Development and Psychopathology **29** (2017), 837–843

doi:10.1017/S0954579416000511

Extremely low birth weight babies grown up: Gene–environment interaction predicts internalizing problems in the third and fourth decades of life

AYELET LAHAT, RYAN J. VAN LIESHOUT, KAREN J. MATHEWSON, JAMES MACKILLOP, SAROJ SAIGAL, KATHERINE M. MORRISON, MICHAEL H. BOYLE, AND LOUIS A. SCHMIDT
McMaster University

Abstract

Extremely low birth weight (ELBW; <1000 g) infants have been exposed to stressful intrauterine and early postnatal environments. Even greater early adversity has been experienced by ELBW survivors who were also born small for gestational age (SGA; <10th percentile for GA) compared to those born appropriate for GA (AGA). ELBW survivors, particularly those born SGA, face increased risk for internalizing problems compared to normal BW (NBW; ≥2500 g) controls. Internalizing problems are related to allelic variations in the promoter region of the serotonin transporter linked polymorphic region gene (*5-HTTLPR*). We followed the oldest longitudinal cohort of ELBW survivors to adulthood. Participants provided buccal cells and reported on internalizing problems, using the Young Adult Self-Report when they were in their mid-20s (ELBW/SGA, $N = 28$; ELBW/AGA, $N = 60$; NBW, $N = 81$) and mid-30s (ELBW/SGA, $N = 27$; ELBW/AGA, $N = 58$; NBW, $N = 76$). The findings indicate that ELBW/SGAs carrying the *5-HTTLPR* short allele reported increased internalizing problems, particularly depression, during the third and fourth decades of life. This is the first known report on gene–environment interactions predicting psychopathology among ELBW survivors. Our findings elucidate putative neurobiological pathways that underlie risk for psychopathology.

Infants born at extremely low birth weight (ELBW; <1000 g) are the tiniest and most vulnerable babies and are exposed to negative environmental stressors in prenatal and early postnatal life. ELBW infants provide an experiment in nature that can illustrate how exposure to extreme early adversity may play a role in gene–environment interactions. Preterm infants, and particularly those born at ELBW, are exposed to suboptimal intrauterine conditions, which create an adverse early environment. These infants spend their first weeks or months of life in the neonatal intensive care unit (NICU), an extremely stressful environment, where these infants require numerous invasive medical procedures to diagnose and treat life-threatening conditions. This environment contrasts greatly from the calm and protective maternal intrauterine environment that they were to experience during this period of development (Chau et al., 2014). However, premature birth has brought these fragile neonates to repeated exposure of highly stressful and potentially painful experiences that are developmentally unexpected.

Later in development, survivors of ELBW are at increased risk for a wide range of neurosensory impairments, behavioral and emotional problems, decrements in intellectual performance, and poorer general health than those born at normal BW (NBW; BW ≥ 2500 g; Levy-Shiff et al., 1994; Rickards, Kelly, Doyle, & Callanan, 2001; Saigal & Doyle, 2008; Saigal et al., 1996; Saigal, Hoult, Streiner, Stoskopf, & Rosenbaum, 2000; Saigal, Rosenbaum, Szatmari, & Campbell, 1991; Saigal, Szatmari, Rosenbaum, Campbell, & King, 1990, 1991).

Because of advances in fetal and neonatal medicine, the first generation of ELBW survivors born in the early era of neonatal intensive care has reached adulthood. This cohort of survivors was born in the late 1970s–early 1980s, prior to many medical advancements, including the use of surfactant for proper lung functioning. Thus, rates of ELBW survival were substantially lower than today. In the present prospective, longitudinal study, we examined whether gene–environment interactions predicted internalizing problems among survivors of ELBW in their mid-20s and mid-30s. To the best of our knowledge, this is the first study to report on the impact of gene–environment interactions on psychopathology among ELBW adult survivors.

Studies that have followed infants born at ELBW or very low BW (VLBW; <1500 g), and extreme prematurity

This research was supported by Canadian Institutes of Health Research Grants CIHR:TMH-103145 (to L.A.S) and CIHR:MOP42536 (to S.S.) and National Institute of Child Health and Human Development Grant NICHD:1-R01HD40219 (to S.S.). This research was also supported by a Social Sciences and Humanities Research Council Banting Post-Doctoral Fellowship (to A.L.) and the Peter Boris Chair in Addictions Research (to J.M.). We thank the study participants and their families for their continued participation in this work and Paz Fortier, Sue McKee, Vladimir Miskovic, Jordana Waxman, and Shirien Yunus for their help with data collection and data entry. We also thank Jane Foster for performing the genotyping.

Address correspondence and reprint requests to: Ayelet Lahat, Department of Psychology, Neuroscience & Behaviour, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada; E-mail: lahata@mcmaster.ca.

