HHS Public Access

Author manuscript

Cancer Epidemiol. Author manuscript; available in PMC 2017 December 01.

Published in final edited form as:

Cancer Epidemiol. 2016 December ; 45: 47–57. doi:10.1016/j.canep.2016.09.007.

MicroRNA-Related Polymorphisms and Non-Hodgkin Lymphoma Susceptibility in the Multicenter AIDS Cohort Study

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The authors declare no conflicts of interest.

Declaration of Interest: 'Conflicts of interest: none'.

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Abstract

Background—MicroRNAs, small non-coding RNAs involved in gene regulation, are implicated in lymphomagenesis. We evaluated whether genetic variations in microRNA coding regions, binding sites, or biogenesis genes (collectively referred to as miRNA-SNPs) were associated with risk of AIDS-associated non-Hodgkin lymphoma (AIDS-NHL), and serum levels of four lymphoma-related microRNAs.

Methods—Twenty-five miRNA-SNPs were genotyped in 180 AIDS-NHL cases and 529 HIV-infected matched controls from the Multicenter AIDS Cohort Study (MACS), and real-time polymerase chain reaction was used to quantify serum microRNA levels. Adjusted odds ratios (ORs) measured using conditional logistic regression evaluated associations between miRNA-SNPs and AIDS-NHL risk. A semi-Bayes shrinkage approach was employed to reduce likelihood of false-positive associations. Mean ratios (MR) calculated using linear regression assessed associations between miRNA-SNPs and serum microRNA levels.

Results—*DDX20* rs197412, a non-synonymous miRNA biogenesis gene SNP, was associated with AIDS-NHL risk (OR=1.34 per minor allele; 95% CI: 1.02–1.75), and higher miRNA-222 serum levels nearing statistical significance (MR=1.21 per minor allele; 95% CI: 0.98–1.49). MiRNA-196a2 rs11614913 was associated with decreased central nervous system (CNS) AIDS-NHL (CT vs. CC OR=0.52; 95% CI: 0.27–0.99). The minor allele of *HIF1A* rs2057482, which creates a miRNA-196a2 binding site, was associated with systemic AIDS-NHL risk (OR=1.73 per minor allele; 95% CI: 1.12–2.67), and decreased CNS AIDS-NHL risk (OR=0.49 per minor allele; 95% CI: 0.25–0.94).

Conclusions—This study suggests that a few miRNA-SNPs are associated with AIDS-NHL risk and may modulate miRNA expression. These results support a role for miRNA in AIDS-NHL and may highlight pathways to be targeted for risk stratification or therapeutics.

Keywords

DDX20; AIDS-NHL; microRNA; SNPs; microRNA-SNP; Lymphoma; Epidemiology

1.1. Introduction

Non-Hodgkin Lymphoma (NHL) is the most common hematological cancer in adults worldwide, and most frequently diagnosed AIDS-defining cancer among HIV-infected individuals [1–4]. AIDS-related NHL (AIDS-NHL) represents a significant source of morbidity in those infected with HIV-1 and accounts for approximately one-quarter of all AIDS-associated deaths [5–7]. AIDS-NHLs are thought to arise in part due to the loss of immunoregulatory control over Epstein-Barr virus (EBV) -infected B cells resulting from depletion of $CD4^+$ T cells [8–11], and in part due to the chronic B cell activation that accompanies chronic HIV-1 infection [12–14]. The molecular mechanisms for NHL development in the setting of HIV-1 infection are not fully understood, although prior studies suggest that microRNAs (miRNAs) may be important [15–19].

miRNAs are small non-coding RNA sequences that regulate gene expression on the post transcriptional level through interaction with the 3′ untranslated region (UTR) of the target gene messenger RNA [20–23]. miRNAs undergo modifications during their biogenesis through interactions with other molecules, with each interaction highly sequence dependent [24–28]. The importance of miRNAs in lymphomagenesis has been further defined in recent years [29, 30], and it is evident that miRNAs have critical roles in both lymphopoiesis and lymphoma pathogenesis and progression [31, 32]. Aberrant miRNA expression or function has been defined for nearly all lymphomas [33], and distinct miRNA signatures highlight the potential for miRNAs to serve as diagnostic biomarkers [34, 35]. Burkitt lymphoma is characterized by a loss of the oncogene miR-155 [31, 36], while diffuse large B-cell lymphoma overexpresses this miRNA which also plays a critical role in B cell development [37]. Moreover, tumor associated miRNAs have been reported in serum of patients with DLBCL [38], and in blood B cells of patients who develop AIDS-NHL [39]. Genetic variations in microRNA coding regions, binding sites, or biogenesis genes (collectively referred to as miRNA-SNPs) are also able to induce aberrant miRNA expression and function to influence lymphomagenesis and outcomes related to progression [40], treatment response and survival [41, 42]. However, studies evaluating the role of miRNA-related polymorphisms (SNPs) and AIDS-NHL susceptibility are lacking.

In recent work, we found that miRNA expression is associated with subsequent AIDS-NHL development [43–45]. We propose that miRNA-related SNPs disrupt miRNA biogenesis or miRNA binding to target messenger RNA, and may be susceptibility loci for AIDS-NHL [46–50]. To test our hypothesis that miRNA-related SNPs are associated with AIDS-NHL risk, we designed a candidate gene case-control study nested within the Multicenter AIDS Cohort Study (MACS). Further, we sought to assess the potential association between SNPs in miRNA biogenesis genes and serum levels of four miRNAs (miR-21, miR-122, miR-222, and miR-223) with known associations with AIDS-NHL [44].

2.1. Materials and Methods

2.1.1. Study design and population

We conducted a nested case-control study within the MACS [51, 52]. In brief, the MACS is an ongoing multicenter longitudinal study of the natural and treated history of HIV/AIDS.

The MACS includes 7,087 men who have sex with men who have been recruited beginning in 1985 from four U.S. cities (Baltimore, Chicago, Los Angeles, and Pittsburgh). Participants have been re-contacted semi-annually for in-person interview, physical exam, and specimen collection for up to thirty-one years.

The study was approved by the Institutional Review Boards associated with four MACS sites, including the University of California at Los Angeles, USA.

2.1.2. Case and control definitions

Cases were defined as HIV-1 infected MACS participants diagnosed with NHL as of July 2010 with archived cell pellets available for DNA extraction. Cases were based on confirmation by medical records and pathology reports, or in some cases identified by pathology reports at autopsy. One-hundred and eighty-three NHL cases were identified for this study.

