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Title

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Permalink
https://escholarship.org/uc/item/74t798vr

Journal
Nursing research, 66(2)

ISSN
0029-6562

Authors
Smoot, Betty
Kober, Kord M
Paul, Steven M
et al.

Publication Date
2017-03-01

DOI
10.1097/nnr.0000000000000203

Peer reviewed

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HHS Public Access

Author manuscript

Nurs Res. Author manuscript; available in PMC 2018 March 01.

Published in final edited form as:

Nurs Res. 2017 ; 66(2): 85–94. doi:10.1097/NNR.0000000000000203.

Potassium Channel Candidate Genes Predict the Development of Secondary Lymphedema Following Breast Cancer Surgery

Betty Smoot, PT, DPTSc, MAS [Associate Professor],
School of Medicine, University of California San Francisco

Kord M. Kober, PhD [Assistant Professor],
School Nursing, University of California San Francisco

Steven M. Paul, PhD [Principal Statistician],
School Nursing, University of California San Francisco

Jon D. Levine, MD [Professor],
School of Medicine, University of California San Francisco

Gary Abrams, MD [Professor],
School of Medicine, University of California San Francisco

Judy Mastick, RN, MN [Project Director],
School Nursing, University of California San Francisco

Kimberly Topp, PT, PhD [Professor],
School of Medicine, University of California San Francisco, San Francisco, CA

Yvette Conley, PhD, FAAN [Professor], and
School of Nursing, University of Pittsburgh, Pittsburgh PA

Christine Miaskowski, PhD, RN, FAAN [Professor]
School Nursing, University of California San Francisco

Abstract

Background—Potassium (K+) channels play an important role in lymph pump activity, lymph formation, lymph transport, and the functions of lymph nodes. No studies have examined the relationship between K+ channel candidate genes and the development of secondary lymphedema (LE).

Corresponding Author: Christine Miaskowski, PhD, RN, FAAN, Professor, 2 Koret Way, Room 631F, Box 0601, School of Nursing, University of California San Francisco, San Francisco, CA (chris.miaskowski@nursing.ucsf.edu).
Betty Smoot, PT, DPTSc, MAS, is Associate Professor, School of Medicine, University of California San Francisco.
Kord M. Kober, PhD, is Assistant Professor, School Nursing, University of California San Francisco.
Steven M. Paul, PhD, is Principal Statistician, School Nursing, University of California San Francisco.
Jon D. Levine, MD, is Professor, School of Medicine, University of California San Francisco.
Gary Abrams, MD, is Professor, School of Medicine, University of California San Francisco.
Judy Mastick, RN, MN, is Project Director, School Nursing, University of California San Francisco.
Kimberly Topp, PT, PhD, is Professor, School of Medicine, University of California San Francisco.
Yvette Conley, PhD, FAAN, is Professor, School of Nursing, University of Pittsburgh, Pittsburgh, PA.
Christine Miaskowski, PhD, RN, FAAN is Professor, School of Nursing, University of California San Francisco.

The authors have no conflicts of interest to report.

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Objective—The study purpose was to evaluate for differences in genotypic characteristics in women who did (*n* = 155) or did not (*n* = 387) develop upper extremity LE following breast cancer treatment based on an analysis of single nucleotide polymorphisms (SNPs) and haplotypes in 10 K + channel genes.

Methods—Upper extremity LE was diagnosed using bioimpedance resistance ratios. Logistic regression analyses were used to identify those SNPs and haplotypes that were associated with LE while controlling for relevant demographic, clinical, and genomic characteristics.

Results—Patients with LE had a higher body mass index, a higher number of lymph nodes removed, had more advanced disease, received adjuvant chemotherapy, received radiation therapy, and were less likely to have undergone a sentinel lymph node biopsy. One SNP in a voltage-gated K+ channel gene (*KCNA1* rs4766311); four in two inward rectifying K+ channel genes (*KCNJ3* rs1037091 and *KCNJ6* rs2211845, rs991985, rs2836019); and one in a two pore K+ channel gene (*KCNK3* rs1662988) were associated with LE.

Discussion—These preliminary findings suggest that K+ channel genes play a role in the development of secondary LE.

Keywords

breast cancer; candidate gene association study; lymphedema; potassium channel

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Lymphedema (LE) is the accumulation of interstitial fluid as a result of dysfunction within the lymphatic system (Ridner, 2013). Recent evidence suggests that 1 in 5 women will develop upper extremity LE as a result of their breast cancer treatment (DiSipio, Rye, Newman, & Hayes, 2013), which means that over 600,000 women have or will develop this chronic and potentially disabling condition. On average, LE occurs within 14 months after breast cancer surgery. However, some cases are reported 20 years after surgery. Untreated, LE worsens and leads to significant disability and reduced quality of life (Kwan et al., 2002). Identification of risk factors for and mechanisms that contribute to the development and progression of LE will facilitate earlier treatment, and, in turn, reduce the significant negative sequelae associated with this condition.

