper B, Schmidt B, Langford D, Cataldo J, Aouizerat BE. Associations between pro- and anti-inflammatory cytokine genes and breast pain in women prior to breast cancer surgery. J Pain. 2012 May; 13(5):425-37.

3. Illi J, Miaskowski C, Cooper B, Levine JD, Dunn L, West C, Dodd M, Dhruva A, Paul SM, Baggott C, Cataldo J, Langford D, Schmidt B, Aouizerat BE. Association between pro- and anti-inflammatory cytokine genes and a symptom cluster of pain, fatigue, sleep disturbance, and depression. Cytokine. 2012 Jun; 58(3):437-47

Accepted Publications:

1. Dunn, LB, Aouizerat, BE, Langford, DJ, Cooper, BA, Dhruva, A, Cataldo, JK, Baggott, CR, Merriman, JD, Dodd, M, West, C, Paul, SM, Miaskowski C. Cytokine gene variation is associated with depressive symptom trajectories in oncology patients and family caregivers. Accepted to *European Journal of Oncology Nursing, October 2012*.

2. Merriman JD, Aouizerat BE, Langford DJ, Cooper BA, Baggott CR, Cataldo JK, Dhruva A, Dunn LB, West C, Paul SM, Ritchie CS, Swift PS, Miaskowski C. Preliminary evidence of an association between an interleukin 6 promoter polymorphism and self-reported attentional function in oncology patients and their family caregivers. Accepted to *Biological for Nursing, September 2012*.

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Paper/Poster Presentations:

1. "Preliminary evidence of an association between candidate channel genes and preoperative breast pain in women with breast cancer." 31st Annual Scientific Meeting of the American Pain Society, Honolulu, HI.	Poster presentation Paper presentation (Young Investigator poster award)
2. "Association between cytokine gene polymorphisms and sleep disturbance in patients with breast cancer." State of the Science Congress on Nursing, Washington D.C.	Paper presentation
3. "Association between cytokine gene polymorphisms and depressive symptoms in patients with breast cancer." State of the Science Congress on Nursing, Washington D.C.	Paper presentation

<u>Conclusions</u>

Enrollment and data collection are proceeding ahead of schedule, with genotyping currently occurring at the UCSF Genome Core for the first 236 breast cancer patients. Preliminary analyses will occur after genotype scoring and quality controls have been implemented. In the meantime, I have had invaluable research experience using existing data sets as well as data collected for the current project, including: genetic analyses of a sample of breast cancer patients followed for six months following surgery, intensive training in genetic analyses, biostatistics (including sophisticated statistical analyses of longitudinal data), database management, and protocol development, and the preparation and submission of manuscripts for peer-review. In Year 2, I will complete the ATCR certificate program, receive training and become proficient in cluster analysis, and conduct preliminary analyses using genetic data.

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3. Miaskowski C, Dodd M, Lee K, et al: Preliminary evidence of an association between a functional interleukin-6 polymorphism and fatigue and sleep disturbance in oncology patients and their family caregivers. J Pain Symptom Manage 40:531-544

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5. Cleeland CS, Bennett GJ, Dantzer R, et al: Are the symptoms of cancer and cancer treatment due to a shared biologic mechanism? A cytokine-immunologic model of cancer symptoms. Cancer 97:2919-2925, 2003

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