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Full-length Article

Interaction between childhood maltreatment on immunogenetic risk in depression: discovery and replication in clinical case-control samples

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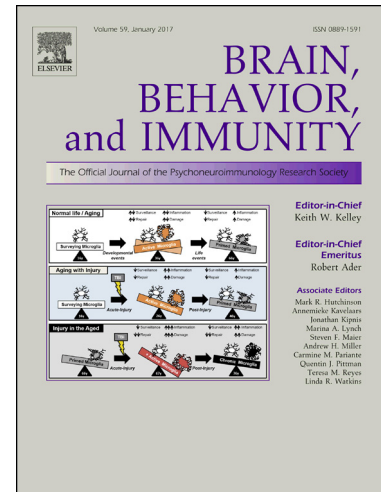
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Interaction between childhood maltreatment on immunogenetic risk in depression: discovery and replication in clinical case-control samples.

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0. Abstract

Major depressive disorder (MDD) is a prevalent disorder with moderate heritability. Both MDD and interpersonal adversity, including childhood maltreatment, have been consistently associated with elevated inflammatory markers. We investigated interaction between exposure to childhood maltreatment and extensive genetic variation within the inflammation pathway (*CRP*, *IL1b*, *IL-6*, *IL11*, *TNF*, *TNFR1*, and *TNFR2*) in relation to depression diagnosis. The discovery RADIANT sample included 262 cases with recurrent DSM-IV/ICD-10 MDD, and 288 unaffected controls. The replication Münster cohort included 277 cases with DSM-IV MDD, and 316 unaffected controls. We identified twenty-five single nucleotide polymorphisms (SNPs) following multiple testing correction that interacted with childhood maltreatment to predict depression in the discovery cohort. Seven SNPs representing independent signals (rs1818879, rs1041981, rs4149576, rs616645, rs17882988, rs1061622, and rs3093077) were taken forward for replication. Meta-analyses of the two samples presented evidence for interaction with rs1818879 (*IL6*) (RD=0.059, SE=0.016, $p < 0.001$), with the replication Münster sample approaching statistical significance in analyses restricted to recurrent MDD and controls following correction for multiple testing ($q=0.066$). The *CRP* locus (rs3093077) showed a similar level of evidence for interaction in the meta-analysis (RD=0.092, SE=0.029, $p=0.002$), but less compelling evidence in the replication sample alone (recurrent MDD $q=0.198$; all MDD $q=0.126$). Here we present evidence suggestive of interaction with childhood maltreatment for novel loci in *IL-6* (rs1818879) and *CRP* (rs3093077), increasing risk of depression. Replication is needed by independent groups, targeting these specific variants and interaction with childhood maltreatment on depression risk.

1. Introduction

Major depressive disorder (MDD) is a highly prevalent disorder that represents a substantial economic as well as clinical burden (Mortality and Causes of Death, 2016). Heritability has been well-established and estimates from population-based studies range between 31-42% (Sullivan et al., 2000), with higher estimates of around 70% based on clinical or more severe cases (McGuffin et al., 2007). Many studies have attempted to identify genes associated with the disorder, however despite significant improvements in genotyping platforms, and large-scale increases in sample sizes, consistently replicated genetic variants remain elusive (Major Depressive Disorder Working Group of the Psychiatric Consortium et al., 2013). This may be for multiple reasons, including failure of most association studies to take into account the role of stress in the development of MDD (Cohen-Woods et al., 2013; Hosang et al., 2012; Thapar et al., 2012).

The first reported specific gene–environment (GE) interaction in MDD was with the serotonin transporter polymorphism, *5HTTLPR* and this has proven controversial (Uher and McGuffin, 2010). Recent meta-analyses indicate that the variable being measured (i.e. childhood stress vs. adult recent stress vs. medical illness) can impact findings, and highlight the need for replication (Karg et al., 2011), with the most recent failing to confirm a robust GE interaction with the *5HTTLPR* and life events (Culverhouse et al., 2017). GE studies face significant methodological challenges with recommendations for future candidate gene-environment studies including: reporting *all* statistical tests conducted for a novel candidate GE study, applying appropriate corrections for statistical testing, and replication (Duncan et al., 2014).

Elevated inflammation has been associated with both MDD and exposure to childhood traumatic events. Our study focuses on genes where proteins in the periphery have been associated with both MDD *and* childhood maltreatment (*CRP*, *IL-6*, *TNF*), genes where GE interactions have previously been reported that include childhood or adolescent stressors (*IL-1b*, *IL6*), or genes associated with anti-depressant response in caucasian MDD patients (*IL-11*); poor anti-depressant response has been reported in individuals with a history of childhood maltreatment (Williams et al., 2016). Elevations of many inflammatory proteins have been reported in patients with MDD including C-reactive protein (CRP), tumour necrosis factor (TNF), and interleukin-6 (IL-6) (Dowlati et al., 2010; Howren et al., 2009). Further, psychological stressors, including early-life maltreatment, have been consistently associated with elevated inflammatory markers (Baumeister et al., 2016; Danese et al., 2008; Fagundes et al., 2013; Kiecolt-Glaser et al., 2011), supported by animal models (Ganguly and Brenhouse, 2015).