Findings on the specific risk factors for the development of LE are highly variable. Axillary lymph node dissection (ALND), higher body mass index (BMI), more lymph nodes removed, field of radiation, postoperative seroma, infection, early edema, and surgery plus radiation are linked to the development of LE (Bevilacqua et al., 2012; Dominick, Madlensky, Natarajan, & Pierce, 2013; Paskett, Dean, Oliveri, & Harrop, 2012). However, it is unclear why—in the presence of similar phenotypic risk factors—some women develop LE and others do not.

In our previous studies (Leung et al., 2014; Miaskowski et al., 2013), increased BMI, increased number of lymph nodes removed, higher stage of disease at diagnosis, and having had an ALND, were associated with an increased risk for LE. Preliminary findings from our research group (Leung et al., 2014; Miaskowski et al., 2013) and others (Finegold et al., 2012; Finegold et al., 2008; Newman et al., 2012) suggest that variations in lymphatic, angiogenic, and pro-inflammatory cytokine genes are associated with increased risk for the

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development of LE after breast cancer treatment. However, these associations did not explain all of the variability in the occurrence of LE. Therefore, additional research is needed to identify other biological pathways that contribute to the development and progression of secondary LE. Since the etiology of LE is multifactorial, information on genetic variations in other biological pathways involved in the lymphatic system that are associated with LE can lead to the identification of high-risk patients.

Intrinsic lymphatic pump mechanisms facilitate the transport of lymph (Stanton, Modi, Mellor, Levick, & Mortimer, 2009) and play a key role in the function of the lymphatic system. Lymph flow is facilitated by phasic contractions that occur in the collecting lymphatic vessels. These contractions, and resultant pumping action, are an intrinsic property of lymphatic smooth muscles and are a result of action potentials in the smooth muscle cells in the vessel walls. Potassium (K+) channels, the most widely distributed types of ion channels, span cell membranes of vascular smooth muscle cells in the microcirculation, and are selective for K+ ions. K+ channels participate in the sodium-K+ pump that generates an electrochemical gradient across cell membranes. Movement of K+ across these membranes sets or resets the resting membrane potential and helps shape the action potential. Ion movement and cell excitability are essential components of the intrinsic pump mechanism, which facilitates lymph transport. Deficits in this pumping mechanism may compromise lymph flow (Telinius et al., 2014; von der Weid et al., 2012).

Preclinical models suggest that ATP-activated K+ channels in lymphatic smooth muscle and endothelium mediate lymphatic pump activity (Kousai, Mizuno, Ikomi, & Ohhashi, 2004). Telinius et al. (2014) found that the major classes of K+ channels have a role in the regulation of spontaneous contractions in the thoracic duct in humans. Because of their role in establishing and re-establishing normal voltage differentials across the cell membrane, K+ channels play an important role in lymph pump activity and lymph transport. Yet, no studies were identified that evaluated for associations between polymorphisms in K+ channel genes and the development of LE following breast cancer surgery. Therefore, the purpose of this study was to determine if variations in K+ channel genes were associated with the development of LE following breast cancer treatment.

Methods

Study Samples and Procedures

Demographic, clinical, and genomic data from a cross-sectional study (i.e., LE Study: NR0101282) and a longitudinal study (i.e., Breast Symptoms Study [CA107091 and CA118658]) were combined for these analyses. Both studies used the same subjective and objective measures. The University of California San Francisco (UCSF) Committee on Human Research and the Clinical and Translational Science Institute (CTSI) Clinical Research Center Advisory Committee approved both studies. Detailed information about methods used in this study are available in Leung et al. (2014) and Miaskowski et al. (2013).

LE Study—The LE study used a cross-sectional design to evaluate for differences in phenotypic and genotypic characteristics in women with ($n = 70$) and without a diagnosis of LE ($n = 71$). Women who were 18 years of age or older, six months or more posttreatment

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for unilateral breast cancer, with or without upper extremity LE, were recruited. Women were excluded for bilateral breast cancer, current upper extremity infection, lymphangitis, preexisting LE prior to breast cancer surgery, current breast cancer, or contraindications to bioimpedance spectroscopy (BIS) testing. Women were recruited through the National Lymphedema Network website, San Francisco Bay area hospitals, and breast cancer or LE support groups and conferences from 2007 to 2009. Women were evaluated in the Clinical Research Center at UCSF. After obtaining written informed consent, women completed the study questionnaires. Following the completion of the questionnaires, the research staff performed the objective measurements: height, weight, and BIS. A blood sample was drawn for genomic analyses.

Breast Symptoms Study—The Breast Symptoms Study used a longitudinal design to evaluate neuropathic pain and LE following breast cancer surgery. Women were recruited from breast care centers located in a comprehensive cancer center, two public hospitals, and four community practices from 2004 to 2008. Patients were eligible to participate if they: were adult women 18 years of age or older who would undergo breast cancer surgery on one breast; able to read, write, and understand English; agreed to participate; and gave written informed consent. Patients were excluded if they were: having breast cancer surgery on both breasts and/or had known distant metastasis at the time of diagnosis.

