Replication of the diathesis-stress model for depression in Generation Scotland.

Running title: **The diathesis-stress model for depression in Generation Scotland.**

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ABSTRACT

Depression has well-established influences from both genetic and environmental factors. A popular theory of depression aetiology in psychiatry and psychology is the *diathesis-stress* theory, which assumes a multiplicative gene-by-environment interaction (GxE) effect on risk. Recently, *Colodro-Conde et al* empirically tested it, reporting GxE effects additively contributing to liability.

We replicate that study on an independent sample of 4 919 unrelated individuals who reported stressful life events (SLE) over the 6 months immediately before a self-reported measure of depressive symptoms, and test for sex-specific differences.

We identified significant but weak positive GxE using the total number of SLE reported in the full cohort (R^2 = 0.08%, $p = 4.87 \times 10^{-2}$) and in women (R^2 = 0.19%, $p = 1.66 \times 10^{-2}$), but not in men ($p > 0.05$). We also detected significant GxE effects, but only in women, when SLE were split into those in which the respondent may play an active role ("dependent" SLE, R^2 = 0.15%, $p = 3.85 \times 10^{-2}$) or a passive role ("independent" SLE, R^2 = 0.16%, $p = 3.32 \times 10^{-2}$). Further, in women who experienced no SLE, the *diathesis* effect showed a protective effect (β = -0.061, s.e. = 0.029, p = 0.037), suggesting a possible role of genetic plasticity in risk variants.

Our study replicates Colodro-Conde *et al.*, reinforcing the presence of additional risk in the aetiology of depression due to GxE effects. Furthermore, these results support possible differences between sexes and effects of SLE subtypes. However, more power is required to robustly replicate these findings.

INTRODUCTION

Adversity faced during stressful life events (SLE) has been consistently recognized as a determinant of depressive symptoms with many studies reporting significant associations between SLE and major depressive disorder (MDD)[1-7]. Some studies suggest that severe adversity is present before the onset of illness in over 50% of individuals with depression[8] and may characterize a subtype of cases[9]. However, some individuals facing severe stress never present symptoms of depression[10]. This has led to a proposal that interaction between stress and an individual's vulnerability, or *diathesis*, is a key element in the development of depressive symptoms. Such vulnerability can be conceived as a set of biological factors that predispose to illness. This idea was first conceptualized into the *diathesis-stress* model to explain the development of schizophrenia back in the 1960s[11]. Later in the 1980s it was used to explain the origins of depression[12-14] and since then several *diathesis-stress* models have been applied to many psychopathologies[15-19]. The *diathesis-stress* model proposes that a latent *diathesis* may be activated by stress before psychopathological symptoms manifest. Some levels of *diathesis* to illness are present in everybody, with a threshold over of which they will present symptoms. Exceeding such illness threshold depends on the interaction between *diathesis* and the degree of adversity faced in SLE, which increase the liability of depression beyond the combined additive effects alone[15]. Inherent genetic risk factors can be conceived as a genetic *diathesis*. Thus, this genetically driven effect produced by the *diathesis-stress* interaction can be seen as a gene-by-environment interaction (GxE).

MDD is characterized by a highly polygenic architecture composed of common variants with small effect and/or rare variants[20]. Therefore, GxE interactions in depression are

also expected to be highly polygenic. In recent years, with the increasing success of GWAS, GxE studies in depression have shifted towards hypothesis-free genome-wide and polygenic approaches that capture liability to depression using molecular data[21-29]. Recent advances in genomics and the massive effort from national institutions to collect genetic, clinical and environmental data on large population-based samples now provide an opportunity to empirically test the *diathesis-stress* model in depression. A novel paradigm to quantify genetic *diathesis* into a single genetic measure, to study GxE effects with more predictive power than any single variant, is the construction of polygenic risk scores (PRS)[30-33]. PRS are genetic indicators of the aggregate effect from risk alleles carried by an individual weighted by their allelic effect estimated from Genome-Wide Association Studies (GWAS).

This polygenic approach to assess the *diathesis-stress* model for depression has been tested with childhood trauma[21,23,29] and SLE[22,27,29], as measures of environmental adversity.

Recently, Colodro-Conde *et al.*[27] provided a direct test of the *diathesis-stress* model for recent SLE and depressive symptoms. In this study, Colodro-Conde *et al.* used PRS weighted by the most recent genome-wide meta-analysis conducted by the Psychiatric Genetics Consortium (PGC; N = 159 601), having a substantially larger sample size than any discovery sample used previously, and measures of three environmental exposures: lack of social support, "personal" SLE (PSLE), and "network" SLE. Colodro-Conde *et al.* reported a significant additive risk on liability to depression due to a GxE effect in individuals who combine a high genetic predisposition to MDD and a high number of reported PSLE, mainly driven by effects in women. No significant interaction was reported in males. They found no significant interaction with "network" SLE or

social support. They concluded that the effect of stress on risk of depression was dependent on an individual's *diathesis,* supporting the *diathesis-stress* theory. In addition, they suggested possible sex-specific differences in the aetiology of depression. However, Colodro-Conde *et al.* findings have not, to our knowledge, been independently replicated.

In the present study we aim to replicate Colodro-Conde *et al.* and assess differences between women and men in an independent sample of 4 919 unrelated white British participants from a further longitudinal assessment from Generation Scotland: Scottish Family Health Study, who along with a self-reported diagnosis of depressive symptoms, self-reported SLE over the preceding 6 months.

