

ORIGINAL ARTICLE

Exploring the association of interleukin polymorphisms with aggression and internalizing behaviors in children and adolescents

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Abstract

Background: There is growing evidence that inflammation influences mental health. Blood interleukin levels, which regulate inflammation, have been linked to aggression and internalizing behaviors. We performed a hypothesis-driven genetic study to (1) evaluate the association of *IL1B*, *IL2*, and *IL6* gene variants with aggression and internalizing behaviors and (2) explore gene–environment interactions with childhood adversity in a deeply phenotyped childhood-onset aggression sample including 255 cases and 226 controls of European ancestry.

Methods: We evaluated the association of putative functional and tag SNPs within *IL1B*, *IL2*, and *IL6* with aggression case status, parent-reported internalizing problems, self-reported anxiety symptoms, and self-reported depressive symptoms in our sample. We also performed exploratory GxE analyses within cases, testing for statistical interaction between interleukin SNP genotype and childhood adversity for depressive symptoms.

Results: No significant association was observed between any of the interleukin SNPs and childhood-onset aggression. We observed association of *IL6* variant rs2069827 with depressive symptoms ($p = 7.15 \times 10^{-4}$), and trends for an interaction between severe childhood adversity and SNPs in *IL1B* and *IL2* for depressive symptoms.

Conclusions: Our findings provide preliminary evidence that common variation in *IL6* may be associated with depressive symptoms in children and adolescents, and that common variation in interleukin genes may sensitize individuals to the depressogenic effects of traumatic life experiences. Replication in independent samples is needed.

KEYWORDS

adversity, aggression, child, cytokine, gene, inflammation, interleukin, internalizing, trauma

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1 | INTRODUCTION

Behavioral problems in young children generally fall into two broad categories: externalizing behaviors (problems with attention, self-regulation, and noncompliance including inattention/hyperactivity, antisocial, and aggressive behaviors) and internalizing behaviors (depression, withdrawal, anxiety, and somatic complaints) (Achenbach et al., 1987; Campbell, 1995). Both externalizing and internalizing behaviors in childhood and adolescence are associated with long-term difficulties, including poor functional and mental health outcomes (Achenbach et al., 1995; Campbell and Ewing, 1990; Hughes et al., 2008; Johnson et al., 2018; Moffitt, 1990; Pine et al., 1998). Among children, aggression and internalizing behaviors (including depression and anxiety) are the most common reasons for referral to psychiatric care (Pikard et al., 2018), and we have therefore focused our attention on these clinical entities in the present study while recognizing that aggression alone does not represent the full spectrum of externalizing behaviors.

Currently, the causes of childhood-onset aggression and internalizing disorders are not fully understood, and treatment options remain limited. While certain psychotherapy modalities (e.g., parent management training (Michelson et al., 2013) and cognitive-behavioral therapy (Sukhodolsky et al., 2000)) have been shown to improve aggressive and internalizing disorders in children, they are not widely available. As a result, second-generation antipsychotics (SGAs) and selective serotonin +/- norepinephrine reuptake inhibitors (SSRIs and SNRIs) are often used in children and adolescents to treat severe aggression and depression/anxiety, respectively, despite limited evidence of efficacy (Locher et al., 2017; Pringsheim and Gorman, 2012) and significant side-effect burden (Locher et al., 2017; Pringsheim et al., 2011) particularly for SGAs. An improved understanding of the causes of childhood-onset aggression and internalizing behaviors is needed to facilitate improvements in prevention, detection, and management. Notably, both aggression and internalizing behaviors are heritable ($h^2 \sim 0.5$ (Bergen et al., 2007; Rhee and Waldman, 2002)) making them amenable to genetic study.

There is accumulating support for an “inflammatory hypothesis” of aggression and internalizing behaviors, whereby some complex interplay of biological, psychological, and social risk factors triggers an overactive inflammatory response, which then predisposes an individual to develop aggression, depression, and anxiety (Costello et al., 2019; Miller and Raison, 2016; Takahashi et al., 2018). Initial support for this inflammatory hypothesis came from the observation in cross-sectional studies that peripheral inflammation was present among patients with certain medical and psychiatric disorders—including aggression and internalizing behaviors (Costello et al., 2019; Miller and Raison, 2016; Takahashi et al., 2018). Longitudinal studies have also found that baseline blood levels of inflammatory biomarkers—including C-reactive protein (CRP), interleukin 1 beta (IL1B), and interleukin 6 (IL6)—predict the development of both aggressive and internalizing behaviors in animal and human studies (Costello et al., 2019; Graham et al., 2006; Lamers et al., 2019; Valkanova et al., 2013). Furthermore, there is a

growing body of literature identifying elevated peripheral inflammatory biomarkers, such as CRP and IL6, in children and adolescents with externalizing (Mitchell and Goldstein, 2014; Pham et al., 2022; Slopen et al., 2013) and internalizing behaviors (Belem da Silva et al., 2017; Copeland et al., 2012; Mitchell and Goldstein, 2014; Pham et al., 2022; Slopen et al., 2013). A recent prospective study of 482 infants found that higher glycoprotein acetyls (GlycA, a measure of cumulative inflammation) levels at birth were associated with externalizing and internalizing problems at age two, underscoring the impact of inflammation during sensitive periods of early brain development (Pham et al., 2022). Overall, it appears that inflammation may be an important biological pathway underlying aggressive and internalizing behaviors in at least a subset of children and adolescents. For these young people, various combinations of environmental exposures and genetic vulnerability lead to peripheral inflammation, which ultimately may contribute to their psychiatric symptoms.

