Research paper

Increased depressive and anxiety symptoms in non-heterosexual individuals: Moderation by childhood factors using a twin design

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A B S T R A C T

Background: Evidence indicates that minority stress does not sufficiently explain mental health disparities in non-heterosexual compared to heterosexual individuals. We investigated alternative mechanisms whereby childhood factors (childhood gender nonconformity, early-life adversities and parent-child interactions) moderate the relationships between sexual orientation and depressive and anxiety symptoms.
Methods: The sample comprised twin pairs from the Finnish Genetics of Sexuality and Aggression cohort (n = 3166 individuals, mean age = 37.5 ± 2.93 years). Twin analyses using structural equation modelling was performed in OpenMx. Specifically, we tested whether childhood factors differentially moderated the underlying genetic and environmental influences on the relationships between sexual orientation, and depressive and anxiety symptoms.
Results: The associations between non-heterosexuality, and depressive and anxiety symptoms (*r* = 0.09, 0.10 respectively) were significantly influenced by both genetic and environmental factors. The genetic influences explaining the relationships of sexual orientation with depressive and anxiety symptoms were maximal at high levels of childhood gender nonconformity (β_A = 0.09 and 0.11 respectively) whereas the individual-specific environmental influences on these relationships were maximal at lower levels of childhood gender nonconformity (β_E = -0.10).
Limitations: Childhood factors were assessed retrospectively in a cross-sectional design.
Conclusions: Childhood gender nonconformity is associated with increased genetic and decreased individual-specific environmental influences on mental health among non-heterosexual individuals. Childhood gender nonconformity may, thus, enhance genetic risk and non-genetic protective processes for depressive and anxiety symptoms among non-heterosexual individuals.

1. Introduction

One out of every four individuals will experience a mental health disorder in their lifetimes, with the most common being depressive and anxiety disorders (Steel et al., 2014). Despite increasing acceptance of lesbian, gay and bisexual individuals (Russell and Fish, 2016), they remain nearly twice as likely as heterosexual individuals to report depressive and anxiety symptoms (King et al., 2008; Semlyen, et al., 2016). These disparities have been partly explained by minority stress including discrimination and internalized stigma due to non-heterosexuality (Hatzenbuehler, 2009; Meyer, 2013).

Alternatively, these mental health disparities may reflect exposure to higher levels of childhood adversities (sexual, emotional and physical abuse and neglect, and poor parent-child relationships) among non-heterosexual compared to heterosexual individuals (D'Augelli et al., 2006; Roberts et al., 2013) which can differentially increase (positively moderate) their risk for mental health problems later in life. These adverse experiences may be partly attributed to childhood gender nonconformity (i.e., children behaving in ways typical of the opposite sex) which is higher among non-heterosexual individuals (Bailey and Zucker, 1995; Li et al., 2017) and associated with both childhood adverse experiences (D'Augelli et al., 2006; Roberts et al., 2013) and later mental health problems (Oginni et al., 2019; Roberts et al., 2013). For example, Alanko et al. (2009), using a subsample of the present

dataset showed that heterosexual individuals who were gender conforming in childhood had lower levels of depressive and anxiety symptoms compared to non-heterosexual individuals who reported childhood gender nonconformity. Similarly, non-heterosexual students who had experienced more early-life adversities showed a trend towards higher odds of adverse mental health outcomes (suicidal ideation) compared to students who had no such experiences and heterosexual students who had experienced these adversities (Clements-Nolle et al., 2018). Another study found that although non-heterosexual young adults were significantly more likely to use illicit drugs compared to their heterosexual peers, this risk was significantly less among those who had closer relationships with their fathers (Magette et al., 2018). Together, these findings suggest that the correlation between sexual orientation and adverse mental health outcomes may be stronger at higher levels of these childhood factors (i.e., positive moderation); however, these relationships are phenotypic.

Considering the genetic and environmental correlations between sexual orientation and depressive/anxiety symptoms (Ganna et al., 2019; Zietsch et al., 2012), it is possible that these moderation effects manifest differently across the latent genetic and environmental influences underlying these phenotypic associations. However, to date, no studies have looked at the extents to which the phenotypic moderation of the relationship between sexual orientation and mental health problems by childhood stressors are differentially driven by moderation of the underlying genetic and environmental influences. What we do know from current research is that the genetic influences on depressive and anxiety disorders are positively moderated by both early-life adversities (Nugent et al., 2011) and poor parent-child relationships (Hayden et al., 2010) which may manifest as larger genetic variation at higher levels of the moderators in a population-based twin design (Dick, 2011).

Findings from genetically sensitive designs to dissect these moderator effects can stimulate further research into identifying specific environmental and/or genetic mechanisms for these effects. These, if known, can in turn guide intervention designs which may include identifying those at highest risk for adverse effects of minority stress as adults or stratifying risk for adverse mental health outcomes among non-heterosexual adults.

In the present twin study, we therefore investigated i. whether childhood stressors (childhood gender nonconformity, early-life adversities and dysfunctional parent-child interactions) moderate the phenotypic associations between sexual orientation and depressive/anxiety symptoms; and ii. the extents to which these observed (phenotypic) moderation effects are explained by differential moderation of the underlying genetic and environmental influences. Since these models also allow incorporation of moderator effects on the variances, we investigated iii. whether the three childhood factors moderate the genetic and environmental variance components of depressive and anxiety symptoms. Finally, we tested iv. sex differences in these effects.

