Polygenic phenotypic plasticity moderates the effects of severe childhood abuse on depressive symptom severity in adulthood: A 5-year prospective cohort study

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ABSTRACT

Objective To test the phenotypic plasticity framework using a polygenic approach in a prospective depression cohort of primary care attendees with and without histories of severe childhood abuse. Methods Depressive symptoms were assessed at baseline and annually for 5 years post-baseline using the Primary Care Evaluation of Mental Disorders Patient Health Questionnaire-9 (PHQ-9) among 288 adult primary care attendees. Twelve polymorphisms in nine genes were genotyped and polygenic phenotypic plasticity allelic load (PAL) calculated. Linear mixed models assessed differences in depressive symptom severity over the 5-year follow-up period by PAL and history of severe childhood abuse. Results A higher PAL conferred greater depressive symptom severity among those with a history of severe childhood abuse but conferred significantly lower symptom severity among those without this history. Importantly, this interaction withstood adjustments for important covariates (e.g., antidepressant use, comorbid anxiety) and was stable over the 5 years of observation. Conclusions Aligned with the phenotypic plasticity framework, depressive symptom severity was dependent on the interaction between PAL and history of severe childhood abuse in a “for better and for worse” manner. Measures of polygenic phenotypic plasticity, such as ours, may serve as a trait marker of sensitivity to negative and potentially positive environmental influences.

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Introduction

Abusive experiences in childhood are well-established predictors of depressive phenotypes in adulthood yet variation in phenotypic expression, such as symptom severity, among those with a history of abusive experiences is not uncommon. In fact, some individuals appear to be “vulnerable” while others are quite “resilient” to the effects of abuse. This dichotomy is the foundation of the diathesis-stress framework (Monroe and Simons 1991) that postulates an individual’s vulnerability is dependent on the biological context (e.g., genotype) in which adversity (e.g., abuse) is encountered. However, this traditional thinking has been challenged by the differential susceptibility framework (Belsky 1997) which, suggests an individual’s biological context moderates sensitivity to both negative and positive environmental influences and views traditionally labelled “vulnerable” individuals as “plastic/malleable” individuals.

In the past decade, a number of studies have provided support for the differential susceptibility framework by demonstrating traditional “risk” alleles in several polymorphisms could be better defined as “phenotypic plasticity” alleles in that they predict outcomes in a “for better and for worse” manner depending on the nature (i.e., positive or negative) of the environmental influence (Belsky et al. 2009). However, few studies have used a polygenic approach and none have examined the stability of this framework over time (see reviews Belsky and Pluess 2009, 2013). As such, we sought to test the differential susceptibility framework using a polygenic approach in a longitudinal depression cohort of primary care attendees with and without histories of severe childhood abuse. We hypothesised that a higher phenotypic plasticity allelic load (PAL) would confer greater depressive symptom severity among those with a history of severe childhood abuse but significantly lower symptom severity among those without this history.
Material and methods

Study population

Participants were enrolled in the Diagnosis, Management and Outcomes of Depression in Primary Care (diamond) study, an ongoing prospective cohort that commenced in 2005 (Gunn et al. 2008). Details of the methods have been published elsewhere (Gunn et al. 2008, 2013; Potiriadis et al. 2008). Briefly, primary care patients were recruited from 30 general practice sites and were eligible for the diamond cohort if they were: (1) aged 18–75 years, (2) able to read English, (3) not terminally ill, (4) did not reside in a nursing home, and (5) scored 16 or higher on the Centre for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977). Participants were assessed annually using postal surveys as well as computer-assisted telephone interviews. In 2011 (cohort year 6), participants enrolled in the cohort were invited to provide a saliva sample for DNA extraction and genotyping.

Depressive symptom measures

Depressive symptoms were assessed at baseline and annually for 5 years post-baseline using the self-administered Primary Care Evaluation of Mental Disorders Patient Health Questionnaire-9 (PHQ-9; Kroenke et al. 2001). The PHQ-9 is based directly on the nine signs and symptoms of major depressive disorder (MDD) as described in the DSM-IV (American Psychiatric Association 2000) and has been validated to screen and monitor depression severity in the primary care setting (Spitzer et al. 1999). The PHQ-9 asks respondents to rate their symptoms over the past 2 weeks and is scored on a scale of zero (“not at all”) to three (“nearly every day”) for each item with a range of zero to 27 (Kroenke et al. 2001). Scores of 5, 10, 15 and 20 on the PHQ-9 represent cut-points for mild, moderate, moderately severe, and severe depression, respectively (Kroenke et al. 2001). DSM-IV criteria (American Psychiatric Association 1994) for MDD was assessed using the Composite International Diagnostic Interview (CIDI) Auto version 2.1 (WHO 1997) by a trained research assistant.