After obtaining informed consent, the patient completed the enrollment questionnaires, prior to surgery. Following the completion of the questionnaires, the research nurse performed the objective measurements: height, weight, and BIS. A blood sample was drawn for genomic analyses. Patients were contacted two weeks after surgery to schedule the first postsurgical appointment. The research nurse met with the patients either in their home or in the Clinical Research Center at 1, 2, 3, 4, 5, 6, 8, 10, and 12 months after surgery. In the second through fifth years of the study, patients were seen every four months. During each of the study visits, the women completed the study questionnaires and had the objective measures done by the research nurse.

Self-Report Measures

A demographic questionnaire was used to obtain information on age, marital status, education, ethnicity, employment status, living situation, and financial status. Functional status was evaluated using the Karnofsky Performance Status (KPS) scale; KPs scores have well-established validity and reliability (Karnofsky, 1977). The KPS scale ranged from 30 = *I feel severely disabled and need to be hospitalized*) to 100 = *I feel normal; I have no complaints or symptoms*. Patients were asked to indicate if they exercised on a regular basis (*yes*/*no*). Clinical information was obtained from patient interviews and medical record reviews.

The Self-Administered Comorbidity Questionnaire (SCQ) is a short and easily understood instrument that was developed to measure comorbidity in clinical and health service research settings (Sangha, Stucki, Liang, Fossel, & Katz, 2003). The questionnaire consists of 13 common medical conditions that were simplified into language that could be understood without any prior medical knowledge. Patients were asked to indicate if they had the

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condition using a *yes*/*no* format. If they indicated that they had a condition, they were asked if they received treatment for it and whether it limited their activities. For each condition, a patient can receive a maximum of three points. Total SCQ scores can range from 0 to 39. SCQ scores have well-established validity and reliability and has been used in studies of patients with a variety of chronic conditions (MacLean, Littenberg, & Kennedy, 2006; Sangha et al., 2003; Smith, Zimmerman, Williams, & Zebrack, 2009).

Objective Measures

For both studies, BIS measurements of the affected and unaffected arm were done using the procedures described by Cornish et al. (2000) and Hayes, Cornish, and Newman (2005). Patients were instructed not to exercise or take a sauna within eight hours of the assessment. In addition, they were asked to refrain from drinking alcohol for 12 hours prior to the assessment. In the LE study, the LE diagnosis was known based on a previous diagnosis by the patient's healthcare provider. In the LE study, BIS measurements were taken using the SFB7 device (ImpediMed, San Diego, CA). The Quantum X Bioelectrical Impedance Device (RJL Systems, Clinton Township, MI) was used in the Breast Symptoms Study. Women removed all jewelry, and their skin was prepped with an alcohol wipe prior to surface electrode placement. Patients laid supine on a massage table with their arms 30 degrees from the body and legs not touching for at least 10 minutes prior to the BIS measurements. Electrodes were placed on the dorsum of the wrists adjacent to the ulnar styloid process, the dorsum of the hands just proximal to the third metacarpophalangeal joint, anterior to the ankle joints between the malleoli, and over the dorsum of the feet over the third metatarsal bone just proximal to the third metatarsophalangeal joint. Two "measurement" electrodes were placed at either end of the 40 cm length over which the circumference measurements were made and the 'drive' electrodes were placed 8–10 cm distal to these measurement electrodes. Two readings of resistance were obtained from the affected and unaffected arms, and each arm averaged for subsequent analyses.

While cases and noncases of LE were known in the LE study, for the Breast Symptoms Study, LE cases were determined based on the procedures of Cornish et al. (2000) and Hayes et al. (2005) using all of the data obtained from each woman during her participation in the study. A woman was defined as a LE case if the ratio of the resistance in the untreated arm to the resistance in the treated arm was >1.139 for those women who had surgery on the dominant side, or >1.066 for those women who had surgery on the nondominant side, at any of the BIS assessments.

Analysis of Phenotypic Data

Descriptive statistics and frequency distributions were generated on the sample characteristics. Independent sample *t*-tests, χ^2 tests for independence, and Mann-Whitney *U* tests were done to evaluate for differences in demographic and clinical characteristics between patients with and without LE. Data were analyzed using SPSS Version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Macintosh, Version 22.0. Armonk, NY: IBM Corp).

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Analysis for Genomic Data

Gene selection—K+ channel genes known to have a role in lymphatic pump mechanisms were included in this analysis. Ten K+ channel genes were selected across the three main types of K+ channels. Three of the selected genes encode for voltage-gated K+ channels (i.e., *KCNA1, KCND2, KCNS1*); four encode for inward rectifying K+ channels (i.e., *KCNJ3, KCNJ5, KCNJ6, KCNJ9*); and three encode for two-pore domain K+ channels (i.e., *KCNK2, KCNK3, KCNK9*). All genes are identified according to the approved symbol in the Human Genome Organization (HUGO) Gene Nomenclature Committee database (http://www.genenames.org/).

Blood collection and genotyping—Of the 542 patients in this study (387 without LE and 155 with LE), DNA was recovered from the archive buffy coat of 407 patients (i.e., 110 with and 297 without LE) who provided a blood sample. Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). Samples were genotyped using the Golden Gate genotyping platform (Illumina, San Diego, CA) and processed using GenomeStudio (Illumina, San Diego, CA). Genotyping was performed blinded to LE status, positive and negative controls were included, and Hardy-Weinberg equilibrium was assessed for each single nucleotide polymorphism (SNP).

