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West, Claudia
Elboim, Charles
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Cytokine Candidate Genes Predict the Development of Secondary Lymphedema Following Breast Cancer Surgery

Geraldine Leung, RN, MS,[1] Christina Baggott, RN, PhD,[1] Claudia West, RN, MS,[1] Charles Elboim, MD,[4]
Steven M. Paul, PhD,[1] Bruce A. Cooper, PhD,[1] Gary Abrams, MD,[2] Anand Dhruva, MD,[2]
Brian L. Schmidt, DDS, MD, PhD,[5] Kord Kober, PhD,[1] John D. Merriman, RN, PhD(c),[1]
Heather Leutwyler, RN, PhD,[1] John Neuhaus, PhD,[2] Dale Langford, PhD,[1] Betty J. Smoot, PhD,[2]
Bradley E. Aouizerat, PhD,[1,3*] and Christine Miaskowski, RN, PhD,[1*]

Abstract

Background: Lymphedema (LE) is a frequent complication following breast cancer treatment. While progress is being made in the identification of phenotypic risk factors for the development of LE, little information is available on the molecular characterization of LE. The purpose of this study was to determine if variations in pro- and anti-inflammatory cytokine genes were associated with LE following breast cancer treatment.
Methods and Results: Breast cancer patients completed a number of self-report questionnaires. LE was evaluated using bioimpedance spectroscopy. Genotyping was done using a custom genotyping array. No differences were found between patients with ($n = 155$) and without LE ($n = 387$) for the majority of the demographic and clinical characteristics. Patients with LE had a significantly higher body mass index, more advanced disease, and a higher number of lymph nodes removed. Genetic associations were identified for three genes (i.e., interleukin (IL4) 4 (rs2227284), IL 10 (rs1518111), and nuclear kappa factor beta 2 (NFKB2 (rs1056890)) associated with inflammatory responses.
Conclusions: These genetic associations suggest a role for a number of pro- and anti-inflammatory genes in the development of LE following breast cancer treatment.

Introduction

Lymphedema (LE) is a frequent complication of breast cancer treatment. LE is caused by a disruption in the lymphatic system that results in the accumulation of fluid in the interstitial space.[1] LE manifests as swelling of the affected limb and is associated with chronic pain, disfigurement, reduced mobility, functional impairment, predisposition to infections, and increased health care costs.[2–5]

The true incidence of breast cancer-related LE is unknown, though estimates range from 6% to 83%.[6] This wide variation is due to differences in diagnostic criteria, measurement techniques, timing of measurements, duration of follow-up, and sample characteristics.[7,8] In a recent review of 11 prospective cohort studies,[9] the median incidence rate for

LE within 3 years of breast cancer treatment was 20%. In the United States, this rate would mean that more than 500,000 breast cancer survivors are affected by this incurable condition.[10]

Research is often directed at identifying risk factors for LE with the hope of developing interventions to reduce its incidence.[11] In our previous study,[12] we identified both phenotypic and genotypic differences between women who did and did not develop LE following breast cancer treatment. The phenotypic characteristics associated with LE were increased body mass index (BMI), increased number of lymph nodes removed, higher stage of disease, and having had a sentinel lymph node biopsy (SLNB). In addition, a number of candidate genes in the lymphatic and angiogenesis pathways were identified as being associated with LE (i.e., lymphocyte

Schools of Nursing[1] and Medicine[2] and Institute for Human Genetics,[3] University of California, San Francisco, California.
[4]Redwood Regional Medical Group, Santa Rosa, California.
[5]School of Dentistry, New York University, New York, New York.
*Shared senior authorship.

cytosolic protein 2 (LCP2), neuropilin-2 (NRP2), protein tyrosine kinase (SYK), Forkhead box protein C2 (FOXC2), vascular cell adhesion molecule 1 (VCAM1), and vascular endothelial growth factor C (VEGFC)). While this study was novel in uncovering associations between LE and lymphatic and angiogenic candidate genes, further investigation is warranted to identify additional molecular pathways.

Several studies have suggested that cytokines may be involved in the pathophysiology of LE.[13,14] Cytokines play a key role in modulating inflammatory responses, which may subsequently lead to lymphatic dysfunction and LE.[13] In a study that used a specific bioassay and performed transcriptional microarray analysis on human skin,[15] a number of cytokine genes (i.e., interleukin (IL) 4, IL6, IL10, IL13) were upregulated in LE specimens. In another study that investigated the role of inflammation in the regulation of fibrosis and lymphatic dysfunction,[14] the blockade of T-helper 2 cytokines, including IL-4 and IL-13, prevented T-cell differentiation and its subsequent inflammatory response in a mouse-tail model of LE. This blockade resulted in less fibrosis and improved lymphatic function. Findings from these studies suggest that variations in cytokine genes may account for some of the differences in the development of LE. Therefore, the purpose of this study was to determine if variations in pro-and anti-inflammatory cytokine 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. Both studies were approved by the Committee on Human Research at the University of California, San Francisco (UCSF) and the Clinical Translational Science Institute's (CTSI) Clinical Research Center Advisory Committee.

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 ($n = 71$) LE. Women who were ≥18 years of age and ≥6 months post-treatment 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, 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. Women were evaluated in the Clinical Research Center at UCSF. After obtaining written informed consent, women completed the study questionnaires. Following the completion of these 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.[12,16–18] Women were recruited from Breast Care Centers located in a Comprehensive Cancer Center, two public hospitals, and four community practices. Patients were eligible to participate if they were adult women (≥18 years) who would undergo breast cancer surgery on one breast; were 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 distant metastasis at the time of diagnosis. A total of 516 patients were approached to participate, 410 were enrolled in the study (response rate 79.5%), and 398 completed the preoperative assessment. The major reasons for refusal were: too busy, overwhelmed with the cancer diagnosis, or insufficient time available to do the enrollment assessment prior to surgery.

During the patient's preoperative visit, a clinician explained the study, determined the patient's willingness to participate, and introduced the patient to the research nurse. The research nurse met with the woman, determined eligibility, and obtained written informed consent prior to surgery. After obtaining written informed consent, the patient completed the study questionnaires prior to surgery. Following the completion of the questionnaires, the research nurse performed the objective measurements: height, weight, and bioimpedance spectroscopy (BIS). A blood sample was drawn for genomic analyses. Patients were contacted 2 weeks after surgery to schedule the first post-surgical 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 4 months. During each of the study visits, the women completed the study questionnaires and had the objective measures done by the research nurse.

Subjective measures

A demographic questionnaire obtained 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 that has well established validity and reliability.[19,20] Patients rated their functional status using the KPS scale that 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 Co-morbidity Questionnaire (SCQ) is a short and easily understood instrument that was developed to measure co-morbidity in clinical and health service research settings.[21] 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 condition using a "yes/no" format. If they indicated that they had a condition, they were asked if they received treatment for it and did it limit their activities. Patients were given the option to add two additional conditions not listed on the instrument. For each condition, a patient can receive a maximum of 3 points. Because there are 13 defined medical conditions and 2 optional conditions, the maximum score totals 45 points if the open-ended items are used and 39 points if only the closed-ended items are used. The SCQ has well-established validity and

reliability and has been used in studies of patients with a variety of chronic conditions.[21–25]

Objective measures

Bioimpedance Spectroscopy (BIS) of LE—BIS measurements of the affected and unaffected arms, were done using the procedures described by Cornish and colleagues.[26–28] Patients were instructed not to exercise or take a sauna within 8 h of the assessment. In addition, they were asked to refrain from drinking alcohol for 12 h prior to the assessment. BIS measurements were taken using a single channel BIS device (i.e., SFB7 device; ImpediMed, San Diego, CA in the LE study, or the Quantum × Bioelectrical Impedance Device; RJL Systems, Clinton Township, MI in the Breast Symptoms Study).

