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RESEARCH ARTICLE

Genome-wide transcriptome differences associated with perceived discrimination in an urban, community-dwelling middle-aged cohort

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Abstract

Discrimination is a social adversity that is linked to several age-related outcomes. However, the molecular drivers of these observations are poorly understood. Social adverse factors are associated with proinflammatory and interferon gene expression, but little is known about whether additional genes are associated with discrimination among both African American and White adults. In this study, we examined how perceived discrimination in African American and White adults was associated with genome-wide transcriptome differences using RNA sequencing. Perceived discrimination was measured based on responses to self-reported lifetime discrimination and racial discrimination. Differential gene expression and pathway analysis were conducted in a cohort ($N = 59$) stratified by race, sex, and overall discrimination level. We found 28 significantly differentially expressed genes associated with race among those reporting high discrimination. Several of the upregulated genes for African American versus White adults reporting discrimination were related to immune function *IGLV2-11*, *S100B*, *IGKV3-20*, and *IGKV4-1*; the most significantly downregulated genes were associated with immune modulation and cancer, *LUCAT1*, *THBS1*, and *ARPIN*. The most enriched gene ontology biological process between African American and White men reporting high discrimination was the regulation of cytokine biosynthetic processes. The immune response biological process was significantly lower for African American women compared to White women reporting high discrimination. Discrimination was associated with the expression of small nucleolar RNAs, long noncoding RNAs, and microRNAs associated with energy homeostasis, cancer, and actin. Understanding the pathways through which adverse social factors like discrimination are associated with gene expression is crucial in advancing knowledge of age-related health disparities.

KEYWORDS
discrimination, gene expression, psychosocial stress, race, RNA sequencing

1 | INTRODUCTION

Recently, attention has focused on the significance of adverse social and environmental factors in the determination of health outcomes. Studies have shown that low socioeconomic status (SES), poor housing, poverty, and social stress can increase an individual's susceptibility to a variety of adverse chronic health conditions, including chronic kidney disease, cardiovascular disease, cancer, and mortality.[1–4] Race, a social construct, is another important social determinant of health, as multiple studies reveal differences in life expectancy and excess mortality among different ethnic and racial groups within the United States.[5,6]

In attempts to understand the relationship between social factors and health disparities, previous research has identified alterations in molecular and physiological processes associated with social determinants of health. Continued work in social genomics may allow for a better understanding of the biological processes connected with social adversity. In longstanding work from Steven Cole and colleagues, similarities were observed in the gene expression profiles of the immune cells derived from individuals facing a variety of adverse social conditions. The ubiquity of the pattern across different animal species has given rise to the conserved transcriptional response to adversity (CTRA).[7,8] CTRA is characterized as an increase in expression of genes regulating inflammation in conjunction with a decrease in expression of innate antiviral response and antibody production genes.[7] Thus far, various aspects of social adversity have been examined in the context of CTRA including loneliness,[8] low SES,[9] psychological well-being,[10] and grief.[11] Most of these studies were conducted in cohorts where the majority were White individuals or different race/ethnicities were included but not examined separately. Recent data examining the stress of neighborhood violence on Black mothers indicated that CTRA genes were not altered by this stress, but rather higher levels of genes regulated by the glucocorticoid receptor (GR).[12] Transcriptomic analysis of whole blood from African American adults indicated that five genes of the CTRA had good predictive power for SES while 55 other genes had the best predictive power for SES.[13] In addition, previous work from our group found that expression levels of both long noncoding RNA and mRNAs were altered with poverty status and age in African American and White middle-aged men.[14] Collectively, current data indicate that social adversity may be associated with different transcriptional programs in African American and White adults and that it is important to consider race when comparing transcriptional changes.

Discrimination is a chronic, psychosocial stressor that targets social status, such as racial and gender discrimination, whether it may be day-to-day or lifetime discrimination[15] and can be characterized on multiple levels from the individual perspective to society-at-large.[16] In addition to psychological distress, perceived discrimination or the experience of discriminatory treatment is associated with a host of negative health outcomes, including longitudinal increases in blood pressure,[17] inflammation,[18] coronary artery calcification,[19] cardiovascular reactivity,[20] poorer sleep,[21] increased depression, and worse self-reported health,[22] in addition to the incidence of metabolic syndrome[23] and cardiovascular disease. Our previous work has extensively explored perceived discrimination via various physiological measures. We have found that in African American adults, perceived discrimination was associated with red blood cell oxidative stress,[24] poor kidney function,[25] and greater white matter lesion volume.[26]

Thus far, limited data exist on whether the psychological stressor, discrimination, is associated with transcriptomic changes. In a cohort of HIV-positive ($n=37$) and HIV-negative ($n=35$) African American and White individuals, NF-κB and AP-1 pro-inflammatory pathways, IRF factors involved in type I IFN signaling and GR signaling pathways were upregulated in African American versus White individuals.[27] Subsequently, the authors found that perceived discrimination partially mediated the race-related differences in gene expression in some but not all of the identified pathways.[27] Higher CTRA gene expression was reported in Black and Latino men who have sex with men in those who had experienced homophobic victimization[28] and in Black trauma survivors ($n=94$) who experienced high levels of racial discrimination following an acute traumatic injury.[29] Notably, a recent report compiled a set of "socially sensitive genes" ($n=1854$),[30] but no significant genes were associated with discrimination. In a follow-up study, the authors found that discrimination was associated with inflammation/immune response and inflammation-related gene sets.[31] Therefore, a gap still remains in our knowledge about which biological processes may be most affected by discrimination. More comprehensive exploratory/discovery analyses are needed.

The field can benefit from transcriptome-wide analyses that provide a comprehensive assessment of the genes and transcriptional processes important to discrimination. This broad overview extends beyond CTRA and may lead to the discovery of novel molecular pathways. In this study, we applied this framework to examine genome-wide transcriptional changes associated with racial and lifetime discrimination in an urban cohort of African American and White middle-aged adults.

2 | METHODS

2.1 | Cohort selection

Participants were selected from the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study of the National Institute on Aging Intramural Research Program (NIA IRP).[32] The goal of the HANDLS study is to longitudinally examine age-associated health disparities in relation to race and poverty status in middle-aged urban-dwelling African American and White adults living in Baltimore, Maryland. Poverty status was defined as living above or below 125% of the US Federal Poverty Guidelines based on household size and income at enrollment.[33] HANDLS has received approval from the Institutional Review Board of the National Institutes of Health and all participants have given written, informed consent. Participants for the RNA sequencing cohort had completed a series of questionnaires and submitted a blood sample during the first wave of HANDLS data collection (2004–2009). Individuals with HIV/AIDS or hepatitis B/C diagnoses were excluded in establishing the cohort, and only participants aged 45–64 years were eligible. The final cohort ($n = 60$) was selected using a factorial design across sex at birth and race (African American or White) for those with high or low perceived discrimination (Table 1).

TABLE 1 Description of study cohort ($n = 60$) from the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study.

	Low perceived discrimination ($n = 28$)	High perceived discrimination ($n = 32$)
Race, *n* (%)		
African American	14 (50)	16 (50)
White	14 (50)	16 (50)
Sex, *n* (%)		
Men	14 (50)	16 (50)
Women	14 (50)	16 (50)
Poverty status, *n* (%)		
Above	21 (75)	22 (69)
Below	7 (25)	10 (31)
Age in years, mean (SD)	55.3 (5.6)	53.4 (5.6)
Lifetime discrimination score, mean (SD)	2 (0)	6.3 (1.0)
Racial discrimination score, mean (SD)	6 (0)	10.2 (1.1)

Note: Final sequencing cohort was 59 participants due to one sequencing sample having low quality.

