An established body of research indicates that discrimination is associated with increased symptoms of anxiety and negative affect. However, the association cannot be interpreted unambiguously as an exposure effect because a common set of genetic factors can simultaneously contribute to increased liability for symptoms of anxiety, negative affect, and the perception of discrimination. The present study elucidates the association between discrimination and anxiety/negative affect by implementing strict genetic controls in a large sample of adults. We used data from the biomarker project of the Study of Midlife Development in the United States (MIDUS), a national probability sample of noninstitutionalized, English-speaking respondents aged 25 to 74 y. Participants who consented to provide genetic data were biologically unrelated and of European ancestry as determined by genotype principal components analysis (n = 1,146). A single structural regression model was fit to the data with three measures of discrimination specified to load onto a latent factor and six measures of anxiety and negative affect specified to load onto a second latent factor. After accounting for potential genetic confounds—polygenic scores for anxiety, depression, and neuroticism and the first five genetic principal components—greater discrimination was associated with greater anxiety/negative affect (β = 0.53, SE = 0.04, P < 0.001). Findings suggest that measures of perceived discrimination should be considered environmental risk factors for anxiety/negative affect rather than indices of genetic liability for anxiety, depression, or neuroticism. Clinical interventions and prevention measures should focus on ways to mitigate the impact of discrimination to improve mental health at the population level.

Significance

Genetic factors can concurrently influence perceptions of threatening and stressful events, like discriminatory experiences, and increase liability for anxiety and negative affect. The question has remained unsettled as to whether genetic susceptibility to anxiety and negative affect confounds the relationship between discrimination exposure and these phenotypes. In a national probability sample of noninstitutionalized, English-speaking respondents (n = 1,146), we found that discrimination was positively associated with anxiety and negative affect, operationalized by a common latent factor, even after accounting for genetic confounds. These findings suggest that discrimination is a risk factor for anxiety and related disorders rather than a result of common genetic liability alone. Reducing exposure to discrimination has the potential to improve mental health at the population level.

Author contributions: A.G.C., F.D.M., D.R.W., and R.F.K. designed research; F.D.M. analyzed data; A.G.C. and F.D.M. wrote the paper; and A.G.C., F.D.M., D.R.W., and R.F.K. revised the manuscript for important intellectual content.

Reviewers: S.R.H.B., University of Georgia; Y.P., Deakin University; and J.Y.T., Columbia University Medical Center.

The authors declare no competing interest.

Published under the PNAS license.

1To whom correspondence may be addressed. Email: dwilliam@hsph.harvard.edu.

Published December 31, 2020.

PNAS 2021 Vol. 118 No. 1 e2017224118

https://doi.org/10.1073/pnas.2017224118

Downloaded at Steenbock Memorial Library on February 2, 2021

Discrimination and anxiety: Using multiple polygenic scores to control for genetic liability

Adolfo G. Cuevas, Frank D. Mann, David R. Williams, and Robert F. Krueger

*Department of Community Health, Tufts University, Medford, MA 02155; †Department of Family, Population, and Preventive Medicine, Program in Public Health, Stony Brook University, Stony Brook, NY 11794; ‡Department of Social and Behavioral Sciences, Harvard T.H. Chan School of Public Health, Boston, MA 02115; §Department of African and African American Studies, Harvard University, Cambridge, MA 02138; and ¶Department of Psychology, University of Minnesota, Minneapolis, MN 55455

Contributed by David R. Williams, November 12, 2020 (sent for review August 14, 2020; reviewed by Steven R. H. Beach, Yin Paradies, and Jacquelyn Y. Taylor)

Anxiety disorders (ADs) are the most common mental illness in the United States, affecting over 40 million adults in the United States every year (1). ADs represent a variety of different disorders, including generalized anxiety disorder (GAD), panic disorder (PD), or phobias, that are generally characterized by excessive, persistent, and impairing worry or fear (2). Symptoms of ADs are also a common associated feature of depressive disorders, and those who meet diagnostic criteria for one or more AD are likely to meet criteria for a depressive disorder, a common pattern of comorbidity thought to be underpinned by genetic risk factors for neuroticism or emotional liability (2). Overall, ADs account for a substantial burden of morbidity and mortality as well as long-term work disability and absenteeism (3–5). For instance, ADs are associated with several chronic health conditions, including heart disease, hypertension, and diabetes (6, 7). Although the etiology of anxiety and related disorders remains unclear, familial and genetic factors have been established as risk factors.

