

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

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### 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 ( $\pm 5$  years) and gender. The study was approved by the Institutional Review Boards at Yale University (New Haven, CT), the

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

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**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   |                                  |                     |   |             |       |       |         |
| <i>CCL1</i>                     | Chemokine (C–C motif) ligand 1   | 17q12               | rs2282691, rs12603965, rs365654, rs408121, rs7502772  | 0.608       | 0.150 | 0.736 | 0.044   |
| <i>CCL2</i>                     | Chemokine (C–C motif) ligand 2   | 17q11.2-q12         | rs17652343, rs1860190, rs2857653, rs991804  | 0.890       | 0.816 | 0.351 | 0.341   |
| <i>CCL5</i>                     | Chemokine (C–C motif) ligand 5   | 17q11.2-q12         | rs2107538, rs4795095  | 0.474       | 0.564 | 0.349 | 0.832   |
| <i>CCL7</i>                     | Chemokine (C–C motif) ligand 7   | 17q11.2-q12         | rs3091237, rs2190970, rs3091322, rs3091324, rs8081047   | 0.321       | 0.263 | 0.750 | 0.549   |
| <i>CCL8</i>                     | Chemokine (C–C motif) ligand 8   | 17q11.2             | rs3138037, rs1233653, rs3138034, rs3138035, rs3138039, rs8082480, rs885691  | 0.600       | 0.247 | 0.058 | 0.134   |
| <i>CCL11</i>                    | 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   |
| <i>CCL13</i>                    | Chemokine (C–C motif) ligand 13  | 17q11.2             | rs1431991, rs442319   | 0.055       | 0.220 | 0.423 | 0.003   |
| <i>CCL18</i>                    | Chemokine (C–C motif) ligand 18  | 17q11.2             | rs2015070, rs11080372, rs8073066, rs854462, rs854477  | 0.204       | 0.622 | 0.288 | 0.048   |
| <i>CCL20</i>                    | Chemokine (C–C motif) ligand 20  | 2q36.3              | rs11694155, rs13034664, rs13389224, rs1827924, rs3138119, rs6749704, rs940339   | 0.144       | 0.269 | 0.594 | 0.088   |
| <i>CCL24</i>                    | Chemokine (C–C motif) ligand 24  | 7q11.23             | rs11465307, rs2302004, rs13340490, rs13340508, rs17361077, rs7797547  | 0.502       | 0.194 | 0.541 | 0.931   |
| <i>CCL26</i>                    | Chemokine (C–C motif) ligand 26  | 7q11.23             | rs2240478, rs11465352, rs11465353   | 0.846       | 0.560 | 0.831 | 0.148   |
| Chemokine (C–C motif) receptors |                                  |                     |   |             |       |       |         |
| <i>CCR1</i>                     | Chemokine (C–C motif) receptor 1 | 3p21                | rs17283264, rs3136671, rs3136673, rs3181077, rs7617872  | 0.706       | 0.535 | 0.743 | 0.408   |
| <i>CCR3</i>                     | 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   |
| <i>CCR4</i>                     | Chemokine (C–C motif) receptor 4 | 3p24                | rs2228428, rs6770096  | 0.991       | 0.586 | 0.707 | 0.815   |
| <i>CCR6</i>                     | 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   |
| <i>CCR7</i>                     | Chemokine (C–C motif) receptor 7 | 17q12-q21.2         | rs2023906, rs2290065, rs3136685, rs588019   | 0.260       | 0.259 | 0.357 | 0.272   |
| <i>CCR8</i>                     | Chemokine (C–C motif) receptor 8 | 3p22                | rs2853699, rs17038748, rs872066   | 0.375       | 0.012 | 0.611 | 0.971   |
| <i>CCR9</i>                     | 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

| SNPs                   | NHL overall |       |      |             |       | DLBCL |      |             |         | FL    |      |             |      | CLL/SLL |      |             |        |
|------------------------|-------------|-------|------|-------------|-------|-------|------|-------------|---------|-------|------|-------------|------|---------|------|-------------|--------|
|                        | Controls    | Cases | OR   | 95% CI      | P     | Cases | OR   | 95% CI      | P       | Cases | OR   | 95% CI      | P    | Cases   | OR   | 95% CI      | P      |
| <i>CCL13 rs1431991</i> |             |       |      |             |       |       |      |             |         |       |      |             |      |         |      |             |        |
| 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 |
| <i>CCR8 rs2853699</i>  |             |       |      |             |       |       |      |             |         |       |      |             |      |         |      |             |        |
| 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.

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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  $\chi^2$  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 con-

ducted 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 DLBCL ( $P = 0.012$ ), 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

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