LYMPHOID NEOPLASIA

Brief report

Genetic variation in CXCR4 and risk of chronic lymphocytic leukemia

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A genome-wide linkage scan has provided evidence for a chronic lymphocytic leukemia (CLL) susceptibility locus at 2q21 to which the chemokine receptor CXCR4 gene maps. Recent data provide some evidence for common variation in CXCR4 according to the polymorphic variant rs2228014 defining CLL risk. To examine the role of genetic variation in CXCR4 on CLL risk, we screened 188 familial CLL cases and 213 controls for germline mutations in the coding regions of CXCR4 and genotyped rs2228014 in 1058 CLL cases and 1807 controls. No association between rs2228014 and risk of CLL was seen (P = .83). One truncating (W195X) and 2 missense mutations with possible functional consequences (V139 and G335S) were identified among 186 familial cases and 0 in 213 controls sequenced. Our analysis provides no evidence that common variation in CXCR4 defined by rs228014 influences the risk of CLL, but that functional coding mutations in CXCR4 may contribute to familial CLL. (Blood. 2009;114:4843-4846)

Introduction

Although the etiology of chronic lymphocytic leukemia (CLL) is largely unknown, the disease is characterized by a strong familial basis with an 8-fold increased risk observed in relatives of cases.1 Familial clustering of CLL and related B-cell lymphoproliferative disorders has provided the motivation for seeking to identify a susceptibility gene through genetic linkage. A recent linkage scan of 206 CLL families has provided evidence for a disease locus at 2q21, conferring a moderate-high risk of disease.2 The genetic basis of this linkage signal is, however, presently unknown. The gene encoding chemokine receptor 4 (CXCR4) maps to 2q21 and represents a potential candidate for the basis of the linkage signal. CXCR4 plays a key role in B lymphopoiesis by retaining immature B cells in the bone marrow. In addition, CXCR4 is important for trafficking and survival of CLL and other leukemia cells, through attachment to CXCL12-secreting stromal and nurse-like cells,3 and has extensively been studied as a coreceptor for HIV-1.4 Moreover, CXCR4 is up-regulated by interferon regulatory factor 4,5 and a recent genome-wide association study has shown that variation in interferon regulatory factor 4 influences the development of CLL.6 A recent association study of the single nucleotide polymorphism (SNP) rs2228014, which maps to exon 2 of CXCR4, provides some support for CXCR4 having a role in CLL risk, albeit marginally significant (P < .002, Padjusted = .09).7

To further examine the role of genetic variation in CXCR4 in CLL, we conducted a comprehensive analysis of the coding sequence and intron-exon boundaries in 188 familial CLL cases and 213 controls. In addition, we compared the genotype frequency of rs2228014 in 1058 cases and 1807 controls.

Methods

Mutational analysis of the coding and splice site regions of CXCR4 was conducted in 188 CLL patients (134 patients with family history of CLL only, 54 with family history of CLL and other B-cell lymphoproliferative disorders) ascertained through the International CLL Linkage Consortium. Mouthwash samples were available for 63 of the cases. The association study was based on 1058 CLL cases collected through International CLL Linkage Consortium and the Royal Marsden National Health Service Hospitals trust. The diagnosis of CLL was based on World Health Organization guidelines.8 Peripheral blood samples obtained from 1807 healthy persons, recruited through the National Study of Colorectal Cancer Genetics,9 were used as controls. Cases and controls were British residents and self-reported to be of European Ancestry. All biologic samples were obtained from patients and controls with informed consent, in accordance with the tenets of the Declaration of Helsinki, and approval from the Institute of Cancer Research ethical review board.

A search for mutations in the coding regions and splice sites of CXCR4 was performed by sequencing amplified polymerase chain reaction (PCR) fragments using BigDye Terminator chemistry implemented on an ABI 3730xl sequencer (Applied Biosystems). PCR primers were designed using Primer 3 software to facilitate the investigation of all intron-exon boundaries (supplemental Table 1, available on the Blood website; see the Supplemental Materials link at the top of the online article). Sequence traces were aligned and compared with the gene consensus sequence using Mutation Surveyor (Version 3.0; SoftGenetics). Two in silico algorithms, PolyPhen and SIFT, were used to predict the putative impact of missense variants on protein function. Scores were classified as tolerated or deleterious according to proposed criteria.10 12

Genotyping of rs2228014 was conducted by competitive allele-specific PCR KASPar chemistry (KBiosciences Ltd). Primers (supplemental Table 1) were designed using PrimerPicker software (KBiosciences Ltd).

