Evidence for plasticity genotypes in a gene–gene–environment interaction: the TRAILS study


Interdisciplinary Center for Psychiatric Epidemiology, Department of Psychiatry and Unit of Genetic Epidemiology & Bioinformatics, Department of Epidemiology (HRI), University Medical Center Groningen, University of Groningen, The Netherlands

*Corresponding author: E. Nederhof, PhD, Interdisciplinary Center for Psychiatric Epidemiology, Department of Psychiatry, CC72, University Medical Center Groningen, PO Box 30 001, 9700 RB Groningen, The Netherlands. E-mail: e.nederhof@med.umcg.nl

The purpose was to study how functional polymorphisms in the brain derived neurotrophic factor gene (BDNF val66met) and the serotonin transporter gene linked promoter region (5-HTTLPR) interact with childhood adversities in predicting Effortful Control. Effortful Control refers to the ability to regulate behavior in a goal-directed manner and is an interesting endophenotype for psychopathology because of its heritability and the association of low Effortful Control with both internalizing and externalizing problems. In a longitudinal population-based study Effortful Control was assessed with the parent version of the Early Adolescent Temperament Questionnaire at age 11. Pregnancy and delivery adversities and childhood events were assessed in a parent interview at age 11. Long-term difficulties until age 11 were assessed with a parent questionnaire at age 13.5. Blood or buccal cells were collected at age 16 for genotyping the rs6265 and rs25531 SNPs and the 5-HTTLPR length polymorphism. The study included 1032 complete data sets. Effortful Control was significantly predicted by the interaction between BDNF val66met, 5-HTTLPR and childhood events. The BDNF val66met val/val –5-HTTLPR genotype was unaffected by childhood events, while having either at least one BDNF val66met met or 5-HTTLPR s′ allele (I′/I′-met-carrier; I′/s′-val/val; s′/s′-val/val) made children sensitive to childhood events. Predictions of Effortful Control by pregnancy and delivery adversities and long-term difficulties were largely independent of genotype. We concluded that the I′/I′-met-carrier, I′/s′-val/val and the s′/s′-val/val genotypes showed greatest plasticity while the I′/I′-val/val genotype was unaffected by childhood events.

Keywords: SERT, SLC6A4, stressful life events, temperament, personality

Received 29 April 2010, revised 11 June 2010 and 16 July 2010, accepted for publication 21 July 2010

The focus of the present study is the interaction between childhood adversity and genetic predispositions on the prediction of Effortful Control in pre-adolescence. Childhood adversities have a high predictability for later psychopathology, although there are large inter-individual differences in outcomes. Possibly, individuals with certain genotypes have higher plasticity or sensitivity to environmental influences than others (Belsky et al. 2009). Specifically, we hypothesize that a polymorphism in the brain derived neurotrophic factor gene (BDNF val66met) interacts with a polymorphism on the serotonin transporter gene linked promoter region (5-HTTLPR). The BDNF valine (val) to methionine (met) substitution at codon 66 leads to lower levels of the protein BDNF compared to the BDNF val66met val allele (Bath & Lee 2006). The 5-HTTLPR short (s) allele has lower expression of serotonin transporter, leading to higher concentrations of serotonin in the synaptic cleft compared to the long (I) allele (Lesch et al. 1996).

There are many evidences that the protein BDNF interacts with serotonin (Dell’Osso et al. 2008; Lang et al. 2005). Strength of interaction effects has been linked to 5-HTTLPR and BDNF val66met genotypes. Larger differences in serotonin transporter availability and greater morphological differences have been found between individuals with the BDNF val66met val/val genotype compared to BDNF val66met met-carriers in several brain regions (Henningsson et al. 2009; Pezawas et al. 2008). It has also been shown that the influence of BDNF protein concentrations on serotonin uptake is strongest in the 5-HTTLPR I/I genotype (Mossner et al. 2000) and that individuals with the 5-HTTLPR I/I genotype have larger variation in neuroticism scores depending on BDNF val66met genotype compared to s-carriers (Terracciano et al. 2010). Thus, the BDNF val66met val/val and the 5-HTTLPR I/I genotypes seem to have higher plasticity depending on genotype of the other gene. Specifically, we hypothesize that the high-expression 5-HTTLPR and BDNF val66met genotypes (I/I and val/val) show greatest plasticity in combination with a low-expression allele of the other gene (s and met), but not in combination with the high-expression genotype.

