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# Genome-Wide Association Study of Loneliness Demonstrates a Role for Common Variation

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Loneliness is a complex biological trait that has been associated with numerous negative health outcomes. The measurement and environmental determinants of loneliness are well understood, but its genetic basis is not. Previous studies have estimated the heritability of loneliness between 37 and 55% using twins and family-based approaches, and have explored the role of specific candidate genes. We used genotypic and phenotypic data from 10 760 individuals aged ⩾50 years that were collected by the Health and Retirement Study (HRS) to perform the first genome-wide association study of loneliness. No associations reached genome-wide significance ($p > 5 \times 10^{-8}$). Furthermore, none of the previously published associations between variants within candidate genes (*BDNF*, *OXTR*, *RORA*, *GRM8*, *CHRNA4*, *IL-1A*, *CRHR1*, *MTHFR*, *DRD2*, *APOE*) and loneliness were replicated ($p > 0.05$), despite our much larger sample size. We estimated the chip heritability of loneliness and examined coheritability between loneliness and several personality and psychiatric traits. Our estimates of chip heritability (14–27%) support a role for common genetic variation. We identified strong genetic correlations between loneliness, neuroticism, and a scale of 'depressive symptoms.' We also identified weaker evidence for coheritability with extraversion, schizophrenia, bipolar disorder, and major depressive disorder. We conclude that loneliness, as defined in this study, is a modestly heritable trait that has a highly polygenic genetic architecture. The coheritability between loneliness and neuroticism may reflect the role of negative affectivity that is common to both traits. Our results also reflect the value of studies that probe the common genetic basis of salutary social bonds and clinically defined psychiatric disorders.
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## INTRODUCTION

Humans are fundamentally social animals who form bonds with others for mutual aid and protection. For social species, the perception of being socially isolated even when in the presence of others signals danger and evokes a dysphoric state termed loneliness in humans (Cacioppo *et al*, 2014, 2015a). A variety of biological mechanisms have evolved that capitalize on aversive signals to motivate people to act in ways that are essential for reproduction and survival. Just as physical pain is an aversive signal that alerts us of potential tissue damage and motivates us to take care of our physical body, loneliness—triggered by a discrepancy between an individual's preferred and actual social relations—is part of a biological warning system that has evolved to alert us of threats or damage to our social body.

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A substantial literature now shows that loneliness is a major risk factor for adverse physical (Holt-Lunstad *et al*, 2015) and mental (Cacioppo *et al*, 2015c) health outcomes. A recent meta-analysis of 70 independent prospective studies involving >3 million people who were followed for an average of 7 years shows that loneliness increases the odds of mortality by 26% even after controlling for objective social isolation and other potentially confounding factors (Holt-Lunstad *et al*, 2015). For instance, using longitudinal data from the Health and Retirement Study (HRS), Luo *et al* (2012) found that loneliness in 2002 predicted mortality over the subsequent 6 years even after controlling for demographic factors, health behaviors, and objective social isolation.

Investigations have found loneliness to be stable over years (see, eg, Boomsma *et al*, 2005) and to differ from other personality factors such as extraversion, neuroticism, depressive symptomatology shyness, and anxiety (see, eg, Cacioppo *et al*, 2006, 2010). Studies designed to identify the *mechanisms* underlying the association between loneliness and mortality have found that loneliness is associated with increased hypothalamic–pituitary–adrenocortical (HPA)

activity (Adam *et al*, 2006; Cacioppo *et al*, 2006; Doane and Adam, 2010; Glaser *et al*, 1985; Kiecolt-Glaser *et al*, 1984; Steptoe *et al*, 2004), altered gene expression indicative of decreased inflammatory control and increased glucocorticoid insensitivity (Cole *et al*, 2007, 2011), increased inflammation, elevated vascular resistance, and blood pressure (Hackett *et al*, 2012; Hawkley *et al*, 2006, 2010b; Jaremka *et al*, 2013), higher rates of metabolic syndrome (Whisman, 2010), diminished immunity (Dixon *et al*, 2006; Glaser *et al*, 2005; Kiecolt-Glaser *et al*, 1984; Pressman *et al*, 2005; Straits-Tröster *et al*, 1994), increased risk for age-related cognitive decline and dementia (Wilson *et al*, 2007), and increased sleep fragmentation (Cacioppo *et al*, 2002; Hawkley *et al*, 2010a; Jacobs *et al*, 2006; Kurina *et al*, 2011). Cross-lagged panel analyses have also shown that loneliness has also been associated with changes in psychological states that can contribute to morbidity and mortality, including increased depressive symptomatology (Booth, 2000; Cacioppo *et al*, 2006, 2010; VanderWeele *et al*, 2011), lower subjective well-being (Kong and You, 2013; VanderWeele *et al*, 2012), heightened vigilance for social threats (Cacioppo *et al*, 2015b), and decreased executive functioning (Baumeister and DeWall, 2005; Cacioppo *et al*, 2000; Hawkley *et al*, 2009).

The heritability of loneliness has been documented in twin and other studies using both children (Bartels *et al*, 2008; McGuire and Clifford, 2000) and adults (Boomsma *et al*, 2005, 2006, 2007). For instance, in an early longitudinal study of 8387 young adult and adult Dutch twins who participated in longitudinal surveys, Boomsma *et al*, (2005) analyzed variations in loneliness with genetic structural equation models. The estimate of genetic contributions to variation in loneliness in adults was 48%, similar to the heritability estimates reported by McGuire and Clifford (2000) in their study of children. Boomsma *et al* (2005) found no evidence for sex or age differences in heritability. Subsequent twin studies have yielded heritability estimates ranging from 37 to 55% (Boomsma *et al*, 2005, 2006; Distel *et al*, 2010; McGuire and Clifford, 2000; Waaktaar and Torgersen, 2012). Candidate gene studies for loneliness have concentrated primarily on systems related to monoamine neurotransmitters (eg, dopamine, serotonin) and other signaling pathways associated with human attachment (eg, oxytocin) (Goossens *et al*, 2015). Typical of candidate gene studies, they used modest sample sizes and therefore implicitly assumed relatively large effect sizes for the alleles being studied, a scenario that is inconsistent with the results of genome-wide association studies (GWAS) for numerous disease and nondisease traits (Hart *et al*, 2013).

