The role of TPH2 variant rs4570625 in shaping infant attention to social signals

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ABSTRACT

TPH2, the rate-limiting enzyme in the synthesis of serotonin, has been connected to several psychiatric outcomes. Its allelic variant, rs4570625, has been found to relate to individual differences in cognitive and emotion regulation during infancy with T-carriers of rs4570625 showing a relatively heightened attention bias for fearful faces. A significant gene-environment interaction was also reported with the T-carriers of mothers with depressive symptoms showing the highest fear bias.

We investigated these associations in a sample of 8-month old infants (N=330), whose mothers were prescreened for low/high levels of prenatal depressive and/or anxiety symptoms. Attention disengagement from emotional faces (neutral, happy, fearful, and phase-scrambled control faces) to distractors was assessed with eye tracking and an overlap paradigm. Maternal depressive symptoms were assessed at several time points during pregnancy and postpartum. The mean levels of symptoms at six months postpartum and the trajectories of symptoms from early pregnancy until six months postpartum were used in the analyses (N=274).

No main effect of the rs4570625 genotype on attention disengagement was found. The difference in fear bias between the genotypes was significant but in an opposite direction compared to a previous study. The results regarding the interaction of the genotype and maternal depression were not in accordance with the previous studies.

These results show inconsistencies in the effects of the rs4570625 genotype on attention biases in separate samples of infants from the same population with only slight differences in age.

Keywords: Attention; Face perception; Infancy; Maternal depression; Gene-environment interaction (GxE)

INTRODUCTION

The neurotransmitter serotonin (*5-HT*) is implicated in the pathophysiology of many common neuropsychiatric disorders such as attention-deficit hyperactivity disorder, anxiety, and major depression (Hariri & Weinberger, 2011; Hirvonen et al., 2008; Karlsson et al., 2013). Alterations in 5-HT synthesis influence the structure and/or function of brain regions involved in cognitive and emotional processing and may result in higher stress susceptibility and psychopathology especially in the presence of other risk factors, such as additive or interactive effects of different genes or adverse environmental factors (Ottenhof, Sild, Lévesque, Ruhé, & Booij, 2018; van den Buuse & Hale, 2019).

The tryptophan hydroxylase-2 (*TPH2*) gene is expressed in the central nervous system (CNS) and functions as the first step and rate-limiting enzyme in the biosynthesis of serotonin (Genetics Home Reference, https://ghr.nlm.nih.gov/gene/TPH2; https://www.ncbi.nlm.nih.gov/gene/121278). *TPH2* expression is restricted to 5-HT neurons, and its highest expression is detected in the pons in humans and the raphe nuclei in rodents (Mosienko & Alenina, 2019). However, the TPH2 protein can be detected at several serotonin projection sites such as the cortex, striatum, hippocampus, and cerebellum (Mosienko & Alenina, 2019). It is considered a key factor in maintaining normal serotonin transmission in the CNS (Gao et al., 2012), as *Tph2* deficiency in rodents leads to extreme reductions specifically in central serotonin levels but not in other neurotransmitters in the brain. Therefore, *TPH2* may contribute to the pathogenesis of psychiatric disorders, for instance, by altering the serotonergic circuit formation (e.g., by inhibiting or promoting sprouting of serotonin axon terminals or influencing neuronal apoptosis) and the availability of serotonin in the brain or by influencing the functions of other neurotransmitter systems and brain circuits important for cognitive and emotion regulation (Gizatullin, Zaboli, Jönsson, Åsberg, & Leopardi, 2008; Mosienko & Alenina, 2019; Ottenhof et al., 2018). Animal studies show clear developmental, physiological, and behavioral consequences of *Tph2* and central serotonin deficiency starting from overall physical growth delays to more fine-grained influences on social behavior, stress sensitivity, and anxiety. These studies have

furthered our understanding of the possible mechanisms underlying human neuropsychiatric disorders (Mosienko & Alenina, 2019).

Previous studies in humans have found that allelic variations in *TPH2* in healthy individuals influence serotonin synthesis rates in frontal-limbic brain circuits and the functional connectivity between limbic-striatal circuits important for emotional and cognitive regulation (Booij et al., 2012; Furmark et al., 2016). Acute tryptophan depletion, in turn, has been connected to subtle changes in a wide array of emotional and cognitive processing and regulation, such as in difficulties in the ability to recognize facial expressions or a greater attentional bias to negative information (Ottenhof et al., 2018). A recent review and meta-analysis found evidence for a consistent role of variations in the *TPH2* gene in mood disorders, suicide, and schizophrenia (Ottenhof et al., 2018). Of the wide range of *TPH2*-related single nucleotide polymorphisms (SNPs) that were reviewed, the G allele or GG genotype compared to T of the rs4570625 SNP, located in the promoter area, was the most consistently associated with psychiatric disorders suggesting a common or transdiagnostic mechanism of this polymorphism implicated in a wide range of psychopathologies. Contributing to the research on the associations between the rs4570625 genotype and psychiatric outcomes, neuroimaging studies in healthy adult participants have shown a connection between the rs4570625 T-allele and enhanced amygdala activity in response to emotional stimuli (Brown et al., 2005; Canli et al., 2005). Also, the T-allele has been found to be abundant among adults high in the personality trait “harm avoidance,” which is related to anxiety-proneness (Waider et al., 2011) and is connected with smaller amygdalar and hippocampal volumes (Inoue et al., 2010). Thus, variation in the *TPH2* rs4570625 SNP may lead to individual differences in the structural and functional development of the brain’s key emotion processing networks via influences on serotonin availability that predispose some individuals to be at risk for self-regulation problems.