We will investigate this plasticity hypothesis using Effortful Control as the outcome. Effortful Control refers to the ability to regulate behavior in a goal-directed manner while suppressing behavior distracting from that goal (e.g. Rothbart et al. 2003). Effortful Control is an endophenotype for mental health problems as it is to a large extent heritable (Lemery-Chalfant et al. 2008; Yamagata et al. 2005) and low Effortful Control predicts both internalizing and externalizing problems, whereas other temperament traits predict either internalizing or externalizing problems (Eisenberg et al. 2007; Oldenhinkel et al. 2004; Ormel et al. 2005). Additionally, Effortful Control is predicted by environmental influences such as...
family environment (Nakao et al. 2000), positive and negative life events (Costa et al. 2000), and childhood adversity (Rosenman & Rodgers 2006).

In conclusion, we hypothesize that children with the BDNF val66met val/val genotype show greatest variability in Effortful Control depending on the amount of childhood adversities (i.e. greatest plasticity) when carrying one or two 5-HTTLPR s alleles, and that children with the 5-HTTLPR l/l genotype show greatest plasticity in combination with a BDNF val66met met allele.

Methods

Sample

Data from the first, second and third wave of the TRacking Adolescents’ Individual Lives Survey (TRAILS) were used. TRAILS is a prospective cohort study of Dutch adolescents for which three data collection waves have been completed (for more information about the cohort see De Winter et al. 2005; Huisman et al. 2008). Mean ages were 11.09 (SD = 0.59) years at the first wave, 13.55 (SD = 0.54) years at the second wave and 16.13 (SD = 0.59) years at the third wave. All procedures have been approved by the Central Committee on Research Involving Human Subjects. All participants and their parents signed informed consent before participation.

Procedures

At the first assessment wave well-trained interviewers visited one of the parents or guardians (preferably the mother, 95.6%) at their homes to administer an interview covering a wide range of topics, including the TRAILS Family History Interview during which prenatal and perinatal risks and the occurrence of stressful events were assessed. Besides the interview, the parent was asked to fill out questionnaires including the parent version of the Revised Early Adolescent Temperament Questionnaire (EATQ-R; Oldehinkel et al. 2004). At the second wave, the parents filled out a questionnaire including questions about long-term difficulties affecting the child. At the third wave 1599 samples with blood or buccal cells were collected for DNA analysis.

Pregnancy and delivery adversities

Prenatal and perinatal risks were assessed during the first assessment wave with the TRAILS Family History Interview. The variable pregnancy and delivery adversities (PAdv) was created based on questions about maternal prenatal smoking, maternal prenatal alcohol use, birth weight, gestational age, and pregnancy and delivery complications. For birth weight, prenatal smoking, and pregnancy and delivery complications, the same criteria were used as Buschgens et al. (2009) did. For maternal prenatal alcohol use three groups were created: no alcohol use, mild alcohol use (up to three glasses per week) and heavy alcohol use (four glasses per week or more). Gestational age was also recoded into two groups: normal (between 34 and 42 weeks) and abnormal (33 weeks or less, or more than 42 weeks). The sum score of these variables was used as an indication of PAdv.

Childhood events

Stressful events that occurred until the first wave (mean age 11.09) were assessed at the TRAILS Family History Interview (T1). The variable childhood events (CE) was a sum of the occurrence of severe disease of the mother, severe disease of the father, threatening disease of a sibling, death of a direct family member, divorce of the parents and absence from home for three months or longer.

Long-term difficulties

Long-term difficulties (LTD) were assessed with a questionnaire filled out by the parents at the second assessment wave. LTD was the sum score of the occurrence of chronic disease or handicap of the child, chronic disease or handicap of a direct family member, the child is being bullied, long-lasting conflicts of the child with a direct family member and long-lasting conflicts of the child with someone else, all until the age of 11.

Effortful control

At the first assessment wave the parents completed the Dutch version of the EATO-R about their child. Effortful Control was measured with a 11-item sub-scale with high internal consistency, α = 0.86 (Oldehinkel et al. 2004). The scale includes questions about voluntary attentional control (‘My child pays close attention when told how to do something’) and voluntary behavioral control (‘My child starts right away with difficult assignments’). Answers were given on a 1–5 scale with 1 = ‘almost never’ and 5 = ‘almost always’. Six items were reverse coded. Average item score on Effortful Control was used in the present study.

