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Original Article

Common sequence variants in chemokine-related genes and risk of breast cancer in post-menopausal women

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Abstract: Chemokines are small molecules that when secreted by tissues under pathological conditions such as inflammation are believed to be involved in carcinogenesis. Recent reports have found that the genetic variation in chemokine encoding genes are associated with risk of breast cancer. Methods: Using data from a population-based case-control study of 845 invasive breast cases and 807 controls, we genotyped 34 single nucleotide polymorphisms (SNPs) in 8 chemokine candidate genes (*CCL3, CCL4, CCL5, CCL20, CCR5, CCR6, CXCL12* and *CXCR4*). Associations with breast cancer were computed for individual SNPs, groups of SNPs within genes, and in a gene-set analysis. We also performed a meta-analysis of *CXCL12* rs1801157 and a haplotype analysis for two SNPs: *CXCR4* rs2228014 and *CXCL12* rs1801157. Results: We found no significant associations between the risk of breast cancer and any individual SNPs, single genes, or combined gene sets. Some individual variants were marginally associated with some histologic subtypes, but these associations were not significant after adjustment for multiple comparisons. In the meta-analysis of five studies of European ancestry, *CXCL12* rs1801157 was marginally associated with breast cancer risk (OR=1.14, 95% CI: 1.00, 1.30). Conclusions: Our findings suggest that genetic variants in the 8 candidate genes coding for chemotactic cytokines have little influence in the risk of breast cancer in postmenopausal women. Additional examination of the relationship between *CXCL12* rs1801157 and breast cancer risk is warranted.

Keywords: Breast cancer, chemokines, genetic variation, epidemiology

Introduction

It is well established that certain inflammatory processes, such as obesity [1] and diabetes [2], increase the risk of breast cancer in post-menopausal women. Chemokines are small chemotactic cytokines that are important for migration of cells, and under pathological conditions, such as inflammation, they contribute to leukocyte migration to the injured tissue [3]. Chemokines may regulate immune function against developing tumors [4]. Although many genome wide association studies (GWAS) have been performed in breast cancer, most platforms have not included single nucleotide polymorphism (SNP) rs1801157 located in the chemokine gene *CXCL12* (http://www.broadinstitute.org/mpg/snap/ldsearch.php), which has been associated with several cancers [5]. Therefore, the candidate gene approach provides information not accounted for in most GWAS.

The association between *CXCL12* rs1801157 and breast cancer has been inconsistent. While some studies found that *CXCL12* rs1801157 was associated with a significant increased risk of breast cancer [6, 7], others found no association [8-11]. However, all these studies were small (less than 300 cases each) and conduct-

Table 1. Association between common sequence variants in chemokine-related genes and breast cancer risk in postmenopausal women

				Controls		Cases				
Gene	Variant	Function	Genotype	N=807	(%)	N=845	(%)	OR*	(95% CI)*	*P*-value#
CXCR4										0.22
	rs2228014	Synonymous						0.70	(0.47-1.06)	
			CC	689	(92.24)	723	(94.39)			
			TC	56	(7.50)	43	(5.61)			
			TT	2	(0.27)	0	(0.00)			
	rs2680880	5'UTR						1.04	(0.91-1.19)	
			TT	259	(32.33)	267	(31.79)			
			AT	388	(48.44)	397	(47.26)			
			AA	154	(19.23)	176	(20.95)			
	rs2471859	Intron						0.88	(0.75-1.03)	
			AA	451	(56.30)	507	(60.36)			
			AG	300	(37.45)	285	(33.93)			
			GG	50	(6.24)	48	(5.71)			
CCL20										0.32
	rs6749704	Flanking 5'UTR						0.92	(0.79-1.07)	
			AA	385	(48.06)	430	(51.19)			
			AG	347	(43.32)	339	(40.36)			
			GG	69	(8.61)	71	(8.45)			
	rs940339	Flanking 3'UTR						1.09	(0.95-1.25)	
			AA	203	(25.37)	210	(25.00)			
			AG	415	(51.88)	405	(48.21)			
			GG	182	(22.75)	225	(26.79)			
CCR5										0.85
	rs11575816	Flanking 3'UTR						0.99	(0.86-1.14)	
			GG	318	(39.7)	340	(40.48)			
			AG	376	(46.94)	387	(46.07)			
			AA	107	(13.36)	113	(13.45)			
CCR6										0.57
	rs3093027	Flanking 5'UTR						1.22	(0.94-1.59)	
			AA	682	(85.14)	692	(82.48)			
			AC	112	(13.98)	144	(17.16)			
			CC	7	(0.87)	3	(0.36)			
	rs3093026	Flanking 5'UTR						0.92	(0.8-1.05)	
			GG	215	(26.84)	235	(28.01)			
			AG	388	(48.44)	422	(50.30)			
			AA	198	(24.72)	182	(21.69)			
	rs3093023	Flanking 5'UTR						1.04	(0.9-1.19)	
			GG	274	(34.21)	263	(31.31)			
			AG	368	(45.94)	420	(50.00)			
			AA	159	(19.85)	157	(18.69)			
	rs3798315	Intron						1.05	(0.85-1.3)	
			GG	639	(79.78)	664	(79.05)			
			AG	150	(18.73)	162	(19.29)			
			AA	12	(1.50)	14	(1.67)			
	rs3093012	Intron						1.01	(0.88-1.16)	
			GG	271	(33.83)	279	(33.21)			
			AG	390	(48.69)	415	(49.40)			
			AA	140	(17.48)	146	(17.38)			

