Brief report

Single nucleotide polymorphisms and inherited risk of chronic lymphocytic leukemia among African Americans

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The incidence of chronic lymphocytic leukemia (CLL) is significantly lower in African Americans than whites, but overall survival is inferior. The biologic basis for these observations remains unexplored. We hypothesized that germline genetic predispositions differ between African Americans and whites with CLL and yield inferior clinical outcomes among African Americans. We examined a discovery cohort of 42 African American CLL patients ascertained at Duke University and found that the risk allele frequency of most single nucleotide polymorphisms known to confer risk of development for CLL is significantly lower among African Americans than whites. We then confirmed our results in a distinct cohort of 68 African American patients ascertained by the CLL Research Consortium. These results provide the first evidence supporting differential genetic risk for CLL between African Americans compared with whites. A fuller understanding of differential genetic risk may improve prognostication and therapeutic decision making for all CLL patients. (*Blood*. 2012;120(8): 1687-1690)

Introduction

Chronic lymphocytic leukemia (CLL) is a malignant lymphoproliferative disorder affecting approximately 15 000 Americans per year that is characterized by the progressive clonal expansion of $CD5^+$ B cells.[1-3] Although CLL can follow a relatively indolent disease course, it remains incurable with approximately 4390 attributable deaths per year in the United States.[4] Despite years of research effort, the cause of CLL remains unknown. Risk factors for the development of CLL have been identified, including advanced age, male sex, white ethnicity, and a family history of CLL or other lymphoproliferative disorder.

Epidemiologic data compiled by the Survey Epidemiology and End Results identifies important differences in incidence and survival for African Americans with CLL.[4] Although the incidence of CLL is lower among African Americans than whites (4.4 and 6.1 per 100 000 men, respectively), age-adjusted survival is inferior. The overall 5-year relative survival for 1999-2006 from 17 Survey Epidemiology and End Results geographic areas by race and sex was 77.0% for white men, 81.1% for white women, 62.4% for African American men, and 68.3% for African American women. The biologic basis for these observations remains almost entirely unexplored.[5]

The 8% to 10% of CLL patients have a family history of CLL, suggesting an inherited predisposition. The relative risk of CLL among first-degree relatives of CLL patients is between 5.5 and 7.0.[6,7] Thus, CLL has a heritability that is twice that of common solid tumors, such as breast and colon cancer.[6,8] Approximately 10% of the inherited risk for CLL is attributable to "common variants," single nucleotide polymorphisms (SNPs) with a population prevalence $> 5\%$.[9] These SNPs were identified in genome-wide association (GWA) studies[9-14] and individually confer relatively small disease risk. The CLL GWA studies were undertaken in almost exclusively white study populations, so the importance of these SNPs in other racial groups is undetermined. Further, the allele frequencies for some of these SNPs vary considerably between different racial and ethnic groups.[15] Although the function of these SNPs in CLL pathogenesis is incompletely understood, identifying SNPs that vary in allele frequency between races might facilitate discovery of the effects of these variants.

We therefore hypothesized that there are underlying genetic differences that affect disease pathogenesis between African Americans and whites with CLL. We explored this hypothesis by determining the risk allele frequencies of 15 SNPs known to confer risk of CLL in white in a cohort of African American CLL patients.

Methods

The study population included a total of 112 African American CLL patients, identified by self-reported race. This study did not directly consent any study subjects. The biospecimens were ascertained under institutional review board–approved parent protocols at the respective institutions. Forty-two patients were from Duke University Medical Center and the Durham Veterans Administration Medical Center, and a validation cohort of 70 African American CLL patients was obtained from CLL Research Consortium (CRC) sites. At Duke, CLL cells were enriched by negative selection using Rosette-Sep for B cells (StemCell Technologies) depleting monocytes, neutrophils, erythrocytes, and T cells with antibodies and gradient sedimentation typically yielding CLL cells with $> 98\%$ purity.[16]

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Table 1. Ancestry informative genotyping

SNP	Chromosome	Allele	HapMap CEU	HapMap ASW	Δ (CEU-ASW)	HapMap YRI	African American control[20,21]	Duke African American CLL	CRC African American CLL	Duke/CRC African American CLL	P*	P†
rs798443	2	A	0.82	0.14	0.68	0.035	0.18	0.15	0.18	0.17	.75	.72
rs1462309	3	T	0.84	0.22	0.62	0.028	0.15	0.21	0.15	0.17	.67	.32
rs10041728	5	G	0.93	0.23	0.71	0.031	0.21	0.29	0.25	0.26	.10	.66
rs4896780	6	A	0.17	0.86	0.69	0.99	0.85	0.85	0.79	0.81	.25	.42
rs4885162	13	C	0.75	0.12	0.63	0.014	0.14	0.14	0.13	0.14	1	.99
rs2246695	14	T	0.82	0.16	0.67	0.003	0.16	0.21	0.18	0.20	.17	.71
rs12594483	15	A	0.071	0.88	0.81	1	0.82	0.85	0.75	0.79	.19	.12
rs7187359	16	A	0.21	0.84	0.62	0.99	0.84	0.80	0.85	0.83	.81	.49

*Comparison of allele frequencies of Duke/CRC African American CLL with MD Anderson controls.
†Comparison of allele frequencies of Duke African American CLL with CRC African American CLL.

