UCSF

Title

Minority stress and leukocyte gene expression in sexual minority men living with treated HIV infection

Permalink

https://escholarship.org/uc/item/83k9794w

Authors

Flentje, Annesa
Kober, Kord M
Carrico, Adam W
et al.

Publication Date

2018-05-01

DOI

10.1016/j.bbi.2018.03.016

Peer reviewed

Minority Stress and Leukocyte Gene Expression in Sexual Minority Men Living with Treated HIV Infection

Annesa Flentje[1], **Kord M. Kober**[2,8], **Adam W. Carrico**[3], **Torsten B. Neilands**[4], **Elena Flowers**[2,5], **Nicholas C. Heck**[6], and **Bradley E. Aouizerat**[7]

[1]Community Health Systems, School of Nursing, University of California, San Francisco

[2]Physiological Nursing, School of Nursing, University of California, San Francisco

[3]School of Medicine, University of Miami

[4]Center for AIDS Prevention Studies, University of California, San Francisco

[5]Institute for Human Genetics, University of California, San Francisco

[6]Department of Psychology, Marquette University

[7]Bluestone Center for Clinical Research, College of Dentistry, New York University

[8]Institute for Computational Health Sciences, University of California, San Francisco, United States

Abstract

Sexual minority (i.e., non-heterosexual) individuals experience poorer mental and physical health, accounted for in part by the additional burden of sexual minority stress occurring from being situated in a culture favoring heteronormativity. Informed by previous research, the purpose of this study was to identify the relationship between sexual minority stress and leukocyte gene expression related to inflammation, cancer, immune function, and cardiovascular function. Sexual minority men living with HIV who were on anti-retroviral medication, had viral load <200 copies/mL, and had biologically confirmed, recent methamphetamine use completed minority stress measures and submitted blood samples for RNA sequencing on leukocytes. Differential gene expression and pathway analyses were conducted comparing those with clinically elevated minority stress (n=18) and those who did not meet the clinical cutoff (n=20), covarying reactive urine toxicology results for very recent stimulant use. In total, 90 differentially expressed genes and 138 gene set pathways evidencing 2-directional perturbation were observed at false discovery rate (FDR) < 0.10. Of these, 41 of the differentially expressed genes and 35 of the 2-directionally perturbed pathways were identified as functionally related to hypothesized mechanisms of inflammation, cancer, immune function, and cardiovascular function. The neuroactive-ligand receptor pathway (implicated in cancer development) was identified using signaling pathway

---

Corresponding Author: Annesa Flentje, PhD, Community Health Systems, School of Nursing, University of California, San Francisco, 2 Koret Way, N505, San Francisco, CA 94143, (415)502-0697, (415)502-0697.

impact analysis. Our results suggest several potential biological pathways for future work investigating the relationship between sexual minority stress and health.

Keywords

Gene expression; Sexual Minority Men; Minority Stress; HIV; Stimulant Use; Immune Activation; Inflammation; Cancer; Cardiovascular

1. Introduction

Sexual minority (non-heterosexual) individuals experience higher rates of both mental (Cochran, Ackerman, Mays, & Ross, 2004; Cochran, Keenan, Schober, & Mays, 2000; Cochran, Sullivan, & Mays, 2003; Hughes & Eliason, 2002; King et al., 2008; McCabe, Boyd, Hughes, & d'Arcy, 2003; Stall et al., 2001) and physical health problems (Cochran & Mays, 2007; Conron, Mimiaga, & Landers, 2010). Meyer's model of minority stress related to sexual minority status (hereafter termed "sexual minority stress"), which includes experiences and expectations of discrimination, internalization of societal stigma, and concealment of sexual orientation, has garnered significant empirical support in explaining the the health disparities that are observed among sexual minority individuals (Institute of Medicine, 2011; McCabe, Bostwick, Hughes, West, & Boyd, 2010; Meyer, 2003). Studies also document relationships between sexual minority status and incidence of negative health outcomes such as cardiovascular disease (Conron et al., 2010; Fredriksen-Goldsen, Kim, Barkan, Muraco, & Hoy-Ellis, 2013), obesity (Conron et al., 2010; Fredriksen-Goldsen et al., 2013), and diabetes (Fredriksen-Goldsen et al., 2013), though studies are generally limited due to delays in the inclusion of sexual orientation in many large-scale health data collection efforts (Sell & Holliday, 2014), and findings often differ between sexual minority subgroups. Compared to heterosexual men, sexual minority men evidence pre-clinical indicators of risk for negative health outcomes such as high levels of C-reactive protein, diastolic blood pressure, faster heart rate (Hatzenbuehler, McLaughlin, & Slopen, 2013), and hypertension (Everett & Mollborn, 2013). Research is needed to begin to investigate the mechanisms that underly these health disparities among sexual minority people.

Studies are beginning to document associations between sexual minority stress processes and biological and physiological outcomes. For example, Frost, Lehavot, and Meyer (2015) found that the experience of a prejudice event related to sexual minority status predicts subsequent report of a physical health problem, including problems such as hypertension, illness, or cancer. Other studies have examined cortisol responsiveness within the context of sexual minority stress. For example, lesbian, gay, and bisexual people raised in environments where minority sexual orientations are more highly stigmatized show blunted cortisol responses to social stress (Hatzenbuehler & McLaughlin, 2014). In addition, openness about one's sexual orientation in the workplace is related to higher overall cortisol levels on workdays among sexual minority men (Huebner & Davis, 2005). Further, the glucocorticoid resistance that can occur in response to chronic stress is related to both a failure to downregulate inflammation and a less effective immune response to a viral challenge (Cohen et al., 2012). Inflammation, in turn is an important predictor of other health

conditions (e.g., high blood pressure; Chae, Lee, Rifai, & Ridker, 2001) as well as greater risk for mortality for HIV-positive individuals (Tien et al., 2010) and among the general population (Kabagambe et al., 2011), while impaired immunity can increase vulnerability to disease. Sexual minority stress may also impact physiology through behavioral pathways, for example sexual minority stress is related to substance use (Hatzenbuehler, Nolen-Hoeksema, & Erickson, 2008; McCabe et al., 2010), which also impacts immune and inflammatory response (Cabral, 2006; Carrico et al., 2008; Cole, Korin, Fahey, & Zack, 1998; Pandrea, Happel, Amedee, Bagby, & Nelson, 2010; Sacerdote, Franchi, & Panerai, 2012) and cardiovascular function (Darke, Kaye, McKetin, & Duflou, 2008; Lange & Hillis, 2001) In sum, while the exact mechanisms need further exploration, existing research on minority stress and chronic stress suggests that minority stress may be related to inflammation, as well as immune and cardiovascular function, which in turn may be related to poorer health outcomes observed among sexual minority people.

Most of the existing research demonstrating associations between sexual minority stress and biological outcomes has been conducted with HIV-positive populations. In the era prior to highly active anti-retroviral therapy, HIV-positive men who concealed their sexual minority status experienced accelerated progression of HIV, including faster progression to AIDS, AIDS mortality, and lower CD4+ T-cell counts (Cole, Kemeny, Taylor, Visscher, & Fahey, 1996). Concealment of sexual minority status was also linked to greater likelihood of subsequent cancer or infection (Cole, Kemeny, Taylor, & Visscher, 1996). Similarly, discomfort with situations in which one's minority sexual orientation could be evident is associated with faster progression to AIDS, AIDS mortality, and lower CD4+ T-cell counts for sexual minority men living with HIV, though less so if they conceal their sexual orientation (Cole, Kemeny, & Taylor, 1997). Even after the widespread use of highly active antiretroviral therapy, the experience of victimization due to sexual orientation was shown to be related to greater HIV viral load, and internalized stigma was related to lower CD4+ T-cell counts (Norcini Pala, Hart, & Steca, 2015). In the modern era of HIV treatment, studies with those who are receiving effective HIV treatment have the potential to elucidate the biological pathways whereby stress may contribute to inflammation and residual immune dysregulation (Lederman, Funderburg, Sekaly, Klatt, & Hunt, 2013). In sum, the existing research among HIV positive persons supports the hypothesis that minority stress may be related to immune function, while also suggesting that cancer development may be related to minority stress. Inflammation, immune function, cancer, and cardiovascular function are all areas that may be considered in relationship to minority stress, based on previous work on minority stress and the health implications of chronic stress.

Emerging research indicates that our social surroundings, the stressors associated with those environments, and our perceptions of those stressors can change the expression of our genes (Slavich & Cole, 2013), thereby altering complex biological processes. For instance, we know that early adverse childhood experiences can influence subsequent inflammation (Miller & Cole, 2012) and that interpersonal stress (Miller, Rohleder, & Cole, 2009) and social isolation (Cole et al., 2007) are related to expression of genes that drive inflammatory processes. Stress exposure is related to increased expression of pro-inflammatory genes and reduced expression of interferon response genes that are important for coordinating the immune response to novel pathogens (Cole, Hawkley, Arevalo, & Cacioppo, 2011; Slavich

& Cole, 2013; i.e., genes that regulate against viral infections). Gene expression may have superior sensitivity in measuring biological stress responses over serum markers, as evidenced by sensitivity to stressors or interventions for stress management when no significant effects were detected in plasma inflammatory markers such as interleukin 6 (IL-6) and C-reactive protein (Creswell et al., 2012; Miller et al., 2009; Murphy, Slavich, Rohleder, & Miller, 2012). Gene expression may allow us to index responses to stress with greater precision because of the upstream role it plays within the cascade of biological events within the organism. Assessment of gene expression is a promising approach for tracking the impact of psychological stress on health as it is capable of measuring both biological responses to adversity (Slavich & Cole, 2013), and the reversal of those responses through interventions (Antoni et al., 2012; Black et al., 2013; Creswell et al., 2012).

The purpose of this exploratory study is to identify if sexual minority stress is related to differential expression of genes. Consistent with previous research and the minority stress model, genes implicated in inflammatory processes, immune function, cancer, and cardiovascular risk were examined. This model was tested within sexual minority men living with HIV who were receiving effective treatment for HIV, as the potentially deleterious effects of minority stress may be most evident in this population. We hypothesized that the association of minority stress with leukocyte gene expression would be independent of recent stimulant use, which is the primary focus of a companion paper to this paper (Carrico et al., Under Review).

2. Methods

Sexual minority men living with HIV with recent biologically verified methamphetamine use (verified through urine or hair toxicology testing) were recruited from substance abuse treatment programs, HIV medical clinics, AIDS service organizations, the community, and referrals from other participants. Data collected for this study were collected at the baseline visit of a randomized controlled trial and were collected prior to randomization (Methods also described in Carrico et al., under review). Participants met with study staff to provide informed consent, which included specific consent for blood specimen banking and analysis of gene expression. Inclusion criteria were: 1) 18 years of age or older; 2) identify as gay or bisexual or are sexually active with men (*i.e.*, sexual minority); 3) provide documentation of HIV-positive serostatus; and 4) provide a urine or hair (only collected for those whose urine toxicology was negative) sample that is reactive for methamphetamine at the screening visit, which occurred prior to the baseline visit described here. Participants were selected for this substudy to undergo RNA sequencing if they were on anti-retroviral treatment (ART), had viral load less than 200 copies per mL, and completed measures of sexual minority stress.

