Infant Behavior & Development 47 (2017) 22–26

Contents lists available at ScienceDirect

Infant Behavior and Development

The *BDNF*$_{val66met}$ polymorphism and individual differences in temperament in 4-month-old infants: A pilot study

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ARTICLE INFO

Article history:
Received 17 November 2016
Received in revised form
23 December 2016
Accepted 26 January 2017
Available online 21 March 2017

Keywords:
BDNF
Genetics
Infant
Polymorphism
Temperament

ABSTRACT

Individual differences in infants' temperament are under genetic control. We investigated the association between brain-derived-neurotrophic-factor (BDNF$_{val66met}$) polymorphism and temperament in 63 full-term infants. *Met*-carriers ($N = 25$) had lower Regulatory capacities compared to *val*-homozygotes ($N = 38$). These findings suggest that the BDNF polymorphism affects early temperament individual differences.

Temperament has been defined as biologically based individual differences in reactivity and self-regulation (Posner & Rothbart, 2009). Temperamental characteristics are thought to emerge early in life and infants give evidence of precocious individual differences during the first months of life (Rothbart, Ahadi, & Evans, 2000). Genetic variations (i.e., polymorphisms) might affect individual differences in temperament, suggesting that the temperament should be considered, at least partially, under genetic control (Papageorgiou & Ronald, 2013). Indeed, previous research has documented that structural DNA changes (i.e., polymorphisms) in candidate genes, such as those coding for the serotonin transporter (5-HTTLPR) and the dopamine receptor (DRD4), have implications in early temperamental differences (Auerbach, Faroy, Ebstein, Kahana, & Levine, 2001). Nonetheless, in a recent review of literature (Papageorgiou & Ronald, 2013), inconsistency arose suggesting that other polymorphisms might be potentially involved.

The brain-derived neurotrophic factor (BDNF) is mainly expressed in prefrontal and hippocampal brain regions and promotes neuronal survival, development and synaptic plasticity (Goggi, Pullar, Carney, & Bradford, 2003; White et al., 2016). Consistently, it has been proposed that variations in BDNF availability might associate with individual differences in behavioral regulation and reactivity potentially through altered patterns of cortical and hippocampus-amygdala neural circuits connectivity (Ninan, 2014; Sandi & Richter-Levin, 2009). This polymorphism consists in a valine (*val*) to methionine (*met*) substitution at codon 66 in the BDNF gene (Chen et al., 2004). The *met* allele of the *BDNF*$_{val66met}$ is associated with reduced BDNF availability and higher risk of poorer regulation, mood disorders and stress susceptibility in adults (Kim et al., 2007; Lang et al., 2005; Wichers et al., 2008). Research on adults documented an association between reduced BDNF

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http://dx.doi.org/10.1016/j.infbeh.2017.01.006
0163-6383/

availability and higher scores of harm avoidance, which is a feature of adult temperament (Mandelli et al., 2010; Minelli et al., 2011). Importantly, these findings were not replicated when looking at $BDNF_{val66met}$ genotype in adults (Tsutsumi et al., 2011). As such, it seems plausible to assume that BDNF has a role in temperament, but a direct effect of genetic variations of the $BDNF_{val66met}$ availability has not been demonstrated in previous adult research. Recently 7-year-old children with the *met* allele of $BDNF_{val66met}$ have been found to show altered stress susceptibility to a cognitive task (Chau, Cepeda, Devlin, Weinberg, & Grunau, 2015), which suggests that the effects of BDNF genetic variability on temperament could be better observed during early stages of development.

To the best of our knowledge, there are no studies investigating the role of $BDNF_{val66met}$ with regard to individual differences in temperament during infancy, a period in which genetically-based behavioral traits are hypothesized to be more easily observed (Posner & Rothbart, 2009; Rothbart et al., 2000). Hence, the purpose of the current study was to explore the association between the $BDNF_{val66met}$ polymorphism and individual differences in temperament in a sample of healthy 4-month-old full-term infants.

Sixty-three 4-month-old healthy full-term infants (33 females) and their mothers were enrolled at the Sacra Famiglia Hospital in Erba (CO, Italy). Infants from mothers with documented psychopathology, drug abuse or underage were excluded. All participant mothers singed an informed consent and the study was approved by the Ethics Committee of the Scientific Institute IRCCS Eugenio Medea in Bosisio Parini (LC, Italy). Mothers were first contacted during the last trimester of pregnancy. Lab sessions were scheduled by telephone-call when infants were reaching 4-month age. All infants and mothers were Caucasian. Genotyping occurred by collecting epithelial cells from infants' oral cavity using an appropriate non-noxious oral brush rubbed on internal cheek surfaces. Mothers were asked to fill in a socio-demographic form and a questionnaire on infants' temperament (see below). Moreover, as maternal depressive symptomatology has been found to associate with individual differences in mother-reported infants' temperament (Murray, Stanley, Hooper, King, & Fiori-Cowley, 1996), maternal depressive symptoms were measured (see below).