CRC samples are PBMCs purified by density centrifugation, typically yielding > 90% CLL phenotype cells.[17]

Eight ancestry-informative SNPs and 15 CLL risk SNPs (Tables 1 and 2) were genotyped using TaqMan SNP genotyping assays (Invitrogen) by the DNA Analysis/Automated Sequencing Core Facility at the Duke Cancer Institute. Only SNP calls of > 95% quality were included in the analysis. The 8 ancestry-informative SNPs were selected based on availability of HapMap allele frequencies for CEU (Utah residents with Northern and Western European ancestry from the CEPH collection), ASW (African ancestry in Southwest United States), and YRI (Yoruban in Ibadan, Nigeria) populations; location on different chromosomes to eliminate the possibility of linkage disequilibrium between markers; and a minimum allele frequency difference of > 0.60 between CEU and ASW populations. CLL risk allele frequencies were compared with pooled results from previously published white CLL patients.[9,10,12-14,20,21] Control African American allele frequencies were obtained from published GWA data (474 African American patients from MD Anderson[18,19]) and from the HapMap database[15] (http://hapmap.ncbi.nlm.nih.gov/). Control allele frequencies were not available for rs1050979.

Statistical analyses comparing differences in allele frequencies were performed using Yates' corrected χ^2 for comparisons. A P value less than .05 was considered significant. Analyses were performed using JMP Version 9.0.1 (SAS).

Results and discussion

CLL patients were ascertained for this study based on self-reported race. Ancestry was evaluated by genotyping 8 ancestry-informative markers[22] (Table 1). Ancestry genotyping excluded 2 patients whose haplotypes matched CEU ancestry (14 of 16 alleles for both patients). We elected to include 4 patients whose haplotypes were divided between CEU alleles and ASW alleles because these patients were presumed to be of biracial ancestry, and self-identified as African American. Ancestry SNPs were compared between the Duke and CRC populations, and to the MD Anderson control African American population, with no statistically significant difference among the groups (Table 1).

Fifteen SNPs associated with inherited risk of CLL were genotyped in a cohort of 42 African American patients ascertained at Duke University (Table 2). Of the 15 genotyped SNPs, 9 showed a statistically significant lower risk allele frequency in African American CLL compared with previously reported white CLL patients (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article):

Table 2. CLL risk allele genotyping

SNP	Locus	Gene	Risk allele	White CLL RAF*	African American CLL RAF (Duke)	African American CLL RAF (CRC)	African American CLL RAF (Duke/CRC)	P†	P‡	African American control RAF (GWA + HapMap)§	P‖
rs17483466	2q13	ACOXL	G	0.26	0.11	0.07	0.09	< .001	.54	0.06	.18
rs13397985	2q37.1	SP140	G	0.25	0.17	0.13	0.15	.002	.61	0.10	.06
rs757978	2q37.3	FARP2	A	0.13	0.10	0.10	0.10	.22	1	0.08	.46
rs872071	6p25.3	IRF4	G	0.56	0.15	0.12	0.13	< .001	.56	0.09	.13
rs1050976	6p25.3	IRF4	T	0.48	0.15	0.12	0.13	< .001	.56	0.18	.27
rs1050979	6p25.3	IRF4	G	0.47	0.15	0.12	0.13	< .001	.56	—	—
rs2456449	8q24.21	—	G	0.41	0.22	0.21	0.21	< .001	.95	0.18	.27
rs735665	11q24.1	SF3A3P2	A	0.27	0.10	0.07	0.08	< .001	1	0.05	.09
rs7169431	15q21.3	—	A	0.11	0.24	0.21	0.22	< .001	.69	0.23	.82
rs4777184	15q23	—	T	0.47	0.32	0.29	0.31	< .001	.78	0.29	.69
rs783540	15q25.2	CPEB1	G	0.42	0.31	0.47	0.41	.93	.03	0.43	.67
rs305061	16q24.1	IRF8	T	0.70	0.82	0.90	0.87	< .001	.11	0.85	.34
rs391525	16q24.1	IRF8	A	0.75	0.63	0.67	0.66	.004	.67	0.61	.23
rs1036935	18q21.1	—	T	0.26	0.33	0.40	0.38	< .001	.36	0.32	.10
rs11083846	19q13.32	PRKD2	A	0.27	0.08	0.13	0.11	< .001	.46	0.07	.09

