The Association Between 5-HTTLPR Gene Polymorphism and Behavioral Inhibition in Chinese Toddlers

ABSTRACT: As one of the fundamental individual characteristics, behavioral inhibition in early childhood has considerable implications for the development of social, cognitive, and psychological adjustment. The purpose of this study was to examine the relation between the 5-HTTLPR polymorphism and behavioral inhibition in Chinese children using a cross-sectional design. A sample of 263 2-year-old children (134 boys and 129 girls of Han ethnicity; ages ranging from 24 to 26 months) in China participated in the study. Behavioral inhibition was assessed through laboratory observations, and genomic DNA was collected with buccal swabs. The results of analysis of covariance (ANCOVA) indicated that the homozygous short 5-HTTLPR allele was associated with lower levels of behavioral inhibition, which was different from most of the findings based on individuals in Western countries. The results suggest that social and cultural factors may be involved in shaping links between the 5-HTTLPR polymorphism and children’s specific behaviors.

INTRODUCTION

Researchers have observed substantial individual differences in children’s reaction to unfamiliar or challenging situations. Whereas some children are relaxed and spontaneous and display relatively little distress, others tend to be vigilant, anxious, and fearful (e.g., Asendorpf, 1991; Kagan, Snidman, Kahn, & Towsley, 2007; Fox, Henderson, Marshall, Nichols, & Ghera, 2005). The term behavioral inhibition has been used to characterize individual differences in this type of reaction (García-Coll, Kagan, & Reznick, 1984; Rubin, Hastings, Stewart, Henderson, & Chen, 1997). Behavioral inhibition is a significant phenomenon in early childhood that plays an important role in social, cognitive, and psychological development (Fox et al., 2005; Kagan, Reznick, & Snidman, 1988). For example, behavioral inhibition is associated with later problems such as social withdrawal, academic difficulties, and feelings of loneliness and depression (e.g., Asendorpf, 1991; Asendorpf, Denissen, & van Aken, 2008; Caspi et al., 2003a; Coplan, Prakash, O’Neil, & Armer, 2004; Kagan et al., 2007; Prior, Smart, Sanson, & Oberklaid, 2000; Schmidt, Fox, Rubin, Hu, & Hamer, 2002). Thus, it has been argued that early inhibition represents a risk factor for maladaptive
development (Pennebaker, 1993; Rubin, Coplan, & Bowker, 2009).

One of the biological systems that has received heightened attention in the study of behavioral inhibition is the serotonergic system. Researchers have focused on the serotonin transporter gene linked polymorphic region (5-HTTLPR), which is believed to be associated with predispositions to negative emotionality such as anxiety and fear (Fox et al., 2005; Murphy, Lerner, Rudnick, & Lesch, 2004). Serotonin transporter (5-HTT) regulates the reuptake of released serotonin following neuronal stimulation. Transcriptional activity of human 5-HTT is modulated by 5-HTTLPR, which is located on chromosome 17q12 and composed of 14 exons. The 5-HTTLPR gene consists of two alleles, 14-repetitive short (s) allele and 16-repetitive long (l) allele. The “s” allele is associated with diminished 5-HTT transcription, lower transporter levels, and reduced serotonin uptake (Hariri et al., 2002), whereas the “l” variant is associated with more rapid initial platelet 5-HT uptake (Greenberg et al., 1999). Individuals with the l/l and s/l genotypes exhibit significantly higher platelet 5-HT levels than those with the s/s genotype (Hanna et al., 1998).

Researchers have conducted numerous studies with adults to examine relations between 5-HTTLPR polymorphism and human behavioral functioning, especially of an internalizing nature, such as anxiety and social fear, which has been found to be associated with behavioral inhibition in early childhood (e.g., Schmidt & Buss, 2010; Schwartz, Snidman, & Kagan, 1999), and the results are sometimes inconsistent with each other (see Munafo et al., 2003; Serretti, Calati, Mandelli, & De Ronchi, 2006 for reviews). Despite the inconsistencies and confusions in the literature, a general pattern emerged indicating that when significant results were found, adult humans with the s allele of 5-HTTLPR appeared to be more inhibited, displaying higher anxiety and distress in reaction to novel and challenging situations (e.g., Furmark et al., 2004; Lesch et al., 1996; Munafo et al., 2003; Whisman, Richardson, & Smolen, 2011). Moreover, individuals with the s allele tended to display greater amygdala activity when facing threats and challenges (Bertolino et al., 2005; Furmark et al., 2004; Hariri et al., 2002; Heinz et al., 2005).