SNP selection—A combination of tag-single nucleotide polymorphisms (SNPs) and literature driven SNPs were selected for analysis. Tag-SNPs were common (minor allele frequency .05) in public databases. SNPs with call rates <95% or a Hardy-Weinberg *p*-value of <.001 were excluded. As shown in the table in Supplemental Digital Content, a total of 155 SNPs and 100 haplotypes across 10 candidate genes (*KCNA1*: 1 SNP; *KCND2*: 9 SNPs and 2 haplotypes; *KCNS1*: 4 SNPs and 6 haplotypes; *KCNJ3*: 28 SNPs and 16 haplotypes; *KCNJ5*: 8 SNPs and 3 haplotypes; *KCNJ6:* 58 SNPs and 41 haplotypes; *KCNJ9*: 2 SNPs; *KCNK2*: 22 SNPs and 17 haplotypes, *KCNK3*: 6 SNPs and 5 haplotypes; *KCNK9*: 17 SNPs and 10 haplotypes) passed all quality control filters and were included in the genetic association analyses.

Regional annotations were identified using the University of California Santa Cruz (UCSC) Human Genome Browser GRCh37/hg19 (http://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19). Potential regulatory involvement of SNPs was investigated using the ENCODE data tracks for Transcription Factor ChIP-seq (i.e., wgEncodeAwgTfbsUniform, wgEncodeRegTfbsClusteredV30, H3K27Ac Mark (wgEncodeRegMarkH3k27ac), H3K4Me1 Mark (wgEncodeRegMarkH3k4me1), DNaseI Hypersensitivity Clusters (i.e, wgEncodeRegDnaseClustered), and the TargetScan miRNA Regulatory Sites (i.e., targetScanS) tracks (Karolchik et al., 2014).

Statistical analyses of genetic data—Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by using χ^2 or Fisher's exact tests. Measures of linkage disequilibrium (LD; i.e., D′ and r2) were computed from patient genotypes with Haploview 4.2. The LD-based haplotype block definition was based on the D′ confidence interval (Gabriel et al., 2002).

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For SNPs that were members of the same haploblock, haplotype analyses were conducted in order to localize the association signal within each gene and to determine if haplotypes improved the strength of the association with the phenotype. Haplotypes were constructed using the program PHASE version 2.1 (Stephens, Smith, & Donnelly, 2001). In order to improve the stability of haplotype inference, the haplotype construction procedure was repeated five times using different seed numbers with each cycle. Only haplotypes that were inferred with probability estimates of .85, across the five iterations, were retained for downstream analyses. Haplotypes were evaluated assuming a dosage model (i.e., analogous to the additive model).

Ancestry informative markers (AIMS) were used to minimize confounding due to population stratification (Hoggart et al., 2003; Tian, Gregersen, & Seldin, 2008). Homogeneity in ancestry among patients was verified by principal component analysis (Price et al., 2006), using Helix Tree (Golden Helix, Bozeman, MT). Briefly, the number of principal components (PCs) that distinguished the major racial/ethnic groups in the sample by visual inspection of scatter plots of orthogonal principal components (PCs; i.e., PC 1 versus PC2, PC2 versus PC3) were sought. This procedure was repeated until no discernible clustering of patients by their self-reported race/ethnicity was possible (data available on request). One hundred and six AIMs were included in the analysis. The first three PCs were selected to adjust for potential confounding due to racial/ethnic population substructure by including the three PCs as covariates in all regression models.

For association tests, three genetic models were assessed for each SNP: additive, dominant, and recessive. Barring trivial improvements (i.e., delta <10%), the genetic model that best fit the data (i.e., the model with the smallest *p*-value) was selected for each SNP. Logistic regression analysis that controlled for significant covariates, as well as genomic (i.e., three PCs)and self-reported race/ethnicity, was used to evaluate the relationship between genotype and LE group membership. A backwards stepwise approach was used to create the most parsimonious model. Genetic model fit and both unadjusted and covariate-adjusted odds ratios were estimated using STATA version 14.

As was done in our previous studies (Illi et al., 2012; McCann et al., 2012), based on recommendations in the literature (Hattersley & McCarthy, 2005; Rothman, 1990), the implementation of rigorous quality controls for genomic data, the nonindependence of SNPs/haplotypes in LD, and the exploratory nature of the analyses, adjustments were not made for multiple testing. In addition, significant SNPs identified in the bivariate analyses were evaluated further using regression analyses that controlled for differences in phenotypic characteristics, potential confounding due to population stratification, and variation in other SNPs/haplotypes within the same gene. Only those SNPs that remained significant were included in the final presentation of the results. Therefore, the significant independent associations reported are unlikely to be due solely to chance. Unadjusted (bivariate) associations are reported for all SNPs and haplotypes passing quality control criteria are shown (see Table; Supplementary Digital Content 1) to allow for subsequent comparisons and meta-analyses.