In this study we have performed the first GWAS for loneliness. The UCLA loneliness scale is the most commonly used measure in the literature and has very good psychometric properties, including internal reliability, temporal stability, discriminant validity, convergent validity, construct validity, and predictive validity (Cacioppo *et al*, 2006; Russell, 1980, 1996). Importantly, the term 'loneliness' does not appear in this scale because respondents, especially males, have been found to be reluctant to report feeling lonely (Russell *et al*, 1980). Hence, the measurement of this phenotype is not dependent on the respondents' ability or willingness to self-report being lonely. Since 2002, the HRS has included a 3-item version of the UCLA loneliness scale that has also been shown to have very good psychometric properties, including internal reliability, concurrent validity (eg, $r = 0.88$ with the full 20-item UCLA loneliness scale; Hughes *et al*, 2004), convergent and discriminant validity (Hughes *et al*, 2004), and predictive validity (eg, predicts mortality in the HRS sample over a 6-year period; Hughes *et al*, 2004; Luo *et al*, 2012). We used a genomic restricted maximum likelihood (GREML) method implemented in the Genetic Complex Trait Analysis (GCTA) software (Yang *et al*, 2011) to examine chip heritability that is specifically due to the additive effect of genotyped (or imputed) common variants. Loneliness and objective social isolation are often weakly correlated (Coyle and Dugan, 2012; Holt-Lunstad *et al*, 2015), although loneliness does tend to be lower in individuals who are married than those who are unmarried (eg, Hawkley *et al*, 2008). Analyses were therefore performed including marital status as a covariate. We also determined whether previously reported candidate gene associations could be replicated in the HRS. Finally, using polygenic risk scoring and estimates of genetic correlation, we were able to begin to probe possible shared genetic influences between personality traits and psychiatric diagnoses and loneliness.

## MATERIALS AND METHODS

### Subjects

The University of Michigan HRS is a longitudinal study that began in 1992 and includes more than 26 000 Americans who are $>50$ years of age (Health_and_Retirement_Study, 2012). Our study included genotype data (both directly genotyped and imputed) obtained from dbGaP (accession number phs000428.v1.p1) on a total of 12 454 subjects from HRS that were genotyped by the Center for Inherited Disease Research (CIDR). Permission to use the HRS data set was obtained through application to dbGaP by JTC. Phenotypic data were collected by the HRS on subjective experiences of loneliness during the 2006 and 2008 data collection waves.

### Loneliness Phenotype

Loneliness was assessed using the 3-Item Leave Behind Questionnaire (LBQ) as part of a larger written questionnaire administered as part of the HRS (Hughes *et al*, 2004). Respondents were asked three questions. 'How often do you feel that you lack companionship? 'How often do you feel left out?' and 'How often do you feel isolated from others?' Possible answers were 'hardly ever, or never' (scored as 1); 'some of the time' (scored as 2); or 'often' (scored as 3). Thus, higher scores represent greater self-reported loneliness (Supplementary Figure S1). The total score on this 3-item loneliness scale has been shown to be highly correlated ($r = 0.88$) with the total score on the UCLA loneliness scale. Only participants who responded to all the three questions were included in our study. Pairwise correlation coefficients between questions were obtained using Spearman's correlation statistics in SAS version 9.3 (SAS Institute, Cary, NC).

We derived three phenotypes from the loneliness scale for subsequent genetic analyses: (1) 'linear'—a continuous phenotype obtained by summing the scores from all three questions, thus yielding a score between 3 (least lonely) and 9 (most lonely); (2) 'multivariate'—a single score for each question ranging from 1 (least lonely) to 3 (most lonely); and

(3) 'case/control'—a dichotomous score in which participants who answered 1 on all three items were considered controls (totally loneliness score = 3) and individuals with a loneliness score of $\geqslant 6$ were considered cases (participants with scores of 4 or 5 were treated as missing). There were nine subjects who answered the loneliness questions twice (first in 2006 and again in 2008); we used an average of their two scores. Frequency distributions by ancestry for the linear trait and case/control labels are shown in Supplementary Figure S2.

We considered sex, age (continuous), and marital status (categorical) as covariates in our analyses. Marital status was ascertained such that it consisted of six levels (married, annulled, separated, divorced, widowed, and never married). For the genetic analyses, we summarized these six levels into a binary variable (married or unmarried). Subjects for whom any of these three covariates were missing were excluded from all of our analyses.

## SNP Genotyping and Quality Control

Genotyping of HRS subjects was performed by the NIH CIDR (http://www.cidr.jhmi.edu/), using the Illumina Human Omni-2.5 Quad BeadChip. Genotyping quality control was performed by the Genetics Coordinating Center at the University of Washington, Seattle, WA. Further information is available from the HRS website (http://hrsonline.isr.umich.edu/sitedocs/genetics/HRS_QC_REPORT_MAR2012.pdf). Additional more stringent QC was conducted for SNP-based heritability analyses, as models that include the joint effect of all SNPs are known to be sensitive to technical artifacts. For the directly genotyped data, we applied the recommended SNP quality filter provided by CIDR, yielding 1 681 327 SNPs. Quality control of the imputed genotype data was performed with the QCTOOL software package (http://www.well.ox.ac.uk/~gav/qctool/#overview). SNPs with call rate $>95\%$, MAF $>1\%$, Hardy–Weinberg equilibrium $P>10^{-6}$, and an imputation info score $>0.5$ were retained for further analysis. Subject-level QC was performed with the GTOOL software package (http://www.well.ox.ac.uk/~cfreeman/software/gwas/gtool.html) and included iteratively removing one subject of any pair whose kinship coefficient was $>0.1$. The numbers of SNPs available after QC for each analysis are shown in Supplementary Table S1.