Both serotonin excess and deficiency may modulate structural and functional brain development and lead to altered behavioral phenotypes in later life (Booij, Tremblay, Szyf, & Benkelfat, 2015; Ottenhof et al., 2018). The less common T-variant of rs4570625 has been associated with decreased mRNA expression rates in the raphe nuclei leading to decreased serotonin, reduced inhibition of axonal growth, and higher levels of structural connectivity in the brain networks during development (Markett et al., 2017). Furthermore, *Tph2*-deficient mice have been found to be more susceptible to stress and more affected by early life stress (Mosienko & Alenina, 2019) indicating a key role of serotonin in interaction with the environment in the development of stress regulation. However, due to limited knowledge of the role of *TPH2* polymorphisms and rs4570625 for emotional and cognitive regulation in humans, especially during infancy when the cognitive and emotional brain systems are developing rapidly, there is an obvious need to study the role of this allelic variant in early human development.

Deficits in attention control may play a key role in the development and maintenance of psychopathology, as both problems in general control of attention as well as deviances in social-emotional information processing likely underlie the problems observed, for instance, in depressive and anxiety disorders (Armstrong & Olatunji, 2012; Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburgh, & van IJzendoorn, 2007; Leppänen, 2006). The ability to control attention matures rapidly during the first months of life (Hunnius, Geuze, & van Geert, 2006; Matsuzawa, & Shimojo, 1997), and by the enabling of efficient engagement, disengagement, and shifting of attention provides a foundation for self-regulation (Keehn, Müller, & Townsend, 2012; Posner & Rothbart, 2009). Serotonin is implicated in normal cognitive and affective processing, and in line with this, allelic variation in *TPH2* has been associated with various psychological traits of emotion regulation during adulthood, for instance, in the variation in the ability to inhibit negative emotional content when processing emotional material (Latsko et al., 2016). Mappings between individual variations in genes and behavioral traits may be more direct during infancy, as the environment has had less influence

on development compared to during later childhood or adulthood (Johnson & Pasco Fearon, 2011). Recognition of the precisely defined components of cognition during early development might help in identifying developmental endophenotypes underlying the risk for developmental psychopathologies (Johnson & Pasco Fearon, 2011; Papageorgiou & Ronald, 2017). Specifically, individual differences in attention disengagement from emotional facial expression, such as threat-alerting fearful faces, may be one of the early-developing phenotypes that is relevant for self-regulation and associated risk for rumination and mood disorders (Armstrong & Olatunji, 2012; Bar-Haim et al., 2007; Leppänen, 2006).

Two previous studies have investigated the T/G base substitution of rs4570625 in *TPH2* with attentional processing during infancy. Both studies used an overlap paradigm to assess infant attention disengagement from emotional faces, i.e., neutral, happy, fearful and phase-scrambled control faces, to lateral distractors. Leppänen et al. (2011) studied 7-month old infants (N=66) and reported a main effect of the rs4570625 polymorphism on the number of missing attention shifts. The T-carriers (GT/TT) had an increased number of missing attentions shifts from centrally presented faces to distractors as compared to GG-carriers. This effect was not specific to a particular facial expression, although there was a tendency for the missing attention shifts in the T-allele carrier to be more pronounced when affectively salient, i.e., happy or fearful, faces were presented. Also, this pattern of attention was negatively related to infant self-regulation, i.e., parent-reported soothability, consistent with previous research showing that relatively slower attention disengagement is associated with reduced soothability in infants (Johnson et al., 1991). The authors concluded that variations in serotonin may modulate the early emerging attention systems foundational for more mature emotion regulation, and that these differences were related to overall attention disengagement compared to emotion-specific processes (Leppänen et al., 2011). Forssman et al. (2014), in their replication study with a larger 5–7 month old infant sample (N=139), did not find a main effect of rs4570625 genotype on overall disengagement probabilities but instead noticed that the T-carriers displayed difficulty

disengaging specifically from fearful faces. They also investigated the possibly moderating role of early-life environment, namely mother-reported concurrent depressive symptoms and recent negative life-events, and found a significant gene-environment effect. A higher bias for fearful compared to happy, neutral, or control faces, i.e., fear bias, was especially pronounced among T-carriers of mothers with high compared to low self-reported concurrent depressive symptoms. Allelic variance of rs4570625 may associate with early deviances in attention orienting/holding processes. This may be related specifically to emotional processing, especially in the presence of early environmental risk factors. Thus, genetic research during infancy potentially offers an opportunity to identify variations that may be relevant for understanding the risk for later psychopathologies, while more studies are needed to investigate the associations in larger samples with well-defined infant phenotypes.

Vulnerability to common psychiatric disorders is multifactorial with a complex genetic architecture and multiple environmental factors contributing to their emergence (Gonda et al., 2018; Hariri & Weinberger, 2011; Waider et al., 2011). Consequently, most positive findings, for instance, in candidate polymorphism and depression studies, have emerged, when the interaction between genetic risk and adverse psychological exposures have been investigated. Among environmental risk factors, maternal mental health during the pre- and postnatal periods potentially exert a crucial impact on the child's developing self-regulation and mental health (Junge et al., 2017; Meaney, 2018). Recent studies have shown less frequent disengagement from fearful or angry compared to happy or neutral faces, i.e., a higher fear bias in infants of mothers with heightened depressive or anxiety symptoms during the pre- and/or postnatal periods (Forssman et al., 2014; Kataja et al., 2018, 2019; Morales et al., 2017). Thus, maternal depression may be a potential candidate "stressor" that is well suitable for studies inspecting gene-environment interactions (Dick et al., 2015). A heightened fear bias, in turn, may be a useful candidate for an intermediate phenotype, as it has been suggested to be a risk factor for problems in emotion regulation during childhood (Nakagawa & Sukigara, 2012) and later in life affective disorders (Bar-Haim et al., 2007). Additionally, prior studies propose that a heightened fear

bias may be related already to prenatal maternal symptoms suggesting that early-life programming, genetic factors, or both influence its emergence during infancy (Kataja et al. 2018, 2019).