MATERIALS AND METHODS

Sample description

The present study was conducted using data available on 4 919 unrelated individuals (mean age at questionnaire: 47.2, s.d. = 12.2, range 22-95; *females*: *n* = 2 990 - 60.8%, mean age 56.1, s.d. = 12.4; *males*: *n* = 1 929 - 39.2%, mean age 58.7, s.d. = 11.8) from a further longitudinal assessment from Generation Scotland: Scottish Family Health Study (GS:SFHS; www.ed.ac.uk/generation-scotland/using-resources/scottish-family-health-study) funded by a Wellcome Trust Strategic Award "STratifying Resilience and Depression Longitudinally" (STRADL) reference 10436/Z/14/Z. STRADL[34] is a project aimed to investigate the aetiology underlying depression by re-contacting participants from GS:SFHS to collect new and updated mental health questionnaires covering a wide range of psychiatric symptoms and SLE measures, among others. Further details on the recruitment procedure and GS:SFHS profile are described in detail elsewhere[35-39]. In 2014, 21 525 GS:SFHS participants eligible for re-contact were sent self-reported questionnaires. 9 618 GS:SFHS re-contacted participants (44.7% response rate) agreed to provide new measures to STRADL mental health follow-up[34]. Those participants: duplicated, with diagnoses of bipolar disorder or with missing data on reported SLE, population outliers, with sex discrepancies, or with more than 2% missing genotypes, were removed. SNPs with more than 2% genotype missingness, Hardy-Weinberg Equilibrium test $p < 1 \times 10^{-6}$, or minor allele frequency lower than 1%, were excluded. After applying quality control, individuals were filtered by degree of relatedness (pi-hat < 0.05) using PLINK v1.9[40], maximizing retention of those participants reporting higher numbers of SLE (see phenotype assessment below). The final dataset comprised 4 919 unrelated individuals and 560 351 SNPs after quality control. 20 principal components

were calculated for the final "full cohort" dataset (N = 4 919), "females" dataset (N = 2 990) and "males" dataset (N = 1 929). All participants provided written consent. All components of GS:SFHS and STRADL obtained ethical approval from the Tayside Committee on Medical Research Ethics on behalf of the National Health Service (reference 05/s1401/89). GS:SFHS & STRADL data is available to researchers on application to the Generation Scotland Access Committee (access@generationscotland.org).

Phenotype assessment

Individual's current depressive symptoms were assessed using the 28-item scaled version of The General Health Questionnaire (GHQ)[41,42]. GHQ is a reliable and validated psychometric screening tool to detect common psychiatric and non-psychotic conditions (GHQ Cronbach alpha coefficient: 0.82 – 0.86)[43]. GHQ assesses symptoms over the last two weeks through 28 yes/no questions (items). At same time, each symptom/item is rated on a four-point Likert scale from 0 to 3 to assess its degree or severity (e.g. *Have you recently felt that life is entirely hopeless?* "Not at all", "No more than usual", "Rather more than usual", "Much more than usual"), resulting on an 84-point scale *depression* score. *Depression* score was log transformed to reduce the effect of positive skew/provide a better approximation to a normal distribution. For a better interpretation, *depression* score was scaled to a mean of 0 when required (see Figure 3).

Data from a brief life events questionnaire (BLEQ), based on the List of Threating Experiences (LTE)[44] and self-reported by STRADL participants, was used to construct a measure of common SLE over the previous 6 months. LTE is a reliable psychometric

device to measure psychological "stress"[45,46]. The BLEQ consists of a 12-item questionnaire to assess SLE with considerable long-term contextual effects (e.g. *Over last 6 months, did you have a serious problem with a close friend, neighbour or relatives?*). A final score reflecting the total number of SLE (TSLE) ranging from 0 to 12 was constructed by summing yes responses. Additionally, TSLE was split into two categories based on BLEQ-items measuring SLE in which the individual may play and active role, and therefore in which the SLE is influenced by genetic factors and thus subjected to be "dependent" on an individual's own behaviour or symptoms (DSLE; 6 BLEQ-items, e.g. *a serious problem with a close friend, neighbour or relatives* may be subject to a respondent's own behaviour), or SLE that are not influenced by genetic factors, likely to be "independent" on a participant's own behaviour (ISLE; 5 BLEQ-items, e.g. *a serious illness, injury or assault happening to a close relative* is potentially independent of a respondent's own behaviour)[44,47]. The BLEQ-item *"Did you/your wife or partner give birth?"* was excluded from this categorization. In addition, for each SLE measure, individuals were categorized in three categories based on the amount of SLE experienced (0 SLE = "missing", 1 or 2 SLE = "low", and 3 or more SLE = "high").

Polygenic profiling & statistical analysis

Polygenic risk scores (PRS) were generated by PRSice[48], whose functionality relies entirely in PLINK v1.9[40], and calculated using the genotype data of STRADL participants (i.e. target sample) and summary statistics for MDD from the PGC-MDD2 GWAS release (July 2016, discovery sample) used by Colodro-Conde *et al*. with the added contribution from QIMR cohort and the exclusion of Generation Scotland participants,

resulting in summary statistics for MDD derived from a sample of 50 455 cases and 105 411 controls.

Briefly, PRSice removed strand-ambiguous SNPs and clump-based pruned (r^2 = 0.1, within a 10Mb window) our target sample to obtain the most significant independent SNPs (n) in approximate linkage equilibrium. Independent risk alleles were then weighted by the allelic effect sizes estimated in the independent discovery sample were aggregated into PRS. PRS were generated for eight *p* thresholds (*p* thresholds: < 5 x 10^{-8}, < 1 x 10^{-5}, < 0.001, < 0.01, < 0.05, < 0.1, < 0.5, <=1) determined by the discovery sample. (See Supplementary Table 1 for summary of PRS).

Following Colodro-Conde *et al.,* covariates (i.e. age, age^2, sex, age-by-sex and age^2-by-sex interactions, and 20 principal components) were regressed from PRS (PRS') and SLE scores (i.e. TSLE, DSLE and ISLE; SLEs') before fitting models in GCTA 1.26.0[49] to guard against confounding influences on the PRS-by-SLEs interactions[50]. PRS' and SLEs' were standardized to a mean of 0 and a standard deviation of 1. A genetic relatedness matrix (GRM) was calculated for each dataset (i.e. *full cohort*, *males* and *females*) using GCTA.

Mixed linear models using the GRM were used to estimate the variance in *depression* score explained by PRS', SLEs' and their interaction, and stratified by sex.

