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Prevalence of BCL-2/J(H) Translocation in Healthy African Americans

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Abstract

Objective—The translocation t(14;18)(q32;q21) (BCL-2/J(H)) is present in over 80% of all follicular lymphomas and is detectable in peripheral blood lymphocytes (PBL) of healthy individuals. The prevalence of this translocation has not been studied in African Americans (AAs). Given the higher incidence of follicular lymphomas in whites compared to AAs in the United States (US), we hypothesized that the translocation prevalence in the blood of AAs would be lower.

Methods—DNA was isolated from PBL from blood samples collected from participants from Florida. Polymerase chain reaction was performed on the BCL-2/J(H) major (MBC) and minor breakpoint cluster (mBC) regions.

Findings—Eight of the 77 (10.4%) blood samples from AA participants were positive for MBC (95% CI, 4.6%–19.5%), and three (3.9%) were positive for mBC (95% CI, 0.81%–10.97%) of BCL-2/J(H), with a total of 11 (14.3%) participants with positive samples (95% CI, 7.35%–

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Conflicts of Interest:

Dr. Colon-Otero discloses research funding with Novartis pharmaceuticals. Dr. Ailawadhi discloses that he is a consultant for Novartis, Amgen, Pharmacyclics, and Takeda pharmaceuticals. Dr. Fonseca has received a patent for the prognostication of MM based on genetic categorization by FISH of the disease. He has a patent application pending for the use of calcium isotopes as biomarkers for bone metabolisms (Primary site and investigators Arizona State University). He has received consulting fees from Celgene, BMS, Bayer, Novartis, Sanofi, Janssen, Millennium a Takeda Company, and AMGEN. He is also a member of the Scientific Advisory Board of Applied Biosciences.

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24.13%). In 167 white patient samples, 22 (13.2%; 95% CI, 8.44%–19.26%) were positive for MBC, and five (3.0%; 95% CI, 0.98%–6.85%) were positive for mBC, with a total 25 (15%) participants with positive samples (CI, 9.93%–21.30%).

Interpretation—The prevalence of t(14;18)(q32;q21) is not significantly different among AAs and whites from the US. The lower prevalence of follicular lymphomas in AAs compared with whites is likely a result of differences in secondary molecular alterations involved in follicular lymphoma development. This study is the first report of prevalence of t(14;18) in an AA cohort.

Keywords

lymphomas; genetics; minorities; population

Introduction

Two of the most common non-Hodgkin lymphomas in the United States (US) are diffuse large B-cell lymphomas (DLBCLs) and follicular lymphomas (FL) [1]. The t(14;18)(q32;q21) chromosomal translocation involving IgH and BCL-2 genes (BCL-2/J[H]) is present in over 80% of FL and 20% to 30% of DLBCL tumor specimens [2,3]. The incidence of FL is higher in the US compared to Japan, and within the US, the rates (age standardized, per 100,000) are highest in whites (3.8), intermediate in Hispanics (2.8), and lowest in Asian/Pacific Islanders (1.7) and African Americans (AAs) (1.5). Within these groups, rates are slightly higher in men compared to women [4,5]. In the general population, the t(14;18) translocation is also detectable in the peripheral blood of presumably healthy individuals, and the prevalence increases with age, is higher in men, and appears to be higher in people of European ancestry than in those of East Asian ancestry (Table 1) [6,8]. This molecular alteration is likely an early causative event in the development of this malignancy. Recent data from Rouillard et al [9] estimated that the presence of t(14;18) in >1/10,000 total peripheral blood leukocytes in healthy persons is predictive of a 23-fold greater risk of subsequent FL development. This study demonstrated the potential value of t(14;18) in the peripheral blood as the first predictive marker for FL.

No previous studies have evaluated the prevalence of t(14;18) in the peripheral blood of AAs. In part, this is because AAs are underrepresented in cancer clinical trials due to lack of research awareness, limited access to trials, fear, distrust, cultural beliefs, and discrimination [10,11]. The limited access to trials can be attributed to several factors, including receiving care from facilities that are not involved in research, lack of access to facilities that perform research, and a higher percentage of patients with comorbidities, making them ineligible for participation in research [10,11]. As stated above, the prevalence of FL in AAs is also significantly lower than that observed in whites [4,5,12]. Also, AAs with multiple myeloma have a better prognosis compared to whites, perhaps related to a lower prevalence of IgH translocations, which are associated with poor prognosis [13]. Based on these findings, we hypothesized that the prevalence of t(14;18) in the peripheral blood of AAs would be lower than the prevalence in whites.