In terms of relevant environmental exposures predisposing to early life aggression and internalizing behaviors, early life stress is a well-established risk factor (Dodge et al., 1990; Nemeroff, 2016). Childhood neglect and physical/emotional/sexual abuse influence mental health throughout life, including increasing severity of both externalizing and internalizing symptoms (Anda et al., 2006; Cole et al., 2012; Janusek et al., 2017; Tursich et al., 2014). While the neurobiological mechanisms by which childhood adversity influences mental health are not fully understood, it is clear that early-life stress has cumulative and lasting effects on both the brain (Anda et al., 2006) and the immune system (Cole et al., 2012; Saarinen et al., 2021). An emerging theory is that childhood adversity increases cross-talk between the immune system and the brain throughout the lifespan, resulting in sensitization of the brain's threat responses (cortico-amygdala circuit) and attenuation of reward responses (cortico-basal circuit) and executive control (prefrontal cortex) via pro-inflammatory cytokines—including IL1B and IL6—acting on these brain regions (Nusslock and Miller, 2016). Thus, childhood adversity appears to influence the development of aggressive and internalizing behaviors, at least in part, by altering levels of circulating inflammatory biomarkers that influence the developing brain.

Regarding genetic risk factors, large-scale genome-wide association studies (GWASs) of childhood aggression (Pappa et al., 2016) and internalizing behaviors (Benke et al., 2014; Jami et al., 2020; Trzaskowski et al., 2013) have revealed that these traits are polygenic and share genetic risk factors with adult-onset disorders, but have not yet identified robustly associated genetic variants. To increase statistical power for variant discovery in these GWASs, the results of many smaller studies were pooled by meta-analysis in order to achieve massive sample sizes ($N_{\text{range}} = 2,000\text{--}60,000$). Trade-offs for increasing statistical power by pooling smaller studies include limited phenotypic data and increased clinical heterogeneity. Hypothesis-driven studies are an alternative strategy to GWASs, which can be used to explore biological hypotheses that require more detailed phenotypic data, such as gene-environment interactions (Caspi et al., 2002). Due to the smaller sizes typical of deeply phenotyped samples, hypothesis-driven genetic

studies are susceptible to false negatives (type II error), as well as spurious findings (type I error) unless there is stringent correction for multiple testing, and are therefore exploratory until robust replication in independent studies has been achieved (Dick et al., 2015). Given the demonstrated role of IL1B, interleukin 2 (IL2), and IL6 in stress-induced aggression and internalizing behaviors in feline (Bhatt et al., 2005; Hassanain et al., 2003) and rodent models (Chourbaji et al., 2006; Koo and Duman, 2008; Niraula et al., 2019), respectively, polymorphisms in these genes are of particular interest for follow-up in hypothesis-driven gene–environment studies.

To date, there has been limited investigation of the relationship between childhood adversity, genetic variation, and aggressive or internalizing behaviors. Previous studies have identified potential interactions between childhood adversity and polymorphisms in *IL1B* and *IL6* in regulating risk for internalizing symptoms (Kovacs et al., 2016; Ridout et al., 2014). Here we performed a hypothesis-driven genetic study to evaluate the association of *IL1B*, *IL2*, and *IL6* gene variants with aggression and internalizing behaviors in children and adolescents, and to explore gene–environment interactions with childhood adversity.

2 | MATERIALS AND METHODS

2.1 | Clinical sample

Our sample comprised 314 cases and 287 controls recruited in an ongoing study of childhood-onset aggression that has been previously described (Beitchman et al., 2004; Beitchman et al., 2006; Malik et al., 2014). Briefly, cases comprised children and adolescents aged 6–16 years referred to outpatient psychiatric care for persistent, extreme aggression at the Centre for Addiction and Mental Health (a tertiary care hospital providing inpatient and outpatient psychiatric services located in Toronto, Ontario, Canada) and Youthdale Treatment Centre (a community-based mental health agency providing inpatient and outpatient services in Toronto, Ontario, Canada). Inclusion criteria for cases consisted of a minimum 2-year history of aggressive behavior, and scores at the 90th percentile or higher on the aggressive subscale of the Achenbach Child Behaviour Checklist (CBCL) by two independent raters, which could include any of: the parent or guardian (CBCL); the teacher (teacher report form, TRF); the participant (youth self-report form, YSR) (Achenbach and Rescorla, 2001). Healthy controls comprised children and adolescents aged 6–16 years scoring within the normal range on the Achenbach CBCL by two independent raters (Achenbach and Rescorla, 2001) with no history of disruptive behavior, recruited from the community at the Ontario Science Centre (Toronto, Ontario, Canada) and Science North Education Centre (Sudbury, Ontario, Canada). Exclusion criteria for cases and controls included intelligence quotient below 70 as measured using a 2-subtest shortened form of the Wechsler Intelligence Scale for Children—Third Edition (Wechsler, 1991), history of chronic medical illness, psychosis, mania, neurological disorder, or pervasive developmental disorder. Ethnicity was assessed by parental self-report, and we included only

European subjects in our analyses to avoid potential confounding by population stratification.