	rs3093010	Intron						1.00	(0.86-1.15)	
			CC	365	(45.57)	375	(44.64)			
			AC	336	(41.95)	369	(43.93)			
			AA	100	(12.48)	96	(11.43)			
	rs3093007	Synonymous						0.93	(0.78-1.11)	
			AA	534	(66.67)	577	(68.69)			
			AG	239	(29.84)	234	(27.86)			
			GG	28	(3.50)	29	(3.45)			
	rs3093006	3'UTR						1.06	(0.87-1.3)	
			GG	608	(75.91)	625	(74.40)			
			AG	179	(22.35)	201	(23.93)			
			AA	14	(1.75)	14	(1.67)			
	rs3093005	3'UTR						1.04	(0.64-1.7)	
			AA	768	(96.00)	805	(95.83)			
			AG	32	(4.00)	35	(4.17)			
			GG	0	(0.00)	0	(0.00)			
	rs11575083	3'UTR						0.99	(0.58-1.67)	
			GG	773	(96.50)	811	(96.55)			
			AG	28	(3.50)	29	(3.45)			
			AA	0	(0.00)	0	(0.00)			
	rs3093002	Flanking 3'UTR						0.90	(0.78-1.05)	
			GG	355	(44.38)	395	(47.08)			
			AG	371	(46.38)	378	(45.05)			
			AA	74	(9.25)	66	(7.87)			
	rs3093001	Flanking 3'UTR						1.13	(0.98-1.29)	
			AA	208	(26.10)	194	(23.23)			
			AC	399	(50.06)	416	(49.82)			
			CC	190	(23.84)	225	(26.95)			
	rs367523	Flanking 3'UTR						0.92	(0.8-1.06)	
			CC	303	(37.83)	334	(39.76)			
			CG	392	(48.94)	410	(48.81)			
			GG	106	(13.23)	96	(11.43)			
	rs4710189	Flanking 3'UTR						0.92	(0.8-1.07)	
			AA	340	(42.45)	377	(44.88)			
			AG	374	(46.69)	380	(45.24)			
			GG	87	(10.86)	83	(9.88)			
CXCL12										0.68
	rs266093	3'UTR						1.07	(0.93-1.24)	
			GG	299	(40.46)	304	(39.79)			
			GC	338	(45.74)	333	(43.59)			
			CC	102	(13.8)	127	(16.62)			
	rs1029153	3'UTR						0.91	(0.79-1.05)	
			AA	391	(48.81)	427	(50.89)			
			AG	322	(40.20)	337	(40.17)			
			GG	88	(10.99)	75	(8.94)			
	rs1801157	3'UTR						1.04	(0.87-1.23)	
			GG	515	(64.29)	523	(62.26)			
			AG	251	(31.34)	288	(34.29)			
			AA	35	(4.37)	29	(3.45)			
	rs266087	Intron						0.93	(0.81-1.08)	
			GG	325	(40.68)	360	(42.91)			
			AG	371	(46.43)	378	(45.05)			