Because the participants in the HRS were a mixture of European Americans (EA, $n=7556$), African Americans (AA, $n=1155$), and Hispanic Americans (HA, $n=695$), we calculated the first 10 principal components (PCs) from the genotype data to use as covariates. After manual inspection, we concluded that 1354 subjects did not clearly fit any of these categories (see Supplementary Figure S3) and were therefore excluded from both GREML and polygenic analyses.

## Genome-Wide Association Study

We used Linear Mixed Model (LMM) or Multivariate Linear Mixed Models (MLMM) implemented in the Genome-wide Efficient Mixed Model Association (GEMMA) software package to further correct for residual population structure due to ancestry or cryptic relatedness in our GWAS (Zhou and Stephens, 2012). We examined the linear, multivariate, and case/control phenotype models using either directly genotyped or a combination of genotyped and imputed SNP data, adjusting for sex, age, and marital status (binary). We excluded SNPs with MAF $<0.01$. For the case/control studies, controls were coded as 0 and cases were coded as 1, as suggested in the GEMMA documentation (Zhou and Stephens, 2012). The association analyses were performed using all 10 760 subjects and separately in the subset of the 7556 EA subjects. We implemented the leave-one-chromosome-out (LOCO) method within the mixed model framework in order to avoid a loss of statistical power due to 'proximal contamination' or inclusion of the candidate marker (or markers in LD with the candidate marker) in the genetic relationship matrix (GRM) (Cheng *et al*, 2013; Yang *et al*, 2014).

## Analysis of Candidate Variants Identified in Prior Studies

We identified 13 variants in 11 genes that have previously been associated with loneliness phenotypes in the published literature (Chou, 2010, 2014; Connelly *et al*, 2014; Lan *et al*, 2012; Lucht *et al*, 2009; Terracciano *et al*, 2010; Tsai *et al*, 2012; van Roekel *et al*, 2010, 2011, 2013; Verhagen *et al*, 2014; Wang *et al*, 2013). We examined the association of each of these SNPs with loneliness (linear, multivariate, and case/control) in the results from the GWAS described above. For those candidate SNPs that were not directly genotyped or imputed in our study, we identified proxy SNPs with $r^2>0.8$ whenever possible. For SNPs that yielded $p<0.05$, we determined whether the direction of the association was consistent between the prior and current studies. We did not apply any correction for multiple comparisons.

## Estimation of Variance in Loneliness Explained by the Genotyped SNPs ('Chip Heritability')

To estimate the proportion of phenotypic variance explained ('chip heritability'; $h^2_g$), we used the GREML method implemented in GCTA (Yang *et al*, 2010). The purpose of the GREML method is to estimate the proportion of variation in a phenotype that is due to all SNPs. The GREML method is well established, has been described in detail, and exploits the fact that genotypic similarity (ie, 'relatedness', measured using genotyped SNPs) will be correlated with phenotypic similarity for phenotypes that are influenced by genetic variation. Additional individual-level quality control was implemented and distantly related individuals with pair-wise relationships were further filtered at two thresholds ($K_{IBS}<0.05$ and $K_{IBS}<0.025$). Covariates included in the GREML analysis were age (continuous), self-reported sex (male/female), marital status (married/not married), and top 10 PCs. GREML analyses were run using only directly genotyped SNPs to construct the GRM. We obtained estimates of heritability for both the linear trait and the case/control classification in the EA subset ($N=7556$).

## Polygenic Risk Score (PRS) Analysis

We investigated whether the genetic risk for loneliness overlaps with the genetic risk for several personality traits and psychiatric diseases (Supplementary Table S2). For each set ('discovery sample') of GWAS results (eg, SCZ2), we

identified SNPs that were also genotyped in our HRS loneliness data ('target sample') and then used PLINK to LD-prune the SNPs ($r^2 < 0.2$; using the '–indep-pairwise' command). The target sample was restricted to EAs to avoid confounding due to residual population stratification. SNPs with association $p$-values passing predetermined significance thresholds ($p < 10^{-5}$, $10^{-4}$, $10^{-3}$, 0.01, 0.05, 0.1, 0.3, and 0.5, respectively) in the discovery sample were extracted along with their risk alleles and odds ratios. For each significance threshold, a quantitative aggregate risk score was calculated for each EA individual in the target sample, defined as the sum of the number of risk alleles present at each locus weighted by the log of the odds ratio for that locus estimated from the discovery sample (as implemented in the PLINK '—score' command (Purcell *et al*, 2007). The relationship between aggregate risk score in relation to three phenotypes (eg, linear, multivariate, and case/control status) in the target sample was examined at each significance threshold using linear regression, multivariate regression, or logistic regression correspondingly.

### LD Score Regression (LDSC)

To further investigate the genetic overlap between loneliness and various other traits (Supplementary Table S2), we used LDSC (Bulik-Sullivan *et al*, 2015a, b). We limited our analysis to the EA subjects and used the results from the case/control analysis shown in Figure 1. To standardize the input files, we followed quality controls as implemented by the LDSC python software package (https://github.com/bulik/ldsc). We used precalculated LD scores ('eur_w_ld_chr/' files; Finucane *et al*, 2015) for each SNP using individuals of European ancestry from the 1000 Genomes project that are suitable for LD score analysis in European populations. To restrict the analysis to well-imputed SNPs, the SNPs were filtered to HapMap3 SNPs (International Hapmap Consortium *et al*, 2010), and were required to have a MAF above 1%. INDELs, structural variants, strand-ambiguous SNPs, and SNPs with extremely large effect sizes ($\chi^2 > 80$) were removed.

## RESULTS

### Demographics

The distributions of responses to each question are shown in Supplementary Figure S1 and the sum of the three questions is shown in Supplementary Figure S2. Table 1 displays population characteristics according to loneliness status. Consistent with prior studies, loneliness was influenced by ancestry, decreased slightly but significantly with age, and did not differ by gender. In addition, consistent with prior studies, marital status had a large impact on our quantitative measure of loneliness, with all non-married categories showing greater loneliness compared with individuals who were married (Table 1).