Given that the association between *TPH2* rs4570625 and attention disengagement from fearful faces in infants may reflect an early-emerging genetic effect on functionally significant attentional processes (i.e., attention to negative emotion), but has so far been relatively little studied, we sought to examine the replicability of this association in a new sample of 8-month old infants (N=330 and N=274 for genetic and gene x environment analyses, respectively). Infant attention was assessed with eye tracking and an attention disengagement paradigm with neutral, happy, fearful, and phase-scrambled control faces and distractors. Maternal depression was assessed through self-report at several timepoints during pregnancy and during the postpartum period. The following hypotheses were tested:

1. The T-carriers (GT/TT) compared to GG would show a higher probability of missing attention shifts from the central faces towards the lateral distractors (Leppänen et al., 2011).
2. The T-carriers would show a higher fear bias (Forssman et al., 2014).

3a. The fear bias would be highest in the T-carriers of mothers with higher depressive symptoms with the mean level of symptoms assessed at six months postpartum, i.e., the most recent assessment point for eye tracking (Forssman et al., 2014).

3b. The fear bias would be associated with the *TPH2* genotype and the trajectories of maternal depressive symptoms from early pregnancy until six months postpartum (Kataja et al., 2018a) and would be highest in the T-carriers of mothers with increasing and decreasing compared to those with low symptom levels.

METHODS

Participants

The current sample was formed with 330 infants from an ongoing longitudinal FinnBrain Birth Cohort Study for whom both eye-tracking data and *TPH2* genotype information were available (Demographic characteristics, Table 1). The eye-tracking assessments were conducted between the years 2013–16 as part of the Child Development and Parental Functioning Lab study visits for FinnBrain children and their mothers. N=11 infants were born prematurely (<37 gestational weeks, gwk), and the analyses were run both with and without these infants. This did not significantly alter the results, and thus the results are reported from the whole sample. The mothers gave informed consent on behalf of their infant. They were also informed about the study details and their option to withdraw from the testing at any time without providing a specific reason.

The subjects for the present study belong to a nested case-control population embedded in the main Cohort, i.e., the Focus Cohort, designed to investigate the effects of maternal prenatal distress on child development. The inclusion for the Focus Cohort required scoring in the highest or lowest approximately 25th percentiles on depression, anxiety, and/or pregnancy-related anxiety symptom questionnaires across pregnancy (see Karlsson et al. 2018 for details of the Cohort). The sample is a part of the sample (N=363) reported in Kataja et al.2018 and consists of infants from whom both eye tracking and genetic data were available. Out of the N=330 mother-infant dyads, N=114 (34.5%) were “cases” that reported heightened levels of prenatal distress, and N=178 were controls that reported low levels of distress. The sample also included infants, who had participated in a previous FinnBrain assessment, e.g., an infant MRI or a pediatric visit, and thus N=32 (11.5%) were outside the Focus Cohort. The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol. The study was conducted in full compliance with the Helsinki Declaration.

Eye tracking

During the eye-tracking procedure, the infant sat on the parent's lap at the distance of 50–70 cm from the eye tracker (EyeLink1000+, SR Research Ltd, Toronto, Ontario, Canada). A sampling frequency of 500Hz was used. Before every measurement, a five-point calibration procedure, with an audiovisual animation sequentially presented in five locations on the screen, was used. The calibration could be repeated before actual testing and also during the measurement when necessary. Small breaks were allowed during measurement, if necessary. The eye-tracking laboratory was dimly lit, and the researcher sat on an independent host computer next to the infant-parent dyads but was separated by a curtain to avoid interference.

Gaze acquisition and raw data processing

Similarly to Leppänen et al. (2011) and Forssman et al. (2014), the overlap paradigm was used to study infant attention disengagement from a centrally presented face or a scrambled face control stimulus to a lateral distractor. Photographs of two different women portraying happy, fearful, and neutral faces together with scrambled non-face control pictures were shown (Peltola et al., 2008). A set of 48 trials were presented, 12 trials per condition, i.e., each emotion and the control picture, comprising 18 photographs of each woman and 12 non-face control pictures in a semi-random order.

A fixation stimulus, i.e., a brief animation, was presented on the screen before each trial to attract the participant's attention on the screen and to center gaze position prior to the start of the trial. When the infant was attending towards the fixation stimulus, the trial started by a picture of a face or a non-face control stimulus in the center of the screen for 1000 ms (Figure 1). Then, a lateral distractor appeared on either left or right side of the face at a visual angle of 13.6° for 3000 ms simultaneously with the face. The lateral distractor was a pattern showing vertically arranged black and white rectangles (as shown in Figure 1) or circles. The distractor measured 15.4° vertically and 4.3°

horizontally. One trial lasted for 4000 ms. The sizes of the emotion-depicting pictures and distractor stimuli were 15.4° x 10.8° and 15.4° x 4.3°, respectively. The order of the central stimuli was semi-randomized with a constraint that the same stimulus was not presented more than three times in a row. The lateral stimulus was selected and presented randomly for each trial.