Controls were selected from all HIV-1 infected MACS participants who were NHL-free and who had archived cell pellets. Up to 3 controls were matched to each NHL case on recruitment year (1984–1985 or 1987–1991), cancer-free HIV-1 infection-duration time since first HIV-1-positive MACS study visit (i.e., controls had to be followed at least as long as the cases), race (white versus non-white), and $CD4^+$ T-cell count at NHL diagnosis date or, in controls, matched time-point reflecting infection-duration since first HIV-1-positive MACS study visit (0–49 mm^3, 50–99 mm^3, 100–199 mm^3, 200–349 mm^3, 350–499 mm^3, and ≥500 mm^3). Additionally, cases that seroconverted during follow-up were matched to controls that also seroconverted and on time since seroconverting. A total of 533 HIV-1 infected controls were selected.

2.1.3. miRNA-related SNP Selection

Genes and SNPs of high interest given putative biologic plausibility were selected using a combination literature-based and bioinformatic approach. First, we identified miRNAs repeatedly associated with cancer in the literature, including: miR-196a2, miR-26a1, miR-27a, miR-300, and pre-miR-146a [18, 50, 53, 54]. Some of these (e.g., miR-146a and miR-26a1) had previous implications in AIDS-NHL or lymphoma [45, 55, 56]. We used *in silico* approaches to identify SNPs in these miRNA coding regions, and SNPs in target genes of these miRNA that also had some link to AIDS-NHL, including *WWOX*, *IL6R*, *CXCL12* [57, 58], or overall cancer [18, 59]. Next, we identified other key genes associated with NHL or tumorigenic mechanisms, and looked *in silico* for miRNAs that bound to those target genes, and for SNPs within those miRNA coding regions [60–64]. We also selected SNPs from within key-regulatory genes involved in miRNA biogenesis (*DROSHA, XPO5, RAN, DICER1, AGO, DDX20, GEMIN4*), some of which have been implicated in lymphomagenesis [17, 48, 65–68].

For the *in silico* analyses, we used TargetScan 5.2 as the miRNA target prediction algorithm [69–73], UCSC Genome Browser “Blat” to map genomic sequences, and NCBI dbSNP to identify SNPs in target genes and sequences [74]. Only common SNPs were included (minor allele frequency [MAF] ≥5%). In total, 25 SNPs were genotyped within miRNA coding

regions (n=5), miRNA biogenesis genes (n=8), and within or near predicted miRNA target sites (n=12; Table 1).

2.1.4. SNP genotyping

Genomic DNA was extracted from archived peripheral blood mononuclear cell pellets using the QIAamp DNA blood mini kit (Qiagen, Valencia, CA), purified, and then whole genome amplified using the REPLI-g Mini Kit (Qiagen, Valencia, CA).

Genotyping was performed with a customized Fluidigm Dynamic 96.96 Array™ assay [75]. This assay used allele-specific polymerase chain reaction (PCR) SNP detection chemistry with integrated fluidic circuits to perform high throughput SNP genotyping. Tagged, allele specific PCR primers were employed alongside a common reverse primer. A universal probe set was used in every reaction, producing uniform fluorescence. Additionally, Fluidigm provided locus-specific primer sequences that allowed one to confirm target locations.

For quality control, positive controls (samples with known genotypes) and negative controls (samples with no DNA) were included in each reaction plate to evaluate appropriate genotype calling. Further, 5% of study samples were randomly selected and plated in replicate to evaluate genotype concordance. All identifying information for the tested samples, including the identity of the quality control replicates, was unknown to the lab technicians.

2.1.5. Quantification of serum miRNA

miRNAs found to be associated with AIDS-NHL in a previous study were measured for a subset of 77 cases and controls in our study [44]. In brief, serum RNA was extracted using TRIzol LS reagent from Life Technologies (Carlsbad, CA). RNA was resuspended and quantified using Quant-iT RiboGreen RNA Reagent and kit (Molecular Probes, Eugene, OR). Exiqon's Serum/Plasma Focus miRNA PCR panel (Exiqon, Vedbaek, Denmark) was used to measure serum miRNA expression in participants as an initial screening method. Differentially expressed miRNAs were identified from this initial screen using the MultiExperiment Viewer software v4.8. Individual miRNAs found to be differentially expressed were validated using TaqMan miRNA Reverse Transcription kit and TaqMan miRNA Assay kit (Applied Biosystems). Each sample was assayed in triplicate, and the levels of all serum miRNAs were normalized to miR-16 (consistent with screening protocol), and cellular miRNAs were normalized to RNU 48 (small nucleolar RNA), using the following equation: $dCt = Ct_{miRNA}\text{-}Ct_{miR\text{-}16\ (or\ RNU\ 48)}$. The relative expression of miRNAs was calculated using: 2^{-dCt} [44]. Relative miRNA expression data from the validated TaqMan assays were utilized in this analysis.

2.1.6. Statistical Analysis

We excluded SNPs from analysis if they had: (a) genotyping call rate <95%; (b) Hardy-Weinberg equilibrium (HWE) *P*-value <0.002; or (c) duplicate sample genotype concordance <95%. Samples with poor DNA quality were identified and excluded if the sample yielded a SNP call proportion <90%.

Differences in participant characteristics were assessed using Pearson's Chi-square tests (categorical variables) or Student's two-sample T-tests (continuous variables) to estimate reported *P*-values. We calculated adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between miRNA-related SNPs and AIDS-NHL risk using conditional logistic regression. ORs were also calculated for AIDS-NHL subgroups by site, systemic versus primary central nervous system (CNS). We calculated genotype specific ORs, and ORs for a log-additive genetic model. Covariates were included in the models if they were historically associated with AIDS-NHL risk and included age, HIV-1 viral load at set-point (the time point at which there is a natural equilibrium between viral replication and viral clearance, and which is usually achieved several months after initial infection in individuals who are unexposed to anti-retroviral treatment), having a prior AIDS diagnosis, anti-retroviral therapy use, $CD4^+$ T-cell count, race, and hepatitis C infection [9, 76–82]. Missing data for HIV-1 viral load at set-point (n=128) and $CD4^+$ T-cell count (n=56) was imputed using the median values of the control group [83, 84]. The reference date for covariate values (age, $CD4^+$ T-cell count, history of anti-retroviral therapy, and hepatitis C infection) was NHL diagnosis date (cases) or matched date reflecting infection-duration since first HIV-1-positive MACS study visit (controls). For comparison, unadjusted results are presented in Supplemental Table 1.