There are only a few studies that directly examined relations between 5-HTTLPR polymorphism and behavioral inhibition in children. The results indicated that children with the s/s 5-HTTLPR allele tended to be more inhibited than children with the l/l or l/s 5-HTTLPR allele (e.g., Auerbach, Faroy, Ebstein, Kahana, & Levine, 2001; Battaglia et al., 2005; Hayden et al., 2007; Lakatos et al., 2003). The results were consistent with some recent meta-analyses that showed significant associations between the s allele of 5-HTTLPR and individual vulnerability to stress including the HPA-axis reactivity and psychopathological symptoms (e.g., Serretti et al., 2006; Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2013). Nevertheless, different results have been reported. For example, Arbelle et al. (2003) found in a study with school age children that the l/l allele of 5-HTTLPR was positively associated with shyness assessed using questionnaires, and Schmidt et al. (2002) failed to find an association between the 5-HTTLPR polymorphism and shyness.

Researchers have conducted studies comparing the relative frequencies of 5-HTTLPR alleles between East Asian and Western populations. The results showed that higher proportions of Chinese and Japanese people possessed the s/s and l/s allele than those of White people (Kumakiri et al., 1999; Nakamura et al., 1997; Tsai, Hong, & Cheng, 2002). Researchers also examined behavioral inhibition in different social contexts, and found that it might have functional meanings in Asian children that were different from those in Western children. Behavioral inhibition refers to individual reactivity, as manifested in vigilant, wary, and anxious behaviors, to stressful or challenging novel settings (García Coll et al., 1984; Fox et al., 2005). Researchers (e.g., Chen et al., 1998; Chen, Chen, Li, & Wang, 2009; Rubin et al., 2006) found behavioral patterns reflecting the construct of behavioral inhibition in unfamiliar or challenging situations in Chinese children. However, the relations between behavioral inhibition and adult and peer attitudes in Chinese children were different from those in Western children. For example, whereas inhibition in toddlerhood was positively associated with maternal rejection and punishment orientation in Canada, it was positively associated with maternal positive attitudes such as warmth and acceptance in China. Unlike their counterparts in Euro-American societies who are often viewed less favorably by others (e.g., Fox et al., 2005), inhibited children in China were regarded as well behaved and socially competent (e.g., Chen & French, 2008; Chen, DeSouza, Chen, & Wang, 2006). Moreover, Chen et al. (2009) found that early childhood inhibition predicted later social and school adjustment in Chinese children; inhibited Chinese children displayed more socially desirable behaviors and were better adjusted socially and psychologically than uninhibited children in early adolescence. Similar results were found in other studies in Chinese and other Asian societies (e.g., Chen, Rubin, Li, & Li, 1999; Eisenberg, Pidada, & Liew, 2001).

Given this background, an interesting and important question is whether 5-HTTLPR polymorphism is associated with children’s behavioral inhibition in the same manner across contexts. Research on the relations between gene polymorphism and behaviors in different
contexts is important for us to understand genetic and environmental influences and their interactions on human behaviors (e.g., Chiao & Ambady, 2007; Miller & Kinsbourne, 2012). Genetic dispositions provide a foundation for the development of human behavior. Social and cultural practices, particularly socialization, serve to regulate the developmental processes and patterns. Numerous studies have indicated gene–environmental interactions in predicting human behaviors. Sheese, Voelker, Rothbart, & Posner (2007), for example, found that the association between DRD4 gene polymorphism and sensation seeking is moderated by the quality of parenting. The presence of the DRD4 7 repeat allele was positively associated with aggregated measures of sensation-seeking among children with low quality parenting, but the association was negative among children who experienced high quality parenting. Similar results have been reported in other studies such as those concerning interactions between DRD4 and parenting in predicting externalizing behaviors (Bakermans-Kranenburg & van IJzendoorn, 2006) and interactions between 5-HTTLPR and stressors in predicting depression (Caspi et al., 2003b; Hankin, Jenness, Abela, & Smolen, 2011). From the differential susceptibility perspective, researchers (e.g., Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2011) have argued that children with certain genes such as DRD4 and 5-HTTLPR may be particularly sensitive to the influence of environmental factors. For example, the DRD4 7 repeat allele and the s/s allele of 5-HTTLPR may confer heightened sensitivity to environmental influences, both positive and negative. Most of the research on gene–environment interactions has focused mainly on environmental variations within a society. It would be interesting to examine whether variations at the societal- or cultural-contextual level affect the relations between gene polymorphism and individual behaviors.

The purpose of the present study was to examine the association in a sample of Chinese toddlers. In keeping with the general findings in the literature (e.g., Battaglia et al., 2005), we hypothesized that the homozygous s allele of 5-HTTLPR would be associated with higher levels of behavioral inhibition. The study is intended to enhance our understanding of the relations between gene polymorphisms and children’s behaviors.