The relationship between categorical variables was determined by Fisher exact test. Deviation of the genotype frequencies in the controls from those expected under Hardy-Weinberg equilibrium was calculated using the χ2 test statistic. The risk of CLL associated with rs2228014 was calculated by deriving allelic, heterozygous, and homozygous odds ratios by unconditional logistic regression. All statistical manipulations were undertaken using STATA (Version 8.0; Stata Corporation).

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Results and discussion

Complete sequence data of the coding and splice site regions of CXCR4 were generated for 186 of the 188 patient samples submitted for mutational analysis. Mouthwash DNA genotypes were completely concordant with blood DNA genotypes in 100% of cases (data not shown). Together with the fact that CXCR4 do not map to any of the regions of the genome commonly associated with copy number variation in CLL, highlights against bias from differential genotyping as a consequence of allelic imbalance influencing study findings.

Mutational analysis of the coding regions of CXCR4 in the 186 CLL cases identified 8 sequence variants (Table 1). Two of these, rs2228014 (414C>T) and rs56400844 (I53L), have previously been documented by dbSNP as polymorphisms. The frequency of rs2228014 was identical in the 213 controls screened. V139I, W195X, and G335S were identified in single cases and one in the controls. The truncating mutation was more probable as a basis for a role in CLL development. Loss of CXCR4 expression has been shown to lead to more progressive CLL. Moreover, inactivation of CXCR4 in hematopoietic stem cells has been documented to cause excessive hematopoietic stem cell proliferation.

Table 1. Sequence variants identified in CXCR4

<table>
<thead>
<tr>
<th>Nucleotide change*</th>
<th>dbSNP 56400844</th>
<th>MAF 0.005 (1)</th>
<th>Protein change</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs65400844 157A&gt;C</td>
<td>No data†</td>
<td>I53L</td>
<td>0.005 (1)</td>
<td></td>
</tr>
<tr>
<td>rs2228014 414C&gt;T</td>
<td>0.043</td>
<td>Synonymous</td>
<td>0.037 (13)</td>
<td></td>
</tr>
<tr>
<td>415G&gt;A</td>
<td>—</td>
<td>V139I</td>
<td>0.005 (1)</td>
<td></td>
</tr>
<tr>
<td>584A&gt;G</td>
<td>—</td>
<td>W195X</td>
<td>0.005 (1)</td>
<td></td>
</tr>
<tr>
<td>1003A&gt;G</td>
<td>—</td>
<td>G335S</td>
<td>0.005 (1)</td>
<td></td>
</tr>
<tr>
<td>1295C&gt;G</td>
<td>—</td>
<td>3’ UTR</td>
<td>0.005 (1)</td>
<td></td>
</tr>
<tr>
<td>1350G&gt;A</td>
<td>—</td>
<td>3’ UTR</td>
<td>0.005 (1)</td>
<td></td>
</tr>
<tr>
<td>1430C&gt;T</td>
<td>—</td>
<td>3’ UTR</td>
<td>0.005 (1)</td>
<td></td>
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</tbody>
</table>

Controls (n = 213)

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<tr>
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<tbody>
<tr>
<td>153T&gt;A</td>
<td>—</td>
<td>Synonymous</td>
<td>0.004 (1)</td>
<td></td>
</tr>
<tr>
<td>294C&gt;T</td>
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<td>Synonymous</td>
<td>0.004 (1)</td>
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<tr>
<td>rs2228014 414C&gt;T</td>
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<td>Synonymous</td>
<td>0.08 (19)</td>
<td></td>
</tr>
<tr>
<td>783C&gt;T</td>
<td>—</td>
<td>Synonymous</td>
<td>0.004 (1)</td>
<td></td>
</tr>
<tr>
<td>1336G&gt;A</td>
<td>—</td>
<td>3’ UTR</td>
<td>0.009(2)</td>
<td></td>
</tr>
</tbody>
</table>

MAF indicates minor allele frequency of SNP in whites (as reported in dbSNP); —, not applicable; and UTR, untranslated region.

*Nucleotide position is taken from the first base of the start codon; dbSNP accession number is shown where available.
†No frequency data available for whites.

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Authorship

Contribution: D.C.-S. designed and performed research, analyzed data, and wrote the paper; M.Q. performed research; M.J.S.D., E.M., and C.D. performed sample acquisition; D.C. obtained funding; R.S.H. designed research, obtained funding, and wrote the paper; and all authors contributed to the final paper.

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Figure 1. Nonsynonymous mutations in CXCR4 in CLL and WHIM syndrome. Nonsynonymous mutations identified in CLL (chronic lymphocytic leukemia) patients are indicated at their relative positions as sequence traces with corresponding amino acid substitutions. Sites of truncating mutations associated with WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome are also shown.15,18 Cross-species sequence conservation is shown for the SNPs postulated to be functionally deleterious.

References

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