(<26 weeks), have reported increased risks of psychopathology during childhood and adolescence, particularly inattention and anxiety (Botting, Powls, Cooke, & Marlow, 1997; Farooqi, Hägglöf, Sedin, Gothefors, & Serenius, 2007; Indredavik, Vik, Heyerdahl, Kulseng, & Brubakk, 2005; Indredavik et al., 2004; Johnson et al., 2010; Saigal, Pinelli, Hoult, Kim, & Boyle, 2003; Stevenson, Blackburn, & Pharoah, 1999; Szatmari, Saigal, Rosenbaum, & Campbell, 1993; Szatmari, Saigal, Rosenbaum, Campbell, & King, 1990). However, very few studies have followed ELBW survivors into adulthood. Nonetheless, the studies that have followed them reported increased levels of anxiety and depression in their mid-20s and mid-30s (Boyle et al., 2011), as well as shyness, timidity, behavioral inhibition, and risk aversion (Schmidt, Miskovic, Boyle, & Saigal, 2008; Waxman, Van Lieshout, Saigal, Boyle, & Schmidt, 2013). These internalizing problems may be particularly prominent among ELBW survivors who were born small for gestational age (SGA; BW < 10th percentile for GA) compared to those born at an appropriate weight for GA (AGA; BW > 10th percentile for GA; Boyle et al., 2011; Van Lieshout, Boyle, Saigal, Morrison, & Schmidt, 2015). In a study with adults aged 18–27, who were born at VLBW, only those born SGA were at elevated risk for emotional instability and depression compared to those born at VLBW and AGA (Raikkonen et al., 2008; Strang-Karlsson et al., 2008). Thus, intrauterine growth restriction and size for GA are factors that may modify the risk of developing psychopathology. According to a cumulative risk model, individuals born at both ELBW and SGA are placed at the greatest risk for negative developmental outcomes, including psychopathology.

Internalizing problems are also related to allelic variations in the promoter region of the serotonin transporter linked polymorphic region gene (*5-HTTLPR*; Lesch et al., 1996). The short allele of *5-HTTLPR* is associated with lower transcriptional efficiency compared to the long allele, with functional effects on neural circuits regulated by serotonin (Hariri et al., 2002). This reduction is linked to a predisposition for depression, anxiety, and negative emotionality (Munafo et al., 2003). In addition, carriers of the short allele are more likely than carriers of the long allele to develop depression or depressive symptoms in response to adverse or stressful life events (Benjet, Thompson, & Gotlib, 2010; Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Caspi et al., 2003; Taylor et al., 2006; Uher & McGuffin, 2008). Taken together, these studies suggest that the risk for internalizing problems following exposure to significant psychological and/or physiological stress is elevated among carriers of *5-HTTLPR* short allele and diminished among those carrying the long allele. Thus, given the early pre- and postnatal (e.g., experiences in the NICU) adversity to which ELBW survivors, particularly SGAs, are exposed, it is likely that similar genetic variations leading to internalizing problems will be observed among these survivors.

Here, we examined the combined effect of BW status (a proxy of intrauterine and early postnatal environmental exposure to stress) and *5-HTTLPR* on internalizing problems among survivors of ELBW during their mid-20s and mid-30s. We prospectively followed the oldest known longitudinal cohort of ELBW survivors and a matched group of NBW controls. ELBW participants were further stratified into SGA and AGA groups. To the best of our knowledge, this is the first study to examine the interaction effect of BW status *and 5-HTTLPR* on adult psychopathology among survivors of ELBW. Given that ELBW survivors, particularly those born SGA, are at increased risk for internalizing problems in adulthood and because carrying the short allele of *5-HTTLPR* has been linked with these problems as well, our study focused on internalizing problems in the mid-20s and 30s. Participants were classified into either *5-HTTLPR* short ($SS/SL_G/SL_A$) or long (L_AL_G/L_AL_A) alleles. Based on previous research (Boyle et al., 2011; Van Lieshout et al., 2015), we expected that survivors of ELBW who carry the short allele would report increased internalizing problems. Furthermore, we expected cumulative risk for ELBW/SGA short allele carriers and predicted that this group will report the most internalizing problems.

Method

Participants and cohort overview

The study followed 397 predominantly Caucasian infants who were born at ELBW (501–1000 g) between 1977 and 1982 to residents of a geographically defined region in central-west Ontario, Canada. Infants were weighed at birth, and GA was estimated from maternal report of the first day of the last menstrual period. Using Canadian norms (Kramer et al., 2001), infants with BWs < 10th percentile for GA were classified as SGA and the remainder as AGA. Follow-up assessments were conducted when participants were 22–26 and 29–36 years old. Of the original 397 infants, 179 survived to hospital discharge. There were 13 later deaths, and 166 survived to adulthood. At age 22–26, Young Adult Self-Report (YASR; Achenbach, 1997) data were collected on 142 of the 166 adult survivors. At age 29–36, 100 ELBW participants completed the YASR. At age 29–36, 92 ELBW participants provided buccal cell samples, with 89 providing an adequate amount for processing. There was 1 participant who did not complete the age 22–26 assessment, but completed the age 29–36 assessment.

The NBW control group was recruited when both groups were 8 years old. This group comprised 145 children born at term according to maternal report, between 1977 and 1981. The control sample was matched with the ELBW cohort on child age, sex, and socioeconomic status (Saigal, Szatmari, et al., 1991). At age 22–26, YASR data were collected on 133 of the 145 NBW control participants. During the 29–36 year assessment, 89 NBW participants completed the YASR. At age 29–36, 89 participants provided buccal cell samples, with 81 participants providing an adequate amount for processing. The study was approved by the Hamilton Integrated Research Ethics Board, and informed consent was obtained from all participants.

https://doi.org/10.1017/S0954579416000511 Published online by Cambridge University Press

Measures

YASR. The YASR (Achenbach, 1997) was completed during both the mid-20s and mid-30s assessments. The YASR contains problem items rated from 0 (*not true*) to 2 (*very true or often true*). Our study focused only on scales related to internalizing problems. Based on experts' ratings of the items' consistency with classifications in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; American Psychiatric Association, 1994), the items were grouped into DSM-oriented scales (Achenbach, Bernstein, & Dumenci, 2005). We analyzed continuous scores for the depressive (mid-20s $\alpha = 0.88$; mid-30s $\alpha = 0.87$), anxiety (mid-20s $\alpha = 0.77$; mid-30s $\alpha = 0.82$), somatic (mid-20s $\alpha = 0.74$; mid-30s $\alpha = 0.77$), and avoidant personality (mid-20s $\alpha = 0.76$; mid-30s $\alpha = 0.80$) problems scales. In addition, we analyzed a higher order internalizing problems scale (mid-20s $\alpha = 0.93$; mid-30s $\alpha = 0.93$), which was composed of all other scales.