Based on reports of higher levels of these childhood stressors among non-heterosexual individuals (D'Augelli et al., 2006; Roberts et al., 2013) and their associations with higher risk for mental health problems later in life (Fogelman and Canli, 2019), we hypothesized that the relationships between sexual orientation and depressive and anxiety symptoms will be stronger at higher levels of childhood gender nonconformity, early-life adversities and dysfunctional parent-child interactions. We also hypothesized that the genetic correlation between sexual orientation and depressive/anxiety symptoms will be stronger at higher levels of childhood stressors (i.e., a positive moderation). This was based on evidence indicating increased genetic susceptibility to depressive and anxiety symptoms in the presence of adverse childhood experiences (Nugent et al., 2011) which are higher among non-heterosexual individuals (D'Augelli et al., 2006; Li et al., 2017). Considering that these childhood stressors also increase susceptibility to the psychopathologic impact of other life stressors, we also hypothesized that childhood stressors will positively moderate environmental influences on the relationship between sexual orientation and

depressive/anxiety symptoms. Consistent with prior research (Bandoli et al., 2017; Nugent et al., 2011; Uher, 2014), we also hypothesized that the genetic and environmental influences on the variance in depressive and anxiety symptoms would be positively moderated by early-life adversities and poor parent-child interactions. We expected similar moderation effects of childhood gender nonconformity based on its positive associations with early-life adversities and poor parent-child interactions. Our investigation of sex differences in all analyses were exploratory.

2. Materials and methods

2.1. Sample

The present study sample comprised participants in the first wave of the Finnish Genetics of Sexuality and Aggression twin cohort who were recruited in 2005. Monozygotic and dizygotic twin pairs were identified from the Central Population Registry of Finland (see Johansson et al., 2013 for further details of recruitment). Questionnaires were mailed to 5,000 twin pairs of which 3604 individuals responded. Of these, 46 were discarded due to incomplete data, giving a final sample size of 3558. For the present analyses, 392 participants were further excluded (107 incomplete responses, and 285 with undefined zygosity - ascertained by two questions about physical resemblance as in Sarna et al., 1978). This gave a total of 3166 individuals including 867 complete twin pairs (87 monozygotic male, 105 dizygotic male, 232 monozygotic female, 255 dizygotic female and 188 opposite sex twins). Incomplete twin pairs were included in the variance component models as "singletons" to facilitate the estimation of the variances and covariances (Neale and Cardon, 1992). Ethical approval was obtained from the Ethics Committee of the Department of Psychology, Åbo Akademi, Finland and informed consent was obtained from all participants.

We note that the present dataset has previously been analysed to demonstrate a bidirectional causal relationship between childhood gender nonconformity and parent-child relationships (Alanko et al., 2011), a genetic correlation between sexual orientation and childhood gender nonconformity (Alanko et al., 2010), phenotypic moderation of the association between childhood gender nonconformity and mental health by parent-child relationships (Alanko et al., 2008), and phenotypic moderation of the relationship between sexual orientation and psychiatric symptoms by childhood gender nonconformity (Alanko et al., 2009). Of these, only the latter study overlaps with one of our objectives. However, in that study, only a subsample of the dataset was used, the moderating effects of early-life adversities and parent-child relationships were not investigated, and the genetic structure of the data was not incorporated as was done in the present analyses.

2.2. Measures

Sexual orientation was assessed by four questions from the 12-item Sell assessment of sexual orientation (Sell, 1996). The selected questions elicited same-sex sexual attraction and behavior in women as follows (the wordings were appropriately adjusted for men): "How many different women (men for male participants) have you been sexually attracted to in the past year?"; "With how many different women (men for male participants) have you engaged in sexual activity in the past year?"; "How often have you, on average, felt sexual attraction towards women (men for male participants) in the past year?"; and "How often have you, on average, engaged in sexual activity with a woman (man for male participants) in the past year?". The first two questions were scored on an 8-point scale ranging from "None" (0) to "100 or more" (7), and the other two questions were scored on a 7-point scale which ranged from "Never" (0) to "Every day" (6). The mean of the scores on all four questions was derived and used in analyses. Possible scores ranged between 0 and 6.5 with higher scores indicating higher non-heterosexuality. The Cronbach's alpha for the items in this study

was 0.89.

Depressive and anxiety symptoms. These were respectively assessed using the depression and anxiety subscales of the 18-item self-report Brief Symptom Inventory (Derogatis, 2001). Each subscale consisted of six questions each scored on a 5-point Likert scale ranging from "Not at all" (scored 0) to "Extremely" (scored 4). The scores in each subscale were summed and used in analyses with higher scores indicating higher levels of symptoms. The Cronbach's alphas for the depression and anxiety subscales in the present study were 0.84 and 0.83 respectively.

2.2.1. Moderators

Childhood gender nonconformity before the age of 12 years was retrospectively assessed using a shortened version of the Recalled Childhood Gender Identity/Gender Role Questionnaire comprising 13 items (Alanko et al., 2010; Zucker et al., 2020). Each item was rated on a 5-point Likert scale ranging from 1 to 5 with higher values indicating greater gender-nonconforming behavior. Responses that did not apply to the participants were scored 0 and were not used in the calculation of the total scores. We derived the total score by calculating the mean of the responses excluding scores of 0 and multiplying this by the total number of items, this final score was used in analyses. The Cronbach's alpha in the present study was 0.91.