			AA	103	(12.89)	101	(12.04)		
	rs2839695	3'UTR						1.07	(0.91-1.27)
			AA	504	(62.92)	528	(62.86)		
			AG	270	(33.71)	264	(31.43)		
			GG	27	(3.37)	48	(5.71)		
	rs2236534	Intron						0.98	(0.83-1.16)
			CC	493	(61.55)	530	(63.10)		
			AC	276	(34.46)	268	(31.90)		
			AA	32	(4.00)	42	(5.00)		
	rs3780891	Intron						0.90	(0.71-1.13)
			GG	647	(80.77)	692	(82.38)		
			AG	144	(17.98)	140	(16.67)		
			AA	10	(1.25)	8	(0.95)		
CCL5									0.62
	rs2107538	Flanking 5'UTR						0.95	(0.8-1.14)
			GG	541	(67.54)	590	(70.24)		
			AG	240	(29.96)	218	(25.95)		
			AA	20	(2.50)	32	(3.81)		
CCL3									0.93
	rs9972960	Flanking 5'UTR						1.00	(0.87-1.16)
			GG	307	(38.33)	330	(39.29)		
			AG	395	(49.31)	398	(47.38)		
			AA	99	(12.36)	112	(13.33)		
	rs1634502	Flanking 5'UTR						0.98	(0.85-1.12)
			AA	286	(35.75)	294	(35.00)		
			AT	372	(46.50)	412	(49.05)		
			TT	142	(17.75)	134	(15.95)		
CCL4									0.92
	rs10491121	Flanking 5'UTR						0.97	(0.85-1.11)
			GG	288	(35.96)	298	(35.48)		
			AG	368	(45.94)	406	(48.33)		
			AA	145	(18.10)	136	(16.19)		
	rs1634517	Intron						1.05	(0.89-1.24)
			CC	472	(59.97)	492	(59.78)		
			AC	282	(35.83)	284	(34.51)		
			AA	33	(4.19)	47	(5.71)		
	rs17679451	Flanking 3'UTR						1.03	(0.72-1.47)
			GG	736	(91.89)	770	(91.67)		
			AG	63	(7.87)	68	(8.10)		
			AA	2	(0.25)	2	(0.24)		
	rs1619526	Flanking 3'UTR						1.05	(0.89-1.24)
			GG	472	(58.93)	492	(58.57)		
			AG	292	(36.45)	297	(35.36)		
			AA	37	(4.62)	51	(6.07)		

*Odds ratios (ORs) and 95% confidence intervals (CI) are computed using a multiplicative model (additive on log scale). If genotype counts were less than 5 for any of the three cells, a dominant model was used. ORs and 95% CI are age adjusted (age as a continuous linear variable). #*P*-value adjusted for multiple comparisons within a gene using a P-min permutation test.

ed in different populations (Greece, Iran, China, Brazil and Poland). A meta-analysis using data from most of the above studies reported a significant association [12]. In addition, Lin *et al.* found a reduced breast cancer risk with the combination of *CXCL12* rs1801157 and other SNPs including *CXCR4* rs2228014 [9].

Using data from a population-based case-control study, we performed a comprehensive anal-

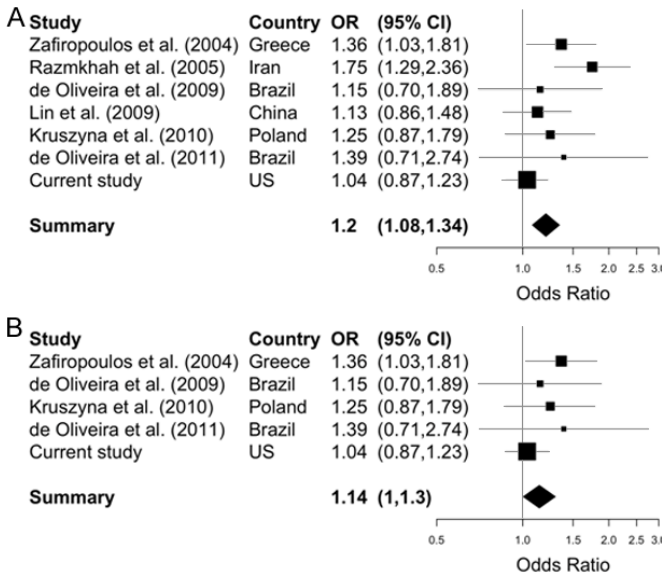

Figure 1. Forest plot summarizing odds ratios (ORs) and 95% confidence intervals (CIs) for the association between tagSNP rs1801157 and breast cancer risk. A. Pooled effect estimated from 7 case-control studies conducted in 6 different countries using fixed-effect meta-analytic models (I^2=40.6% [0%, 75%]; Q: 10.09, P=0.121). B. Pooled effect estimated from 5 case-control studies of European ancestry using fixed-effect meta-analytic models (I^2=0% [0%, 75%]; Q: 3.32, P=0.505).

ysis of the association between the common genetic variation in eight chemokines or chemokine receptors and the risk of breast cancer in post-menopausal women.