Women removed all jewelry and their skin was prepped with an alcohol wipe prior to surface electrode placement. Patients lay supine on a massage table with their arms 30 degrees from the body and legs not touching for at least 10 min 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 averaged for subsequent analyses.

While cases and non-cases of LE were known in the LE study, for the Breast Symptoms Study, LE cases were determined based on the procedures of Cornish and colleagues[26–28] 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 resistance ratio for the untreated arm/treated arm was >1.139 or >1.066 for those women who had surgery on the dominant or nondominant side, respectively at any of the BIS assessments.

Methods of analysis for phenotypic data

Data were analyzed using SPSS Version 19.[29] Descriptive statistics and frequency distributions were generated on the sample characteristics. Independent sample *t*-tests, Chi-square analyses, and Mann Whitney U tests were done to evaluate for differences in demographic and clinical characteristics between patients with and without LE.

Methods of analysis for genomic data

Gene selection.   Cytokines and their receptors are classes of polypeptides that mediate inflammatory processes.[30] These polypeptides are divided into pro- and anti-inflammatory cytokines. Pro-inflammatory cytokines promote systemic inflammation and include interferon gamma (IFNG) 1, IFNG receptor 1 (IFNGR1), IL1R1, IL2, IL8, IL17A, nuclear factor kappa beta (NFKB1), NFKB2, and tumor necrosis factor alpha (TNFA).[30,31] Anti-inflammatory cytokines suppress the activity of pro-inflammatory cytokines and include IL1R2, IL4, IL10, and IL13.[30,31] Of note, IFNG1, IL1B, and IL6 possess pro- and anti-inflammatory functions.[31]

Blood collection and genotyping.   Genomic DNA was extracted from archived buffy coats using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). Of the 543 patients recruited for this study, DNA was recovered from the archive buffy coat of 407 patients (i.e., 110 with and 297 without LE) who provided a blood sample. Genotyping was performed blinded to LE status, and positive and negative controls were included. DNA was quantitated with a Nano-drop Spectrophotometer (ND-1000) and normalized to a concentration of 50 ng/μL (diluted in 10 mM Tris/1 mM EDTA). Samples were genotyped using the GoldenGate genotyping platform (Illumina, San Diego, CA) and processed according to the standard protocol using GenomeStudio (Illumina). Signal intensity profiles and resulting genotype calls for each single nucleotide polymorphism (SNP) were visually inspected by two blinded reviewers. Disagreements were adjudicated by a third reviewer.

SNP selection.   A combination of tagging SNPs and literature driven SNPs were selected for analysis. Tagging SNPs were required to be common (i.e., estimated to have a minor allele frequency ≥0.05) in public databases (e.g., HapMap). In order to ensure robust genetic association analyses, quality control filtering of SNPs was performed. SNPs with call rates of <95% or Hardy-Weinberg *p* values of <0.001 were excluded.

As shown in Table 1, a total of 86 SNPs among the 15 candidate genes (IFNG1: 5 SNPs, IFNGR1: 1 SNP; IL1B: 12 SNPs; IL1R1: 4 SNPs; IL1R2: 3 SNPs; IL2: 5 SNPs; IL4: 3 SNPs; IL6: 9 SNPs; IL8: 3 SNPs; IL10: 8 SNPs; IL13: 4 SNPs; IL17A: 5 SNPs; NFKB1: 11 SNPs; NFKB2: 4 SNPs; TNFA: 9 SNPs) passed all quality control filters and were included in the genetic association analyses. Potential functional roles of SNPs associated with LE were examined using PUPASuite 2.0,[32] a comprehensive search engine that tests a series of functional effects.

Statistical analyses

Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the Chi-square or Fisher Exact tests. Measures of linkage disequilibrium ((LD) i.e., D' and r^2) were computed from the patients' genotypes with Haploview 4.2. LD-based haplotype block definition was based on D' confidence interval.[33]

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.[34] 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 >0.85, 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.[35–37] Homogeneity in ancestry among patients was verified by principal component analysis,[38] using Helix Tree (Golden

TABLE 1. CYTOKINE GENES AND SINGLE NUCLEOTIDE POLYMORPHISMS ANALYZED
for LYMPHEDEMA VERSUS NO LYMPHEDEMA

Gene	SNP	Position	Chr	MAF	Alleles	Chi Square	p-value	Model
IFNG1	rs2069728	66834051	12	.101	G > A	0.606	.739	A
IFNG1	rs2069727	66834490	12	.397	A > G	0.369	.831	A
IFNG1	rs2069718	66836429	12	.489	C > T	0.719	.698	A
IFNG1	rs1861493	66837463	12	.278	A > G	0.615	.735	A
IFNG1	rs1861494	66837676	12	.285	T > C	1.192	.551	A
IFNG1	rs2069709	66839970	12	.002	G > T	n/a	n/a	n/a
IFNG1	HapA3					0.685	.710	
IFNG1	HapA5					0.412	.814	
IFNGR1	rs9376268	137574444	6	.262	G > A	0.387	.824	A
IL1B	rs1071676	106042060	2	.189	G > C	2.856	.240	A
IL1B	rs1143643	106042929	2	.385	G > A	2.190	.335	A
IL1B	rs1143642	106043180	2	.080	C > T	0.918	.632	A
IL1B	rs1143634	106045017	2	.187	C > T	2.776	.250	A
IL1B	rs1143633	106045094	2	.393	G > A	2.876	.238	A
IL1B	rs1143630	106046282	2	.110	C > A	0.332	.847	A
IL1B	rs3917356	106046990	2	.457	G > A	0.622	.733	A
IL1B	rs1143629	106048145	2	.380	T > C	2.479	.290	A
IL1B	rs1143627	106049014	2	.386	T > C	3.397	.183	A
IL1B	rs16944	106049494	2	.380	G > A	4.658	.097	A
IL1B	rs1143623	106050452	2	.278	G > C	1.003	.606	A
IL1B	rs13032029	106055022	2	.455	C > T	0.590	.745	A
IL1B	HapA1					3.917	.141	
IL1B	HapA4					2.127	.345	
IL1B	HapA6					2.964	.227	
IL1B	HapB1					8.064	.018	
IL1B	HapB6					1.013	.602	
IL1B	HapB8					1.053	.591	
IL1R1	rs949963	96533648	2	.211	G > A	7.695	.021	A
IL1R1	rs2228139	96545511	2	.054	C > G	0.391	.823	A
IL1R1	rs3917320	96556738	2	.048	A > C	n/a	n/a	n/a
IL1R1	rs2110726	96558145	2	.336	C > T	1.720	.423	A
IL1R1	rs3917332	96560387	2	.184	A > T	2.612	.271	A
IL1R1	HapA1					1.827	.401	
IL1R1	HapA2					2.792	.248	
IL1R1	HapA3					2.683	.261	
IL1R2	rs4141134	96370336	2	.378	T > C	2.388	.303	A
IL1R2	rs11674595	96374804	2	.254	T > C	4.848	.089	A
IL1R2	rs7570441	96380807	2	.411	G > A	2.978	.226	A
IL1R2	HapA1					2.406	.300	
IL1R2	HapA2					2.292	.318	
IL1R2	HapA4					4.803	.091	
IL2	rs1479923	119096993	4	.302	C > T	0.540	.763	A
IL2	rs2069776	119098582	4	.264	T > C	1.245	.536	A
IL2	rs2069772	119099739	4	.247	A > G	0.251	.882	A
IL2	rs2069777	119103043	4	.053	C > T	0.747	.688	A
IL2	rs2069763	119104088	4	.275	T > G	0.770	.680	A
IL2	HapA1					1.805	.406	
IL2	HapA2					0.245	.885	
IL2	HapA3					1.980	.372	
IL4	rs2243248	127200946	5	.087	T > G	1.061	.588	A
IL4	rs2243250	127201455	5	.244	C > T	n/a	n/a	n/a
IL4	rs2070874	127202011	5	.224	C > T	n/a	n/a	n/a
IL4	rs2227284	127205027	5	.366	C > A	FE	.010	R
IL4	rs2227282	127205481	5	.368	C > G	n/a	n/a	n/a
IL4	rs2243263	127205601	5	.127	C > G	3.268	.195	A
IL4	rs2243266	127206091	5	.216	G > A	n/a	n/a	n/a
IL4	rs2243267	127206188	5	.217	G > C	n/a	n/a	n/a
IL4	rs2243274	127207134	5	.239	G > A	n/a	n/a	n/a
IL6	rs4719714	22643793	7	.252	A > T	0.190	.910	A
IL6	rs2069827	22648536	7	.071	G > T	0.771	.680	A
IL6	rs1800796	22649326	7	.123	C > G	n/a	n/a	n/a