Early-life adversities were defined as emotional, physical and sexual abuse and neglect before the age of 18 years (Duffy et al., 2018) and assessed using the 28-item Childhood Trauma Questionnaire (Bernstein and Fink, 1998). Each item was scored on a 5-point Likert scale ranging from 1 ("Never True") to 5 ("Very Often True"). Item responses were summed (excluding three items constituting a minimization or denial scale as recommended when scoring), and total scores used in analyses. The Cronbach's alpha in this study was 0.90 and higher scores indicated higher levels of early-life adversities.

Parent-child interactions with each parent (mothers and fathers separately) were assessed using the 15-item Measure of Parenting Styles (MOPS) questionnaire (Parker et al., 1997) which retrospectively assesses dysfunctional parent-child interactions including overprotection and low parental care (Alanko et al., 2008) before the age of 16 years. The items were worded negatively (e.g. "My father tried to induce feelings of guilt") and each item was scored on a Likert scale ranging from 1 ("Describes me very well") to 4 ("Does not describe me well at all"). Responses to individual items were summed with lower scores indicating more negative or dysfunctional parent-child interactions. Considering the significant positive correlations between ratings for paternal and maternal interactions as previously reported (Parker et al., 1997) and found in the present analyses ($r = 0.59$, $p < 0.001$); an average of the paternal and maternal interaction scores was derived which was used in analyses. Where only one parent was present, the MOPS score for this parent was used. The Cronbach's alpha for the combined scores was 0.94; and 0.92 and 0.91 for separate paternal and maternal ratings respectively.

2.2.2. Covariates

These included age in years, which was assessed using a single question; and (birth) sex which was ascertained from the Finnish Central Population Registry.

2.3. Statistical analyses

2.3.1. Data preparation and summary statistics

We carried out model-fitting analyses using OpenMx in R (Boker et al., 2011). Participants with item-missingness greater than 75% per variable were excluded from analyses. Missingness ranged between 0.4-9.0% (Supplementary Table S0) and completely at random (Little's test $\chi^2_{[618]} = 618.30$, $p = 0.49$), a requirement for multiple imputation. Multiple imputation was carried out in SPSS version 25 (IBM Corp, 2017) to maximize the data on moderation variables in complete twin pairs because missingness on these variables would lead to exclusion of

valuable data points on all variables. Expectation-maximization was specifically used because of its compatibility with maximum likelihood estimation methods (Dong and Peng, 2013). Potential confounding effects of covariates were partialled out by residualizing all the study variables. Controlling for age further helped exclude any unmeasured cohort effects. The residuals were log-transformed to ensure a normal distribution and minimize scale effects before use in subsequent analyses. For each moderation model, we further adjusted for the main effects of the moderator score (for each twin and their cotwin) on sexual orientation and depressive and anxiety symptoms (Purcell, 2002; van der Sluis et al., 2012) to prevent false positive moderation effects.

2.3.2. Structural Equation Modelling (SEM) on twin data

Phenotypic correlations between sexual orientation, depressive and anxiety symptoms and the moderator variables were preliminarily calculated using maximum likelihood estimation. Consistent with genetic model-fitting assumptions, we constrained means, and within-person variances and correlations to be equal across birth order and zygosity in this and subsequent models.

Using the classical twin design, genetic models were used to preliminarily estimate the latent genetic and environmental influences on the variances of depressive and anxiety symptoms and their covariances with sexual orientation. This design estimates the relative contributions of latent genetic (***A***) and environmental (shared – ***C*** and non-shared or individual-specific – ***E***) influences to the variances (individual differences) and covariances (unstandardized correlations) of variables by comparing the observed correlations across monozygotic and dizygotic twin pairs (Rijsdijk and Sham, 2002). This design assumes that monozygotic and dizygotic twin pairs are 100% and 50% genetically similar respectively; do not share their individual-specific environments; and when raised together, are equally influenced by their shared environment.

Phenotypic moderation analyses investigated the first objective (and tested the first hypothesis) by specifying three separate phenotypic moderation models – one each for the three moderators (early-life adversities, childhood gender nonconformity and parent-child interactions) using a trivariate Cholesky model (see genetic model in Fig. 1).

Genetic moderation analyses investigated the second and third objectives (and the corresponding hypotheses) by testing moderation of the ***A, C***, and ***E*** influences on the relationships between sexual
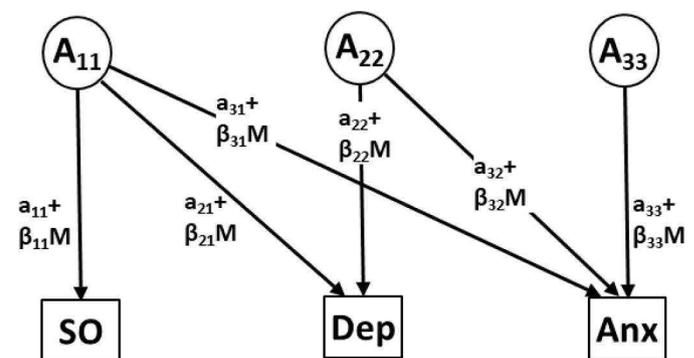