Family and twin studies reveal that 20 to 40% of the variance contributing to ADs is heritable (8–10). For instance, Hettema et al. meta-analyzed two large twin studies and found the ~32% of the variance for liability to GAD was attributable to additive genetic factors and that the same genes predispose men and women to GAD (8). With recent advances in molecular genetic studies, such as the genome-wide association studies (GWAS), researchers have identified chromosomal risk loci and susceptibility genes for ADs. A meta-analysis of seven GWAS with a total of nine samples of European ancestry (n = 17,310) found that certain single-nucleotide polymorphisms are associated with a lifetime diagnosis of GAD, PD, agoraphobia, social anxiety disorder, or specific phobias (11). Together, however, common familial environments and genetic predisposition do not fully explain liability to ADs, suggesting that the remaining variance is due to individual-specific environmental exposures.

Over the last two decades, research has shown that exposure to unfair treatment, often referred to as perceived discrimination, has deleterious effects on mental health (12, 13). In the first national study to assess the distribution, prevalence, and mental health correlates of major and everyday discrimination, Kessler et al. (14) found that discrimination was relatively common in the total population. Of the 3,032 US adults in the study (ages ranging from 25 to 74 y), ~34% of participants reported experiencing at least one type of major discrimination, which is characterized as acute and observable discriminatory experiences (e.g., being denied a bank loan or having a promotion withheld). Approximately 61% of participants reported experiencing at least one type of everyday discrimination, which is a more minor form of interpersonal transgressions (e.g., being treated with less courtesy than other people). Using the same measures of discrimination, a national 2015 survey by the American Psychological Association documented that the prevalence of self-reported discrimination remains high, with 61% of American adults reporting everyday discrimination | anxiety | negative affect | internalizing | polygenic scores
Methods

Interventions. have high potential impact for prevention efforts and clinical factors. Identifying whether and to what extent the discrim-present study was to examine the association between perceived anxiety and perception of discrimination. The purpose of the genetic risk of anxiety on the relationship between discrimination and anxiety. When a genetic variant directly affects more than one threatening and stressful environmental events, like discrimina-
tors, depression, and posttraumatic stress disorders (12, 13, 18–23). The evidence for the link between discrimination and ADs, however, cannot be interpreted unequivocally as shared genetic vulnerabilities could be confounding factors that explain the associations between perceived discrimination and ADs.

Expression of certain genes may influence emotional arousal and vigilance even to nonemotional and neutral stimuli (24, 25). This, therefore, opens the possibility that the perception of threatening and stressful environmental events, like discrimination experiences of everyday discrimination almost every day or at least once a week. People also experience discrimination based on factors that traverse racial demarcations, including gender, age, sexual orientation, physical appearance, and religion (16, 17).

In the study by Kessler et al., both major and everyday discrimination were positively associated with psychological distress and major depression but were not associated with GAD (14). Subsequent laboratory and community-based studies found extensive evidence that discrimination is adversely associated with a broad range of psychiatric disorders, including anxiety disorders, depression, and posttraumatic stress disorders (12, 13, 18–23). The evidence for the link between discrimination and ADs, however, cannot be interpreted unequivocally as shared genetic vulnerabilities could be confounding factors that explain the associations between perceived discrimination and ADs.

Expression of certain genes may influence emotional arousal and vigilance even to nonemotional and neutral stimuli (24, 25). This, therefore, opens the possibility that the perception of threatening and stressful environmental events, like discrimination experiences of everyday discrimination almost every day or at least once a week. People also experience discrimination based on factors that traverse racial demarcations, including gender, age, sexual orientation, physical appearance, and religion (16, 17).