Materials and methods

The study population and methods have been described elsewhere [13]. Briefly, a population-based case-control study was conducted in King, Pierce and Snohomish counties in western Washington State. Cases were identified via the Cancer Surveillance System (CSS), a population-based cancer registry that participates in the Surveillance Epidemiology and End Results (SEER) program [14]. Cases were 65-79 years old at diagnosis of a first primary invasive breast cancer between April 1, 1997 and May 31, 1999. Controls were identified from the general population using Health Care Financing Administration records and frequency matched to cases in 5-year age groups. Estrogen receptor (ER) and progesterone receptor (PR) status of the tumor were available from the CSS.

TagSNPs in eight candidate chemokine-related genes (*CCL3, CCL4, CCL5, CCL20, CCR5, CCR6, CXCL12* and *CXCR4*) were selected using the Genome Variation Server (http://gvs.gs.washington.edu/GVS/) if they had minor allele frequency (MAF) greater than 5% and $r^2 \leq 0.80$ in the CEU-HapMap population. In addition, SNPs located in exons or the untranslated region (UTR) that had MAF of at least 2% were selected. The SNAGGER algorithm was used to select a subset of the tagSNPs, which yielded similar coverage, resulting in the attempted genotyping of 48 SNPs [15]. Genotying was performed using the Illumina GoldenGate multiplex platform and was successful for 33 SNPs. One SNP from *CXCR4* (rs2228014) that failed on the Illumina platform and one SNP from *CXCL12* (rs266093) that was not suited for the Illumina panel were genotyped by a parallel Taqman assay (KASPAR, Kbioscience Inc). One of the resulting 35 SNPs was monomorphic and was not included in the analysis.

Computations were carried out using the R software environment (version 2.15.0 for Macintosh). Analysis was restricted to women of European ancestry, as determined by self-report and principal component analysis as described previously [13]. Linkage disequilibrium (LD) between variants and departures from Hardy-Weinberg equilibrium were computed in controls [16]. Odds ratios (OR) and 95% confidence intervals (CI) were computed using logistic regression adjusting for age as a continuous linear variable and assuming an additive model of inheritance. The analysis of the combined set of genes included in the study was performed using the GRASS algorithm [17]. For single gene tests, we corrected for multiple tagSNPs within a gene with a P-min procedure using 10,000 permutations [18]. Gene-based *P*-values were computed separately for the main and subgroup analyses. Fixed-effects analysis was done to obtain meta-analytic estimates of the summary OR across studies looking at the association between *CXCL12* rs1801157 and breast cancer risk using the *rmeta* package. Haplotype analysis was performed to explore the association between tagSNPs *CXCR4* rs2228014 and *CXCL12* rs1801157 with breast of risk cancer using the *haplo.stats* R library.

Table 2. Association between common sequence variants in chemokine-related genes and breast cancer risk in postmenopausal women by tumor subtypes