Fig. 1. Moderation of the genetic (A) influences on the variances of Depressive (Dep) and Anxiety symptoms (Anx) and their covariances with Sexual Orientation (SO) by moderator M. A_{22} and A_{33} are genetic components influencing Depressive and Anxiety symptoms respectively. a_{22} and a_{33} denote their respective path coefficients while a_{21} and a_{31} denote the genetic influences on the covariance between Sexual Orientation, and Depressive and Anxiety symptoms respectively. a_{32} denotes genetic influence on the covariance between Depressive and Anxiety symptoms. β indicates the respective moderation parameter for each path coefficient. Note: The common (C) and individual-specific (E) environment factor structures were modelled in a similar way to A but omitted from this figure for clarity.

orientation and depressive and anxiety symptoms (objective 2) and the variances of depressive and anxiety symptoms (objective 3) by adapting the bivariate moderation model described by Purcell (2002) (Fig. 1). As with phenotypic moderation analyses, we fitted a trivariate moderation model for each moderator separately.

Significance of the moderation coefficients was determined using 95% maximum likelihood confidence intervals with intervals containing zero indicating statistical non-significance. The unstandardized variances were plotted as functions of the values of the moderator (mean ± 1-2 standard deviations) to visualize moderation effects.

2.3.3. Sex Differences

We investigated sex differences at all levels of analyses (fourth objective) by comparing models in which estimates were freed to differ by sex against models in which estimates were constrained to be equal in males and females. Models were compared using Chi-squared tests and the results reported in the supplementary material.

3. Results

3.1. Preliminary results

3.1.1. Descriptive Statistics

The mean age of the sample was 37.5 (± 2.93) years (Supplementary Table S1). The mean scores for childhood gender nonconformity, early-life adversity and parent-child interaction were 26.4 (± 8.13; Range: 12.3-60.0), 37.4 (± 12.23; Range: 24.8-110.0) and 49.1 (± 6.96; Range: 20.0-60.0) respectively. The median sexual orientation score was 0.1 (Range: 0-6). Majority (93.2%) of the participants had a sexual orientation score of 0 (no same-sex sexuality) while progressively fewer proportions had increasing scores (Supplementary Table S2). The median scores for depressive and anxiety symptoms were 3.9 (Range: 0-24) and 2.9 (Range 0-24) respectively.

3.1.2. Phenotypic correlations

There were significant positive correlations between sexual orientation, and depressive and anxiety symptoms (r = 0.09 and 0.10 respectively, 95% CIs: 0.05-0.14; Table 1) such that higher non-heterosexuality scores were associated with higher depressive and anxiety symptom scores. Similarly, the correlations with moderator

Table 1
Within-person and cross-twin correlations of sexual orientation, depressive and anxiety symptoms with 95% confidence intervals per zygosity group.

	SO (1)	Dep (2)	Anx (3)
Within person			
(1)	1		
(2)	0.10	1	
	(0.07, 0.14)		
(3)	0.09	0.66	1
	(0.05, 0.12)	(0.64, 0.68)	
Cross-twin			
Monozygotic			
(1)	0.54		
	(0.45, 0.60)		
(2)	0.07	0.34	
	(0.01, 0.13)	(0.24, 0.42)	
(3)	0.10	0.29	0.34
	(0.04, 0.16)	(0.21, 0.36)	(0.25, 0.43)
Dizygotic			
(1)	0.07		
	(-0.02, 0.15)		
(2)	0.03	0.12	
	(-0.03, 0.09)	(0.04, 0.20)	
(3)	0.08	0.12	0.19
	(0.02, 0.14)	(0.04, 0.18)	(0.11, 0.27)

SO: Sexual orientation, Dep: Depressive symptoms, Anx: Anxiety

variables were all statistically significant (Supplementary Table S3). Those with childhood gender nonconformity and early-life adversity were positive (r: 0.05 to 0.30, 95% CIs: 0.02-0.34) while those with parent-child interactions were negative (r: -0.26 to -0.09, 95% CIs: -0.31–0.06). Thus, non-heterosexuality, and depressive and anxiety symptom scores were significantly associated with higher childhood gender nonconformity and early-life adversities, and more dysfunctional parent-child relationships. Similarity of twins for sexual orientation and depressive and anxiety symptoms is reflected by the monozygotic and dizygotic cross-twin correlations.

3.1.3. Genetic and environmental influences on covariances and variances

Depressive and anxiety symptom scores were significantly influenced by genetic (A) factors which explained 32% and 25% of their variances respectively (95% CIs: 0.16-0.39 and 0.07-0.40 respectively; Table 2) while individual-specific environmental (E) factors explained 68% and 67% of their respective variances (95% CIs: 0.61-0.77, 0.59-0.75 respectively). The covariances of sexual orientation with both depressive and anxiety symptoms were mostly due to genetic influences (73% and 100% respectively), however, these were not statistically significant (95% CIs: -0.01-1.41 and 0.00-2.38 respectively). C influences on these relationships were mostly estimated to be null and were excluded from the genetic models without significant loss in fit ($\chi^2_{[6]}$ = 2.71, p = 0.84); hence, subsequent moderation analyses were based on A and E influences only.

3.2. Moderation

3.2.1. Phenotypic relationships between sexual orientation, and depressive and anxiety symptoms, and their genetic and environmental components

The phenotypic relationship between sexual orientation and depressive symptoms was not significantly moderated by childhood gender nonconformity, early-life adversities and parent-child interactions (Table 3, Fig. 2). However, further genetic analyses indicated significant moderation of the genetic influences on this relationship whereby genetic influences on this relationship were lowest at low (-2SD) to medium (Mean) levels of childhood gender nonconformity and highest (2SD) at the highest levels of childhood gender nonconformity (β_A = 0.11, 95% CI: 0.04-0.17). In contrast, moderation of individual-specific environmental influences on this relationship was not statistically significant.