DNA extraction and genotyping. Participants provided buccal cell samples during the mid-30s visit. DNA was isolated from buccal swabs using the QIAamp DNA Investigator kit from Qiagen. Genotyping for *5-HTTLPR* was done in a two-step process. First, the gene was amplified using polymerase chain reaction (PCR). Second, a portion of the PCR product underwent restriction fragment length polymorphism analysis where a restriction enzyme is used to digest the DNA into fragments that can translate into genotype. Genotyping was performed without knowledge of the participant's BW group. Either a 484- or a 528-base pair fragment was generated using forward primers 5′-GGCGTTGCCGCTCTGAATGC and reverse primer 5′-GAGGGACTGAGCTGGACAACCAC (Lesch et al., 1996). AccuPrime GC-Rich DNA polymerase (invitrogen 12337-016) was used to amplify the gene. The 25 μl amplification mixture contained 100 ng of genomic DNA, 0.2 μM of each primer, 1X AccuPrime GC-Rich Buffer A, and 1 U of AccuPrime GC-Rich DNA polymerase. The cycling conditions were as follows: initial denaturation at 95 °C for 3 min; followed by seven cycles of 95 °C for 30 s, 68 °C for 30 s, and 72 °C for 1 min; then seven cycles of 95 °C for 30 s, 67 °C for 30 s, and 72 °C for 1 min; and another seven cycles of 95 °C for 30 s, 66 °C for 30 s, and 72 °C for 1 min. Final extension took place at 72 °C for 10 min. The uncut product was run on a 1.5% agarose gel at 50 V for 90 min. Twelve microliters of the PCR product was then cut using the restriction enzyme MSPI (New England Biolabs) for 3 hr at 37 °C (Praschak-Rieder et al., 2007). The cut product was run on a 4%–20% tris/borate/EDTA gel at 14 mA for 100 min to separate the bands (invitrogen EC6225BOX). *5-HTTLPR* is found on chromosome 17. *5-HTTLPR* is a 44 base pair deletion in the promoter region at base pairs 1212 to 1255, resulting in a short or long allele (Heils et al., 1996). Within the extra 44 base pairs of the long variant there is also a single nucleotide polymorphism, which is a substitution of adenosine to guanine present at nucleotide 6 (Hu et al., 2006). The resulting band pattern for the cut PCR product was 340 base pairs for long-A, 297 base pairs for short, and 166 + 174 base pairs for long-G (Praschak-Rieder et al., 2007). The participants were classified according to allele into either short ($SS/SL_G/SL_A$) or long (L_AL_G/L_AL_A). No significant differences were found between the BW groups in the distribution of short or long *5-HTTLPR* alleles (all *p*s > .38). The genotypes were within Hardy–Weinberg equilibrium.

Results

Preliminary analyses

At both the mid-20s and mid-30s assessments, participants who were included and not included in the analyses did not significantly differ on age (all *p*s > .45), BW (all *p*s > .24), GA (all *p*s > .08), or level of education (all *p*s > .48). Significant gender differences were found between participants included and not included in the analysis at both the mid-20s assessment, χ^2 (1) = 9.53, $p < .01$, and mid-30s assessment, χ^2 (1) = 14.48, $p < .0001$, with more females (mid-20s, $N = 103$; mid-30s, $N = 103$) than males (mid-20s, $N = 63$; mid-30s, $N = 57$) included in both visits.

Statistical analyses included participants who had both YASR and *5-HTTLPR* data available. Data were examined for outliers and participants with more than 3 *SD* from the mean were removed from all analyses. These outliers were removed because they can affect the mean dramatically and not represent the majority of the group. This resulted in one ELBW/SGA and two ELBW/AGA participants being excluded from the analysis on the internalizing scale at the mid-20s assessment. As well, one ELBW/SGA participant was excluded from the analysis on the internalizing scale at the mid-30s assessment. Table 1 indicates the number of participants who were included in each analysis.

To ascertain that the three BW groups were comparable on sociodemographic measures (Hollingshead, 1969), a series of analyses comparing ELBW/SGA, ELBW/AGA, and NBW participants were carried out (Table 1). A univariate analysis of variance revealed significant age differences during the mid-20s assessment, F (2, 163) = 3.89, $p < .05$, $\eta_p^2 = 0.05$, such that ELBW/SGAs were significantly younger than NBWs (Table 1). Therefore, participant age was entered as a covariate in all further analyses. No other significant sociodemographic differences emerged between the three groups. Next, we analyzed each scale using separate analyses of variance for the 22–26 and 29–36 time points. Birth weight group (ELBW/SGA, ELBW/AGA, NBW) and *5-HTTLPR* efficiency (short, long) were between-subject factors, and age was included as a covariate.