The mixed linear model used to assess the effects of PRS' is as follows:

$$Depression = \beta_0 + \beta_1 PRS' + GRM + Covariates$$

Mixed linear models used to assess the effect of the stressors (=SLEs') are as follows:

$$Depression = \beta_0 + \beta_1 TSLE' + GRM + Covariates$$

$$Depression = \beta_0 + \beta_1 DSLE' + GRM + Covariates$$

$$Depression = \beta_0 + \beta_1 ISLE' + GRM + Covariates$$

The Mixed linear models (i.e. the diathesis-stress model) used to assess PRS' interactions with SLEs' are as follows:

$$Depression = \beta_0 + \beta_1 PRS' + \beta_2 TSLE' + \beta_3 PRS' x TSLE' + GRM + Covariate$$

$$Depression = \beta_0 + \beta_1 PRS' + \beta_2 DSLE' + \beta_3 PRS' x DSLE' + GRM + Covariate$$

$$Depression = \beta_0 + \beta_1 PRS' + \beta_2 ISLE' + \beta_3 PRS' x ISLE' + GRM + Covariate$$

Covariates fitted in the models above were age, age^2, sex, age-by-sex, age^2-by-sex and 20 principal components. Sex and its interactions (age-by-sex and age^2-by-sex) were not included when stratifying by sex. All parameters from the models were estimated using GCTA and the significance of the effect (β) from fixed effects assessed using Student's *t*-test at *p*-value threshold = 0.05.

Using linear regressions we applied a least squares approach to assess PRS' effects on *depression* score in each SLE category (i.e. "missing stress", "low stress" "high stress") where significant GxE were detected (significance at *p*-value < 0.05).

RESULTS

Depression PRS' significantly predicted the *depression* score in the whole sample (β = 0.080, s.e. = 0.014, p = 7.53 x 10^{-9}) explaining 0.64% of the variance at its best p threshold (= 0.1; see Figure 1a). Stratifying by sex, PRS' significantly predicted the *depression* score in both sexes, explaining 0.59% in men and 0.67% in women (*men*: p-threshold = 0.1, β = 0.077, s.e. = 0.022, p = 2.09 x 10^{-4}; *women*: p-threshold = 0.1, β = 0.082, s.e. = 0.018, p = 4.93 x 10^{-6}; see Figure 1a). Self-reported SLE over the last 6 months (TSLE, mean = 1.3 SLE), significantly predicted symptoms of depression (*depression* score) in the whole sample and stratified by sex: *full cohort*: variance explained = 4.91%, β = 0.222, s.e. = 0.014, p = 9.98 x 10^{-59}; *men*: 4.19%, β = 0.205, s.e. = 0.021, p = 2.23 x 10^{-22}; *women*: 5.33%, β = 0.231, s.e. = 0.018, p = 7.48 x 10^{-38} (Figure 1b). However, the variance in *depression* score explained by the TSLE appears lower than the variance explained by the measure of PSLE used in Colodro-Conde *et al*. (12.9%). There was no significant difference in the direct effect of TSLE between women and men. Although questions about "dependent" SLE (DSLE, mean = 0.4 SLE) represented over 28% of the TSLE-items reported, the main effect of DSLE explained approximately 93% of the amount of variance explained by TSLE (*full cohort*: variance explained = 4.56%, β = 0.212, s.e. = 0.014, p = 1.73 x 10^{-54}; *men*: 3.74%, β = 0.193, s.e. = 0.021, p = 9.66 x 10^{-21}; *women*: 5.07%, β = 0.225, s.e. = 0.018, p = 8.09 x 10^{-35}; see Figure 1b). "Independent" SLE (ISLE, mean = 0.85 SLE), which represented over 69% of TSLE-items, explained approximately 57% of the amount of variance explained by TSLE (*full cohort*: variance explained = 2.80%, β = 0.167, s.e. = 0.014, p = 1.32 x 10^{-33}; *men*: 2.44%, β = 0.156, s.e. = 0.022, p = 2.88 x 10^{-13}; *women*: 3.02%, β = 0.174, s.e. = 0.018, p = 5.20 x 10^{-22}; see Figure 1b). When DSLE and ISLE were combined together in a single

model, DSLE explained 3.34% of the variance of depressive score compared to 1.45% of the variance being explained by ISLE. This suggests that DSLE have a greater effect on liability to depressive symptoms than ISLE.

We detected significant, albeit weak, GxE effects on *depression* score as conceptualized in the *diathesis-stress* model (see figure 2). The PRS interaction with TSLE was significant in the full cohort ($\beta = 0.028$, s.e. = 0.014, $R^2 = 0.08\%$, $p = 4.87 \times 10^{-2}$) and slightly stronger in women ($\beta = 0.044$, s.e. = 0.018, $R^2 = 0.19\%$, $p = 1.66 \times 10^{-2}$; see figure 2a). The best-fit threshold was much lower in women (p-threshold = 1×10^{-5}) compared to the full cohort (p-threshold = 0.01). GxE effects estimated in women and men at p-threshold = 1×10^{-5} where significantly different (GxE*sex $p = 0.017$), but not at the best p-threshold in the full cohort (p-threshold = 0.01, GxE*sex $p = 0.32$). In women, GxE effect with DSLE predicted *depression* score (p-threshold = 1×10^{-5}; $\beta = 0.039$, s.e. = 0.019, $R^2 = 0.15\%$, $p = 3.85 \times 10^{-2}$; see figure 2b), as did the GxE effect with ISLE (p-threshold = 1×10^{-5}; $\beta = 0.040$, s.e. = 0.019, $R^2 = 0.16\%$, $p = 3.32 \times 10^{-2}$; see figure 2c). No significant interaction was detected in men (best-fit p-threshold = 0.1) using either TSLE ($\beta = 0.039$, s.e. = 0.022, $R^2 = 0.15\%$, $p = 7.19 \times 10^{-2}$; see figure 2a), DSLE ($\beta = 0.024$, s.e. = 0.022, $R^2 = 0.06\%$, $p = 0.28$; see figure 2b) or ISLE ($\beta = 0.043$, s.e. = 0.022, $R^2 = 0.18\%$, $p = 5.47 \times 10^{-2}$; see figure 2c).