Most studies to date have focused on protein analyses, and are limited in number; many more studies have genetic data available rather than serum or plasma, which is necessary for peripheral protein analyses. For this reason investigating genetic variants that have potential to impact gene- and protein- expression in the context of stressful life events is of value. There is also some evidence for interaction with genetic variants in *IL-1b* and *IL-6* and chronic recent stress exposure predicting depressive scores in Australian youth (Tartter et al., 2015), and *IL-1b* predicting depressive symptoms in preschoolers exposed to childhood maltreatment (Ridout et

al., 2014), and IL-11 with anti-depressant response (Uher et al., 2010). Other genes in the inflammation pathway have been associated with anti-depressant response (e.g. (Barnes et al., 2017; Wong et al., 2008), however as our populations are caucasian, for this study we restricted to genes identified in Caucasian populations. Thus overall there are sound reasons for exploring genes involved in inflammation in GE research, looking at depressive disorder onset, course and exposure to stressors including childhood maltreatment (Baumeister et al., 2016; Grosse et al., 2016; Kiecolt-Glaser et al., 2015; Miller and Raison, 2016).

In this paper we present the first investigation of the interaction between exposure to childhood stress and genetic variation, as captured by array, within the inflammation pathway. We focus on genes based on proteins and/or genes previously implicated in depression *and* with childhood maltreatment directly (*CRP*, *IL1b*, *IL-6*, *IL11*, and *TNF*), and indirectly (*TNFR1*, and *TNFR2*) (Camara et al., 2015; Danese et al., 2008; Goldsmith et al., 2016; Kiecolt-Glaser et al., 2015). This study aimed to address the recommendations by Duncan et al. (Duncan et al., 2014): including previously implicated GE loci (IL-6 (rs1800795) and IL-1b (rs16944)) (Tartter et al., 2015), by clearly reporting *all* statistical tests run, robust multiple testing correction and reporting, and independent replication, extended to a meta-analysis.

2. Material and methods

2.1 Participants

2.1.1 Discovery sample

Individuals were drawn from the Radiant MDD cohort recruited in the United Kingdom from three sites (Birmingham, Cardiff, and London), described in detail previously (Fisher et al., 2013; Lewis et al., 2010). In brief, MDD cases experienced a minimum of two DSM-IV/ICD-10 depressive episodes of moderate to severe severity (First, 1994), ascertained by interview in person with the Schedules Clinical Assessments Neuropsychiatry (SCAN) (Wing et al., 1990); DNA was extracted from whole blood. Exclusion criteria were history of substance-related disorders, mania or hypomania, mood-incongruent psychosis, and a first or second-degree relative with bipolar or psychotic disorder. Control individuals were screened to ensure they had no psychiatric history themselves, or in their first-degree relatives. The Radiant sample assessed for childhood trauma consisted of 262 cases (190 females, and 72 males; mean age = 44.79 (± 12.38)) and 288 controls (119 females, and 169 males; mean age = 47.49 (± 9.24)); DNA was extracted from saliva. Controls were excluded if they had a personal or first-degree relative with a history of any psychiatric disorder. All participants were white European, with parents and grandparents of white European origin, and aged 18 years or over. This study was approved by the local University and NHS Ethics Committees at each site and conformed to the Declaration of Helsinki (1975). All participants provided written informed consent.

2.1.2 Replication sample

To replicate findings, we used an independent cohort, the Münster Depression cohort described in detail elsewhere (Power et al., 2016). In brief, MDD cases were identified to have experienced a minimum of one DSM-IV/ICD-10 depressive episode, ascertained using the Structured Clinical Interview for DSM Disorders (SCID) (Wing et al., 1990). Exclusion criteria were any neurologic abnormalities,

substance-related disorders, psychotic symptoms, and/or a history of mania or hypomania. Control individuals were screened to have no psychiatric history themselves, also using the SCID. Exclusion criteria were scores ≥ 10 on the Beck Depression Inventory (BDI), any neurological abnormalities, history of seizures, head trauma or unconsciousness, intake of any psychotropic medication. The replication cohort included 277 adult cases (159 females, and 118 males; mean age = 40.27 (± 11.89)) and 316 adult controls (177 females, and 139 males; mean age = 34.30 (± 11.21)). Of the cases, 78% ($n = 215$) had experienced recurrent episodes of depression (125 females, and 90 males; mean age = 40.62 (± 11.74)) which represented our primary replication sample as this most closely reflected our discovery sample. All participants were white European, with parents and grandparents of white European origin, and aged 18 years or over. DNA was extracted from whole blood for all participants. This study was approved by the local University Ethics Committees and conformed to the Declaration of Helsinki (1975). All participants provided written informed consent.