TABLE 1. (CONTINUED)

Gene	SNP	Position	Chr	MAF	Alleles	Chi Square	p-value	Model
IL6	rs1800795	22649725	7	.316	C > G	0.357	.837	A
IL6	rs2069835	22650951	7	.061	T > C	n/a	n/a	n/a
IL6	rs2066992	22651329	7	.124	G > T	FE	.023	D
IL6	rs2069840	22651652	7	.323	C > G	0.585	.746	A
IL6	rs1554606	22651787	7	.343	G > T	2.265	.322	A
IL6	rs2069845	22653229	7	.343	A > G	1.893	.388	A
IL6	rs2069849	22654236	7	.021	C > T	n/a	n/a	n/a
IL6	rs2069861	22654734	7	.072	C > T	1.140	.566	A
IL6	rs35610689	22656903	7	.254	A > G	4.146	.126	A
IL6	HapA1					0.158	.924	
IL6	HapA2					0.285	.867	
IL6	HapB1					7.655	.022	
IL6	HapB2					4.402	.111	
IL6	HapB6					1.555	.460	
IL8	rs4073	70417508	4	.450	T > A	2.672	.263	A
IL8	rs2227306	70418539	4	.371	C > T	2.868	.238	A
IL8	rs2227543	70419394	4	.375	C > T	2.117	.347	A
IL8	HapA1					3.305	.192	
IL8	HapA4					2.564	.278	
IL10	rs3024505	177638230	1	.129	C > T	2.112	.348	A
IL10	rs3024498	177639855	1	.210	A > G	2.556	.279	A
IL10	rs3024496	177640190	1	.413	T > C	0.778	.678	A
IL10	rs1878672	177642039	1	.412	G > C	0.460	.795	A
IL10	rs3024492	177642438	1	.199	T > A	2.986	.225	A
IL10	rs1518111	177642971	1	.299	G > A	FE	.014	D
IL10	rs1518110	177643187	1	.296	G > T	FE	.010	D
IL10	rs3024491	177643372	1	.403	G > T	1.190	.552	A
IL10	HapA1					7.517	.023	
IL10	HapA2					4.372	.112	
IL10	HapA9					3.360	.186	
IL13	rs1881457	127184713	5	.229	A > C	1.229	.541	A
IL13	rs1800925	127185113	5	.243	C > T	1.163	.559	A
IL13	rs2069743	127185579	5	.017	A > G	n/a	n/a	n/a
IL13	rs1295686	127188147	5	.259	G > A	0.654	.721	A
IL13	rs20541	127188268	5	.213	C > T	1.273	.529	A
IL13	HapA1					0.572	.751	
IL13	HapA4					1.067	.586	
IL17A	rs4711998	51881422	6	.337	G > A	3.022	.221	A
IL17A	rs8193036	51881562	6	.321	T > C	0.625	.732	A
IL17A	rs3819024	51881855	6	.366	A > G	0.613	.736	A
IL17A	rs2275913	51882102	6	.359	G > A	0.443	.801	A
IL17A	rs3804513	51884266	6	.019	A > T	n/a	n/a	n/a
IL17A	rs7747909	51885318	6	.215	G > A	0.013	.994	A
NFKB1	rs3774933	103645369	4	.416	T > C	0.568	.753	A
NFKB1	rs170731	103667933	4	.362	A > T	0.480	.786	A
NFKB1	rs17032779	103685279	4	.009	T > C	n/a	n/a	n/a
NFKB1	rs230510	103695201	4	.409	T > A	1.640	.441	A
NFKB1	rs230494	103706005	4	.434	A > G	0.043	.979	A
NFKB1	rs4648016	103708706	4	.007	C > T	n/a	n/a	n/a
NFKB1	rs4648018	103709236	4	.015	G > C	n/a	n/a	n/a
NFKB1	rs3774956	103727564	4	.437	C > T	0.023	.989	A
NFKB1	rs10489114	103730426	4	.015	A > G	n/a	n/a	n/a
NFKB1	rs4648068	103737343	4	.359	A > G	1.605	.448	A
NFKB1	rs4648095	103746914	4	.052	T > C	FE	.853	A
NFKB1	rs4648110	103752867	4	.175	T > A	0.334	.846	A
NFKB1	rs4648135	103755716	4	.061	A > G	FE	.605	A
NFKB1	rs4648141	103755947	4	.174	G > A	1.474	.478	A
NFKB1	rs1609798	103756488	4	.339	C > T	1.789	.409	A
NFKB1	HapA1					1.435	.488	
NFKB1	HapA4					0.934	.627	
NFKB1	HapA9					0.248	.883	
NFKB2	rs12772374	104146901	10	.170	A > G	0.972	.615	A