In addition to case status, information on the following clinical characteristics were analyzed:

Internalizing problems. Severity of overall internalizing problems was assessed by parent or guardian report using the Achenbach CBCL internalizing problems score (Achenbach and Rescorla, 2001). The internalizing problems score captures the anxious-depressed, withdrawn-depressed, and somatic complaints syndrome scales of the CBCL. Internalizing problems scores were available for 253 cases and 218 controls.

Anxiety. Severity of anxiety was assessed by self-report using Revised Children's Manifest Anxiety Scale (RCMAS) total scores (Reynolds and Richmond, 1985). RCMAS total scores were available for 241 cases and 226 controls.

Depression. Severity of depressive symptoms was assessed by self-report using Children's Depression Inventory (CDI) total scores (Kovacs, 1985). CDI total scores were available for 234 cases and 224 controls.

Exposure to severe childhood adversity. Subjects aged 12 and older completed the Massachusetts Youth Screening Instrument—Version 2 (MAYSI-2), and we assessed history of trauma using the self-reported MAYSI-2 Traumatic Experiences scale (Grisso and Barnum, 2000). This scale indicates the number of traumatic experiences endorsed using the following questions: (1) Have you ever in your whole life had something very bad or terrifying happen to you? (2) Have you ever been badly hurt, or been in danger of getting badly hurt or killed? (3) Have you ever been raped, or been in danger of being raped? (4) Have you had a lot of bad thoughts or dreams about a bad or scary event that happened to you? (5) Have you ever seen someone severely injured or killed (in person—not in movies or on TV)? Traumatic life experience data were available for 124 cases and 83 controls. Based on the distribution of scores in our sample (Table 1), we defined severe childhood adversity as a MAYSI-2 Traumatic Experiences scale score ≥ 4 .

2.2 | Genotyping and quality control

2.2.1 | Discovery sample

Tag single-nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) ≥ 0.05 within 10 kb upstream and 3 kb downstream of each interleukin gene were identified using the database of common SNPs available in the International HapMap Project's Phase III European (CEPH) sample (International HapMap 3 Consortium, 2010) and Haploview version 4.2 (Barrett et al., 2005). SNPs were prioritized for selection based on evidence of regulatory function in the Encyclopedia of DNA Elements (Dunham et al., 2012) as queried using HaploReg (Ward and Kellis, 2012) and RegulomeDB (Boyle et al., 2012). In total, 16 putative functional and tag SNPs within *IL1B* (rs4849127, rs13032029, rs16944, rs3136558, rs1143634, rs1143643), *IL2* (rs2069762, rs2069772, rs2069778, rs2069779), and *IL6* (rs2069827, rs2069837, rs2066992, rs2069840, rs2069861,

TABLE 1 Demographic and clinical characteristics of the sample

Characteristic	Cases (n = 255)	Controls (n = 226)	p
Male (%)	152 (60)	125 (55)	0.39 ^a
Age at recruitment ± SD (years)	12.06 ± 3.0	11.25 ± 2.45	5.06 × 10 ⁻⁵ b
IQ ^c	97.94 ± 14.0	113.52 ± 14.05	1.79 × 10 ⁻¹⁰ d
CBCL ^e total	74.06 ± 6.7	48.31 ± 10.7	5.52 × 10 ⁻¹¹⁹ d
CBCL externalizing	74.89 ± 7.5	46.86 ± 9.8	1.78 × 10 ⁻⁷² b
CBCL internalizing	68.66 ± 9.7	49.80 ± 10.2	2.02 × 10 ⁻⁵² b
CDI total ^f	54.73 ± 13.5	43.25 ± 6.6	8.45 × 10 ⁻²⁸ b
RCMAS total ^g	52.96 ± 12.4	42.35 ± 10.4	1.55 × 10 ⁻²¹ d
History of abuse (%) ^h	138 (69)	39 (47)	9.92 × 10 ⁻⁴ a

^achi-square test.

^bKruskal–Wallis test.

^cIntelligence quotient (IQ) measured using a 2-subtest shortened form of the Wechsler Intelligence Scale for Children—Third Edition was available for 236 cases and 42 controls.

^dANOVA test.

^eProblem behaviors measured using the Achenbach Child Behaviour Checklist (CBCL) completed by child's parent(s) or guardian were available for 253 cases and 218 controls.

^fSelf-reported depressive symptoms measured using the Children's Depression Inventory (CDI) were available for 234 cases and 224 controls.

^gAnxiety symptoms measured using the Revised Children's Manifest Anxiety Scale (RCMAS) were available for 241 cases and 226 controls.

^hDefined as history of childhood abuse based on physician assessment, which was available for 201 cases and 83 controls.

rs10242595) were genotyped. For a summary of the regulatory function of the selected SNPs, see Supplementary Table 1.