At the baseline visit, enrolled participants completed a battery of psychosocial measures, provided an additional urine sample for on-site toxicology testing to index recent (~72 hours) stimulant use, and provided a peripheral venous blood sample collected in two 2.5 mL PAXgene® vacutainers (Qiagen, Inc.) for gene expression analyses and in two vacutainers containing the preservative EDTA to measure HIV disease markers and for specimen banking. This study was approved by the Institutional Review Boards for the

University of California, San Francisco, University of Miami, and Northwestern University. A certificate of confidentiality was obtained from the National Institute on Drug Abuse.

2.1 Measures

2.1.1 Demographics, health and psychiatric status, and HIV markers—

Participants completed a demographic questionnaire assessing age, race, ethnicity, and health-related factors such as current HIV medication regimen. The Addiction Severity Index (ASI) was used to measure severity of drug and alcohol use (McLellan et al., 1992). The 20-item Centers for Epidemologic Studies Depression Scale (CES-D) measured depression (Radloff, 1977). Possible Post Traumatic Stress Disorder (PTSD) was assessed using th PTSD Checklist-Civilian Version (PCL-C; Wilkins, Lang, & Norman, 2011). The Abbott Real Time HIV-1 assay (Abbott Molecular, Inc.; Des Plaines, IL), with a lower limit of detection of 40 copies/mL was used to assess HIV viral load. CD4+ T-cell count was assessed by Quest Diagnostics using a clinical assay.

2.1.2 Sexual Minority Stress—

Sexual minority stress was measured using the five-item sexual minority stress subscale of the Cultural Assessment of Risk for Suicide (CARS) scale (Chu et al., 2013a), which is validated for use with sexual minority people. This subscale captures distress related to sexual minority stress, and includes items that reference constructs of concealment, internalized stigma, and rejection from others. For example one item on this scale referencing concealment is "The decision to hide or reveal my sexual or gender orientation to others causes me significant distress," while an item referencing rejection and stigma is "I was rejected by a family member or friend after telling him/her my sexual or gender orientation." Each item of the CARS is rated on a scale from 1 to 6, ranging from "strongly disagree" to "strongly agree." Scores were calculated as specified within the CARS manual by summing the five items, with one item reverse coded (Chu et al., 2013b). The sexual minority stress subscale of the CARS was validated in the context of the relationship between minority stress and suicidality (Chu et al., 2013a), and cutoff scores were created to determine the level of clinical concern based on responses to the CARS items (Chu et al., 2013b). Cutoff scores were derived based on the relationships between these cutoff scores and increased risk of suicidal ideation, suicide attempts, and hopelessness (Chu et al., 2013b). Within this sample, internal consistency of this scale was 0.71. Participants were divided into low and high sexual minority stress groups using cutoff scores deemed to be of clinical concern according to the CARS manual: 13, corresponding to "minimal stress" and 14, corresponding to "moderate" or "severe" stress (Chu et al., 2013b).

2.1.3. On-site urine screening—

On-site toxicology screening using iCup (Redwood Biotech, Inc., Santa Rosa, CA) was conducted on urine samples. This was used to identify individuals who had used methamphetamine or cocaine in the previous ~72 hours.

2.1.4. Pro-inflammatory cytokines in plasma—

Plasma levels of tumor necrosis factor-alpha (TNF-α) and IL-6 were examined within this study. IL-6 was selected due to its relationship with stress and mortality (Papanicolaou, 1998), and because IL-6 is associated with mortality specifically among persons living with HIV (Kuller et al., 2008). TNF-α was

selected because both TNF-α and IL-6 monocyte expression can be altered by stimulant (*i.e.*, cocaine) use (Irwin et al., 2007). TNF-α and IL-6 levels were obtained via undiluted plasma samples using the Human Quantikine Immunoassay (R&D Systems, Minneapolis, MN). Results were $\log_{10}$ transformed prior to use in analysis.

2.2. RNA sample preparation, sequencing, and analysis

2.2.1. Sample preparation and sequencing—Total RNA was isolated from samples in PAXgene tubes using the PAXgene blood miRNA kit (Qiagen). Library preparation and sequencing was done by a Core Facility, the Vincent J. Coates Genomics Sequencing Laboratory at the University of California, Berkeley. Ribosomal RNA was reduced using Ribo-Zero (Illumina Inc., San Diego, CA). RNAs were then prepared for Illumina sequencing on an Apollo 324™ with PrepX™ RNAseq Library Prep reagents (WaferGen Biosystems, Fremont, CA) following the manufacturer's protocol. Thirteen cycles of PCR amplification were used for single 6 base pair index addition and library fragment enrichment. Prepared libraries were then quantified on a Roche LightCycler 480II (Roche Diagnostics Corp., Indianapolis, IN) using KAPA Illumina library quantitative PCR reagents (Roche Diagnostics Corp., Indianapolis, IN). Sequencing was done on an Illumina HiSeq 4000 apparatus (Illumina Inc., San Diego, CA). RNA-sequencing as a methodology has been validated against other methodologies in multiple studies (see Fang & Cui, 2011 for a discussion or Marioni, Mason, Mane, Stephens, & Gilad, 2008 for previous validation).

In total, 72 samples were sequenced across 8 lanes, including 10 technical replicates, for a total of 62 unique samples from 62 different participants. Of these 62 participants, 39 had completed measures of minority stress, with 38 of these eligible for inclusion in the analyses in this study. Nine samples were multiplexed per lane for 100 cycles of paired end reads with a 1% PhiX v3 control library spike in (Illumina Inc., San Diego, CA). Post-sequencing basecall files (bclfiles) were demultiplexed and converted into FASTQ file format using the bcl2fastq v2.17 software (Illumina Inc., San Diego, CA). Specific data processing procedures are outlined in supplemental materials (S1). One sample from each replicate pair was excluded using a coin-flip. Data exploration was performed with multi-dimensional scaling (MDS) plots for all samples, and was used to identify potential batch effects due to technical artifacts (e.g., RNA quality, processing laboratory, technician, sequencing lane).

RNA-sequencing measurement of gene expression has been found to be a reliable and reproducible technology, with the measurement of relative expression levels in high agreement with qPCR and microarrays (Su et al., 2014). To evaluate the reproducibility of our measurements (Allison, Cui, Page, & Sabripour, 2006), the 10 technical replicates were generated from independent library preparations and sequenced in different lanes (Z. Fang & Cui, 2011). The reliability of the sequencing data was evaluated through alignment performance and MDS plots between replicate pairs. The reproducibility of replicate count estimates was evaluated by Pearson correlation.

2.3. Statistical Analyses

Chi-square analyses and t-tests were used to examine differences in demographic and clinical characteristics between low and high sexual minority stress groups, including: age,

time since HIV diagnosis, CD4+ T-cell count, number of days in the past 30 that methamphetamine was used, severity of drug use, severity of alcohol use, depression (both continuous and using cutoff scores of 16 consistent with a widely used clinical cutoff for depression, Vilagut, Forero, Barbaglia, & Alonso, 2016), PTSD symptoms, sexual orientation, race, proportion on different ART regimens, proportion with undetectable HIV viral load (< 40 copies/mL), and toxicology that was positive or negative for stimulants. Differences in these demographic and clinical characteristics were also examined between participants who had RNA sequenced who had received minority stress measures and those who had participated in the study before these measures were integrated into the protocol. T-tests were used to examine differences in $\log_{10}$ transformed plasma levels of TNF-α and IL-6 by low and high sexual minority stress. Levels of TNF-α and IL-6 are reported here to identify if results of gene expression assays that pertain to inflammation are also observed in plasma. Differential expression was examined using a variance modeling strategy in edgeR to address the over-dispersion observed in gene expression count data (Landau & Liu, 2013), which performs well relative to other strategies (Rapaport et al., 2013; Soneson & Delorenzi, 2013). Our workflow for this project was based on best practices previously described in detail elsewhere (Anders et al., 2013; Love, Anders, Kim, & Huber, 2015). General linear models were used to estimate overall, gene-wise, and tag-wise dispersion, using the Cox-Reid-adjusted likelihood method (Cox & Reid, 1987; McCarthy, Chen, & Smyth, 2012). Negative-binomial generalized linear models (GLMs) were fit for each gene examining differences by high and low minority stress groups, and accounting for urine toxicology positive or negative for stimulants. Gene identifiers were annotated with Entrez gene IDs and names were annotated using the HUGO Gene Nomenclature Committee database ("HGNC database of human gene names | HUGO Gene Nomenclature Committee," n.d.). Surrogate variable analysis was used to capture unspecified heterogeneity (Leek, Johnson, Parker, Jaffe, & Storey, 2012), and surrogate variables were retained unless highly correlated with the sexual minority stress phenotype ($p<.10$). Likelihood ratio tests were used to test differences between the high and low minority stress groups. The Benjamini-Hochberg procedure was used to estimate the false discovery rate (FDR) with an FDR cut-off of 0.10.

Gene set or pathway analyses, examining differences in perturbations among groups of genes were completed using Generally Applicable Gene-set Enrichment (GAGE, Luo, Friedman, Shedden, Hankenson, & Woolf, 2009), using a competitive method to identify if a given pathway of interest is differentially expressed more often between high and low minority stress groups when compared to a complementary set of genes (Emmert-Streib & Glazko, 2011; Goeman & Bühlmann, 2007) using an FDR cutoff of 0.10. Additional gene set analyses were conducted using signaling pathway impact analysis (SPIA, Tarca et al., 2009) to identify purturbations in a biological pathway considering both the level of differential expression of individual genes as well as the topology of the pathway. The Kyoto Encyclopedia of Genes and Genomes (Kanehisa, Goto, Sato, Furumichi, & Tanabe, 2011; KEGG: Kyoto Encyclopedia of Genes and Genomes, n.d.) database was used to define gene sets.

Once results were obtained, existing research on each of the differentially expressed genes was reviewed for relevance to the hypothesized mechanisms of inflammation, immune

function, cancer, or cardiovascular function. Pathways were categorized for potential relationships to each of these hypothesized mechanisms.

3. Results

3.1. Participants

Thirty-eight of 62 participants met all eligibility criteria and had RNA sequencing data available for analysis. The current study utilized data from the 39 participants who completed minority stress measures; the 23 participants lacking minority stress measures were enrolled prior to the integration of these measures into the study protocol. There were no differences in demographic or clinical characteristics among those who underwent RNA sequencing who received or did not receive minority stress measures, with the exception that participants who received minority stress measures were more likely to have a higher CD4+ T-cell count (M=730) than those who did not (M=474, p<.001). One additional sample was removed from the 39 who had sequencing data because the participant's viral load was greater that 200 copies per mL, thus they did not meet our inclusion criteria, for a total of 38 participants within this study. Of the 38 participants, 20 were designated low sexual minority stress and 18 were designated high sexual minority stress. There were no differences between low versus high minority stress groups on age, time since HIV diagnosis, CD4+ T-cell count, number of days within the past 30 that methamphetamine was used, drug or alcohol use severity, depression, PTSD symptoms, sexual orientation, race/ethnicity, proportion with undetectable viral load, proportion on different ART regimens, or proportion with a positive urine toxicology screen for stimulants. There were no differences in plasma levels of TNF-α and IL-6 in the low versus high minority stress groups. Participant demographic information and p-values of chi-square analyses and t-tests of demographic and clinical variables by low and high minority stress groups are found in Table 1.