Mid-20s assessment

The results for the internalizing scale revealed a main effect for BW group, F (2, 159) = 3.70, $p < .05$, $\eta_p^2 = 0.04$, with ELBW/SGA participants ($M = 20.48$, $SE = 2.03$) reporting more internalizing problems than NBW participants ($M = 14.09$, $SE = 1.24$), $p < .05$. ELBW/AGA participants

https://doi.org/10.1017/S0954579416000511 Published online by Cambridge University Press

Table 1. *Comparison of demographic variables by birth weight group at the mid-20s (age 22–26) and mid-30s (age 29–36) assessments*

Variable	Mid-20s Assessment			Mid-30s Assessment		
	ELBW/SGA	ELBW/AGA	NBW	ELBW/SGA	ELBW/AGA	NBW
N (males, females)	28 (7, 21)	60 (26, 34)	81 (31, 50)	27 (6, 21)	58 (26, 32)	76 (26, 50)
Mean (*SD*)						
Age (years)	23.05[a] (1.15)	23.22 (1.14)	23.64 (1.04)	32.02 (1.46)	32.15 (1.77)	32.50 (1.33)
BW (g)	832.86[b] (129.24)	820.92[b] (128.75)	3343.02 (423.89)	830.37[b] (131.02)	824.05[b] (130.08)	3329.09 (422.77)
GA (weeks)	30.07[b,c] (1.98)	25.85[b] (1.36)	40.00 (0.00)	30.11[b,c] (2.01)	25.88[b] (1.38)	40.00 (0.00)
Highest education level	4.62 (1.30)	4.42 (1.24)	4.81 (1.42)	5.48 (1.12)	5.43 (0.97)	5.49 (1.06)
SES at 8 years of age	3.12 (0.95)	3.10 (0.85)	2.99 (1.02)	3.12 (0.97)	3.07 (0.86)	3.01 (0.99)

Note: ELBW, Extremely low birth weight; SGA, small for gestational age; AGA, average for gestational age; NBW, normal birth weight; SES, socioeconomic status (Hollingshead, 1969). Highest level of education ranges from 1 to 7, with 7 being the highest score; SES ranges from 1 to 5, with 5 being the lowest score.
[a]Compared with NBW participants, $p < .05$.
[b]Compared with NBW participants, $p < .0001$.
[c]Compared with ELBW/AGA participants, $p < .0001$.

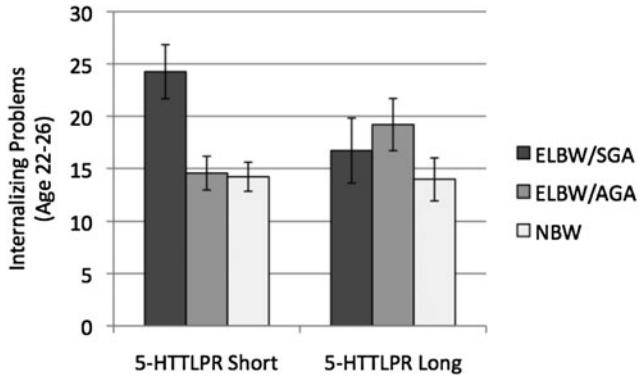

Figure 1. Internalizing problems at age 22–26 as a function of birth weight group and *5-HTTLPR* allele. Bars indicate standard errors.

reported intermediate levels of internalizing problems ($M = 16.87$, $SE = 1.49$). This main effect was qualified by a significant Birth Weight Group × *5-HTTLPR* interaction, F (2, 159) $= 2.98$, $p < .05$, $\eta_p^2 = 0.04$. Pairwise comparisons indicated that among short allele carriers, ELBW/SGA participants reported more internalizing problems than both ELBW/AGA and NBW participants (all ps $< .005$), which did not significantly differ from each other. No significant differences were found between the three BW groups among carriers of the long allele. No significant differences were found between short and long allele carriers within each BW group (Figure 1).

The results for the depressive problems scale revealed a significant Birth Weight Group × *5-HTTLPR* interaction, F (2, 157) $= 4.37$, $p < .01$, $\eta_p^2 = 0.05$. Pairwise comparisons indicated that among short allele carriers, ELBW/SGA participants reported more depressive problems than both ELBW/AGA and NBW participants (all ps $< .005$), which did not significantly differ from each other. No significant differences were found between the three BW groups among carriers of the long allele. Furthermore, among ELBW/SGA participants, those carrying the short allele reported significantly more depressive problems than those carrying the long allele, $p < .05$. No significant differences were found between short and long allele carriers within the ELBW/AGA or NBW groups (Figure 2).

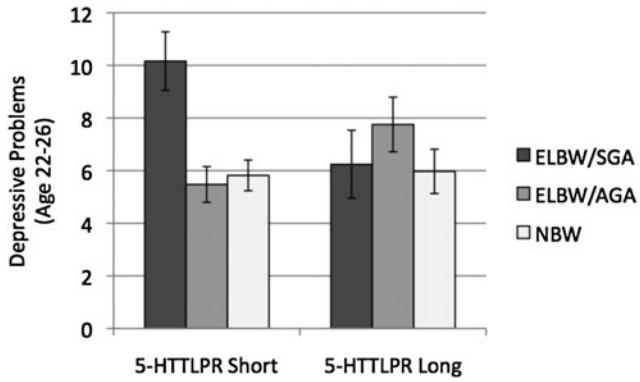

Figure 2. Depressive problems at age 22–26 as a function of birth weight group and *5-HTTLPR* allele. Bars indicate standard errors.

https://doi.org/10.1017/S0954579416000511 Published online by Cambridge University Press

The results for the anxiety problems scale revealed a main effect for BW group, F (2, 159) = 3.78, $p < .05$, $\eta_p^2 = 0.05$, with ELBW/SGA participants ($M = 4.66$, $SE = 0.50$) reporting significantly more anxiety problems than NBW participants ($M = 3.21$, $SE = 0.30$), $p < .05$. ELBW/AGA participants reported intermediate levels of anxiety problems ($M = 4.15$, $SE = 0.36$). No other significant effects were found for anxiety problems during the mid-20s assessment.