We used the semi-Bayes (SB) approach to minimize the reporting of false positive results. The SB approach is a shrinkage-based, penalized-likelihood method which regresses estimates toward zero in a manner inversely proportional to their prior variances and proportional to estimated variances [85–89]. Using this method, we augmented our dataset with a prior dataset of null association [$\beta \sim N(0, 0.5)$] to obtain posterior semi-Bayes estimates (OR) and 95% posterior CIs for each estimate [90]. The SB posterior estimates tended to be closer to the null than maximum likelihood estimates from the conditional logistic regression models, with narrower 95% posterior CIs [90]. We present the SB OR estimates and 95% posterior CIs for all miRNA-SNP NHL associations. To account for the possibility of population stratification, we also report SB OR estimates and 95% posterior CIs restricted to self-reported non-Hispanic Whites (NHW) for significant miRNA-SNP NHL associations found among the entire study population. We discuss results that remained statistically significant after SB correction, and note results that remained of marginal (i.e., borderline) significance.

To investigate the associations between SNPs within miRNA biogenesis genes and miRNA serum levels, we calculated mean ratios (MR) and 95% CIs using linear regression, adjusted for AIDS-NHL case status, race, and $CD4^+$ T-cell count at date of serum sample collection. To estimate the mean ratio of miRNA serum levels by genotype, the mean natural log transformed ($\log_e$) miRNA serum level among those with one or more copies of the risk allele was compared to the mean $\log_e$(miRNA) serum level among those with the wild-type genotype, then exponentiated for interpretation. All analyses were conducted using SAS v9.1.3 software (SAS Institute, Cary NC).

3.1. Results

All plated blank and water samples were confirmed as “no calls” using the Fluidigm Genotyping Analysis software. Genotype calls for positive control samples were 100% concordant with known genotypes. All genotyping call rates were above 98% and none of the SNPs in this study were in LD at an r^2 value greater than 0.80 with any other included SNP (Table 1). Three SNPs (*TAB3* rs3816757, *WWOX* rs12828 and *DROSHA* rs10719) and six study participants that did not meet the quality control thresholds were excluded. We investigated 22 SNPs genotyped from 709 MACS participants (180 cases and 529 matched controls).

Table 2 shows select characteristics of the study population. A higher proportion of Hispanic ethnicity and a lower proportion of NHWs were observed among cases than controls (*P*=0.02). Most NHL cases were diagnosed during the period 1984–1995, although 15% were diagnosed during 1996–2006. Cases tended to be older than controls (*P*=0.04). The $CD4^+$ T-cell count was similarly distributed for cases and controls due to the matching criteria. A higher proportion of cases had a prior AIDS diagnosis compared to controls (*P*=0.01), although a similar proportion of cases and controls (6% and ~9%, respectively) were treated with a potent combination of anti-retroviral drugs. Cases had higher plasma HIV-1 RNA levels compared to controls (*P*=0.004). The majority of AIDS-NHL tumors were systemic (68.1%), most of which were diffuse large B-cell lymphomas. Fewer than 50% of cases had adequate tumor tissue available for EBV testing; however the majority of tested NHLs (67%) were EBV positive.

Among SNPs located within miRNA coding regions, individuals with at least one copy of the minor allele (T) of microRNA-196a2 rs11614913 experienced a decrease in CNS AIDS-NHL risk (CT vs. CC: OR=0.52; 95% CI: 0.27–0.99; Table 3). This result remained statistically significant among NHWs (CNS AIDS-NHL CT vs. CC: OR=0.46; 95% CI: 0.23–0.94). While not statistically significant, individuals with one or more copies of the minor allele (C) of microRNA-27 rs895819 experienced a suggested elevation in AIDS-NHL risk (OR=1.29 per minor allele; 95% CI: 0.97–1.73), which was attenuated in NHWs (OR=1.27 per minor allele; 95% CI: 0.92–1.75).

Among SNPs located within miRNA biogenesis genes, individuals with one or more copies of the minor (C) allele of *DDX20* rs197412 were at increased risk of developing AIDS-NHL (OR=1.34 per minor allele; 95% CI: 1.02–1.75). These results remained similar, although not statistically significant, among NHWs (OR=1.33 per minor allele; 95% CI: 0.99–1.78). While not statistically significant, an increased risk was suggested between the minor (C) allele of *GEMIN4* rs7813 and systemic AIDS-NHL (OR=1.26 per minor allele; 95% CI: 0.92–1.72). This association became more pronounced among NHWs, reaching statistical significance (OR=1.47 per minor allele; 95% CI: 1.04–2.08).

Individuals with one or more copies of the minor (T) allele of *HIF1A* rs2057482 were at an increased risk of developing systemic AIDS-NHL (OR=1.73 per minor allele; 95% CI: 1.12–2.67; NHWs OR=1.72 per minor allele; 95% CI: 1.06–2.79). A decreased risk of CNS

AIDS-NHL was evident in association with *HIF1A* rs2057482 (OR=0.49 per minor allele; 95% CI: 0.25–0.94; NHWs OR=0.51 per minor allele; 95% CI: 0.26–1.02).

While *IL15* rs10519613 did not reach a level of statistical significance in adjusted analyses, this miRNA-SNP was suggested to increase risk of AIDS-NHL in unadjusted analyses (uOR=1.40 per minor allele; 95% CI: 0.97–2.03; Supplemental Table 1); a result more pronounced among systemic AIDS-NHL cases (uOR=1.64 per minor allele; 95% CI: 1.08–2.50). Last, the association between *TP53INP1* rs896849 and systemic AIDS-NHL did not reach a level of statistical significance in adjusted analyses, however was suggested to increase risk of systemic AIDS-NHLs in unadjusted analyses (uOR=1.36 per minor allele; 95% CI: 0.93–1.98).

The associations between select SNPs within miRNA biogenesis genes and miRNA-21, miRNA-122, miRNA-222, and miRNA-223 serum levels are presented in Table 4. The minor allele (C) of *DDX20* rs197412 was associated with higher miRNA-21 serum levels, albeit not at a statistically significant level (MR=1.24 per minor allele; 95% CI: 0.97–1.59). Individuals with the homozygous minor genotype exhibited miR-21 relative expression levels of 17.9% compared to 10.8% relative expression in individuals with the homozygous major allele genotype (data not shown). Further, the minor allele (C) of *DDX20* rs197412 was associated with higher miRNA-222 serum levels; a result that neared statistical significance (MR=1.21 per minor allele; 95% CI: 0.98–1.49). Individuals with the homozygous minor allele genotype exhibited miR-222 relative expression levels of 13.2% compared to 7.5% relative expression in individuals with the homozygous major allele genotype (data not shown). Last, while not statistically significant the minor allele (C) of *DDX20* rs197412 was also associated with higher miRNA-223 serum levels (MR=1.31 per minor allele; 95% CI: 0.96–1.78).