2.2 | Measurement of discrimination

Two assessments were combined for an overall perceived discrimination level: lifetime discrimination and racial discrimination. Lifetime discrimination was assessed through a two-item questionnaire asking if discrimination interfered with the participant's life and if life was harder due to discrimination with possible responses of "not at all," "a little," "some," and "a lot," which were, respectively, assigned scores of 1, 2, 3, and 4.[34] Racial discrimination was assessed through a six-item questionnaire asking if the participant experienced discrimination at school, at work, from the police/court, or when obtaining employment, housing, and medical care with possible responses of "no" and "yes," which were, respectively, assigned scores of 1 and 2.[35] The scores for each questionnaire were summed with possible values of 2–8 for lifetime discrimination and 6–12 for racial discrimination. Low overall perceived discrimination was classified as minimal scores for both questionnaires; 2 for lifetime discrimination and 6 for racial discrimination. High overall perceived discrimination was classified as a lifetime discrimination score ≥5 and a racial discrimination score ≥9.

2.3 | Total RNA Sequencing

Peripheral blood mononuclear cells (PBMCs) were isolated from fasting blood samples prior to storage at −80°C.[36] Total RNA from PBMCs was isolated using TRIzol® (Life Technologies) in accordance with manufacturer's guidelines, with the addition of DNase treatment and overnight precipitation. Each RNA pellet was resuspended in 12 μL of RNase-free water and stored at −80°C until further use. Total RNA quantity and quality were analyzed using an Agilent TapeStation.

Total RNA samples were next depleted of both cytoplasmic and mitochondrial rRNA using Ribo Zero Gold (TruSeq Stranded Total RNA Library Prep Kit with

Ribo-Zero Gold; Illumina) and then fragmented using divalent cations under elevated temperatures. Next-generation RNA-seq was conducted using HiSeq® SBS Kit v4 (FC-401-4002), HiSeq® SR Cluster Kit v4 – cBot™ (GD-401-4001), and TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero Gold Set B (RS-122-2302). The products were then purified using RNAClean XP Beads. First-strand cDNA was generated using SuperScript II Reverse Transcript (Thermo Fisher Scientific). Second-strand synthesis was achieved using DNA Polymerase I and RNaseH and the products were purified using AMPure XP Beads.

The double-stranded cDNAs were prepared for ligation of the multiple indexing adapters by adenylating the 3′ blunt ends of the fragments. One adenine (A) nucleotide was added to the blunt fragments to prevent them from ligating each other during the adapter ligation reaction. One corresponding thymine (T) nucleotide present on the 3′ end of each of the indexing adapters provides a complementary overhang for ligating to the fragment. This strategy ensures a low rate of chimera (concatenated template) formation. The fragments were then purified using a two-stage AmpPure XP bead cleanup. Only fragments with an adapter on both ends can both bind to the surface-bound primers on a flow cell and form clusters.

To enrich the DNA for these fragments, and to amplify the amount of DNA in the library, 15 cycles of PCR were used followed by an additional AmPureXP bead cleanup. All 60 libraries were pooled and then clustered using an eight-lane high-density flow cell (HiSeq® SR Cluster Kit v4 – cBot™), on an Illumina cBot-2. These clustered flow cells were then sequenced on an Illumina HiSeq 2500 DNA sequencer for 141 cycles with an additional eight sequencing cycles to identify the sample indexes (HiSeq® SBS Kit v4). The libraries were sequenced on Illumina HiSeq 2500 and the images were processed with the corresponding Illumina RTA software version v1.18.66.3 and base-calling was performed using bcl-2fastq v2.18.0.12. Libraries were generated with single-end sequencing.

2.4 | Bioinformatics analyses

FASTQ files were trimmed for adapter sequences using Cutadapt version v1.15.[37] FastQC (v0.11.9) was then performed on the FASTQ files to evaluate quality control metrics.[38] The FASTQ files were aligned to human genome GRCh38 (hg38) Ensembl v100 using Spliced Transcripts Alignment to a Reference (STAR) software version 2.7.3a.[39] featureCounts from the subread module version 2.0.3 were used to create gene counts, with the strand-specific option set to reverse strand.[40] Sequence alignment information including the number of input reads and mapping information are included in Table S1. Out of the 60 samples that were sequenced, one sample was removed due to a low percentage of uniquely mapped reads. The final sequencing cohort was 59 samples. Differential expression analysis of the gene counts was done using DESeq2 version 1.30.1.[41] Only genes with greater than or equal to 10 counts in every sample were included for differential expression analysis (total of 13 217 genes; Table S2) as recommended by the DESeq2 vignette (https://bioconductor.org/packages/devel/bioc/vignettes/DESeq2/inst/doc/DESeq2.html#pre-filtering). The grouping method for examining interactions as recommended by DESeq2 was used for differential gene expression analysis (https://github.com/thelovelab/DESeq2/blob/devel/R/results.R) and a priori contrasts for comparisons of interest were set across multiple variables. The false discovery rate (FDR) was used to adjust *p*-values for multiple comparison testing. Significant, differentially expressed genes were defined as having a fold change absolute value of ≥2, and FDR-adjusted *p*-value <.05. RNA-sequencing data are located in GEO database # GSE244654.

Parametric analysis of gene set enrichment (PAGE) was used to discover significant gene ontology (GO) terms.[42] For each comparison of interest, the gene names and $\log_2$-transformed fold change values for all 13 217 genes that met our threshold of detection were extracted from the differential gene expression results table. These genes were then used as input for the pathway analysis (per respective comparison of interest) and for data quality control assessment. PAGE was used to ascertain significant gene ontology (GO) terms as previously described ([43] and references within). Significance for GO terms was defined as having at least three genes and a maximum of 300 genes in the gene set, and a *p*-value and its corrected FDR both <.05. Ingenuity pathway analysis (IPA) was utilized to identify disease and function-related pathways using the same significant gene selection cutoff process described above. Significant diseases and function pathways were defined as having a Benjamini–Hochberg (B–H) *p*-value <.05.

3 | RESULTS

Differentially expressed genes (DEGs) were first examined by overall discrimination status (high vs. low). No significant DEGs were identified in high versus low discrimination status groups, even when adjusted for race, sex, poverty status, and age. There were also no significant DEGs associated with poverty status and discrimination

status. We then examined significant DEGs by sex and discrimination status and found that no significant DEGs were identified in men with high discrimination compared to men with low discrimination, or in women with high discrimination compared to women with low discrimination. In men with high discrimination compared to women with high discrimination, only one gene, mitochondrially encoded cytochrome C oxidase 1 pseudogene 12 (*MTCO1P12*), was significantly decreased ($\log_2$ fold change −2.83, Table S3).

There were no significant differences in White participants with high discrimination compared to White participants with low discrimination, or in African American participants with high discrimination compared to African American participants with low discrimination. However, we identified 28 significant DEGs (10 decreased expression, 18 increased expression) in African American participants with high discrimination compared to White participants with high discrimination (Figure 1A, Table S3). Of these 28 DEGs, seven were associated with immune responses and inflammation (*IGLV2-11*, *IGKV3-20*, *IGKV4-1*, *IGHV1-46*, *IGKV3-11*, *IGKV3-15*, *IL3RA*, and *LUCAT1*). The others were associated with tumorigenesis, energy homeostasis, calcium binding, and mitochondrial function.

There were six significant DEGs (four decreased and two increased) identified in African American women with high discrimination compared to White women with high discrimination (Figure 1B, Table S3). Two of these six DEGs have been previously associated with cancer (*SNORD3C* and *MIR155HG*). In African American men with high discrimination compared to White men with high discrimination, five significant DEGs were identified (Figure 1C, Table S3), and associated with immune responses and inflammation. In White men with high discrimination compared to White women with high discrimination, only one gene, *IFIT2*, was significantly decreased ($\log_2$ fold change −1.63, Table S3). Two significant DEGs, *IGHG2* ($\log_2$ fold change 3.05) and snoRNA gene *SNORD3C* ($\log_2$ fold change −6.22), were found in African American men with high discrimination compared to African American women with high discrimination (Table S3).