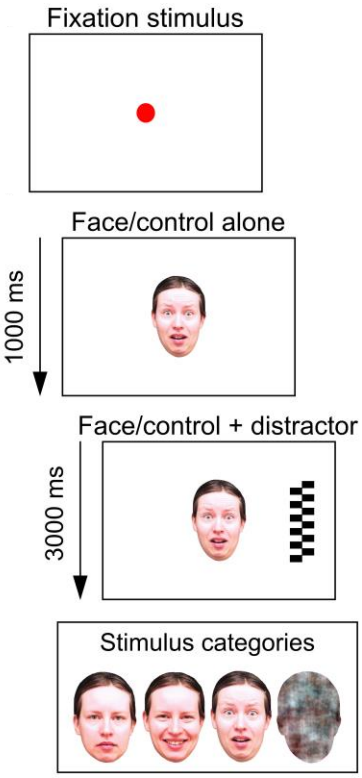

Figure 1. The overlap paradigm. A face or a control stimulus was presented in the center of the screen after the infant fixated on a fixation stimulus. A distractor appeared to the right or to the left side of the central stimulus after 1,000 ms from face/control onset. The central stimulus was presented until the end of each trial (4,000 ms) overlapping in time with the distractor (Figure reproduced from Yrttiaho, Forssman, Kaatiala, & Leppänen, 2014, https://doi.org/10.1371/journal.pone.0100811.g001 under the terms of the creative commons attribute license).

The trial data comprising of timestamps for the onset times of central and lateral pictures and the xy coordinates of the participants' gaze position, at 500 samples per second, were stored as text files and analyzed offline using a library of Matlab (Mathworks, Natick, MA) scripts (Leppänen, Forssman, Kaatiala, Yrttiaho, & Wass, 2015). We used the following quality control criteria based on prior

studies (Leppänen et al., 2015) to retain trials for the analysis. First, trials had to have sufficient looking at the central stimulus of greater than 70% during a time interval that started at the onset of the trial, i.e., appearance of the face or non-face on the screen, and extended to the end of the analysis period, i.e., gaze disengagement from the central to lateral stimulus or if a gaze disengagement was not observed, 1000 ms after the appearance of the lateral distractor. Secondly, trials had to have a sufficient number of valid samples in the gaze data with no gaps greater than 200 ms. This meant that gaps in the data were extrapolated by the analysis script by carrying the last recorded sample forward, but if the gap was greater than 200 ms, the trial was flagged as invalid and excluded from subsequent analyses. Thirdly, if disengagement occurred during the trial, the exact timing of the eye movement from the central to the lateral stimulus was required to be known for the trial to be included in the analysis, i.e., if the eye movement occurred during a period of missing or extrapolated gaze data, the trial was rejected.

The proportion of invalid trials was 20.8% corresponding to previous infant studies using the same methodology in 7- to 11-month old infants (21.6-28.2%, Leppänen et al., 2015).

Maternal depressive symptoms

Maternal depressive symptoms were assessed at gwk 14, 24, and 34 and three and six months postpartum with the Edinburgh Postnatal Depression Scale (EPDS). The EPDS consists of 10 questions scored on a 4-point Likert scale (from 0 to 3) (Cox et al., 1987). Mothers completed the EPDS along with other questionnaires covering a wide range of information related to maternal well-being during the perinatal period and early child development. The questionnaires were either mailed to the participants or filled out online. Data from N=274 mothers were available for the current infant sample (Table 1). The mothers who did not return the questionnaire at six months postpartum tended to be younger (t[322] = 1.83, p = .028) and less educated (χ^2 [2] = 7.59, p = .023), but they did not differ in terms of parity (χ^2 [1] = .63, p = .43) or the mean level of depressive symptoms during

pregnancy (gwks 14, 24, or 34) or three months postpartum (t test, *p* values >.32 for all assessment points of maternal symptoms).

The EPDS showed good internal consistency in our study (α=0.87). Two different analysis strategies were applied for maternal symptoms to test Hypotheses 3a and 3b. First, the EPDS sum score at six months postpartum, being our closest assessment point of maternal symptoms for infant eye tracking, was used to evaluate depressive symptoms, and a median-split of 4.5 points (similarly to the analyses of Forssman et al., 2014) was used to compare groups of infants of mothers with lower or higher levels of depressive symptoms. Second, the trajectories of maternal depressive symptoms from gwk 14 to six months postpartum, which was modelled with Latent growth mixture modelling (see Kataja et al., 2018), were used to compare groups of infants of mothers with increasing (N=29), decreasing (N=39), and low and stable (N=238) symptom levels.

Table 1. The demographic characteristics of mother-infant dyads participating to the study

Characteristic	*TPH2 x eye tracking N=330*	*TPH2 x maternal depression x eye tracking N=274*
Age, mother Mean (SD)	31.08 (4.25)	31.31 (4.10)
Infant sex		
Female	54.9%	55.2%
Male	45.1%	44.8%
Education level, mother		
High school/vocational	33.1%	27.7%
Polytechnics	31.9%	34.5%
University	34.9%	37.9%
Primiparity	51.8%	58.1%
TPH2 rs4570625 (N);		
GG vs. GT/TT	205/125	171/103
EPDS, Mean (SD), gw 14	4.64 (4.02)	4.80 (4.14)
EPDS, Mean (SD), gw 24	4.61 (4.43)	4.70 (4.60)
EPDS, Mean (SD), gw 34	4.59 (4.60)	4.65 (4.64)
EPDS, Mean (SD), 3 months	3.89 (3.77)	3.97 (3.75)

EPDS, Mean (SD), 6 months	4.14 (4.43)	4.59 (4.65)

DNA extraction and genotyping

DNA samples were extracted according to standard procedures at the National Institute for Health and Welfare. DNA samples were genotyped at the Estonian Genome Center using Illumina Infinitium beadchips (PsychArray and Global Screening Array). Quality control (QC) was performed with PLINK 1.9 (http://www.cog-genomics.org/plink/1.9/; Chang et al 2015). Markers were removed for missingness (>5%) and the Hardy-Weinberg equilibrium (p-value < 1 x 10^{-6}). Individuals were checked for missing genotypes of greater than 5%, relatedness by identical by descent calculation, PI_HAT>0.2, and population stratification through multidimensional scaling. Genotyped data was imputed with SHAPEIT2 (Delaneau et al. 2013) and IMPUTEv2 (Howie et al. 2009) using the 1000 Genomes project phase 3 haplotypes and a haplotype set of 1941 whole-genome sequenced Finnish individuals as reference panels.