4.1. Discussion

We examined the association between 22 miRNA-SNPs and AIDS-NHL susceptibility, and miRNA serum levels. *DDX20* rs197412 was associated with an increase in risk of AIDS-NHL, and marginally (i.e., nearing a statistically significant level) associated with higher levels of miRNA-21, miRNA-222, and miR-223. *DDX20* rs197412 is a non-synonymous miRNA-SNP resulting in a residue change within a RNA helicase gene in the DEAD-box protein family. These suggested associations are in support of growing literature demonstrating that germline variation within miRNA-biogenesis genes may contribute to tumorigenesis [16]. Indeed, miRNA-21, miRNA-222, and miR-223 serum levels have been previously associated with AIDS-NHL risk, and our results suggest that these may have an underlying inherited genetic component [44].

DDX20, also known as GEMIN3, directly binds to and negatively regulates p53, blocking normal tumor suppressive function. Further, DDX20 also directly binds to EBV nuclear antigen 2 and EBV nuclear antigen 3C [91]. Genetic variation in *DDX20* has been shown to affect RNA transport, RNA metabolism and decay, ribosome biogenesis, and RNA translation [92–96]. Although bioinformatic algorithms such as PolyPhen-2 and SIFT suggest that *DDX20* rs197412 is a “benign” and “tolerated” SNP (respectively) [97, 98], and

no other common SNPs appear to be in LD with it, there may be other functionality of this SNP or correlated (minor) SNPs that may be responsible for the observed associations.

The variant allele (T) of microRNA-196a2 rs11614913 was associated with decreased risk of CNS AIDS-NHL. In support of our finding, a recent meta-analysis investigating this miRNA-SNP across 32 studies observed a decrease in overall cancer risk associated with the variant (T) allele of miR-196a2 rs11614913 (T vs. C OR=0.89; 95% CI: 0.84–0.94) [99]. To our knowledge, no studies have investigated miR-196a2 rs11614913 in relation to lymphoma or AIDS-NHL. miR-196a2 is composed of two mature miRNA sequences processed from the same stem-loop, with microRNA-196a2 rs11614913 located within the precursor strand of what becomes the 3′ passenger strand of the primary and mature sequence [54]. A SNP in this location may interfere with the formation of the secondary stem-loop structure, resulting in less efficient miRNA biogenesis and maturation from the precursor miRNA [18]. In fact, prior studies have shown that the minor allele of microRNA-196a2 rs11614913 is associated with decreased mature miR-196a2 levels *in vitro* [54]. Given that this SNP decreases target gene regulation, lowers mature miR-196a2 levels, and is inversely associated with overall cancer development, the tumor suppressive potential of miR-196a2 rs11614913 to impact AIDS-NHL, as seen in our study, is biologically plausible. As with *DDX20* rs197412, no common SNPs were identified to be in high LD with miR-196a2 rs11614913.

The minor (T) allele of *HIF1A* rs2057482 was positively associated with systemic AIDS-NHL risk and inversely associated with CNS AIDS-NHL risk. Per the miRNA-SNP analytic tool PolymirTS-3.0, the minor allele of *HIF1A* rs2057482 creates novel miRNA binding sites for miR-196a-5p, miR-196b-5p and miR-921, among others, opening up the possibility for differential messenger RNA regulation across alleles [100]. Although associations have been suggested between this SNP and other cancers (lung, non-small cell lung, and rectal cancers), this SNP was not observed to have an overall cancer effect in a recent meta-analysis [101]. *HIF1A* encodes a subunit of the heterodimeric transcription factor, hypoxia-inducible factor 1 (HIF1) which is involved in oxygen homeostasis and activates the expression of over 60 genes, including *BCL-XL,* contributing to cell regulation, proliferation and survival [102–105]. In cancer cells an accumulation of genetic alterations are induced by *HIF1A* over-expression, suggesting that *HIF1A* may provide selective advantages for the survival and promotion of cancer cells [106–108]. As, *HIF1A* is over-expressed in lymphoma cells, it is plausible that AIDS-NHL susceptibility may be influenced by SNPs within *HIF1A*.

This study represents the largest composition of AIDS-NHL cases from a single cohort study, and the first investigation into miRNA-related SNPs and AIDS-NHL. However, an important shortcoming is the small number of cases that limited our power to detect modest associations with more rare SNPs, and to discern heterogeneous effects by tumor site and histology [109–111]. Given the modest sample size, there is the possibility that some of these findings could be due to chance. Due to these limitations, our findings need to be replicated in a larger validation cohort and follow-up with functional studies would be beneficial. Furthermore, we recognize that two covariates were imputed; however our results remained robust across analyses comparing estimates from the complete-case analysis,

median imputed analysis, and analyses not adjusting for the imputed covariates (data not shown).

The major strength of our study was the nested case-control design and the selection of AIDS-NHL cases and HIV-1-infected controls from an established, longitudinal prospective cohort with rich biological and epidemiological data. The ability for us to supplement our genotypic data with expression levels of miRNA further enhanced our result interpretation. Detailed covariate data collected at multiple time points helped to account for confounding factors. Last, despite our modest sample size, the application of the semi-Bayesian approach decreased biases due to sparse-data and multiple comparisons by pulling our findings toward the null, while adding confidence to the report of associations that remained statistically significant after this correction.

5.1. Conclusion

We observed a few miRNA-SNPs that were associated with AIDS-NHL susceptibility, and suggest that some SNPs within miRNA biogenesis genes may influence miRNA expression. As the processes of miRNA biogenesis, regulation and target determination are all critically dependent on sequence complementation, SNPs located within these regions have the ability to alter normal miRNA function through interrupting or impairing sequence interaction. Identification of miRNA-SNPs associated with cancer risk is relevant to define potential markers of susceptibility, and may lead to the development of a high-risk intervention strategy for the HIV-infected population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank all of the Multicenter AIDS Cohort Study participants for their time and efforts in supporting this study. The authors also thank our funding sponsors for making this study possible. This research was supported by the National Institutes of Health [T32CA009142 to ZFZ, R01CA168482 to OMM, and K07CA140360 to SKH]; and the UCLA AIDS Institute [FDP-NIH AI028697]. Specimens and data used for this research were collected by the Multicenter AIDS Cohort Study (MACS), which is supported by the U.S. National Institutes of Health (NIH), with centers at: Johns Hopkins University Bloomberg School of Public Health (Joseph Margolick, PI), U01-AI35042; Northwestern University (Steven Wolinsky, PI), U01-AI35039; University of California, Los Angeles (Roger Detels, Otoniel Martinez-Maza, PIs), U01-AI35040; University of Pittsburgh (Charles Rinaldo, PI), U01-AI35041; the Center for Analysis and Management of MACS, Johns Hopkins University Bloomberg School of Public Health (Lisa Jacobson, PI), UM1-AI35043. The MACS is funded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), with additional co-funding from the National Cancer Institute (NCI). MACS data collection is also supported by UL1-TR 001079.

