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14. ABSTRACT

The proposed project is a cross-sectional observational study of 300 women undergoing active chemotherapy treatment for breast cancer at the University of California, San Francisco (UCSF) Comprehensive Cancer Center, El Camino Hospital, and Alta Bates Summit Medical Center. The purpose of the proposed project is to identify common genetic variations (i.e., single nucleotide polymorphisms [SNPs] and haplotypes) in cytokine genes, as well demographic, clinical, and psychological characteristics that are associated with the severity of pain and co-occurring symptoms. Pain is a multidimensional experience that is influenced by intrinsic and extrinsic factors. In addition, other symptoms commonly co-occur with pain, including fatigue, sleep disturbance, and depressive symptoms. Previous candidate gene studies by our group found that variation in cytokine candidate genes were associated with the severity of fatigue, sleep disturbance, and depression 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, suggesting that the cytokine signaling pathway may play an important role in mediating these symptoms. The identification of factors (i.e., demographic, clinical, psychological, genetic) that contribute to variability in the experience of pain and associated symptoms may provide valuable information that will improve our ability to identify patients at higher risk of more severe symptoms. Such factors may also represent novel targets for pain prevention and management in women with breast cancer.

15. SUBJECT TERMS
breast cancer, pain, chemotherapy, cytokine gene variation, fatigue, sleep disturbance, depressive symptoms, latent class profile analysis

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Introduction

Long-term survival of patients with breast cancer has increased dramatically with more effective anticancer therapies. However, approximately 35-70% of breast cancer patients undergoing active treatment[1], and approximately 50% of survivors[2,3] experience pain as a result of the disease and/or its treatment. Research on factors that contribute to inter-individual variability in pain is important because unrelieved pain is associated with decreases in breast cancer patients' quality of life (QOL) and ability to function[3].

Pain is a multidimensional experience that is influenced by intrinsic and extrinsic factors. In addition, other symptoms commonly co-occur with pain, including fatigue, sleep disturbance, and depressive symptoms. The identification of factors (i.e., demographic, clinical, psychological, genetic) that contribute to variability in the experience of pain and associated symptoms may provide valuable information that will improve our ability to identify patients at higher risk of more severe symptoms. Such factors may also represent novel targets for pain prevention and management in women with breast cancer.

Previous candidate gene studies by our group found that variation in cytokine candidate genes were associated with the severity of fatigue[4], sleep disturbance[5], and depression[6] 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[7,8], suggesting that the cytokine signaling pathway may play an important role in mediating these symptoms.

The proposed project is a cross-sectional observational study of 300 women undergoing active chemotherapy treatment for breast cancer at the University of California, San Francisco (UCSF) Comprehensive Cancer Center, El Camino Hospital, and Alta Bates Summit Medical Center. The purpose of the proposed project is to identify common genetic variations (i.e., single nucleotide polymorphisms [SNPs] and haplotypes) in cytokine genes, as well demographic, clinical, and psychological characteristics that are associated with the severity of pain and co-occurring symptoms.

Body

Participant Recruitment, Enrollment, and Data Collection

Participant screening and recruitment continued as described previously. Briefly, at UCSF, where the majority of participants were recruited, we (PI and research assistants) screen pharmacy charts at the Infusion Center daily to identify eligible patients to approach the next day. At El Camino and Alta Bates, weekly schedules are screened for potentially eligible patients based on type of infusion, and nurses or physicians verify participant suitability before the patient is approached to participate in the study.

To date, 574 women were approached, 193 (33.6%) refused to participate, mainly due to feeling overwhelmed or being too busy, and 381 women were enrolled. Of these women, 52 (13.6%) withdrew from the study after enrollment. Complete data (blood specimen and

questionnaire data) were collected for 323 breast cancer patients, recruited from UCSF (n=227), Alta Bates (N=50), and El Camino Hospital (N=46). Blood specimens were obtained from 6 other patients who were enrolled at the end of September. We expect to receive their completed questionnaires shortly. On average, participants were 53.1 years old (standard deviation = 10.9; range: 27 – 86). For details regarding ethnicity and race, see Table 1 below.

Table 1. Breakdown of current total sample by ethnic and racial group.