The frequency of the minor T-allele was 21%.

Statistical analyses

The infants were divided to groups based on their allelic variance of rs4570625 in TPH2: G/G homozygotes, N=205; and T-allele carriers, T/T, N=14 and G/T, N=111. The allele frequencies were in Hardy-Weinberg equilibrium (p>0.2).

The probability of no attention shift (PNA) was defined as the infants' probability to *not* disengage their attention from the central, e.g., a fearful, happy or neutral face, or a non-face, to the lateral distractor stimulus. The PNAs for a population average infant were estimated by the mixed effects logistic regression (MELR) models with random intercept for each infant using, trial by trial, a binary disengagement variable, being disengagement or no disengagement, as the dependent variable.

Fear bias describes the difference between the infants' tendency to disengage from the non-fearful face conditions, i.e., control, happy or neutral faces, and their tendency to disengage from fearful faces. The children who were less likely to disengage from fearful faces, compared to the other conditions, had a higher fear bias. Technically, fear bias was defined as the ratio of the geometric average odds to disengage from the non-fearful conditions to the odds to disengage from the fearful condition.

The associations between PNAs and the predictors were analyzed using mixed-effects logistic regression (MELR) models with the binary attention shift variable that indicated whether there was an attention shift or not as the response. Only the valid trials, i.e., trials passing the quality control/pre-processing criteria, were used in the analysis. All our MELR models had a one-child-specific effect, a random effect, for each condition, i.e., four random effects per infant were included. Furthermore, as the PNAs depended strongly on trial number, i.e., the PNAs increased over the course of the experiment, see Kataja et al., 2018 and Leppänen et al., 2011, we controlled for it in all our models by including trial number as a fixed effect in the analysis. The trial number dependency was modeled by a natural cubic spline (de Boor, 1978) with one cut-point between trials 24 and 25, as curvilinear associations are recommended to be modeled by splines (Harrell, 2001) and setting the cut point at 24.5 trials gave a good fit with the data.

Our first hypothesis was tested using a model (Model 1) with the fixed effects

$$Condition + TPH2 + TNS$$

where *Condition* is a categorical variable with four values, e.g., neutral, happy, fearful and control faces, *TPH2* is a binary variable, indicating whether an infant was a T-carrier or not, and *TNS* means the two trial number spline terms. To analyze our second hypothesis (Model 2), a model with the fixed effects

$$Condition + TPH2 + Condition \times TPH2 + TNS$$

was used. The third hypothesis was analyzed using a model (Model 3a) with the fixed effects,

$$Condition + TPH2 + EPDS + Condition \times TPH2 + Condition \times EPDS + TPH2 \times EPDS + Condition \times TPH2 \times EPDS + TNS$$

where *EPDS* is a binary variable indicating if a mother has an EPDS score at six months postpartum under or over 4.5 points. Furthermore, at any time when fear bias was analyzed, we used such contrast coding for *Condition* that we were able to compare the average of the happy, neutral and control conditions against the fearful condition.

Finally, Hypothesis 3b was analyzed with a model equal to Model 3 except that the continuous variable *EPDS* was replaced by a categorical variable being *EPDS trajectory* with levels: Increasing, Decreasing, and Low.

All the statistical analyzes were performed in R 3.5.2 (R Core Team, 2018) using package lme4 (Bates, et al, 2015) for analyzing the MELR models and ggplot2 (Wikham, 2016) for making Figures 2, 3, and 4.

RESULTS

In the whole data, the non-disengagement ratios, defined as the number of no disengagements / number of valid trials for each condition, were: control: 623/2801 = 0.22, neutral: 1171/2825 = 0.41, happy: 1163/2832 = 0.41, and fearful: 1586/2889 = 0.55. The probability of no disengagement (PNA) was dependent on the trial number such that PNA increased over the course of the experiment (for an illustration, see Supplementary Materials, Figure S1).

Testing Hypothesis 1 revealed a non-significant main effect of *TPH2* SNP rs4570625 (-703 G/T) on the PNAs, $p = .73$ (Figure 2a).

Analysis of Hypothesis 2 showed a significant difference in fear bias between the T- and GG-carriers ($p = .02$). However, the result was in the *opposite direction* compared to the results shown in Forssman et al. (2014) as in our sample the T-carriers showed a lower fear bias (Figure 2b).

Analyzing Hypothesis 3a revealed that the T-carriers of the mothers with high depressive symptoms did not have a higher fear bias as compared to other infant groups classified by the genotype and maternal symptom levels (Table 2, Figure 2c). Instead, as a *post-hoc* result, we found that the T-carriers of the mothers with low depressive symptoms had significantly lower fear bias as compared to other groups (p values < .05) (Table 2, Figure 2d). Finally, analyzing Hypothesis 3b revealed no significant differences in fear bias between the groups (all p's > .05; Figure 3).