Abbreviations

miRNA	MicroRNA
miRNA-SNPs	MicroRNA-related polymorphisms
AIDS-NHL	AIDS-associated non-Hodgkin lymphoma
MACS	Multicenter AIDS Cohort Study

EBV	Epstein-Barr virus
SNPs	Single nucleotide polymorphisms
OR	Adjusted odds ratios
CIs	Confidence intervals
CNS	Central Nervous System
SB	semi-Bayes
PL	Posterior limits
NHW	non-Hispanic Whites
MR	Adjusted mean ratios
NHL	Non-Hodgkin Lymphoma
UTR	Untranslated region
MAF	Minor allele frequency
HWE	Hardy-Weinberg equilibrium
LD	Linkage disequilibrium
PCR	Polymerase chain reaction
$\log_e$	Natural log transformed
uOR	unadjusted OR

7.1. References

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Highlights

- This study generated data on novel miRNA-related susceptibility loci for AIDS-NHL.
- *DDX20* rs197412 in a miRNA biogenesis gene increases AIDS-NHL risk and miRNA serum levels.
- *HIF1A* rs2057482 creates a miRNA-196a2 binding site and influences AIDS-NHL risk.
- These results suggest biomarkers and miRNA pathways for AIDS-NHL risk stratification.

Table I

Description of genotyped SNPs

Gene	dbSNP ID	Allele; Amino Acid Change	Location and function of SNP	dbSNP MAF Global	Study MAF Overall
miRNA coding regions					
miR-196a2	rs11614913	C→T	Downstream of *HOXC8* and *HOXC9*/Noncoding RNA	0.38	0.38
miR-26a1	rs7372209	C→T	Intron variant within *CTDSPL*/Noncoding RNA	0.25	0.27
miR-27a	rs895819	T→C	Downstream of miR-181c/d and *NANOS3*/Noncoding RNA	0.36	0.33
miR-300	rs12894467	C→T	Exon Chromosome 14q 32.31/Noncoding RNA	0.43	0.41
pre-miR-146a	rs2910164	G→C	Upstream of miR-3142 and *PTTG1* genes/Noncoding RNA	0.38	0.22
miRNA biogenesis genes					
AGO2	rs4961280	C→A	Intron variant	0.14	0.19
DICER1	rs3742330	A→G	3′ UTR	0.15	0.09
DROSHA	rs10719	C→T	3′ UTR	0.47	------
DDX20	rs197412	T→C; [Ile⇒Thr]	Chr1: 112308953/Missense	0.48	0.42
GEMIN4	rs2740348	G→C; [Gln⇒Glu]	Chr17: 648186/Missense	0.12	0.17
GEMIN4	rs7813	C→T[a]; [Arg⇒Cys]	Chr17: 744946/Missense	0.31	0.39
RAN	rs14035	C→T	3′ UTR	0.30	0.31
XPO5	rs11077	A→C	3′ UTR	0.38	0.42
Genes with SNPs near or within a predicted miRNA binding site[b]					
CDK6	rs42031	A→T	3′ UTR; Within 30 base pairs of a putative miR-26 binding site	0.15	0.20
CXCL12	rs1804429	T→G	3′ UTR; Within a putative miR-23a/b binding site	0.05	0.03
E2F2	rs2075993	G→A[a]	3′ UTR; Within 30 base pairs of a putative Let-7 binding site	0.42	0.49
HIF1A	rs2057482	C→T	3′ UTR; Within a putative miR-196a binding site	0.21	0.16
IL15	rs10519613	C→A	3′ UTR; Within a putative miR-203 binding site	0.20	0.10
IL6R	rs4072391	C→T	3′ UTR; Within 30 base pairs of a putative miR-34 Family/miR-23 binding site	0.22	0.20
KRAS	rs9266	C→T	3′ UTR; Within 15 base pairs of a putative miR-181 binding site	0.45	0.47
RCHY1	rs2126852	A→G	3′ UTR; Within 2 base pairs of a putative miR-153 binding site	0.26	0.28
TAB3	rs3816757	C→G	3′ UTR; Within a putative miR-23 binding site	0.21	0.22
TP53INP1	rs7760	T→G	3′ UTR; Within 30 base pairs of a putative miR-24/miR-153 binding site	0.17	0.13

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Gene	dbSNP ID	Allele; Amino Acid Change	Location and function of SNP	dbSNP MAF Global	Study MAF Overall
TP53INP1	rs896849	T→C	3′ UTR; Within a putative miR-155 binding site	0.20	0.17
WWOX	rs12828	A→G	3′ UTR; Within a putative miR-153 binding site	0.44	0.42

[a] Allele changes presented represent those found on HAPMAP/dbSNP. The minor alleles of two SNPs, *E2F2* rs2075993 and *GEMIN4* rs7813, as described in HAPMAP/dbSNP, were found to be the major alleles in our post-genotyped samples. We suspect this is due to the fact that the documented HAPMAP/dbSNP MAFs hovered around 0.50 (rs2075993: HAPMAP MAF A=0.47, major to minor GG/AG/AA; Our data MAF G=0.49 and rs7813: HAPMAP MAF T=0.49; major to minor CC/CT/TT; Our data MAF C=0.39).

[b] miRNA identification and binding location predicted using Target Scan 5.2 in combination with SNP basepair location as identified in dbSNP.

MAF: minor allele frequency.

UTR: untranslated region.

Table II

Demographic Characteristics of AIDS-NHL Cases and HIV$^+$ Controls in the Multicenter AIDS Cohort Study

	All AIDS-NHL Cases	HIV$^+$ Controls	*P*-value[a]
Total, N	180	529	
Reference Year, n (%)[b]			
1984–1989	47 (26.1)	144 (27.2)	
1990–1995	106 (58.9)	302 (57.1)	
1996–2000	21 (11.7)	62 (11.7)	
2001–2006	6 (3.3)	21 (4.0)	0.99
Study Site, n (%)			
Baltimore	44 (24.4)	119 (22.5)	
Chicago	44 (24.4)	129 (24.4)	
Pittsburgh	22 (12.2)	85 (16.1)	
Los Angeles	70 (38.9)	196 (37.1)	0.65
Age, n (%)[c]			
<30	13 (7.2)	33 (6.3)	
30–39	65 (36.1)	231 (43.7)	
40–49	70 (38.9)	211 (39.9)	
≥50	32 (17.8)	54 (10.2)	**0.04**
Self-reported Race/Ethnicity, n (%)			
White, non-Hispanic	152 (84.4)	479 (90.6)	
Black, non-Hispanic	10 (5.6)	21 (4.0)	
White, Hispanic	18 (10.0)	25 (4.7)	
Other	0 (0.0)	4 (0.7)	**0.02**
AIDS diagnosis, n (%)[d]			
No	86 (47.8)	312 (59.0)	
Yes	94 (52.2)	217 (41.0)	**0.01**
HIV-1 RNA levels at set-point (log10 scale; VC/mL; n (%))[e]			
Less than 3 (VC/mL)	2 (1.1)	21 (4.0)	
3–5 (VC/mL)	108 (60.0)	362 (68.4)	
More than 5 (VC/mL)	26 (14.4)	62 (11.7)	
Missing	44 (24.4)	84 (15.9)	0.10
Mean HIV-1 RNA levels at set-point (log10 scale; VC/mL; mean, range; SD)[e]	4.5 (2.6–6.0; 0.6)	4.39 (2.5–5.8; 0.7)	**0.004**
CD4$^+$ T-cell count (mean, range; SD)[e]	176.9 (2.0–923.0; 211.8)	184.1 (3.0–1361.0; 224.7)	0.78
HAART therapy, n (%)[d]			
Never	170 (94.4)	483 (91.3)	
Yes	10 (5.6)	46 (8.7)	0.26
Years from first HAART date to reference date, mean (range; SD)	3.8 (0.1–9.7; 3.0)	2.9 (0.1–9.5; 2.8)	0.29