Methods

This project was part of a Mayo Clinic Institutional Review Board-approved translational cancer research pilot study aimed at increasing awareness on the importance of cancer research among the Northeast Florida AA population [10,11]. The study took place in AA churches located in Northeast Florida and at the Volunteers in Medicine Clinic, a clinic that serves low-income, working, uninsured patients and is located in inner-city Jacksonville, Florida [10,11]. Blood samples were collected from consented adult subjects during educational sessions in a mobile research unit, a converted recreational vehicle that has sample collection, processing, and storage capabilities [11]. All samples were collected, stored, and transported back to Mayo Clinic in the mobile research unit. Race and ethnicity assignment of participants was based on self-identification through a study questionnaire provided to each patient by a clinical research coordinator. Blood samples from age-matched, self-identified whites from Northeast Florida from the Mayo Clinic Biobank were used as controls. Participants' demographic characteristics are summarized in Table 2.

DNA was isolated from the buffy coat fractions (peripheral blood leukocytes), and a polymerase chain reaction (PCR) quality control assay targeting a 293bp fragment of an HLA class II gene was used to determine acceptable DNA quality and quantity. Next, a two-step seminested PCR was performed on the t(14;18) major breakout cluster regions (MBC) and minor breakpoint cluster regions (mBCR) using about 500 ng of DNA for each assay. A consensus JH primer was used in combination with primers complementary to BCL-2 sequences for both the MBC and mBC. PCR products were run on a 1% agarose gel. In order to confirm the positive results, positive PCR products were analyzed by direct sequence by the Sanger method and subsequently assembled to the human genome using the Basic Local Alignment Search Tool from the US National Library of Medicine.

Results

During the education sessions at the AA churches and the Volunteers in Medicine Clinic, 77 blood samples were taken from consenting AA participants. The AA samples were analyzed for the presence of t(14;18). A total of eight (10.4%) samples were positive for the MBC (95% CI, 4.6%–19.5%) and three (3.9%) were positive for the mBC (95% CI, 0.81%–10.97%), for a total of 11 (14.3%) participants with positive samples (95% CI, 7.35%–24.13%). In 167 age-matched white patient samples from the Mayo Clinic Biobank, a total of 22 (13.2%; 95% CI, 8.44%–19.26%) were positive for MBC and five (3.0%; 95% CI, 0.98%–6.85%) were positive for mBC, with a total of 25 (15.0%) positive samples (95% CI, 9.93%–21.30%), with two (8.0%) of the 25 positive for both mBC and MBC. The odds ratio [AA vs. white] adjusted for age and sex was 0.98 (95% CI, 0.40–2.42; $P=.97$) for positive MBC and 1.26 (95% CI, 0.56–2.85, $P=.58$) for any positive sample (Table 1). All cases were confirmed by Sanger sequencing.

Discussion

We found a lower prevalence of t(14;18) in our white participants than had been previously reported. A plausible explanation for this finding is the high heterogeneity of approaches

used across studies (different DNA amounts, different methodologies), which affect the assay sensitivity (Table 3). For example, several of the reported series used either real-time quantitative PCR or nested PCR, and the amount of DNA analyzed varied from 1.2–10 µg per sample. The amount of DNA analyzed per patient in our sample was 0.5 µg, which is lower than the amount use in previously reported series (Table 3).

The similar prevalence of t(14;18) among whites and AAs suggests that the difference in prevalence of FL among these populations may reflect differences in prevalence of subsequent molecular events needed for FL development. Multiple secondary chromosomal aberrations are present in FL, including deletions of chromosomes 1, 6, and 17 and partial trisomies of chromosomes 1, 7, 8, and 18 [9]. Further studies on the molecular changes seen in FL in different populations defined on race/ethnicity or geography may help identify potential pathogenetic environmental factors.

Two of our study participants had more than one t(14;18) rearrangement (one MBC and one mBC) noted in their peripheral blood. The concomitant presence of MBC and mBC rearrangements of BCL-2/J(H) in patients with FL was previously described in a study by Weinberg et al [20]. Eight (4.3%) of 185 patients with FL that were positive for a breakpoint rearrangement were positive for both MBC and mBC in that series. This study suggested that a comprehensive PCR assay to probe for additional breakpoint rearrangements aside from MBC should be performed in all patients with FL. Further studies on the presence of MBC and mBC in patients with FL and histologic grade, prognosis, and response to therapy may help identify the clinical significance of double breakpoint rearrangements.

Limitations of this study include the fact that a larger patient sample may have revealed smaller significant differences in prevalence among the different populations. A larger patient sample and an assay utilizing multiple replicates may have increased the sensitivity of the detection of t(14;18) and may have increased the possibility of detecting smaller differences between the two study groups.