No significant main effects or interactions were found for somatic or avoidant personality problems during the mid-20s assessment.

Mid-30s assessment

The results for the internalizing scale revealed a main effect for BW group, F (2, 151) = 4.25, $p < .05$, $\eta_p^2 = 0.05$, with ELBW/SGA participants ($M = 18.61$, $SE = 2.16$) reporting more internalizing problems than NBW participants ($M = 11.97$, $SE = 1.38$), $p < .05$. ELBW/AGA participants reported intermediate levels of internalizing problems ($M = 16.69$, $SE = 1.65$). No other significant effects or interactions were found for internalizing problems during the mid-30s assessment. The interaction between BW group and *5-HTTLPR* was not significant.

The results for the depressive problems scale revealed a main effect for BW group, F (2, 146) = 3.09, $p < .05$, $\eta_p^2 = 0.05$. Pairwise comparisons probing this main effect were not significant. The main effect of BW group was qualified by a significant Birth Weight Group × *5-HTTLPR* interaction, F (2, 146) = 3.05, $p < .05$, $\eta_p^2 = 0.04$. Pairwise comparisons indicated that among short allele carriers, ELBW/SGA participants reported more depressive problems than NBW participants, $p < .05$, but did not significantly differ from ELBW/AGA participants. No significant differences were found between the three BW groups among carriers of the long allele. No significant differences were found between short and long allele carriers within each BW group (Figure 3).

The results for the avoidant personality problems scale revealed a main effect for BW group, $F(2, 149) = 3.07$, $p < .05$, $\eta_p^2 = 0.04$. Pairwise comparisons probing this main effect were not significant. No other significant effects or interactions were found for avoidant personality problems during the mid-30s assessment.

No significant main effects or interactions were found for anxiety or somatic problems during the mid-30s assessment.

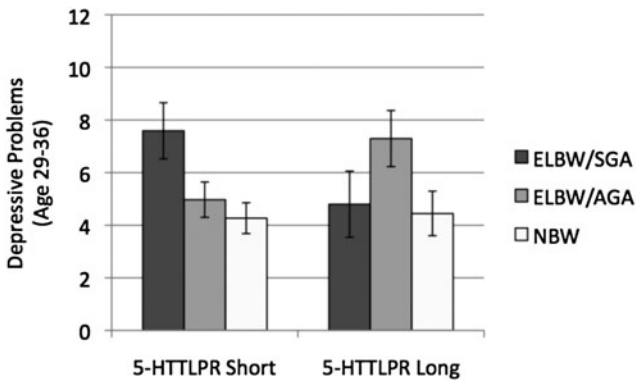

Figure 3. Depressive problems at age 29–36 as a function of birth weight group and *5-HTTLPR* allele. Bars indicate standard errors.

Stability of internalizing problems

In order to examine whether internalizing problems were stable between the two assessments points, Pearson correlations were conducted on outcome variables. The findings revealed that scores on the YASR scale during the mid-20s assessment were significantly positively correlated with these scores during the mid-30s assessment: internalizing problems, $r(153) = .68$, $p < .0001$; depressive problems, $r(150) = .61$, $p < .0001$; anxiety problems, $r(154) = .59$, $p < .0001$; somatic problems, $r(151) = .39$, $p < .0001$; avoidant personality problems, $r(152) = .67$, $p < .0001$.

Discussion

The present study is the first known to examine gene–environment interactions predicting psychopathology among ELBW survivors. As expected, the findings suggest that increased levels of internalizing problems are found among ELBW survivors through their early to mid-30s. These internalizing problems were particularly increased among ELBW survivors who were also born SGA. For the first time, we also report an interaction between BW group and *5-HTTLPR*. Across their 20s and 30s, ELBW/SGAs carrying the short allele of *5-HTTLPR* reported more internalizing problems, particularly depression, as compared to ELBW/AGAs and NBW controls. Taken together, these data suggest that ELBW/SGA survivors are at increased risk for internalizing problems, particularly those carrying the short allele of *5-HTTLPR*. These findings were obtained when these survivors were in their third decade of life and remained stable into their fourth decade. Internalizing scores during the mid-20s assessment were significantly and substantially positively correlated with internalizing scores during the mid-30s assessment, suggesting that these problems were stable between ages 22–26 and 29–36.

The present study is consistent with previous studies suggesting increased internalizing problems among adults born at ELBW (Boyle et al., 2011; Schmidt et al., 2008; Van Lieshout et al., 2015; Waxman et al., 2013). Our findings extend this body of research by suggesting that *5-HTTLPR* is a factor in the development of internalizing problems among survivors of ELBW. In particular, our findings support a cumulative risk model, according to which ELBWs who were also born SGA were at the greatest risk for internalizing problems during their mid-20s and mid-30s. These findings underscore the importance of examining moderating neurobiological factors that contribute to developmental outcomes and risk for psychopathology among survivors of prematurity.