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Results

Demographic and Clinical Characteristics

Demographic and clinical characteristics that differed between groups are presented in Table 1. Women with LE had a higher BMI, lower KPS score, were more likely to have lung disease, and had more advanced disease. They were less likely to have had a sentinel lymph node (SLNB) biopsy and more likely to have had ALND. Women with LE had a higher number of lymph nodes removed and had more positive nodes. They were more likely to have received adjuvant chemotherapy (CTX), radiation therapy (RT), and/or a combination of therapies.

Candidate Gene Analyses for the Development of LE

The genotype frequency was significantly different between those who did and did not develop LE for 10 SNPs and one haplotype spanning 4 K+ channel genes (*KCNA1, KCNJ3, KCNJ6, KCNK3;* see Table, Supplementary Digital Content 1). For the SNP rs4766311 in the voltage-gated K+ channel gene *KNCA1*, the recessive model fit the data best ($p = .02$). For the SNP rs1037091 in the inward-rectifying K+ channel gene *KCNJ3*, the dominant model fit the data ($p = .02$). One haplotype, HapE3, in *KCNJ3* was found to be associated with LE ($p = .05$).

Seven SNPs in the inward-rectifying K+ channel gene *KCNJ6* were associated with LE: rs858010 ($p = .04$); rs2211843 ($p = .04$); rs2211845 ($p = .004$); rs4817896 ($p = .04$); rs991985 ($p = .02$); rs2836019 ($p = .03$); and rs3787870 ($p = .02$). For rs858010, rs4817896, rs991985, and rs3787870, a recessive model fit the data best. For rs2211843, rs2211845, rs2836019, a dominant model fit the data best.

In the two-pore K+ channel gene *KCNK3*, for the SNP rs1662988, a dominant model fit the data best ($p = .04$). No associations were found between the occurrence of LE and polymorphisms in *KCND2, KCNS1, KCNJ5, KCNJ9, KCNK2,* or *KCNK9.*

Regression Analyses of *KCNA1, KCNJ3, KCNJ6*, and *KCNK3* Genotypes and the Development of LE

Multivariate logistic regression models were fit to better estimate the magnitude of the association between genotype and the development of LE. As shown in Table 2, in addition to genotype, the phenotypic characteristics included in the regression models were ethnicity, BMI, stage of disease, having an SLNB, and number of lymph nodes removed. Receipt of CTX and RT, while not significant after the inclusion of genomic and self-reported race/ethnicity, were retained in all of the regression models for face validity.

As shown in Figures 1, Panels A through F, the only genetic associations that remained significant in the multivariate logistic regression analyses were for *KNCA1* rs4766311, *KCNJ3* rs1037091, *KCNJ6* rs2211845, *KCNJ6* rs991985, *KCNJ6* rs2836019, and *KCNK3* rs1662988.

In the regression analysis for *KNCA1* rs4766311, carrying two doses of the rare T allele (i.e., CC+CT vs. TT) was associated with 59% lower odds of being in the LE group. In the

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regression analysis for *KCNJ3* rs1037091, having one or two doses of the rare allele A (i.e., GG vs. GA+AA) was associated with 48% lower odds of being in the LE group. For the 3 *KCNJ6* SNPs, each was included individually in its own regression, and then together in a single regression. For *KCNJ6* rs2211845, having one or two doses of the rare C allele (i.e., TT vs. TC+CC) was associated with a 1.9 fold greater odds of being in the LE group. For *KCNJ6* rs991985, having two doses of the rare A allele (i.e., CC+CT vs. AA) was associated with almost a 2.2 fold higher odds of being in the LE group. For *KCNJ6* rs2836019, having one or two doses of the rare T allele (i.e., CC vs. CT+TT) was associated with 46% lower odds of being in the LE group. In the regression analysis for *KCNK3* rs1662988, having one or two doses of the rare T allele (i.e., CC vs. CT+TT) was associated with 46% lower odds of being in the LE group.

Discussion

This study is the first to evaluate for associations between polymorphisms in K+ channel genes and the development of LE following breast cancer surgery. Details on phenotypic differences in this sample were reported previously (Leung et al., 2014; Miaskowski et al., 2013). Therefore, this discussion will focus on the K+ channel genes found to have a significant association with LE. After controlling for significant phenotypic characteristics, as well as self-reported and genomic estimates of race/ethnicity, 6 SNPs across 4 K+ channel genes were found to be associated with the occurrence of LE. Interestingly, SNPs were identified in genes for each of three families of K+ channels that were evaluated: voltage-gated, inward rectifying, and two-pore K+ channel genes.