The development of new, highly effective, targeted agents for the treatment of FL, including agents that target BCL-2, may lead to the development of effective treatments that may prevent the development of FL with acceptable toxicity. One of these newly approved oral agents, venetoclax (Venclexta), has shown significant clinical activity against B-cell lymphomas [21]. It is also conceivable that the elimination of t(14;18) clones in healthy individuals prior to the development of an overt lymphoma may have an impact on the incidence of DLBCL because a percentage of patients with aggressive B-cell lymphomas have a concomitant or preceding FL and because t(14;18) is seen in 20% of all DLBCLs. This may have a major impact on the mortality of the most common lymphomas and will represent a potential chemopreventive intervention for this common, frequently fatal illness.

Conclusions

The prevalence of BCL-2/J(H) translocations in white and AAs from the US were similar. This suggests that the observed lower prevalence of FL in AAs compared to whites in the US is likely due to differences in the development of secondary genetic changes needed for

lymphomagenesis. This study represents the first report of the prevalence of t(14;18) in an AA population.

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References

1. SEER Cancer Statistics Factsheets. Non-Hodgkin Lymphoma. National Cancer Institute; Bethesda, MD: 2016. Available at: <http://seer.cancer.gov/statfacts/html/nhl.html> [Accessed July 19, 2016]
2. Kridel R, Sehn LH, Gascoyne RD. Pathogenesis of follicular lymphoma. *J Clin Invest*. 2012; 122:3424–3431. [PubMed: 23023713]
3. van Dongen JJ, Langerak AW, Brüggemann M, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia*. 2003; 17:2257–2317. [PubMed: 14671650]
4. Chihara D, Ito H, Matsuda T, et al. Differences in incidence and trends of haematological malignancies in Japan and the United States. *Br J Haematol*. 2014; 16:536–545.
5. Flowers CR, Sinha R, Vose JM. Improving outcomes for patients with diffuse large B-cell lymphoma. *CA Cancer J Clin*. 2010; 60:393–408. [PubMed: 21030533]
6. Liu Y, Hernandez AM, Shibata D, Cortopassi GA. BCL2 translocation frequency rises with age in humans. *Proc Natl Acad Sci USA*. 1994; 91:8910–8914. [PubMed: 8090743]
7. Ji W, Qu GZ, Ye P, Zhang XY, Halabi S, Ehrlich M. Frequent detection of bcl-2/JH translocations in human blood and organ samples by a quantitative polymerase chain reaction assay. *Cancer Res*. 1995; 55:2876–2882. [PubMed: 7796416]
8. Yasukawa M, Bando S, Dölken G, et al. Low frequency of BCL-2/J(H) translocation in peripheral blood lymphocytes of healthy Japanese individuals. *Blood*. 2001; 98:486–488. [PubMed: 11435322]
9. Roulland S, Kelly RS, Morgado E, et al. t(14, 18) translocation: A predictive blood biomarker for follicular lymphoma. *J Clin Oncol*. 2014; 32:1347–1355. [PubMed: 24687831]
10. Colon-Otero G, Smallridge RC, Solberg LA Jr, et al. Disparities in participation in cancer clinical trials in the United States : a symptom of a healthcare system in crisis. *Cancer*. 2008; 112:447–454. [PubMed: 18085590]
11. Colon-Otero G, Albertie M, Lesperance M, et al. A pilot program in collaboration with African American churches successfully increases awareness of the importance of cancer research and participation in cancer translational research studies among African Americans. *J Cancer Educ*. 2012; 27:294–298. [PubMed: 22072126]
12. Li Y, Wang Y, Wang Z, Yi D, Ma S. Racial differences in three major NHL subtypes: descriptive epidemiology. *Cancer Epidemiol*. 2015; 39:8–13. [PubMed: 25560974]
13. Baker A, Braggio E, Jacobus S, et al. Uncovering the biology of multiple myeloma among African Americans: A comprehensive genomics approach. *Blood*. 2013; 121:3147–3152. [PubMed: 23422747]
14. Summers KE, Goff LK, Wilson AG, Gupta RK, Lister TA, Fitzgibbon J. Frequency of the Bcl-2/IgH rearrangement in normal individuals: Implications for the monitoring of disease in patients with follicular lymphoma. *J Clin Oncol*. 2001; 19:420–424. [PubMed: 11208834]
15. Dolken G, Dolken L, Hirt C, Fusch C, Rabkin CS, Schuler F. Age-dependent prevalence and frequency of circulating t(14;18)-positive cells in the peripheral blood of healthy individuals. *J Natl Cancer Inst Monogr*. 2008; 39:44–47.
16. Nambiar M, Raghavan SC. Prevalence and analysis of t(14;18) and t(11;14) chromosomal translocations in healthy Indian population. *Ann Hematol*. 2010; 89:35–43. [PubMed: 19488754]
17. Hirt C, Weitmann K, Schuler F, et al. Circulating t(14;18)-positive cells in healthy individuals: Association with age and sex but not with smoking. *Leuk Lymphoma*. 2013; 54:2679–2684.