Socio-demographic data, including maternal age, educational level (i.e., years of study) and occupational status as well as infants' gestational age, birth weight and Apgar scores were obtained. Family socio-economic status was scored according to Hollingshead (1978) classification.

Genomic DNA was extracted from buccal cells using Gentra Puregene Handbook (Qiagen, Venlo, NL) according to the manufacturer's instructions. A 563 bp fragment was amplified by PCR using forward primer 5′-ACCAGGTGAGAAGAGTGATG-3′ and reverse primer 5′-GGGAGTTCCAATGCCTTTTG-3′. Amplification was performed in a total volume of 25 μl containing 50 ng of genomic DNA, GoTaq® 5X Flexi Buffer, $MgCl_2$ 2 mM, dNTP 4 mM (Fermentas Inc) and GoTaq® DNA polymerase (1 U; Promega Co). The reaction mixture was subjected to 30 cycles of amplification (30 s at 94 °C, 30 s at 62 °C and 30 s at 72 °C) followed by a 5 min extension at 72 °C. After amplification, 5 μl of PCR product were treated with 2 units of *Eco72I* (Fermentas) at 37 °C for 4 h. Digested products (two fragments of 351 and 212 bp in the case of *val* allele and one fragment of 563 bp in the case of *met* allele) were analysed by electrophoresis on 3% agarose gel stained with ethidium bromide.

The mothers completed the 191-item Infant Behavior Questionnaire-Revised (IBQ-R; Putnam, Helbig, Gartstein, Rothbart, & Leerkes, 2014), which has proved satisfactory reliability and validity (Cozzi et al., 2013). Items are rated on a 7-point Likert scale and they are resumed into 14 temperament scales, resumed by three main factors: Surgency (scales: approach, activity level, high-intensity pleasure, perceptual sensitivity, smile and laughter, vocal reactivity), Negative Emotionality (scales: distress, fear, falling reactivity, sadness), and Orienting/Regulatory Capacity (scales: cuddliness, orienting, low-intensity pleasure, soothability).

The Beck Depression Inventory (BDI-II; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) is a 21-item scale questionnaire widely used to assess depressive symptomatology. Each item is rated on a 4-point Likert scale. The BDI-II is commonly used in research on non-clinically depressed samples.

Infants' $BDNF_{val66met}$ genotype was dichotomized as follows: *val*-homozygotes (i.e., infants with two high-expression *val* alleles; $N = 38$, 17 females) and *met*-carrier (i.e., infants with at least one low-expression *met* allele; $N = 25$, 15 females; 5 *met*-homozygotes, infants with two low-expression *met* alleles). Genotype groups were evaluated for any differences in socio-demographic variables and maternal depressive symptoms (i.e., BDI score) with χ^2 and *t*-tests, as appropriate. A multivariate general linear model (GLM) were used to test the effect of $BDNF_{val66met}$ genotype (0 = *val*-homozygotes; 1 = *met*-carriers) on IBQ-R factors. For factors significantly associated with $BDNF_{val66met}$ genotype, a similar GLM was used to test associations with factor's scales. All analyses were carried at $p < 0.05$ through SPSS 20.0 (Armonk, NY, USA).

The allelic distribution was in Hardy–Weinberg equilibrium. Descriptive statistics and comparisons are reported in Table 1. Sex distribution did not differ significantly between genotypes, $\chi^2 = 0.002$, $p > 0.10$. The $BDNF_{val66met}$ genotype was associated with differences in IBQ-R factors, $F(3,59) = 3.13$, $p < .05$, $\eta^2_p = .13$. *Met*-carriers had lower Orienting/Regulatory Capacity scores than *val*-homozygous counterpart (Table 1). No differences emerged for Surgency and Negative Emotionality. Moreover, genotype-related differences emerged for Orienting/Regulatory Capacity scales, $F(4,58) = 2.20$, $p < .10$, $\eta^2_p = .13$. Compared to *val*-homozygotes, *met*-carrier infants had lower scores in orienting and low-intensity pleasure scales (Table 1).

The aim of the current study was to investigate the association between the $BDNF_{val66met}$ polymorphism and 4-month-old infants' temperament. Allelic variation of the $BDNF_{val66met}$ predicted individual differences in regulatory capacities. Specifically, infants carrying at least one *met* allele (i.e., *met*-carriers) were rated by the mothers as having minor capacity of orienting as well as reduced responsivity to low intensity stimulations. These findings appear to be consistent with previous studies on animal models (Vasconcelos•, Stein, & Almeida, 2015) and humans (Ninan, 2014). Elevated scores in low-intensity