Gene	SNP	Position	Chr	MAF	Alleles	Chi Square	p-value	Model
NFKB2	rs7897947	104147701	10	.215	T > G	0.872	.647	A
NFKB2	rs11574849	104149686	10	.064	G > A	1.036	.596	A
NFKB2	rs1056890	104152760	10	.311	C > T	FE	.049	R
NFKB2	HapA1					1.759	.415	
NFKB2	HapA2					0.899	.638	
NFKB2	HapA3					0.723	.697	
TNFA	rs2857602	31533378	6	.361	T > C	0.223	.894	A
TNFA	rs1800683	31540071	6	.377	G > A	1.527	.466	A
TNFA	rs2239704	31540141	6	.356	G > T	0.175	.916	A
TNFA	rs2229094	31540556	6	.273	T > C	0.452	.798	A
TNFA	rs1041981	31540784	6	.371	C > A	1.116	.572	A
TNFA	rs1799964	31542308	6	.220	T > C	0.271	.873	A
TNFA	rs1800750	31542963	6	.016	G > A	n/a	n/a	n/a
TNFA	rs1800629	31543031	6	.146	G > A	0.121	.941	A
TNFA	rs1800610	31543827	6	.103	C > T	3.613	.164	A
TNFA	rs3093662	31544189	6	.071	A > G	3.566	.168	A
TNFA	HapA1					1.579	.664	
TNFA	HapA6					0.683	.711	
TNFA	HapA8					2.767	.251	

A, additive model; Chr, chromosome; D, dominant model; Hap, haplotype; IFNG, interferon gamma; IL, interleukin; MAF, minor allele frequency; n/a, not assayed because SNP violated Hardy-Weinberg expectations ($p < 0.001$) or because MAF was $< .05$; NFKB, nuclear factor kappa beta; R, recessive model; SNP, single nucleotide polymorphism; TNFA, tumor necrosis factor alpha.

Helix, Bozeman, MT). Briefly, the number of principal components (PCs) was sought which distinguished the major racial/ethnic groups in the sample by visual inspection of scatter plots of orthogonal PCs (i.e., PC 1 vs. PC2, PC2 vs. PC3). This procedure was repeated until no discernible clustering of patients by their self-reported race/ethnicity was possible (data not shown). One hundred and six AIMs were included in the analysis. The first three PCs were selected to adjust for potential confounding due to population substructure (i.e., race/ethnicity) by including the three 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, by maximizing the significance of the p value, was selected for each SNP. Logistic regression analysis that controlled for significant covariates, as well as genomic estimates of 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 a parsimonious model. Genetic model fit and both unadjusted and covariate-adjusted odds ratios were estimated using STATA version 9.[39]

As was done in our previous studies,[17,40,41] based on recommendations in the literature,[42,43] the implementation of rigorous quality controls for genomic data, the non-independence 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 passing quality control criteria in Table 1 to allow for subsequent comparisons and meta-analyses.

Results

Differences in demographic and clinical characteristics

As shown in Table 2, no differences were found between patients with and without LE for the majority of the demographic and clinical characteristics.[12] In the bivariate analyses, patients with LE had a significantly higher BMI and a lower KPS score, and were more likely to report lung disease. In addition, patients with LE had a higher number of lymph nodes removed, a higher number of positive nodes, more advanced disease at the time of diagnosis, were less likely to have had a SLNB, were more likely to have had an axillary lymph node dissection (ALND), had received chemotherapy (CTX) prior to or following surgery, and had received radiation therapy (RT) following surgery.

Candidate gene analyses for the development of LE

As summarized in Table 1, no associations with the occurrence of LE were found in the SNPs evaluated for INFG1, INFGR1, IL1R2, IL2, IL8, IL13, IL17, NFKB1, and TNFA. However, the genotype frequency was significantly different between those who did and did not develop LE for six SNPs and three haplotypes spanning six genes (i.e., IL1B, IL1R1, IL4, IL6, IL10, and NFKB2). One haplotype (HapB1, $p = 0.018$) was identified for IL1B. For the SNP in IL1R1 (rs949963), an additive model fit the data best ($p = 0.021$). For the SNP in IL4 (rs2227284), a recessive model fit the data best ($p = 0.010$). One SNP (rs2066992) and 1 haplotype (HapB1, $p = 0.022$) were identified in IL6. For rs2066992, a dominant model fit the data best ($p = 0.023$). Two SNPs (rs1518111, rs1518110) and 1 haplotype (HapA1, $p = 0.023$) were identified in IL10. For both

SNPs, a dominant model fit the data best ($p=0.014$, 0.010, respectively). For the SNP in NFKB2 (rs1056890), a recessive model fit the data best ($p=0.049$).

Regression analyses of IL1B, IL4, IL6, IL10, and NFKB2 genotypes and haplotypes and the development of LE

In order to better estimate the magnitude (i.e., odds ratio, OR) and precision (95% confidence interval, CI) of genotype on the development of LE, multivariate logistic regression models were fit. As shown in Table 3, in addition to genotype, the phenotypic characteristics included in the regression models were ethnicity (i.e., White, Black, Asian, Hispanic/ Mixed ethnic background/Other), BMI, stage of disease, having a SLNB, and number of lymph nodes removed. Receipt of CTX and RT, while not significant after the inclusion of genomic estimates of and self-reported race/ethnicity,[12] were retained in all of the regression models for face validity.

The only genetic associations that remained significant in the multivariate logistic regression analyses were for IL4 rs2227284, IL10 rs1518111, IL10 rs1518110, and NFKB2 rs1056890 (Table 3 and Fig. 1). In the regression analysis for IL4 rs2227284, carrying two doses of the rare allele (i.e., CC+CA vs. AA) was associated with a 69% decrease in the odds of developing LE (Figure 1A). In the regression analysis for IL10 rs1518111, carrying one or two doses of the rare allele (i.e., GG vs. GA+AA) was associated with 51% decrease in the odds of developing LE (Fig. 1B). The analyses for the second SNP in IL10, namely rs1518110, revealed that it is a perfect surrogate for IL10 rs1518111. IL10 rs1518111 was selected to represent the two surrogate SNPs. In the regression analysis for NFKB2 rs1056890, carrying two doses of the rare allele (i.e., CC+CT vs. TT) was associated with a 3.06-fold increase in the odds of developing LE (Fig. 1C).

Discussion

This study is the first to evaluate for variations in pro- and anti-inflammatory cytokine genes and the development of LE following breast cancer treatment. In brief, in the bivariate analyses (Table 2), the phenotypic predictors of LE included: a higher BMI, lower KPS score, having lung disease, increased number of lymph nodes removed, increased number of positive lymph nodes, a higher stage of disease at the time of diagnosis, not having a SLND, having an ALND, and receiving CTX or RT. However, in the multivariate analysis (Table 3), KPS score, having lung disease, number of positive nodes removed, and having an ALND were not retained in the final model (Table 3). In addition, when genomic estimates of and self-reported race/ethnicity were included in the multivariate logistic regression analysis,[12] neither receipt of CTX nor receipt of RT remained significant predictors of LE.

The complex molecular pathways that underlie the development of LE following breast cancer treatment are being uncovered. In our previous study,[12] variations in seven genes that play a role in lymphatic development and angiogenesis were associated with the development of LE. In this study, we extend this work and evaluated for variations in pro- and anti-inflammatory cytokine genes and their association with the development of LE.