ELBW infants, particularly those who were also born SGA, have been exposed to a stressful intrauterine and early postnatal environment. Birth weight and GA are surrogate

https://doi.org/10.1017/S0954579416000511 Published online by Cambridge University Press

markers of the fetal environment (Boyle et al., 2011). This environment may become stressful due to a variety of factors, including maternal genes, health behavior during pregnancy, and adverse maternal exposure. This stressful intrauterine environment, in combination with the stressful NICU environment, exposes these infants to adverse early experiences. Thus, our findings are also consistent with previous research suggesting that in response to negative life events, individuals carrying the short allele of *5-HTTLPR* are more likely than those carrying the long allele to experience internalizing symptoms, particularly depression (e.g., Caspi et al., 2003, 2010; Uher & McGuffin, 2008).

It is important to note that our findings were significant for the depressive problems scale at both the mid-20s and mid-30s assessments, as well as for the internalizing problems scale at the mid-20s assessment. We did not find significant interactions for the other scales that we analyzed. These findings suggest that the gene–environment interaction observed in this study was stable across the two adult assessment points for depression problems only. The gene–environment interaction for the internalizing problems scale was not stable over time. The reason is that this scale comprises all other internalizing scales, for which no interaction effects were observed, other than the depression problems scale. The finding that *5-HTTLPR* interacts with adverse environmental influences in predicting depression is consistent with previous studies (Benjet et al., 2010; Caspi et al., 2003, 2010; Taylor et al., 2006; Uher & McGuffin, 2008), which suggest that early adversity, in combination with the short allele of *5-HTTLPR*, plays a role in the development of depression.

Several limitations to the present study should be noted. First, our participants were born and raised in a developed country, where medical care and education are universally available.

Second, it is important to note that our sample size is relatively small for a gene–environment study. However, given the uniqueness of our sample, and the fact that our results are consistent with prior gene–environment studies (Benjet et al., 2010; Caspi et al., 2003, 2010; Taylor et al., 2006; Uher & McGuffin, 2008), we believe this limitation does not undermine the importance of our findings.

Third, although our ELBW and NBW groups were matched on age, gender, and socioeconomic status, and the participants in the mid-20s and mid-30s assessments did not differ on important variables, our sample has undergone attrition due to loss to follow-up over the past 30 years. However, no significant sociodemographic differences were found between those who continued in the study and those who dropped out.

Fourth, our participants were born before the use of surfactant, at a time when survival rate of ELBW infants was lower, and therefore in order to survive, SGAs, on average, had a higher GA than AGAs. Although our sample does not include ELBW/SGAs with an earlier GA (because they did not survive), our findings are in line with research suggesting that late preterm infants face increased risk for physical and mental health problems, as well as mortality, compared to full-term infants (e.g., Engle, Tomashek, & Wallman, 2007; van Baar, Vermaas, Knots, de Kleine, & Soons, 2009). Furthermore, although our sample was born before 1990, when surfactant became widely used in neonatal intensive care, the literature suggests that mental health problems, particularly internalizing problems, are also prevalent among ELBW samples born after 1990 (Anderson, Doyle, & Group, 2003; Conrad, Richman, Lindgren, & Nopoulos, 2010; Farooqi et al., 2007; Hack et al., 2009; Methúsalemsdóttir, Egilson, Guðmundsdóttir, Valdimarsdóttir, & Georgsdóttir, 2013; Taylor, Margevicius, Schluchter, Andreias, & Hack, 2015).

Fifth, our sample is unique because it includes survivors of ELBW who are currently in their fourth decade of life. Hence, replication of this study is currently not possible given the uniqueness of this sample. However, our findings are in line with research on methylation of the serotonin transporter gene (solute carrier family C6, member 4 [*SLC6A4*]) in a cohort of children who were born very preterm (24- to 32-weeks GA; Chau et al., 2014). The findings from that work suggest that greater methylation of *SLC6A4* among very preterm school-age children was significantly associated with total problems on the Child Behavior Checklist (Achenbach & Rescorla, 2001). Although findings from the present study and Chau et al.'s (2014) study point to similar conclusions, future research should attempt to replicate the findings of the present study in other samples of ELBW adult survivors.

In sum, this is the first known study to examine gene–environment interactions in predicting internalizing problems among adult survivors of ELBW. Using the oldest known longitudinally followed cohort of ELBW survivors, we found increased internalizing problems among ELBW survivors who were also born SGA and carried the short allele of *5-HTTLPR*. Our findings are novel and possibly elucidate the neurobiological pathways underlying risk for psychopathology. Understanding the factors playing a role in developmental trajectories leading to psychopathology in these survivors can provide valuable insights for prevention and intervention efforts particularly given the worldwide increase in rates of premature birth.

References

Achenbach, T. M. (1997). *Manual for the Young Adult Self-Report and Young Adult Behavior Checklist*. Burlington, VT: University of Vermont, Department of Psychiatry.

Achenbach, T. M., Bernstein, A., & Dumenci, L. (2005). DSM-oriented scales and statistically based syndromes for ages 18 to 59: Linking taxonomic paradigms to facilitate multitaxonomic approaches. *Journal of Personality Assessment*, *84*, 49–63.