### GWAS of Loneliness

Figure 1 shows the results of our GWAS for the 7556 EA-only cohort using both quantile–quantile (QQ) and Manhattan plots. None of the GWAS yielded genome-wide significant associations ($p < 5 \times 10^{-8}$). The most significant results are listed in an Excel spreadsheet that is included in the Supplementary Material. We also performed these analyses using the full set of 10 760 subjects, and the results were not meaningfully different (Supplementary Figures S4–S6).

### Previously Studied Candidate Genes

Table 2 shows that we did not replicate any of the associations between loneliness and specific candidate genes that had been previously reported. None of these SNPs showed significant evidence for association ($p \leqslant 0.05$ without correction for multiple comparisons), with the exception of the gene *MTHFR* (Table 2), for which the direction of the association in our data was opposite to what was reported previously (Lan *et al*, 2012). Therefore, none of the previously reported associations could be replicated, despite our large sample size.

### Heritability Estimates for Loneliness

We found that loneliness had a significant chip heritability (case/control 0.27, SE = 0.12, $p = 0.01$; linear trait PVE = 0.16, SE = 0.06, $p = 0.002$; Table 3). Because loneliness was significantly associated with self-reported ethnicity, we focused on the EA subset for heritability estimates to avoid confounding. To guard against any within-EA structure, we calculated heritability after adjusting for the top 10 PCs from the genotype data and also after additionally eliminating individuals with $K_{IBS} > 0.05$ and $K_{IBS} > 0.025$. Results were robust to these different approaches. Although the $h^2_g$ estimate for the case/control phenotype was higher than the linear trait, the overlapping standard errors indicate that these estimates are not significantly different.

### Polygenic Score Analyses

For these analyses, we used the 6924 distantly related/unrelated EA subjects. Results for neuroticism (Table 4 and Supplementary Tables S3 and S4) showed unambiguously significant positive coheritability. We observed modest evidence for negative coheritability with extraversion (Supplementary Table S5); the multivariate analysis suggested that the questions 'How often do you feel that you lack companionship?' and 'How often do you feel left out?' showed the greatest coheritability with extraversion. We also observed modest evidence for negative coheritability with schizophrenia (SCZ1 and SCZ2, Supplementary Tables S6 and S7) and bipolar disorder (Supplementary Table S8); for these diseases the multivariate analysis suggested that the question 'How often do you feel left out?' showed the greatest coheritability. There was no evidence for coheritability with major depressive disorder (Supplementary Table S9) but a no-clinical trait called 'depressive symptoms' did show significant positive coheritability with loneliness (Supplementary Table S10); the multivariate analysis suggested that all three questions contributed to the observed coheritability.

### Genetic Correlation

The results for LDSC analysis used the case/control loneliness GWAS summary statistics and produced results that were broadly similar to the results from the PRS. The genetic correlation between loneliness and personality traits was