Genomic DNA was extracted from blood, saliva, or buccal swab samples and DNA was extracted using the high-salt method (Lahiri and Nurnberger, 1991). Genotyping was done blind to case status using the Taqman® OpenArray® Genotyping system (Applied Biosystems Inc, Foster City, CA) according to manufacturer's protocols.

2.2.2 | Publicly available genome-wide association study (GWAS) and phenome-wide association study (PheWAS) data

To further investigate interleukin SNPs of interest (i.e., those that were associated with internalizing behaviors in our discovery sample), we evaluated their association with internalizing behaviors in previously published publicly available GWASs of adult depression (Wray et al., 2018) and adult anxiety (Otowa et al., 2016). These GWASs have been described in detail elsewhere (Otowa et al., 2016). Briefly, the samples comprising these GWASs included a total of 135,458 adult cases and 344,901 controls for depression (Wray et al., 2018) and 17,016 adult cases and 14,745 controls for anxiety (Otowa et al., 2016).

2.3 | Power analysis

Power calculations for main effects were done post-hoc using Quanto version 1.2.4, assuming an additive model and MAF = 0.23 (mean MAF of SNPs included in our study). Assuming a population prevalence of 10% for childhood-onset aggression (cases were defined as children

scoring above the 90th percentile on the CBCL), we had over 80% power to detect SNPs with an OR of 1.39 in association with aggression in the sample of 212 cases and 213 controls. Assuming a population mean CBCL internalizing problems score of 59.93 ± 13.7, we had over 80% power to detect a difference in mean internalizing problems of 3.28 between genotypes in the total sample of 425 subjects. Assuming a population mean CDI total score of 49.12 ± 12.1, we had over 80% power to detect a difference in mean CDI of 2.91 between genotypes in the total sample of 425 subjects. Assuming a population mean RCMAS total score of 47.82 ± 12.6, we had over 80% power to detect a difference in mean RCMAS of 3.02 between genotypes in the total sample of 425 subjects.

2.4 | Statistical analysis

Linkage-disequilibrium (LD) structure, including the proportion of common variation in each of the interleukin genes, was determined using Haploview version 4.2 (Barrett et al., 2005). Descriptive analysis and data visualization were done using R version 3.1 statistical software (R Core Team, 2012) with the *rms* package (Harrell, 2014). After evaluating differences in demographic and clinical variables between cases and controls (Table 1), association analyses were done for each of the 14 SNPs individually using PLINK version 1.90b6.7v (Chang et al., 2015). Gene-environment interaction analyses were done for each of the 14 SNPs individually using R version 3.1 statistical software (R Core Team, 2012) with the *rms* package (Harrell, 2014).

We used logistic regression to test for association with aggression as a binary trait and linear regression to test for association with internalizing problems, anxiety, and depression as quantitative traits. We

adjusted for age and sex in all analyses a priori, as these variables have previously been associated with aggression and internalizing behaviors (Martel, 2013). Given the non-normal distribution of quantitative variables (i.e., CBCL internalizing score, RCMAS total scores, CDI total scores) in the total sample, approximately normal distribution of these variables within cases and controls, and significant differences in these variables between cases and controls (Table 1), we analyzed quantitative variables in cases and controls separately and then conducted inverse variance-weighted fixed effects meta-analysis using METAL (Willer et al., 2010). Finally, we evaluated gene-environment interactions between each of the interleukin SNPs and traumatic life experiences. For these gene-environment interaction analyses, we looked only within cases. The significance of gene-environment interactions was tested using linear regression for CBCL internalizing score, CDI total scores, and RCMAS total scores using a multiplicative model with adjustment for the main effects of each SNP and trauma history with age and sex as covariates.

To correct for multiple testing, we determined that the interleukin SNPs analyzed were equivalent to 13 independent tests using the single-nucleotide polymorphism spectral decomposition method (Nyholt, 2004). We tested these 13 SNPs for association with four phenotypes, and also for gene-environment interactions with childhood adversity. We therefore used a significance threshold of $\alpha < 7.69 \times 10^{-4}$ ($0.05/13$ genotypes * 5 outcomes). This significance threshold is conservative, as the overarching phenotype being investigated was internalizing behavior and three of the outcome measures used to study this phenotype were significantly correlated (Internalizing-CDI Kendall's $r = 0.33$; $p = 4.55 \times 10^{-24}$, Internalizing-RCMAS Kendall's $r = 0.28$; $p = 2.42 \times 10^{-18}$, CDI-RCMAS Kendall's $r = 0.49$; $p = 1.91 \times 10^{-52}$).

3 | RESULTS

3.1 | Genetic architecture of *IL1B*, *IL2*, and *IL6* variants analyzed

We excluded one SNP (rs3136558) with poor clustering in allelic discrimination plots. Among the remaining 15 SNPs, call rates were > 85%. Duplicate samples (10%) were used to check genotyping accuracy, and > 99% concordance was observed. Two SNPs violated Hardy-Weinberg equilibrium and were removed from further analysis (rs13032029, $p = 1 \times 10^{-4}$ and rs2069779, $p = 1 \times 10^{-56}$). The remaining 13 SNPs were in Hardy-Weinberg equilibrium ($p > 0.01$). The 13 SNPs available for analysis captured 83% of common alleles (MAF > 0.05) in *IL1B*, 60% of common alleles in *IL2*, and 24% of common alleles in *IL6*.