None of the phenotypic relationships between sexual orientation, and depressive and anxiety symptoms were significantly moderated by the childhood factors. Only childhood gender nonconformity significantly moderated the genetic and environmental influences on this relationship (β_A = 0.09, 95% CI: 0.03, 0.15; β_E = -0.10, 95% CI: -0.16–0.03). Specifically, genetic influences on this relationship were largest and smallest at higher (2SD) and lower (-2SD) levels of childhood gender nonconformity respectively (Fig. 2, Table 4). In contrast, the individual-specific environmental influences on the relationship between sexual orientation and anxiety symptoms followed a U-shaped curve (decreasing and then increasing) as childhood gender nonconformity increased.

3.2.2. Genetic and environmental influences on variances of depressive and anxiety symptoms

The genetic influences on the variances of depressive and anxiety symptoms were maximal at higher levels (2SD) of childhood gender nonconformity and lowest at lower levels (-2SD) of childhood gender nonconformity (Fig. 2) and this moderation effects were statistically significant (β_A = 0.21 and -0.24; 95% CIs: 0.05-0.31 and -0.29- -0.17 respectively; Table 4). Similar moderation effects were observed for early-life adversities on the genetic influences on depressive symptoms (β_A = -0.27; 95% CIs: -0.31- -0.21; Table 4) and non-shared environmental influences on both depressive and anxiety symptoms (β_E = 0.07 and 0.09; 95% CIs: 0.03-0.08 and 0.06-0.09 respectively; Table 4). Non-

Table 2

Standardized ACE variance-covariance components of sexual orientation, depressive and anxiety symptoms with 95% confidence intervals.

Component	A[a]			C[a]			E[a]		
	SO	Dep	Anx	SO	Dep	Anx	SO	Dep	
SO	**0.45**			0.00			**0.55**		
	(0.34, 0.53)			(0.00, 0.05)			**(0.47, 0.64)**		
Dep	0.73	**0.32**		0.00	0.00		0.27	**0.68**	
	(-0.01, 1.41)	**(0.16, 0.39)**		(-0.33, 0.41)	(0.00, 0.11)		(-0.33, 0.89)	**(0.61, 0.77)**	
Anx	1.16	**0.42**	**0.25**	0.21	0.00	0.09	-0.37	**0.58**	**0.67**
	(0.00, 2.38)	**(0.19, 0.54)**	**(0.07, 0.40)**	(-0.42, 1.02)	(-0.05, 0.17)	(0.00, 0.22)	(-1.31, 0.31)	**(0.48, 0.69)**	**(0.59, 0.75)**

Note: SO - Sexual Orientation, Dep - Depressive symptoms and Anx – Anxiety, A, C and E: Additive genetic effects, Shared environmental effects and Individual-specific environmental effects respectively.

Model fit indices: -2LL = 28742.95, degrees of freedom = 9477, AIC = 9788.95.

Model fit indices when C parameters are dropped: -2LL = 28745.67, degrees of freedom = 9483, AIC = 9779.67, model comparison: $\Delta\chi^2[6] = 2.71$, $p = 0.84$

[a] Diagonals of each matrix represent the proportion of variance for each component, while the off-diagonals represent the proportion of covariance.

Table 3

Unstandardized moderation effects on the phenotypic variance-covariances of sexual orientation, depressive and anxiety symptoms with 95% confidence intervals.

Moderator	SO-Dep covariance	SO-Anx covariance	Dep variance	Anx variance	-2LL (df)
	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
CGN[a]	-0.02 (-0.04, 0.01)	-0.02 (-0.04, 0.01)	**0.09 (0.06, 0.11)**	**0.05 (0.04, 0.07)**	23144.07 (9474)
ELA[b]	0.02 (0.00, 0.05)	0.02 (-0.01, 0.02)	**0.12 (0.12, 0.12)**	**0.10 (0.09, 0.10)**	20911.88 (9474)
PCI[c]	-0.06 (-0.15, 0.03)	-0.04 (-0.13, 0.06)	**-0.53 (-0.59, -0.52)**	**0.43 (0.38, 0.48)**	24300.06 (9474)

Note: SO - Sexual Orientation, Dep - Depressive symptoms and Anx – Anxiety, CGN – Childhood Gender Nonconformity, ELA – Early-Life Adversity, PCI – Parent-Child Interaction, -2LL - -2 Log Likelihood, df – degrees of freedom.

[a] Model with CGN as moderator

[b] Model with ELA as moderator

[c] Model with PCI as moderator

shared environmental influences on depressive and anxiety symptoms were significantly moderated by parent-child interactions, being maximal at low scores (i.e., more dysfunctional interactions; $\beta_E = -0.29$ and -0.43; 95% CIs: -0.46- -0.15 and -0.54- -0.43 respectively); and the genetic influences on depressive symptoms were similarly moderated ($\beta_A = -0.54$; 95% CI: -0.75- -0.25).