Voltage-Gated K+ Genes

While genes for three types of voltage-gated K+ channels were evaluated in this study, only *KCNA1* was associated with LE classification. Specifically, for the SNP rs4766311, carrying two doses of the rare T allele was associated with lower odds of being in the LE group. In humans, *KCNA1* encodes for the K+ voltage-gated channel subfamily A, member 1 (Kv1.1). A variety of voltage-gated K+ channels are expressed across the cell membranes of smooth muscles cells in the microcirculation. During the action potential, voltage-gated K+ channels are essential in shaping the action potential or returning the depolarized membrane to its resting state (Jackson, 2005; Miller, 2000). These functions act to regulate the excitability of smooth muscle cells. Voltage-gated K+ channels may regulate the frequency and the amplitude of the spontaneous smooth muscle contractions in lymphatic collectors and ducts (Telinius et al., 2014). Polymorphisms in *KCNA1* have been linked to episodic ataxia and myokymia (D'Adamo et al., 2015). *KCNA1* rs4766311, located in the 3′ untranslated region of the gene, has no known function.

Inward-Rectifying K+ Genes

Of the four inward-rectifying genes evaluated, *KCNJ3* and *KCNJ6* were found to be associated with LE. For *KCNJ3* rs1037091 having one or two doses of the rare allele A was associated with a lowered odds of being in the LE group. *KCNJ3* encodes for the G-protein inwardly rectifying potassium channel, subfamily J, member 3p (Kir3.1 or GIRK1).

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For *KCNJ6* rs2211845, having one or two doses of the rare C allele was associated with an increased odds of being in the LE group. For *KCNJ6* rs991985, having two doses of the rare A allele was associated with an increased odds of being in the LE group. For *KCNJ6* rs2836019 having one or two doses of the rare T allele was associated with a lower odds of being in the LE group. *KCNJ6* encodes for the G-protein inwardly rectifying potassium channel, subfamily J, member 6p (Kir3.2 or GIRK2).

In contrast to voltage-gated K+ channels, which are outward rectifying, the inward-rectifying channels conduct more K+ current into the cell than outward. These channels function to stabilize the cell's resting membrane potential near the K+ equilibrium potential until the threshold potential is reached. Alterations in GIRK1 channels have been associated with epilepsy (Chioza et al., 2002) and atrial fibrillation (Brundel et al., 2001).

In this same sample, different polymorphisms in both the GIRK1 and GIRK2 genes were associated with the occurrence of breast pain (Langford et al., 2015; Langford et al., 2014). Polymorphisms in the GIRK2 gene have been associated with postoperative pain, pain sensitivity, and addiction (Nishizawa et al., 2014). The significant SNPS in *KCNJ3* and *KCNJ6* identified in this study are located in the intronic region of the genes and have no known function.

Two-Pore K+ Channel Gene

Of the three two-pore K+ channel genes evaluated, only *KCNK3* was associated with LE. For *KCNK3* rs1662988, having one or two doses of the rare T allele was associated with a lower odds of being in the LE group. *KCNK3* encodes for the two-pore potassium channel, domain subfamily K, member 3 (TASK-1). Two-pore K+ channels give rise to background, or leak, K+ currents, which play a role in the maintenance of the resting membrane potential. TASK-1 channels are sensitive to changes in extracellular pH and are inhibited by increased extracellular acidity (Lesage & Lazdunski, 2000). *KCNK3* rs1662988 is located in the intronic region of the gene, with no known function.

Limitations

Study limitations warrant consideration. Our sample size precluded analysis of interaction effects and genome-wide association studies. Larger sample sizes may reveal additional associations between polymorphisms in K+ channel genes and LE. In addition, we did not evaluate every type of potassium channel gene. Future studies are needed to confirm these preliminary findings and to evaluate the functional effects of these polymorphisms.

Conclusions

Despite these limitations, the preliminary findings from this study suggest that polymorphisms in K+ channel genes are associated with the occurrence of LE following breast cancer surgery. Phasic contractions of the smooth muscles in lymphatic vessels contribute to the intrinsic lymph pump mechanism, which facilitates lymph transport. These contractions depend in part on K+ channel regulation of the smooth muscle cell resting membrane potential and the shaping of the action potential. Alterations in K+ channels may affect the excitability of the smooth muscle cell membrane and modify lymph pump activity.