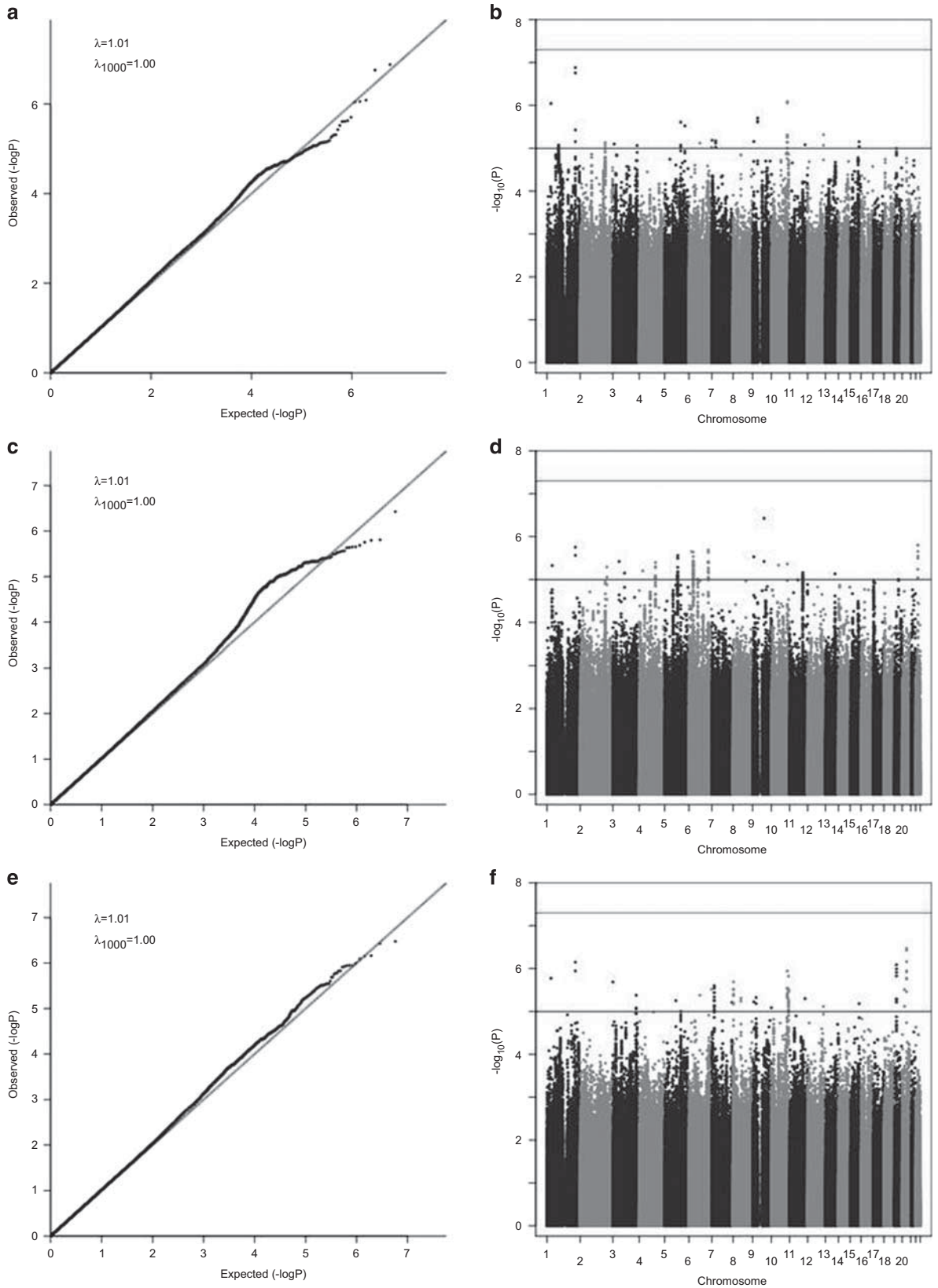


**Figure 1** QQ and Manhattan plots of genome-wide association studies for loneliness in EA-only subjects using the imputed SNPs, calculated by mixed regression models with adjustments for age, gender, and marital status. (a, b) Linear mixed model ($n = 7556$); (c, d) multivariate mixed model ($n = 7556$), and (e, f) case/control mixed model ($n = 5228$).

**Table 1** Participants' Characteristics of Health and Retirement Study (HRS) and Associations with Loneliness[a]

| Characteristic | Linear trait (3–9; $n = 10\,760$) | | | | Case/control (cases = 2853, controls = 4583) | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean (SE)/N (%) | β | SE | *P* | Case | Control | OR (95% CI) | *P* |
| Age[b] | 67.2 (10.3) | −0.01 | 0.0016 | **<0.0001** | 66.6 (10.8) | 67.4 (9.9) | 0.98 (0.98–0.99) | **<0.0001** |
| *Gender*[c] | | | | | | | | |
| Women | 6376 (50.6) | — | — | | 1067 (37.4) | 2007 (43.8) | — | |
| Men | 4384 (40.7) | −0.001 | 0.03 | 0.98 | 1786 (62.6) | 2576 (56.2) | 1.02 (0.92–1.14) | 0.67 |
| *Self-reported ethnicity*[c] | | | | | | | | |
| European Americans | 8490 | — | — | | 2075 (72.73) | 3875 (82.59) | — | |
| African Americans | 1228 | 0.22 | 0.05 | **<0.0001** | 427 (14.97) | 398 (8.68) | 1.54 (1.32–1.79) | **<0.0001** |
| Hispanic Americans | 867 | 0.25 | 0.06 | **<0.0001** | 294 (10.30) | 327 (7.14) | 1.52 (1.28–1.80) | **<0.0001** |
| Other/ Unknown | 175 | 0.18 | 0.12 | 0.13 | 57 (2.00) | 73 (1.59) | 1.39 (0.97–1.99) | 0.07 |
| *Marital status*[c] | | | | | | | | |
| Married | 7120 (66.2) | — | — | | 1538 (53.9) | 3496 (76.3) | — | |
| Annulled | 364 (3.4) | 0.16 | 0.09 | 0.06 | 100 (3.5) | 165 (3.6) | 1.26 (0.98–1.64) | 0.08 |
| Separated | 132 (1.2) | 1.06 | 0.14 | **<0.0001** | 63 (2.2) | 31 (0.7) | 4.34 (2.81–6.71) | **<0.0001** |
| Divorced | 991 (9.2) | 0.85 | 0.05 | **<0.0001** | 394 (13.8) | 264 (5.8) | 3.30 (2.79–3.91) | **<0.0001** |
| Widowed | 1892 (17.6) | 0.75 | 0.05 | **<0.0001** | 655 (23.0) | 551 (12.0) | 3.29 (2.84–3.80) | **<0.0001** |
| Never married | 261 (2.4) | 0.75 | 0.10 | **<0.0001** | 103 (3.6) | 76 (1.7) | 3.02 (2.23–4.09) | **<0.0001** |
| *Binary marital status*[c] | | | | | | | | |
| Married | 7120 (66.2) | — | — | | 1538 (53.9) | 3496 (76.3) | — | |
| Unmarried | 3640 (33.8) | 0.72 | 0.03 | **<0.0001** | 1315 (46.1) | 1087 (23.7) | 2.91 (2.62–3.23) | **<0.0001** |

β (β coefficient), SE, and *P*-values were obtained from linear regression models, adjusting for age, sex, and marital status. Odds ratios (ORs) and 95% confidence intervals (CIs) were assessed with a logistic regression model using the same covariates. *P*-values ⩽ 0.05 are shown in bold, whereas *P*-values between 0.05 and 0.1 are underlined.
[a]Higher score means more loneliness.
[b]Continuous variables are presented as mean and SD.
[c]Categorical variables are presented as counts and percentages (gender, ethnicity, and marital status).

significantly positive for all three neuroticism data sets (see Figure 2, $r_g = 0.39$, $p = 4.1 \times 10^{-4}$; $r_g = 0.40$, $p = 8.4 \times 10^{-5}$; $r_g = 0.41$, $p = 2.5 \times 10^{-3}$, respectively) and negative for extraversion ($r_g = -0.34$, $p = 0.013$). Unlike the modest evidence from the PRS analysis, schizophrenia and bipolar disorder did not show any significant results. Whereas there was absolutely no evidence for coheritability with major depression in the PRS analysis, there was a trend toward a positive correlation in the LDSC analysis ($r_g = 0.25$, $p = 0.08$). Similar to the PRS analysis, the 'depressive symptoms' trait was strongly positively correlated with loneliness ($r_g = 0.39$, $p = 2.9 \times 10^{-4}$). We also included height as a negative control in the LDSC analysis; as expected, there was no coheritability between loneliness and height ($p > 0.05$).