3.2. Gene expression results

Mean RNA integrity numbers were 7.2 (range 6.3-9.1). High replicability was supported with clustering of technical replicates in MDS plots and with mean within-replicate Pearson correlations coefficients of 0.999 between technical replicates across different lanes. After filtering out genes that were lowly expressed within the sample (less than 1 count per million in 10 of the samples), 19,541 genes remained for consideration. Mean library size was 14,020,862 after filtering. Eight surrogate variables were found, 7 were used in modeling, with the remaining 1 removed from models due to high correlation with sexual minority stress groupings.

3.3. Differential Gene Expression

In total, 90 genes were differentially expressed at an FDR of 0.10. Of these 90 genes, 69 were upregulated and 21 were downregulated. Genes differentially expressed at an FDR of 0.10 with suspected relevance to inflammation or the biological functions that were shown to be previously related to sexual minority stress (i.e., immune function, cancer, or cardiovascular function; 41 genes in total), are listed in Table 2. The complete list of genes is available within supplementary materials (S2).

3.4. Pathway Analysis

In GAGE analysis, there were 138 two-directional perturbed gene sets. A comprehensive list of two-directional perturbed pathways is included as a supplementary material (S3). Selected 2 directional pathways that are related to inflammation or previously observed relationships between sexual minority stress and biological function (i.e., immune function, cancer, cardiovascular) are listed in Table 3. Of note, as can be seen in supplementary materials, metabolic pathways from the KEGG database emerged frequently in two directional perturbation gene set analyses. SPIA analysis found one significant pathway: the neuroactive ligand-receptor interaction (KEGG ID: 04080), which was activated in the high sexual minority stress group (p_{GFDR}=0.008). The significance of this pathway was driven by perturbation (p_{pert}=0.000005), and not an enrichment of the number of differentially expressed genes (p_{NDE}=0.97). This pathway was also detected in 2 dimensional perturbation analyses using GAGE (q=0.0000007).

4. Discussion

This study found that sexual minority stress is related to differential expression of individual genes and pathways in leukocytes that are implicated in inflammation, immune function, cancer, and cardiovascular function. In total, 90 single genes were differentially expressed at an FDR of less than 0.10, of which 41 are related to hypothesized mechanisms underlying minority stress: inflammation, immune function, cancer, and cardiovascular function. Notably, these relationships between minority stress and differential gene expression were observed after controlling for recent stimulant use. Furthermore, 138 pathways evidenced 2-directional perturbation, 35 of which are related to hypothesized mechanisms. The neuroactive ligand receptor interaction pathway was identified through both enrichment pathway analysis and perturbation analysis, driven by multiple genes evidencing perturbation on this pathway. These results add to the emerging body of research that has identified relationships between minority stress and biological functioning (e.g., Cole, Kemeny, Taylor, & Visscher, 1996; Cole, Kemeny, Taylor, Visscher, et al., 1996; Frost et al., 2015; Huebner & Davis, 2005) and suggest that continued investigation of these relationships is warranted. In this study, the approach employed to assess differential gene expression and pathway analysis builds on prior studies, which examined the relationship between sexual minority stress and HIV progression and mortality, cancer or infection, and cortisol. This study provides a first step in exploring the biological processes whereby sexual minority stress may influence the health of sexual minority people.

Relevant to the health and immune function of individuals living with HIV, this study found that 7 genes and 16 pathways related to inflammation or immune functioning were differentially expressed between low and high minority stress groups. For example, the differentially expressed genes include *BTLA*, whose proteins are associated with homeostasis of inflammatory responses within cells (Bekiaris, Šedý, Macauley, Rhode-Kurnow, & Ware, 2013), *KLRB1*, which inhibits natural killer cell cytotoxicity, and *CD69*, which has a role in the regulation of immune function and inflammation (Sancho, Gomez, & Sanchez-Madrid, 2005). Interestingly, although we found differential perturbation in the cytokine-cytokine receptor interaction pathway (KEGG 04060) of which *TNF* and *IL-6* are

members, and that TNF-α and IL-6 are common inflammatory markers, we did not see a difference between groups in the levels of either in plasma or in gene expression. The relationship between circulating cytokines and gene expression is not always consistent, as some studies have observed differences in circulating cytokines when no difference was observed in gene expression (Moldoveanu, Shephard, & Shek, 2000), while other studies have, similar to the study reported here, found evidence for differences in gene expression related to inflammation when no differences were observed in circulating cytokines such as IL-6 (Creswell et al., 2012; Miller et al., 2009; Murphy, Slavich, Rohleder, & Miller, 2012). Moreover, *de novo* gene expression is but one of several means of regulation (*e.g.*, sequestration of proteins in complexes such as inflammaosomes) and thus differences in gene expression may not immediately result in differences in protein levels and vice versa. Finally, given that circulating cytokines can be impacted by multiple factors (*e.g.*, co-occurring mental illness, Hoge et al., 2009; Schiepers, Wichers, & Maes, 2005, or viral infection, Yu et al., 2011), a larger sample size may be required to detect differences in circulating cytokines in relation to minority stress. This supports the hypothesis that although immune function and inflammation are associated with minority stress, a broader set of biomarkers should be investigated in future studies. While these results corroborate previous research that has found a relationship between minority stress and HIV related outcomes such as faster progression to AIDS, AIDS mortality, and lower CD4 T-cell count (Cole et al., 1997; Pala et al., 2015), more research is needed to elucidate the specific mechanisms by which minority stress may be related to poorer HIV outcomes. Our results suggest that dysregulation of the immune and inflammatory systems could be a potential area for further investigation.

This study found that between low and high sexual minority stress groups, 32 differentially expressed genes and 8 perturbed pathways were related to cancer. These results corroborate two studies which previously found a relationship between minority stress and development of cancer (Cole, Kemeny, Taylor, & Visscher, 1996; Frost et al., 2015). To appropriately contextualize these results, it is important to note that we included genes related to cancer irrespective of their tissue of origin or the directionality of this relationship. Furthermore these results may in part be biased by the amount of cancer research that is reflected in the literature; while many genes are being documented as related to cancer development (*e.g.*, cell survival and proliferation, biological functions which exist outside of cancer), their roles are likely more complex than solely based on their contribution to cancer pathogenesis. In time our understanding of the complex networks of cancer development will have greater specificity. In the meantime, we observed differential expression of genes related to cancer development of viral origins, such as *ZNF426* (Yang, Wen, Minhas, & Wood, 2009; Yang & Wood, 2007), which is important to the replication of Kaposi's sarcoma associated herpes virus, and those that have been shown to be related to cancer development (e.g., *SKIL*, Annala et al., 2015; Hagerstrand et al., 2013; Shinozuka et al., 2013; Zhu et al., 2016) or aggressiveness (e.g. *EIF5A2*, Li et al., 2014; Luo et al., 2009; Meng et al., 2015). Notably, the relationship observed in this study between minority stress and differential expression of *ZNF426* support prior research that illustrated the role of autonomic nervous system activity in the reactivation of Kaposi's sarcoma associated herpes virus (Chang et al., 2005). The neuroactive-ligand receptor interaction (KEGG ID: 04080) also appears to be associated

with cancer (e.g., Fang et al., 2013; Liu, Wang, & Sun, 2015; Wei et al., 2015; Wei, Tang, & Li, 2012), though this pathway has also been associated with other phenomena (e.g., chronic fatigue syndrome as in Fang et al., 2006, or viral infection as in Huang et al., 2008). Of note, the measure of minority stress that we used within this study likely detects persistent minority stress, as opposed to acute minority stress (e.g., exposure to a single instance of discrimination), and it is likewise expected that greater cancer risk would be conferred over time rather than through acute exposure. More research is needed to understand the specific cascade of biological events that may result after acute exposure to sexual minority stressors, through prolonged exposure to minority stressors, and to understand if those responses can be modified through interventions.

The genes and pathways documented here in relationship to minority stress warrant replication in order to further refine which genes would be most useful as outcomes in future studies examining minority stress or interventions to reduce the impacts of this unique, pernicious form of stress. Future research is needed to validate the candidate genes identified in this study in an independent sample of participants. The genes and pathways identified here and in replication studies could function as outcomes within studies examining the efficacy of cognitive or behavioral interventions intended to reduce the deleterious impacts of minority stress. These genes or pathways may even be considered as measures that could enable titration or adjustment of these interventions (e.g., changes in the expression of these genes would indicate efficacy, while lack of change would warrant adjustment of the intervention).

While unable to examine it here, the complex paths by which sexual minority stress and its relationship with gene expression may interact with psychological processes such as rumination, hopelessness, or self-schemas (i.e., expansion of work by Hatzenbuehler, 2009) is an important area of future research. Within this model, it is anticipated that gene expression, as an active biological process responsible for the biological behavior of the organism, may both be impacted by and reciprocally impact these psychological processes. In addition to psychological processes, behavioral processes associated with sexual minority stress (e.g., smoking or substance use, Livingston, Flentje, Heck, Szalda-Petree, & Cochran, 2017) are important to consider. Additionally, social processes which may impact biological functioning (e.g., childhood adversity, Miller & Cole, 2012, or social isolation, Cole et al., 2007) and may have reciprocal relationships with minority stress (e.g., the higher rates of childhood victimization reported among sexual minority people, Friedman et al., 2011) will also be important to consider within this model. Identifying ways in which the response to sexual minority stressors may impact subsequent biological functioning will be an important area for future research. Sexual minority stress was considered here while controlling for recent stimulant use. As sexual minority stress is predictive of substance use (Hatzenbuehler et al., 2008; Livingston et al., 2017), additional studies with larger samples are needed to examine whether or not there is a mediating or moderating effect for substance use in the relationship between sexual minority stress and expression of genes on these pathways. Of note, none of the differentially expressed genes reported here overlapped with those found to be related to stimulant use in our companion paper resulting from this project (Carrico et al., under review), which suggests that the relationship between sexual minority stress and gene expression may be unique and not a direct result of stimulant use. Future research is also

needed to examine if these same candidate genes or pathways are responsive to stress in general, and sexual minority stress in particular, and whether or not these patterns of gene expression may be modified through intervention, either through modification of health behaviors or by targeting the stress directly.

There are limitations to consider in contextualizing these results and considering future directions for research. First, this study was within individuals who had used methamphetamine recently, though not all participants had used methamphetamine or other stimulants in the days preceding the study visit. Stimulant use that had occurred in the days just prior to the study visit was biologically confirmed via urinalysis within this sample, and we controlled for this variable within analysis to control for the effects of stimulants. This study was also conducted among sexual minority men living with HIV, and the effects among people living with HIV may be more robust than effects among persons who are living without a co-occurring disease process. Much of the literature to date that has documented a relationship between minority stress and biological functioning appears to be among individuals with HIV, and it remains unknown if that is because people living with HIV are generally more studied, or if it is because the same relationships have not been observed among individuals who do not have HIV.

