Cancer Epidemiology, Biomarkers & Prevention

Null Results in Brief

Single-Nucleotide Polymorphisms in Genes Encoding for CC Chemokines were Not Associated with the Risk of Non-Hodgkin Lymphoma

Qiong Chen[1,2], Tongzhang Zheng[2], Qing Lan[3], Catherine Lerro[2], Nan Zhao[2], Qin Qin[4], Xiaobin Hu[2,5], Huang Huang[2], Jiaxin Liang[2], Theodore Holford[2], Brian Leaderer[2], Peter Boyle[6], Stephen J. Chanock[3,7], Nathaniel Rothman[3], and Yawei Zhang[2]

Abstract

Background: Chemokines play a pivotal role in immune regulation and response, and previous studies suggest an association between immune deficiency and non-Hodgkin lymphoma (NHL).

Methods: We evaluated the association between NHL and polymorphisms in 18 genes (*CCL1*, *CCL2*, *CCL5*, *CCL7*, *CCL8*, *CCL11*, *CCL13*, *CCL18*, *CCL20*, *CCL24*, *CCL26*, *CCR1*, *CCR3*, *CCR4*, *CCR6*, *CCR7*, *CCR8*, and *CCR9*) encoding for the CC chemokines using data from a population-based case–control study of NHL conducted in Connecticut women.

Results: *CCR8* was associated with diffuse large B-cell lymphoma (DLBCL; $P = 0.012$), and *CCL13* was associated with chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL; $P = 0.003$) at gene level. After adjustment for multiple comparisons, none of the genes or single-nucleotide polymorphisms (SNP) were associated with risk of overall NHL or NHL subtypes.

Conclusions: Our results suggest that the genes encoding for CC chemokines are not significantly associated with the risk of NHL, and further studies are needed to verify these findings.

Impact: Our data indicate that CC chemokine genes were not associated with NHL risk. *Cancer Epidemiol Biomarkers Prev; 22(7); 1332–5. ©2013 AACR.*

Introduction

CC chemokines, a chemokine subfamily contains 4 or 6 cysteines, play an important role in the development of immune response due to their leukocyte migration function (1). There is growing evidence that CC chemokines and their receptors play a role in the pathogenesis of non-Hodgkin lymphoma (NHL), and chemokine receptors have been shown to be overexpressed in certain NHL subtypes. For example, CCR4 was associated with improved survival in patients with diffuse large B-cell lymphoma (DLBCL), and CCR7 expression was higher in cases of mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL; ref. 2).

Although a direct relationship between the genetic polymorphisms of CC chemokine genes and risk of NHL have never been reported, genetic variations in these genes have been associated with risk of HIV-1 infection and autoimmune disorders (3–6), which are established risk factors for NHL (7).

Using data from a population-based case–control study conducted in Connecticut women, we examined the association between genetic polymorphisms in 18 genes encoding for CC chemokines and risk of NHL.

Materials and Methods

Detailed descriptions of this study population and methods have been previously described (8, 9). Briefly, a total of 601 incident cases and 717 population-based controls were enrolled and completed in-person interviews. All cases were histologically confirmed by 2 independent study pathologists and classified into NHL subtypes according to the World Health Organization classification system. Population-based controls were frequency matched to the cases by age (±5 years) and gender. The study was approved by the Institutional Review Boards at Yale University (New Haven, CT), the

Authors' Affiliations: [1]Henan Province Cancer Hospital, Office for Cancer Control and Prevention, Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, China; [2]Yale University School of Public Health, New Haven, Connecticut; [3]Division of Cancer Epidemiology and Genetics, Department of Health and Human Services, National Cancer Institute, NIH, Rockville, Maryland; [4]Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, Maine; [5]Lanzhou University School of Public Health, Lanzhou, China; [6]International Preventive Research Institute, Lyon, France; and [7]Core Genotyping Facility, Advanced Technology Center, National Cancer Institute, NIH, DHHS, Gaithersburg, Maryland.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Yawei Zhang, Department of Environmental Health Sciences, Yale University School of Public Health, 60 College Street, LEPH 440, New Haven, CT 06520. Phone: 203-785-6210; Fax: 203-737-6023; E-mail: yawei.zhang@yale.edu