2.2 Measures

2.2.1 Childhood maltreatment

Self-reported emotional (EA), physical (PA), and sexual (SA) abuse, emotional (EN) and physical (PN) neglect during childhood were recorded using the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003) in both samples. We used standard coding methods detailed previously (Fisher et al., 2013) to classify each type of maltreatment and the overall maltreatment score into: 0 = None, 1 = Mild, 2 = Moderate or Severe.

2.2.2 Genotyping of RADIANT discovery sample

Sample collection and DNA extraction methods have been described previously (Lewis et al., 2010). Genotyping was completed on the Illumina HumanHap610-Quad BeadChips (Illumina Inc., San Diego, CA, USA) by the Centre National de Génotypage, and underwent stringent quality control as previously described. Quality control has been described in detail elsewhere (Lewis et al., 2010). Single nucleotide polymorphisms (SNPs) were imputed based on genotyping data collected using the HumanOmniQuad Array with 1000 Genome data as the reference panel with the IMPUTE.v2 program (Howie et al., 2009; Marchini and Howie, 2010). This employs combined reference panels of known phased haplotypes provided by HapMap 3 (Feb 2009), 1000 Genomes Project (Mar 2010) (National Centre for Biotechnology Information build 36 coordinates) and the study's sample genotypes. Data were extracted for the inflammation genes and ± 5 Kbs, and then converted to genotypes. SNPs with missing rates over 10%, info scores below 0.5, were monomorphic, with minor allele frequency below 5% and/or failed Hardy-Weinberg equilibrium ($p < 0.009$) were excluded from analyses.

2.2.2 Genotyping of Münster replication sample

Genotyping of six of the replication SNPs in the Münster cohort was completed by the Australian Genome Research Facility (AGRF) using the Sequenom MassARRAY[®] iPLEX Gold assay (rs1041981, rs4149576, rs616645, rs17882988,

rs1061622, and rs3093077). We genotyped in-house one SNP using the TaqMan[®] SNP genotyping platform (rs1818879) due to incompatibility within the Sequenom multiplex. 99% of samples genotyped for SNPs rs4149576, rs17882988, rs1061622, and rs3093077, 98% for SNP rs1041981, and 97% for SNP rs1818879. One SNP failed to cluster (rs616645).

2.3 Analysis

Analyses were primarily conducted using Stata version 11.0. The main effects and interaction between childhood maltreatment and genotype on the presence/absence of recurrent unipolar depression were examined using a generalized linear model with the binomial distribution and identity link function specified (WACHOLDER, 1986) to estimate risk differences (RD) and 95% confidence intervals (CI); analyses were adjusted for gender. Tests that failed to converge in Stata were run in R Studio Version 0.99.491 using the blm function (Kovalchik et al., 2013), a constrained maximum likelihood procedure that is operationally comparable to the iterative reweighted least squares (IRLS) algorithm of generalized linear models. We restricted analyses to additive interactions in additive genetic models in inflammation candidate genes. Additive models have been argued to provide the best representation of a biologically plausible interaction (Schwartz & Susser, 2006; Rothman et al., 1980) and are likely to be the most useful from a public health perspective (Kendler & Gardner, 2010). Meta-analyses were run using the *metafor* package in R. As no significant heterogeneity between studies was observed ($p > 0.05$) we used a random effects model.

In the discovery samples we applied multiple testing correction using SNPSpD (<http://gump.qimr.edu.au/general/daledN/SNPSpD/>) (Nyholt, 2004) to evaluate the number of independent markers within each gene. SNPSpD applies Nyholt's method to estimate the *Meff-Li p-value*, accounting for non-independence between markers and avoiding overly conservative correction in the discovery sample (Nyholt, 2004). In total we analysed 120 SNPs across 7 genes (see Supplementary Table 4 for details including *Meff-Li p-value* thresholds) for interaction with childhood maltreatment in our discovery RADIANT sample. All SNPs targeted for replication were independent, thus the false discovery rate (Benjamini and Hochberg, 1995) was applied across SNPs analysed in the replication study.

We tested for gene-environment correlation by testing association between genotype and childhood maltreatment category, in the discovery and replication samples, as replicated G-E correlations with SNPs also identified to be interacting SNPs significantly impacts conclusions we can draw (Dick, 2011).

3. Results

3.1 RADIANT Discovery sample

All forms of childhood maltreatment were significantly associated with greater risk of recurrent depression (Supplementary Table 1 for association, and Supplementary Table 10 for frequencies). All genotypes were in Hardy Weinberg Equilibrium, and there were no significant main effects of genotype on recurrent depression in the discovery Radiant sample, including adjustment for gender (Supplementary Table 2). No significant differences in overall severity of maltreatment was found by genotype amongst cases or controls following adjustment for gender and correction for multiple testing in *IL-1b*, *IL-6*, *TNF*, and *CRP*.