3.2 | Sample characteristics

Genetic data was available for 255 cases and 226 controls of European ancestry. Overall, 58% of the sample was male. As a group, cases were older than controls, were more likely to have a history of trauma,

and had lower IQ as well as higher symptom scores. Demographic and clinical details of the sample are provided in Table 1.

We excluded 56 individuals, 43 cases, and 13 controls, with > 20% missing genotype rate (i.e., missing genotypes for more than 3 of the 13 interleukin SNPs). After quality control, 212 cases and 213 controls of European ancestry were available for analysis, resulting in a total sample of 425 individuals. The number of individuals included in each analysis varied depending on the missing data rate of the phenotypes analyzed.

3.3 | Association of interleukin SNPs with childhood-onset aggression

We investigated the association of each of the 13 interleukin SNPs with childhood-onset aggression in our sample of 212 cases and 213 controls. Given that age and gender have been previously associated with aggression (Martel, 2013), we included these as covariates in all association tests. No association was observed between any of the interleukin SNPs and childhood-onset aggression in our sample (all $p > 7.69 \times 10^{-4}$, Table 2).

3.4 | Association of interleukin SNPs with internalizing behaviors in children

Next, we evaluated the association of each interleukin SNP with continuous measures of childhood internalizing problems (CBCL subscale), depressive symptoms (CDI total score), and anxiety symptoms (RCMAS total score). We performed association testing separately in cases and controls, and then combined the results using inverse variance-weighted fixed effects meta-analysis. Similar to our case-control analysis, we adjusted for age and gender in all analyses of internalizing behaviors.

We observed significant association of *IL6* variant rs2069827 with depressive symptoms (effect size among cases, $\beta_{CAS} = -4.98$ for each copy of the T allele; effect size among controls, $\beta_{CON} = -2.80$; inverse variance-weighted fixed effects meta-analysis of effect in cases and controls, $p\text{-value}_{META} = 7.15 \times 10^{-4}$), and a trend for association of rs2069827 with anxiety ($\beta_{CAS} = -3.66$ for each copy of the T allele; $\beta_{CON} = 0.07$; $p_{META} = 0.006$). No other interleukin SNPs showed association with any of the internalizing behaviors investigated (all remaining $p > 7.69 \times 10^{-4}$, Table 3).

To further explore the association of *IL6* variant rs2069827 with depression and anxiety, we evaluated the main effect of this SNP in independent GWASs of these traits (Otowa et al., 2016; Wray et al., 2018). Notably, the GWASs were of adults meeting clinical criteria for major depressive disorder (MDD) and anxiety disorders. Thus, these GWASs were not true replication samples as they comprised a different population than our discovery sample of children who did not meet clinical criteria for MDD or anxiety disorders, but were experiencing a variable number of depressive and anxious symptoms. There was no significant association of rs2069827 with MDD (OR = 1.01; $p = 0.22$)

TABLE 2 Association of interleukin SNPs with childhood-onset aggression

Gene	SNP (chr:bp ^a)	Genotype ^b	MAF	OR ^c	p
<i>IL1B</i>	rs4849127 (chr2:112844982)	A/G	0.05	0.79	0.43
	rs16944 (chr2:112837290)	A/G	0.33	1.06	0.69
	rs1143634 (chr2:112832813)	A/G	0.22	0.98	0.92
	rs1143643 (chr2:112830725)	T/C	0.36	1.18	0.24
<i>IL2</i>	rs2069762 (chr4:122456825)	C/A	0.27	1.34	0.05
	rs2069778 (chr4:122454980)	A/G	0.15	0.87	0.48
	rs2069772 (chr4:122451978)	C/T	0.27	0.86	0.33
<i>IL6</i>	rs2069827 (chr7:22725837)	T/G	0.08	0.69	0.16
	rs2069837 (chr7:22728408)	G/A	0.07	1.29	0.37
	rs2066992 (chr7:22728630)	T/G	0.07	0.78	0.37
	rs2069840 (chr7:22728953)	G/C	0.33	0.93	0.64
	rs2069861 (chr7:22732035)	T/C	0.12	0.98	0.93
	rs10242595 (chr7:22734612)	A/G	0.31	1.16	0.32

^aLocations based on Genome Reference Consortium Build 37, hg19.

^bGenotypes are reported as A1/A2 correspond to Minor/Major alleles.

^cEffect sizes are reported with respect to the minor allele (A1) as the risk allele in all analyses.

or anxiety (OR = 1.09; $p = 0.56$) in these samples, and the direction of effect was the opposite of that observed in our discovery sample with the T allele more common among cases than controls.

3.5 | Interaction between interleukin SNPs and childhood adversity

In our sample of early life aggression cases, severe childhood adversity (MAYSI-2 Traumatic Experiences scale score ≥ 4) was associated with greater severity of depressive symptoms ($\beta = 10.35$, $p = 4.38 \times 10^{-6}$) but not severity of internalizing problems ($p = 0.08$) or anxiety ($p = 0.07$) when adjusting for age and gender as covariates.