## DISCUSSION

Traditionally, the emphasis in research on loneliness has been on environmental predictors and determinants. In the past decade, a series of twin studies have provided estimates of the heritability ($h^2$) of loneliness. Here we report the first GWAS of loneliness. We have produced the first estimates of the chip heritability ($h^2_g$) of loneliness (Table 3; 14–27%) that appear to account for approximately half of the heritability estimated from twin and family studies (37–55%). We did not identify any genome-wide significant associations (Figure 1 and Supplementary Figures S4–S6), presumably reflecting the very modest contributions of individual variants. Previous studies have reported associations between polymorphisms in a handful of candidate genes and loneliness (Chou, 2010; Chou *et al*, 2014; Connelly *et al*, 2014; Lan *et al*, 2012; Lucht *et al*, 2009; Terracciano *et al*, 2010; Tsai *et al*, 2012; van Roekel *et al*, 2010, 2011, 2013; Verhagen *et al*, 2014; Wang *et al*, 2013); our study did not provide even nominal evidence for replication, despite our much larger sample size (Table 2). Finally, we identified varying levels of evidence for coheritability between personality traits (positive for neuroticism and negative for extraversion) and psychiatric disease traits (negative for schizophrenia and bipolar disorder and positive for depression). This latter result is especially interesting in light of the behavioral research showing that loneliness and psychiatric illness are related in other contexts (Cacioppo *et al*, 2015c), and provides novel evidence that such associations may reflect genetic as well as environmental influences.

**Table 2** Association between Loneliness Phenotypes and Candidate Gene Associations Reported in Prior Studies

| Gene | Chr | Reported SNP | Function | Population | Sample size | References | *P* for association (all subjects/EA-only)[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Linear | Multivariate | Case/control |
| *BDNF* | 11 | rs6265 | Val66Met | Dutch | 305 | Verhagen *et al* (2014) | 0.78/0.56 | 0.49/0.66 | 0.64/0.70 |
| | | rs53576[b] | Intron | | | Connelly *et al* (2014) | NA | NA | NA |
| *OXTR* | 3 | rs2254298[b] | Intron | UK/ Germany | 7723/285/89 | Lucht *et al* (2009) | 0.61/0.71 | 0.87/0.98 | 0.98/0.48 |
| | | rs2228485[b] | Synonymous | | | van Roekel *et al* (2013) | NA | NA | NA |
| *RORA* | 15 | rs12912233 | Intron | Italy + US | 3972 + 839 | Terracciano *et al* (2010) | 0.60/0.35 | 0.90/0.53 | 0.86/0.60 |
| *GRM8* | 7 | rs17864092 | Intron | | | | 0.77/0.77 | 0.41/0.29 | 0.98/0.83 |
| *CHRNA4* | 20 | rs1044396 | Synonymous | Taiwan | 192 | Tsai *et al* (2012 | 0.75/0.95 | 0.76/0.77 | 0.73/0.82 |
| *IL-1A* | 2 | rs1800587 | 5' UTR | Taiwan | 192 | Wang *et al* (2013) | 0.33/0.55 | 0.25/0.52 | 0.69/0.68 |
| *CRHR1* | 17 | rs1876831 | Intron | UK | 1,374 | Chou *et al* (2014) | 0.28/0.09 | 0.54/0.41 | 0.14/0.06 |
| | | rs242938 | Intron | | | | 0.74/0.46 | 0.87/0.74 | 0.14/0.20 |
| *MTHFR* | 1 | rs1801133 | Ala222Val | Taiwan | 323 | Lan *et al* (2012) | 0.046/0.15 | 0.08/0.31 | 0.036/0.052 |
| *DRD2* | 11 | rs1800497 | Glu713Lys | The Netherlands | 307 | van Roekel *et al* (2011) | 0.45/0.09 | 0.75/0.22 | 0.46/0.16 |
| *APOE* | 19 | rs7412 (ε2) | Arg176Cys | Taiwan | 979 | Chou, (2010) | 0.37/0.69 | 0.43/0.43 | 0.74/0.96 |
| *SLC6A4* | 17 | Insertion/5-HTTLPR | | The Netherlands | 306 | van Roekel *et al* (2010) | NA | NA | NA |

[a]*P*-values are not corrected for multiple comparisons. We used multivariate or logistic regression models (GEMMA) to account for relatedness. Adjustments include sex, age, and marital status. *P*-values before '/' are for all the 10 760 participants, whereas those after '/' are for 7556 European Americans only. For the gene *MTHFR* (rs1801133), the direction of effect was opposite to what had been reported previously. [b]For those candidate SNPs that were not genotyped/imputed in our study, we identified proxy SNPs with $r^2 > 0.8$ that were genotyped or imputed in our study based on HapMap2. When no proxy SNP could be identified we report NA rather than a *p*-value.

**Table 3** Chip Heritability Estimates in European Americans (EAs)

| Threshold of $K_{IBS}$ | | Linear trait | | | | Case/control | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | PVE | SE | *P* | N | PVE | SE | *P* |
| All European Americans[a] | No PCs[b] | 7556 | 16% | 6 | **0.002** | 5228 | 27% | 12 | **0.01** |
| | With 10 PCs[b] | 7556 | 16% | 6 | **0.003** | 5228 | 26% | 13 | **0.02** |
| Excluding closely related pairs ($K_{IBS} < 0.05$)[c] | | 7381 | 16% | 6 | **0.006** | 5113 | 25% | 14 | **0.04** |
| Excluding closely related pairs ($K_{IBS} < 0.025$)[c] | | 6924 | 14% | 7 | **0.02** | 4796 | 25% | 15 | **0.05** |

Abbrevations: IBS, identical by descent; PC, principal component; PVE, percent variance explained; SE, standard error.
*P*-values of $\leqslant 0.05$ are in bold.
[a]Using the full GRM, $K_{IBS}$ on all individuals.
[b]No PCs: covariates including gender, age (continuous), and marital status (binary). With 10 PCs: covariates included the first 10 PCs of genotype data.
[c]The GRM includes only distantly related pairs ($K_{IBS} < 0.05$ or 0.025). One individual from each relative pair was excluded.