DNA extraction and genotyping

DNA was extracted from blood samples (n = 1238) or buccal swabs (Cytobrush® (n = 361) using a manual salting out procedure as described by Miller et al. (1988). Genotyping procedures have been described earlier (Nederhof et al. 2010). In 1460 subjects at least 80% of all SNPs could be genotyped and length polymorphisms were successfully determined in 1445 subjects. Duplicates for blood and buccal samples gave 100% concordance (n = 53). Call rates were 99.5% for BDNF val66met rs6265 and 91.6% for the (A > G) substitution present in the 5-HTTLPR long (l) allele (rs25531). Because the lG polymorphism represents low serotonin expression compared to the s allele, s and lG alleles were recoded s′, lA was recoded l′. Only children of Dutch ancestry were included in the analyses. Twelve participants were excluded from analyses because they had a sibling participating in TRAILS.

Statistical analyses

Means and standard deviations were calculated for Effortful Control. Frequency analyses were performed on genetic data and childhood adversities. Allele frequencies were compared to the Hardy–Weinberg equilibrium using χ²-tests with 1 degree of freedom. Biserial correlations were calculated between genotype and childhood adversities.

Three separate hierarchical linear regression equations were calculated to (1) investigate the main effects of childhood adversities, (2) the interaction between the two genotypes and (3) two- and three-way interactions between each category of childhood adversities and genotypes with Effortful Control as the outcome measure. Each childhood adversity with significant gene–environment interactions was modeled separately as a post hoc analysis to facilitate interpretation. Significance level was set at P < 0.05. Genotypes were recoded into dummy variables with the BDNF val66met val/val and the 5-HTTLPR l/l genotype as reference categories.

Results

Scores on Effortful Control were obtained for 1986 children, mean score was 3.2 (SD = 0.7). Scores for PAdv, CE and LTD were completed for 2119, 2120 and 1899 children, respectively. Distributions of childhood adversities can be found in Fig. 1. BDNF val66met and 5-HTTLPR genotypes were available for 1452 and 1414 children, respectively. A total of 1032 complete datasets was obtained.

The SNP within the l allele of the 5-HTTLPR (rs25531) was in Hardy–Weinberg equilibrium. Frequencies of the bi-allelic polymorphisms were l/l′, l′/l 274, l′/s′ 524 and s′/s′ 234. The BDNF val66met polymorphism (rs6265) was also
Factors printed in bold significantly predict Effortful Control (P < 0.05).

BDNF met = met allele carriers at the brain derived neurotrophic factor gene; 5-HTTLPR /'s', carriers of one long (lA) and one functional short (s or lG) allele on the serotonin transporter gene linked promotor region; /'s', carriers of two functional short alleles on the 5-HTTLPR gene.

Table 3: Linear regression model with BDNF val66met and 5-HTTLPR genotypes on Effortful Control

<table>
<thead>
<tr>
<th>Unstandardized coefficients</th>
<th>B</th>
<th>SE</th>
<th>Standardized β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>3.19</td>
<td>0.05</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>BDNF met</td>
<td>0.16</td>
<td>0.06</td>
<td>0.03</td>
<td>0.45</td>
</tr>
<tr>
<td>5-HTTLPR /'s' /'s'</td>
<td>0.02</td>
<td>0.07</td>
<td>0.01</td>
<td>0.81</td>
</tr>
<tr>
<td>met * /'s' /'s'</td>
<td>-0.17</td>
<td>0.12</td>
<td>-0.07</td>
<td>0.15</td>
</tr>
<tr>
<td>met * /'s' /'s'</td>
<td>-0.11</td>
<td>0.10</td>
<td>-0.06</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Factors printed in bold significantly predict Effortful Control (P < 0.05). BDNF val66met = met allele carriers at the brain derived neurotrophic factor gene; 5-HTTLPR /'s', carriers of one long (lA) and one functional short (s or lG) allele on the serotonin transporter gene linked promotor region; /'s', carriers of two functional short alleles on the 5-HTTLPR gene.