Examination of the significant GxE detected by SLE categories (i.e. “missing”, “low” and “high”) suggests that in participants reporting TSLE the risk of depressive symptoms was higher when greater numbers of SLE were reported; whereas in participants who reported no SLE over the preceding 6 months, the risk of depressive symptoms was the same regardless of their *diathesis* risk (“missing”: PRS’ $\beta = 0.021$, s.e. = 0.022, $p = 0.339$; “low”: PRS’ $\beta = 0.043$, s.e. = 0.021, $p = 0.039$; “high”: PRS’ $\beta = 0.142$, s.e. = 0.039,

$p = 2.86 \times 10^{-4}$; see Table 1 and figure 3a). Although a similar pattern by increasing SLE was observed in women (see Table 1, figure 3b and Supplementary figure 2), we only detected a significant *diathesis* effect on *depression* score in those women who did not experienced TSLE over the last 6 months. This effect was negative (PRS' $\beta = -0.061$, s.e. $= 0.029$, $p = 0.037$, $R^2 = 0.3\%$; see figure 3b), suggesting a protective effect of increasing PRS' in those women reporting no SLE, and suggesting that the contributing alleles may make an individual sensitive to both positive and negative environmental effects (i.e. "plasticity alleles" rather than "risk alleles")[51,52].

DISCUSSION

The findings reported in this study replicate those of Colodro-Conde *et al*. results in an independent sample from Generation Scotland of similar sample size and study design, and suggest possible sex-specific differences in genetic risk of MDD in response to "dependent" SLE.

We identified significant, albeit weak, GxE effect in liability to depression at the population level ($p = 4.87 \times 10^{-2}$) and in women ($p = p = 1.66 \times 10^{-2}$), but not in men ($p = 7.19 \times 10^{-2}$). Both Colodro-Conde *et al.* and our studies suggest that individuals with an inherent genetic predisposition to MDD, reporting high number of recent SLE, are at additional risk of depressive symptoms due to GxE effects, supporting the *diathesis-stress* theory. However, these interactions did not survive multiple testing correction and the power of these studies to draw robust conclusions remains limited[53]. With increased power these studies could more accurately determine both the presence and magnitude of a gene-by-environment interaction in depression.

In the full cohort, the total variance of depressive score explained by the PRS' main effect and the GxE effect jointly was 0.34%, of which 0.07% was attributed to the GxE effect (p-threshold = 0.01; PRS $p = 1.19 \times 10^{-4}$, GxE $p = 4.87 \times 10^{-2}$; both derived from the full diathesis-model with TSLE); lower than the proportion of variance attributed to common SNPs (8.9%) in the full PGC-MDD analysis[20]. As Colodro-Conde *et al.* noted, this result aligns with estimates from experimental organisms suggesting that around 20% of the heritability may be typically attributed to the effects of GxE[54], although it is inconsistent with the majority of human traits with the potential exception of depression[55].

We saw concordance between our results and those of Colodro-Conde *et al.*[27] Consistent with PRS predicting PSLE in Colodro-Conde *et al.,* PRS for MDD predicted SLE in our study (see Supplementary Figure 1), although not at the *p*-threshold at which significant GxE effects were detected. *Depression* score was positive correlated with self-reported SLE scores (TSLE: $r^2 = 0.214$, DSLE: $r^2 = 0.211$, ISLE: $r^2 = 0.169$; all $p < 2.2 \times 10^{-16}$). Genetic and phenotypic correlations, added to the usage of self-reported screening to construct the measures fitted into the analysis, suggest possible confounding i.e. self-report bias. Genetic correlation between SLE and MDD may be driven by genetic factors which results in greater exposure to highly stressful/risky environments, or via personality traits, such as neuroticism, which shows positive associations with negative response to and greater reporting of negative life events[56,57]. This genetic correlation implies that the SLE effect is unlikely to act solely as an environmental risk factor, and genetic factors predisposing to MDD may also increase exposure to/reporting of SLE. This hinders to interpret our findings as pure GxE effects. To solve this limitation and assess this aspect, Colodro-Conde *et al.* broke down the PSLE measure into SLE in which the individual may played an active role (PSLE-active) or a passive role (PSLE-passive)[47,58]. Equivalently, we split the TSLE measure of 12-items into SLE that are potentially either SLE "dependent" (DSLE; 6-items) on a participant's own behaviour (i.e. therefore potentially driven by genetic factors) or not ("independent" SLE, ISLE; 5-items)[44,47]. PSLE-active and DSLE are reported to be more heritable and have stronger associations with MDD than PSLE-passive or ISLE[47,58,59]. Thus, if the GxE detected were driven by ISLE it would point towards a more pure GxE rather than a subtle genotype-by-genotype (GxG) interaction or genotype-by-genotype-by-environment (GxGxE) interaction. We observed in our

study that although DSLE was significantly heritable (h^2_{SNP} = 0.131, s.e. = 0.071, p = 0.029) and reporting ISLE was not significantly heritable (h^2_{SNP} = 0.000, s.e. = 0.072, p = 0.5), in women significant GxE was seen for both. In Colodro-Conde *et al.,* PSLE-active explained most of the variance explained by PSLE. PSLE-passive score explained a marginal amount of variance (0.77%) compared to the PSLE measure (10.5%). In addition, conversely to our findings in which we identified significant GxE effects within women using all three measures of SLE, and explaining similar amount of variances (0.15% – 0.19%; see figure 2), Colodro-Conde *et al*. did not identify significant GxE using PSLE-passive.

Despite the replicated findings supporting the *diathesis-stress* model, there are a few differences between our study and Colodro-Conde *et al.* to consider. First, differences in PRS profiling may have affected replication power. We used the same equivalent PGC-MDD2 GWAS as discovery sample. However, whereas Colodro-Conde *et al.* generated PRS in their target sample containing over 9.5M imputed SNP, in this study we generated PRS in a target sample of over 560K genotyped SNPs (see Supplementary table 1 for comparison). This potentially results in a less informative PRS in our study, with less predictive power, although the variance explained by our PRS' was slightly larger (0.64% vs. 0.46%). The size of the discovery sample is key to constructing an accurate predictive PRS, but to exploit the most of the variants available may be an asset[53].