Prior behavioral genetic studies have used adoption designs (McGuire and Clifford, 2000), twin designs (Boomsma *et al*, 2005; Waaktaar and Torgersen, 2012), a family-based design including nontwin siblings (Boomsma *et al*, 2006), and an extended twin designs to include the partners and parents of twins (Distel *et al*, 2010) to estimate the heritability of loneliness in a variety of populations (Goossens *et al*, 2015). The heritability estimates across these various designs have ranged from 37 to 55%. These estimates reflect the contributions of both common and rare variants. In contrast, estimates of chip heritability only capture the additive contributions of common variation (Yang *et al*, 2013), and are therefore expected to be lower. As such, they provide insight into the genetic architecture of loneliness, namely that it is polygenic in nature and is likely to be influenced by many common genetic variants of small effect. Our estimates of chip heritability add to existing heritability estimates and also reinforce the notion that both genetic and environmental factors influence loneliness. Future studies might increase heritability by utilizing more environmentally homogeneous populations or by including more environmental variables as covariates.

Our study did not identify any genome-wide significant associations. Although the sample size of slightly $>10\,000$ individuals provides appreciably greater statistical power than had been available previously, numerous disease and nondisease phenotypes that are known to be heritable have also yielded negative results with similar sample sizes

**Table 4** Associations between Polygenic Scores for Neuroticism from SSGAC (Okbay *et al*, 2016) and Loneliness in Health and Retirement Study (HRS)[a]

| *P*-value threshold | Num. of SNPs | Linear trait[b] | | | Multivariate traits[c] | | | | | | | Case/control[d] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | β | SE | *P* | Q1:companion | | Q2: left out | | Q3: isolated | | *P* for overall | OR | 95% CI | *P* |
| | | | | | *β1* | *P1* | *β2* | *P2* | *β3* | *P3* | | | | |
| $1\times10^{-5}$ | 33 | 0.03 | 0.02 | 0.08 | 0.01 | 0.14 | 0.01 | 0.12 | 0.01 | 0.14 | 0.37 | 1.08 | 1.01–1.14 | **0.02** |
| $1\times10^{-4}$ | 113 | 0.03 | 0.02 | 0.10 | 0.01 | 0.12 | 0.009 | 0.18 | 0.009 | 0.20 | 0.43 | 1.08 | 1.02–1.15 | **0.01** |
| $1\times10^{-3}$ | 515 | 0.04 | 0.02 | **0.02** | 0.01 | 0.07 | 0.01 | **0.03** | 0.01 | 0.07 | 0.16 | 1.08 | 1.01–1.15 | **0.02** |
| 0.01 | 3223 | 0.07 | 0.02 | $\mathbf{9.1\times10^{-5}}$ | 0.02 | **0.001** | 0.03 | **0.0001** | 0.02 | **0.003** | **0.001** | 1.15 | 1.08–1.22 | $\mathbf{1.2\times10^{-5}}$ |
| 0.05 | 13 036 | 0.08 | 0.02 | $\mathbf{9.3\times10^{-6}}$ | 0.03 | $\mathbf{3.5\times10^{-4}}$ | 0.03 | $\mathbf{9.9\times10^{-6}}$ | 0.02 | **0.0008** | $\mathbf{9.9\times10^{-5}}$ | 1.17 | 1.10–1.24 | $\mathbf{1.5\times10^{-6}}$ |
| 0.1 | 24 251 | 0.09 | 0.02 | $\mathbf{4.1\times10^{-6}}$ | 0.03 | $\mathbf{4.1\times10^{-5}}$ | 0.03 | $\mathbf{2.9\times10^{-5}}$ | 0.02 | **0.0005** | $\mathbf{6.7\times10^{-5}}$ | 1.17 | 1.10–1.24 | $\mathbf{1.4\times10^{-6}}$ |
| 0.3 | 65 722 | 0.09 | 0.02 | $\mathbf{4.1\times10^{-7}}$ | 0.03 | $\mathbf{3.0\times10^{-5}}$ | 0.03 | $\mathbf{3.9\times10^{-6}}$ | 0.03 | $\mathbf{3.3\times10^{-5}}$ | $\mathbf{1.0\times10^{-5}}$ | 1.17 | 1.10–1.25 | $\mathbf{3.6\times10^{-7}}$ |
| 0.5 | 105 444 | 0.10 | 0.02 | $\mathbf{1.6\times10^{-8}}$ | 0.03 | $\mathbf{2.3\times10^{-6}}$ | 0.03 | $\mathbf{4.7\times10^{-7}}$ | 0.03 | $\mathbf{3.0\times10^{-6}}$ | $\mathbf{5.3\times10^{-7}}$ | 1.20 | 1.12–1.27 | $\mathbf{1.6\times10^{-8}}$ |

The testing set was an independent set using the data of HRS; the polygenic scores have been standardized, and hence the β coefficients from the Neuroticism linear regression model correspond to a 1 SD change in the polygenic score. *P*-values $\leqslant 0.05$ are in bold, whereas $0.05 < P\text{-values} \leqslant 0.1$ are underlined.
[a]The polygenic model was developed using SNPs with *p*-values below the indicated threshold from Neuroticism obtained from Social Science Genetic Association Consortium (SSGAC, Neuroticism_Full.txt ).
[b]Using linear regression model for 6924 unrelated EAs; adjustments included sex, age, and marital status; further adjusting for the top 3 PCs had little impact.
[c]Using multivariate regression model for 6924 unrelated EAs, same adjustments as above.
[d]Using logistic regression model for 4796 unrelated EAs (cases/controls = 1632 : 3164), same adjustments as above.

(see, eg, Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium *et al*, 2013).