To understand the potential biological pathways associated with discrimination, we performed PAGE analysis to identify GO biological processes and GO molecular functions. There were 123 significant GO biological processes and 88 GO molecular functions identified in the African American men with high discrimination versus

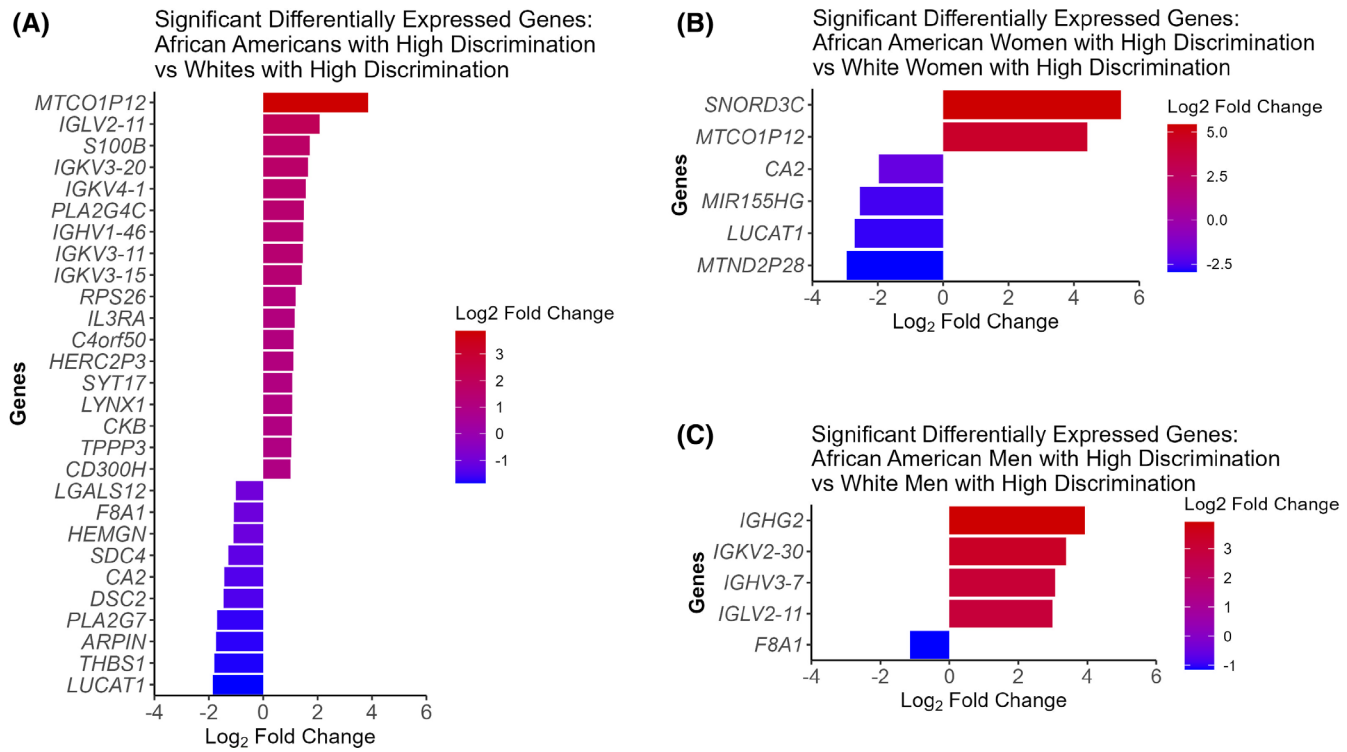

FIGURE 1 Differentially expressed genes associated with high discrimination. Gene names (x-axis) are plotted by $\log_2$ fold change values (y-axis) for differentially expressed genes identified in (A) African Americans with high discrimination compared to Whites with high discrimination, (B) African American women with high discrimination compared to White women with high discrimination, and (C) African American men with high discrimination compared to White men with high discrimination. For the complete list and details of significant, differentially expressed genes for all high discrimination comparison groups, refer to Table S3.

White men with high discrimination comparison group (Table S4). There were 89 significant GO biological processes and 52 significant GO molecular functions in the African American women with high discrimination versus White women with high discrimination comparison group (Table S5). In the White men with high discrimination versus White women with high discrimination comparison group, there were 95 significant GO biological processes and 72 GO molecular functions (Table S6). In the African American men with high discrimination versus African American women with high discrimination comparison group, there were 67 significant GO biological processes and 46 GO molecular functions (Table S7).

We then evaluated overlapping and exclusive GO biological processes and molecular functions per respective comparison group to discover pathway differences associated with high discrimination by race and sex relative to low discrimination by race and sex. Only four GO biological processes (Figure 2A) and seven molecular functions (Figure 2C) overlapped between the African American men with high discrimination versus White men with high discrimination group, African American women