This study did not account for important medical comorbidities that could be occurring among people living with HIV, though the lack of observed differences in CD4 T cell count and IL-6 levels suggests relatively comparable health status of the high and low minority stress groups. Comorbidities can be particularly deleterious for health among those living with HIV and are linked to greater risk of morbidity (Lohse et al., 2011; Rodriguez-Penney et al., 2013). These comorbidities should be accounted for in subsequent research. Given the modest size of the sample, it is likely that additional differences in gene expression and circulating cytokines were not identified (*i.e.*, false negatives). This study consisted only of men who were assigned birth sex of male; future work should examine the relationship between minority stress and expression of genes among sexual minority women and gender minority people. For example, sexual minority women are at greater risk for being overweight or obese (Boehmer, Bowen, & Bauer, 2007) suggesting that biological pathways between stress related to sexual minority status and health may be different (*e.g.*, metabolic) among this population. This study also focused solely on minority stress, while future work should explore the complex relationships between minority stress and other psychosocial factors that may be related to both gene expression and minority stress such as childhood adversity and social isolation. This study included sexual minority men irrespective of their sexual identity. Sexual minority stressors may be uniquely experienced by sexual minority men who identify as heterosexual (as one did in this study).

In conclusion, our study is among the first to observe a relationship between sexual minority stress and differential expression of single genes as well as sets of genes that are functionally related to inflammation, immune function, cancer, and cardiovascular function. Our results suggest several potential biological pathways for future work investigating the relationship between sexual minority stress and health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

Alix-Panabières C, Cayrefourcq L, Mazard T, Maudelonde T, Assenat E, Assou S. Molecular Portrait of Metastasis-Competent Circulating Tumor Cells in Colon Cancer Reveals the Crucial Role of Genes Regulating Energy Metabolism and DNA Repair. Clinical Chemistry. 2017; 63(3):700–713. https://doi.org/10.1373/clinchem.2016.263582. [PubMed: 28007957]

Allison DB, Cui X, Page GP, Sabripour M. Microarray data analysis: from disarray to consolidation and consensus. Nature Reviews Genetics. 2006; 7(1):55.

Anders, S., McCarthy, DJ., Chen, Y., Okoniewski, M., Smyth, GK., Huber, W., Robinson, MD. Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. ArXiv Preprint ArXiv:1302.3685. 2013. Retrieved from https://arxiv.org/abs/1302.3685

Annala M, Kivinummi K, Tuominen J, Karakurt S, Granberg K, Latonen L, Nykter M. Recurrent SKIL-activating rearrangements in ETS-negative prostate cancer. Oncotarget. 2015; 6(8):6235–6250. https://doi.org/10.18632/oncotarget.3359. [PubMed: 25749039]

Antoni MH, Lutgendorf SK, Blomberg B, Carver CS, Lechner S, Diaz A, Cole SW. Cognitive-behavioral stress management reverses anxiety-related leukocyte transcriptional dynamics. Biological Psychiatry. 2012; 71(4):366–372. [PubMed: 22088795]

Arthurs C, Murtaza BN, Thomson C, Dickens K, Henrique R, Patel HRH, Ahmed A. Expression of ribosomal proteins in normal and cancerous human prostate tissue. PloS One. 2017; 12(10):e0186047. https://doi.org/10.1371/journal.pone.0186047. [PubMed: 29016636]

Baik IH, Jo GH, Seo D, Ko MJ, Cho CH, Lee MG, Lee YH. Knockdown of RPL9 expression inhibits colorectal carcinoma growth via the inactivation of Id-1/NF-κB signaling axis. International Journal of Oncology. 2016; 49(5):1953–1962. https://doi.org/10.3892/ijo.2016.3688. [PubMed: 27633352]

Behler-Janbeck F, Takano T, Maus R, Stolper J, Jonigk D, Tort Tarrés M, Maus UA. C-type Lectin Mincle Recognizes Glucosyl-diacylglycerol of Streptococcus pneumoniae and Plays a Protective Role in Pneumococcal Pneumonia. PLoS Pathogens. 2016; 12(12):e1006038. https://doi.org/10.1371/journal.ppat.1006038. [PubMed: 27923071]

Bekiaris V, Šedý JR, Macauley MG, Rhode-Kurnow A, Ware CF. The inhibitory receptor BTLA controls γδ T cell homeostasis and inflammatory responses. Immunity. 2013; 39(6):1082–1094. https://doi.org/10.1016/j.immuni.2013.10.017. [PubMed: 24315996]

Black DS, Cole SW, Irwin MR, Breen E, Cyr NMS, Nazarian N, Lavretsky H. Yogic meditation reverses NF-κB and IRF-related transcriptome dynamics in leukocytes of family dementia caregivers in a randomized controlled trial. Psychoneuroendocrinology. 2013; 38(3):348–355. [PubMed: 22795617]

Boehmer U, Bowen DJ, Bauer GR. Overweight and Obesity in Sexual-Minority Women: Evidence From Population-Based Data. American Journal of Public Health. 2007; 97(6):1134–1140. https://doi.org/10.2105/AJPH.2006.088419. [PubMed: 17463369]

Cabral GA. Drugs of Abuse, Immune Modulation, and AIDS. Journal of Neuroimmune Pharmacology. 2006; 1(3):280–295. https://doi.org/10.1007/s11481-006-9023-5. [PubMed: 18040805]

Carrico AW, Johnson MO, Morin SF, Remien RH, Riley ED, Hecht FM, Fuchs D. Stimulant use is associated with immune activation and depleted tryptophan among HIV-positive persons on anti-retroviral therapy. Brain, Behavior, and Immunity. 2008; 22(8):1257–1262.

Chae CU, Lee RT, Rifai N, Ridker PM. Blood Pressure and Inflammation in Apparently Healthy Men. Hypertension. 2001; 38(3):399–403. https://doi.org/10.1161/01.HYP.38.3.399. [PubMed: 11566912]

Chang M, Brown HJ, Collado-Hidalgo A, Arevalo JM, Galic Z, Symensma TL, Cole SW. β-Adrenoreceptors Reactivate Kaposi's Sarcoma-Associated Herpesvirus Lytic Replication via PKA-Dependent Control of Viral RTA. Journal of Virology. 2005; 79(21):13538–13547. https://doi.org/10.1128/JVI.79.21.13538-13547.2005. [PubMed: 16227274]

Chen J, Wei Y, Feng Q, Ren L, He G, Chang W, Qin X. Ribosomal protein S15A promotes malignant transformation and predicts poor outcome in colorectal cancer through misregulation of p53 signaling pathway. International Journal of Oncology. 2016; 48(4):1628–1638. https://doi.org/10.3892/ijo.2016.3366. [PubMed: 26847263]

Chen X, Dong C, Law PTY, Chan MTV, Su Z, Wang S, Xu H. MicroRNA-145 targets TRIM2 and exerts tumor-suppressing functions in epithelial ovarian cancer. Gynecologic Oncology. 2015; 139(3):513–519. https://doi.org/10.1016/j.ygyno.2015.10.008. [PubMed: 26472353]

Chen YL, Chan SH, Lin PY, Chu PY. The expression of a tumor suppressor gene JDP2 and its prognostic value in hepatocellular carcinoma patients. Human Pathology. 2017; 63:212–216. https://doi.org/10.1016/j.humpath.2017.03.003. [PubMed: 28315425]

Cheng L, Wang P, Yang S, Yang Y, Zhang Q, Zhang W, Zhang Q. Identification of genes with a correlation between copy number and expression in gastric cancer. BMC Medical Genomics. 2012; 5:14. https://doi.org/10.1186/1755-8794-5-14. [PubMed: 22559327]

Chu J, Floyd R, Diep H, Pardo S, Goldblum P, Bongar B. A tool for the culturally competent assessment of suicide: The Cultural Assessment of Risk for Suicide (CARS) Measure. Psychological Assessment. 2013a; 25(2):424. [PubMed: 23356681]

Chu, J., Floyd, R., Diep, H., Pardo, S., Goldblum, P., Bongar, B. A tool for the culturally competent assessment of suicide: The Cultural Assessment of Risk for Suicide (CARS) Measure Manual. 2013b. Retrieved from http://psycnet.apa.org/journals/pas/25/2/424/

Cochran SD, Ackerman D, Mays VM, Ross MW. Prevalence of non-medical drug use and dependence among homosexually active men and women in the US population. Addiction. 2004; 99(8):989–998. [PubMed: 15265096]

Cochran SD, Keenan C, Schober C, Mays VM. Estimates of alcohol use and clinical treatment needs among homosexually active men and women in the US population. Journal of Consulting and Clinical Psychology. 2000; 68(6):1062. [PubMed: 11142540]

Cochran SD, Mays VM. Physical health complaints among lesbians, gay men, and bisexual and homosexually experienced heterosexual individuals: results from the California Quality of Life Survey. American Journal of Public Health. 2007; 97(11):2048–2055. [PubMed: 17463371]

Cochran SD, Sullivan JG, Mays VM. Prevalence of mental disorders, psychological distress, and mental health services use among lesbian, gay, and bisexual adults in the United States. Journal of Consulting and Clinical Psychology. 2003; 71(1):53. [PubMed: 12602425]

Cohen S, Janicki-Deverts D, Doyle WJ, Miller GE, Frank E, Rabin BS, Turner RB. Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. Proceedings of the National Academy of Sciences. 2012; 109(16):5995–5999.

Cole SW, Hawkley LC, Arevalo JM, Cacioppo JT. Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes. Proceedings of the National Academy of Sciences. 2011; 108(7):3080–3085.

Cole SW, Hawkley LC, Arevalo JM, Sung CY, Rose RM, Cacioppo JT. Social regulation of gene expression in human leukocytes. Genome Biol. 2007; 8(9):R189. [PubMed: 17854483]

Cole SW, Kemeny ME, Taylor SE. Social identity and physical health: Accelerated HIV progression in rejection-sensitive gay men. Journal of Personality and Social Psychology. 1997; 72(2):320–335. https://doi.org/10.1037/0022-3514.72.2.320. [PubMed: 9107003]

Cole SW, Kemeny ME, Taylor SE, Visscher BR. Elevated physical health risk among gay men who conceal their homosexual identity. Health Psychology. 1996; 15(4):243. [PubMed: 8818670]

Cole SW, Kemeny ME, Taylor SE, Visscher BR, Fahey JL. Accelerated course of human immunodeficiency virus infection in gay men who conceal their homosexual identity. Psychosomatic Medicine. 1996; 58(3):219–231. [PubMed: 8771621]

Cole SW, Korin YD, Fahey JL, Zack JA. Norepinephrine accelerates HIV replication via protein kinase A-dependent effects on cytokine production. The Journal of Immunology. 1998; 161(2):610–616. [PubMed: 9670934]

Conron KJ, Mimiaga MJ, Landers SJ. A population-based study of sexual orientation identity and gender differences in adult health. American Journal of Public Health. 2010; 100(10):1953–1960. [PubMed: 20516373]

Cox DR, Reid N. Parameter Orthogonality and Approximate Conditional Inference. Journal of the Royal Statistical Society Series B (Methodological). 1987; 49(1):1–39.

Creswell JD, Irwin MR, Burklund LJ, Lieberman MD, Arevalo JM, Ma J, Cole SW. Mindfulness-based stress reduction training reduces loneliness and pro-inflammatory gene expression in older adults: a small randomized controlled trial. Brain, Behavior, and Immunity. 2012; 26(7):1095–1101.