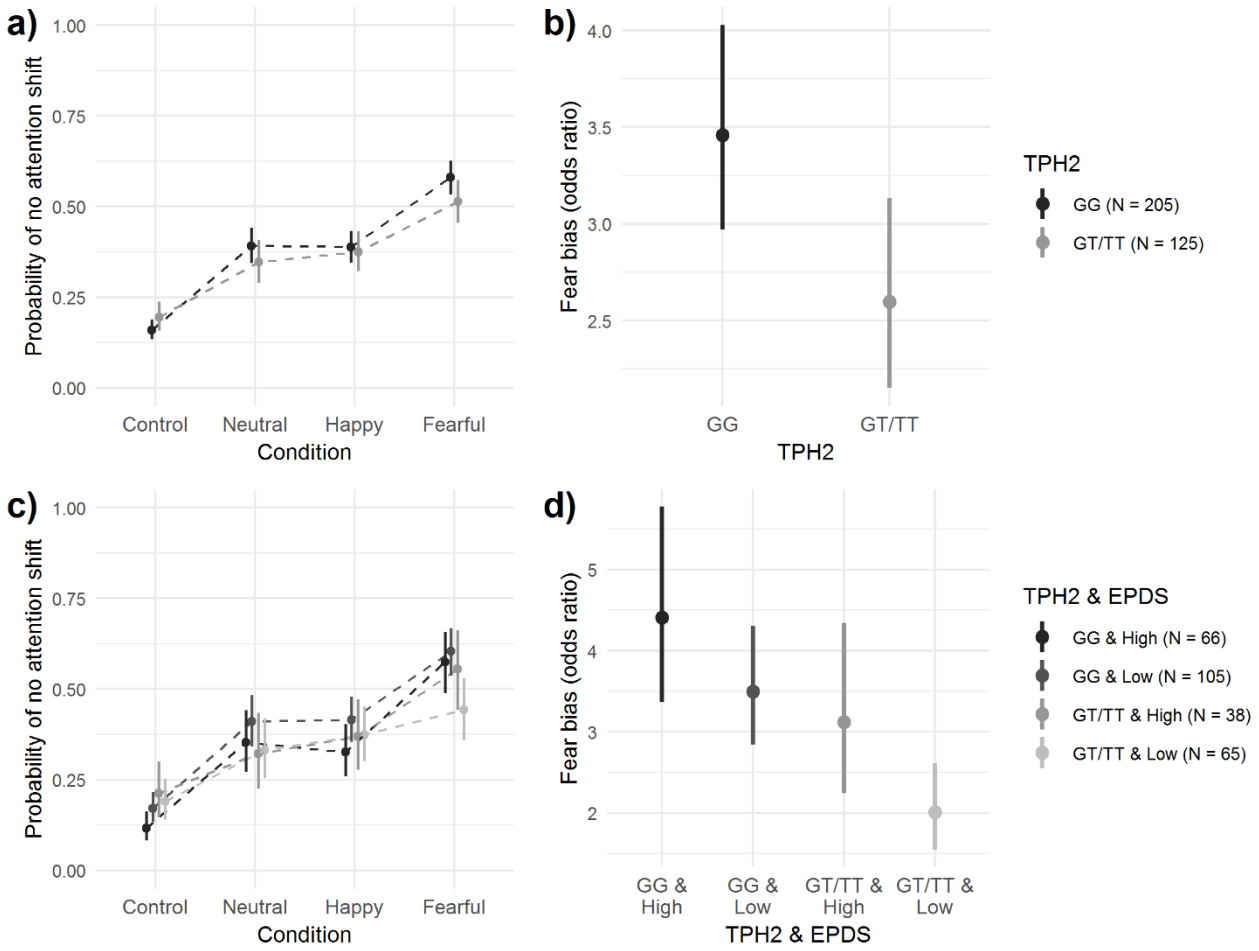

Figure 2a: The probability of no attention shift (PNA) from the central picture to the lateral distractor in the overlap paradigm grouped by the central facial expression and the TPH2 SNP rs4570625 (-703 G/T; G/G N=205; T-carriers, N=125).
Figure 2b: The effect of TPH2 SNP rs4570625 (-703 G/T) genotypes on the fear bias (i.e., the difference between infants' tendency to disengage from the fearful condition and their tendency to disengage from neutral/happy condition).
Figure 2c: The PNAs from the central picture to the lateral distractor grouped by maternal depressive symptoms (N=274; the Edinburgh Postnatal Depression Scale, EPDS, six months postpartum, median-split high *vs.* low at 4.5 points) and the *TPH2* SNP rs4570625 (-703 G/T, G/G, low EPDS, N=105; G/G, high EPDS, N=66; T-carriers, low EPDS, N=65; T-carriers, high EPDS, N=38).
Figure 2d: The fear bias in infants grouped by maternal depressive symptoms (EPDS, six months postpartum). In all the figures, error bars represent 95% confidence intervals.

Table 2. The Fear Bias (Odds Ratio with 95% CI) among the GG- and T-carriers (GT, TT) and among the GG- and T-carriers (GT, TT) of mothers with high or low self-reported depressive symptoms

Group	*Fear bias OR (95% CI)*

GG (N=205)	3.5 (3.0, 4.0)
GT/TT (N=125)	2.6 (2.2, 3.1)
GG & High (N=66)	4.4 (3.4, 5.8)
GT/TT & High (N=38)	3.1 (2.2, 4.3)
GG & Low (N=105)	3.5 (2.8, 4.3)
GT/TT & Low (N=65)	2.0 (1.5, 2.6)