In total 120 SNPs were analysed across the 7 genes; Supplementary Table 4 presents interactions between all genotypes tested and over-all childhood maltreatment on the presence of recurrent depression. We report significant interactions with overall childhood maltreatment severity with 25 SNPs in 5 genes predicting risk of depression following multiple-testing correction (Table 1). The interactions observed represented two groups: those where the minor allele increased risk in those exposed to maltreatment (SNPs in *IL-6* and *CRP*), and those where the major allele increased risk in the exposed group (SNPs in *TNF*, *TNFR1*, and *TNFR2*). Seven of the 25 SNPs represented independent signals (LD < 0.80; see Supplementary Table 5): rs1818879 (*IL-6*), rs1041981 (*TNF*); rs4149576 (*TNFR1*); rs616645 (*TNFR2*); rs17882988 (*TNFR2*); rs1061622 (*TNFR2*); and rs3093077 (*CRP*). No evidence for G-E correlation with childhood maltreatment and these SNPs is reported (Supplementary Table 3).

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Gene	Meff-Li <i>p</i> -value	rsID	Ref	Alt	Ref Freq	Risk Difference (95% CI)	<i>p</i> -value
<i>CRP</i>	≤0.009	rs3093077	T	G	0.94	0.098 (0.024 - 0.172)	0.009
<i>IL-6</i>	≤0.006	rs1818879*	G	A	0.68	0.060 (0.017 - 0.103)	0.006
<i>TNF</i>	≤0.006	rs1041981	C	A	0.64	0.070 (-0.119 - -0.021)	0.005
<i>TNFR1</i>	≤0.015	rs4149576	G	A	0.6	-0.064 (-0.106 - -0.021)	0.004
<i>TNFR2</i>	≤0.005	rs616645*	A	C	0.81	0.083 (0.033 - 0.134)	0.001
<i>TNFR2</i>	≤0.005	rs17882988	G	A	0.86	0.086 (0.034 - 0.137)	0.001
<i>TNFR2</i>	≤0.005	rs1061622*	T	G	0.77	0.053 (0.015 - 0.090)	0.006

Table 1. SNPs presenting evidence for significant interaction with childhood maltreatment in prediction of risk of reporting recurrent depressive episodes in our discovery Radiant cohort. Analyses were adjusted for gender. rsID = rs number; Ref = reference allele; Alt = alternative allele; Ref Freq = frequency of reference allele; CI = confidence intervals. Meff-Li *p*-value represents SNPSpD identified *p*-value threshold for each gene. Only SNPs representing independent signals ($R^2 \leq 0.80$) included, for full data please see Supplementary Table 4. * = a constrained maximum likelihood procedure was applied, operationally comparable to the iterative reweighted least squares algorithm of generalized linear models; only if unconstrained analyses failed to converge

3.2 Münster replication sample

The 7 SNPs identified in the discovery sample passed quality control in our replication sample, except rs616645 (*TNFR2*) which was then excluded. Our primary replication analyses restricted the Münster replication sample to case individuals that had suffered multiple depressive episodes at recruitment (i.e. recurrent depression), more closely representing the discovery sample. Secondary analyses included the entire replication sample, allowing us to investigate if findings extended to individuals that had only experienced a single episode of depression. The replication sample confirmed all forms of childhood maltreatment were highly associated with greater risk of depression (Supplementary table 6 for association, and Supplementary Table 10 for frequencies). All genotypes were in Hardy Weinberg Equilibrium, and there were no differences in the distribution of genotypes between depressed cases and controls, with no main effects of genotype on recurrent depression (Supplementary Table 7). No significant differences in overall severity of maltreatment was found by genotype amongst cases or controls following adjustment for gender (Supplementary Table 8). Data presented in supplementary tables 6 - 8 demonstrate these effects are seen in both the primary recurrent depression replication Münster cohort, and the broader cohort that included individuals that had only experienced a single episode of depression. A SNP in *IL-6* (rs1818879) presented evidence for interaction with overall childhood maltreatment severity to predict recurrent depression in the replication cohort, although this fell just short of significance following FDR correction (see Table 2). Nonetheless, the effect was in the same direction as in the discovery sample (see Figure 1). Meta-analysis of this locus with the two samples corroborated initial findings, indicating interaction with rs1817889 (*IL-6*) and childhood maltreatment (see Figure 1(C): RD = 0.059, SE = 0.016, $p < 0.001$).

Gene	rsID	Recurrent Depression			Depression (Recurrent and Single Episode)		
		Risk Difference (95% CI)	p-value	q-value	Risk Difference (95% CI)	p-value	q-value
<i>CRP</i>	rs3093077	0.083 (-0.006 - 0.172)	0.066	0.198	0.102 (0.015 - 0.189)	0.021	0.126
<i>IL-6</i>	rs1818879	0.058 (0.013 - 0.102)	0.011	0.066	0.044 (0.001 - 0.087)	0.043	0.129
<i>TNF</i>	rs1041981	-0.002 (-0.079 - 0.074)	0.952	0.952	0.011 (-0.059 - 0.081)	0.762	0.762
<i>TNFR1</i>	rs4149576	-0.007 (-0.077 - 0.064)	0.854	0.952	-0.015 (-0.082 - 0.052)	0.668	0.762
<i>TNFR2</i>	rs1061622	0.010 (-0.064 - 0.084)	0.793	0.952	0.014 (-0.058 - 0.085)	0.705	0.762
<i>TNFR2</i>	rs17882988	-0.015 (-0.107 - 0.077)	0.747	0.952	-0.021 (-0.110 - 0.068)	0.639	0.762