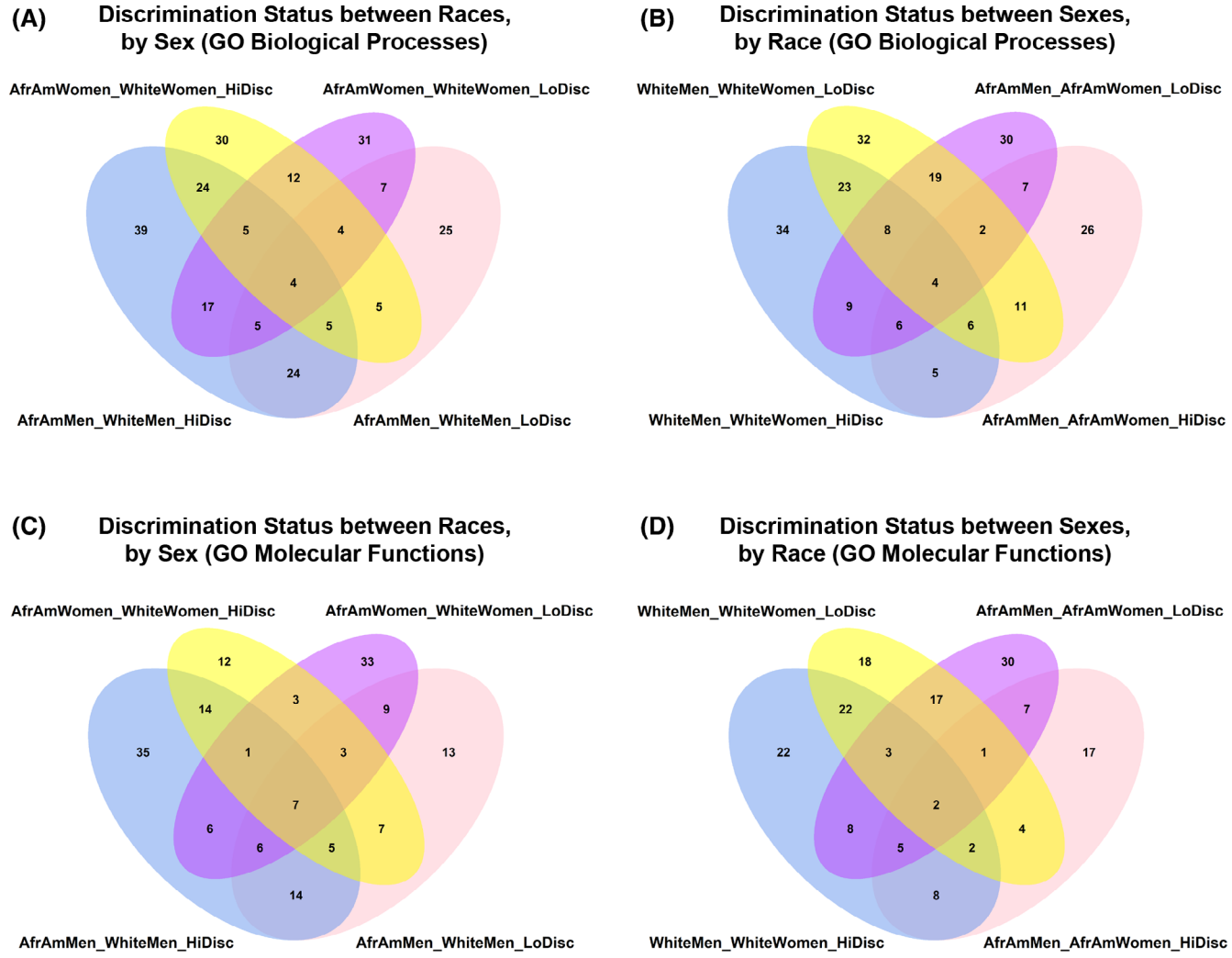

FIGURE 2 Comparison of PAGE pathway overlaps by race, sex, and discrimination. Panels (A) and (C) are Venn diagrams comparing gene ontology (GO) biological processes (A) and molecular functions (C) identified from African American men with high discrimination versus White men with high discrimination ("AfrAmMen_WhiteMen_HiDisc," blue), African American women with high discrimination versus White women with high discrimination ("AfrAmWomen_WhiteWomen_HiDisc," yellow), African American women versus White women with low discrimination ("AfrAmWomen_WhiteWomen_LoDisc," purple), and African American men with low discrimination versus White men with low discrimination ("AfrAmMen_WhiteMen_LoDisc," pink). Panels (B) and (D) are Venn diagrams comparing GO biological processes (B) and molecular functions (D) identified from White men with high discrimination versus White women with high discrimination ("WhiteMen_WhiteWomen_HiDisc," blue), White men with low discrimination versus White women with low discrimination ("WhiteMen_WhiteWomen_LoDisc," yellow), African American men with low discrimination versus African American women with low discrimination ("AfrAmMen_AfrAmWomen_LoDisc," purple), and African American men with high discrimination versus African American women with high discrimination ("AfrAmMen_AfrAmWomen_HiDisc," pink).

with high discrimination versus White women with high discrimination group, African American men with low discrimination versus White men with low discrimination group, and the African American women with low discrimination versus White women with low discrimination group. Four GO biological processes (Figure 2B) and two molecular functions (Figure 2D) overlapped between the White men with high discrimination versus White women with high discrimination group, African American men with high discrimination versus African American women with high discrimination group, White men with low discrimination versus White women with low discrimination group, and African American men with low discrimination versus African American women with low discrimination group.

We then examined exclusive pathways by discrimination status between races by sex, and found that 39 GO biological processes were exclusively identified in African American men with high discrimination versus White men with high discrimination comparison group relative to the other three respective comparison groups (Figures 2A and 3A). GO biological processes exclusively identified in the African American men with high discrimination versus White men with high discrimination comparison group were associated with a wide variety of biological processes including immune response, inflammation, DNA metabolism, estrogen receptor signaling, ion transport, and cholesterol transport. Most pathways were downregulated (Figure 3A). Immune response and inflammation-related pathways were both up- and downregulated but predominantly downregulated. Regulation of cytokine biosynthetic processes was the most upregulated pathway among African American men with high discrimination compared to White men with high discrimination; however, most immune response-related pathways were significantly downregulated including the Toll-like receptor signaling pathway, cytokine, and chemokine medicated signaling and positive regulation of interleukin-6 biosynthesis (Figure 3A).

We identified 30 GO biological processes exclusively in African American women with high discrimination versus White women with high discrimination comparison group relative to the other three respective comparison groups (Figures 2A and 3B). GO biological processes exclusively identified in the African American women with high discrimination versus White women with high discrimination comparison group were also varied with many fewer pathways that are linked to immune response or inflammation. While 10 of the 39 pathways were identified among African American men with high discrimination (26%), just two of the 30 biological processes were related to immunity, chemotaxis, and immune response among African American women. Both biological processes were significantly downregulated. The other significant processes among African American women with high discrimination were stress-related processes (e.g., stress-activated MAPK cascade, response to radiation, cytochrome C oxidase complex assembly) (Figure 3B).

When assessing pathways by discrimination status between sexes by race, we found that 34 GO biological processes were exclusively found in the White men with high discrimination versus White women with high discrimination comparison group relative to the other three respective comparison groups (Figures 2B and 4A). We found GO biological processes associated with immunity and inflammation exclusive to the White men with high discrimination versus White women with high discrimination comparison group (Figure 4A). Regulation of I kappa B kinase or NF kappa B, B-cell costimulation, and Toll-like receptor signaling pathway and B-cell-mediated immunity were upregulated in White men with high discrimination relative to White women with high discrimination.

We identified 26 GO biological processes exclusively identified in the African American men with high discrimination versus African American women with high discrimination comparison group relative to the other three respective groups (Figures 2B and 4B). Immunity and inflammation-related GO biological processes exclusive to the African American men with high discrimination versus African American women with high discrimination comparison group were both up- and downregulated. Ten of the 26 biological processes were related to immune response (38%) with three of the 10 pathways upregulated and seven downregulated including B-cell activation, regulation of cytokine production, and natural killer cell activation among others (Figure 4B).

Thirty-five molecular function pathways were exclusively identified in the African American men with high discrimination versus White men with high discrimination comparison group relative to the other three respective groups (Figures 2C and 5A). The cell signaling molecular functions, SH3 or SH2 adaptor activity and insulin-like growth factor binding were the most upregulated while heme binding and G-protein-coupled receptor activity were among the downregulated molecular functions.

There were 12 molecular function pathways exclusively identified in the African American women with high discrimination compared to White women with high discrimination comparison group relative to the other three respective groups (Figures 2C and 5B). The most upregulated molecular functions included ribonuclease P activity involved in RNA metabolism while the most downregulated molecular function in this context was interleukin-1 receptor activity.