doi: 10.1158/1055-9965.EPI-13-0328

Table 1. Genes and SNPs evaluated

Genes	Name	Chromosome location	SNP Database ID	MinP test *P*			
				NHL	DLBCL	FL	CLL/SLL
Chemokine (C–C motif) ligands							
CCL1	Chemokine (C–C motif) ligand 1	17q12	rs2282691, rs12603965, rs365654, rs408121, rs7502772	0.608	0.150	0.736	0.044
CCL2	Chemokine (C–C motif) ligand 2	17q11.2-q12	rs17652343, rs1860190, rs2857653, rs991804	0.890	0.816	0.351	0.341
CCL5	Chemokine (C–C motif) ligand 5	17q11.2-q12	rs2107538, rs4795095	0.474	0.564	0.349	0.832
CCL7	Chemokine (C–C motif) ligand 7	17q11.2-q12	rs3091237, rs2190970, rs3091322, rs3091324, rs8081047	0.321	0.263	0.750	0.549
CCL8	Chemokine (C–C motif) ligand 8	17q11.2	rs3138037, rs1233653, rs3138034, rs3138035, rs3138039,rs8082480, rs885691	0.600	0.247	0.058	0.134
CCL11	Chemokine (C–C motif) ligand 11	17q21.1-q21.2	rs12948058, rs16969415, rs17735961, rs3091328, rs4795895, rs4795896, rs4795904, rs714910	0.910	0.826	0.608	0.672
CCL13	Chemokine (C–C motif) ligand 13	17q11.2	rs1431991, rs442319	0.055	0.220	0.423	0.003
CCL18	Chemokine (C–C motif) ligand 18	17q11.2	rs2015070, rs11080372, rs8073066, rs854462, rs854477	0.204	0.622	0.288	0.048
CCL20	Chemokine (C–C motif) ligand 20	2q36.3	rs11694155, rs13034664, rs13389224, rs1827924, rs3138119, rs6749704, rs940339	0.144	0.269	0.594	0.088
CCL24	Chemokine (C–C motif) ligand 24	7q11.23	rs11465307, rs2302004, rs13340490, rs13340508, rs17361077, rs7797547	0.502	0.194	0.541	0.931
CCL26	Chemokine (C–C motif) ligand 26	7q11.23	rs2240478, rs11465352, rs11465353	0.846	0.560	0.831	0.148
Chemokine (C–C motif) receptors							
CCR1	Chemokine (C–C motif) receptor 1	3p21	rs17283264, rs3136671, rs3136673, rs3181077, rs7617872	0.706	0.535	0.743	0.408
CCR3	Chemokine (C–C motif) receptor 3	3p21.3	rs13073976, rs13326331, rs3091309, rs6441948, rs12489891, rs1388604, rs1907635, rs1979671, rs1979672, rs9842716	0.989	0.130	0.696	0.729
CCR4	Chemokine (C–C motif) receptor 4	3p24	rs2228428, rs6770096	0.991	0.586	0.707	0.815
CCR6	chemokine (C–C motif) receptor 6	6q27	rs3093010, rs3093012, rs367523, rs11575089, rs1855025, rs3093002, rs3093003, rs3093006, rs3093007, rs3093009, rs3093024, rs3798315, rs9459883	0.630	0.914	0.806	0.366
CCR7	Chemokine (C–C motif) receptor 7	17q12-q21.2	rs2023906, rs2290065, rs3136685, rs588019	0.260	0.259	0.357	0.272
CCR8	Chemokine (C–C motif) receptor 8	3p22	rs2853699, rs17038748, rs872066	0.375	0.012	0.611	0.971
CCR9	Chemokine (C–C motif) receptor 9	3p21.3	rs12638201, rs17714101, rs17765088, rs2236938, rs7614342, rs1471962, rs1860264, rs4683147, rs6441929, rs875890, rs9842595, rs9868455	0.521	0.664	0.709	0.855

Abbreviation: FL, follicular lymphoma.

Table 2. Associations between CC chemokine gene polymorphisms and risk of NHL overall and its common subtypes including DLBCL, follicular lymphoma, and CLL/SLL

		NHL overall				DLBCL				FL				CLL/SLL			
SNPs	**Controls**	**Cases**	**OR**	**95% CI**	***P***	**Cases**	**OR**	**95% CI**	***P***	**Cases**	**OR**	**95% CI**	***P***	**Cases**	**OR**	**95% CI**	***P***
CCL13 rs1431991																	
AA	135	141				46				36				23			
AG	240	209	0.82	(0.61–1.11)	0.21	68	0.83	(0.83–1.28)	0.39	44	0.68	(0.68–1.11)	0.12	22	0.54	(0.29–1.00)	0.05
GG	107	74	0.66	(0.45–0.97)	0.033	23	0.64	(0.64–1.12)	0.12	21	0.72	(0.72–1.32)	0.29	4	0.22	(0.07–0.65)	0.0061
AG or GG	347	283	0.77	(0.58–1.03)	0.08	91	0.77	(0.77–1.16)	0.21	65	0.69	(0.69–1.09)	0.11	26	0.44	(0.24–0.79)	0.0065
Trend	482	424	0.81	(0.67–0.98)	0.032	137	0.8	(0.80–1.06)	0.12	101	0.83	(0.83–1.12)	0.23	49	0.49	(0.31–0.77)	0.0021
CCR8 rs2853699																	
CC	236	193				58				51				22			
CG	216	194	1.10	(0.84–1.45)	0.48	56	1.07	(1.07–1.62)	0.75	46	1.01	(1.01–1.57)	0.97	24	1.18	(0.64–2.17)	0.6
GG	30	37	1.51	(0.90–2.53)	0.12	23	3.18	(3.18–5.91)	0.00025	4	0.61	(0.61–1.81)	0.37	3	1.08	(0.30–3.84)	0.9
CG or GG	246	231	1.15	(0.89–1.50)	0.29	79	1.33	(1.33–1.95)	0.15	50	0.96	(0.96–1.48)	0.84	27	1.17	(0.65–2.11)	0.61
Trend	482	424	1.17	(0.95–1.44)	0.15	137	1.52	(1.52–2.04)	0.0049	101	0.91	(0.91–1.30)	0.60	49	1.11	(0.69–1.79)	0.67

Abbreviation: FL, follicular lymphoma.