Methods

Sample. Participants in the present study enrolled in the Study of Midlife in the United States (MIDUS) Biomarker Project (27). Additional information on MIDUS and its recruitment methods and data collection is found elsewhere (28). Biologically unrelated adults of European ancestry—as determined by genotype principal components analysis (29)—were considered for this study (n = 1,189). Participants with missing data for educational attainment were excluded from the analyses, resulting in a final analytic sample of 1,146 participants.

Measures.

Discrimination. Three self-report scales were used to measure discrimination and other forms of social exclusion. Everyday discrimination (30) was measured using a nine-item scale asking participants how often on a daily basis they experience different forms of interpersonal transgressions, including “being treated with less courtesy than other people,” “treated with less respect than other people,” and “receiving poorer service than other people at restaurants or stores.” Each item was rated on a 4-point scale (1 = Often, 2 = Sometimes, 3 = Rarely, 4 = Never). Scale scores were constructed by taking the sum of reverse-coded values, such that higher scores reflect higher levels of everyday discrimination.

Major discrimination (14) was measured by asking participants how many times in their lives they have been discriminated against based on their race, ethnicity, gender, age, religion, physical appearance, and other social identities. Items included being “discouraged by a teacher or advisor from seeking higher education,” being “denied a scholarship,” and being “prevented from renting or buying a home in the neighborhood you wanted.” Scale scores for major discrimination were constructed by taking the sum of endorsed questions irrespective of frequency (i.e., 1 = event occurred one or more times, 0 = event never occurred).

Chronic job discrimination was measured using a 12-item adapted scale (31, 32), assessing discrimination exposure in the workplace. Items included how often participants think they were unfairly treated or sexual slurs or jokes. Questions were rated on a 5-point scale (1 = Once a week or less, 2 = A few times a month, 3 = A few times a year, 4 = Less than once a year, 5 = Never). Scale scores were calculated by taking the sum of the values of reverse-coded items, such that higher scores reflected higher levels of chronic job discrimination. All data are publicly available on the MIDUS Colectica portal, along with extensive documentation regarding how each measure was calculated (https://midus.colectica.org).

Anxiety and negative affect. Six scales were used to measure anxiety and negative affect: the Spielberger trait anxiety scale (33), the Liebowitz social anxiety scale (34), GAD (35), neuroticism (36), negative affect (37), and the neg-ative affect subscale of the Positive and Negative Affect Schedule (PANAS) (38).

Trait anxiety was measured using a 20-item scale asking participants to rate the frequency of occurrence of state anxiety and trait anxiety. Items included feeling tired, lacking self-confidence, and being in a state of ten-sion, worry, feeling emotionally “down,” feeling distressed, feeling restless or fidgety, not being able to quiet down, and feeling nervous. Scale scores were constructed by calculating the mean across the sets of items. For both scales, items were reverse-coded before computing summed scale scores, such that higher values on the scale indicate higher levels of trait anxiety.

Social anxiety was measured by asking participants to rate how much fear or anxiety they generally feel in certain situations including talking to people in authority, talking with people they don’t know very well, and returning goods to a store. Scales scores were computed as the mean of all items.

GAD was measured by asking participants how often—over the past 12 mo—they experienced symptoms of anxiety, including feeling restless because of worry, feeling “keyed up, on edge, or had a lot of nervous energy,” and having sore or aching muscles because of tension. All questions were rated on a 4-point scale (1 = most days, 2 = about half the days, 3 = less than half the days, 4 = never). A nominal variable was created, such that partic-

Neuroticism was measured using a self-report scale, whereby participants who answered “most of the time” to three or more items were coded as having GAD (>2% = Yes; ~98% = No).

Neuroticism was measured using a self-report scale, whereby participants were asked how much a series of temperament accurately describe them, specifically “moody,” “worrying,” “nervous,” and “calm.” All adjectives were rated on a 4-point scale (1 = A lot, 2 = Some, 3 = A little, 4 = Not at all). The necessary items were reverse-coded before calculating mean scores, such that higher values indicate higher levels of neuroticism.