Table 2: Linear regression model with main effects for the three categories of childhood adversities on Effortful Control

<table>
<thead>
<tr>
<th>Unstandardized coefficients</th>
<th>B</th>
<th>SE</th>
<th>Standardized β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>3.43</td>
<td>0.03</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>PDadv</td>
<td>-0.07</td>
<td>0.02</td>
<td>-0.11</td>
<td>0.00</td>
</tr>
<tr>
<td>CE</td>
<td>-0.03</td>
<td>0.02</td>
<td>-0.04</td>
<td>0.12</td>
</tr>
<tr>
<td>LTD</td>
<td>-0.13</td>
<td>0.02</td>
<td>-0.13</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Factors printed in bold significantly predict Effortful Control (P < 0.05).
Table 4: Final regression model (n = 1096) for childhood events predicting child’s Effortful Control as assessed by the parent

<table>
<thead>
<tr>
<th>Unstandardized coefficients</th>
<th>Constant</th>
<th>CE</th>
<th>BDNF met</th>
<th>5-HTTLPR l/s’</th>
<th>met * l/s’</th>
<th>met * s/s’</th>
<th>CE * met</th>
<th>CE * l/s’</th>
<th>CE * s/s’</th>
<th>CE * met * l/s’</th>
<th>CE * met * s/s’</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>3.13</td>
<td>0.07</td>
<td>0.08</td>
<td>0.15</td>
<td>-0.34</td>
<td>-0.32</td>
<td>-0.20</td>
<td>-0.22</td>
<td>-0.18</td>
<td>0.29</td>
<td>0.24</td>
</tr>
<tr>
<td>SE</td>
<td>0.07</td>
<td>0.06</td>
<td>0.10</td>
<td>0.10</td>
<td>0.15</td>
<td>0.13</td>
<td>0.10</td>
<td>0.08</td>
<td>0.09</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td>Standardized β</td>
<td>0.00</td>
<td>0.19</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>0.02</td>
<td>0.05</td>
<td>0.00</td>
<td>0.04</td>
<td>0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When no genotype is indicated, the numbers represent the effects in the reference genotypes, which are BDNF val66met val/val and 5-HTTLPR l/l’. Factors printed in bold significantly predict Effortful Control (P < .05). met, carriers of one or two valine to methionine substitutions at the brain derived neurotrophic factor gene; l/s’, carriers of one long (lA) and one functional short (s or l) allele on the serotonin transporter gene linked promoter region (5-HTTLPR); s/s’, carriers of two 5-HTTLPR functional short alleles.

Discussion

Our hypothesis was confirmed with CE as a predictor for Effortful Control in those children with either a BDNF val66met met allele or one or two 5-HTTLPR s’ alleles (l/l’, met-carrier, l/s’-val/val or s/s’-val/val). This shows that these genotypes have greater plasticity as children with these genotypes have higher Effortful Control in the absence of CE but lower Effortful Control with increasing numbers of CE. This is an important finding, given the gateway function of Effortful Control in the path to psychopathology (Eisenberg et al. 2009; Oldeninkel et al. 2007).

The combination of BDNF val66met val/val and 5-HTTLPR l/l’ could be regarded the unaffected genotype, as the effect of CE on Effortful Control was smallest in this genotype.

Future studies should investigate whether these genotypes also have differential plasticity to positive environmental influences. Interpreting findings of many gene–environment interaction studies, Belsky et al. (2003) suggested that genotypes sensitive to negative aspects of the environment could very well also be more sensitive to positive aspects of the environment. A first indication for differential plasticity in a gene–gene–environment interaction was found by Kaufman et al. (2006) who showed that individuals with both the BDNF val66met val/val and the 5-HTTLPR l/l’ genotypes did not profit from social support, which is in line with our finding that l/l’-val-val was the unaffected genotype.

Children with both a BDNF val66met met allele and one or two 5-HTTLPR s alleles (l/l’, met-carrier, s/s’-met-carrier) also showed greater plasticity, but in an unexpected direction. Children with those genotypes were predicted to have higher Effortful Control with an increasing number of CE but lower Effortful control in the absence of CE. Although it has previously been reported that the low serotonin responsive BDNF val66met met allele mitigates the effect of the 5-HTTLPR genotype (Pezawas et al. 2008) and that the 5-HTTLPR s allele mitigates effects of BDNF val66met genotype (Terracciano et al. 2010), we did not predict a protective effect of childhood adversities.