Our study provided an efficient means of testing previously reported associations between SNPs in candidate genes and loneliness. Despite an active literature in this area, we did not find support for any of the previously reported candidate gene associations. This is consistent with our previous experience with candidate gene-based studies (Hart *et al*, 2013). Several of the previously published candidate gene studies reported effect sizes that are much larger than those typically observed in genome-wide association studies that in hindsight should have generated more skepticism about those results. Although our findings cast doubt on the previously reported associations—or at least on the original effect sizes that were identified—there are potentially important differences between our study design and the previously published candidate gene studies. For instance, our population was based in the United States and was made up of older adults, many of whom were in stable long-term relationships, whereas approximately half of the candidate gene studies utilized samples of adolescents from the Netherlands or Germany (Lucht *et al*, 2009; van Roekel *et al*, 2010, 2011, 2013; Verhagen *et al*, 2014). Therefore, although our study benefited from a larger sample size than any of the previously reported candidate gene studies, we cannot discount the possibility that differences in the methodologies or the population under study led to our failure to replicate the previously published results. The phenotyping procedure used in the current study has been found to correlate highly ($r = 0.88$) with a more in-depth phenotype for loneliness (Hughes *et al*, 2004), and the candidate gene studies using older adults from the United Kingdom and Taiwan provided no greater evidence for replication than the studies using adolescents (Chou, 2010, 2014; Connelly *et al*, 2014; Lan *et al*, 2012; Tsai *et al*, 2012; Wang *et al*, 2013).

We observed strong genetic correlations between loneliness and two personality dimensions: neuroticism and extraversion (Table 4, Figure 2, and Supplementary Tables S3–S5). The direction of these effects was consistent with the correlations identified previously: greater loneliness has been shown to be positively correlated with neuroticism and negatively correlated with extraversion (see, eg, Cacioppo *et al*, 2006; Mund and Neyer, 2015). Neuroticism is characterized by high negative affectivity, a characteristic also seen in loneliness. Although prior research has shown loneliness and neuroticism to be stochastically and functionally separable, the results from the current study suggest there may be a shared genetic predisposition that contributes to both phenotypes. The multivariate PRS analyses provide information regarding the coheritability between loneliness and extraversion. The results show that whereas all three questions contributed to the genetic correlation with neuroticism (Table 4 and Supplementary Tables S3 and S4), only the items regarding lack of companionship and feeling left out contributed to the genetic correlation with extraversion (Supplementary Tables S5).

We see our study as being a part of an important trend that attempts to relate the genetic causes of psychiatric disease diagnoses to continuously variable traits that represent heritable personality characteristics. It is widely accepted that humans have varying degrees of sensitivity to social isolation; however, the question of whether or not the genetic basis of this variability also underlies the risk for common psychiatric diseases remains largely unexplored. We have previously reported that genetic variation in the initial sensitivity to the euphoric effects of amphetamine is genetically correlated with the risk for both schizophrenia and ADHD (Hart *et al*, 2014). That provocative finding

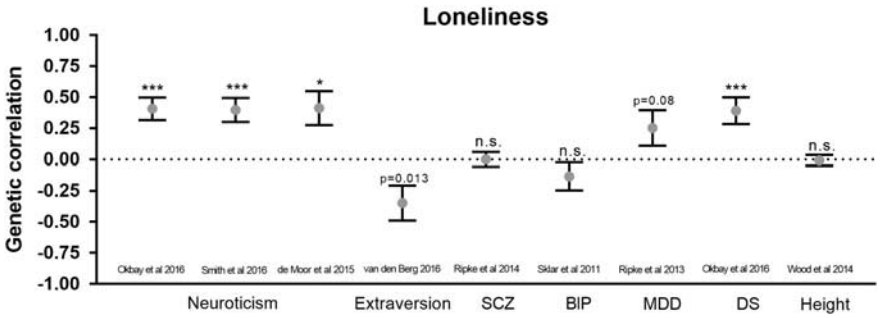


**Figure 2** Genetic correlations between loneliness (EA-only, case/control) and nine additional traits: personality traits (neuroticism, extraversion), psychiatric conditions (schizophrenia (SCZ), bipolar disorder (BP), and major depression disorder (MDD)), a depressive symptoms scale (DS), and height. Error bars represent SE. *$P<0.01$, ***$p<0.0001$, NS $p>0.05$.

provides an example of using genetic variation in a nondisease trait to obtain novel insights into the genetic basis of psychiatric diseases. In the present study, we explored whether or not genetic risk for loneliness mapped onto genetic risk for major psychiatric diseases. Our results provided limited support for this hypothesis (Figure 2 and Supplementary Tables S6–S9). The linear phenotype did not show any evidence for coheritability with schizophrenia, bipolar disorder, or major depression. However, when we used a multitrait mapping approach, which allowed us to consider each of the three questionnaire items independently, we saw suggestive evidence for coheritability between the second question ('how often do you feel left out?') and both schizophrenia and bipolar disorder. The relationship between loneliness and these two diseases was very weakly negative, meaning that being lonelier is associated with reduced risk of disease. We also used summary statistics from the case/control GWAS to perform LDSC that did not support coheritability with schizophrenia or bipolar disorder, but did show a trend toward a positive genetic correlation with major depression ($p=0.08$; lonelier was associated with greater risk for depression). The nonpsychiatric trait termed 'depressive symptoms' was more robustly positively correlated with loneliness in both the PRS and the LDSC analyses (lonelier was genetically correlated with more depressive symptoms). Because of the number of tests performed and the modest levels of significance for the psychiatric diseases, those results should be considered suggestive until they are replicated. Although we assume that few participants in the HRS study would be diagnosed with schizophrenia, such data were not available; therefore, we cannot exclude the possibility that our findings are secondary to the effects of schizophrenia on self-reported loneliness. The direction of the effect suggests that greater genetic risk for loneliness is negatively associated with these psychiatric diseases. We have previously hypothesized that loneliness reflects an adaptive drive toward social interaction that is consistent with the direction of the observed correlation. Future studies of loneliness and other social behavior traits may continue to inform our understanding of the role of social behavior in psychiatric health and disease (Cacioppo *et al*, 2014).

In summary, we have performed the first GWAS of loneliness. Our study has identified significant evidence for heritability, but did not identify specific loci associated with loneliness nor was it able to replicate previously reported candidate gene associations. Finally, we identified strong evidence for coheritability between loneliness and neuroticism, extraversion, and 'depressive symptoms', and suggestive evidence for coheritability between loneliness and schizophrenia, bipolar disorder, and major depressive disorder. We believe that future studies of loneliness, as well as additional studies of other social neuroscience phenotypes, may continue to enrich our understanding of the ways in which our genetic inheritance fundamentally influences individual and social behavior.

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The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)