Consistent with preclinical and clinical studies that identified a role for IL4 in the molecular pathway of LE development,[14,15] patients who were homozygous for the rare allele in IL4 rs2227284 had a 69% decrease in the odds of developing LE. IL4 is a multifunctional cytokine that is known to induce T-helper 2 (Th2) cell immune responses in asthma and scleroderma. IL4 plays a regulatory role in apoptosis and cell proliferation, as well as in the expression of numerous genes in macrophages, lymphocytes, fibroblasts, endothelial cells, and epithelial cells.[14,15] In addition, IL4 has the ability to activate macrophages differentially into M2 macrophages rather than M1 macrophages. M2 macrophages function in tissue repair, fibrosis, and the regulation of inflammation. A subset of M2 macrophages produce the chemokine CCL18, that has both direct effects on fibroblasts and indirect effects on T cells that result in fibrotic inflammatory diseases, including hypersensitivity pneumonitis and idiopathic pulmonary fibrosis.[44] In addition, IL4 activated M2 macrophages increase the production of transforming growth factor beta (TGF-β), a tissue activator that leads to fibroblast production and collagen synthesis.[45] One can hypothesize that dysregulation in the production of IL4 could lead to the development of soft tissue fibrosis and lymphatic dysfunction associated with LE. This hypothesis is supported by a recent preclinical study that demonstrated that inhibition of Th2 differentiation using IL4 prevented the initiation and progression of LE by decreasing tissue fibrosis and increasing lymphatic function.[14]

IL4 rs2227284 is located in the intronic region of chromosome 5 in a region of the gene that undergoes DNA methylation. While no studies were identified that evaluated for an association between this SNP and the development of LE, in one study of Japanese women, individuals who were homozygous for the rare allele had a decreased risk for the development of rhinoconjunctivitis.[46] In another study of Chinese children who were vaccinated for hepatitis B, the rare allele was associated with a poor humoral response to the vaccine.[47] Taken together, these findings suggest that rs2227284 or a SNP(s) in linkage disequilibrium with rs2227284 may modulate a variety of inflammatory and immune responses. Additional research is warranted to confirm these findings in an independent cohort of breast cancer patients with LE.

In our study, patients who were heterozygous or homozygous for the rare allele in IL10 rs1518111 had a 51% decrease in the odds of developing LE. IL10 rs1518111 is located in the intronic region of chromosome 1 in a region that undergoes DNA methylation. In addition, this SNP is known to influence active transcription factor binding sites (i.e., PU-box binding protein (PU.1), RNA polymerase (Pol2)). The influence of IL10 rs1518111 on PU.1 binding is of particular interest because PU.1 plays a role in lymphangiogensis.[48] In addition, this SNP was associated with ischemic stroke,[49] benign prostate hyperplasia,[50] and Behcet's disease (i.e., a chronic vasculitis that affects the skin, joints, lungs, and central nervous system[51]). Findings from these studies suggest that variations in the expression of IL10 may result in increased inflammation and contribute to these diseases. In addition, in a sample of healthy controls who were homozygous for the rare allele in IL10 rs1518111, mRNA expression and protein production of IL10 were decreased.[52]

Recent evidence has implicated IL10 in the development of LE.[15] In addition to its anti-inflammatory effects, Shi et al.[53] demonstrated, using human dermal fibroblasts, that IL10 has

TABLE 2. DIFFERENCES IN DEMOGRAPHIC AND CLINICAL CHARACTERISTICS BETWEEN
PATIENTS WITH (N=155) AND WITHOUT (N=387) LYMPHEDEMA

Characteristic	No lymphedema mean (SD)	Lymphedema mean (SD)	Statistics
Age (years)	54.9 (11.1)	56.2 (10.8)	NS
Education (years)	16.0 (2.7)	15.8 (2.8)	NS
Age at menopause (years)	47.8 (7.2)	46.7 (9.1)	NS
Body mass index (kg/m^2)	26.1 (5.6)	28.2 (6.7)	$P=0.001$
Karnofsky Performance Status score	93.3 (9.7)	91.1 (11.1)	$P=0.028$
Comorbidity score	4.0 (2.9)	4.5 (3.3)	NS
Number of nodes removed	5.8 (6.3)	10.9 (9.0)	$P<0.0001$
Number of positive nodes	0.7 (1.7)	1.7 (3.4)	$P=0.009$
	% (n)	% (n)	
Ethnicity			
White	68.8 (265)	72.9 (113)	
Black	7.5 (29)	9.7 (15)	NS
Asian/Pacific Islander	13.0 (50)	7.1 (11)	
Hispanic/Mixed ethnic background/Other	10.6 (41)	10.3 (16)	
Lives alone			
Yes	23.0 (88)	28.9 (44)	NS
No	77.0 (295)	71.1 (108)	
Married/partnered			
Yes	47.4 (182)	52.0 (79)	NS
No	52.6 (202)	48.0 (73)	
Employed			
Yes	51.4 (197)	49.7 (76)	NS
No	48.6 (186)	50.3 (77)	
Handedness			
Right	88.8 (341)	88.9 (136)	
Left	8.1 (31)	9.2 (14)	NS
Both	3.1 (12)	2.0 (3)	
Occurrence of comorbid conditions (% and number of women who reported each co-morbid condition from the Self-Administered Comorbidity Questionnaire)			
Heart disease	5.6 (21)	6.0 (9)	NS
High blood pressure	27.0 (103)	34.9 (53)	NS
Lung disease	3.7 (14)	8.1 (12)	$p=0.04$
Diabetes	6.6 (25)	7.4 (11)	NS
Ulcer	3.7 (14)	4.7 (7)	NS
Kidney disease	1.6 (6)	2.0 (3)	NS
Liver disease	2.1 (8)	4.8 (7)	NS
Anemia	7.2 (27)	9.5 (14)	NS
Depression	21.8 (81)	26.7 (39)	NS
Osteoarthritis	19.2 (72)	26.7 (40)	NS
Back pain	29.3 (110)	31.5 (47)	NS
Rheumatoid arthritis	3.5 (13)	4.7 (7)	NS
Diagnosed with mastitis			
Yes	13.1 (50)	11.3 (17)	NS
No	86.9 (332)	88.7 (134)	
Diagnosed with cystic breast disease			
Yes	21.5 (81)	23.3 (34)	NS
No	78.5 (295)	76.7 (112)	
Breastfed			
Yes	49.4 (190)	45.1 (69)	NS
No	50.6 (195)	54.9 (84)	
Surgery on affected breast not related to cancer			
Yes	9.3 (36)	14.8 (23)	NS
No	90.7 (351)	85.2 (132)	
Surgery to the affected arm not related to cancer			
Yes	3.1 (12)	5.2 (8)	NS
No	96.9 (375)	94.8 (147)	

TABLE 2. (CONTINUED)