Secondly, different screening tools were used to measure both current depression and recent environmental stressors across the two studies. Both studies transformed their data, using item response theory or by log-transformation, to improve the data distribution. However, neither study used depression scores that were normally

distributed; and although both screening methods have been validated and applied to detect depressive symptoms, different questions may cover and emphasise different features of the illness and may result in different results[54,60]. The same applies to the measurement of environmental stressors. We used a measure of 12-items adapted from the List of Threating Experiences (LTE) to assess SLE over the past 6 months. However, Colodro-Conde *et al.* used a self-reported PSLE measure over the last 12 months, thus covering a longer time-period of exposure to SLE prior to the assessment of current depressive symptoms than in our study. PSLE included the same 12-item LTE we assessed. However, they added seven items concerning serious problems getting along with others (i.e. spouse, someone living with you, a close friend, etc), a range of incidents highly subject to an individual's own behaviour. Thus, PSLE contains a greater number of DSLE/PSLE-active-like items than ISLE/PSLE-passive-like items compared to the original 12-item LTE (and TSLE). Although the overall number of DSLE-items reported in our study represented less than 30% of the overall number of TSLE-items reported, DSLE explained over 90% of the equivalent variance explained by TSLE (see figure 2b). However, ISLE explained (~57%) far more than the remaining variance. Both DSLE and ISLE effects jointly assessed in a single model showed that DSLE explained 70% of the variance of depressive score explained by TSLE, compared to only 30% of such variance being explained by ISLE. Therefore, both covering longer time-period and upweighting by DSLE/PSLE-active-like items may explain the increased explanatory power of PSLE (12.9%) used by Colodro-Conde *et al*. to predict d*epression* score compared to our TSLE measure (4.91%).

Finally, the LTE used to construct stress measures in both studies, despite being a validated and robust screening tool, does not cover a wide range of life events with

marked long-term, or mild to moderate contextual stress, that could impact on the final adversity faced by an individual. These unmeasured aspects of the environmental exposure or its impact may also contribute to lack of stronger replication and positive findings. However, these sources of bias (e.g. self-reporting bias) may be solved soon by extracting data directly from national population-based electronic records (i.e. medical, criminal, financial, historical, etc). Not only time-point measures but also longitudinal scores may be constructed over lifespan to study GxE under a life-course approach. Some studies suggests that the presence of environment-by-environment (ExE) and gene-by-environment-by-environment (GxExE) interactions over lifespan are responsible of the development of mental disorders[61,62]. Therefore, a life-course approach using linkable datasets is likely to help to better detect GxE effects, while reducing bias, and thus provide a better understanding of both genetics and environmental factors involved into the genetic-environment interplay underlying depression, and mental illness in general.

In women (p-threshold = 1×10^{-5}, $p = 1.66 \times 10^{-2}$), the GxE effect estimates were significantly higher ($p = 0.017$) than that in men, suggesting possible differences in the aetiology of depression between sexes. However, such difference was not significant ($p = 0.32$) at p-threshold (0.01) where significant GxE effect was detected in the full cohort. This may explain previous differences seen between sexes such as in: associated risk (i.e. approximately 1.5 - 2-fold higher in women), symptoms reported and/or coping strategies (e.g. whereas women tend to cope through verbal and emotional strategies, men tend to cope by doing sport and consuming alcohol)[63-67]. This also aligns with an increased risk associated with a lack of social support seen in women compared to men[27]. Furthermore, although we do not know whether

participants experienced recent events with positive effects, some genetic variants associated with MDD may operate as 'plasticity alleles' and not just as 'risk alleles', which could provide a protective effect in those women who did not experienced recent SLE (and who may or may not have experienced positive environments; p = 0.037)[51,52]. This would be consistent with a differential-susceptibility model[68,69] of depression. This has been suggested for the interaction effects seen between the serotonin transporter linked promoter region gene (5-HTTLPR) locus and family support and liability to adolescent depression in boys[70]. However, our result is only nominally significant and will require replication in larger samples. Conversely, in the full cohort, our findings were consistent with a latent *diathesis* being activated by the presence of stress to manifest symptoms of depression as proposed by the *diathesis-stress* model. Future GxE studies of depression should assess the full range of life events (i.e. positive and negative).

Empirically demonstrating the *diathesis-stress* theory for depression would validate recent[24-26,28] and future studies using a genome-wide approach to identify genetic mechanisms and interactive pathways involved in GxE underpinning the causative effect of "stress" in the development of depressive symptoms and mental illness in general. Furthermore, sex-specific differences may improve personalized treatments and therapies such as better coping strategies. This study adds to our understanding of gene-by-environment interactions, although larger samples will be required to confirm the suggested differences in *diathesis-stress* effects between men and women.

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FINANCIAL DISCLOSURE

The authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1. a) Association between polygenic risk scores (PRS) and depressive score (main effects, one-sided tests). PRS were generated at 8 *p*-threshold levels using summary statistics from the Psychiatric Genetic Consortium MDD GWAS (released July 2016) with the exclusion of Generation Scotland participants. Depression score was derived from The General Health Questionnaire. Y-axis represents the % of variance of depression score explained by PRS main effects. Full cohort (yellow) was split into men (blue) and women (red). **b)** Association between number of SLE reported and depression score (main effect, one-sided tests, results expressed in % of depression score explained). SLE were self-reported through a brief life events questionnaire based on the List of Threating Experiences and categorized into: total number of SLE reported (TSLE), "dependent" SLE (DSLE) or "independent" SLE (ISLE). Full cohort (yellow) was split into men (blue) and women (red).

Figure 2. Association between GxE effect and depression score. Results represent percentage of depression score explained by the interaction term (two-sided tests) fitted in linear mixed models to empirically test the *diathesis-stress* model. Red numbers show significant interactions *p*-values. Full cohort (yellow) was split into men (blue) and women (red). PRS were generated at 8 *p*-threshold levels using summary statistics from the Psychiatric Genetic Consortium MDD GWAS (released July 2016) with the exclusion of Generation Scotland participants. The interaction effect was tested with **a)** the number of SLE reported (TSLE), **b)** "dependent" SLE (DSLE) and **c)** "independent" SLE (ISLE).