An incidental finding was the significant moderation of the phenotypic relationship between depressive and anxiety symptoms. This relationship was strongest at the highest levels of childhood gender nonconformity and early-life adversities ($\beta = 0.05$ and 0.10 respectively, 95% CI: 0.04-0.10) and more dysfunctional parent-child interactions ($\beta = -0.43$, 95% CI: -0.49- -0.43). However, only early-life adversities and dysfunctional parent-child interactions significantly moderated the genetic influences on this relationship ($\beta_A = -0.26$ and -0.59 respectively, 95% CIs: -0.85- -0.21).

3.3. Sex differences

The distributions of sexual orientation, depressive and anxiety symptoms scores were comparable in male and female participants (Wilcoxon's rank sum coefficient = -0.27, 0.03 and 0.75 respectively; $p > 0.05$; Supplementary Table S2). The phenotypic correlations between sexual orientation, and depressive and anxiety symptoms were comparable across sexes and were consistent with the main results (Supplementary Table S4). There were significant sex differences in the

etiological influences on the correlations ($\chi^2_{[18]} = 144.68$, $p < 0.001$) with **C** and **A** influences being respectively larger in male and female participants (Supplementary Tables S5). Moderation of the genetic and environmental influences on depressive and anxiety symptoms and their relationships with sexual orientation by all three moderators were comparable in men and women (Supplementary Table S7).

4. Discussion

This study utilized a population-based twin design to demonstrate positive correlations between non-heterosexuality and depressive and anxiety symptoms. The phenotypic moderation effects of all childhood factors were small. However, childhood gender nonconformity further moderated the genetic and individual-specific environmental influences on these relationships – positively and negatively respectively. All three childhood factors had significant moderation effects on the genetic and environmental variance components of depressive and anxiety symptoms to varying extents, and all moderation effects were comparable in male and female participants.

Consistent with the literature, higher non-heterosexuality scores were phenotypically associated with higher depressive and anxiety symptoms (King et al., 2008; Plöderl and Tremblay, 2015; Semlyen et al., 2016). There were significant genetic and individual-specific environmental influences on depressive and anxiety symptoms (Kendler et al., 2018; Legrand et al., 1999). The genetic and environmental influences on their relationships with sexual orientation, though non-significant, were comparable to earlier reports (Zietsch et al., 2012). In line with previous research, the moderators were significantly associated with higher non-heterosexuality scores (Busseri et al., 2008; Xu et al., 2020), possibly reflecting indirect effects of childhood gender nonconformity (D'Augelli et al., 2006; Landolt et al., 2004). The moderators' associations with increased depressive and anxiety symptoms (Alanko et al., 2008; 2009; Hannigan et al., 2018; Hughes et al., 2017; Roberts et al., 2013) may reflect early manifestations of minority stress (D'Augelli et al., 2006) and stress-related epigenetic processes (Heim and Binder, 2012).

4.1. Moderation of the relationships of sexual orientation with depressive and anxiety symptoms (and of their genetic and environmental components)

The phenotypic relationships between sexual orientation, and depressive and anxiety symptoms were generally stronger at higher levels of childhood gender nonconformity, early-life adversities and dysfunctional parent-child interactions but these effects were all small and not statistically significant. These small and non-significant effects are consistent with previous negative findings from an analysis of a subset of the present sample (Alanko et al., 2009) and a different study (Roberts et al., 2013). It is also possible that there are true but small
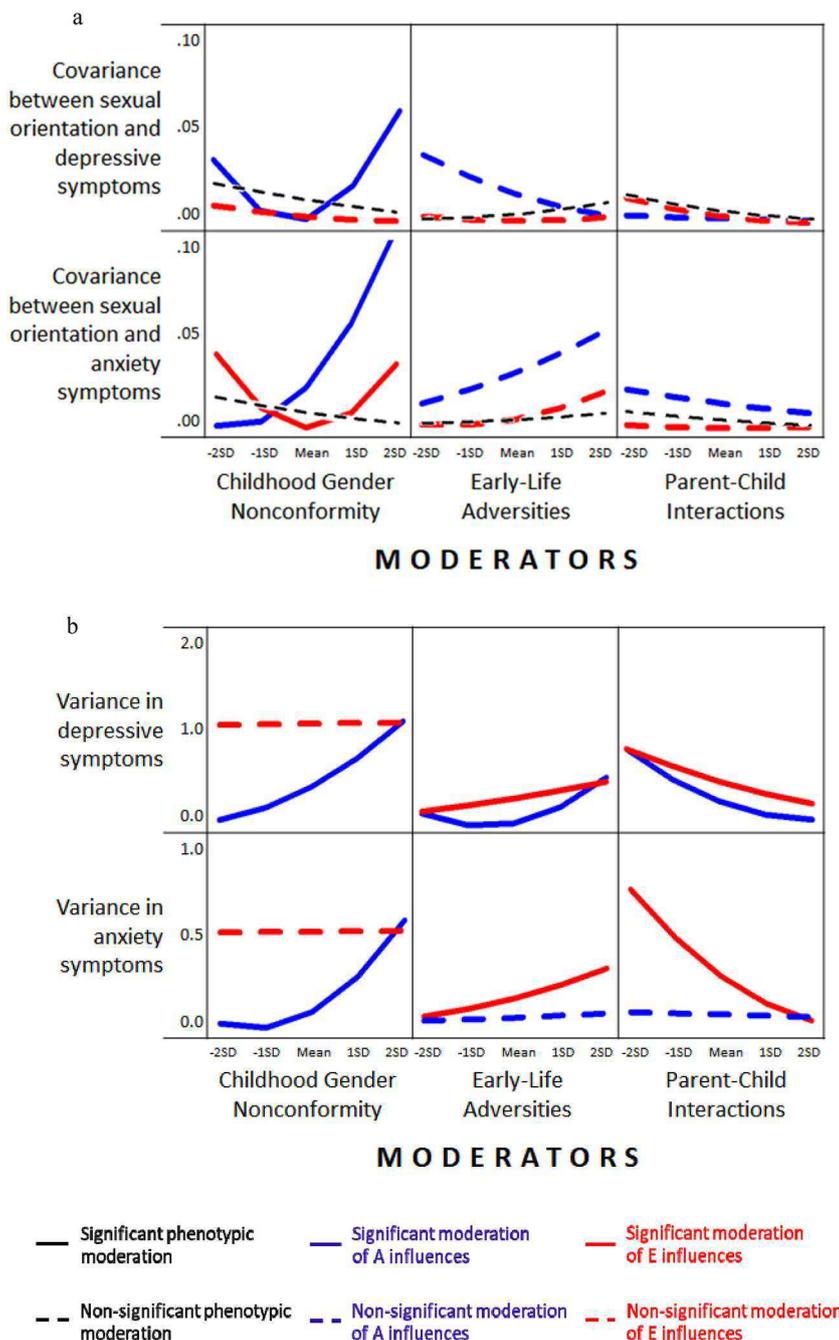
Fig. 2. Moderation of the relationships between sexual orientation and depressive and anxiety symptoms including genetic and individual-specific environmental influences on these relationships (2a) and the genetic and individual-specific environmental influences on depressive and anxiety symptoms (2b). Changes in the covariances and the A and E influences on these (*y-axis*) are plotted against values for each moderator ranging from -2SD (standard deviations) through the mean to 2SD (*x-axis*). A and E = Additive genetic and Individual-specific environmental influences respectively.