Childhood abuse

Exposure to physical and sexual childhood abuse was measured using the Child Maltreatment History Self-Report (CMHSR; MacMillan et al. 1997). The CMHSR consists of 11 items, seven pertaining to physical abuse and four to sexual abuse. The physical abuse questions have a four-point response option (never, rarely, sometimes, often), whereas the sexual abuse questions have a yes/no response option. Severe abuse was defined using the scoring algorithm previously described (MacMillan et al. 1997).

Phenotypic plasticity allelic load

DNA was recovered from stabilised saliva samples. Twelve polymorphisms in nine genes along with 60 unlinked ancestry informative markers (AIMs; Supplementary Table S1 available online) were genotyped with the Sequenom MassARRAY MALDI-TOF genotyping system (Sequenom Inc., San Diego, CA) as described in detail elsewhere (Bousman et al. 2013). In addition, the 5HTTLPR and its A-to-G internal SNP (rs25531) were genotyped by capillary electrophoresis using an AB3730 Genetic Analyser fitted with a 36-cm array, with sizing determined against a Genescan LIZ500 molecular weight marker. Individuals were categorised as s/s if they were s/s, s/I_G or I_G/I_G. Those who had s/I_A or I_A/I_A were categorised as s/I and individuals with I_A/I_A were categorised as I/I.

The genes and polymorphisms were selected a priori based on evidence of interaction with life events in previous depression studies (Mandelli and Serretti 2013) and in part represent serotoninergic, dopaminergic, noradrenergic, glutamatergic, and/or GABAergic pathways, all of which have been implicated in the pathophysiology of depression. For the SLC6A2 and CNR1 genes two polymorphisms were selected but r-squared values were less than 0.35, suggesting weak linkage disequilibrium. For each of the 12 selected polymorphisms, a phenotypic plasticity allele was designated based on previous literature and the total number of phenotypic plasticity alleles present (valid range 0–24) for each individual was calculated to derive a phenotypic PAL (Table 1).

Potential confounding variables

At baseline, potential confounding factors measured were age, sex, education, smoking status, and self-rated health status (Ware et al. 1996). Panic and other anxiety syndromes were also assessed using the anxiety module of the PHQ (Spitzer et al. 1999). Alcohol and drug abuse/dependence (i.e., cannabis, opioid, sedative, cocaine, amphetamine, hallucinogens, inhalants) was measured using the CIDI Auto version 2.1 (WHO 1997). The current use of antidepressants, anxiolytics, and antipsychotics, as well as self-reported visits in the past 12 months to a psychiatrist and/or psychologist were also assessed.
Statistical analysis

PLINK (Purcell et al. 2007) was used to detect departures from Hardy–Weinberg equilibrium and determine minor allele frequency. To estimate the presence of population stratification, the 60 AIMs were used to assign each participant to the HapMap ancestral group (Northern/Western European, Han Chinese and Yoruba in Nigeria) for which they carried the greatest proportion of that population’s AIMs.

Repeated measures linear mixed models were used to determine differences in PHQ-9 depressive symptom severity over the 5-year follow-up period by PAL and history of severe childhood abuse. The mixed models approach enables use of all repeated measurements, accounts for clustering of participants within general practise sites, and provides unbiased estimates in the presence of missing data. Prior to building our model, PAL and history of severe childhood abuse as well as covariates were centred (Kraemer and Blasey 2004). Unadjusted models included fixed effects for time, PAL, and abuse as well as time × PAL, time × abuse, PAL × abuse, and time × PAL × abuse interaction terms. Random effects included individual, general practise site, intercept (baseline PHQ-9) and slope (time). Covariance structures used for the random and repeated effects were unstructured and first-order autoregressive, respectively. Missing data of longitudinal measurements were assumed missing at random.

Adjusted models were fitted to account for any bias from baseline characteristics or potential confounders. Adjusted models included all terms in the unadjusted model along with relevant covariates as well as covariate × time, covariate × PAL, and covariate × abuse interaction terms as recently recommended (Keller 2014). To improve the fit of our adjusted model, a backward stepwise penalised likelihood model selection strategy was employed. This process involved starting with the most complex model and removing the term with the largest $P$ value above 0.05 using unrestricted maximum likelihood estimations. If removal of a covariate term increased the Bayesian information criterion compared to previous more complex model the term was retained. When no further terms in the model could be removed, restricted maximum likelihood estimation was used to calculate the final reduced covariate-adjusted model as this method produces unbiased estimates of covariance parameters. Finally, a leave-one out sensitivity analysis was conducted in which each polymorphism was excluded from the PAL calculation to determine if any of the polymorphisms were preferentially affecting results. All analyses were performed using SPSS 21.0 (IBM, Armonk, NY).