To evaluate whether *IL1B*, *IL2*, or *IL6* genotype moderated the influence of childhood adversity on severity of depressive symptoms, we undertook exploratory gene–environment interaction analyses within cases. These analyses also adjusted for age and gender as covariates. Interactions with severe childhood adversity did not survive multiple testing correction for any of the interleukin SNPs (all $p > 7.69 \times 10^{-4}$, Table 4). We observed suggestive evidence of interaction effects between severe childhood adversity and *IL1B* variant rs1143643 and *IL2* variant rs2069778 ($p < 0.01$, Figure 1). Notably, these interaction effects were driven by a small number of individuals carrying the minor alleles for these variants in our sample ($n < 10$, Figure 1).

4 | DISCUSSION

In this study, we provide an initial exploration of common cytokine gene variants in childhood-onset aggression and internalizing behaviors in a deeply phenotyped Canadian sample. We performed a hypothesis-driven study to evaluate the association of *IL1B*, *IL2*, and *IL6* gene

variants with aggression and internalizing behaviors in children and adolescents, and to explore gene–environment interactions with childhood adversity.

Our findings provide preliminary evidence that *IL6* variant rs2069827 may be associated with severity of depressive symptoms in children and adolescents. Additional studies of rs2069827 in independent cohorts capturing early life depressive symptoms are needed to validate whether or not this association is robust. Notably, rs2069827 has not been previously associated with any human trait in published genome-wide or phenome-wide association studies (Buniello et al., 2019; Denny et al., 2013). However, rs2069827 was previously identified as nominally significant in a Dutch prospective cohort study of longevity investigating 102 SNPs in 16 genes; in this study, the T allele (which had a protective effect on depressive symptoms in our sample) was associated with increased longevity (Soerensen et al., 2013). Although rs2069827 is located in an *IL6* intron and within a putative transcription factor binding site, rs2069827 was not associated with differences in *IL6* expression in RNA expression studies (Soerensen et al., 2013) and was not identified as an expression quantitative trait locus (eQTL) for *IL6* in the Genotype-Tissue Expression (GTEx) project (GTEx Consortium, 2017). Thus, if the preliminary association we observed between rs2069827 and early life depressive symptoms is replicated in future studies, the biological mechanism by which this SNP influences depressive symptoms may not be via altered *IL6* expression.

We also demonstrate the potential contribution of applying hypothesis-driven gene–environment interaction analyses to understand childhood-onset behavior problems. While we did not observe significant interactions between interleukin SNPs and childhood adversity in moderating depressive symptoms, we did observe nominally significant interaction effects for rs1143643 (*IL1B*) and rs2069778 (*IL2*). These SNPs have not been identified as associated

TABLE 3 Association of interleukin SNPs with internalizing behaviors

Gene	SNP	Internalizing problems						Anxiety						Depression					
		Cases			Controls			Cases			Controls			Cases			Controls		
		β_{CAS}	p_{CAS}	p_{CON}	β_{CON}	p_{CON}	p_{META}	β_{CAS}	p_{CAS}	p_{CON}	β_{CON}	p_{CON}	p_{META}	β_{CAS}	p_{CAS}	p_{CON}	β_{CON}	p_{CON}	p_{META}
IL1B	rs4849127	0.54	0.78	0.65	1.05	0.65	0.61	-0.51	0.81	0.06	4.24	0.06	0.90	-3.85	0.11	3.18	0.02	0.56	
	rs16944	-0.19	0.84	0.54	0.60	0.54	0.77	-1.46	0.22	1.07	0.28	0.94	-0.45	0.71	0.61	0.31	0.64		
	rs1143634	-0.11	0.92	0.16	0.16	0.89	0.98	0.44	0.75	1.85	0.11	0.17	-1.74	0.22	0.39	0.58	0.65		
	rs1143643	0.36	0.70	0.66	-0.45	0.66	0.98	-0.17	0.88	-1.00	0.33	0.42	1.49	0.22	-0.76	0.22	0.98		
IL2	rs2069762	-0.85	0.40	0.24	1.33	0.24	0.83	0.05	0.97	1.14	0.31	0.45	0.002	0.99	0.85	0.23	0.39		
	rs2069778	-0.52	0.69	0.12	-2.23	0.12	0.17	0.88	0.60	-0.68	0.63	0.99	1.17	0.49	-0.57	0.51	0.99		
	rs2069772	2.56	0.01	0.45	-0.90	0.45	0.21	0.32	0.81	-0.09	0.94	0.91	0.80	0.54	-0.36	0.62	0.94		
	rs2069827	1.01	0.53	0.75	-0.63	0.75	0.82	-3.66	0.07	-4.09	0.04	0.006	-4.98	0.01	-2.80	0.02	7.15×10^{-4}		
IL6	rs2069837	-0.07	0.97	0.76	-0.57	0.76	0.81	-0.56	0.82	0.60	0.75	0.94	2.70	0.34	0.35	0.76	0.38		
	rs2066992	1.24	0.49	0.04	4.19	0.04	0.05	1.77	0.43	4.28	0.03	0.04	2.98	0.16	2.40	0.05	0.02		
	rs2069840	0.49	0.61	0.52	-0.72	0.52	0.93	1.35	0.26	-0.56	0.61	0.67	1.11	0.35	0.80	0.24	0.13		
	rs2069861	-1.43	0.29	0.90	-0.19	0.90	0.40	-0.66	0.71	1.54	0.32	0.65	-2.59	0.14	0.26	0.79	0.40		
rs10242595	0.38	0.70	0.08	1.85	0.08	0.13	1.52	0.22	0.67	0.53	0.19	2.09	0.10	0.17	0.79	0.18			