Table 2. Interaction of discovery SNPs with childhood maltreatment in prediction of risk of reporting recurrent depressive episodes in our replication Münster cohort. Analyses were adjusted for gender. rsID = rs number; CI = confidence intervals; q-value represents false discovery rate corrected p-value. Bold value: p-/q-value < 0.05.

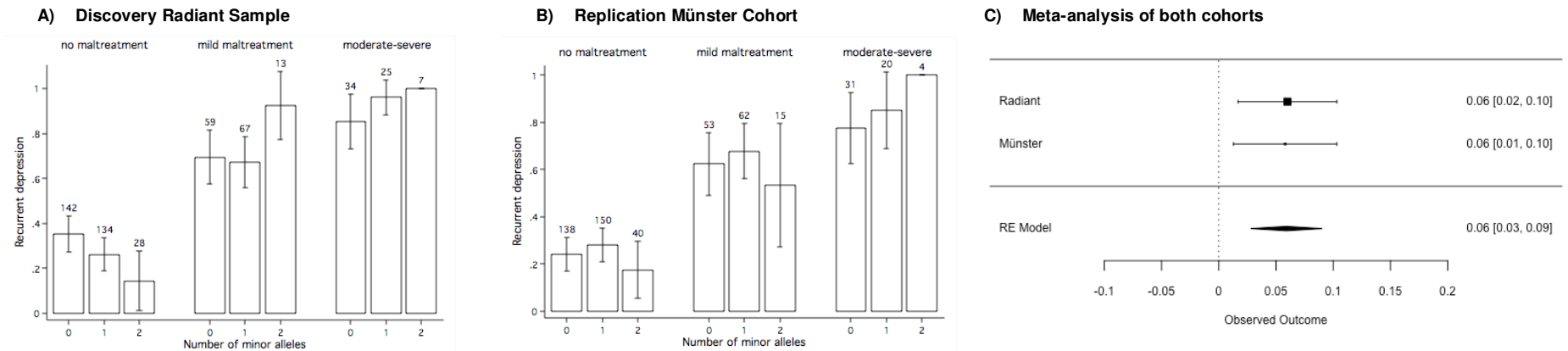


Figure 1. Proportion of individuals with recurrent depression in (A) the discovery sample and (B) replication sample, by severity of different types of childhood maltreatment and *IL-6* rs1818879 (A/G) genotype, and (C) forest plot and meta-analysis of both samples. (A) and (B): y-axis shows probability of reporting recurrent depressive episodes; maltreatment severity is based on the Childhood Trauma Questionnaire (None, Mild, Moderate or Severe), with a rounded average score of all domains for overall maltreatment; the number of individuals included in each subgroup is given above each bar; underlined allele is the minor allele.

When the replication analysis was expanded to include the entire Münster replication sample, including those without a history of recurrent episodes, significance dropped drastically (Table 2).

CRP (rs3093077) was close to significance, which reached non-corrected significance when analyses were expanded to include the whole replication sample and not just recurrent MDD individuals; however this was not significant with multiple testing correction ($q > 0.10$; see Table 2). Figure 2 presents visually the proportion of participants with recurrent depression, and any depression, for each level of childhood maltreatment severity by genotype analysed for *CRP* (rs3093077) in both the discovery and replication samples, showing that the effect was in the same direction. Meta-analysis of this locus with the two samples corroborated initial findings, indicating interaction with rs3093077 (*CRP*) and childhood maltreatment (see Figure 2(C): $RD = 0.092$, $SE = 0.029$, $p = 0.002$).

No other SNPs presented evidence in the replication sample for the originally observed GE interactions in the discovery sample.