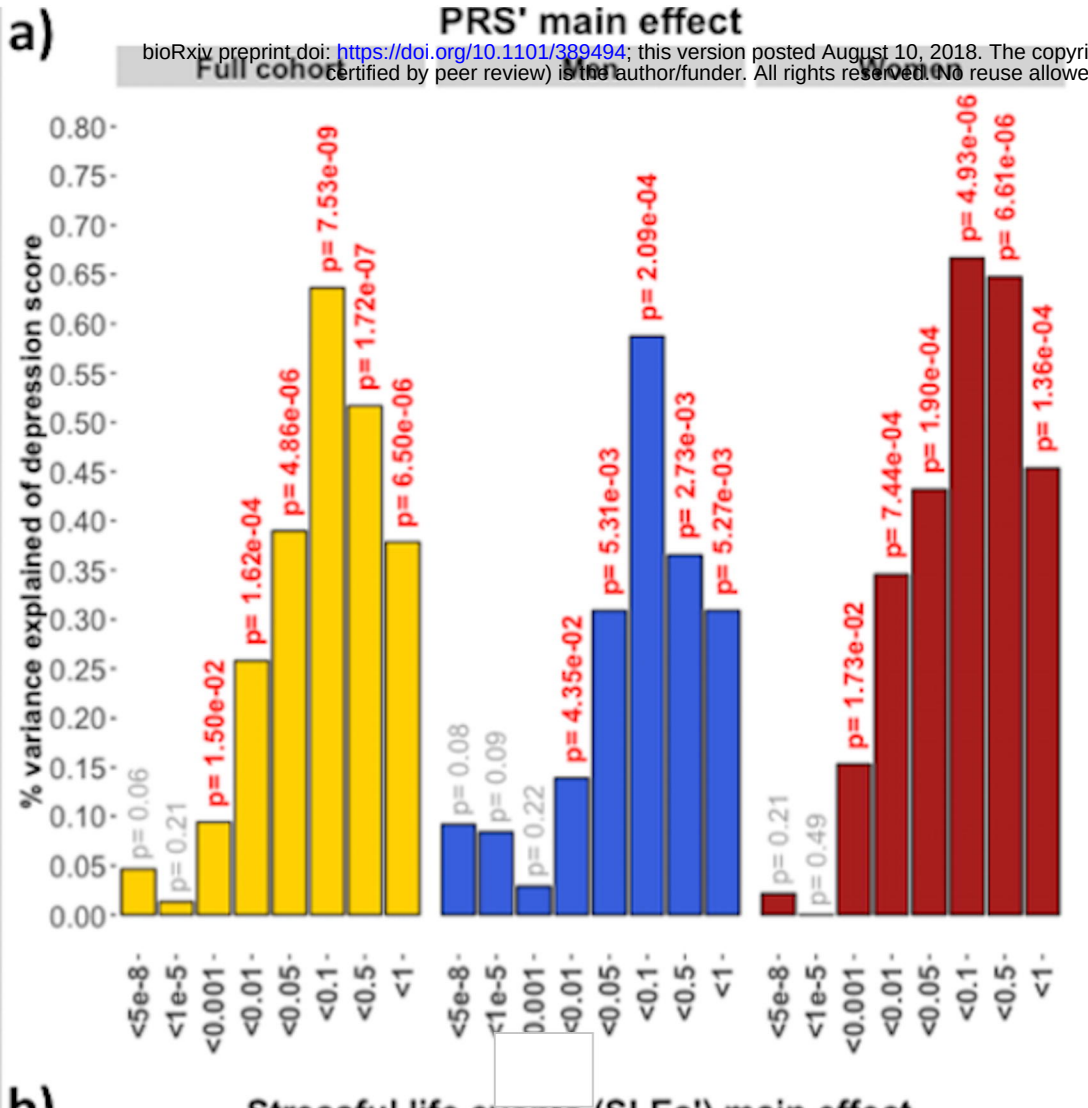
Figure 3. Scatterplot of significant *diathesis-stress* interactions on the risk of depressive symptoms **a)** in full cohort and **b)** in women. X-axis represents the direct effect of PRS