— indicates not applicable.
*White risk allele frequencies (RAF) were abstracted and compiled from previously published reports; reported allele frequencies are for the disease risk-associated allele (supplemental Table 1).
†Comparison of RAF from pooled Duke and CRC African American CLL cohorts with published white CLL RAF.
‡Comparison of Duke African American CLL with CRC African American CLL.
§African American control RAF were pooled from HapMap data (n = 56-57) and genotypes from African American controls participating in a prostate cancer genome-wide SNP association study (n = 474).[20,21]
‖Comparison of RAF from pooled Duke and CRC African American CLL cohorts with pooled African American control CLL RAF.

rs17483466, rs872071, rs1050976, rs1050979, rs2456449, rs735665, rs4777184, rs391525, and rs11083846. Two SNPs showed a significantly higher frequency in African American CLL than in white CLL: rs7169431 and rs305061. To validate our findings in a geographically distinct patient population, a cohort of 70 African American CLL patients enrolled at CRC consortium sites were subsequently genotyped. After excluding the 2 aforementioned patients, 68 CRC patients were included in the analysis. Among the 15 SNPs, only rs783540 significantly differed in allele frequency between Duke and CRC cohorts. Ten of the 11 statistically significant differences in allele frequencies were confirmed through comparison between the CRC cohort and white CLL patients. The only difference that was not confirmed was rs391525, where $P = .052$ for the comparison between CRC and white CLL (all group comparisons are provided in supplemental Table 1).

Given the high degree of concordance between the Duke and CRC cohorts for both ancestry and CLL specific genotyping, we then pooled genotyping results of these 2 groups to increase statistical power for comparisons. Through pooling of Duke and CRC cohorts, we were able to detect additional differences. Of the 15 genotyped SNPs, 10 SNPs (the 9 statistically significantly SNPs noted in the prior paragraph plus rs13397985) showed a statistically significant lower risk allele frequency in African American CLL compared with previously reported white CLL patients (Table 1). Three SNPs showed a significantly higher frequency in African American CLL than in white CLL: rs7169431 and rs305061 as well as rs1036935. rs757978 and rs783540 did not statistically differ in allele frequency between African American and white CLL patients. Hence, in the majority of SNPs examined, the observed allele frequencies are not similar to the values determined in prior reports derived from predominantly white patient populations.

Eleven of the 14 SNPs evaluated had allele frequencies that were numerically higher comparing African American CLL patients with African American controls, suggesting that these alleles may contribute to African American disease pathogenesis; however, these comparisons did not reach statistical significance in any case. In general, the prevalence of these SNPs was lower in African American cases than white, suggesting that these SNPs contribute relatively little CLL risk to African American populations. The absence of significant differences in allele frequency between affected African American versus controls could be the result of relatively low sample size. Assuming a risk allele frequency among controls of 0.15, then this study had 80% power to detect an allele frequency difference of 0.115 between African American cases ($n = 110$) and controls ($n = 530$) with $\alpha = 0.05$.

High concordance of allele frequencies was observed between the Duke African American CLL and CRC African American CLL patients, although this study was not powered to detect differences between these 2 groups. The one exception to this was rs783540, which showed a higher allele frequency in CRC patients than in the Duke cohort (0.47 and 0.31, respectively; $P = .026$). This observation could be the result of chance given the number of SNPs under investigation, to regional differences in allele frequencies, or ascertainment/referral bias. Evidence against regional variation was that there were no differences in the allele frequency among the ancestry SNPs that were studied (Table 1). Overall, we think that the multicenter study design is an important strength as it limits the potential that observed differences are because of regional differences or ethnic subpopulations.

Among African American CLL patients, all SNPs associated with inherited risk for CLL had an allele frequency similar to African American control populations. We therefore conclude that the majority of SNPs known to confer risk of CLL in whites do not contribute significant risk for CLL to African Americans. Nonetheless, because a number of SNPs show differences in allele frequency that trend toward established CLL risk and because only approximately 10% of the inherited risk of CLL has been discovered to date, we think that our results support future race-specific application of GWA studies in a larger, unique cohort of African American CLL. Such studies may define specific SNPs important for the development of CLL in different racial and ethnic populations, ultimately yielding a better understanding of disease pathogenesis and treatment options for all patients with CLL.

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Authorship

Contribution: M.C.L. designed research; C.C.C. and M.C.L. performed research; S.L.S. contributed vital analytical tools; T.J.K., L.Z.R., A.F., L.F., S.S.S., and J.B.W. contributed samples and data; C.C.C. collected data; C.C.C., S.L.S., T.J.K., and M.C.L. analyzed and interpreted data; C.C.C., T.J.K., J.B.W., and M.C.L. wrote the manuscript; and all authors reviewed and approved the manuscript.

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