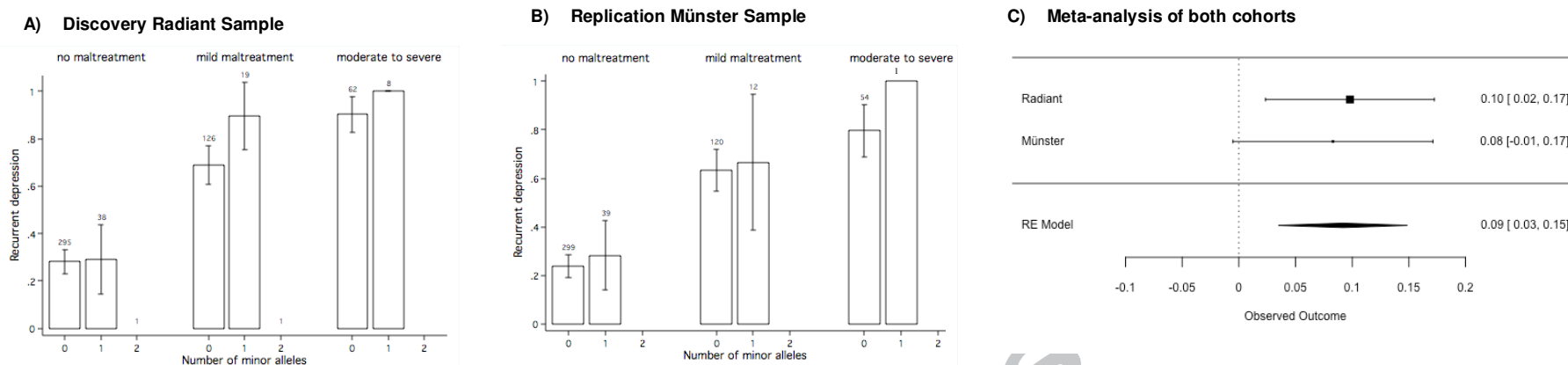


Figure 2. Proportion of individuals with recurrent depression in the (A) discovery sample and (B) replication sample, by severity of different types of childhood maltreatment and *CRP* rs3093077 (G/T) genotype, and (C) forest plot and meta-analysis of both samples. (A) and (B): y-axis shows probability of reporting recurrent depressive episodes; maltreatment severity is based on the Childhood Trauma Questionnaire (None, Mild, Moderate or Severe), with a rounded average score of all domains for overall maltreatment; the number of individuals included in each subgroup is given above each bar; underlined allele is the minor allele.

Stratified analyses on the basis of overall childhood maltreatment exposure (none, mild, and moderate to severe groups), support an effect of rs1818879 and rs3093077 only in individuals with exposure to moderate to severe childhood maltreatment, with minor alleles increasing risk (Table 3). In the Radiant discovery sample there was suggestive evidence for a protective effect of rs1818879 in individuals who experienced no significant maltreatment in childhood, however this was not seen in the replication.

CM category	Discovery RADIANT Sample		Replication Münster Sample	
	Risk Difference (95% CI)	p-value	Risk Difference (95% CI)	p-value
rs1818879				
None	-0.093 (-0.164 - -0.021)	0.011	-0.011 (-0.081 - 0.058)	0.747
Mild	0.088 (-0.022 - 0.197)	0.115	-0.003 (-0.129 - 0.124)	0.967
Moderate to Severe	0.052 (0.012 - 0.092)	0.013	0.103 (0.03 - 0.175)	0.006
rs3093077				
None	0.009 (-0.131 - 0.150)	0.899	0.055 (-0.095 - 0.204)	0.475
Mild	0.108 (-0.064 - 0.280)	0.218	0.021 (-0.258 - 0.300)	0.882
Moderate to Severe	0.104 (0.029 - 0.179)	0.007	0.294 (0.078 - 0.511)	0.008

Table 3. Post hoc analyses investigating SNP associations stratified by levels of childhood maltreatment with the risk of recurrent depressive episodes in our discovery Radiant, and replication Münster samples. Analyses were adjusted for gender. CM category = childhood maltreatment category; CI = confidence intervals. Bold value: p-value < 0.05

We also investigated sub-types of childhood maltreatment as post-hoc exploratory analyses (sexual abuse, physical abuse, emotional abuse, physical neglect, and emotional neglect) in loci suggestive of significance. We report significant interactions between all the childhood maltreatment sub-types and rs1818879 (*IL-6*) (Sexual Abuse: RD = 0.088, SE = 0.037, p = 0.018; Physical Abuse: RD = 0.198, SE = 0.026, p < 0.001; Emotional Abuse: RD = 0.074, SE = 0.022, p = 0.001; Emotional Neglect: RD = 0.086, SE = 0.024, p < 0.001; Physical Neglect: RD = 0.054, SE = 0.023, p = 0.017), and risk for recurrent depression in the discovery Radiant cohort, however this is not seen in the replication Münster cohort (NS). In contrast, rs3093077 (*CRP*) presented no significant interactions with maltreatment sub-types in the discovery study, although there was some evidence for significant interactions with physical abuse (RD = 0.146, SE = 0.047, p = 0.002), and emotional abuse (RD = 0.133, SE = 0.042, p = 0.002) in the replication study. For complete maltreatment sub-type analyses see Supplementary Table 9.