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This study identified six polymorphisms across four K+ channel genes (i.e., *KCNA1, KCNJ3, KCNJ6, KCNK3*) that were significantly associated with the occurrence of LE after breast cancer surgery. At this time, these polymorphisms do not have known functional consequences. However, they may be in linkage disequilibrium with functional polymorphisms. Given that associations were found between the occurrence of LE and polymorphisms in K+ channel genes, cytokine genes, and genes involved in lymphangiogenesis and angiogenesis, the mechanisms that underlie the development of LE are likely to be multifactorial. Therefore, additional research with larger samples is needed to evaluate the relative contribution of these different pathways in the development of LE. However, the identification of genetic risk factors for secondary LE may allow for the early detection and implementation of risk reduction strategies for those women at higher risk for this chronic and potentially disabling condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge this study was funded by grants from the National Cancer Institute (NCI, CA107091 and CA118658) and the National Institute of Nursing Research (NR0101282). Dr. Christine Miaskowski is an American Cancer Society Clinical Research Professor and is funded by a K05 award from the NCI (CA168960). This project was supported by NIH/NCRR UCSF-CTSI Grant Number UL1 RR024131. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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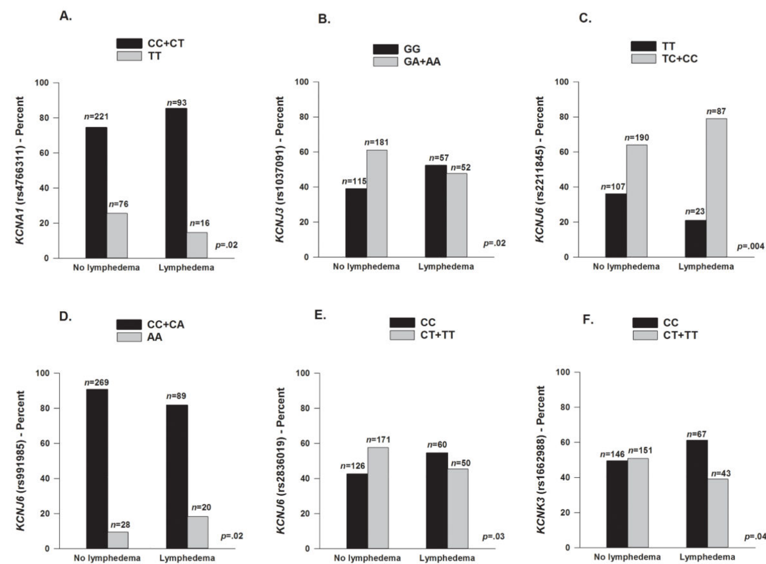

FIGURE 1.

Differences between the lymphedema and no lymphedema groups. Panel A shows differences between the lymphedema and no lymphedema groups in the percentages of patients who were homozygous or heterozygous for the common allele (CC+CT) or homozygous for the rare allele (TT) for rs4766311 in KCNA1. Panel B shows differences between the lymphedema and no lymphedema groups in the percentages of patients who were homozygous for the common allele (GG) or heterozygous or homozygous for the rare allele (GA+AA) for rs1037091 in KCNJ3. Panel C shows differences between the lymphedema and no lymphedema groups in the percentages of patients who were homozygous for the common allele (TT) or heterozygous or homozygous for the rare allele (TC+CC) for rs2211845 in KCNJ6. Panel D shows differences between the lymphedema and no lymphedema groups in the percentages of patients who were homozygous or heterozygous for the common allele (CC+CA) or homozygous for the rare allele (AA) for rs991985 in KCNJ6. Panel E shows differences between the lymphedema and no lymphedema groups in the percentages of patients who were homozygous for the common allele (CC) or heterozygous or homozygous for the rare allele (CT+TT) for rs2836019 in KCNJ6. Panel F shows differences between the lymphedema and no lymphedema groups in the percentages of patients who were homozygous for the common allele (CC) or heterozygous or homozygous for the rare allele (CT+TT) for rs1662988 in KCNJ3.

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TABLE 1

Demographic and Clinical Characteristics in Patients With and Without Lymphedema[a]

Characteristic	Lymphedema (n = 155)		No Lymphedema (n = 387)		p
	M	(*SD*)	*M*	(*SD*)	
Body mass index (kg/m^2)	28.2	(6.7)	26.1	(5.6)	.001
Karnofsky Performance Status (score)	91.1	(11.1)	93.3	(9.7)	.03
Nodes removed (number)	10.9	(9.0)	5.8	(6.3)	.001
Positive nodes (number)	1.7	(3.4)	0.7	(1.7)	.001
	n	(%)	*n*	(%)	
Lung disease (yes)(reported on the SCQ)	12	(8.1)	14	(3.7)	.04
Stage of disease[b]					.001
0	8	(5.2)	70	(18.1)	
I	51	(32.9)	155	(40.0)	
IIA and IIB	75	(48.4)	137	(35.4)	
IIIA, IIIB, IIIC, and IV	21	(13.5)	25	(6.5)	
Sentinel lymph node biopsy (yes)	108	(69.7)	313	(80.9)	.006
Axillary lymph node dissection (yes)	106	(69.3)	152	(39.3)	.001
Adjuvant chemotherapy (yes)	92	(59.7)	142	(36.7)	.001
Adjuvant radiation therapy (yes)	110	(71.0)	221	(57.1)	.003
Treatment combinations					.001
Only surgery	13	(8.4)	92	(23.8)	
Surgery and radiation therapy	50	(32.3)	153	(39.5)	
Surgery and chemotherapy	32	(20.6)	74	(19.1)	
Surgery, radiation therapy, and chemotherapy	60	(38.7)	68	(17.6)	

Note. SCQ = Self-Administered Comorbidity Questionnaire, *SD* = standard deviation.

[a]Additional information about demographic and clinical characteristics is available in Leung et al. (2014) and Miaskowksi et al. (2013).

[b]Stage of disease was classified using American Joint Committee on Cancer staging system.