Characteristic	No lymphedema mean (SD)	Lymphedema mean (SD)	Statistics
Surgery on the affected hand not related to cancer			
Yes	5.2 (20)	7.1 (11)	NS
No	94.8 (367)	92.9 (144)	
Injury to the affected arm			
Yes	17.3 (67)	22.6 (35)	NS
No	82.7 (320)	77.4 (120)	
Injury to the affected hand			
Yes	17.1 (66)	17.4 (27)	NS
No	82.9 (321)	82.6 (128)	
Side of cancer surgery			
Dominant	49.9 (193)	41.9 (65)	NS
Nondominant	50.1 (194)	58.1 (90)	
Type of surgery			
Breast conservation	75.2 (291)	70.3 (109)	NS
Mastectomy	24.8 (96)	29.7 (46)	
Stage of disease			
Stage 0	18.1 (70)	5.2 (8)	
Stage I	40.1 (155)	32.9 (51)	$p < 0.0001$
Stage IIA and IIB	35.4 (137)	48.4 (75)	
Stage IIIA, IIIB, IIIC, and IV	6.5 (25)	13.5 (21)	
Sentinel lymph node biopsy			
Yes	80.9 (313)	69.7 (108)	$p = 0.006$
No	19.1 (74)	30.3 (47)	
Axillary lymph node dissection			
Yes	39.3 (152)	69.3 (106)	$p < 0.0001$
No	60.7 (235)	30.7 (47)	
Reconstruction at the time of surgery			
Yes	21.6 (68)	22.2 (18)	NS
No	78.4 (247)	77.8 (63)	
Adjuvant chemotherapy			
Yes	36.7 (142)	59.7 (92)	$p < 0.0001$
No	63.3 (245)	40.3 (62)	
Adjuvant radiation therapy			
Yes	57.1 (221)	71.0 (110)	$p < 0.0001$
No	42.9 (166)	29.0 (45)	
Combinations of treatments			
Only surgery	23.8 (92)	8.4 (13)	
Surgery and radiation therapy	39.5 (153)	32.3 (50)	$p < 0.0001$
Surgery and chemotherapy	19.1 (74)	20.6 (32)	
Surgery, radiation therapy, and chemotherapy	17.6 (68)	38.7 (60)	
Exercise on a regular basis			
Yes	73.7 (283)	75.2 (115)	NS
No	26.3 (101)	24.8 (38)	

kg, kilograms; m^2, meter squared; NS, not significant; SD, standard deviation. See Ref. 12.

anti-fibrotic properties and can inhibit excessive deposition of collagen and the transformation of fibroblasts to myofibroblasts. In addition, polymorphisms in several candidate genes in IL10 and the IL10 receptor, which were not evaluated in this study, were associated with the development of LE following infection with filarial parasites.

Patients who were homozygous for the rare allele in NFKB2 rs1056890 had a 3.1-fold increase in the odds of developing LE. NF-κB transcription factors play a role in diverse cellular processes including the regulation of angiogenesis, metastasis, cell proliferation, tumor promotion, suppression

of apoptosis, and inflammation.[54] The NF-κB signaling pathway leads to the transcription of pro-inflammatory molecules, such as cytokines and chemokines. Alterations in NF-κB regulation are linked to diseases of chronic inflammation (e.g., Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus). NF-κB2 (p52 and its precursor p100) is one of two subunits that contributes to dimeric NF-κB and is responsible for activating the non-canonical pathway of NF-κB.[55] NF-κB2 functions within an autoregulatory loop in which the precursor protein p100 is processed to become the active NF-κB2 subunit known as p52, which can upregulate

Table 3. Multiple Logistic Regression Analyses for IL4, IL10, and NFKB2 Genotypes
to Predict the Development of Lymphedema

Predictor	Odds Ratio	Standard Error	95% CI	Z	P value
IL4 genotype	0.31	0.156	0.119, 0.829	−2.34	0.019
BMI	1.06	0.022	1.014, 1.102	2.61	0.009
Stage of disease					
Stage 0 versus I	3.22	1.927	0.996, 10.404	1.95	0.051
Stage 0 versus II	4.27	2.714	1.229, 14.838	2.28	0.022
Stage 0 versus III and IV	6.38	4.714	1.500, 27.145	2.51	0.012
SLNB	0.41	0.140	0.206, 0.796	−2.62	0.009
Number of nodes removed	1.09	0.022	1.047, 1.132	4.24	<0.0001
Any chemotherapy	1.11	0.344	0.604, 2.038	0.33	0.738
Any radiation therapy	1.23	0.366	0.685, 2.204	0.69	0.489
Overall model fit: $\chi^2 = 83.69$, p<0.0001, $R^2 = 0.1865$					
IL10 genotype	0.49	0.139	0.282, 0.857	−2.51	0.012
BMI	1.05	0.022	1.012, 1.099	2.53	0.011
Stage of disease					
Stage 0 versus I	2.64	1.553	0.836, 8.359	1.65	0.098
Stage 0 versus II	3.25	2.027	0.954, 11.039	1.88	0.059
Stage 0 versus III and IV	5.78	4.227	1.378, 24.234	2.40	0.016
SLNB	0.40	0.138	0.204, 0.786	−2.66	0.008
Number of nodes removed	1.08	0.022	1.043, 1.128	4.07	<0.0001
Any chemotherapy	1.27	0.399	0.687, 2.354	0.77	0.444
Any radiation therapy	1.41	0.422	0.781, 2.531	1.14	0.256
Overall model fit: $\chi^2 = 84.06$, p<0.0001, $R^2 = 0.1876$					
NFKB2 genotype	3.06	1.338	1.299, 7.209	2.56	0.011
BMI	1.06	0.022	1.015, 1.103	2.69	0.007
Stage of disease					
Stage 0 versus I	2.91	1.725	0.912, 9.301	1.80	0.071
Stage 0 versus II	3.81	2.406	1.108, 13.135	2.12	0.034
Stage 0 versus III and IV	6.23	4.570	1.479, 26.233	2.49	0.013
SLNB	0.40	0.137	0.203, 0.783	−2.67	0.008
Number of nodes removed	1.08	0.021	1.043, 1.126	4.08	<0.0001
Any chemotherapy	1.15	0.361	0.624, 2.129	0.45	0.650
Any radiation therapy	1.36	0.406	0.755, 2.439	1.02	0.307
Overall model fit: $\chi^2 = 84.16$, p<0.0001, $R^2 = 0.1876$					

For each model, the first three principal components identified from the analysis of ancestry informative markers as well as self-report race/ethnicity (i.e., White, Black, Asian/Pacific Islander, Hispanic/Mixed ethnic background/Other) were retained in all models to adjust for potential confounding due to race or ethnicity (data not shown). Predictors evaluated in each model included genotype (IL4 rs2227284: CC+CA versus AA; IL10 rs1518111: GG versus GA+AA; NFKB2 rs1056890: CC+CT versus TT), BMI (kilograms/meter squared), 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; IL, interleukin; NFKB, nuclear factor kappa beta; SLNB, sentinel lymph node biopsy.

p100 expression. p100 can repress p52 activity, which acts as a negative feedback control loop.[56] This autoregulatory loop is tightly controlled.

In one study, Yang et al.[57] found that p52 transgenic mice that were deficient in the p100 precursor protein developed fatal lung inflammation characterized by diffuse alveolar damage with localized fibrosis. The lung tissue of the mice demonstrated high level induction of the Th1 cytokine IFN-γ and its inducible inflammatory chemokines, which are known to activate macrophages and result in a cycle of inflammatory processes and tissue damage. In addition, the transgenic mice displayed a significant increase in TNF-α which acts synergistically with IFN-γ to activate macrophages and regulate fibroblast proliferation and activation.

NFKB2 rs1056890 is located in the 3' UTR region of NFKB2 on chromosome 10. In one study of Chinese patients with multiple myeloma, who were treated bortezomib,[54] individuals who were heterozygous or homozygous for the rare

allele had an overall lower response rate and decreased survival. Although rs1056890 has no known function, its association with response to bortezomib treatment,[54] suggests that this SNP may be in LD with a unmeasured functional SNP(s).

Several limitations of this study need to be acknowledged. Although the sample size was relatively large, larger samples may reveal additional significant candidate gene associations. In addition, future studies need to confirm the functional effects of these polymorphisms.