Negative affect was measured using two self-reported scales. The first scale asked how often in the past 30 d they experienced negative emo-tions, including feeling “restless or fidgety” and feeling “hopeless.” The second scale asked participants how often they felt afraid, jittery, irritable, ashamed, and upset. All items measuring negative affect were rated on a 5-point scale (1 = All of the time, 2 = Most of the time, 3 = Some of the time, 4 = A little of the time, 5 = None of the time). Scale scores were constructed by calculating the mean across the sets of items. For both scales, items were reverse-coded before computing mean scores so higher values reflected higher levels of negative affect.

Genotyping, imputation, and polygenic risk scoring. Information about MIDUS’s genotype calling and DNA collection methods is reported in detail elsewhere (29). Briefly, PLINK (39) was used to analyze all genomic data and conduct quality control (Eagle (40) and non-

mac (41) software via the Michigan Imputation Server pipeline and the 1000 Genomes phase 3 reference panel. Single-nucleotide polymorphisms that deviated from Hardy–Weinberg equilibrium (P < 0.001), with ambiguous stand-
orientation, or had greater than 5% missing calls were removed (29). Using the Polygenic Risk Score software, PRSice 2.0, polygenic risk scores were calculated for physical and behavioral health outcomes using an a priori P value threshold of 1.0 (42) and summary statistic weights from current GWAS for each phenotype (11, 43, 44). For this analysis, we selected three polygenic risk scores, one for anxiety and the other two for closely related phenotypes (proxy phenotypes), depression and neuroticism.

Data Analysis. To increase content validity and decrease unsystematic measurement error, focal study variables (i.e., discrimination and anxiety) were operationalized using a confirmatory factor analysis (CFA) model, estimated using robust weighted least squares (45, 46). Latent factors were scaled using unit loading identification by fixing the factor loading of the first indicator to equal 1. Factor variances were freely estimated, and simple structure was assumed, such that every indicator loaded onto only one factor and covariances between residual variances were fixed to zero. The precision of estimated factor loadings, multiple regression coefficients, and residual errors were evaluated using 95% nonparametric bootstrapped (1,000 draws) confidence intervals. Data were prepared for analysis using R version 3.2.1 and exported for inferential analyses using the MplusAutomation package (47). Inferential analyses were conducted using Mplus 8.1 (48).

Table 1. Descriptive statistics for demographic variables, polygenic scores, and indicators of discrimination and anxiety