			Controls (N=807)		Luminal Cases (N=744)					Non-luminal Cases (N=73)				
Gene	Variant	Genotype	n	(%)	n	(%)	OR*	(95% CI)*	*P*-value#	n	(%)	OR*	(95% CI)*	*P*-value#
CXCR4									0.08					0.75
	rs2228014						0.61	(0.39-0.95)				1.28	(0.56-2.93)	
		CC	689	(92.24)	639	(95.09)				62	(89.86)			
		TC	56	(7.50)	33	(4.91)				7	(10.14)			
		TT	2	(0.27)	0	(0.00)				0	(0.00)			
	rs2680880						1.02	(0.89-1.18)				1.04	(0.74-1.47)	
		TT	259	(32.33)	243	(32.88)				19	(26.03)			
		AT	388	(48.44)	341	(46.14)				43	(58.90)			
		AA	154	(19.23)	155	(20.97)				11	(15.07)			
	rs2471859						0.91	(0.77-1.07)				0.80	(0.49-1.31)	
		AA	451	(56.30)	440	(59.54)				45	(61.64)			
		AG	300	(37.45)	255	(34.51)				26	(35.62)			
		GG	50	(6.24)	44	(5.95)				2	(2.74)			
CCL20									0.22					0.28
	rs6749704						0.91	(0.78-1.06)				1.11	(0.77-1.6)	
		AA	385	(48.06)	380	(51.42)				35	(47.95)			
		AG	347	(43.32)	299	(40.46)				29	(39.73)			
		GG	69	(8.61)	60	(8.12)				9	(12.33)			
	rs940339						1.12	(0.97-1.29)				0.79	(0.55-1.11)	
		AA	203	(25.37)	181	(24.49)				24	(32.88)			
		AG	415	(51.88)	356	(48.17)				35	(47.95)			
		GG	182	(22.75)	202	(27.33)				14	(19.18)			
CCR5									0.93					0.77
	rs11575816						0.99	(0.86-1.15)				0.95	(0.67-1.35)	
		GG	318	(39.70)	294	(39.78)				32	(43.84)			
		AG	376	(46.94)	348	(47.09)				30	(41.1)			
		AA	107	(13.36)	97	(13.13)				11	(15.07)			
CCR6									0.51					1.00
	rs3093027						1.22	(0.93-1.60)				1.05	(0.53-2.05)	
		AA	682	(85.14)	609	(82.52)				62	(84.93)			
		AC	112	(13.98)	126	(17.07)				11	(15.07)			
		CC	7	(0.87)	3	(0.41)				0	(0)			
	rs3093026						0.91	(0.79-1.05)				0.92	(0.65-1.28)	
		GG	215	(26.84)	210	(28.46)				20	(27.4)			
		AG	388	(48.44)	369	(50.00)				37	(50.68)			
		AA	198	(24.72)	159	(21.54)				16	(21.92)			
	rs3093023						1.05	(0.92-1.21)				1.05	(0.75-1.47)	
		GG	274	(34.21)	228	(30.85)				22	(30.14)			
		AG	368	(45.94)	370	(50.07)				38	(52.05)			
		AA	159	(19.85)	141	(19.08)				13	(17.81)			
	rs3798315						1.09	(0.87-1.35)				0.86	(0.46-1.61)	
		GG	639	(79.78)	579	(78.35)				60	(82.19)			
		AG	150	(18.73)	147	(19.89)				13	(17.81)			
		AA	12	(1.50)	13	(1.76)				0	(0)			
	rs3093012						1.02	(0.89-1.18)				0.95	(0.67-1.34)	
		GG	271	(33.83)	242	(32.75)				26	(35.62)			
		AG	390	(48.69)	368	(49.80)				35	(47.95)			
		AA	140	(17.48)	129	(17.46)				12	(16.44)			
	rs3093010						1.02	(0.88-1.18)				0.91	(0.64-1.31)	
		CC	365	(45.57)	324	(43.84)				34	(46.58)			