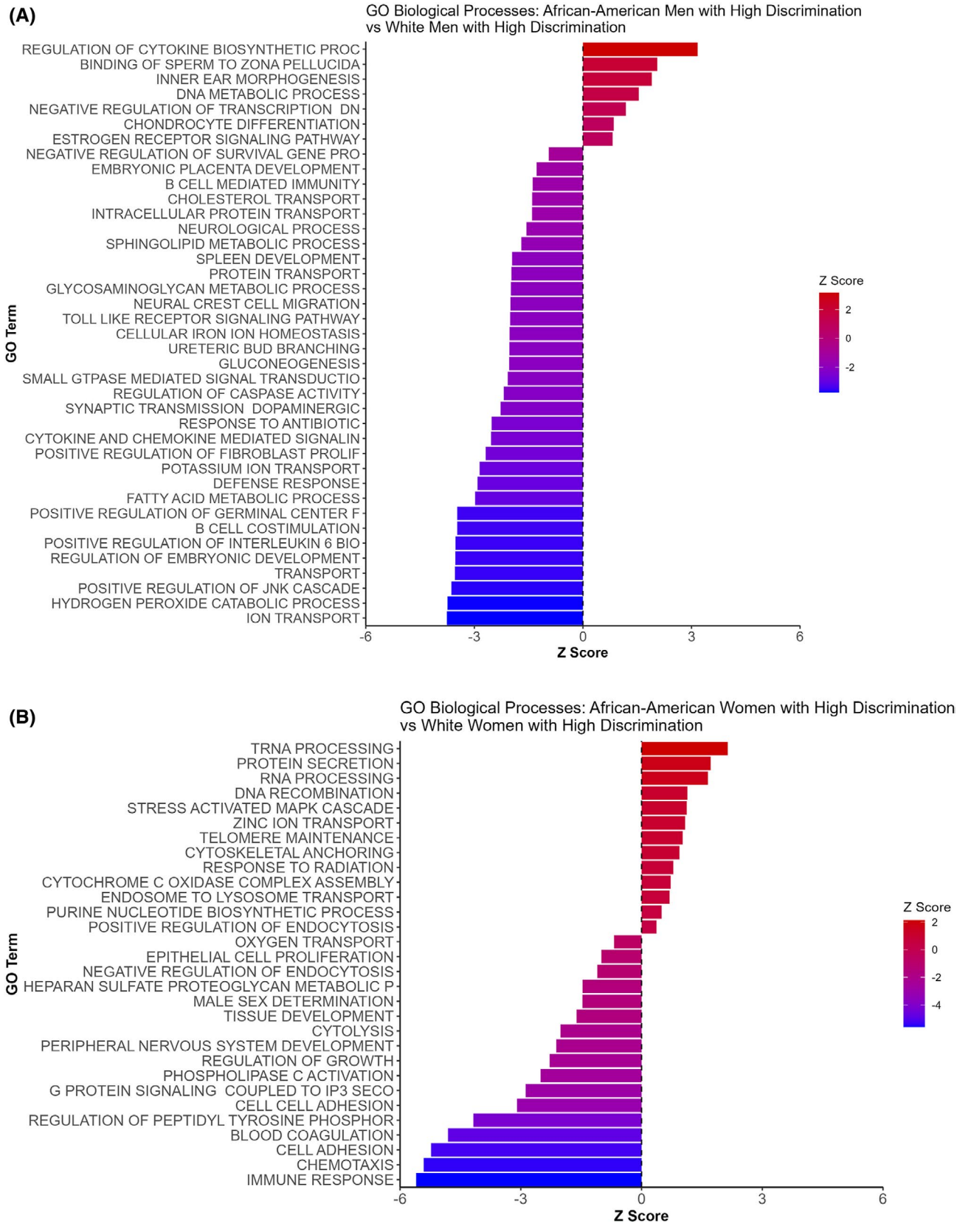

FIGURE 3 Unique biological processes associated with high discrimination between African Americans and Whites. (A) List of gene ontology (GO) biological processes (y-axis) that were exclusively identified in (A) the African American men with high discrimination versus White men with high discrimination comparison group (see Figure 2A), and (B) the African American women with high discrimination versus White women with high discrimination comparison group (see Figure 2A). GO biological processes are plotted by *Z*-score on the x-axis. For complete details on pathway annotations, refer to Tables S4 and S5, respectively.

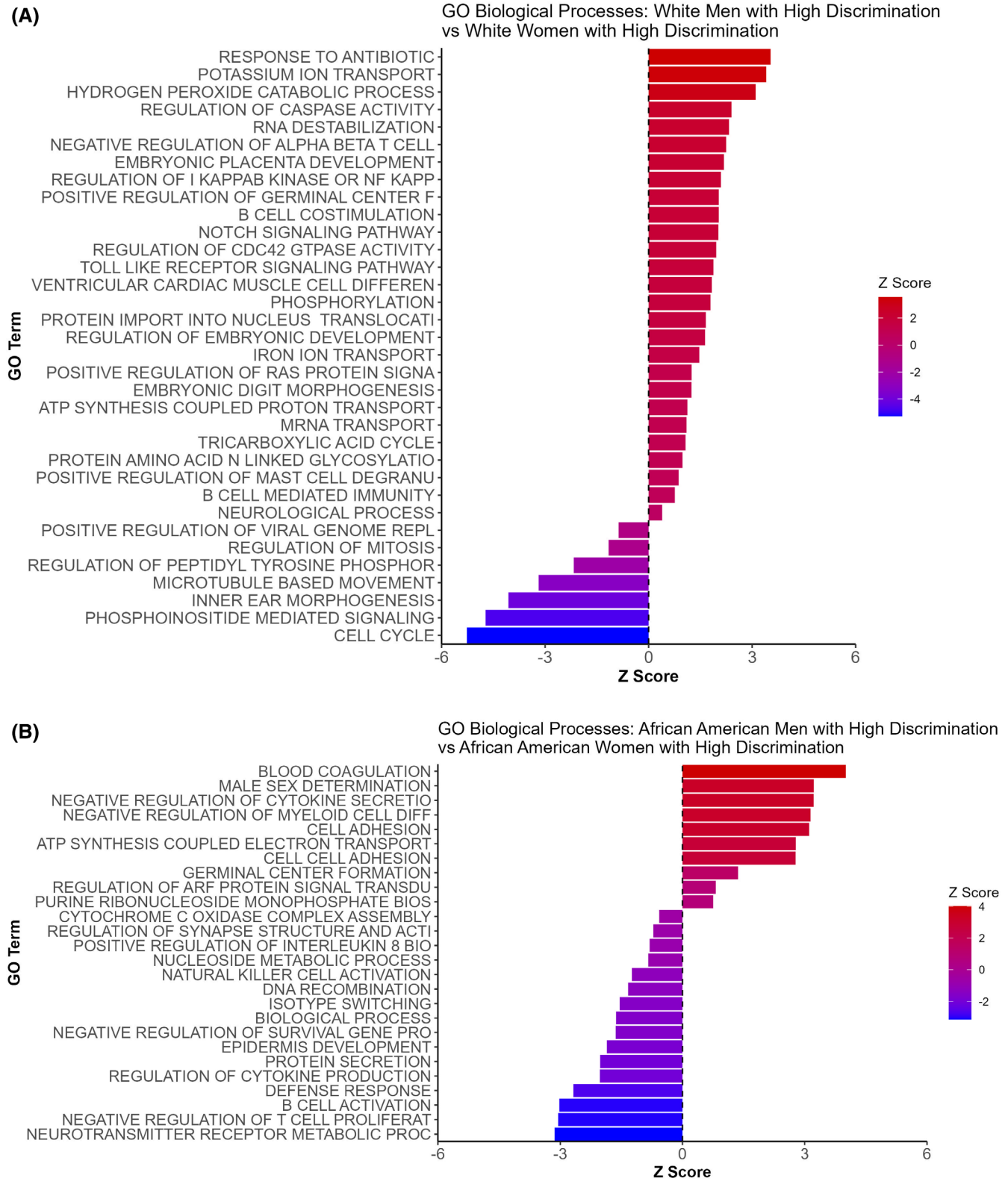

FIGURE 4 Unique biological processes associated with high discrimination between men and women. List of gene ontology (GO) biological processes (y-axis) that were exclusively identified in (A) the White men with high discrimination versus White women with high discrimination comparison group (see Figure 2B), and (B) the African American men with high discrimination versus African American women with high discrimination comparison group (see Figure 2B). GO biological processes are plotted by *Z*-score on the x-axis. For complete details on pathway annotations, refer to Tables S6 and S7, respectively.