Connecticut Department of Public Health, and the National Cancer Institute (Rockville, MD).

DNA was extracted from blood samples (461 cases and 535 controls) using phenol–chloroform extraction method. Genotyping was conducted at the National Cancer Institute Core Genotyping Facility (Advanced Technology Center, Gaithersburg, MD) using an Illumina GoldenGate platform. A total of 448 cases and 525 controls were successfully genotyped. Duplicate samples from 100 study participants and 40 replicate samples from each of the 2 blood donors were interspersed throughout the plates used for genotype analysis for quality control purposes. In total, 103 single-nucleotide polymorphisms (SNP) from 18 genes encoding for CC chemokines were considered. The completion rate for all SNPs was more than 96%, and the concordance rate for quality control samples was more than 95% for all assays.

The χ^2 test was used to assess the Hardy–Weinberg equilibrium (HWE). A minimum P test ("minP") based on permutation resampling was used to test for the association with NHL or subtype (10). This approach adjusts for the number of tag SNPs tested within each gene region as well as underlying linkage disequilibrium pattern. Unconditional logistic regression was used to estimate ORs and 95% confidence intervals (CI) for individual SNPs and NHL, adjusted for age. The models compared the variant allele homozygote and heterozygote with the common allele, which served as the reference group. A linear trend test assuming an additive genetic model was conducted by assigning an ordinal value of 1, 2, or 3 corresponding to the homozygous wild-type, heterozygote, and homozygous variant genotype, respectively. These scores were then modeled as a continuous variable. We examined the haplotype block structure using Haploview version 4.2 (Broad Institute of MIT and Harvard, Cambridge, MA). The false discovery rate method (11) was applied to adjust for multiple comparisons, with significance level of 0.20. Statistical analyses were conducted using R package and Statistical Analysis Software version 9.3 (SAS Institute).

Results

The distribution of age, education level, and family history of cancer in first-degree relatives was similar for cases and controls (8, 9). Of the 103 SNPs examined, 2 SNPs were not in HWE test and were excluded from the analysis (Supplementary Table S1). At the gene level, *CCR8* was associated with DBLCL ($P = 0.012$), and *CCL13* was associated with CLL/SLL ($P = 0.003$; Table 1). After adjustment for multiple comparisons, none of these genes were remained statistically significant.

An increased risk was observed in *CCR8* rs2853699 for DLBCL ($OR_{GG/CC} = 3.18$; 95% CI, 1.71–5.91; $P = 0.00025$; $P_{trend} = 0.0049$). A reduced risk of CLL/SLL was associated with *CCL13* rs1431991 GG genotype ($OR_{GG/AA} = 0.22$; 95% CI, 0.07–0.65; $P = 0.0061$; $P_{trend} = 0.0021$; Table 2 and Supplementary Table S2). However, after adjustment for multiple comparisons, none of these associations remained statistically significant.

Haplotype analyses were consistent with the results of the individual SNP analyses and did not provide additional insight into these associations (data not shown).

Discussion

The study found no statistically significant association between polymorphisms in 18 genes encoding for CC chemokines and the risk of NHL. Potential associations with other CC chemokine genes that were not investigated in this study cannot be ruled out.

Our study was of moderate size for a rare cancer, and statistical power was lacked for consideration of associations in NHL subtype and potential gene–gene interactions. Because of limited SNPs included in our study, future study using the high-throughput analytic methods available (i.e., gene sequencing) may help clarify our findings and provide further insight into the role of CC

chemokines in NHL overall or subtype. In conclusion, our study suggested that genetic variations in CC chemokine gene were not associated with the risk of NHL overall or any subtype. Further studies with larger sample size, larger number of genes examined, and high-quality study design are needed to confirm these findings and investigate the association between additional chemokine genes and risk of NHL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: Q. Chen, T. Zheng, Q. Lan, T.R. Holford, B. Leaderer, P. Boyle, S.J. Chanock, N. Rothman, Y. Zhang
Development of methodology: T. Zheng, Y. Zhang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Q. Chen, T. Zheng, N. Rothman, Y. Zhang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Q. Chen, T. Zheng, Q. Lan, N. Zhao, X. Hu, J. Liang, S.J. Chanock, Y. Zhang
Writing, review, and/or revision of the manuscript: Q. Chen, T. Zheng, Q. Lan, C. Lerro, N. Zhao, Q. Qin, H. Huang, J. Liang, B. Leaderer, P. Boyle, S.J. Chanock, N. Rothman, Y. Zhang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Zheng, Y. Zhang
Study supervision: T. Zheng, Y. Zhang

Grant Support

This work was supported by the NIH grants CA62006, CA165923, 1D43TW008323, 1D43TW007864, and HD70324, and the Intramural Research Program of the National Cancer Institute.

Received March 28, 2013; accepted April 12, 2013; published OnlineFirst May 2, 2013.

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