Genotypes were not correlated with childhood adversities in our sample. This shows that childhood adversities occurred independent of genetic background (Rutter et al. 2006). This provides evidence for differences in sensitivity to childhood adversity between the genotypes.

Studies of gene–gene–environment interactions on depression did not find that the high-expression 5-HTTLPR and BDNF val66met genotypes (l/l’ and val/val) show greatest plasticity in combination with a low-expression allele of the other gene (s and met). Kaufman et al. (2006) found that childhood depression scores were predicted by the interaction between BDNF val66met, 5-HTTLPR and childhood adversities.
maltreatment, with higher depression scores in children with the s/s-val/met genotype. This finding has been replicated in an adult sample with higher depression scores in the i/s-met-carrier genotype (Wichers et al. 2008). In two other studies no differences in plasticity of BDNF val66met–5-HTTLPR genotype in combination with childhood adversity on depressive symptoms were found (Aguilera et al. 2009; Nederhof et al. 2010).

One of the possible reasons for inconsistent findings might be the complexity of psychiatric disease making it difficult, if not impossible, to clearly identify one or two genotypes that make individuals more vulnerable for environmental influences. Gottesman and Gould (2003) pointed out that measurable components underlying complex diseases, or endophenotypes, are more likely to show clear relationships with genetic predisposition both because they are more proximate to the genome and because they are less complex than the disease phenotype (Hasler et al. 2004). These characteristics are all applicable to Effortful Control. We encourage other researchers to confirm the differential plasticity hypothesis with Effortful Control and other endophenotypes for psychiatric illnesses.

Main effects of PDadv and LTD were significant while showing hardly any interaction effects with genotype. The main effect of CE was not significant. Genotypes did not predict large proportions of Effortful Control independent of childhood adversities. These findings can be interpreted as supportive of our conclusions that certain genotypes are more sensitive than others to childhood events such as disease or death of close family members. This differential plasticity does not seem to apply to pre- and perinatal adversities and to more chronic adversities such as chronic disease or handicap of children in the household as PDadv and LTD predicted Effortful Control of all children in our cohort. It is a question for future studies whether it is really the chronicity of the adversities that rule out differential plasticity of specific genotypes, or whether another factor such as person dependency or severity can explain this difference.

We conclude that Effortful Control is predicted by childhood adversity giving support for the endophenotypic approach in gene–environment interaction studies. The BDNF val66met val/val–5-HTTLPR i/i’ genotype was unaffected by childhood events, while the i/i’-met-carrier, i’/s’-val/met and the s’/s’-val/val genotypes showed greatest plasticity.

References


Acknowledgments

This research is part of the TRacking Adolescents’ Individual Lives Survey (TRAILS). Participating centers of TRAILS include various departments of the University Medical Center and University of Groningen, the Erasmus University Medical Center Rotterdam, the University of Utrecht, the Radboud Medical Center Nijmegen and the Parnassia Bavo group, all in the Netherlands. TRAILS has been financially supported by various grants from the Netherlands Organization for Scientific Research NWO (Medical Research Council program grant GB-MW 940-38-011; ZonMW Brainpower grant 100-001-004; ZonMw Risk Behavior and Dependence grants 60-60600-98-018 and 60-60600-97-118; ZonMw Culture and Health grant 261-98-710; Social Sciences Council medium-sized investment grants GB-MaGW 480-01-006 and GB-MaGW 480-07-001; Social Sciences Council project grants GB-MaGW 457-03-018, GB-MaGW 452-04-314, and GB-MaGW 452-06-004; NWO large-sized investment grant 175.010.2003.005); the Sophia Foundation for Medical Research (projects 301 and 393), the Dutch Ministry of Justice (WODC), the European Science Foundation (EuroSTRESS project FP-006), and the participating universities. We are grateful to all adolescents, their parents and teachers who participated in this research and to everyone who worked on this project and made it possible.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1: Linear regression models with main effects for the three categories of childhood adversities on each of the temperamental traits not presented in the main document as measured with the parent version of the Early Adolescent Temperament Questionnaire.

Table S2: Linear regression model with BDNF Val66Met and 5-HTTLPR genotypes on each of the temperamental traits not presented in the main document as measured with the parent version of the Early Adolescent Temperament Questionnaire.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.