Darke S, Kaye S, McKetin R, Duflou J. Major physical and psychological harms of methamphetamine use. Drug and Alcohol Review. 2008; 27(3):253–262. [PubMed: 18368606]

David Sancho, Manual Gomez, Francisco, Sanchez-Madrid. CD69 is an immunoregulatory molecule induced following activation. Trends in Immunology. 2005; 26(3):136–140. https://doi.org/10.1016/j.it.2004.12.006. [PubMed: 15745855]

De Bortoli M, Castellino RC, Lu XY, Deyo J, Sturla LM, Adesina AM, Kim JYH. Medulloblastoma outcome is adversely associated with overexpression of EEF1D, RPL30, and RPS20 on the long arm of chromosome 8. BMC Cancer. 2006; 6:223. https://doi.org/10.1186/1471-2407-6-223. [PubMed: 16968546]

D'Souza S, Kurihara N, Shiozawa Y, Joseph J, Taichman R, Galson DL, Roodman GD. Annexin II interactions with the annexin II receptor enhance multiple myeloma cell adhesion and growth in the bone marrow microenvironment. Blood. 2012; 119(8):1888–1896. https://doi.org/10.1182/blood-2011-11-393348. [PubMed: 22223826]

Dutheil N, Smith SC, Agúndez L, Vincent-Mistiaen ZI, Escalante CR, Linden RM, Henckaerts E. Adeno-associated virus Rep represses the human integration site promoter by two pathways that are similar to those required for the regulation of the viral p5 promoter. Journal of Virology. 2014; 88(15):8227–8241. https://doi.org/10.1128/JVI.00412-14. [PubMed: 24829354]

Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N, Golub TR. Identification of RPS14 as a 5q-syndrome gene by RNA interference screen. Nature. 2008; 451(7176):335–339. https://doi.org/10.1038/nature06494. [PubMed: 18202658]

Emmert-Streib F, Glazko GV. Pathway Analysis of Expression Data: Deciphering Functional Building Blocks of Complex Diseases. PLOS Computational Biology. 2011; 7(5):e1002053. https://doi.org/10.1371/journal.pcbi.1002053. [PubMed: 21637797]

Everett B, Mollborn S. Differences in Hypertension by Sexual Orientation Among U.S. Young Adults. Journal of Community Health. 2013; 38(3):588–596. https://doi.org/10.1007/s10900-013-9655-3. [PubMed: 23397511]

Fang H, Xie Q, Boneva R, Fostel J, Perkins R, Tong W. Gene expression profile exploration of a large dataset on chronic fatigue syndrome. Pharmacogenomics. 2006; 7(3):429–440. https://doi.org/10.2217/14622416.7.3.429. [PubMed: 16610953]

Fang Z, Cui X. Design and validation issues in RNA-seq experiments. Briefings in Bioinformatics. 2011; 12(3):280–287. https://doi.org/10.1093/bib/bbr004. [PubMed: 21498551]

Fang ZQ, Zang WD, Chen R, Ye BW, Wang XW, Yi SH, Ye G. Gene expression profile and enrichment pathways in different stages of bladder cancer. Genetics and Molecular Research: GMR. 2013; 12(2):1479–1489. https://doi.org/10.4238/2013.May.6.1. [PubMed: 23765955]

Fredriksen-Goldsen KI, Kim HJ, Barkan SE, Muraco A, Hoy-Ellis CP. Health Disparities Among Lesbian, Gay, and Bisexual Older Adults: Results From a Population-Based Study. American

Journal of Public Health. 2013; 103(10):1802–1809. https://doi.org/10.2105/AJPH.2012.301110. [PubMed: 23763391]

Friedman MS, Marshal MP, Guadamuz TE, Wei C, Wong CF, Saewyc EM, Stall R. A Meta-Analysis of Disparities in Childhood Sexual Abuse, Parental Physical Abuse, and Peer Victimization Among Sexual Minority and Sexual Nonminority Individuals. American Journal of Public Health. 2011; 101(8):1481–1494. https://doi.org/10.2105/AJPH.2009.190009. [PubMed: 21680921]

Frost DM, Lehavot K, Meyer IH. Minority stress and physical health among sexual minority individuals. Journal of Behavioral Medicine. 2015; 38(1):1–8. [PubMed: 23864353]

Fu XY, Wang HY, Tan L, Liu SQ, Cao HF, Wu MC. Overexpression of p28/gankyrin in human hepatocellular carcinoma and its clinical significance. World Journal of Gastroenterology. 2002; 8(4):638–643. [PubMed: 12174370]

Goeman JJ, Bühlmann P. Analyzing gene expression data in terms of gene sets: methodological issues. Bioinformatics. 2007; 23(8):980–987. https://doi.org/10.1093/bioinformatics/btm051. [PubMed: 17303618]

Grzmil M, Rzymski T, Milani M, Harris AL, Capper RG, Saunders NJ, Norbury CJ. An oncogenic role of eIF3e/INT6 in human breast cancer. Oncogene. 2010; 29(28):4080–4089. https://doi.org/10.1038/onc.2010.152. [PubMed: 20453879]

Hagerstrand D, Tong A, Schumacher SE, Ilic N, Shen RR, Cheung HW, Hahn WC. Systematic interrogation of 3q26 identifies TLOC1 and SKIL as cancer drivers. Cancer Discovery. 2013; 3(9): 1044–1057. https://doi.org/10.1158/2159-8290.CD-12-0592. [PubMed: 23764425]

Hatzenbuehler ML. How does sexual minority stigma "get under the skin"? A psychological mediation framework. Psychological Bulletin. 2009; 135(5):707. [PubMed: 19702379]

Hatzenbuehler ML, McLaughlin KA. Structural stigma and hypothalamic–pituitary–adrenocortical axis reactivity in lesbian, gay, and bisexual young adults. Annals of Behavioral Medicine. 2014; 47(1):39–47. [PubMed: 24154988]

Hatzenbuehler ML, McLaughlin KA, Slopen N. Sexual orientation disparities in cardiovascular biomarkers among young adults. American Journal of Preventive Medicine. 2013; 44(6):612–621. [PubMed: 23683979]

Hatzenbuehler ML, Nolen-Hoeksema S, Erickson SJ. Minority stress predictors of HIV risk behavior, substance use, and depressive symptoms: results from a prospective study of bereaved gay men. Health Psychology. 2008; 27(4):455. [PubMed: 18643003]

HGNC database of human gene names | HUGO Gene Nomenclature Committee. n.d. Retrieved September 20, 2017, from http://www.genenames.org/

Hoge EA, Brandstetter K, Moshier S, Pollack MH, Wong KK, Simon NM. Broad spectrum of cytokine abnormalities in panic disorder and posttraumatic stress disorder. Depression and Anxiety. 2009; 26(5):447–455. https://doi.org/10.1002/da.20564. [PubMed: 19319993]

Huang YCT, Li Z, Hyseni X, Schmitt M, Devlin RB, Karoly ED, Soukup JM. Identification of gene biomarkers for respiratory syncytial virus infection in a bronchial epithelial cell line. Genomic Medicine. 2008; 2(3–4):113–125. https://doi.org/10.1007/s11568-009-9080-y. [PubMed: 19459069]

Huebner DM, Davis MC. Gay and bisexual men who disclose their sexual orientations in the workplace have higher workday levels of salivary cortisol and negative affect. Annals of Behavioral Medicine. 2005; 30(3):260–267. [PubMed: 16336077]

Hughes TL, Eliason M. Substance use and abuse in lesbian, gay, bisexual and transgender populations. Journal of Primary Prevention. 2002; 22(3):263–298.

Institute of Medicine. The Health of Lesbian, Gay, Bisexual, and Transgender People: Building a Foundation for Better Understanding. Washington, D.C.: The National Academies Press; 2011.

Irwin MR, Olmos L, Wang M, Valladares EM, Motivala SJ, Fong T, Cole SW. Cocaine dependence and acute cocaine induce decreases of monocyte proinflammatory cytokine expression across the diurnal period: autonomic mechanisms. Journal of Pharmacology and Experimental Therapeutics. 2007; 320(2):507–515. [PubMed: 17068203]

Jin S, Wang Y, Zhang Y, Zhang HZ, Wang SJ, Tang JQ, Ge HL. Humoral immune responses against tumor-associated antigen OVA66 originally defined by serological analysis of recombinant cDNA

expression libraries and its potentiality in cellular immunity. Cancer Science. 2008; 99(8):1670–1678. https://doi.org/10.1111/j.1349-7006.2008.00860.x. [PubMed: 18754882]

Kabagambe EK, Judd SE, Howard VJ, Zakai NA, Jenny NS, Hsieh M, Cushman M. Inflammation biomarkers and risk of all-cause mortality in the reasons for geographic and racial differences in stroke cohort. American Journal of Epidemiology. 2011:kwr085.

Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Research. 2011; 40(D1):D109–D114. [PubMed: 22080510]

KEGG: Kyoto Encyclopedia of Genes and Genomes. n.d. Retrieved September 20, 2017, from http://www.genome.jp/kegg/

King M, Semlyen J, Tai SS, Killaspy H, Osborn D, Popelyuk D, Nazareth I. A systematic review of mental disorder, suicide, and deliberate self harm in lesbian, gay and bisexual people. BMC Psychiatry. 2008; 8(1):70. [PubMed: 18706118]

Komati H, Maharsy W, Beauregard J, Hayek S, Nemer M. ZFP260 Is an Inducer of Cardiac Hypertrophy and a Nuclear Mediator of Endothelin-1 Signaling. The Journal of Biological Chemistry. 2011; 286(2):1508–1516. https://doi.org/10.1074/jbc.M110.162966. [PubMed: 21051538]

Kottom TJ, Hebrink DM, Jenson PE, Nandakumar V, Wüthrich M, Wang H, Limper AH. The Interaction of Pneumocystis with the C-Type Lectin Receptor Mincle Exerts a Significant Role in Host Defense against Infection. Journal of Immunology (Baltimore, Md: 1950). 2017; 198(9): 3515–3525. https://doi.org/10.4049/jimmunol.1600744.

Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, Nixon D. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Medicine. 2008; 5(10):e203. [PubMed: 18942885]

Landau WM, Liu P. Dispersion Estimation and Its Effect on Test Performance in RNA-seq Data Analysis: A Simulation-Based Comparison of Methods. PLOS ONE. 2013; 8(12):e81415. https://doi.org/10.1371/journal.pone.0081415. [PubMed: 24349066]

Lange RA, Hillis LD. Cardiovascular Complications of Cocaine Use. New England Journal of Medicine. 2001; 345(5):351–358. https://doi.org/10.1056/NEJM200108023450507. [PubMed: 11484693]

Lederman MM, Funderburg NT, Sekaly RP, Klatt NR, Hunt PW. Residual immune dysregulation syndrome in treated HIV infection. Advances in Immunology. 2013; 119:51–83. https://doi.org/10.1016/B978-0-12-407707-2.00002-3. [PubMed: 23886064]

Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics. 2012; 28(6):882–883. [PubMed: 22257669]

Li Y, Fu L, Li JB, Qin Y, Zeng T, Zhou J, Guan XY. Increased expression of EIF5A2, via hypoxia or gene amplification, contributes to metastasis and angiogenesis of esophageal squamous cell carcinoma. Gastroenterology. 2014; 146(7):1701–1713.e9. https://doi.org/10.1053/j.gastro.2014.02.029. [PubMed: 24561231]

Li Z, Lin S, Jiang T, Wang J, Lu H, Tang H, Fan J. Overexpression of eIF3e is correlated with colon tumor development and poor prognosis. International Journal of Clinical and Experimental Pathology. 2014; 7(10):6462–6474. [PubMed: 25400724]

Liu X, Wang J, Sun G. Identification of key genes and pathways in renal cell carcinoma through expression profiling data. Kidney & Blood Pressure Research. 2015; 40(3):288–297. https://doi.org/10.1159/000368504. [PubMed: 26043775]

Liu YY, Patwardhan GA, Bhinge K, Gupta V, Gu X, Jazwinski SM. Suppression of glucosylceramide synthase restores p53-dependent apoptosis in mutant p53 cancer cells. Cancer Research. 2011; 71(6):2276–2285. https://doi.org/10.1158/0008-5472.CAN-10-3107. [PubMed: 21278235]

Liu YY, Patwardhan GA, Xie P, Gu X, Giuliano AE, Cabot MC. Glucosylceramide synthase, a factor in modulating drug resistance, is overexpressed in metastatic breast carcinoma. International Journal of Oncology. 2011; 39(2):425–431. https://doi.org/10.3892/ijo.2011.1052. [PubMed: 21617856]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Livingston N, Flentje A, Heck NC, Szalda-Petree A, Cochran BN. Ecological momentary assessment of daily prejudice experiences and nicotine, alcohol, and drug use among sexual and gender minority individuals. Journal of Consulting and Clinical Psychology. 2017; 85(12):1131–1143. [PubMed: 29189029]

Llibre A, López-Macías C, Marafioti T, Mehta H, Partridge A, Kanzig C, Willberg CB. LLT1 and CD161 Expression in Human Germinal Centers Promotes B Cell Activation and CXCR4 Downregulation. Journal of Immunology (Baltimore, Md: 1950). 2016; 196(5):2085–2094. https://doi.org/10.4049/jimmunol.1502462.

Lohse N, Gerstoft J, Kronborg G, Larsen CS, Pedersen C, Pedersen G, Obel N. Comorbidity Acquired Before HIV Diagnosis and Mortality in Persons Infected and Uninfected With HIV: A Danish Population-Based Cohort Study. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2011; 57(4):334. https://doi.org/10.1097/QAI.0b013e31821d34ed. [PubMed: 21522017]

Love MI, Anders S, Kim V, Huber W. RNA-Seq workflow: gene-level exploratory analysis and differential expression. F1000Research. 2015; 4 Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4670015.1/.

Luo JH, Hua WF, Rao HL, Liao YJ, Kung HF, Zeng YX, Xie D. Overexpression of EIF-5A2 predicts tumor recurrence and progression in pTa/pT1 urothelial carcinoma of the bladder. Cancer Science. 2009; 100(5):896–902. https://doi.org/10.1111/j.1349-7006.2009.01126.x. [PubMed: 19298601]

Luo W, Friedman MS, Shedden K, Hankenson KD, Woolf PJ. GAGE: generally applicable gene set enrichment for pathway analysis. BMC Bioinformatics. 2009; 10(1):161. [PubMed: 19473525]

Mao G, Jin J, Kunapuli SP, Rao AK. Nuclear factor-κB regulates expression of platelet phospholipase C-β2 (PLCB2). Thrombosis and Haemostasis. 2016; 116(5):931–940. https://doi.org/10.1160/TH15-09-0749. [PubMed: 27465150]

Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y. RNA-seq: An assessment of technical reproducibility and comparison with gene expression arrays. Genome Research. 2008; 18(9):1509–1517. https://doi.org/10.1101/gr.079558.108. [PubMed: 18550803]

Maruyama Y, Miyazaki T, Ikeda K, Okumura T, Sato W, Horie-Inoue K, Inoue S. Short hairpin RNA library-based functional screening identified ribosomal protein L31 that modulates prostate cancer cell growth via p53 pathway. PloS One. 2014; 9(10):e108743. https://doi.org/10.1371/journal.pone.0108743. [PubMed: 25285958]

Matta J, Morales L, Dutil J, Bayona M, Alvarez C, Suarez E. Differential expression of DNA repair genes in Hispanic women with breast cancer. Molecular Cancer Biology. 2013; 1(1):54. https://doi.org/10.9777/mcb.2013.10006. [PubMed: 25309843]

McCabe SE, Bostwick WB, Hughes TL, West BT, Boyd CJ. The relationship between discrimination and substance use disorders among lesbian, gay, and bisexual adults in the United States. American Journal of Public Health. 2010; 100(10):1946–1952. [PubMed: 20075317]

McCabe SE, Boyd C, Hughes TL, d'Arcy H. Sexual identity and substance use among undergraduate students. Substance Abuse. 2003; 24(2):77–91. [PubMed: 12766375]

McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Research. 2012; 40(10):4288–4297. [PubMed: 22287627]

McLellan AT, Kushner H, Metzger D, Peters R, Smith I, Grissom G, Argeriou M. The fifth edition of the Addiction Severity Index. Journal of Substance Abuse Treatment. 1992; 9(3):199–213. [PubMed: 1334156]

Meng QB, Kang WM, Yu JC, Liu YQ, Ma ZQ, Zhou L, Zhou WX. Overexpression of eukaryotic translation initiation factor 5A2 (EIF5A2) correlates with cell aggressiveness and poor survival in gastric cancer. PloS One. 2015; 10(3):e0119229. https://doi.org/10.1371/journal.pone.0119229. [PubMed: 25793713]

Meng Y, He L, Guo X, Tang S, Zhao X, Du R, Fan D. Gankyrin promotes the proliferation of human pancreatic cancer. Cancer Letters. 2010; 297(1):9–17. https://doi.org/10.1016/j.canlet.2010.04.019. [PubMed: 20483533]

Meyer IH. Prejudice, social stress, and mental health in lesbian, gay, and bisexual populations: conceptual issues and research evidence. Psychological Bulletin. 2003; 129(5):674. [PubMed: 12956539]

Miller GE, Cole SW. Clustering of depression and inflammation in adolescents previously exposed to childhood adversity. Biological Psychiatry. 2012; 72(1):34–40. [PubMed: 22494534]

Miller G, Rohleder N, Cole SW. Chronic interpersonal stress predicts activation of pro-and anti-inflammatory signaling pathways six months later. Psychosomatic Medicine. 2009; 71(1):57. [PubMed: 19073750]

Moldoveanu AI, Shephard RJ, Shek PN. Exercise elevates plasma levels but not gene expression of IL-1β, IL-6, and TNF-α in blood mononuclear cells. Journal of Applied Physiology. 2000; 89(4): 1499–1504. https://doi.org/10.1152/jappl.2000.89.4.1499. [PubMed: 11007588]

Müllbacher A, Waring P, Tha Hla R, Tran T, Chin S, Stehle T, Simon MM. Granzymes are the essential downstream effector molecules for the control of primary virus infections by cytolytic leukocytes. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96(24):13950–13955. [PubMed: 10570179]

Murphy ML, Slavich GM, Rohleder N, Miller GE. Targeted rejection triggers differential pro-and anti-inflammatory gene expression in adolescents as a function of social status. Clinical Psychological Science. 2012 2167702612455743.

Natrajan R, Little SE, Reis-Filho JS, Hing L, Messahel B, Grundy PE, Jones C. Amplification and overexpression of CACNA1E correlates with relapse in favorable histology Wilms' tumors. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research. 2006; 12(24):7284–7293. https://doi.org/10.1158/1078-0432.CCR-06-1567. [PubMed: 17189400]

Norcini Pala A, Hart RP, Steca P. Minority Stress, Depression and HIV-Progression Biomarkers: An Exploratory Study on a Sample of Italian HIV-Positive Gay and Bisexual Men. Journal of Gay & Lesbian Mental Health. 2015; 19(3):244–260.

O'Connell GC, Treadway MB, Petrone AB, Tennant CS, Lucke-Wold N, Chantler PD, Barr TL. Peripheral blood AKAP7 expression as an early marker for lymphocyte-mediated post-stroke blood brain barrier disruption. Scientific Reports. 2017; 7(1):1172. https://doi.org/10.1038/s41598-017-01178-5. [PubMed: 28446746]

Oliver D, Ji H, Liu P, Gasparian A, Gardiner E, Lee S, Shtutman M. Identification of novel cancer therapeutic targets using a designed and pooled shRNA library screen. Scientific Reports. 2017; 7:43023. https://doi.org/10.1038/srep43023. [PubMed: 28223711]

Otsuki N, Kamimura Y, Hashiguchi M, Azuma M. Expression and function of the B and T lymphocyte attenuator (BTLA/CD272) on human T cells. Biochemical and Biophysical Research Communications. 2006; 344(4):1121–1127. https://doi.org/10.1016/j.bbrc.2006.03.242. [PubMed: 16643847]

Panaccione A, Guo Y, Yarbrough WG, Ivanov SV. Expression Profiling of Clinical Specimens Supports the Existence of Neural Progenitor-Like Stem Cells in Basal Breast Cancers. Clinical Breast Cancer. 2017; 17(4):298–306.e7. https://doi.org/10.1016/j.clbc.2017.01.007. [PubMed: 28216417]

Pandrea I, Happel KI, Amedee AM, Bagby GJ, Nelson S. Alcohol's role in HIV transmission and disease progression. Alcohol Research & Health: The Journal of the National Institute on Alcohol Abuse and Alcoholism. 2010; 33(3):203–218. [PubMed: 23584062]

Papanicolaou DA. The Pathophysiologic Roles of Interleukin-6 in Human Disease. Annals of Internal Medicine. 1998; 128(2):127. https://doi.org/10.7326/0003-4819-128-2-199801150-00009. [PubMed: 9441573]

Peng C, Zhang Z, Wu J, Lv Z, Tang J, Xie H, Zheng S. A critical role for ZDHHC2 in metastasis and recurrence in human hepatocellular carcinoma. BioMed Research International. 2014; 2014:832712. https://doi.org/10.1155/2014/832712. [PubMed: 24995331]

Perez-Oliva AB, Lachaud C, Szyniarowski P, Muñoz I, Macartney T, Hickson I, Alessi DR. USP45 deubiquitylase controls ERCC1–XPF endonuclease-mediated DNA damage responses. The EMBO Journal. 2015; 34(3):326–343. https://doi.org/10.15252/embj.201489184. [PubMed: 25538220]

Phan D, Cheng CJ, Galfione M, Vakar-Lopez F, Tunstead J, Thompson NE, Lin SH. Identification of Sp2 as a transcriptional repressor of carcinoembryonic antigen-related cell adhesion molecule 1 in tumorigenesis. Cancer Research. 2004; 64(9):3072–3078. [PubMed: 15126343]

Qin X, Wang X, Liu F, Morris LE, Wang X, Jiang B, Zhang Y. Gankyrin activates mTORC1 signaling by accelerating TSC2 degradation in colorectal cancer. Cancer Letters. 2016; 376(1):83–94. https://doi.org/10.1016/j.canlet.2016.03.013. [PubMed: 26975632]

Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. Applied Psychological Measurement. 1977; 1(3):385–401.