Association between SNPs and symptom severity was tested by linear regression with adjustment for age and gender as covariates, using an additive genotypic model; p -values shown are not corrected for multiple testing. $p_{cor} = 13^* p$ -value; β_{CAS} , effect size in cases; β_{CON} , effect size in controls; p_{CON} , p -value in cases; p_{CON} , p -value in controls; p_{META} , p -value in inverse variance-weighted fixed effects meta-analysis of effect in cases and controls.

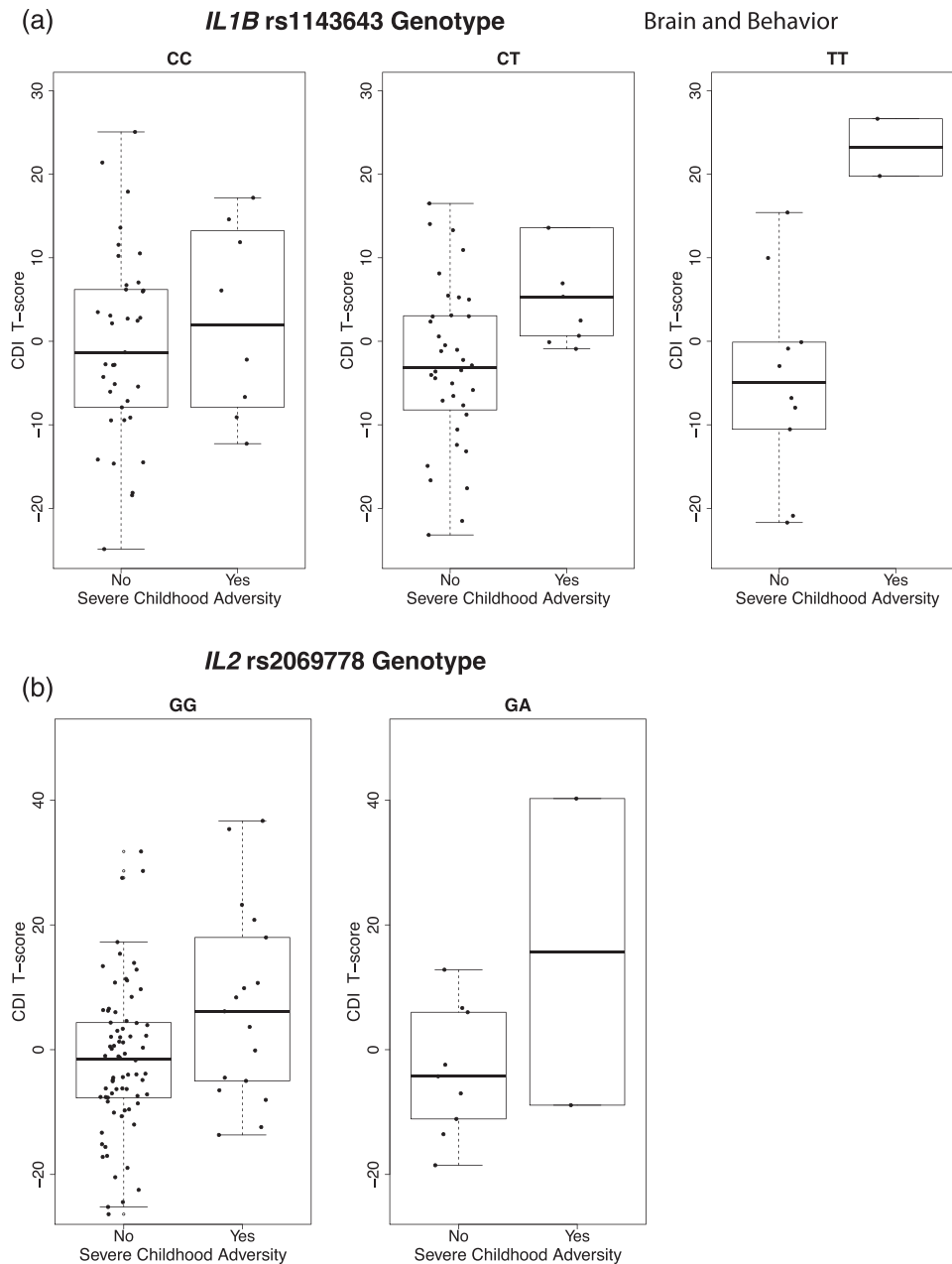


FIGURE 1 Statistical interaction between severe childhood adversity and (a) *IL1B* rs1143643 and (b) *IL2* rs2069778 for depressive symptoms. Depressive symptom severity (measured using the Children's Depression Inventory, CDI) was plotted stratified by exposure to severe childhood adversity (defined as MAYSI-2 Traumatic Experiences scale score ≥ 4) for each genotype at (a) *IL1B* rs1143643 and (b) *IL2* rs2069778. CDI T-scores were plotted after adjustment for age and gender as covariates using linear regression model residuals. The AA genotype for *IL2* rs2069778 is not included in (b), as only one individual in our sample had the AA genotype; this individual did not have a history of severe childhood adversity