		AC	336	(41.95)	328	(44.38)				32	(43.84)		
		AA	100	(12.48)	87	(11.77)				7	(9.59)		
	rs3093007						0.92	(0.77-1.11)				0.94	(0.57-1.58)
		AA	534	(66.67)	510	(69.01)				49	(67.12)		
		AG	239	(29.84)	203	(27.47)				23	(31.51)		
		GG	28	(3.50)	26	(3.52)				1	(1.37)		
	rs3093006						1.04	(0.85-1.29)				1.17	(0.68-2.01)
		GG	608	(75.91)	551	(74.56)				53	(72.6)		
		AG	179	(22.35)	178	(24.09)				18	(24.66)		
		AA	14	(1.75)	10	(1.35)				2	(2.74)		
	rs3093005						0.98	(0.59-1.64)				1.04	(0.31-3.51)
		AA	768	(96.00)	710	(96.08)				70	(95.89)		
		AG	32	(4.00)	29	(3.92)				3	(4.11)		
		GG	0	(0.00)	0	(0.00)				0	(0)		
	rs11575083						0.89	(0.51-1.55)				1.2	(0.35-4.05)
		GG	773	(96.50)	716	(96.89)				70	(95.89)		
		AG	28	(3.50)	23	(3.11)				3	(4.11)		
		AA	0	(0.00)	0	(0)				0	(0)		
	rs3093002						0.90	(0.77-1.05)				1.07	(0.74-1.55)
		GG	355	(44.38)	347	(47.02)				33	(45.21)		
		AG	371	(46.38)	335	(45.39)				31	(42.47)		
		AA	74	(9.25)	56	(7.59)				9	(12.33)		
	rs3093001						1.14	(0.99-1.31)				1.00	(0.71-1.4)
		AA	208	(26.1)	170	(23.16)				17	(23.29)		
		AC	399	(50.06)	363	(49.46)				41	(56.16)		
		CC	190	(23.84)	201	(27.38)				15	(20.55)		
	rs367523						0.90	(0.77-1.05)				1.15	(0.81-1.63)
		CC	303	(37.83)	294	(39.78)				28	(38.36)		
		CG	392	(48.94)	367	(49.66)				31	(42.47)		
		GG	106	(13.23)	78	(10.55)				14	(19.18)		
	rs4710189						0.92	(0.79-1.08)				1.02	(0.71-1.47)
		AA	340	(42.45)	329	(44.52)				34	(46.58)		
		AG	374	(46.69)	340	(46.01)				28	(38.36)		
		GG	87	(10.86)	70	(9.47)				11	(15.07)		
CXCL12									0.69				0.45
	rs266093						1.06	(0.91-1.23)				1.07	(0.75-1.54)
		GG	299	(40.46)	272	(40.54)				24	(35.29)		
		GC	338	(45.74)	286	(42.62)				36	(52.94)		
		CC	102	(13.8)	113	(16.84)				8	(11.76)		
	rs1029153						0.94	(0.80-1.09)				0.81	(0.56-1.18)
		AA	391	(48.81)	370	(50.14)				39	(53.42)		
		AG	322	(40.2)	299	(40.51)				29	(39.73)		
		GG	88	(10.99)	69	(9.35)				5	(6.85)		
	rs1801157						1.03	(0.87-1.24)				1.05	(0.64-1.73)
		GG	515	(64.29)	459	(62.11)				46	(63.01)		
		AG	251	(31.34)	256	(34.64)				25	(34.25)		
		AA	35	(4.37)	24	(3.25)				2	(2.74)		
	rs266087						0.91	(0.78-1.06)				1.14	(0.8-1.62)
		GG	325	(40.68)	325	(44.04)				24	(32.88)		
		AG	371	(46.43)	325	(44.04)				41	(56.16)		
		AA	103	(12.89)	88	(11.92)				8	(10.96)		
	rs2839695						1.07	(0.90-1.27)				1.25	(0.83-1.88)
		AA	504	(62.92)	466	(63.06)				43	(58.9)		
		AG	270	(33.71)	231	(31.26)				25	(34.25)		
		GG	27	(3.37)	42	(6.68)				5	(6.85)		
	rs2236534						1.04	(0.88-1.24)				0.65	(0.38-1.1)

		CC	493	(61.55)	456	(61.71)				52	(71.23)		
		AC	276	(34.46)	242	(32.75)				20	(27.4)		
		AA	32	(4)	41	(5.55)				1	(1.37)		
	rs3780891						0.86	(0.68-1.09)				1.2	(0.67-2.15)
		GG	647	(80.77)	615	(83.22)				57	(78.08)		
		AG	144	(17.98)	116	(15.70)				16	(21.92)		
		AA	10	(1.25)	8	(1.08)				0	(0)		
CCL5									0.56				0.63
	rs2107538						0.94	(0.78-1.14)				1.12	(0.72-1.73)
		GG	541	(67.54)	520	(70.37)				50	(68.49)		
		AG	240	(29.96)	192	(25.98)				18	(24.66)		
		AA	20	(2.5)	27	(3.65)				5	(6.85)		
CCL3									0.99				0.10
	rs9972960						0.99	(0.85-1.15)				1.03	(0.72-1.49)
		GG	307	(38.33)	296	(40.05)				25	(34.25)		
		AG	395	(49.31)	342	(46.28)				41	(56.16)		
		AA	99	(12.36)	101	(13.67)				7	(9.59)		
	rs1634502						1.01	(0.87-1.16)				0.71	(0.5-1.01)
		AA	286	(35.75)	251	(49.80)				33	(45.21)		
		AT	372	(46.5)	368	(16.24)				32	(43.84)		
		TT	142	(17.75)	120	(34.51)				8	(10.96)		
CCL4									0.97				0.06
	rs10491121						1.00	(0.87-1.15)				0.68	(0.48-0.98)
		GG	288	(35.96)	255	(34.51)				33	(45.21)		
		AG	368	(45.94)	361	(48.85)				33	(45.21)		
		AA	145	(18.1)	123	(16.64)				7	(9.59)		
	rs1634517						1.02	(0.86-1.22)				1.57	(1.07-2.32)
		CC	472	(59.97)	437	(60.36)				35	(49.3)		
		AC	282	(35.83)	248	(34.25)				29	(40.85)		
		AA	33	(4.19)	39	(5.39)				7	(9.86)		
	rs17679451						1.06	(0.73-1.52)				0.99	(0.41-2.36)
		GG	736	(91.89)	676	(91.47)				67	(91.78)		
		AG	63	(7.87)	61	(8.25)				6	(8.22)		
		AA	2	(0.25)	2	(0.27)				0	(0)		
	rs1619526						1.04	(0.88-1.23)				1.39	(0.95-2.05)
		GG	472	(58.93)	435	(58.86)				37	(50.68)		
		AG	292	(36.45)	261	(35.32)				30	(41.1)		
		AA	37	(4.62)	43	(5.82)				6	(8.22)		