4. Discussion

We found evidence for interaction between childhood adversity and one SNP in *IL-6* (rs1818879) in the RADIANT sample; the Münster replication sample also presented evidence prior to correction, which reduced to very close to significance following correction for multiple testing. It is notable that for replication, we maintained two-tail significance tests; had a one-tail test been applied this would have reached significance even following correction. Due to the controversies that have arisen in GE depression research to date, we erred on the side of caution using the two-tail statistic which is what was applied in the discovery sample. However meta-analysis of the two samples supported the interaction. It is possible that our replication sample, although closely similar to our discovery sample, possessed reduced statistical power relative to the discovery sample because: i) the discovery Radiant sample control participants were both themselves *and* their first-degree relatives free of psychiatric history, in contrast to the replication sample which did not consider first-degree relative psychiatric history; and ii) the Radiant sample was restricted to case individuals that had experienced moderate to severe episodes of recurrent depression, a criterion not applied to the Münster sample. Thus the discovery sample may have been better powered because the affected individuals represented a more extreme phenotype, and the sample of individuals with recurrent MDD was greater in the discovery relative to the replication sample. When analyses for this SNP were expanded to the entire Münster sample, including individuals that had not suffered recurrent episodes of depression, effects and significance dropped markedly. It is plausible that carrying rs1818879 (*IL-6*) minor alleles in conjunction with exposure to significant childhood maltreatment may increase risk to a more severe and recurrent form of MDD, and robust replication will only be seen in cohorts reflective of this phenotype. Our post-hoc analyses investigating different sub-types of childhood maltreatment suggest that, if a true effect, the genetic risk of the minor allele may be specific to individuals that have experienced abuse-related maltreatment rather than neglect-related maltreatment. However this certainly needs to be further explored in larger cohorts.

Our findings support previous research indicating that *IL-6* is implicated in gene-environment interactions in depression, where rs1800795 interacted with interpersonal stress exposure in Australian youth, predicting depressive symptoms (Tartter et al., 2015). However, rs1800795 was not the same variant that we identified in this cohort. The *IL-6* variant that we found supporting evidence was rs1818879. This is an interesting SNP located downstream of the *IL-6* gene; it has been associated with severe influenza A virus infection (minor allele) (García-Ramírez et al., 2015) and body mass index (major allele within a large haplotype) (Qi et al., 2007). *IL-6* gene-expression, and protein-expression, have been associated with depression and with childhood maltreatment. However, notably although rs1818879 is an eQTL, it is associated with *AC073072.5* expression, an antisense gene to *IL-6* coding for long non-coding RNA (lncRNA). Expression of *AC073072.5* has not been studied although the functional impact of lncRNAs (Pelechano and Steinmetz, 2013), and significance in psychiatric illness such as depression

(Huang et al., 2016) and the stress response (Amaral et al., 2013) are becoming clear.

Although there is evidence for a dysregulation in IL-6 proteins in depressed individuals, the literature isn't completely consistent. This could, in part, be due to the role of childhood maltreatment as IL-6 levels have also been associated with childhood maltreatment exposure. Thus if studies investigated depression in the context of childhood maltreatment exposure, their findings may become more consistent. It could also be due to the impact of this antisense gene, and related lncRNA transcripts, which may directly interact with *IL-6* and/or other inflammation related mRNAs.

The findings for the *CRP* locus (rs3093077) present a similar pattern with the meta-analysis of the two samples showing significant evidence for the GE interaction. However, the data present a less compelling case than that found for the *IL-6* locus. This could be a consequence of low minor allele frequency and thus warrants some discussion. In the replication sample alone rs3093077 showed no GE interaction effect until the sample was expanded to include more broad MDD cases, including individuals that had only experienced single episodes of MDD. This is opposite to the pattern observed with the *IL-6* SNP. This could be due to a low minor allele frequency and thus reduced power resulting in non-detection of effect with the smaller more severe phenotypic sample, or it could simply be reflective of being a chance-finding. Of note, the minor alleles of two SNPs in complete LD with rs3093077 (rs11265260 and rs3093059) are significantly associated with CRP protein abundance in genome-wide association studies (Okada et al., 2011; Reiner et al., 2008); notably one of these studies identified an *IL-6* locus (rs2097677) to also be associated with serum CRP protein abundance. In contrast to IL-6, researchers have investigated CRP protein levels in depressed cohorts in the context of childhood maltreatment and have presented evidence in adolescent and adult cohorts that CRP is elevated in those that are depressed or have a history of depression, and that this elevation is even greater in those that have experienced significant childhood maltreatment (Danese et al., 2008).

Our findings indicate genetic support for the implication of immune pathways in depression, in context of childhood maltreatment. Mechanisms by which gene-environment interactions have their effect have been discussed (e.g. Cohen-Woods et al., 2013), including epigenetic mechanisms. Childhood maltreatment has been demonstrated to alter DNA methylation (Bick et al., 2012); it is plausible that childhood trauma could alter the epigenetic profile of individuals, potentially activating (or switching off) a risk genotype. For example, childhood maltreatment may alter DNA methylation in the *IL-6* promoter or enhancers, potentially altering gene-expression and protein-expression. This may influence risk for inflammation, and in turn, depression.

We must note however that effect sizes are small, as expected in genetic studies, meaning clinical relevance will only be achieved when multiple GE SNPs have been identified, as with a polygenic risk score, to identify those at risk in particular adverse environments. Focusing on specific systems (e.g. functional SNPs and eQTLs in the immune system) could aid this.