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>Missing, %</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Skew</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Continuous variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1,146</td>
<td>3.61</td>
<td>54.49</td>
<td>54.00</td>
<td>12.56</td>
<td>−0.06</td>
<td>−0.69</td>
</tr>
<tr>
<td>Level of education</td>
<td>1,146</td>
<td>3.61</td>
<td>8.15</td>
<td>9.00</td>
<td>2.41</td>
<td>−0.21</td>
<td>−0.98</td>
</tr>
<tr>
<td>Daily discrimination</td>
<td>1,137</td>
<td>4.37</td>
<td>12.61</td>
<td>11.00</td>
<td>4.33</td>
<td>1.25</td>
<td>1.26</td>
</tr>
<tr>
<td>Lifetime discrimination</td>
<td>1,117</td>
<td>6.05</td>
<td>0.89</td>
<td>0.00</td>
<td>1.45</td>
<td>2.16</td>
<td>5.32</td>
</tr>
<tr>
<td>Job discrimination</td>
<td>776</td>
<td>34.73</td>
<td>10.79</td>
<td>10.00</td>
<td>4.56</td>
<td>1.07</td>
<td>0.98</td>
</tr>
<tr>
<td>Trait anxiety</td>
<td>1,146</td>
<td>3.61</td>
<td>34.05</td>
<td>33.00</td>
<td>8.92</td>
<td>0.84</td>
<td>0.46</td>
</tr>
<tr>
<td>Social anxiety</td>
<td>1,146</td>
<td>3.61</td>
<td>1.86</td>
<td>1.80</td>
<td>0.53</td>
<td>0.56</td>
<td>−0.01</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>1,145</td>
<td>3.70</td>
<td>2.04</td>
<td>2.00</td>
<td>0.62</td>
<td>0.45</td>
<td>−0.06</td>
</tr>
<tr>
<td>Negative affect</td>
<td>1,139</td>
<td>4.20</td>
<td>1.50</td>
<td>1.33</td>
<td>0.58</td>
<td>1.82</td>
<td>3.65</td>
</tr>
<tr>
<td>PANAS</td>
<td>1,137</td>
<td>4.37</td>
<td>1.53</td>
<td>1.40</td>
<td>0.51</td>
<td>1.49</td>
<td>2.85</td>
</tr>
<tr>
<td>PRS: anxiety</td>
<td>1,146</td>
<td>3.61</td>
<td>0.00</td>
<td>0.01</td>
<td>1.00</td>
<td>−0.02</td>
<td>−0.09</td>
</tr>
<tr>
<td>PRS: depression</td>
<td>1,146</td>
<td>3.61</td>
<td>0.00</td>
<td>−0.02</td>
<td>1.00</td>
<td>−0.10</td>
<td>−0.11</td>
</tr>
<tr>
<td>PRS: neuroticism</td>
<td>1,146</td>
<td>3.61</td>
<td>0.00</td>
<td>−0.01</td>
<td>1.00</td>
<td>−0.09</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Nominal variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>568</td>
<td>578</td>
<td>68</td>
<td>578</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td>~48%</td>
<td>~49%</td>
<td>~3%</td>
<td>~3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAD</td>
<td>1,125</td>
<td>21</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td>~95%</td>
<td>~2%</td>
<td>~3%</td>
<td>~3%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(0) = does not meet diagnostic criteria, (1) = meets diagnostic criteria. For sex (0) = female, (1) = male. For level of education (0) = No school/some grade school (grades 1 to 6), (2) = Eighth grade/junior high school (grades 7 and 8), (3) = Some high school (grades 9 to 12, No Diploma or GED), (4) = GED (general education diploma), (5) = Graduated from high school, (6) = 1 to 2 y of college, no degree yet, (7) = 3 or 4 y of college, no degree yet, (8) = Graduated from 2 y of college, vocational school, or obtained associate degree, (9) = Graduated from a 4- or 5-y college or obtained a bachelor’s degree, (10) = Attended some graduate school, no graduate degree yet, (11) = Master’s degree, (12) = PhD, EdD, MD, DDS, LLB, LLD, JD, etc.

Results

The average age of participants was ~54 y (SD = 12.61 y; Table 1). Approximately 49% of the sample was female. Approximately 3% of the sample did not graduate from high school, 15% graduated from high school, 15% attended 1 to 2 y of college but did not receive a degree, ~5% attended 3 or 4 y of college but did not receive a degree, ~9% had a vocational or associates degree, 25% had a bachelor’s degree, 4% attended some graduate school but did not receive a degree, 18% had a master’s degree, and 5% had a doctoral degree (i.e., PhD, EdD, MD, DDS, LLB, LLD, JD).

The CFA model showed good fit to the data ($\chi^2 = 334.62$, degrees of freedom = 103, $P < 0.001$; root mean square error of approximation (RMSEA) = 0.044, 90% confidence interval for RMSEA = 0.039, 0.050; comparative fit index (CFI) = 0.923; Tucker–Lewis index (TLI) = 0.900). Standardized estimates are reported in Fig. 2, including factor loadings, residual variances, and the structural regression coefficient that quantifies the magnitude of interdependence between discrimination and anxiety/negative affect, after accounting for variation associated with polygenic risk for anxiety, polygenic risk for depression, polygenic risk for neuroticism, the first five genetic principal components, and demographic covariates (age, biological sex, and educational attainment). Multiple regression coefficients for polygenic risk scores, genetic principal components, and demographic covariates are reported in Table 2.