	All AIDS-NHL Cases	HIV+ Controls	*P*-value[a]
ART therapy, n (%)[d]			
Never	69 (38.3)	154 (29.1)	
Yes	111 (61.7)	375 (70.9)	**0.02**
Years from first ART date to reference date, mean (range; SD)	3.4 (0.1–15.7; 2.6)	3.2 (0.0–17.3; 2.9)	0.52
HCV infection, n (%)[d]			
Never	156 (86.7)	482 (91.1)	
Ever	24 (13.3)	47 (8.89)	0.09
NHL subtypes, n (%)			
Primary Central Nervous System Lymphoma	57 (31.7)	-	
Systemic NHL	123 (68.3)	-	
Diffuse Large B-Cell	36 (29.3)	-	
Diffuse Large B-Cell, Immunoblastic	28 (22.8)	-	
NHL/Lymphoma, Not Specified	32 (26.0)	-	
Burkitt's Lymphoma	21 (17.1)	-	
Others	6 (5.9)	-	
Tumor tested for EBV infection, n (%)			
Tested	86 (47.8)	-	
Not tested	87 (48.3)	-	
Missing	7 (3.9)	-	
Tumor tested positive for EBV infection, n (%)	58 (67.4)	-	

[a]Pearson's Chi-square test or Student's T-test used to estimate *P*-values, as appropriate.

[b]Reference Year: Year of NHL diagnosis in the cases and HIV-infection-duration matched time-point in the controls.

[c]At NHL diagnosis or reference date (date of NHL diagnosis in the cases and HIV-infection-duration matched time-point in the controls).

[d]Prior to NHL diagnosis or reference date.

[e]Before HAART therapy.

VC/mL: Viral copies per milliliter; SD: standard deviation.

Table III

Associations between miRNA-SNPs and AIDS-NHL risk in the Multicenter AIDS Cohort Study

		Overall AIDS-NHL		Systemic AIDS-NHL		CNS AIDS-NHL	
		Ca/Co[a]	OR (95% CI)[b]	Ca/Co[a]	OR (95% CI)[b]	Ca/Co[a]	OR (95% CI)[b]
microRNA coding regions							
miR-196a2 rs11614913	CC	72/196	1.00 (Ref)	41/138	1.00 (Ref)	31/58	1.00 (Ref)
	CT	88/257	0.91 (0.63–1.31)	67/169	1.19 (0.76–1.86)	21/88	**0.52 (0.27–0.99)**[c]
	TT	19/76	0.63 (0.36–1.10)	14/58	0.69 (0.36–1.32)	5/18	0.72 (0.30–1.75)
	Log-Add	179/529	0.80 (0.62–1.05)	122/365	0.89 (0.65–1.23)	57/164	0.62 (0.37–1.02)
miR-26a1 rs7372209	CC	94/276	1.00 (Ref)	61/180	1.00 (Ref)	33/96	1.00 (Ref)
	CT	77/215	1.25 (0.87–1.81)	56/155	1.27 (0.83–1.96)	21/60	1.35 (0.71–2.56)
	TT	9/38	0.84 (0.42–4.67)	6/30	0.78 (0.36–1.69)	3/8	1.16 (0.40–3.40)
	Log-Add	180/529	1.06 (0.79–1.42)	123/365	1.03 (0.74–1.45)	57/164	1.31 (0.74–2.32)
miR-27a rs895819	TT	67/242	1.00 (Ref)	50/170	1.00 (Ref)	17/72	1.00 (Ref)
	TC	92/242	1.23 (0.84–1.82)	58/162	1.12 (0.71–1.75)	34/80	1.41 (0.71–2.78)
	CC	21/43	1.56 (0.85–2.85)	15/32	1.41 (0.70–2.85)	6/11	1.51 (0.60–3.77)
	Log-Add	180/527	1.29 (0.97–1.73)	123/364	1.21 (0.85–1.73)	57/163	1.45 (0.87–2.40)
miR-300 rs12894467	CC	58/183	1.00 (Ref)	38/114	1.00 (Ref)	20/69	1.00 (Ref)
	CT	85/265	0.99 (0.66–1.48)	57/198	0.83 (0.51–1.34)	28/67	1.29 (0.66–2.53)
	TT	37/80	1.31 (0.79–2.17)	28/52	1.40 (0.78–2.52)	9/28	1.09 (0.47–2.55)
	Log-Add	180/528	1.14 (0.87–1.49)	123/364	1.16 (0.84–1.60)	57/164	1.14 (0.70–1.85)
pre-miR-146a rs2910164	GG	110/314	1.00 (Ref)	72/204	1.00 (Ref)	38/110	1.00 (Ref)
	GC	62/193	0.98 (0.67–1.43)	45/145	0.98 (0.63–1.53)	17/48	1.03 (0.54–1.97)
	CC	8/22	0.93 (0.44–1.97)	6/16	0.96 (0.42–2.18)	2/6	1.12 (0.35–3.55)
	Log-Add	180/529	0.97 (0.71–1.32)	123/365	0.97 (0.68–1.40)	57/164	1.08 (0.60–1.94)
Functional SNPs in miRNA biogenesis genes							
AGO2 rs4961280	CC	117/348	1.00 (Ref)	75/241	1.00 (Ref)	42/107	1.00 (Ref)
	CA	56/167	1.00 (0.68–1.49)	43/114	1.23 (0.77–1.97)	13/53	0.64 (0.32–1.26)
	AA	7/14	1.29 (0.57–2.94)	5/10	1.25 (0.50–3.13)	2/4	1.09 (0.34–3.45)