Rao W, Xie G, Zhang Y, Wang S, Wang Y, Zhang H, Ge H. OVA66, a tumor associated protein, induces oncogenic transformation of NIH3T3 cells. PloS One. 2014; 9(3):e85705. https://doi.org/10.1371/journal.pone.0085705. [PubMed: 24633332]

Rapaport F, Khanin R, Liang Y, Pirun M, Krek A, Zumbo P, Betel D. Comprehensive evaluation of differential gene expression analysis methods for RNA-seq data. Genome Biology. 2013; 14:3158. https://doi.org/10.1186/gb-2013-14-9-r95.

Rodriguez-Penney AT, Iudicello JE, Riggs PK, Doyle K, Ellis RJ, Letendre SL, Woods SP. Co-Morbidities in Persons Infected with HIV: Increased Burden with Older Age and Negative Effects on Health-Related Quality of Life. AIDS Patient Care and STDs. 2013; 27(1):5–16. https://doi.org/10.1089/apc.2012.0329. [PubMed: 23305257]

Sacerdote P, Franchi S, E Panerai A. Non-Analgesic Effects of Opioids: Mechanisms and Potential Clinical Relevance of Opioid-Induced Immunodepression. Current Pharmaceutical Design. 2012; 18(37):6034–6042. https://doi.org/10.2174/138161212803582496. [PubMed: 22747543]

Schiepers OJG, Wichers MC, Maes M. Cytokines and major depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2005; 29(2):201–217. https://doi.org/10.1016/j.pnpbp.2004.11.003. [PubMed: 15694227]

Schmitt AO, Specht T, Beckmann G, Dahl E, Pilarsky CP, Hinzmann B, Rosenthal A. Exhaustive mining of EST libraries for genes differentially expressed in normal and tumour tissues. Nucleic Acids Research. 1999; 27(21):4251–4260. [PubMed: 10518618]

Sell RL, Holliday ML. Sexual Orientation Data Collection Policy in the United States: Public Health Malpractice. American Journal of Public Health. 2014; 104(6):967–969. https://doi.org/10.2105/AJPH.2013.301814. [PubMed: 24825192]

Shinozuka E, Miyashita M, Mizuguchi Y, Akagi I, Kikuchi K, Makino H, Takizawa T. SnoN/SKIL modulates proliferation through control of hsa-miR-720 transcription in esophageal cancer cells. Biochemical and Biophysical Research Communications. 2013; 430(1):101–106. https://doi.org/10.1016/j.bbrc.2012.11.005. [PubMed: 23154181]

Shiozawa Y, Havens AM, Jung Y, Ziegler AM, Pedersen EA, Wang J, Taichman RS. Annexin II/annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer. Journal of Cellular Biochemistry. 2008; 105(2):370–380. https://doi.org/10.1002/jcb.21835. [PubMed: 18636554]

Slavich GM, Cole SW. The emerging field of human social genomics. Clinical Psychological Science. 2013 2167702613478594.

Soneson C, Delorenzi M. A comparison of methods for differential expression analysis of RNA-seq data. BMC Bioinformatics. 2013; 14(1):91. [PubMed: 23497356]

Stall R, Paul JP, Greenwood G, Pollack LM, Bein E, Crosby GM, Catania JA. Alcohol use, drug use and alcohol-related problems among men who have sex with men: the Urban Men's Health Study. Addiction. 2001; 96(11):1589–1601. [PubMed: 11784456]

Su Z, Labaj PP, Li S, Thierry-Mieg J, Thierry-Mieg D, Shi W, Thompson JF. A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequencing Quality Control Consortium. Nature Biotechnology. 2014; 32(9):903.

Sun X, Zhang H, Luo L, Zhong K, Ma Y, Fan L, Wan L. Comparative proteomic profiling identifies potential prognostic factors for human clear cell renal cell carcinoma. Oncology Reports. 2016; 36(6):3131–3138. https://doi.org/10.3892/or.2016.5159. [PubMed: 27748938]

Tarca AL, Draghici S, Khatri P, Hassan SS, Mittal P, Kim J, Romero R. A novel signaling pathway impact analysis. Bioinformatics. 2009; 25(1):75–82. https://doi.org/10.1093/bioinformatics/btn577. [PubMed: 18990722]

Tien PC, Choi AI, Zolopa AR, Benson C, Scherzer R, Bacchetti P, Grunfeld C. Inflammation and mortality in HIV-infected adults: analysis of the FRAM study cohort. Journal of Acquired Immune Deficiency Syndromes (1999). 2010; 55(3):316. [PubMed: 20581689]

Vilagut G, Forero CG, Barbaglia G, Alonso J. Screening for Depression in the General Population with the Center for Epidemiologic Studies Depression (CES-D): A Systematic Review with Meta-Analysis. PLOS ONE. 2016; 11(5):e0155431. https://doi.org/10.1371/journal.pone.0155431. [PubMed: 27182821]

Wang D, Berglund AE, Kenchappa RS, MacAulay RJ, Mulé JJ, Etame AB. BIRC3 is a biomarker of mesenchymal habitat of glioblastoma, and a mediator of survival adaptation in hypoxia-driven glioblastoma habitats. Scientific Reports. 2017; 7(1):9350. https://doi.org/10.1038/s41598-017-09503-8. [PubMed: 28839258]

Wang D, Berglund A, Kenchappa RS, Forsyth PA, Mulé JJ, Etame AB. BIRC3 is a novel driver of therapeutic resistance in Glioblastoma. Scientific Reports. 2016; 6:21710. https://doi.org/10.1038/srep21710. [PubMed: 26888114]

Wang L, Luo J, Nian Q, Xiao Q, Yang Z, Liu L. Ribosomal protein S14 silencing inhibits growth of acute myeloid leukemia transformed from myelodysplastic syndromes via activating p53. Hematology (Amsterdam, Netherlands). 2014; 19(4):225–231. https://doi.org/10.1179/1607845413Y.0000000127.

Wei B, Wang L, Du C, Hu G, Wang L, Jin Y, Kong D. Identification of differentially expressed genes regulated by transcription factors in glioblastomas by bioinformatics analysis. Molecular Medicine Reports. 2015; 11(4):2548–2554. https://doi.org/10.3892/mmr.2014.3094. [PubMed: 25514975]

Wei P, Tang H, Li D. Insights into pancreatic cancer etiology from pathway analysis of genome-wide association study data. PloS One. 2012; 7(10):e46887. https://doi.org/10.1371/journal.pone.0046887. [PubMed: 23056513]

Weng MT, Luo J. The enigmatic ERH protein: its role in cell cycle, RNA splicing and cancer. Protein & Cell. 2013; 4(11):807–812. https://doi.org/10.1007/s13238-013-3056-3. [PubMed: 24078386]

Wilkins KC, Lang AJ, Norman SB. Synthesis of the psychometric properties of the PTSD checklist (PCL) military, civilian, and specific versions. Depression and Anxiety. 2011; 28(7):596–606. [PubMed: 21681864]

Wu L, Li X, Xu F, Chang C, He Q, Zhang Z, Zhang Y. Over-expression of RPL23 in myelodysplastic syndromes is associated with apoptosis resistance of CD34+ cells and predicts poor prognosis and distinct response to CHG chemotherapy or decitabine. Annals of Hematology. 2012; 91(10):1547–1554. https://doi.org/10.1007/s00277-012-1486-2. [PubMed: 22580751]

Yamato A, Soda M, Ueno T, Kojima S, Sonehara K, Kawazu M, Mano H. Oncogenic activity of BIRC2 and BIRC3 mutants independent of nuclear factor-κB-activating potential. Cancer Science. 2015; 106(9):1137–1142. https://doi.org/10.1111/cas.12726. [PubMed: 26094954]

Yan SM, Tang JJ, Huang CY, Xi SY, Huang MY, Liang JZ, Du ZM. Reduced expression of ZDHHC2 is associated with lymph node metastasis and poor prognosis in gastric adenocarcinoma. PloS One. 2013; 8(2):e56366. https://doi.org/10.1371/journal.pone.0056366. [PubMed: 23457560]

Yang M, Lewinska M, Fan X, Zhu J, Yuan ZM. PRR14 is a novel activator of the PI3K pathway promoting lung carcinogenesis. Oncogene. 2016; 35(42):5527–5538. https://doi.org/10.1038/onc.2016.93. [PubMed: 27041574]

Yang Z, Wen HJ, Minhas V, Wood C. The zinc finger DNA-binding domain of K-RBP plays an important role in regulating Kaposi's sarcoma-associated herpesvirus RTA-mediated gene expression. Virology. 2009; 391(2):221–231. https://doi.org/10.1016/j.virol.2009.06.014. [PubMed: 19592062]

Yang Z, Wood C. The transcriptional repressor K-RBP modulates RTA-mediated transactivation and lytic replication of Kaposi's sarcoma-associated herpesvirus. Journal of Virology. 2007; 81(12):6294–6306. https://doi.org/10.1128/JVI.02648-06. [PubMed: 17409159]

Yao Y, Liu Y, Lv X, Dong B, Wang F, Li J, Xu Y. Down-regulation of ribosomal protein S15A inhibits proliferation of human glioblastoma cells in vivo and in vitro via AKT pathway. Tumour Biology: The Journal of the International Society for Oncodevelopmental Biology and Medicine. 2016; 37(4):4979–4990. https://doi.org/10.1007/s13277-015-4323-0. [PubMed: 26537582]

Yong WH, Shabihkhani M, Telesca D, Yang S, Tso JL, Menjivar JC, Tso CL. Ribosomal Proteins RPS11 and RPS20, Two Stress-Response Markers of Glioblastoma Stem Cells, Are Novel Predictors of Poor Prognosis in Glioblastoma Patients. PloS One. 2015; 10(10):e0141334. https://doi.org/10.1371/journal.pone.0141334. [PubMed: 26506620]

Yu X, Zhang X, Zhao B, Wang J, Zhu Z, Teng Z, Wu F. Intensive Cytokine induction in Pandemic H1N1 Influenza Virus Infection Accompanied by Robust Production of IL-10 and IL-6. PLOS ONE. 2011; 6(12):e28680. https://doi.org/10.1371/journal.pone.0028680. [PubMed: 22174866]

Yuanhong X, Feng X, Qingchang L, Jianpeng F, Zhe L, Kejian G. Downregulation of AP-1 repressor JDP2 is associated with tumor metastasis and poor prognosis in patients with pancreatic carcinoma. The International Journal of Biological Markers. 2010; 25(3):136–140. [PubMed: 20677166]

Zhang J, Fan D, Jian Z, Chen GG, Lai PBS. Cancer Specific Long Noncoding RNAs Show Differential Expression Patterns and Competing Endogenous RNA Potential in Hepatocellular Carcinoma. PLoS ONE. 2015; 10(10) https://doi.org/10.1371/journal.pone.0141042.