Achenbach, T. M., & Rescorla, L. A. (2001). *Manual for the ASEBA School-Age Forms & Profiles: An integrated system of multi-informant assessment*. Burlington, VT: University of Vermont, Department of Psychiatry.

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington, DC: Author.

Anderson, P., Doyle, L. W., & Group, V. I. C. S. (2003). Neurobehavioral outcomes of school-age children born extremely low birth weight or

https://doi.org/10.1017/S0954579416000511 Published online by Cambridge University Press

very preterm in the 1990s. *Journal of the American Medical Association*, *289*, 3264–3272.
Benjet, C., Thompson, R. J., & Gotlib, I. H. (2010). 5-HTTLPR moderates the effect of relational peer victimization on depressive symptoms in adolescent girls. *Journal of Child Psychology and Psychiatry*, *51*, 173–179.
Botting, N., Powls, A., Cooke, R. W., & Marlow, N. (1997). Attention deficit hyperactivity disorders and other psychiatric outcomes in very low birthweight children at 12 years. *Journal of Child Psychology and Psychiatry*, *38*, 931–941.
Boyle, M. H., Miskovic, V., Van Lieshout, R. J., Duncan, L., Schmidt, L. A., Hoult, L., et al. (2011). Psychopathology in young adults born at extremely low birth weight. *Psychological Medicine*, *41*, 1763–1774.
Caspi, A., Hariri, A. R., Holmes, A., Uher, R., & Moffitt, T. E. (2010). Genetic sensitivity to the environment: The case of the serotonin transporter gene and its implications for studying complex diseases and traits. *American Journal of Psychiatry*, *167*, 509–527.
Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*, *301*, 386–389.
Chau, C. M. Y., Ranger, M., Sulistyoningrum, D., Devlin, A. M., Oberlander, T., & Grunau, R. E. (2014). Neonatal pain and COMT Val158Met genotype in relation to serotonin transporter (SLC6A4) promoter methylation in very preterm children at school age. *Frontiers in Behavioral Neuroscience*. Retrieved from http://dx.doi.org/10.3389/fnbeh.2014.00409
Conrad, A. L., Richman, L., Lindgren, S., & Nopoulos, P. (2010). Biological and environmental predictors of behavioral sequelae in children born preterm. *Pediatrics*, *125*, e83–e89.
Engle, W. A., Tomashek, K. M., & Wallman, C. (2007). "Late-preterm" infants: A population at risk. *Pediatrics*, *120*, 1390–1401.
Farooqi, A., Hägglöf, B., Sedin, G., Gothefors, L., & Serenius, F. (2007). Mental health and social competencies of 10- to 12-year-old children born at 23 to 25 weeks of gestation in the 1990s: A Swedish national prospective follow-up study. *Pediatrics*, *120*, 118–133.
Hack, M., Taylor, H. G., Schluchter, M., Andreias, L., Drotar, D., & Klein, N. (2009). Behavioral outcomes of extremely low birth weight children at age 8 years. *Journal of Developmental and Behavioral Pediatrics*, *30*, 122–130.
Hariri, A. R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., et al. (2002). Serotonin transporter genetic variation and the response of the human amygdala. *Science*, *297*, 400–403.
Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., et al. (1996). Allelic variation of human serotonin transporter gene expression. *Journal of Neurochemistry*, *66*, 2621–2624.
Hollingshead, A. B. (1969). *Two Factor Index of Social Position*. Unpublished manuscript, Yale University.
Hu, X.-Z., Lipsky, R. H., Zhu, G., Akhtar, L. A., Taubman, J., Greenberg, B. D., et al. (2006). Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *American Journal of Human Genetics*, *78*, 815–826.
Indredavik, M. S., Vik, T., Heyerdahl, S., Kulseng, S., & Brubakk, A.-M. (2005). Psychiatric symptoms in low birth weight adolescents, assessed by screening questionnaires. *European Child and Adolescent Psychiatry*, *14*, 226–236.
Indredavik, M. S., Vik, T., Heyerdahl, S., Kulseng, S., Fayers, P., & Brubakk, A. (2004). Psychiatric symptoms and disorders in adolescents with low birth weight. *Archives of Disease in Childhood: Fetal and Neonatal Edition*, *89*, F445–F450.
Johnson, S., Hollis, C., Kochhar, P., Hennessy, E., Wolke, D., & Marlow, N. (2010). Psychiatric disorders in extremely preterm children: Longitudinal finding at age 11 years in the EPICure study. *Journal of the American Academy of Child & Adolescent Psychiatry*, *49*, 453–463.
Kramer, M. S., Platt, R. W., Wen, S. W., Joseph, K. S., Allen, A., Abrahamowicz, M., et al. (2001). A new and improved population-based Canadian reference for birth weight for gestational age. *Pediatrics*, *108*, E35.
Lesch, K.-P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, *274*, 1527–1531.
Levy-Shiff, R., Einat, G., Har-Even, D., Mogilner, M., Mogilner, S., Lerman, M., et al. (1994). Emotional and behavioral adjustment in children born prematurely. *Journal of Clinical Child Psychology*, *23*, 323–333.
Methúsalemsdóttir, H. F., Egilson, S. Þ., Guðmundsdóttir, R., Valdimarsdóttir, U. A., & Georgsdóttir, I. (2013). Quality of life of adolescents born with extremely low birth weight. *Acta Paediatrica*, *102*, 597–601.