with any human trait in published genome-wide or phenome-wide association studies (Buniello et al., 2019; Denny et al., 2013). However, a gene-based study of three SNPs in *IL1B* reported that the C allele of rs1143643 was associated with non-response to antidepressants and reduced amygdala reactivity among 256 patients with depression (Baune et al., 2010). Interestingly, two gene-environment interaction studies have reported that *IL1B* variants interact with stress to modulate depressive symptoms. In a Hungarian sample of 1,053

adult participants, rs1143643 was found to interact with recent life stress to modulate depression and anxiety (Kovacs et al., 2016). More specifically, T allele carriers were protected from depressive and anxiety symptoms when exposed to negative life events (Kovacs et al., 2016); this is the opposite effect observed in our sample of children, where T allele carriers showed greater depressive symptom severity when exposed to severe childhood adversity. In an American sample of 198 preschoolers exposed to maltreatment, the T allele of rs1143633

TABLE 4 Gene–environment interactions between interleukin SNPs and traumatic life experiences

Gene	SNP	β	p
<i>IL1B</i>	rs4849127	9.56	0.36
	rs16944	-4.65	0.32
	rs1143634	-1.30	0.82
	rs1143643	13.94	4.62×10^{-3}
<i>IL2</i>	rs2069762	-0.80	0.88
	rs2069778	18.24	9.23×10^{-3}
	rs2069772	4.95	0.47
<i>IL6</i>	rs2069827	-13.3	0.07
	rs2069837	-22.96	0.11
	rs2066992	14.73	0.06
	rs2069840	-7.80	0.16
	rs2069861	-1.55	0.79
	rs10242595	-0.03	0.99

Note: Statistical interaction between each SNP and severe childhood adversity for depressive symptoms was tested by linear regression using a SNP*adversity interaction term with adjustment for main effects of SNP genotype and adversity as well as covariates age and gender; p -values shown are not corrected for multiple testing, $p_{\text{cor}} = 13 * p$ -value.

(which is in linkage disequilibrium with the T allele of rs1143643, $r^2 = 0.98$ (1000 Genomes Project Consortium, 2015)) was associated with greater depressive symptoms among children experiencing recent life stress, although no such gene–environment interaction was observed between rs1143643 and maltreatment (Ridout et al., 2014). The differences in direction of effect across studies may be accounted for by differences in study population (ancestry, age, clinical presentation) and in environmental and outcome measures. Overall, several previous studies have implicated rs1143643 in depression and in gene–environment interactions in depression, suggesting this variant is a promising target for future gene–environment interaction studies in larger childhood depression samples.

Our study was subject to several limitations. First, early life aggression and internalizing behaviors are complex traits involving hundreds of variants, and our hypothesis-driven study did not consider the potential genetic effects of all interleukin gene variants or other genes outside of the interleukins. Second, the SNPs surviving quality control did not capture all common variations in the interleukin genes studied. Third, although we restricted our analyses to European ancestry individuals, we cannot exclude the possibility of residual population stratification given that ancestry was determined by self-report. This is particularly notable given that rs2069827, the SNP we identified as associated with depressive symptoms in our sample, is polymorphic in European but not Asian or African populations (1000 Genomes Project Conso, 2015). Fourth, given that our sample comprised European ancestry subjects, our findings may not be generalizable to other ancestral populations. Fifth, questionnaire data capturing the outcome variables being studied were incomplete in our sample, resulting in loss

of subjects available for analysis and reducing statistical power (missingness rates varied from 2% for parent-reported problem behaviors captured using the CBCL to 5% for self-reported depressive symptoms captured using the CDI). Finally, as we did not have a suitable replication sample, our study is exploratory in nature and requires validation in independent samples.

In summary, we investigated genetic variation in *IL1B*, *IL2*, and *IL6* as potential risk factors for early life aggression and internalizing behaviors, and explored interactions between these genes and severe childhood adversity in moderating depressive symptoms. Our findings suggest rs2069827 (*IL6*) as a potential risk variant for depressive symptoms in children and adolescents, which should be prioritized for investigation in future studies of childhood mood symptoms. We also observed nominally significant interactions between rs1143643 (*IL1B*) and rs2069778 (*IL2*) and childhood adversity in moderating depressive symptoms, whereby children and adolescents carrying minor alleles for these variants were more sensitive to the depressogenic effects of childhood adversity. While our findings are from exploratory investigations, they may be helpful in revealing insights into the role of interleukins in early life aggression and internalizing behaviors. As larger child and adolescent depression samples become available in the future through international collaboration, it will be important to examine whether our findings are replicated in statistically robust, hypothesis-free GWAS and gene–environment analyses.

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CONFLICT OF INTEREST

James L. Kennedy is a member of the Scientific Advisory Board of Myriad Neuroscience (unpaid). James L. Kennedy and Clement C. Zai are authors on several patents relating to pharmacogenetic tests for psychiatric medications. The remaining authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Genetic data used in this study are not available to be shared due to privacy or ethical restrictions.

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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