Factor loadings onto latent discrimination and anxiety factors were moderate to large (range of $\lambda = 0.47$ and 0.83) and statistically significant ($P < 0.001$). Polygenic risk for neuroticism had a significant and positive effect on anxiety/negative affect ($\beta = 0.09$, SE = 0.03, $P = 0.004$). In addition, age was negatively associated with anxiety/negative affect ($\beta = -0.01$, SE = 0.003, $P < 0.001$) and educational attainment was marginally associated with anxiety/negative affect ($\beta = -0.06$, SE = 0.03, $P = 0.054$). Similarly, age ($\beta = -0.02$, SE = 0.003, $P < 0.001$) and educational attainment ($\beta = -0.18$, SE = 0.03, $P < 0.001$) were negatively associated with discrimination/social exclusion. The third genetic...
The present study used a large sample of genotyped adults to test whether discrimination (and different forms of social exclusions) is associated with the experience of anxiety and negative affect after implementing state-of-the-art genetic controls. Results indicate a high degree of interdependence between discrimination and anxiety, even after accounting for increased genetic liability for anxiety, depression, neuroticism, and other potential genetic and sociodemographic confounds (e.g., genetic principal components, chronological age, biological sex, and education). These findings corroborate systematic reviews that found a strong association between discrimination and psychiatric disorders, including anxiety disorder, depression, and lifetime DSM-IV disorders (12, 13, 18, 49). However, these previous studies did not account for potential genetic confounds, which left open the possibility that the cooccurrence of discrimination and psychiatric disorders was the result of pleiotropy (i.e., the expression of two or more phenotypes from the same set of genetic factors). Individuals with high genetic liability to anxiety may hold beliefs that others view them negatively and have enhanced emotional arousal to threatening and stressful events. This, in turn, may increase the likelihood to perceive and label stressful events as discriminatory. However, results of the present study are more consistent with discrimination acting as an environmental stressor for symptoms of anxiety and negative affect. Consequently, the present study contributes further to the growing body of evidence that discrimination operates like other stressors.

Discriminatory experiences can lead to stress responses characterized by enhanced spontaneous amygdala activity and heightened physiological arousal and negative affect (12, 50, 51). Although the mechanism remains unclear, studies have indicated that greater exposure to discrimination is associated with alterations in cortisol output (52), multisystem physiological dysregulation (53, 54), increase in inflammation (55), shorter telomere length (56), and impairment of the prefrontal cortex's function.
The association between discrimination and anxiety, while controlling for genetic confounds. Although the present study helps rule out common genetic liability as a potential confound of the association between discrimination and anxiety, the cross-sectional design precludes determining the temporal order of discrimination and anxiety. Future longitudinal, genetically informative designs stand to benefit from establishing the temporal direction of effects, in addition to controlling for potential genetic confounds. Finally, polygenic risk scores are continuing to be refined as GWAS grow in size and discover more variants that are associated with the expression of complex phenotypes. Nevertheless, our study lays the foundation for future research to improve model performance and increase the generalizability of findings.

Conclusions

Exposure to discrimination was associated with anxiety, even after adjusting for genetic controls. This suggests that the association between discrimination and anxiety is not explained by known genetic variants of anxiety, depression, or neuroticism. These findings highlight the importance of increased awareness among health researchers and clinicians of discrimination as a potential pathogenic factor for mental illness. Further research is needed to illuminate the psychological and physiological pathways by which discrimination can affect health and to identify the optimal societal interventions to reduce the prevalence of discrimination and the needed psychosocial and clinical interventions to minimize its negative effects.

Data Availability

Data in this manuscript are archival data and may be accessed via the MIDUS Collectica Portal (https://midus.collectica.org/). Data, analysis scripts, and figure files are also available through the Open Science Framework (https://osf.io/ukg8q/).

ACKNOWLEDGMENTS.

The MIDUS study is supported by the John D. and Catherine T. MacArthur Foundation Research Network, National Institute on Aging (P01-AG200166), and the National Institute on Aging (U19-AG051426). Biomarker data collection was further supported by the National Institute of Health National Center for Advancing Translational Sciences (NCATS) Clinical and Translational Science Award (CTSA) program as follows: UL1TR001409 (Georgetown), UL1TR001881 (UCLA), and UL1TR002501 (UW). The development of the manuscript was partially supported by Cancer Disparities Research Network/Geographic Management Program (GMap) Region 4 funded by 3 P30 CA006927-5252 and by the Clinical & Translational Science Institute Mentored Career Development Award (KL2 TR002545).