Ethics

All procedures were conducted in accord with principles expressed in the Declaration of Helsinki and obtained approval from the University of Melbourne Human Research Ethics Committee (Ethics ID 1135247.1).

Results

A total of 789 participants were recruited into the diamond cohort, of whom 498 were enrolled at the time of DNA collection (commencement of 6-year follow-up) and 344 (69%) consented and returned a DNA sample. We excluded from the present analysis, 35 participants who were missing genotype data for one or more of the selected polymorphisms as well as an additional 21 participants missing abuse information. All participants were of Northern/Western European (CEU) ancestry based on 60 unlinked AIMs. A total sample of 288 participants were included in the analysis (Table 2).

The distribution and means of PAL in the full sample and by history of childhood abuse did not differ (distribution: Mann–Whitney U-test = –0.615, $P = 0.538$; means: $t_{286} = 0.430$, $P = 0.667$; Figure 1). Linear mixed model analysis revealed a two-way interaction between PAL and history of severe childhood abuse that withstood covariate adjustment ($P_{\text{adjusted}} = 0.041$; Table 3). For every additional phenotypic plasticity allele carried, individuals with a history of childhood abuse reported

| Gene symbol | Gene name | Polymorphism | Plasticity allele | Current study allele frequency |
|-------------|-----------|--------------|-------------------|-----------------------------|
| BDNF | Brain-derived neurotrophic factor | rs6265 (Val66Met) | Met | 0.17 |
| SLC6A4 | Serotonin transporter | 5-HTTLPR | Short | 0.50 |
| HTR1A | Serotonin receptor 1A | rs878567 | C | 0.51 |
| HTR2A | Serotonin receptor 2A | rs6313 | T | 0.43 |
| HTR3A | Serotonin receptor 3A | rs1062613 | C | 0.78 |
| SLC6A2 | Noradrenaline transporter | rs2242446 | C | 0.28 |
| CNR1 | Cannabinoid receptor 1 | rs7766029 | T | 0.45 |
| GSK3B | Glycogen synthase kinase 3 beta | rs7682799 | C | 0.63 |
| IL18 | Interleukin-18 | rs1946518 | G | 0.61 |
| FKBPS | FKS06 binding protein S | rs1360780 | T | 0.30 |
a 0.54 (SE = 0.25) point increase in PHQ-9 depressive symptom severity while for those with no history there was a 0.57 (SE = 0.08) decrease. The three-way interaction between PAL, history of severe childhood abuse and time was not significant (P = 0.568), suggesting the two-way interaction effect was stable over the 5-year follow-up period (Figure 2). Importantly, sensitivity analysis suggested that removal of any one of the 12 polymorphisms from the PAL had no significant effect on the two-way interaction effect we observed.

Discussion

Aligned with our hypothesis and differential susceptibility thinking, we have preliminarily showed that depressive symptom severity was dependent on the interaction between PAL and history of severe childhood abuse in a “for better and for worse” manner. A higher PAL was associated with greater depressive symptom severity among those with a history of severe childhood abuse but significantly lower symptom severity among those without this history. Importantly, this effect withstood adjustments for important covariates (e.g., antidepressant use, comorbid anxiety) and was stable over the 5 years of observation.

Our findings suggest that an individual’s PAL may serve as a trait marker of sensitivity to negative and positive environmental influences. However, the suitability and robustness of PAL to serve as such a trait marker and the mechanism(s) by which PAL confers this sensitivity, specifically to the presence or absence of severe childhood abuse, remains to be established. In fact, given the diverse biological pathways represented by the genes and polymorphisms used to calculate our PAL, it is difficult to speculate on the

Table 2. Participant characteristics (n = 288).