18. Schmitt C, Balogh B, Grundt A, et al. The bcl-2/IgH rearrangement in a population of 204 healthy individuals: Occurrence, age and gender distribution, breakpoints, and detection method validity. *Leuk Res.* 2006; 30:745–750. [PubMed: 16297448]
19. Ott G, Rosenwald A. Molecular pathogenesis of follicular lymphoma. *Haematologica.* 2008; 93:1773–1776. [PubMed: 19050067]
20. Weinberg OK, Ali WZ, Mariappan MR, Shum C, Levy R, Arber DA. “Minor” BCL2 breakpoints in follicular lymphoma: Frequency and correlation with grade and disease, presentation in 236 cases. *J Mol Diagn.* 2007; 9:530–537. [PubMed: 17652637]
21. Stilgenbauer S, Eichhorst B, Schetelig J, et al. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. *Lancet Oncol.* 2016; 17:768–778. [PubMed: 27178240]

Table 1

Prevalence of MBC, mBC and Odds Ratio Adjusted for Age and Sex

	African American (n = 77)				White (n = 167)			
	No. positive (%)	95%, CI	OR	95%, CI	No. positive (%)	95%, CI	OR	95%, CI
MBC	8 (10.4%)	4.59, 19.45	0.98	0.40-2.42	22 (13.2%)	8.44, 19.26	1.0	-
mBC	3 (3.9%)	0.81, 10.97	-	-	5 (3.0%)	0.98, 6.85	-	-
MBC or mBC	11 (14.3%)	7.35, 24.13	1.26	0.56-2.85	25 (15.0%)	9.93, 21.30	1.0	-

Data are given as the number (percent) of participants with a positive sample along with an exact 95% CI. For the odds ratio, white=reference group.

P values: for positive MBC $P=97$; for either positive MBC or positive mBC $P=58$.

Abbreviations: MBC, major breakpoint cluster; mBC, minor breakpoint cluster; OR, odds ratio

Table 2

Patient Demographics

	Mean Age	Minimum Age	Maximum Age	Females (%)	Males (%)
White	56	18	91	82 (49)	85 (51)
African American	51	31	83	51 (66)	26 (34)

Table 3

Published Series of Prevalence of t(14;18)

Participants' Country of Origin/ Reference	Platform	Tissue ^a	t(14;18) Breakpoints (MBC/mBC)	n	Prevalence	Replicates	DNA µg per Sample	Estimated Cells Screened ^c
Europe^c Roulland 2014 ⁹	qPCR	Total PBL	MBC	218	29%	3	3	0.5 × 10 ⁶
UK Summers 2001 ¹⁴	qPCR	Total PBL	MBC	481	23%	3	1.5	0.25 × 10 ⁶
Germany Dolken 2008 ¹⁵	qPCR	Mono PBL	MBC	644	45%	5	5	0.83 × 10 ⁶
India Nambiar 2010 ¹⁶	Nested PCR	Mono PBL	MBC	253	34%	N/A	N/A	N/A
Japan(J)/Germany(G) Yasukawa 2001 ⁸	Nested PCR	Mono PBL	MBC	241 (J) 75 (G)	16% (J) 52% (G)	0	10	1.7 × 10 ⁶
Germany Hirt 2013 ¹⁷	qPCR	Total PBL	MBC	3966	39%	5	5	0.83 × 10 ⁶
Germany Schmitt 2004 ¹⁸	Nested PCR qPCR	Total PBL	MBC	204	24% 19%	6 4	3 1.2	0.5 × 10 ⁶ 0.2 × 10 ⁶
USA African American Current Study	Semi-Nested PCR	Total PBL	MBC mBC	77	10% 4%	0	0.5	0.08 × 10 ⁶
USA White Current Study	Semi-Nested PCR	Total PBL	MBC mBC	167	13% 3%	0	0.5	0.08 × 10 ⁶

Abbreviations: mBC = minor breakpoint cluster; MCB = major breakpoint cluster; Mono PBL = mononuclear peripheral blood leukocytes; NA, not available; PBL = peripheral blood leukocytes; qPCR = quantitative polymerase chain reaction

^aTotal PBL indicates that mononuclear cells were not separated before assay was performed.

^bCells estimated using the calculation 1 cell = 6 pg DNA (Schmitt et al).¹⁸

^cEPIC cohort = Denmark, France, Germany, Greece, Italy, Netherlands, Norway, Spain, Sweden, and the United Kingdom (England, Wales, Scotland, Ireland).