Munafo, M. R., Clark, T. G., Moore, L. R., Payne, E., Walton, R., & Flint, J. (2003). Genetic polymorphisms and personality in healthy adults: A systematic review and meta-analysis. *Molecular Psychiatry*, *8*, 471–484.
Praschak-Rieder, N., Kennedy, J., Wilson, A. A., Hussey, D., Boovariwala, A., Willeit, M., et al. (2007). Novel 5-HTTLPR allele associates with higher serotonin transporter binding in putamen: A [11 C] DASB positron emission tomography study. *Biological Psychiatry*, *62*, 327–331.
Raikkonen, K., Pesonen, A.-K., Heinonen, K., Kajantie, E., Hovi, P., Jarvenpaa, A.-L., et al. (2008). Depression in young adults with very low birth weight: The Helsinki study of very low-birth-weight adults. *Archives of General Psychiatry*, *65*, 290–296.
Rickards, A. L., Kelly, E. A., Doyle, L. W., & Callanan, C. (2001). Cognition, academic progress, behavior and self-concept at 14 years of very low birth weight children. *Journal of Developmental & Behavioral Pediatrics*, *22*, 11–18.
Saigal, S., & Doyle, L. W. (2008). An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet*, *371*, 261–269.
Saigal, S., Feeny, D., Rosenbaum, P., Furlong, W., Burrows, E., & Stoskopf, B. (1996). Self-perceived health status and health-related quality of life of extremely low-birth-weight infants at adolescence. *Journal of the American Medical Association*, *276*, 453–459.
Saigal, S., Hoult, L. A., Streiner, D. L., Stoskopf, B. L., & Rosenbaum, P. L. (2000). School difficulties at adolescence in a regional cohort of children who were extremely low birth weight. *Pediatrics*, *105*, 325–331.
Saigal, S., Pinelli, J., Hoult, L., Kim, M. M., & Boyle, M. H. (2003). Psychopathology and social competencies of adolescents who were extremely low birth weight. *Pediatrics*, *111*, 969–975.
Saigal, S., Rosenbaum, P. L., Szatmari, P., & Campbell, D. (1991). Learning disabilities and school problems in a regional cohort of extremely low birth weight (<1000 g) children: A comparison with term controls. *Journal of Developmental and Behavioral Pediatrics*, *12*, 294–300.
Saigal, S., Szatmari, P., Rosenbaum, P., Campbell, D., & King, S. (1990). Intellectual and functional status at school entry of children who weighed 1000 grams or less at birth: A regional perspective of births in the 1980s. *Journal of Pediatrics*, *116*, 409–416.
Saigal, S., Szatmari, P., Rosenbaum, P., Campbell, D., & King, S. (1991). Cognitive abilities and school performance of extremely low birth weight children and matched term control children at age 8 years: A regional study. *Journal of Pediatrics*, *118*, 751–760.
Schmidt, L. A., Miskovic, V., Boyle, M. H., & Saigal, S. (2008). Shyness and timidity in young adults who were born at extremely low birth weight. *Pediatrics*, *122*, e181–e187.
Stevenson, C., Blackburn, P., & Pharoah, P. (1999). Longitudinal study of behaviour disorders in low birthweight infants. *Archives of Disease in Childhood: Fetal and Neonatal Edition*, *81*, F5–F9.
Strang-Karlsson, S., Räikkönen, K., Pesonen, A.-K., Kajantie, E., Paavonen, E., Lahti, J., et al. (2008). Very low birth weight and behavioral symptoms of attention deficit hyperactivity disorder in young adulthood: The Helsinki study of very-low-birth-weight adults. *American Journal of Psychiatry*, *165*, 1345–1353.
Szatmari, P., Saigal, S., Rosenbaum, P., & Campbell, D. (1993). Psychopathology and adaptive functioning among extremely low birthweight children at eight years of age. *Development and Psychopathology*, *5*, 345–357.
Szatmari, P., Saigal, S., Rosenbaum, P., Campbell, D., & King, S. (1990). Psychiatric disorders at five years among children with birthweights <1000 g: A regional perspective. *Developmental Medicine and Child Neurology*, *32*, 954–962.
Taylor, H. G., Margevicius, S., Schluchter, M., Andreias, L., & Hack, M. (2015). Persisting behavior problems in extremely low birth weight adolescents. *Journal of Developmental & Behavioral Pediatrics*, *36*, 178–187.
Taylor, S. E., Way, B. M., Welch, W. T., Hilmert, C. J., Lehman, B. J., & Eisenberger, N. I. (2006). Early family environment, current adversity, the serotonin transporter promoter polymorphism, and depressive symptomatology. *Biological Psychiatry*, *60*, 671–676.
Uher, R., & McGuffin, P. (2008). The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: Review and methodological analysis. *Molecular Psychiatry*, *13*, 131–146.
van Baar, A. L., Vermaas, J., Knots, E., de Kleine, M. J., & Soons, P. (2009). Functioning at school age of moderately preterm children born at 32 to 36 weeks' gestational age. *Pediatrics*, *124*, 251–257.
Van Lieshout, R. J., Boyle, M. H., Saigal, S., Morrison, K., & Schmidt, L. A. (2015). Mental health of extremely low birth weight survivors in their 30s. *Pediatrics*, *135*, 452–459.
Waxman, J., Van Lieshout, R. J., Saigal, S., Boyle, M. H., & Schmidt, L. A. (2013). Still cautious: Personality characteristics of extremely low birth weight adults in their early 30s. *Personality and Individual Differences*, *55*, 967–971.