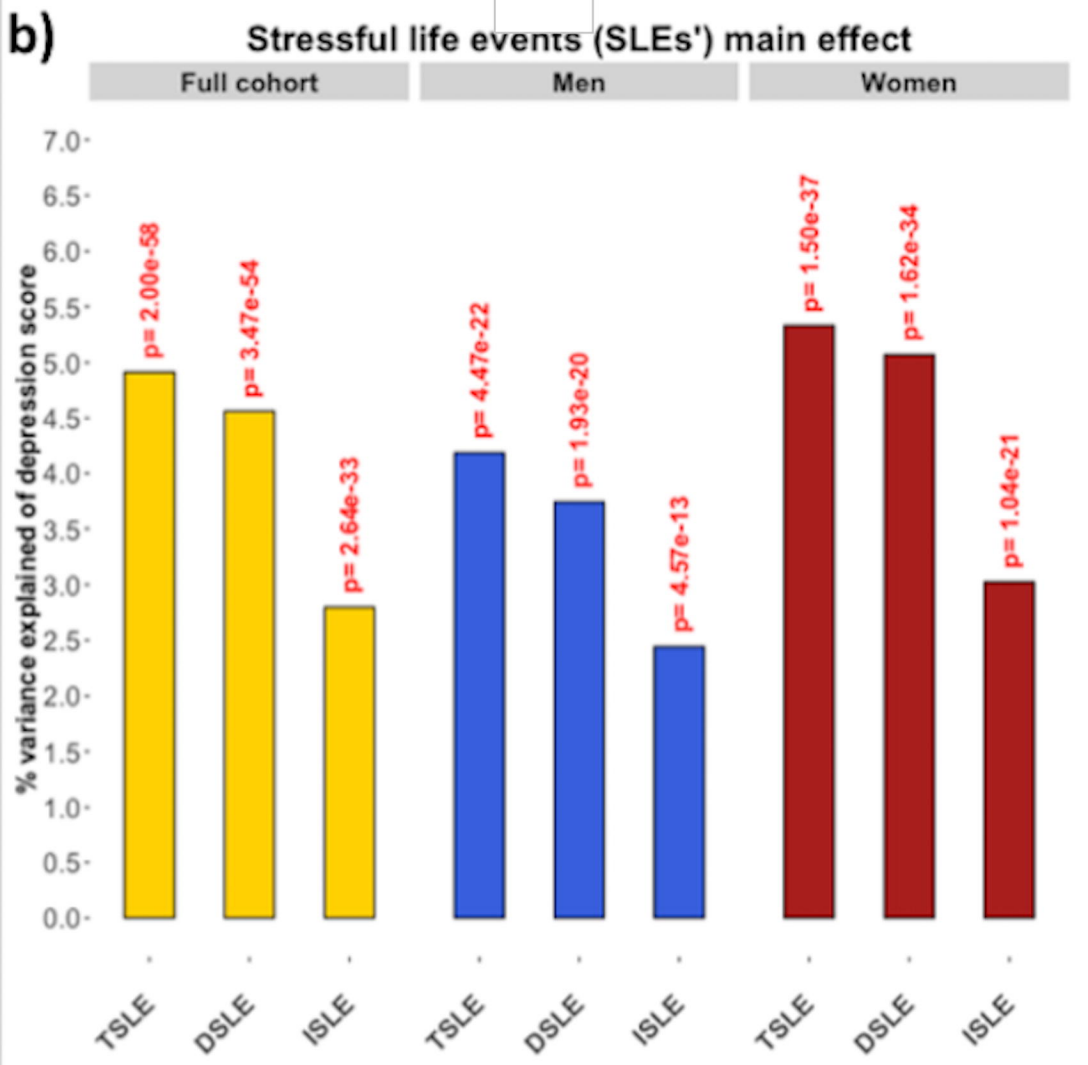
(standard deviation from the mean) based on **a)** *p*-threshold = 0.01 and **b)** *p*-threshold = 1 x 10^{-5}, using the total number of SLE reported (TSLE) by each participant (dot) as environmental exposures at three SLE levels represented by colours. Blue: 0 SLE, "missing stress", n = 1 833/1 041; green: 1 or 2 SLE, "low stress", n = 2 311/1 459; red: 3 or more SLE, "high stress", n = 775/490 in the full cohort and women, respectively. Y-axis reflects log transformed depression score standardized to mean of 0 and standard deviation of 1. Lines represent the increment of risk of depression under a certain degree of "stress" dependent on a genetic predisposition (= *diathesis*).

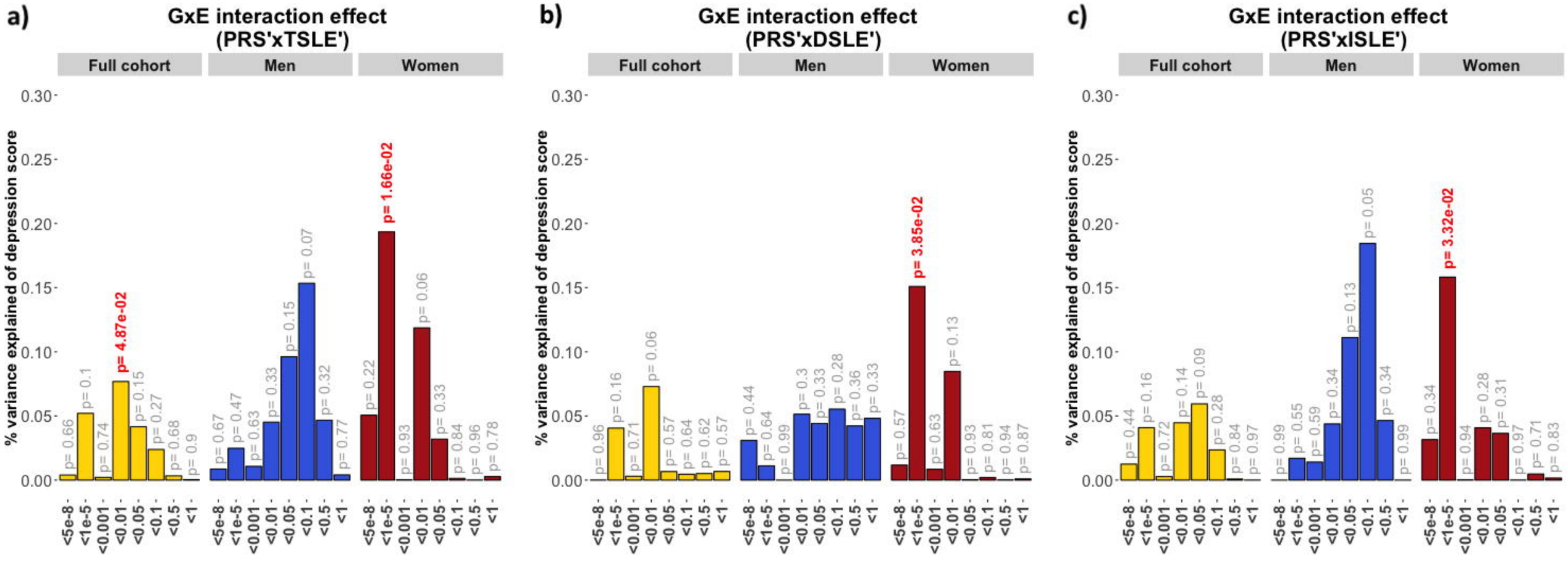
Table 1. ***Diathesis*** **effect under 3 SLE categories at those significant GxE effect detected in the full cohort and in women.**