Zhang L, Huang Q, Lou J, Zou L, Wang Y, Zhang P, Zhang Z. A novel PHD-finger protein 14/KIF4A complex overexpressed in lung cancer is involved in cell mitosis regulation and tumorigenesis. Oncotarget. 2017; 8(12):19684–19698. https://doi.org/10.18632/oncotarget.14962. [PubMed: 28160558]

Zhang W, Tong D, Liu F, Li D, Li J, Cheng X, Wang Z. RPS7 inhibits colorectal cancer growth via decreasing HIF-1α-mediated glycolysis. Oncotarget. 2016; 7(5):5800–5814. https://doi.org/10.18632/oncotarget.6807. [PubMed: 26735579]

Zhao LY, Yao Y, Han J, Yang J, Wang XF, Tong DD, Shao Y. miR-638 suppresses cell proliferation in gastric cancer by targeting Sp2. Digestive Diseases and Sciences. 2014; 59(8):1743–1753. https://doi.org/10.1007/s10620-014-3087-5. [PubMed: 24623314]

Zheng Y, Xu B, Zhao Y, Gu H, Li C, Wang Y, Chang X. CA1 contributes to microcalcification and tumourigenesis in breast cancer. BMC Cancer. 2015; 15:679. https://doi.org/10.1186/s12885-015-1707-x. [PubMed: 26459317]

Zhu Q, Le Scolan E, Jahchan N, Ji X, Xu A, Luo K. SnoN Antagonizes the Hippo Kinase Complex to Promote TAZ Signaling during Breast Carcinogenesis. Developmental Cell. 2016; 37(5):399–412. https://doi.org/10.1016/j.devcel.2016.05.002. [PubMed: 27237790]

- Differential gene expression analysis was used to compare sexual minority men who were low or high in minority stress related to sexual minority status.

- Differential expression of 90 genes was observed between low and high minority stress groups, 41 of these were related to hypothesized biological functions of inflammation, immune function, cancer, and cardiovascular function.

- Two-directional perturbation of 138 gene set pathways was observed, 35 of which were identified as related to hypothesized biological functions of inflammation, immune function, cancer, and cardiovascular function.

- The neuroactive-ligand receptor pathway was identified through both enrichment pathway analysis and perturbation analysis.

Table 1

Demographic information and clinical characteristics of 38 sexual minority men living with HIV by low and high minority stress

	Low sexual minority stress (*n*=20)	High sexual minority stress (*n*=18)	Difference, *p*
	M (SD)	*M (SD)*	
Age	41.6 (8.2)	46.0 (5.3)	0.06
Time since HIV diagnosis	13.8 (8.4)	12.9 (8.2)	0.73
CD4+ T-cell count (cells/mm^3)	708.3 (273.6)	780.4 (280.3)	0.43
Methamphetamine use days (past 30 days)	5.0 (6.5)	8.1 (8.8)	0.22
ASI drug score	0.1 (0.1)	0.2 (0.1)	0.10
ASI alcohol score	0.1 (0.1)	0.2 (0.1)	0.48
CES-D depression	22.6 (13.7)	28.7 (11.8)	0.15
PCL-C PTSD	48.4 (15.0)	52.2 (13.0)	0.41
Log$_{10}$ interleukin-6 (IL-6)	0.3 (0.3)	0.4 (0.3)	0.29
Log$_{10}$ tumor necrosis factor – alpha (TNF-α)	1.7 (0.2)	1.7 (0.2)	0.96
	n (%)	*n (%)*	
Sexual orientation			0.36
Gay	17 (85.0%)	14 (77.8%)	
Bisexual	2 (10.0%)	4 (22.2%)	
Heterosexual	1 (5.0%)	0 (0.0%)	
Race/ethnicity			0.32
Asian,	0 (0.0%)	1 (5.6%)	
African American/Black	2 (10.0%)	2 (11.1%)	
Hispanic/Latino	3 (15.0%)	6 (33.3%)	
Multiracial	2 (10.0%)	3 (16.7%)	
White	13 (65.0%)	6 (33.3%)	
ART Regimen			0.06
INSTI & NRTI	3 (15.8%)	8 (50.0%)	
Boosted PI & NRTI	6 (31.6%)	2 (12.5%)	
NNRTI & NRTI	5 (26.3%)	6 (37.5%)	
Other HAART	4 (21.0%)	0 (0.0%)	
No HAART	1 (5.3%)	0 (0.0%)	
Undetectable HIV viral load (< 40 copies/mL)	16 (80.0%)	14 (77.8%)	0.87
Urine positive for stimulants	8 (40.0%)	12 (66.7%)	0.10

Abbreviations: ART = antiretroviral therapy; ASI = Addiction Severity Index; CES-D = Center for the Epidemiologic Study -Depression Scale; HAART = highly active anti-retroviral therapy; INSTI = integrase strand transfer inhibitor; M = mean; NNRTI = non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; PCL-C = PTSD Checklist – Civilian Version; PI = protease inhibitor; PTSD = post traumatic stress disorder; SD = standard deviation

Table 2

Selected differentially expressed genes (FDR <0.10) between low and high sexual minority stress groups (N=38 sexual minority men living with HIV) grouped by potential biological relevance to inflammation, immune function, cancer, or cardiovascular function, and including citations that suggested this biological relevance

Function	Gene[a]	Log fold difference	FDR adjusted p value
Inflammation			
	PLCB2 (Mao, Jin, Kunapuli, & Rao, 2016)	−0.357	0.076
	BTLA (Bekiaris et al., 2013; Otsuki, Kamimura, Hashiguchi, & Azuma, 2006)	0.582	0.062
Immune			
	CD69 (David Sancho et al., 2005)	0.798	0.072
	CLEC4E (Behler-Janbeck et al., 2016; Kottom et al., 2017)	−0.576	0.083
	KLRB1 (Llibre et al., 2016)	0.569	0.098
	GZMA (Müllbacher et al., 1999)	0.828	0.050
	PPP1R12C (Dutheil et al., 2014)	−0.384	0.088
Cancer			
	ZNF426 (Yang et al., 2009; Yang & Wood, 2007)	0.480	0.006
	RPS15A (Chen et al., 2016; Yao et al., 2016)	1.011	0.050
	CACNA1E (Natrajan et al., 2006)	−1.056	0.050
	TRIM2 (Chen et al., 2015; Panaccione, Guo, Yarbrough, & Ivanov, 2017)	1.230	0.050
	RPL9 (Baik et al., 2016)	1.305	0.050
	ERH (Schmitt et al., 1999; Weng & Luo, 2013)	0.676	0.053
	SKIL (Annala et al., 2015; Hagerstrand et al., 2013; Shinozuka et al., 2013; Zhu et al., 2016)	0.476	0.053
	RPS21 (Arthurs et al., 2017)	0.763	0.053
	PRR14 (M. Yang, Lewinska, Fan, Zhu, & Yuan, 2016)	−0.584	0.057
	BIRC3 (D. Wang et al., 2016, 2017; Yamato et al., 2015)	0.602	0.062
	RPS7 (W. Zhang et al., 2016)	1.028	0.062
	SNRPF (Sun et al., 2016)	0.761	0.076
	RPL23 (Wu et al., 2012)	0.897	0.076
	EIF5A2 (Y. Li et al., 2014; J.-H. Luo et al., 2009; Q.-B. Meng et al., 2015)	0.694	0.076
	ANKRD46 (Cheng et al., 2012)	0.498	0.076
	ANXA2R (D'Souza et al., 2012; Shiozawa et al., 2008)	0.450	0.076
	CA1 (Zheng et al., 2015)	0.727	0.076
	SP2 (Phan et al., 2004; Zhao et al., 2014)[b]	−0.939	0.076
	PSMD10 (Fu et al., 2002; Meng et al., 2010; Qin et al., 2016)	0.435	0.077
	MAPKAPK5-AS1 (Zhang, Fan, Jian, Chen, & Lai, 2015)	0.500	0.083
	RPS14 (Ebert et al., 2008; Wang et al., 2014)	0.589	0.083
	EIF3E (Grzmil et al., 2010; Li et al., 2014)	0.474	0.083
	RPL31 (Maruyama et al., 2014)	1.077	0.083

Function	Gene[a]	Log fold difference	FDR adjusted p value
	UGCG (Liu, Patwardhan, Bhinge, et al., 2011; Liu, Patwardhan, Xie, et al., 2011)	0.345	0.083
	ZDHHC2 (Peng et al., 2014; Yan et al., 2013)	0.420	0.083
	USP45 (Perez-Oliva et al., 2015)	0.393	0.083
	NUDCD1 (Jin et al., 2008; Rao et al., 2014)	0.643	0.087
	RPS20 (De Bortoli et al., 2006; Yong et al., 2015)	0.545	0.089
	PHF14 (Zhang et al., 2017)	0.367	0.089
	FANCM (Alix-Panabières et al., 2017; Matta et al., 2013)	0.449	0.089
	RPL35A (Oliver et al., 2017)	0.578	0.064
	JDP2 (Chen, Chan, Lin, & Chu, 2017; Yuanhong et al., 2010)	−0.493	0.076
Cardiovascular			
	AKAP7 (O'Connell et al., 2017)	0.599	0.077
	ZNF260 (Komati, Maharsy, Beauregard, Hayek, & Nemer, 2011)	0.677	0.083

[a]Gene name is accompanied by references relating it to this biological process or function. References were included irrespective of tissue or direction of relationship with biological process or function.

Table 3

Selected gene sets identified in GAGE analysis to have 2 dimensional perturbation below an FDR<0.10 between low and high sexual minority stress groups in 38 sexual minority men living with HIV with potential relevance to inflammation or observed health disparities among sexual minority people or biological outcomes previously related to minority stress (e.g., cardiovascular risk, inflammation, HIV progression, cancer, or infection/immune)

Function	Database and ID	Name	q value
Inflammation			
	KEGG 04060	Cytokine-cytokine receptor interaction	9.87^{-07}
	KEGG 04062	Chemokine signaling pathway	2.36^{-05}
	KEGG 05322	Systemic lupus erythematosus	0.0008
	KEGG 04610	Complement and coagulation cascades	0.001
	KEGG 04514	Cell adhesion molecules	0.004
	KEGG 04620	Toll-like receptor signaling pathway	0.006
	KEGG 04940	Type I diabetes mellitus	0.006
	KEGG 05310	Asthma	0.010
	KEGG 05332	Graft-versus-host disease	0.010
	KEGG 05145	Toxoplasmosis	0.013
	KEGG 04145	Phagosome	0.019
	KEGG 04664	Fc epsilon RI signaling pathway	0.021
	KEGG 04670	Leukocyte transenothelial migration	0.025
Immune			
	KEGG 04650	Natural killer cell mediated cytotoxicity	5.57^{-05}
	KEGG 05150	Staphylococcus aureus infection	0.003
	KEGG 04660	T cell receptor signaling pathway	0.015
Cancer			
	KEGG 04080	Neuroactive ligand-receptor interaction	6.58^{-07}
	KEGG 05200	Pathways in cancer	1.50^{-05}
	KEGG 05211	Renal cell carcinoma	0.005
	KEGG 05219	Bladder cancer	0.013
	KEGG 05221	Acute myeloid leukemia	0.016
	KEGG 05222	Small cell lung cancer	0.016
	KEGG 05223	Non-small cell lung cancer	0.017
	KEGG 05212	Pancreatic cancer	0.037
Cardiovascular			
	KEGG 04260	Cardiac muscle contraction	0.0003
	KEGG 04270	Vascular smooth muscle contraction	0.005
	KEGG 04614	Renin-angiotensin system	0.013

Function	Database and ID	Name	q value
	KEGG 05414	Dilated cardiomyopathy	0.013