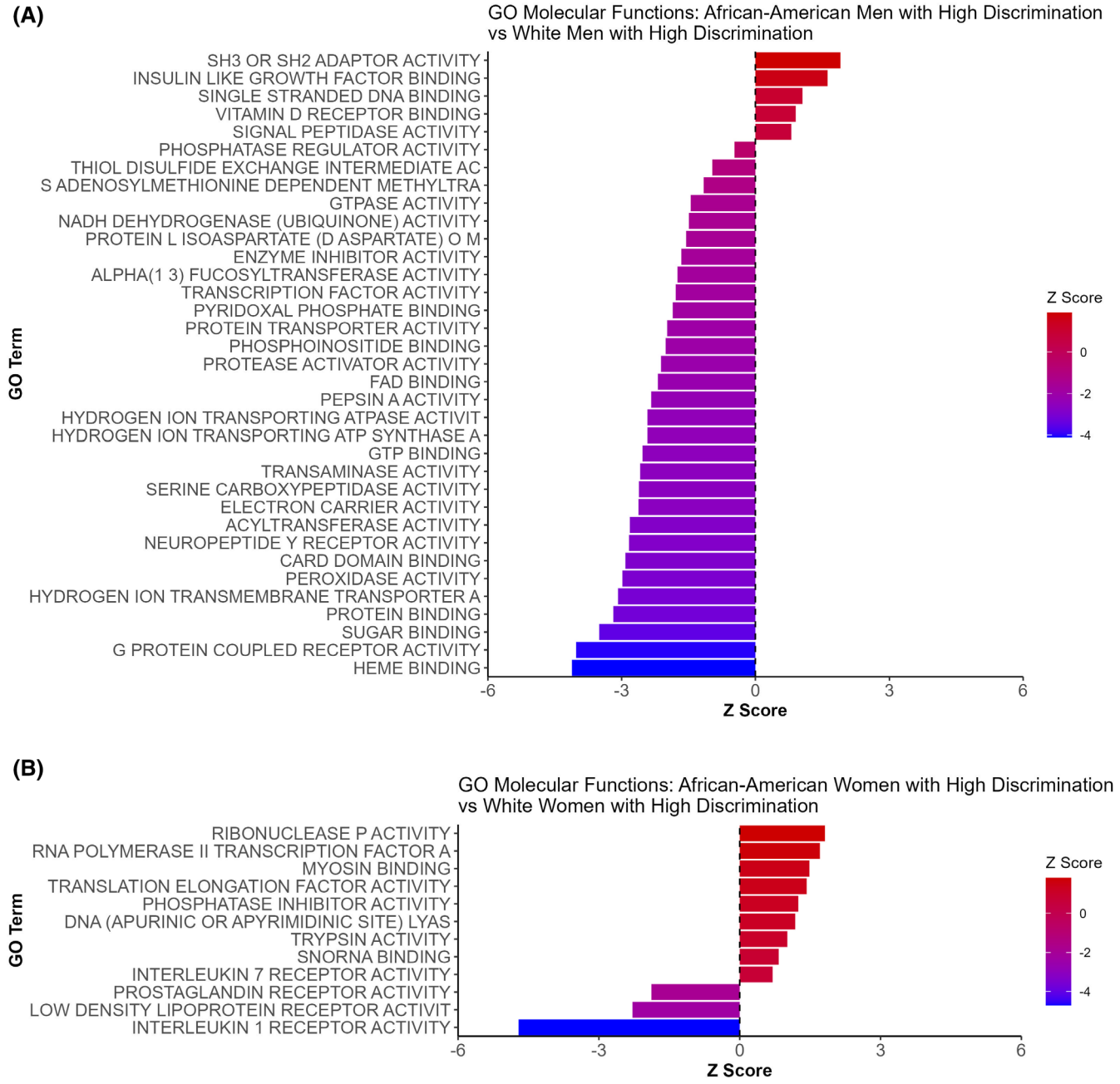

FIGURE 5 Unique molecular functions associated with high discrimination between African Americans and Whites. List of gene ontology (GO) molecular processes (y-axis) that were exclusively identified in (A) the African American men with high discrimination versus White men with high discrimination (see Figure 2C), and (B) the African American women with high discrimination versus White women with high discrimination comparison group (see Figure 2C). GO biological processes are plotted by *Z*-score on the x-axis. For complete details on pathway annotations, refer to Tables S4 and S5, respectively.

There were 22 molecular function pathways exclusively found in the White men with high discrimination versus White women with high discrimination comparison group relative to the other three respective groups (Figures 2D and 6A). The most upregulated molecular functions included hexokinase activity, voltage-gated potassium channel activity, and sugar hydrogen ion transporter activity. Microtubule motor activity, motor activity, and galactosyltransferase activity were the most downregulated molecular function. There were 17 molecular function pathways exclusively found in the African American men with high discrimination versus African American women with high discrimination group relative to the other three comparison groups (Figures 2D and 6B). Among these 17 unique pathways, there were many more downregulated molecular functions in African American men and women with high discrimination when compared to the unique molecular functions identified in White

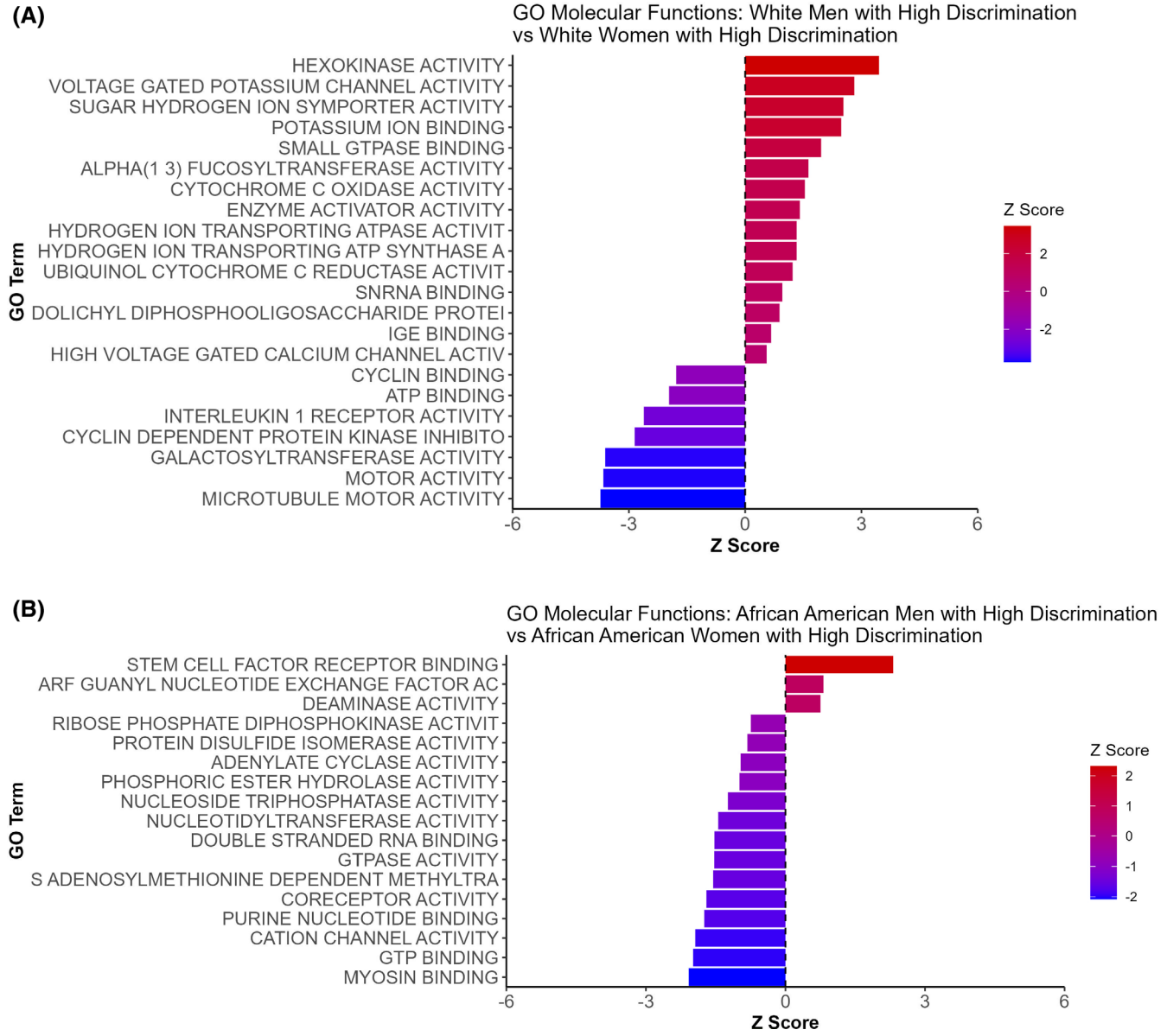

FIGURE 6 Unique molecular functions associated with high discrimination between men and women. List of gene ontology (GO) molecular processes (y-axis) that were exclusively identified in (A) the White men with high discrimination versus White women with high discrimination comparison group (see Figure 2D), and (B) the African American men with high discrimination versus African American women with high discrimination comparison group (see Figure 2D). GO biological processes are plotted by *Z*-score on the x-axis. For complete details on pathway annotations, refer to Tables S6 and S7, respectively.

men and women with high discrimination (Figure 6B). There were only three upregulated molecular functions stem cell factor receptor binding, ARF guanyl nucleotide exchange factor activity, and deaminase activity. The other 14 molecular functions identified were all downregulated.