		Overall AIDS-NHL		Systemic AIDS-NHL		CNS AIDS-NHL	
		Ca/Co[a]	**OR (95% CI)[b]**	**Ca/Co[a]**	**OR (95% CI)[b]**	**Ca/Co[a]**	**OR (95% CI)[b]**
	Log-Add	180/529	1.08 (0.77–1.51)	123/365	1.23 (0.83–1.84)	57/164	0.75 (0.41–1.36)
DICER1 rs3742330	AA	150/430	1.00 (Ref)	102/297	1.00 (Ref)	48/133	1.00 (Ref)
	AG	29/96	0.94 (0.59–1.50)	20/66	0.95 (0.55–1.65)	9/30	0.85 (0.40–1.81)
	GG	1/3	1.01 (0.31–3.32)	1/2	1.04 (0.31–3.48)	0/1	NAC
	Log-Add	180/529	0.95 (0.62–1.47)	123/365	0.97 (0.59–1.62)	57/164	0.84 (0.40–1.77)
DDX20 rs197412	TT	54/189	1.00 (Ref)	40/140	1.00 (Ref)	14/49	1.00 (Ref)
	TC	87/254	1.27 (0.86–1.88)	60/169	1.39 (0.88–2.19)	27/85	0.94 (0.48–1.86)
	CC	39/86	1.67 (1.00–2.80)	23/56	1.48 (0.80–2.72)	16/30	1.68 (0.74–3.81)
	Log-Add	180/529	**1.34 (1.02–1.75)**	123/365	1.31 (0.95–1.80)	57/164	1.39 (0.84–2.29)
GEMIN4 rs2740348	GG	123/376	1.00 (Ref)	81/254	1.00 (Ref)	42/122	1.00 (Ref)
	GC	49/134	1.10 (0.74–1.66)	36/100	1.05 (0.65–1.69)	13/34	1.13 (0.56–2.29)
	CC	8/18	1.02 (0.47–2.25)	6/10	1.30 (0.53–3.18)	2/8	0.81 (0.26–2.46)
	Log-Add	180/528	1.07 (0.77–1.48)	123/364	1.13 (0.77–1.66)	57/164	0.96 (0.53–1.76)
GEMIN4 rs7813	TT	67/200	1.00 (Ref)	41/133	1.00 (Ref)	26/67	1.00 (Ref)
	TC	78/242	1.05 (0.72–1.54)	56/173	1.12 (0.70–1.78)	22/69	0.89 (0.48–1.66)
	CC	35/85	1.33 (0.81–2.17)	26/57	1.51 (0.84–2.71)	9/28	1.09 (0.49–2.43)
	Log-Add	180/527	1.15 (0.89–1.49)	123/363	1.26 (0.92–1.72)[c]	57/164	1.00 (0.65–1.56)
RAN rs14035	CC	85/257	1.00 (Ref)	60/182	1.00 (Ref)	25/75	1.00 (Ref)
	CT	73/222	1.06 (0.72–1.54)	50/154	1.12 (0.72–1.73)	23/68	0.87 (0.45–1.70)
	TT	22/50	1.25 (0.72–2.17)	13/29	1.24 (0.64–2.43)	9/21	1.25 (0.55–2.82)
	Log-Add	180/529	1.12 (0.85–1.46)	123/365	1.14 (0.83–1.59)	57/164	1.09 (0.69–1.72)
XPO5 rs11077	AA	62/175	1.00 (Ref)	44/115	1.00 (Ref)	18/60	1.00 (Ref)
	AC	82/265	0.98 (0.66–1.46)	55/189	0.86 (0.53–1.38)	27/76	1.20 (0.61–2.37)
	CC	34/89	1.10 (0.66–1.82)	23/61	1.02 (0.56–1.85)	11/28	1.18 (0.53–2.63)
	Log-Add	178/529	1.05 (0.80–1.37)	122/365	0.98 (0.71–1.37)	56/164	1.15 (0.72–1.85)
Candidate genes with SNPs near or within a predicted miRNA binding site							
CDK6 rs42031	AA	116/335	1.00 (Ref)	80/237	1.00 (Ref)	36/98	1.00 (Ref)
	AT	55/174	0.96 (0.65–1.41)	37/116	1.01 (0.63–1.62)	18/58	0.87 (0.46–1.64)

		Overall AIDS-NHL		Systemic AIDS-NHL		CNS AIDS-NHL	
		Ca/Co[a]	OR (95% CI)[b]	Ca/Co[a]	OR (95% CI)[b]	Ca/Co[a]	OR (95% CI)[b]
	TT	8/19	1.21 (0.56–2.60)	5/11	1.15 (0.47–2.81)	3/8	1.20 (0.42–3.45)
	Log-Add	179/528	1.03 (0.75–1.42)	122/364	1.06 (0.72–1.56)	57/164	1.00 (0.59–1.69)
CXCL12 rs1804429	TT	162/498	1.00 (Ref)	112/345	1.00 (Ref)	50/153	1.00 (Ref)
	TG	18/30	1.54 (0.84–2.84)	11/20	1.33 (0.66–2.71)	7/10	1.63 (0.63–4.22)
	GG	0/1	NAC	0/0	NAC	0/1	NAC
	Log-Add	180/529	1.37 (0.76–2.47)	123/365	1.33 (0.66–2.71)	57/164	1.26 (0.53–2.98)
E2F2 rs2075993	AA	43/142	1.00 (Ref)	30/102	1.00 (Ref)	13/40	1.00 (Ref)
	AG	92/252	1.33 (0.85–2.08)	63/165	1.39 (0.84–2.32)	29/87	1.19 (0.57–2.46)
	GG	45/134	1.12 (0.67–1.88)	30/97	1.05 (0.59–1.89)	15/37	1.19 (0.51–2.78)
	Log-Add	180/528	1.07 (0.81–1.40)	123/364	1.04 (0.76–1.43)	57/164	1.16 (0.68–1.97)
HIF1A rs2057482	CC	125/369	1.00 (Ref)	79/266	1.00 (Ref)	46/103	1.00 (Ref)
	CT	49/147	1.09 (0.72–1.65)	40/92	**1.67 (1.04–2.70)**	9/55	**0.42 (0.19–0.94)**
	TT	6/12	1.17 (0.49–2.77)	4/6	1.64 (0.57–4.67)	2/6	0.69 (0.24–1.96)
	Log-Add	180/528	1.11 (0.78–1.57)	123/364	**1.73 (1.12–2.67)**[c]	57/164	**0.49 (0.25–0.94)**
IL15 rs10519613	CC	135/433	1.00 (Ref)	89/303	1.00 (Ref)	46/130	1.00 (Ref)
	CA	42/91	1.35 (0.87–2.08)	32/57	1.47 (0.89–2.44)	10/34	0.99 (0.46–2.15)
	AA	2/5	1.07 (0.67–3.15)	2/5	1.11 (0.38–3.29)	0/0	NAC
	Log-Add	179/529	1.29 (0.87–1.91)	123/365	1.38 (0.89–2.15)	56/164	0.99 (0.46–2.15)
IL6R rs4072391	CC	117/337	1.00 (Ref)	86/236	1.00 (Ref)	31/101	1.00 (Ref)
	CT	57/169	1.00 (0.69–1.44)	36/114	0.94 (0.60–1.48)	21/55	1.22 (0.65–2.30)
	TT	6/22	0.79 (0.36–1.73)	1/14	0.50 (0.18–1.37)	5/8	1.38 (0.51–3.73)
	Log-Add	180/528	0.93 (0.68–1.27)	123/364	0.77 (0.52–1.16)	57/164	1.31 (0.79–2.17)
KRAS rs9266	CC	52/151	1.00 (Ref)	37/114	1.00 (Ref)	15/37	1.00 (Ref)
	CT	85/261	0.87 (0.57–1.34)	61/177	1.02 (0.62–1.67)	24/84	0.60 (0.29–1.23)
	TT	42/117	0.90 (0.55–1.47)	24/74	0.89 (0.50–1.60)	18/43	0.84 (0.39–1.80)
	Log-Add	179/529	0.94 (0.72–1.22)	122/365	0.94 (0.69–1.29)	57/164	0.86 (0.54–1.37)
RCHY1 rs2126852	AA	102/272	1.00 (Ref)	75/187	1.00 (Ref)	27/85	1.00 (Ref)
	AG	67/210	0.91 (0.63–1.32)	39/147	0.74 (0.47–1.16)	28/63	1.37 (0.73–2.56)