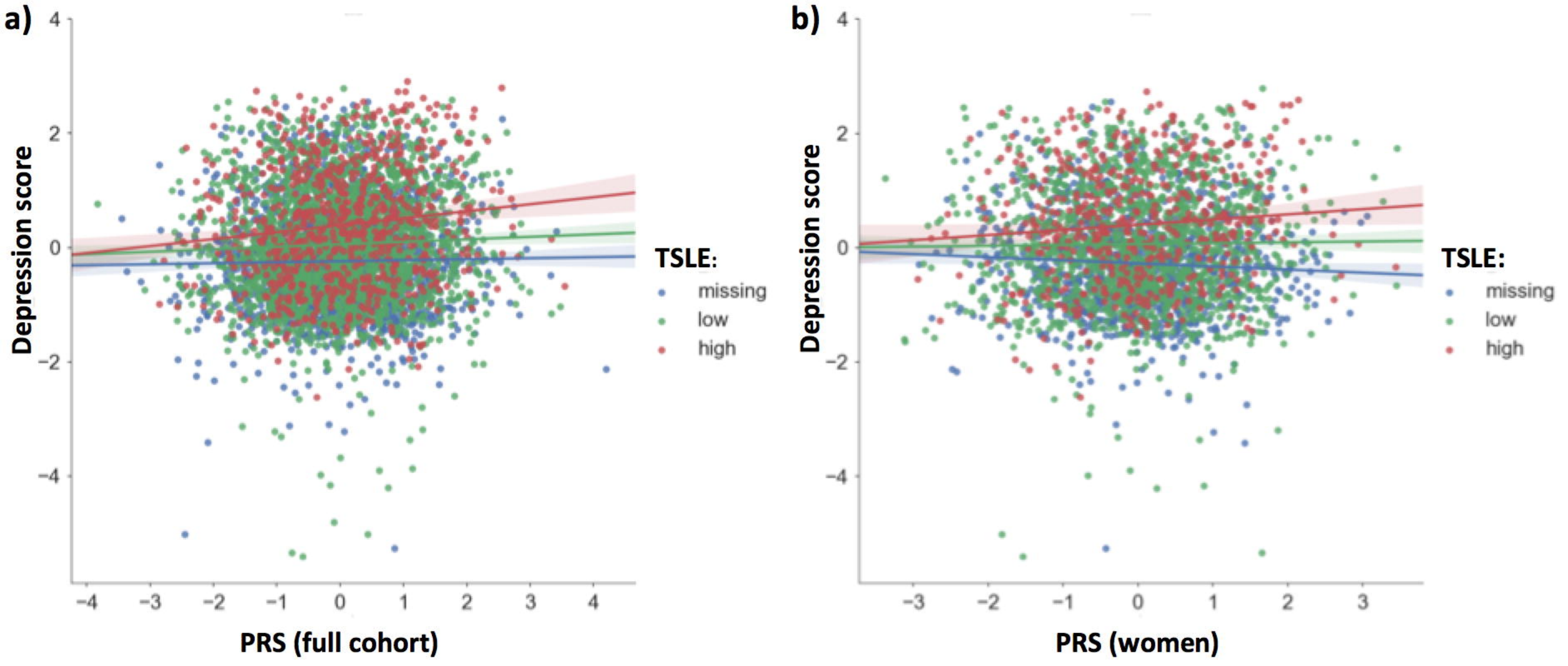
Sample	**FULL COHORT**			**WOMEN**								
diathesis	**PRS at *p* value threshold = 0.01**			**PRS at *p* value threshold = 10-5**								
SLE	**TSLE**			**TSLE**			**DSLE**			**ISLE**		
SLE category	**missing**	**low**	**high**	**missing**	**low**	**high**	**missing**	**low**	**high**	**missing**	**low**	**high**
N	1833	2311	755	1041	1459	490	2111	816	63	1393	1338	259
Effect (β)	0.021	0.043	0.142	-0.061	0.014	0.078	-0.022	0.061	-0.05	-0.044	0.027	0.079
s.e.	0.022	0.021	0.039	0.029	0.027	0.049	0.021	0.038	0.147	0.026	0.027	0.068
t	0.957	2.07	3.644	-2.086	0.541	1.609	-1.055	1.605	-0.337	-1.654	0.978	1.16
***p* value**	0.339	0.039	2.86×10^{-4}	0.037	0.589	0.108	0.292	0.109	0.738	0.098	0.328	0.247
CI (95%)	-0.022 0.065	0.002 0.084	0.065 0.218	-0.119 -0.004	-0.038 0.066	-0.017 0.174	-0.063 0.019	-0.014 0.136	-0.344 0.245	-0.095 0.008	-0.027 0.080	-0.055 0.214

SLE categories (amount of SLE experienced): 0 SLE = “missing”, 1 or 2 SLE = “low”, and 3 or more SLE = “high”. *TSLE, DSLE* and *ISLE* stand for "total", "dependent" and "independent" SLE reported.






a)
GxE interaction effect
(PRS'xTSLE')
Full cohort
Men
Women
% variance explained of depression score
0.30
0.25
0.20
0.15
0.10
0.05
0.00
p= 0.66
p= 0.1
p= 0.74
p= 4.87e-02
p= 0.15
p= 0.27
p= 0.68
p= 0.9
p= 0.67
p= 0.47
p= 0.63
p= 0.33
p= 0.15
p= 0.07
p= 0.32
p= 0.77
p= 0.22
p= 1.66e-02
p= 0.93
p= 0.06
p= 0.33
p= 0.84
p= 0.96
p= 0.78
<5e-8
<1e-5
<0.001
<0.01
<0.05
<0.1
<0.5
<1
b)
GxE interaction effect
(PRS'xDSLE')
Full cohort
Men
Women
% variance explained of depression score
p= 0.96
p= 0.16
p= 0.71
p= 0.06
p= 0.57
p= 0.64
p= 0.62
p= 0.57
p= 0.44
p= 0.64
p= 0.99
p= 0.3
p= 0.33
p= 0.28
p= 0.36
p= 0.33
p= 0.57
p= 3.85e-02
p= 0.63
p= 0.13
p= 0.93
p= 0.81
p= 0.94
p= 0.87
c)
GxE interaction effect
(PRS'xISLE')
Full cohort
Men
Women
% variance explained of depression score
p= 0.44
p= 0.16
p= 0.72
p= 0.14
p= 0.09
p= 0.28
p= 0.84
p= 0.97
p= 0.99
p= 0.55
p= 0.59
p= 0.34
p= 0.13
p= 0.05
p= 0.34
p= 0.99
p= 0.34
p= 3.32e-02
p= 0.94
p= 0.28
p= 0.31
p= 0.97
p= 0.71
p= 0.83


a)
b)
Depression score
PRS (full cohort)
PRS (women)
TSLE:
missing
low
high
4
2
0
−2
−4
−4
−3
−2
−1
0
1
2
3
4