Ethnic category	Number (%) of subjects enrolled
Hispanic or Latino	16 (5.0)
Not Hispanic or Latino	299 (92.6)
Unreported	8 (2.5)
TOTAL:	**323 (100.0)**
Racial category	**Number (%) of subjects enrolled**
White	224 (69.3)
Asian	52 (16.1)
Black or African American	18 (5.6)
Mixed Ethnic Background/Native American/Pacific Islander/Other	24 (7.4)
Unreported	5 (1.5)
TOTAL:	**323 (100.0)**

Of note, this number includes only those participants who gave blood for genomic analysis and who completed demographic questionnaires, as well pain, fatigue, sleep disturbance, and depressive symptom inventories, that provide sufficient data for statistical analyses. Screening and data collection continue to be tracked through a secure study log, maintained by our research coordinator, Ann Murai, and project director, Judy Mastick. Data are scanned and exported periodically to a statistical software package (i.e., SPSS), using Optical Mark Recognition (OMR) technology by research assistants. Our research team continues to meet monthly to discuss progress with regards to recruitment, enrollment, and data collection.

Training in Genetics

In year 2, I received formal training in genetics through my enrollment in the Advanced Training in Clinical Research Certificate Program, where I elected to take courses offered in genetics. These courses, “Molecular and Genetic Epidemiology” and “Statistical Methods in Genetic Epidemiology”, covered basic conceptual issues, as well as specific approaches to the design and interpretation of genetic studies. In particular, I gained an understanding of

various molecular and genetic techniques, approaches to linkage and association studies, gene x environment interactions, population substructure, quality control procedures, and ethics in genetic research. These courses greatly enhanced my knowledge of the field and allowed me to evaluate, interpret, and disseminate our own genetic findings more clearly and proficiently.

I continued to meet regularly with my co-mentor, Dr. Aouizerat. In large part, these meetings were related to the hands-on analysis and interpretation of genetic data for two first-author manuscripts I prepared this year related to the association between potassium channel gene variation and breast pain in women with breast cancer. The first paper, related to the occurrence of preoperative breast pain, was recently resubmitted (with minor revisions) to the *Journal of Neurogenetics*. The second paper, related to persistent postoperative breast pain, will be submitted shortly to *Pain*.

Custom Genotyping Array

As outlined in my Annual Summary for Year 1, due to the timeline of the parent project, as well as the benefit of economy of scale, a larger sample of SNPs across a greater number of cytokine genes will be assessed than initially proposed. In addition, a number of ancestry informative markers (AIMS; SNPs known to vary by ethnicity) that were included in the parent custom array will be used to control for population substructure.

Data from the custom array was received from the UCSF Genome Core Facility in December of Year 2. We are nearing completion of quality control procedures, genotype scoring, and analyses for AIMS to prepare the data for statistical analyses. I will then work with Dr. Aouizerat to extract genotype data for the candidate cytokine genes for the proposed study. The funds budgeted for genotyping in Year 2 contributed to the custom array.

Other Relevant Training

Advanced Training in Clinical Research Certificate (ATCR) Program: As budgeted, In Year 2, I completed the ATCR Certificate Program (see Appendix for certificate) offered by the Department of Epidemiology and Biostatistics at UCSF. This four-quarter program (August 2012 – May 2013) involved intensive training in methodological, clinical, molecular/genetic epidemiology, database management, as well as a series of courses in biostatistics. These courses were incredibly relevant to my research pursuits. In addition, the intensity of the courses allowed me to truly develop and strengthen my skills in these areas. In addition to these didactic courses, I participated in a seminar series that involved the presentation and peer-review of proposals, posters, manuscripts in progress, etc. As a result, I received valuable advice and inspiration to approach study questions and analyses in new and interesting ways.

One-on-one meetings with mentor: Throughout Year 2, I continued to meet regularly with my co-mentor, Dr. Miaskowski, to discuss ongoing analyses, manuscript preparation, career plans, and progress of the proposed research. As a result of these meetings, in addition to

the two aforementioned genetics papers, I prepared a first-author manuscript that describes changes over time in pain qualities, pain interference, grip strength, shoulder mobility, and sensations in the breast scar area among distinct subgroups of women with persistent breast pain following breast cancer surgery. In addition, I am preparing a manuscript that describes parallel analyses for subgroups of women with persistent *arm* pain following breast cancer surgery. These companion papers will be submitted to a pain specialty journal in Year 3.

One-on-one meetings with biostatisticians: I continued to meet with biostatisticians, Dr. Steven Paul and Dr. Bruce Cooper, as needed, in order to ensure appropriate statistical analyses and to gain expertise in new statistical methods. In year 1, I outlined a manuscript related to cumulative life stress in oncology patients. Since its initial submission and review, we recruited several hundred additional patients which afford us the opportunity to do more sophisticated statistical analyses that will better evaluate the relationship between life stress and psychological symptoms. As a result, I have and will continue to meet regularly with Dr. Cooper in order to learn how to conduct structural equation modeling. This manuscript will be submitted in Year 3.