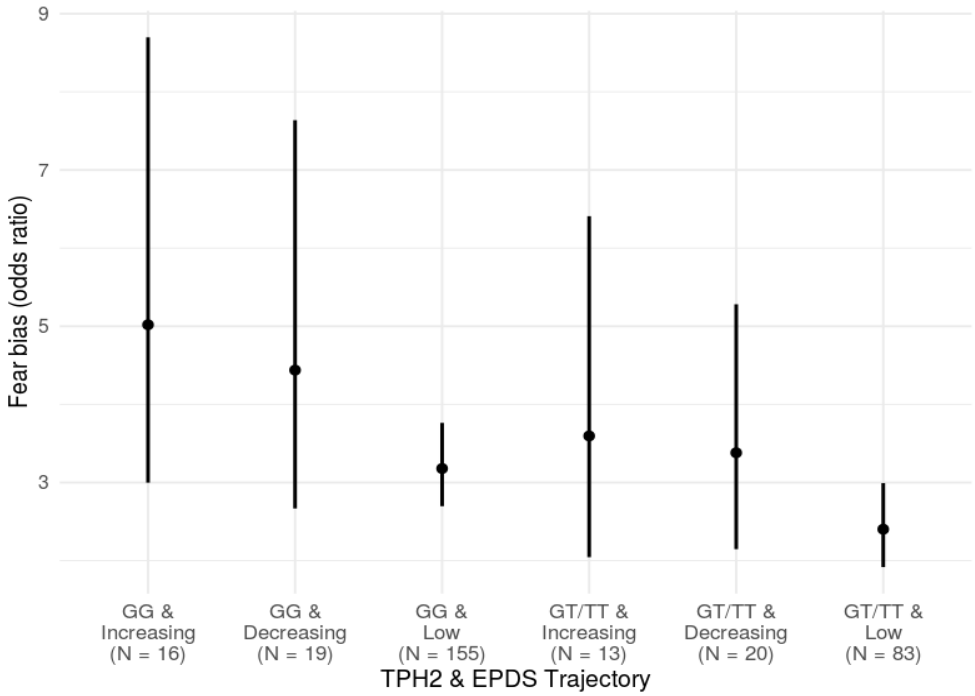

Figure 3. The fear bias in infants grouped by the trajectories of maternal depressive symptoms (EPDS from gwk 14 until six months postpartum; the groups of decreasing, increasing, and low symptom levels). Error bars represent 95% confidence intervals.

DISCUSSION

Two previous studies (Leppänen et al., 2011; Forssman et al., 2014) found that allelic variance of rs4570625 in *TPH2* influences cognitive processing of emotional faces during infancy. The T-carriers (GT/TT) compared to the GG-carriers disengaged their attention less frequently from affectively salient faces, especially from fearful faces, thereby reflecting a potential genetic vulnerability for increased attention to signals of negative emotion. This effect was more pronounced in the presence of an established environmental risk factor being maternal concurrent depressive symptoms (Forssman et al., 2014). In the current study, with a larger infant sample, no main effect of *TPH2* on attention disengagement was found. However, there was a significant difference between the groups in fear bias, as the T-carriers showed lower fear bias in general, and further, the T-carriers of mothers with low depressive symptoms showed a significantly lower fear bias as compared to other groups. The latter finding was not in line with our *a priori* hypotheses and to be considered as a novel result, which should be interpreted cautiously.

Our results are important in showing that the *TPH2* rs4570625 polymorphism is not consistently associated with attention regulation during infancy. There are several possible reasons for the inconsistent findings. One of these may relate to the general limitations of the candidate gene approach. Many studies have found a connection between the rs4570625 polymorphism and psychiatric morbidity during adulthood, but similarly to these findings in infants, the results have been inconsistent with regard to which allele (G or T) is associated with unfavorable outcomes (Laas et al., 2017). It is also possible that the allelic variance in this gene may be related to emotional sensitivity, but the association may be contingent on multiple individual and/or environmental risk factors. For instance, it is possible that the *TPH2* rs4570625 SNP association appears due to the linkage disequilibrium with another SNP (Ottenhof et al., 2018) or through interaction with a polymorphism in another serotonin transportation gene (e.g., 5-HTT; Canli et al., 2005). Moreover, the influence of allelic variance in TPH2 rs4570625 or the serotonin precursor tryptophan in general

on the developing brain and emotion processing systems is not yet understood and could be investigated further. Finally, the inconsistencies in the results may also underscore more general problems in the field of candidate gene research, where small effect sizes, inconsistent results, and failures to replicate observations or data are common (Dick et al., 2015; Duncan & Keller, 2011).

A second possibility is that the differences in the effects of *TPH2* rs4570625 on infant attention arise from the gene effect being affected by subtle differences in the studies. The age ranges of the infants differed between the studies, as they were 5–7 months old in the studies by Leppänen et al. (2011) and Forssman et al. (2014) and 8 months old in the current study. In normative development, preference to faces is strengthened between 6 and 9 months (Frank, Vul, & Johnson, 2009) suggesting that face perception undergoes developmental changes during the second half of first year. Previous studies further show that the fear bias becomes more evident in infants between 5 and 7 months of age (Leppänen, Cataldo, Bosquet Enlow, & Nelson, 2018, although see Forssman et al., 2014). Changes during the second half of the first year have not been documented, but more long-term follow-up studies have shown that biases for faces and fear decline between 7 and 24 months (Peltola, Yrttiaho, & Leppänen, 2018). Considering these findings, it could be speculated that the fear bias during infancy might develop differently among the T- and G-carriers with a higher fear bias being prevalent earlier among the T-carriers (i.e., at 5–7 months as was shown in previous studies) and among the G-carriers at 8 months (as is shown in this study). This possibility may not be unlikely, as the fear bias score, i.e., the primary outcome in the current analyses, has not been found to be stable between short time intervals, i.e., 5 to 7 months during infancy, while individual differences in gaze disengagement *per se* are relatively stable (Yrttiaho et al., 2014). Further, studies in rodents indicate that *Tph2* deficiency may change developmental timing by delaying the maturation of upper cortical layers which normalizes during adulthood (Mosienko & Alenina, 2019). Thus, the alterations in *TPH2* and central serotonin levels in human infants may lead to transient developmental phenotypical differences during early development and again normalize along with their development.