| Parameter                                      | Unadjusted estimates of fixed effects | Adjusted estimates of fixed effects1 |
|------------------------------------------------|--------------------------------------|-------------------------------------|
| Age, mean (SD) years                           | 49 (12)                              | 49 (12)                             |
| Sex, % (n) female                              | 74 (212)                             | 74 (212)                            |
| Education, % (n) 12 years or greater           | 68 (196)                             | 68 (196)                            |
| DSM-IV MDD, % (n)                              | 48 (138)                             | 48 (138)                            |
| Co-morbid psychiatric disorders                | 27 (78)                              | 27 (78)                             |
| PHQ panic/anxiety syndrome, % (n)              | 12 (34)                              | 12 (34)                             |
| Co-morbid substance use                        | 5 (14)                               | 5 (14)                              |
| Smoker, % (n)                                  | 26 (74)                              | 26 (74)                             |
| Alcohol abuse/dependence, % (n)               | 12 (34)                              | 12 (34)                             |
| Any drug abuse/dependence, % (n)              | 5 (14)                               | 5 (14)                              |
| Medication use                                 |                                      |                                     |
| Antidepressant, % (n)                          | 59 (169)                             | 59 (169)                            |
| Anxiolytic, % (n)                              | 24 (70)                              | 24 (70)                             |
| Antipsychotic, % (n)                           | 11 (31)                              | 11 (31)                             |
| Visited counselor/psychologist past 12 months, % (n) | 30 (87)                             | 30 (87)                             |
| Self-rated health, % (n) good to excellent     | 64 (183)                             | 64 (183)                            |
| Childhood abuse, % (n)                         | 39 (113)                             | 39 (113)                            |
| Phenotypic PAL, mean (SD)                      | 11.5 (2.2)                           | 11.5 (2.2)                          |

1n = 280; 2n = 276.

Figure 1. Phenotypic PAL distributions among participants without (A) and with (B) a history of severe child abuse.
mechanism. However, our findings do suggest that multiple genes from multiple biological pathways are likely to be involved in shaping an individual’s sensitivity to environmental influences. This thinking promotes the notion that child abuse disrupts multiple neurobiological pathways and the pattern of this disruption is likely to be heterogeneous. In fact, previous studies have adopted this notion when examining the moderating effects of polygenic phenotypic plasticity. Belsky and Beaver (2011) used a five-gene phenotypic plasticity composite representing serotonergic and dopaminergic pathways and showed phenotypic PAL moderated the relation between parenting and adolescent self-control in a “for better and for worse” manner. Similarly, Cicchetti and Rogosch (2012) showed phenotypic PAL moderated the relationship between child maltreatment and resilient functioning in low-income children, using a four-gene composite representing serotonergic, dopaminergic, oxytocinergic and cortisol pathways. Thus, previous and our current findings suggest future gene × environment interaction studies may benefit from a polygenic approach that encompasses multiple biological pathways; as this approach is likely more aligned with the underlying mechanisms from which the expression of complex phenotypes, such as depressive symptoms severity, emerge.

We also provide novel evidence suggesting that the moderating effect of polygenic phenotypic plasticity is stable over time in an adult primary care population. We are not aware of any other study that has estimated the stability of a gene × environment × depression interaction over time in primary care or any other population. However, the stability we observed will need to be confirmed independently and explored in younger and older populations in which neurodevelopment and neurodegeneration processes could disrupt this stability.

Several caveats should be noted. We used the absence of a severe childhood abuse history as a proxy for “positive” environmental influences. Measures of “positive” exposures in childhood, such as maternal attachment or parental engagement, would be ideal but were not available. However, given the potent long-term effects of severe childhood abuse it could be argued that the mere absence of this toxic exposure is positive. Nonetheless, future work should examine a wider continuum of environmental influences to more comprehensively test the differential susceptibility framework. Furthermore, history of childhood abuse was assessed in adulthood and as such reporting bias is a potential concern. We also only examined sexual and physical childhood abuse, which cautions the generalisation of our results to individuals who have experienced other forms of abuse and/or neglect. Finally, our measure of polygenic phenotypic plasticity was calculated using an unweighted additive method and as such assumed each polymorphism contributed equally to an individual’s sensitivity to environmental influences. Although it is unlikely that all polymorphisms carry the same “weight”, we did not have the sample size and statistical power required to derive weights for each polymorphism and then test our hypothesis. Larger scale studies, such as genome-wide association studies, are well equipped to derive accurate weights and test them with sufficient statistical power.

**Conclusions**

We have provided preliminary evidence suggesting that polygenic phenotypic plasticity moderates the association
between severe childhood abuse and subsequent depressive symptom severity in adulthood. This moderating effect was stable over a 5-year period and was not confounded by antidepressant use, comorbid panic/anxiety, smoking status, or general wellbeing. Our findings contribute to a growing evidence base opposing the traditional conceptualisation of genetic “risk” and “resilience”. Specifically, that high or low depressive symptom severity may not simply be a consequence of an individual’s abundance or absence of “risk” alleles but rather a contingent relationship between the individual’s genetic and environmental context.

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Statement of interest

None to declare.

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