		Overall AIDS-NHL		Systemic AIDS-NHL		CNS AIDS-NHL	
		Ca/Co[a]	**OR (95% CI)[b]**	**Ca/Co[a]**	**OR (95% CI)[b]**	**Ca/Co[a]**	**OR (95% CI)[b]**
	GG	11/47	0.64 (0.33–1.22)	9/31	0.71 (0.34–1.45)	2/16	0.65 (0.23–1.80)
	Log-Add	180/529	0.82 (0.61–1.09)	123/365	0.76 (0.54–1.06)	57/164	0.97 (0.58–1.62)
TP53INP1 rs7760	TT	134/407	1.00 (Ref)	91/285	1.00 (Ref)	43/122	1.00 (Ref)
	TG	42/111	1.02 (0.67–1.54)	29/73	1.15 (0.71–1.86)	13/38	0.76 (0.37–1.57)
	GG	4/11	0.99 (0.38–2.57)	3/7	1.13 (0.40–3.22)	1/4	0.80 (0.24–2.67)
	Log-Add	180/529	1.01 (0.71–1.44)	123/365	1.15 (0.76–1.75)	57/164	0.72 (0.37–1.38)
TP53INP1 rs896849	TT	121/375	1.00 (Ref)	84/266	1.00 (Ref)	37/109	1.00 (Ref)
	TC	50/140	1.03 (0.69–1.52)	32/91	1.02 (0.64–1.63)	18/49	0.96 (0.50–1.86)
	CC	9/13	1.58 (0.70–3.52)	7/7	2.06 (0.82–5.18)	2/6	0.85 (0.28–2.57)
	Log-Add	180/528	1.16 (0.83–1.62)	123/364	1.29 (0.87–1.92)	57/164	0.89 (0.50–1.60)

[a] Cases and controls matched on: cohort (1 or 2), date of infection (continuous); duration of follow-up (continuous); seroconversion status (categorical); race (white or non-white); and $CD4^+$ T-cell count at date of matching (categorical).

[b] Adjusted for age at case diagnosis or reference date in controls (continuous); HIV-1 RNA levels before set point (continuous); AIDS diagnosis prior to case diagnosis or reference date in controls (reference=No); history of ART Treatment (reference=No); $CD4^+$ T-cell at reference date (continuous); race (reference=non-Hispanic White); and history of HCV infection (reference=No).

[c] Result remained statistically significant in self-reported non-Hispanic White (NHW) subgroup analyses.

NAC: not able to calculate due to limited sample size.

Ca/Co: numbers of cases and controls.

CNS: Central nervous system.

Log-Add: Log-additive model used for effect estimation.

Cancer Epidemiol. Author manuscript; available in PMC 2017 December 01.

Table IV

Associations between selected miRNA biogenesis gene SNPs and miRNA serum levels among a subgroup of 77 study participants

		microRNA-21 serum levels	microRNA-122 serum levels	microRNA-223 serum levels	microRNA-222 serum levels
Genotype	***N***	**MR (95% CI)[a]**	**MR (95% CI)[a]**	**MR (95% CI)[a]**	**MR (95% CI)[a]**
DDX20 rs197412					
TT	26	1.00 (Reference)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
TC	37	1.31 (0.89–1.92)	0.98 (0.33–2.91)	1.29 (0.80–2.09)	1.33 (0.96–1.84)
CC	14	1.51 (0.90–2.53)	1.92 (0.44–8.36)	1.72 (0.90–3.31)	1.40 (0.90–2.17)
Log-Add	77	1.24 (0.97–1.59)	1.29 (0.64–2.60)	1.31 (0.96–1.78)	1.21 (0.98–1.49)
RAN rs14035					
CC	38	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
CT	34	0.72 (0.51–1.01)	0.45 (0.17–1.18)	0.69 (0.45–1.06)	0.83 (0.61–1.11)
TT	5	0.85 (0.43–1.68)	0.82 (0.12–5.60)	1.43 (0.61–3.35)	0.98 (0.54–1.77)
Log-Add	77	0.81 (0.62–1.06)	0.64 (0.30–1.36)	0.91 (0.65–1.28)	0.90 (0.72–1.14)
XPO5 rs11077					
AA	22	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
AC	41	0.83 (0.56–1.23)	0.82 (0.27–2.52)	1.46 (0.90–2.37)	0.97 (0.69–1.35)
CC	14	0.75 (0.45–1.23)	0.52 (0.13–2.19)	1.46 (0.78–2.74)	0.80 (0.52–1.23)
Log-Add	77	0.86 (0.67–1.10)	0.73 (0.36–1.48)	1.24 (0.91–1.68)	0.90 (0.73–1.12)

[a]Adjusted for AIDS-NHL case indicator (reference=No); race (reference=non-Hispanic White); and $CD4^+$ T-cell count at date of serum sample (continuous).

MR: Mean ratio, the exponentiated ratio of the mean $\log_e$(miRNA) serum level among those with one or more copies of the risk allele, to the mean $\log_e$(miRNA) serum level among those with the wild-type genotype.