Despite these limitations, the novel findings from this study suggest that genetic variations in pro- and anti- inflammatory cytokine genes may play a role in the development of secondary LE following breast cancer treatment. Although the pathophysiology of LE is complex and largely undetermined, the identified genetic associations may help with risk assessment and the development of targeted molecular therapy for this incurable condition.
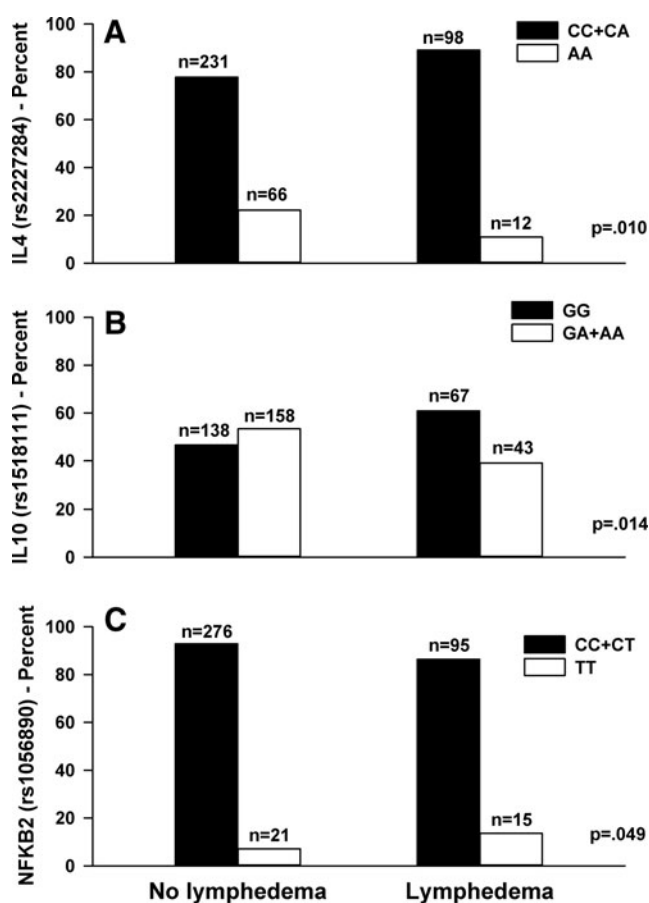
FIG. 1. (A) Differences in the percentages of patient with and without lymphedema who were homozygous or heterozygous for the common allele (CC+CA) or homozygous for the rare allele (AA) for rs2227284 in interleukin 4 (IL4). Values are plotted as unadjusted proportions with the corresponding *p* value. (B) Differences in the percentages of patients with and without lymphedema who were homozygous for the common allele (GG) or heterozygous or homozygous for the rare allele (GA+AA) for rs1518111 in IL13. Values are plotted as unadjusted proportions with the corresponding *p* value. (C) Differences in the percentages of patients with and without lymphedema who were homozygous or heterozygous for the common allele (CC+CT) or homozygous for the rare allele (TT) for rs1056890 in nuclear factor kappa beta 2 (NFKB2). Values are plotted as unadjusted proportions with the corresponding *p* value.

References

1. Paskett ED, Dean JA, Oliveri JM, Harrop JP. Cancer-related lymphedema risk factors, diagnosis, treatment, and impact: A review. J Clin Oncol 2012;30:3726–3733.
2. Shih YC, Xu Y, Cormier JN, et al. Incidence, treatment costs, and complications of lymphedema after breast cancer among women of working age: A 2-year follow-up study. J Clin Oncol 2009;27:2007–2014.
3. Cormier JN, Askew RL, Mungovan KS, Xing Y, Ross MI, Armer JM. Lymphedema beyond breast cancer: A systematic review and meta-analysis of cancer-related secondary lymphedema. Cancer 2010;116:5138–5149.
4. Hayes S, Di Sipio T, Rye S, et al. Prevalence and prognostic significance of secondary lymphedema following breast cancer. Lymphat Res Biol 2011;9:135–141.
5. Brorson H, Ohlin K, Olsson G, Karlsson MK. Breast cancer-related chronic arm lymphedema is associated with excess adipose and muscle tissue. Lymphat Res Biol 2009;7:3–10.
6. Clark B, Sitzia J, Harlow W. Incidence and risk of arm oedema following treatment for breast cancer: A three-year follow-up study. QJM. 2005;98:343–348.
7. Armer JM, Stewart BR. A comparison of four diagnostic criteria for lymphedema in a post-breast cancer population. Lymphat Res Biol 2005;3:208–217.
8. Bernas M. Assessment and risk reduction in lymphedema. Semin Oncol Nurs 2013;29:12–19.
9. Hayes SC, Johansson K, Stout NL, Prosnitz R, Armer JM, Gabram S, Schmitz KH. Upper-body morbidity after breast cancer: Incidence and evidence for evaluation, prevention, and management within a prospective surveillance model of care. Cancer 2012;118:2237–2249.
10. Armer JM. Research on risk assessment for secondary lymphedema following breast cancer treatment. Cancer Epidemiol Biomarkers Prev 2010;19:2715–2717.
11. Morcos B, Ahmad FA, Anabtawi I, Sba AM, Shabani H, Yaseen R. Development of breast cancer-related lymphedema: Is it dependent on the patient, the tumor or the treating physicians? Surg Today 2014;44:100–106.
12. Miaskowski C, Dodd M, Paul SM, et al. Lymphatic and angiogenic candidate genes predict the development of secondary lymphedema following breast cancer surgery. PLoS One 2013;8:e60164.
13. Jensen MR, Simonsen L, Karlsmark T, Bulow J. Microvascular filtration is increased in the forearms of patients with breast cancer-related lymphedema. J Appl Physiol 2013;114:19–27.
14. Avraham T, Zampell JC, Yan A, et al. Th2 differentiation is necessary for soft tissue fibrosis and lymphatic dysfunction resulting from lymphedema. FASEB J 2013;27:1114–1126.
15. Lin S, Kim J, Lee MJ, Roche L, Yang NL, Tsao PS, Rockson SG. Prospective transcriptomic pathway analysis of human lymphatic vascular insufficiency: Identification and validation of a circulating biomarker panel. PLoS One 2012;7:e52021.

16. Miaskowski C, Cooper B, Paul SM, et al. Identification of patient subgroups and risk factors for persistent breast pain following breast cancer surgery. J Pain 2012;13:1172–1187.

17. McCann B, Miaskowski C, Koetters T, et al. Associations between pro- and anti-inflammatory cytokine genes and breast pain in women prior to breast cancer surgery. J Pain 2012;13:425–437.

18. Van Onselen C, Cooper BA, Lee K, et al. Identification of distinct subgroups of breast cancer patients based on self-reported changes in sleep disturbance. Support Care Cancer 2012;20:2611–2619.

19. Karnofsky D. Performance Scale. Kennealey GT, Mitchell MS, editors. New York: Plenum Press; 1977.

20. Karnofsky D, Abelmann WH, Craver LV, Burchenal JH. The use of nitrogen mustards in the palliative treatment of carcinoma. Cancer 1948;1:634–656.

21. Sangha O, Stucki G, Liang MH, Fossel AH, Katz JN, The Self-Administered Comorbidity Questionnaire: A new method to assess comorbidity for clinical and health services research. Arthritis Rheum 2003;49:156–163.