*Odds ratios (ORs) and 95% CI intervals (CI) are adjusted for age. If genotype counts were less than 5 for any of the three cells, a dominant model was used; otherwise, an additive model was used. #*P*-value adjusted for multiple comparisons within a gene using a P-min permutation test.

Table 3. Association between haplotype formed by tagSNPs *CXCR4* rs2228014 and *CXCL12* rs1801157 and breast cancer risk

CXCR4 rs2228014	*CXCL12* rs1801157	Controls (%)	Cases (%)	OR*	(95% CI)*
C	G	(76.35)	(77.15)	1.00	(Ref.)
C	A	(19.63)	(20.04)	1.02	(0.85, 1.21)
T	G	(3.61)	(2.25)	0.63	(0.39, 1.01)
T	A	(0.41)	(0.55)	1.26	(0.21, 7.55)

*Odds ratios (ORs) and 95% confidence intervals (CI) are age adjusted (age as a continuous linear variable).

Results

We analyzed the association between 34 tagSNPs in 8 chemokine-related genes and case status in our population of 845 cases and 807 controls. No departures from Hardy-Weinberg equilibrium were observed among controls. None of the tagSNPs in the chemokine-related

genes studied altered the risk of breast cancer (**Table 1**). When we combined all the genes in a gene-set analysis, no association was observed with the risk breast cancer (p=0.112). In the meta-analysis of five studies of European ancestry looking at the association between *CXCL12* rs1801157 and breast cancer, there was a marginally significant 14% increased risk (OR=1.14, 95% CI: 1.00, 1.30) (**Figure 1B**).

None of the SNPs were related to the risk of luminal and non-luminal breast cancers, after adjusting for multiple comparisons (**Table 2**). Finally, compared to the most common haplotype formed by tagSNPs *CXCR4* rs2228014 and *CXCL12* rs1801157, other haplotypes did not significant alter the risk of breast cancer (**Table 3**).

Discussion

No strong evidence of association between chemokine-related genes and breast cancer was observed in our study, and this was true regardless of the receptor subtypes of the tumors.

Our study has a few limitations that could have influenced our results. Study sample size could have prevented us from detecting a small association. However, our sample size (845 cases and 807 controls) gave us better power than either of the two previous studies (233 cases and 210 controls [6]; 278 cases and 181 controls [7]) that did find a significant association for *CXCL12* rs1801157. Because of the possible small effect of each single SNP on breast cancer, we also combined all the SNPs into gene regions and all the genes in a gene-set analysis, but still were unable to observe an association.

A previous meta-analysis of five studies looking at *CXCL12* rs1801157 and breast caner risk reported a positive association [12]. We performed a meta-analysis to include a Brazilian and our current study and observed a marginal significant increased risk. However, while our study was restricted to postmenopausal women and the risk of invasive breast cancer, other studies included pre- and postmenopausal women as well as all stages, possibly *in situ* and invasive [6-11].

In summary, our study fails to support the hypothesis that genetic variation in chemokine-related genes is involved in breast cancer etiology in postmenopausal Caucasian women.

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Disclosure of conflict of interest

None.

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