To identify potential diseases impacted by high discrimination, we then examined IPA diseases and function pathways in the high discrimination comparison groups (Table S8). We found that several cancer pathways were disrupted in the African American men with high discrimination versus African American women with high discrimination comparison group (Table 2). These were mostly associated with different types of leukemias, head and neck cancer, and breast cancer, all of which have known health disparities in African American individuals.[44] We also found that disease ontologies associated with glaucoma, hypertension, and early-stage diabetic nephropathy were altered in African American women with high discrimination compared to White women with high discrimination (Table 3), in which health disparities for African American women have also been well documented.[45–47]

TABLE 2 IPA diseases and functions associated with African American men with high discrimination compared to African American women with high discrimination.

IPA diseases or functions annotation	B–H *p*-value
Uterine corpus endometrioid carcinoma	.0252
Tumorigenesis of sarcomatoid pancreatic ductal adenocarcinoma	.0252
Myelomonocytic leukemia	.0322
Primary T acute lymphoblastic leukemia	.0326
Transitional-cell carcinoma in urinary tract	.0362
T acute lymphoblastic leukemia	.0372
Poorly differentiated thyroid carcinoma	.0372
Plasma cell myeloma	.0322
Development of head and neck tumor	.0326
Medulloblastoma	.0372
Breast carcinoma	.0372
Breast adenocarcinoma	.0422

TABLE 3 IPA diseases and functions associated with African American women with high discrimination compared to White women with high discrimination.

Diseases or functions annotation	B–H *p*-value
Chronic open-angle glaucoma	.0154
Acute angle-closure glaucoma	.0154
Malignant hypertension	.0154
Childhood hypertension	.0154
Early-stage diabetic nephropathy	.0436

4 | DISCUSSION

In this study, we analyzed genome-wide transcriptome differences associated with perceived discrimination in a cohort of African American and White middle-aged adults. There were 28 significantly differentially expressed genes associated with high discrimination exposure comparing African American to White adults. The most significantly upregulated genes among African Americans with high discrimination exposure were related to immune function *IGLV2-11*, *S100B*, *IGKV3-20*, *IGKV4-1*, and the most significantly downregulated genes were associated with immune modulation and cancer *LUCAT1*, *THBS1*, and *ARPIN*. Pathways related to cytokine biosynthetic processes were higher in African American men compared to White men with high exposure to discrimination. Conversely, immune response was the most significantly differentially downregulated biological process among African American women with high discrimination exposure compared to White women with high discrimination exposure. Of note, we found differential expression among genes and biologic and molecular pathways that are not traditionally included as part of the CTRA. In addition, we did not observe any significant differences in any of our comparisons for the CTRA genes[28] or "socially sensitive genes".[30] However, none of the socially sensitive genes that were compiled to analyze in their study were associated with discrimination after correction for multiple testing. Here, we compared genome-wide transcriptional differences associated with high discrimination between African American and White adults. Many of the studies examining CTRA genes in the context of social adversity were examined in cohorts where the majority were White individuals or different race/ethnicities were included but not examined separately. Such a study design may explain why we did not observe any differences in the CTRA genes in the various comparisons in our study. In addition, we performed a comprehensive analysis and did not perform a specific analysis of the identified 53 CTRA indicator pro-inflammatory, type 1 interferon response, and antibody synthesis genes.[48]

While we did find many genes that play pivotal roles in immune response and inflammatory pathways, including some involved in the antiviral response, we also identified genes whose expression is altered with discrimination with functions beyond immune response and inflammation. For example, expression of the mitochondrially encoded cytochrome C oxidase l pseudogene 12 encoding the *MTCO1P12* gene is upregulated in both African American men and African American women reporting high levels of discrimination when compared to White men and women reporting high discrimination. This gene, believed to contribute to mitochondrial electron transport, has been reported to be associated with major depressive disorder.[49]

In addition, *THBS1* and *ARPIN* are significantly downregulated in African American men with high discrimination compared to White men with high discrimination. Thrombospondin 1, which encodes a glycoprotein that functions as a mediator for cell–cell interaction and binds to fibrinogen and fibronectin, is important in angiogenesis and tumorigenesis.[50] Downregulation of thrombospondin 1 RNA is also associated with disease progression in several cancer subtypes.[50]

The oncogenic long noncoding RNA, *LUCAT1*, is significantly downregulated in both African American men and African American women with high discrimination compared to White men and women with high levels of discrimination exposure. *LUCAT1* regulates tumor proliferation, invasion, and migration, and is highly expressed in a wide variety of tumors including breast, esophageal, gastric, hepatocellular, and pancreatic, each of which have different rates of incidence and mortality among African Americans when compared to White Americans.[44,51] When downregulated, *LUCAT1* suppressed proliferation,

migration, and invasion. In addition, when *LUCAT1* is ablated in myeloid cells, investigators identified a hyper-inflammatory gene signature associated with expression of pro-inflammatory cytokines as well as increased expression of inflammation and immune response pathways.[52]

ARPIN (actin-related protein 2/3 complex inhibitor) is an inhibitor of the Arp 2/3 complex, which functions to assemble actin filaments to generate branched actin networks that regulate cell migration.[53,54] Through its inhibitory function, ARPIN participates in the directional persistence of cell migration.[55] Downregulation of ARPIN mRNA and protein is associated with more aggressive pathology and poor prognosis in breast, gastric, and pancreatic cancers.[56–58]

Among African American women with high discrimination small nucleolar RNA, C/D box 3C (*SNORD3C*), was significantly upregulated compared to White women with high discrimination. Small nucleolar RNAs are noncoding RNAs involved in posttranscriptional modification, ribosomal RNA (rRNA) modification as well as tRNA and mRNA modification.[59] *SNORD3C*, a C/D box snoRNA, performs 2'-O-ribose methylation as part of the processing of rRNA.[59] Recently published data suggested that snoRNAs play a role in tumorigenesis and metastasis.[60] *SNORD3C* has been shown to have altered mRNA expression in breast, cervical, lung, and ovarian cancer.[59–62]

Among African American women with high discrimination in addition to *LUCAT1*, we also found significant downregulation of *MTNDSP28* and the microRNA *MIR155HG*. Mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 2 pseudogene 28 (*MTNDSP28*), a protein coding gene, is a part of the mitochondrial membrane respiratory chain complex I assembly. It has been associated with multiple sclerosis, myocardial infarction, and neurodegenerative diseases including Alzheimer's disease and bladder cancer (https://www.genecards.org/cgi-bin/carddisp.pl?gene=MT-ND2).

MIR155 host gene (*MIR155HG*) is a long noncoding RNA associated with microRNA155-5p and 3p known to play a role in immunity, hematopoietic differentiation, cancer, and inflammation.[63] *MIR155HG* by encoding miRNA-155 plays a role in regulation of the inflammatory response, antiviral response, tumor progression, invasion, and metastasis.[64] In addition, it acts as a prognostic biomarker in several cancers including cholangiocarcinoma, lung adenocarcinoma, glioblastoma multiforme, glioma, as well as renal clear cell carcinoma, and melanoma. It is also associated with colorectal carcinoma, pancreatic carcinoma, and breast cancer.[65] *MIR155HG* is also a mechanosensitive miRNA that is upregulated by shear stress in endothelial cells, is involved in cardiovascular disease, and may contribute to the production of nitric oxide.[66]

There was only one gene significantly downregulated in African American men with high discrimination when compared to White men with high discrimination. Coagulation factor VIII associated 1 (*F8A1*) is an X-chromosome gene that is part of a complex of factor VIII genes that encode the Huntingtin-associated protein 40 (HAP40). Huntingtin-associated protein 40 complexes with the Huntingtin protein and is thought to be involved in the pathogenesis of Huntington's disease although specific functions are still under investigation (for review[67]).