As a result of my meetings with Dr. Paul, I was able to independently conduct mixed effects linear modeling to determine how symptoms, quality of life, and muscle strength and mobility change over time in women with and without preoperative breast pain (I will complete and submit this manuscript in Year 3). I plan to use these findings as the basis for a National Institutes of Health career development award application (K99/R00) to be submitted in February of 2014.

Finally, with the assistance of Dr. Cooper, I am learning how to conduct latent class profile analysis in order to apply these methods to the proposed project. Preliminary analyses, using enrollment data from the parent study, identified three subgroups of patients (i.e., latent classes) with distinct experiences with pain, fatigue, sleep disturbance, and depressive symptoms. However, we will refine these analyses using questionnaire data for the subset of women with a diagnosis of breast cancer.

Oncology Symptom Management Research Group (OSMRG) Meetings: Our OSMRG continues to meet biweekly to discuss the progress of ongoing analyses and manuscript preparation. These meetings have allowed me to share my work with a transdisciplinary team of researchers, who are similarly interested in the genetic and psychological determinants of cancer-related symptoms. As a result of my work with this team, I have served as co-author on 5 manuscripts published in Year 2 (see Key Research Accomplishments).

Reviewer for Peer-Reviewed Scientific Journals

In Year 2, I served as an ad hoc reviewer for several peer-reviewed journals, including: *Journal of Pain*; *General Hospital Psychiatry*; *Pharmacology, Biochemistry, and Behavior*; *Social Neuroscience*; and *Behavioural Processes*. My training in the ATCR program, combined with the careful and thorough review that my mentors apply to my work prepared

me well for these exciting opportunities. Given that peer-review is an important part of an independent research career, learning the peer review process and how to compose a helpful and thoughtful review was invaluable.

Key Research Accomplishments

- Enrolled 381 participants by the end of Year 2 (total projected for the duration of the fellowship: 300)
- Compiled complete data (blood specimen, demographic questionnaires, and symptom inventories) for 323 participants
- Received results of first custom genotyping array from UCSF Genome Core Facility; currently completing scoring and cleaning of genetic data for statistical analyses
- Actively learning latent class profile analysis to cluster patients according to severity of pain, fatigue, sleep disturbance, and depressive symptoms
- Submitted "Variations in Potassium Channel Genes Are Associated with Breast Pain in Women Prior to Breast Cancer Surgery" as first author, based on a sample of 302 patients from a previous study related to pain and lymphedema in women following breast cancer surgery
    - Identified 7 single nucleotide polymorphisms and 1 haplotype across 4 potassium channel genes that were associated with occurrence of preoperative breast pain
    - Recently resubmitted to *Journal of Neurogenetics* with minor revisions
- Presented poster describing association between variations in potassium channel genes and persistent breast pain following breast cancer surgery at the American Pain Society 32nd Annual Scientific Meeting in New Orleans, LA (May, 2013)
    - Identified 7 single nucleotide polymorphisms across 5 potassium channel genes that were associated with persistent postoperative pain
    - Recipient of American Pain Society Young Investigator Travel Award
    - Manuscript prepared and will be submitted shortly (i.e., early in Year 3)
- First author manuscript prepared for submission: "Subgroups of women with persistent pain following breast cancer surgery I: A detailed phenotypic characterization of the experience of breast pain over time"
    - I used mixed linear effects modeling to evaluate changes in pain qualities, pain interference, grip strength, shoulder mobility, and sensations in breast scar area over time in a sample of women with persistent breast pain following breast cancer surgery
    - A companion paper describing parallel analyses for persistent arm pain is currently in preparation

Reportable Outcomes from Year 02

Paper/Poster Presentations:

1.	"Variations in potassium channel genes are associated with persistent breast pain after breast cancer surgery." 32nd Annual Scientific Meeting of the American Pain Society, New Orleans, LA (May, 2013).	Poster presentation (Young Investigator Travel Award)
2.	"Social modulation of pain in laboratory mice." Swiss Laboratory Animal Science Association, Zurich, Switzerland (November, 2013).	Podium presentation

Publications:

1. Alfaro E, Dhruva A, **Langford DJ**, Koetters T, Merriman JD, West C, Dunn LB, Paul SM, Cooper B, Cataldo J, Hamolsky D, Elboim C, Kober K, Aouizerat BE. Associations between cytokine gene variations and self-reported sleep disturbance in women following breast cancer surgery. *European Journal of Oncology Nursing*. 2013 Sep; Epub ahead of print. (PMID: 24012192)

2. Dhruva A, Aouizerat BE, Cooper B, Paul SM, Dodd M, West C, Wara W, Lee K, Dunn LB, **Langford DJ**, Merriman JD, Baggott C, Cataldo J, Ritchie C, Kober K, Leutwyler H, Miaskowski C. Differences in morning and evening fatigue in oncology outpatients and their family caregivers. *European Journal of Oncology Nursing*. 2013 Sep; Epub ahead of print. (PMID: 24012189).

3. Merriman JD, Aouizerat BE, **Langford DJ**, Cooper BA, Baggott CR, Cataldo JK, Dhruva A, Dunn L, 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. *Biological Research for Nursing*. 2013 Mar; Epub ahead of print. (PMID: 23482714)

4. 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. *European Journal of Oncology Nursing*. 2013 Jun; 17(3): 346-353. (PMID: 23187335)

5. Miaskowski C, Cooper B, Paul SM, West C, **Langford D**, Levine JD, Abrams G, Hamolsky D, Dunn L, Dodd M, Neuhaus J, Baggott C, Dhruva A, Schmidt B, Cataldo J, Merriman J, Aouizerat BE. Identification of patient subgroups and risk factors for persistent breast pain following breast cancer surgery. *Journal of Pain*. 2012 Dec; 13(12):1172-1187. (PMID: 23182226)

Papers in Revision

1. **Langford DJ**, West C, Elboim C, Cooper BA, Abrams G, Paul SM, Schmidt BL, Levine JD, Merriman JD, Dhruva A, Neuhaus J, Leutwyler H, Baggott C, Ward Sullivan C, Aouizerat BE, Miaskowski C. Variations in potassium channel genes are associated with breast pain in women prior to breast cancer surgery. Resubmitted to *Journal of Neurogenetics* (October, 2013).

2. **Langford DJ**, Dunn LB, Keagy C, Humphreys J, Dhruva A, Paul SM, Gold M, Cataldo JK, Merriman JD, Baggott C, West C, Schmidt BL, Speyer J, Chen L-M, Aouizerat BE, Miaskowski C. Cumulative life stress in oncology patients receiving chemotherapy. In revision for *Psycho-Oncology*.

Papers in Preparation

1. **Langford DJ**, Paul SM, West C, Mastick J, Cooper BA, Aouizerat BE, Miaskowski C. Subgroups of women with persistent pain following breast cancer surgery I: A detailed phenotypic characterization of the experience of breast pain over time.

2. **Langford DJ**, West C, Elboim C, Cooper BA, Abrams G, Paul SM, Schmidt BL, Levine JD, Merriman JD, Dhruva A, Neuhaus J, Leutwyler H, Baggott C, Ward Sullivan C, Aouizerat BE, Miaskowski C. Variations in potassium channel genes are associated with distinct trajectories of persistent breast pain in women after breast cancer surgery. In preparation for *Pain.*

3. **Langford DJ**, Paul SM, West C, Mastick J, Cooper BA, Aouizerat BE, Miaskowski C. Subgroups of women with persistent pain following breast cancer surgery II: A detailed phenotypic characterization of the experience of arm/shoulder pain over time.

4. **Langford DJ,** Paul SM, West C, Mastick J, Cooper BA, Aouizerat BE, Miaskowski C. Women with pre-operative and persistent post-operative breast pain experience persistent decrements in cancer-related symptoms, quality of life, and physical function.

Conclusions

The proposed sample size of 300 participants was met in Year 2. Pending completion of genotype scoring, quality controls, AIMS, and cluster (or latent profile class) analysis, I will run preliminary statistical analyses to determine the association between variation in cytokine genes and subgroup membership. In the interim, I have had formal training in epidemiology and biostatics, including statistical methods for genetic data, through my participation in the ATCR certificate program. I prepared three first-author papers for submission to peer-review journals, with three more first-author papers in preparation for submission.

In Year 3, I will devote much of my time to statistical analyses of phenotypic and genotypic data and manuscript preparation for the proposed study. In addition, I will develop an application for

a career development award to launch my independent clinical research career in the area of breast cancer.

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