It should also be considered that differential patterns of results may arise from more general changes in task performance in older infants. For example, the task may become less interesting for older infants. In our study, the number of invalid trials (20.8%) was at the same level as compared to previous eye-tracking studies with younger infants, e.g., 7-month old infants (20.6%) (Leppänen et al., 2015). While this does not preclude the possibility that there are developmental changes in how attention is distributed to faces and distractors at different ages, it does suggest that there were no age differences in attention to screen or task completion rates. Of note is also that we used eye tracking to assess attention disengagement, whereas the previous studies used video-based analyses. Venker et al. (2019), when investigating the attention patterns of toddlers with ASD, showed that automatic eye tracking and manual gaze coding produced different rates of data loss, i.e., higher data loss in eye tracking as well as different patterns of results. Direct comparisons of attention disengagement times from eye tracking and manually coded data have shown consistent results (Leppänen et al., 2015), although data retention rates differ between these two methods. The two previous *TPH2* rs4570625 studies reported a manual coding data loss level of 5.6%, which is clearly lower than ours. However, as our eye tracking experiment lasted longer to that of the two previous studies, i.e., 48 vs. 25-30 trials, we were still able to retain more data for our analyses with an average of 8.6 valid trials per condition per infant compared to 4.9 in other studies. While this does not change the fact that the patterns of results may be different between manually coded versus automatic eye tracking, we may only argue that our study should have had better statistical power as compared to the two original studies, i.e., due to a higher number of trials and a larger sample size to detect effects. To explore the possibility that the results are due to differences in the length of the experiment, we also reported the results for Hypotheses 1–3a separately for the first and second half of the experiment in a supplement (Figures S2, S3). The results showed that the effect of genotype on infant attention patterns is similar between the first and second halves of the study but becomes stronger during the second half of the experiment. The T-carriers likely drove this effect more as the reduction in fear bias towards the end

of the experiment, which is reasonable due to the overall habituation to the distractors (Kataja et al., 2018), was higher among the T-carriers with a fear bias OR decreased by 35% compared to GG-carriers with a 16% decrease.

A further difference between the studies is that the assessment time for maternal depressive symptoms differed. Forssman et al. (2014) assessed maternal symptoms concurrently with the infant eye tracking at 5–7 months in contrast to six months postpartum for maternal symptoms and eight months for infant eye tracking in this current study. Maternal depressive symptoms tend to be highly correlated between different stages during the pre- and postnatal period (see Kataja et al. 2018) and are not likely to significantly differ in this study. Still, though, the infant's developing attention systems may be differentially sensitive to maternal symptoms at different developmental time-points, which may additionally lead to differences in study results. Due to this discrepancy, and as we have measured maternal depressive symptoms at several timepoints from early pregnancy on, we also ran the analyses by using a symptom trajectory approach, i.e., latent growth mixture modelling used in our previous study (Kataja et al., 2018). These analyses did not reveal significant differences in fear bias between the infants divided into the groups by their allelic status and maternal symptom trajectories, although the point estimates from this analysis corresponded to the main analyses using maternal symptoms at six months. Thus, our findings with a rather robust measure of maternal depression (i.e., the trajectories of symptoms from gwk 14 to six months postpartum) did not support the assertation that maternal depressive symptoms mediate the interaction between the TPH2 and fear bias. The analyses also showed that the previously reported result showing a difference in fear bias in infants of mothers with low versus increasing/decreasing symptoms (Kataja et al. 2018) was at present relatively independent of the *TPH2* allelic status of the infant. This result may suggest that cognitive-emotional traits during infancy are relatively strongly shaped by environmental factors.

The limitations of the candidate gene approach and the potential sensitivity of the *TPH2* rs4570625 effects to subtle differences in methods and ages are, hence, important to consider when interpreting the results of the current study. However, it is also interesting to speculate on the possible mechanisms that may result in the pattern of results observed in the current study, i.e., a higher fear bias in GG homozygotes. Tryptophan is essential for human body functions, and its role in emotional well-being appears mostly in its relationship with serotonin. Several studies have found a connection between tryptophan deficiency and autism spectrum disorder (ASD) that is likely mediated by impaired serotonin synthesis leading to core social and emotional deficits found in ASD (Kałużna-Czaplińska et al., 2019). Attenburrow et al. (2003) found that nutritionally sourced tryptophan enhances the perception of fearful and weakly happy faces among healthy female participants. This finding corresponded to their previous study of SSRI effects of emotion processing and was a finding that was inverse to their other previous study showing a decreased recognition of fearful faces due to tryptophan depletion. Thus, we may argue that "normal" serotonin levels are needed to recognize fearful facial expressions efficiently, which is an attention feature particularly prominent at the second half of the first year. If the less common T-variant is associated with decreased levels of serotonin in the brain, then the T-carriers might indeed show a lower fear bias due to their lower efficiency of recognizing fear or to differentiate between different emotions. Maternal psychiatric symptoms, in turn, enhance infant fear or threat bias (Forssman et al., 2014; Kataja et al., 2018a, 2019; Morales et al., 2017) even at subclinical levels. Fear processing systems are malleable to environmental factors due to their importance for survival and well-being. The parental caregiving environment has been found to be especially influential on the development of child affective neurobiology, as parents can both buffer/downregulate or amplify/upregulate their children's fear responses in contingency with their own emotional output (Callaghan et al., 2019; van der Bruggen, Stams, & Bögels, 2008). We may speculate that the T-carriers, inherently showing lower fear bias, still respond to their early

caregiving environment by exaggerated fear sensitivity. Here, for instance, some other neuroendocrinological mechanisms, e.g., cortisol, may also play a role.

In summary, our study with a relatively large infant sample does not provide support for a significant main effect of *TPH2* allelic variance on general attention disengagement patterns from emotional faces to distractors. We found evidence for the effect of *TPH2* on fear bias, but the findings were in the opposite direction as compared to previous studies. The *TPH2* effect on fear bias was not contingent on maternal depressive symptoms in the expected way, although a *post-hoc* finding showed that the T-carrier infants of mothers with low depressive symptoms close to the time of testing had a lower fear bias than all the other infant groups in terms of their fear bias. Together, these results show that the effect of the *TPH2* rs4570625 on infant attention is inconsistent across studies possibly reflecting the known limitations of the candidate gene approach or perhaps a complex relationship among the gene effect, infant age, and environmental risk factors.

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