Our data may suggest the need to consider classification of other genes whose expression is influenced by discrimination, particularly when studying a diverse cohort. Our work also highlights the importance of stress or adversity in cancer risk and promotion. Previous work has shown that CTRA was involved in response to cancer treatment and risk of relapse suggesting that the CTRA may be a readout of stress-induced cancer-related physiological response.[68,69] The cancer-related genes we identified may be important genes that also transduce stress as an environmental factor to increase cancer susceptibility via stress physiology pathways. Our work also suggests that pathways other than previously identified stress-related ones are relevant as a readout of chronic lifelong adversity among diverse populations.

In agreement with previous data that inflammatory markers were higher in adults exposed to discrimination,[18,70] we found that biological processes related to cytokine biosynthetic processes were higher in African American men compared to White men exposed to high discrimination. As inflammation is a well-known driver of age-related diseases, including cancer, these differences may underly the health disparities observed between African American and White men with age-related diseases. Immune response was the most significantly downregulated biological process among African American women compared to White women with high discrimination exposure. These results are consistent with data from the multi-ethnic study of atherosclerosis (MESA) study that reported that discrimination was associated with gene sets that were associated with inflammatory and immune response.[30] However, this study did not examine sex or race comparisons.

Our IPA analysis revealed several diseases and conditions that were altered between African American and White women exposed to high discrimination. These diseases and conditions have been described to have health disparities by race and include chronic open-angle glaucoma, acute angle-closure glaucoma, malignant hypertension, childhood hypertension, and early-stage diabetic nephropathy. In addition, we identified cancer pathways in African American men and women with high discrimination that are associated with various cancers which may

be the reason for disparate cancer mortality. It should be noted that here we performed transcriptomic analysis in PBMCs, which are a mixed population of cells. This fact may point to differences in immunosurveillance pathways that may lead to accelerated aging in the immune system and defects in the ability to detect or defend against cancer.

Of note, we observed that significantly differentially expressed genes were not concordant between African American men with high discrimination and African American women with high discrimination when compared to White men and White women with high discrimination, respectively. The cancer-related snoRNA *SNORD3C* and the mitochondrial membrane gene *MTCO1P12* were significantly differentially upregulated in African American women with high discrimination. However, immunoglobulin genes related to immune function and inflammation were significantly differentially upregulated in African American men with high discrimination. The differential impact of discrimination on African American men compared to African American women may explain our gender-based DEGs. There are intersectional aspects to perceived discrimination. African American women and African American men experience discrimination differently. African American women often experience "double jeopardy," or "double" discrimination based on sex and race. That differs qualitatively and quantitatively from the experience of African American men.[71] There is very little research on the biological or health effects of these sex-based differences in experienced discrimination among African American adults. However, research has shown differences in how African American men versus women cope with discrimination. African American women have been shown to be angered and to internalize discrimination more than African American men. However, they are also more likely to address discrimination by acting while African American men were more likely to accept the fact that discrimination is a pervasive factor in their lives.[72] Therefore, these sex-specific patterns in experiencing and coping with the adversity of discrimination may lead to different levels of physiologic stress, activation, or inhibition of different biological and molecular pathways in addition to the differences in gene expression that we have observed. Here, we also examined racial differences in perceived discrimination. There are differences by race in perceived discrimination, as African American adults report higher levels of lifetime discrimination than White adults.[73,74] However, it was reported that minority status was a positive predictor of eudaimonic well-being, relative to white status.[75] Consistent across racial groups perceived discrimination was a consistent negative predictor of psychological well-being for women, but not men. Thus, it is important to include in studies of perceived discrimination in different racial and sex groups.

Discrimination is an important component of chronic external environment exposure shown here to influence transcription of immune and inflammatory genes as well as those important in tumorigenesis and cancer risk. Strong evidence supports the link between cancer and discrimination. For example, there are significant breast cancer disparities between African American and White women born in "Jim Crow" states during the time of legalized racial discrimination.[76] Residential segregation also yields prostate cancer disparities between African American and White men.[77] Our data highlight the importance of a comprehensive exposome approach that includes race, ethnicity, and discrimination as well as systemic and structural racism that influence the internal cellular environment to understand the role of external environmental factors in health and disease.[78,79]

Our study has several strengths. Here, we examined genome-wide transcriptional differences, which builds upon earlier work that focused on how various psychosocial stresses were associated with specific gene sets, that is, CTRA. This approach allowed us to analyze a broader scope of genes in an objective manner. Our inclusion of both African American and White adults allows for comparisons when examining how exposure to discrimination is associated with gene expression and biological pathways differently between both racial groups. Few studies have examined the effects of psychosocial stress on gene expression in African American individuals, which is an important strength of this study. There are also some limitations to our study. The cross-sectional study design impedes any interpretation about causation. Although our cohort size is similar to other cohorts examining psychosocial stress and gene expression,[13,27,28] it is limited in size and power to identify associations. Future studies are warranted to verify the generalizability of our findings, particularly in other groups subjected to discrimination or other chronic stressors. While we looked at a number of covariates (race, sex, poverty status, and age), it is possible that other covariates may also confound the results. Here, we have combined lifetime and racial discrimination into high and low-discrimination groups, which may preclude teasing out the impacts of the different types of discrimination on gene expression.

Additionally, previous studies have shown that various differential gene expression (DGE) analysis tools can produce somewhat different results,[80–82] which can be attributed to differences in each respective tool's underlying statistical models including count distribution modeling and normalization methods.[82,83] While there is no consensus in the field for standardizing DGE analysis,[82] DESeq2, edgeR, and limma-voom are the most widely used tools. DESeq2 and edgeR both rely on a negative binomial model for count distribution but differ in how they estimate

dispersion factors for the mean–variance relationship, while limma-voom integrates precision weights into a log-normal linear counts model followed by an empirical Bayes statistical procedure.[82] Several studies have shown that DESeq2 and edgeR can detect more DEGs (with increased sensitivity to detecting DEGs with low-level expression) compared to limma-voom.[80,82–84] However, previous work has also demonstrated that DESeq2 and edgeR have inflated false discovery rates, while limma-voom does not demonstrate excessive false positives.[80,81,85] Therefore, it is possible that there may be increased false positive results from our analysis strategy. However, some studies have demonstrated as much as 90% DEGs overlapped between DESeq2, edgeR, and limma-voom approaches.[84–86] Future studies should continue to evaluate and standardize DGE analysis tools, including the potential for integrating multiple DGE analysis methods to identify top gene candidates for further validation.[80]

Nevertheless, our findings address some of the gaps in our knowledge about novel genes and pathways that may mediate how perceived discrimination leads to health disparities and negative health outcomes. Understanding the association of perceived discrimination with gene expression and biological pathways can elucidate the mechanism by which psychosocial stress leads to accelerated aging and age-associated disease. These steps can help shape policy and the recognition of the harmful effects, both mentally and physically, of discrimination.

AUTHOR CONTRIBUTIONS

NLP, NNH, DB-M, and MKE conceived and planned the project. SFW, YZ, and SD conducted the sequencing. YZ, NLP, and MM-B analyzed the data. NNH, NAM, ABZ, and MKE designed the cohort. NE performed physical examinations and interpreted laboratory analyses for all participants. NLP, NNH, and MKE contributed to the interpretation of the results. NLP, SFW, NNH, and MKE wrote the manuscript, and all remaining authors provided critical feedback for the manuscript.

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DISCLOSURES

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GEO database #GSE244654. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request through the HANDLS website https://handls.nih.gov/.

CONSENT FOR PUBLICATION

Not applicable.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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