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TABLE 2

Multiple Logistic Regressions: Prediction of Lymphedema from *KCNA1, KCNJ3, KCNJ6*, and *KCNK3* Genotypes

Genotype/covariates	*OR*	(*SE*)	95% CI	*Z*	*p*	Model fit χ^2	Model fit *p*
KCNA1 rs4766311	0.41	(0.147)	[0.20, 0.83]	−2.48	.01	84.61	<.001
BMI (kg/m^2)	1.06	(0.022)	[1.02, 1.10]	2.71	.007		
Stage of disease							
Stage 0 versus I	3.37	(2.008)	[1.05, 10.84]	2.04	.04		
Stage 0 versus II	4.07	(2.581)	[1.17, 14.11]	2.21	.03		
Stage 0 versus III and IV	6.52	(4.815)	[1.54, 27.72]	2.54	.01		
SLNB (yes)	0.41	(0.141)	[0.21, 0.81]	−2.59	.01		
Nodes removed (number)	1.08	(0.021)	[1.04, 1.13]	4.04	<.001		
Any chemotherapy (yes)	1.14	(0.359)	[0.62, 2.12]	0.42	.67		
Any radiation therapy (yes)	1.30	(0.389)	[0.72, 2.34]	0.88	.38		
KCNJ3 rs1037091	0.52	(0.142)	[0.31, 0.89]	−2.40	.02		
BMI (kg/m^2)	1.06	(0.022)	[1.02, 1.11]	2.77	.006	84.91	<.001
Stage of disease							
Stage 0 versus I	2.83	(1.675)	[0.89, 9.02]	1.76	.08		
Stage 0 versus II	3.64	(2.289)	[1.06, 12.49]	2.05	.04		
Stage 0 versus III and IV	6.53	(4.816)	[1.54, 27.72]	2.54	.01		
SLNB (yes)	0.42	(0.148)	[0.22, 0.84]	−2.46	.01		
Nodes removed (number)	1.09	(0.022)	[1.04, 1.13]	4.06	<.001		
Any chemotherapy (yes)	1.22	(0.382)	[0.66, 2.25]	0.63	.53		
Any radiation therapy (yes)	1.34	(0.400)	[0.74, 2.40]	0.97	.33		
KCNJ6 rs2211845	1.92	(0.610)	[1.03, 3.58]	2.06	.04	94.41	<.001
KCNJ6 rs991985	2.17	(0.842)	[1.02, 4.64]	2.00	.05		
KCNJ6 rs2836019	0.56	(0.159)	[0.32, 0.98]	−2.03	.04		
BMI (kg/m^2)	1.07	(0.024)	[1.02, 1.11]	2.85	.004		
Stage of disease							
Stage 0 versus I	3.15	(1.893)	[0.97, 10.23]	1.91	.06		
Stage 0 versus II	3.74	(2.400)	[1.07, 13.15]	2.06	.04		

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Genotype/covariates	*OR*	(*SE*)	95% CI	*Z*	*p*	Model fit	
						χ^2	*p*
Stage 0 versus III and IV	6.34	(4.739)	[1.46, 27.44]	2.47	.01		
SLNB (yes)	0.35	(0.125)	[0.18, 0.71]	−2.95	.003		
Nodes removed (number)	1.08	(0.022)	[1.04, 1.13]	3.93	<.001		
Any chemotherapy (yes)	1.34	(0.427)	[0.72, 2.51]	0.93	.35		
Any radiation therapy (yes)	1.45	(0.446)	[0.80, 2.65]	1.22	.22		
KCNK3 rs1662988	0.54	(0.147)	[0.31, 0.92]	−2.27	.02	83.09	<.001
BMI (kg/m^2)	1.06	(0.022)	[1.01, 1.10]	2.64	.008		
Stage of disease							
Stage 0 versus I	3.15	(1.870)	[0.98, 10.08]	1.93	.05		
Stage 0 versus II	4.23	(2.665)	[1.23, 14.54]	2.29	.02		
Stage 0 versus III and IV	6.46	(4.734)	[1.54, 27.16]	2.55	.01		
SLNB (yes)	0.39	(0.132)	[0.20, 0.76]	−2.78	.006		
Nodes removed (number)	1.08	(0.021)	[1.04, 1.13]	4.07	<.001		
Any chemotherapy (yes)	1.22	(0.378)	[0.66, 2.24]	0.64	0.523		
Any radiation therapy (yes)	1.33	(0.400)	[0.74, 2.40]	0.96	0.337		

Note. In each model, the first three principal components identified from the analysis of ancestry informative markers as well as self-report of race/ethnicity (i.e., White, Black, Asian/Pacific Islander, Hispanic/Mixed ethnic background/Other) were retained to adjust for potential confounding due to race/ethnicity (data not shown). Predictors evaluated in each model included genotype (*KCNA1* rs4766311, recessive model CC + CT versus TT; *KCNJ3* rs1037091, dominant model GG versus GA + AA; *KCNJ6* rs221845 dominant model, TT versus TC + CC; KCNJ6 rs991985, recessive model, CC + CA versus AA; *KCNJ6* rs2836019, dominant model, CC versus CT + TT; *KCNK3* rs1662988, dominant model CC versus CT + TT), BMI, stage of disease, sentinel lymph node biopsy, number of lymph nodes removed, chemotherapy prior to or following surgery, and radiation therapy following surgery. BMI = body mass index; CI =confidence interval; SLNB = sentinel lymph node biopsy.