phenotypic moderation effects which the present study was not powered to detect.

Our findings, however, extend previous research by demonstrating that the small phenotypic moderation effect of childhood gender nonconformity on the relationship between higher non-heterosexuality scores and greater depressive and anxiety symptoms may be due to opposing moderation of genetic and environmental influences on these relationships. The stronger genetic influences on the associations between sexual orientation, and depressive and anxiety symptoms at higher levels of childhood gender nonconformity suggest that among non-heterosexual individuals, genetic risk for depressive and anxiety symptoms manifest more strongly among those who were highly gender-nonconforming in childhood. In contrast, the negative moderation of individual-specific environmental influences by childhood gender nonconformity may indicate compensatory psychological processes which attenuate the association between sexual orientation and mental

health problems. For example, despite experiencing greater discrimination, which was associated with higher depressive symptoms; gender nonconforming non-heterosexual South African men had comparable levels of depressive symptoms to non-heterosexual men who were gender conforming (Cook et al., 2013). This protective effect was explained by greater self-acceptance among the gender nonconforming men (Sandfort et al., 2016). Although this effect was demonstrated for adult gender conformity, the continuity between childhood and adult gender nonconformity (Lippa, 2008) suggests the possibility of a similar effect for childhood gender nonconformity, however, this needs to be specifically tested. As moderation of individual-specific influences followed a U-shaped curve, it is possible that these protective processes (indicated by the initial negative-sloped portion of the curve) are more prominent at lower levels of childhood gender nonconformity, while the subsequent positive-sloped portion of the curve indicates that risk processes predominate at higher levels of childhood gender nonconformity

Table 4
Unstandardized moderation effects on the AE variance-covariances of sexual orientation, depressive and anxiety symptoms with 95% confidence intervals.

Moderator		SO-Dep covariance	SO-Anx covariance	Dep variance	Anx variance	-2LL (df)
		Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	
CGN[a]	β_A	**0.11** **(0.04, 0.17)**	**0.09** **(0.03, 0.15)**	**0.21** **(0.05, 0.31)**	**-0.24** **(-0.29, -0.17)**	22478.35 (9468)
	β_E	-0.02 (-0.11, 0.07)	**-0.10** **(-0.16, -0.03)**	0.00 (-0.04, 0.07)	0.00 (-0.04, 0.05)	
ELA[b]	β_A	-0.03 (-0.11, 0.07)	0.03 (-0.04, 0.13)	**-0.27** **(-0.31, -0.21)**	-0.02 (-0.09, 0.04)	20859.42 (9468)
	β_E	0.02 (-0.05, 0.08)	-0.04 (-0.10, 0.02)	**0.07** **(0.03, 0.08)**	**0.09** **(0.06, 0.09)**	
PCI[c]	β_A	-0.02 (-0. 27, 0.22)	-0.04 (-0.33, 0.25)	**-0.54** **(-0.75, -0.26)**	-0.03 (-0.31, 0.25)	24295.00 (9468)
	β_E	-0.07 (-0.26, 0.14)	0.04 (-0.20, 0.27)	**-0.29** **(-0.46, -0.15)**	**-0.43** **(-0.54, -0.43)**	

Note: SO - Sexual Orientation, Dep - Depressive symptoms and Anx – Anxiety, CGN – Childhood Gender Nonconformity, ELA – Early-Life Adversity, PCI – Parent-Child Interaction, -2LL - -2 Log Likelihood, df – degrees of freedom, β_A, β_E: Moderation coefficient for additive genetic and individual-specific environmental components respectively. Bold font indicates significant coefficients.
[a] Model with CGN as moderator
[b] Model with ELA as moderator
[c] Model with PCI as moderator.