22. MacLean CD, Littenberg B, Kennedy AG. Limitations of diabetes pharmacotherapy: results from the Vermont Diabetes Information System study. BMC Fam Pract 2006;7:50.

23. Cieza A, Geyh S, Chatterji S, Kostanjsek N, Ustun BT, Stucki G. Identification of candidate categories of the International Classification of Functioning Disability and Health (ICF) for a Generic ICF Core Set based on regression modelling. BMC Med Res Methodol 2006;6:36.

24. Smith SK, Zimmerman S, Williams CS, Zebrack BJ. Health status and quality of life among non-Hodgkin lymphoma survivors. Cancer 2009;115:3312–3323.

25. Brunner F, Bachmann LM, Weber U, Kessels AG, Perez RS, Marinus J, Kissling R. Complex regional pain syndrome 1–the Swiss cohort study. BMC Musculoskelet Disord 2008;9:92.

26. Hayes S, Cornish B, Newman B. Comparison of methods to diagnose lymphoedema among breast cancer survivors: 6-month follow-up. Breast Cancer Res Treat 2005;89:221–226.

27. Cornish BH, Chapman M, Hirst C, Mirolo B, Bunce IH, Ward LC, Thomas BJ. Early diagnosis of lymphedema using multiple frequency bioimpedance. Lymphology 2001; 34:2–11.

28. Cornish BH, Chapman M, Thomas BJ, Ward LC, Bunce IH, Hirst C. Early diagnosis of lymphedema in postsurgery breast cancer patients. Ann NY Acad Sci 2000;904:571–575.

29. SPSS. IBM SPSS for Windows (Version 19). Chicago, Illinois: SPSS, Inc.; 2010.

30. Verri WA Jr., Cunha TM, Parada CA, Poole S, Cunha FQ, Ferreira SH. Hypernociceptive role of cytokines and chemokines: Targets for analgesic drug development? Pharmacol Ther 2006;112:116–138.

31. Seruga B, Zhang H, Bernstein LJ, Tannock IF. Cytokines and their relationship to the symptoms and outcome of cancer. Nat Rev Cancer 2008;8:887–899.

32. Conde L, Vaquerizas JM, Dopazo H, Arbiza L, Reumers J, Rousseau F, Schymkowitz J, Dopazo J. PupaSuite: Finding functional single nucleotide polymorphisms for large-scale genotyping purposes. Nucleic Acids Res 2006;34:W621–625.

33. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. Science 2002;296: 2225–2229.

34. Stephens M, Smith NJ, Donnelly P, A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–989.

35. Halder I, Shriver M, Thomas M, Fernandez JR, Frudakis T. A panel of ancestry informative markers for estimating individual biogeographical ancestry and admixture from four continents: Utility and applications. Hum Mutat 2008;29: 648–658.

36. Hoggart CJ, Parra EJ, Shriver MD, Bonilla C, Kittles RA, Clayton DG, McKeigue PM. Control of confounding of genetic associations in stratified populations. Am J Hum Genet 2003;72:1492–1504.

37. Tian C, Gregersen PK, Seldin MF. Accounting for ancestry: Population substructure and genome-wide association studies. Hum Mol Genet 2008;17:R143–150.

38. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006;38:904–909.

39. StataCorp. Stata Statistical Software: Release 9. College Station, Texas: Stata Corporation; 2005.

40. Illi J, Miaskowski C, Cooper B, et al. Association between pro- and anti-inflammatory cytokine genes and a symptom cluster of pain, fatigue, sleep disturbance, and depression. Cytokine 2012;58:437–447.

41. Miaskowski C, Cooper BA, Dhruva A, Evidence of associations between cytokine genes and subjective reports of sleep disturbance in oncology patients and their family caregivers. PLoS One 2012;7:e40560.

42. Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology 1990;1:43–46.

43. Hattersley AT, McCarthy MI. What makes a good genetic association study? Lancet 2005;366:1315–1323.

44. Luzina IG, Keegan AD, Heller NM, Rook GA, Shea-Donohue T, Atamas SP. Regulation of inflammation by interleukin-4: A review of "alternatives". J Leukoc Biol 2012; 92:753–764.

45. Novak ML, Koh TJ. Macrophage phenotypes during tissue repair. J Leukoc Biol. 2013;93:875–881.

46. Miyake Y, Tanaka K, Arakawa M. Polymorphisms in the IL4 gene, smoking, and rhinoconjunctivitis in Japanese women: The Kyushu Okinawa Maternal and Child Health Study. Hum Immunol 2012;73:1046–1049.

47. Wang Y, Xu P, Zhu D, Zhang S, Bi Y, Hu Y, Zhou YH. Association of polymorphisms of cytokine and TLR-2 genes with long-term immunity to hepatitis B in children vaccinated early in life. Vaccine 2012;30:5708–5713.

48. Gordon EJ, Rao S, Pollard JW, Nutt SL, Lang RA, Harvey NL. Macrophages define dermal lymphatic vessel calibre during development by regulating lymphatic endothelial cell proliferation. Development 2010;137:3899–3910.

49. Park HK, Kim DH, Yun DH, Ban JY. Association between IL10, IL10RA, and IL10RB SNPs and ischemic stroke with hypertension in Korean population. Mol Biol Rep 2013;40: 1785–1790.

50. Yoo KH, Kim SK, Chung JH, Chang SG. Association of IL10, IL10RA, and IL10RB polymorphisms with benign prostate hyperplasia in Korean population. J Korean Med Sci 2011; 26:659–664.

51. Xavier JM, Shahram F, Davatchi F, et al. Association study of IL10 and IL23R-IL12RB2 in Iranian patients with Behcet's disease. Arthritis Rheum 2012;64:2761–2772.

52. Remmers EF, Cosan F, Kirino Y, et al. Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behcet's disease. Nat Genet 2010;42:698–0702.

53. Shi JH, Guan H, Shi S, et al. Protection against TGF-beta1-induced fibrosis effects of IL-10 on dermal fibroblasts and its potential therapeutics for the reduction of skin scarring. Arch Dermatol Res 2013;305:341–352.

54. Du J, Huo J, Shi J, et al. Polymorphisms of nuclear factor-kappaB family genes are associated with development of multiple myeloma and treatment outcome in patients receiving bortezomib-based regimens. Haematologica 2011;96:729–737.

55. Vu D, Tellez-Corrales E, Sakharkar P, Kissen MS, Shah T, Hutchinson I, Min DI. Impact of NF-kappaB gene polymorphism on allograft outcome in Hispanic renal transplant recipients. Transpl Immunol 2013;28:18–23.

56. Liptay S, Schmid RM, Nabel EG, Nabel GJ. Transcriptional regulation of NF-kappa B2: Evidence for kappa B-mediated positive and negative autoregulation. Mol Cell Biol 1994;14:7695–7703.

57. Yang L, Cui H, Wang Z, Zhang B, Ding J, Liu L, Ding HF. Loss of negative feedback control of nuclear factor-kappaB2 activity in lymphocytes leads to fatal lung inflammation. Am J Pathol 2010;176:2646–2657.

Address correspondence to:
Christine Miaskowski, RN, PhD, FAAN
Department of Physiological Nursing
University of California
2 Koret Way – N631Y
San Francisco, CA 94143-0610

E-mail: chris.miaskowski@nursing.ucsf.edu