Significantly, the literature does indicate there is a sub-group of MDD individuals that exhibit elevated levels of peripheral inflammation (Raison and Miller, 2011); thus some individuals with MDD show elevated inflammation whilst others do not. It is possible, and plausible, that the interaction we report here is related to an inflammation sub-group; individuals with a history of childhood maltreatment and *IL-6* risk variant, may also exhibit higher levels of inflammation. Further, inflammation-based restriction to MDD groups in studies (i.e. those with elevated inflammation in the periphery separated from those with no elevated inflammation in the periphery) may enhance effect sizes when studying inflammation pathway genes in both genetic and GE studies. This needs to be explored further, although it does require collection of plasma or serum, in addition to blood for genetic testing. Exploring if childhood maltreatment may account for previously reported sub-types of inflammation-based depressions would be valuable, and could also be another pathway by which the reported GE interactions occur in these genes.

It is interesting that we failed to replicate in the other genes where SNPs were identified in the discovery sample (e.g. *TNF*, and its receptors). This could be due to issues with power, however the effect sizes and directions indicates this is unlikely. It is possible that different aspects of the complex inflammation pathway may have differing roles. Interestingly the most convincing GE interaction we report with *IL6* is with an eQTL for the antisense gene to *IL-6* that codes for a lncRNA; perhaps genetic variation within the immune system that alters non-coding RNA is more salient than other variation.

This study addresses many concerns previously described in the literature with gene-environment interaction studies, addressing clear a priori hypotheses, with full reporting of all tests applied (and resulting statistics), and correcting for multiple testing. Limitations however do exist, including relatively limited sample sizes, a focus on additive genetic models (not evaluating multiplicative models due to the burden of multiple testing), the retrospective assessment of childhood maltreatment, and restriction of analyses to clinical depression. Further, our conclusions are restricted only to the genes that we included in our study; broader study of the immune system in context of childhood maltreatment and risk of MDD would be warranted. However, if increasing the number of genes studied we would suggest additional cohorts to improve power, using a meta-analytical framework. Finally, including additional potential confounders in future analyses would be valuable including smoking and body mass index; we did not have this data across all samples in this study.

5. Conclusion

Here we present suggestive evidence for novel interaction loci in *IL-6* (rs1818879) and potentially *CRP* (rs3093077) in the context of childhood maltreatment, increasing risk of recurrent depression (*IL-6*) and non-recurrent depression (*CRP*). Although we have described and discussed the results for both these SNPs, we must highlight that independent replication analyses fell short of significance, although relatively marginal in terms of rs1818879. To address restricted power we also ran meta-analyses of these loci, which present significant support for these interactions. However further

independent replication is necessary. It is important that these loci are pursued in larger samples, potentially with an international meta-analytic collaborative effort, and for case-control studies in the context of moderate to severe recurrent depression as well as more general diagnoses. Further investigation into multiplicative interaction models, and dominant/recessive genetic models in inflammation loci is warranted.

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Conflicts

AF and PM have received consultancy fees and honoraria for participating in expert panels for pharmaceutical companies including GlaxoSmithKline. BB have received honoraria for speaking and participating in expert panels for pharmaceutical companies including Lundbeck, Pfizer, and AstraZeneca. VA declares that over the last three years he has received compensations for his contributions as member of advisory boards and for presentations for the following companies: Astra-Zeneca, Eli Lilly, Janssen-Organon, Lundbeck, Otsuka, Servier, and Trommsdorff. These collaborations have no relevance to the work that is covered in the manuscript.

SCW, HLF, DA, KD, DS, GMH, AK, MO, NC, UD, GB, IWC, CML, RU:
Nothing to declare

Author Contributions: SCW designed the study, genetic analysis, statistical analysis, sample and genotyping of RADIANT and Münster sample, writing of manuscript; DA statistical analysis, and manuscript review; KD bioinformatic data extraction, and manuscript review; HLF childhood maltreatment data management, contributed to data interpretation and manuscript review; DS taqman genotyping, contributed to data interpretation and manuscript review; GMH contributed to data interpretation and manuscript review; AK, MO, NC collected discovery Radiant sample; VA, UD collected replication Münster sample, and manuscript review; BTB collected replication Münster sample, contributed to study design, genotyping of replication sample, and manuscript review; GB contributed to genotyping of RADIANT sample, data interpretation, and manuscript review, IWC and AF collected discovery Radiant sample, contributed to data interpretation and manuscript review; CML data imputation and contributed to statistical analyses, data interpretation, and manuscript review; RU contributed to statistical analyses, data interpretation, and manuscript review; PM contributed to study design, genotyping of discovery sample, data interpretation, and manuscript.

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Highlights

- Gene-environment study focusing on comprehensive analysis of immune candidate genes
- Interaction with childhood maltreatment in predicting recurrent depression
- Loci identified in the discovery sample taken forward to an independent replication sample
- Two loci present some evidence for replication in *IL-6* and *CRP*
- These loci should be targeted for replication in further studies internationally in the future