(D'Augelli et al., 2006; Thoma et al., 2021).

In contrast, the non-significant moderation of the genetic and environmental influences by early-life adversity and dysfunctional parent-child interactions may reflect low power.

4.2. Moderation of the genetic and environmental influences on variance in depressive and anxiety symptoms

The positive moderation of the genetic influences on depressive and anxiety symptoms by early-life adversities and dysfunctional parent-child interactions found in the present study is consistent with stress-diathesis gene-environment interactions demonstrated in previous research (Hayden et al., 2010; Nugent et al., 2011) whereby stressful experiences increase the likelihood of adverse mental health outcomes by increasing sensitivity to genetic influences (Manuck and McCaffery, 2014). We also demonstrate for the first time that gender nonconformity in childhood may be considered an adequate stressor which can increase sensitivity to genetic influences on depressive and anxiety disorders.

In line with previous research, the present study also found that early-life adversities and dysfunctional parent-child interactions were associated with increased individual-specific environmental influences on depression and anxiety symptoms (Fogelman and Canli, 2019). This may reflect epigenetic processes through which neurobiological stress-regulatory processes become more vulnerable to the adverse impacts of later stress (Fogelman and Canli, 2019), however, this effect was not demonstrated for childhood gender nonconformity.

The lower genetic correlation between depressive and anxiety symptoms in the present study (e.g., compared to Kendler et al., 1992; Taporoski et al., 2015) may reflect the effect of incorporating a third variable (i.e., sexual orientation). However, the moderation of the genetic influences on this relationship by early-life adversities and dysfunctional parent-child interactions is consistent with the stress-diathesis framework.

4.3. Sex differences

The larger shared environmental influences on the relationships between sexual orientation, and depressive and anxiety symptoms in male participants may suggest that non-heterosexual men may be more negatively affected by environmental consequences of their sexuality (D'Augelli et al., 2006). However, moderation of the genetic and environmental influences on the relationships between sexual orientation and depressive and anxiety symptoms did not appear to differ by sex.

4.4. Implications

Our study shows for the first time, that similar to early-life adversities and dysfunctional parent-child relationships, childhood gender nonconformity is associated with increased genetic influences on adult depressive and anxiety symptoms in the general population. Our findings further suggest that childhood gender nonconformity may specifically amplify genetic risk for adverse mental health outcomes in non-heterosexual individuals but simultaneously activate individual-specific protective mechanisms. This indicates the need for further research to understand possible risk and protective processes in these relationships; and the need to support gender nonconforming children to reduce the risk of mental health difficulties among both heterosexual and non-heterosexual adults.

While our findings suggest that early-life adversities and dysfunctional parent-child relationships do not substantially increase the risk for adverse mental health outcomes in non-heterosexual individuals, larger future studies are needed to further investigate these relationships.

4.5. Strengths and Limitations

The strengths of our study include the innovative use of a genetically sensitive design and the assessment of study variables using standardized questionnaires with good psychometric properties. However, our findings need to be interpreted in the light of the following limitations. Firstly, the retrospective assessments of childhood gender nonconformity, early-life adversities and parent-child interactions may introduce recall bias. Similarly, the higher levels of depressive and anxiety symptoms among non-heterosexual individuals may make them prone to recall negative autobiographical events (Matt et al., 1992) such as the adverse childhood experiences assessed in the present study. However, findings from prospective designs indicate that children who went on to identify as non-heterosexual adults were more gender nonconforming (Li et al., 2017), experienced more childhood adversities (Xu et al., 2020) and had poorer relationships with their parents (Xu et al., 2019). Hence, the impact of recall bias is likely to be negligible; however, data from longitudinal twin cohorts with childhood assessments of the moderator variables would facilitate more accurate estimates of moderator effects.

Secondly, the population from which the sample was recruited was mostly Caucasian. Hence our findings may not generalize to non-Western cultures where the associations between adult mental health and the childhood factors investigated in the present study may be

different (Cook et al., 2013; Oladeji et al., 2010). Future studies should ensure greater socio-demographic diversity and utilize non-Western samples which may allow for investigating the impacts of socio-cultural differences. Larger samples would also overcome the possibility of type 2 errors, especially for effects with small magnitude such as the phenotypic moderation effects investigated in the present study. Also, as the estimates of genetic and environmental influences on variance from the twin design are time- and population-specific (Neale and Cardon, 1992), it is important to replicate our findings in different populations. Relatedly, the estimation of genetic and environmental influences as latent constructs indicates the need for further research to identify specific genetic and environmental processes.

5. Conclusion

The present study demonstrated that early-life adversities and dysfunctional parent-child interactions increase the likelihood of adverse mental health outcomes by increasing genetic and environmental influences on these adverse outcomes in the general population. We extended this finding by showing that childhood gender nonconformity may similarly increase the likelihood of adverse mental health outcomes by increasing sensitivity to genetic influences on these mental health outcomes in the general population and among non-heterosexual individuals. In contrast, childhood gender nonconformity may also attenuate the relationship between non-heterosexuality and adverse mental health outcomes – possibly by activating protective mechanisms but this needs to be further investigated.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jad.2021.10.095.

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