METHOD

Participants

The participants in the study were 263 2-year-old toddlers (134 boys and 129 girls, mean age = 24.84 months, SD = .74, ranging from 24 to 26 months) and their mothers. In addition, there were 19 children who participated in the behavioral observation paradigm but DNA data could not be collected from them; these children were not included in the present study. A nonsignificant difference was found on behavioral inhibition between those children who had DNA data and those who did not. This sample size, based on power analysis with an effect size of .20, power of .80, and α of .05, would be adequate for the analyses in the study. The participants were from a middle-size city of China. The city has fallen behind other major cities such as Shanghai and Beijing in social and economic development but is currently experiencing rapid social change and therefore more representative of most Chinese cities of its size. The families were recruited randomly from the birth lists of a large central-city hospital’s maternity ward, after mothers gave their birth to the babies, and thus were representative of the population in the city. The families were contacted first by a phone call and then through invitation letters for participation in the study. All toddlers were of Chinese Han, which is the predominant ethnic group in China (over 90% of the population). In the sample, 18.3% of the mothers and 15.2% of the fathers had an educational level of high school or below high school, 35.7% of the mothers and 32.3% of the fathers had an education of community college or professional training school, and the others had an education of university undergraduate or graduate. The average monthly family income was 8,858 yuan (approx. $1,400) (SD = 6,135 yuan).

Eligibility criteria included the following: mother at 18 years of age or older at the time of the child’s birth; first-born child in the family; birth weight of 2500 grams or more; and gestation 37 weeks or more without significant congenital abnormalities.

Procedure

When the toddlers were 24 months old, their parents were invited to visit the laboratory at the university and experience an adapted version of the Behavioral Inhibition Paradigm (e.g., Chen et al., 1998; García Coll et al., 1984; Rubin et al., 1997). Specifically, after each dyad entered an unfamiliar room, the child was allowed to play with an assortment of attractive toys for 10 min. while the mother sat in a chair and filled out a questionnaire concerning demographic information (free play). Then, after a cleanup session, an unfamiliar woman entered the room with a toy dump truck and some blocks. She sat quietly for 1 min, played with the truck for 1 min, and then (if the toddler had not yet approached) encouraged the child to join her in play. Next, the adult brought a toy robot, moving, “smoking,”...
and making noises, into the room. She was silent for 30 s, and then invited the child to play with the robot for 1 min. The child continued to experience other sessions assessing different abilities and behaviors such as reactions to frustration. The data from these sessions were not included in the present study because the data were complex, especially in terms of their relations with biological processes, and were not relevant to the current topic. For the DNA samples, the experimenter or the parent collected cheek cells from the children by rolling a buccal cotton brush gently on both sides of the cheek, approximately 20 times on each side. The buccal swabs were put into 0.9% physiological salt solution and refrigerated immediately. Informed written consent was obtained from parents.

Inhibition Coding
Following procedures described by Garcia-Coll et al. (1984) and Rubin et al. (1997), behavioral inhibition was coded based on the duration of the child’s physical contact with the mother in the free play, the latency to approach the stranger, and the latency to touch the toys. Specific coding and scoring systems are described in Chen et al. (1998). Reliability was computed for 10% of the sample as suggested by other authors (e.g., García Coll et al., 1984). The intercoder reliability for inhibited behavior (percentage of agreement, i.e., dividing the amount of time of agreement by the total amount of time of agreement and disagreement in seconds) was 96%. The procedure has been used and proved to be valid in North America and other countries such as China, Korea, and Italy (e.g., Chen et al., 1998; Rubin et al., 2006; Zappulla & Lo Coco, 2003). Following the instruction described by Chen et al. (1998) and Rubin et al. (1997), the inhibition scores were standardized and aggregated and then used in the analyses.

DNA Analysis
Genomic DNA was extracted from cheek swab samples by standard proteinase K digestion and chloroform extraction. 5-HTTLPR genotypes were determined by polymerase chain reaction (PCR) amplification using primers 5'-CTTGTGGGATTCTCCCGCTGGCTT-3' and 5'-TCGAGGCTGAGCGTCTAGAGGGACTGAGCT-3'. The PCR amplification protocol included denaturation at 95°C for 1 min, followed by 35 cycles at 95°C for 40 s, 63°C for 40 s, and 72°C for 40 s, followed by elongation at 72°C for 10 min. Three 5-HTTLPR genotypes were identified, including homozygous short allele (s/s), homozygous long allele (l/l), and heterozygous genotype (l/s).

RESULTS
Among the 263 samples, 85 were homozygous short allele (s/s), 134 were heterozygous genotype (l/s), and 43 were homozygous long allele (l/l), as shown in Table 1. There was no difference between boys and girls in the frequencies of 5-HTTLPR genotypes, \( \chi^2(2) = 0.82, p > 0.05 \). 5-HTTLPR genotypes were in the Hard–Weinberg equilibrium, \( \chi^2(1) = 0.75, p > 0.05 \). The mean scores of behavioral inhibition were \( -0.07 \) and \( 0.06 \), SDs = 2.11 and 2.06 for boys and girls, respectively.

An analysis of covariance (ANCOVA) was conducted to test the effects of gender, genotype, and their interaction on children’s behavioral inhibition, with parental education and family income as covariates. We coded children’s genotypes as 0 (s/s), 1 (l/s), and 2 (l/l), and gender as 0 (boy) and 1 (girl). The results indicated a significant main effect of the 5-HTTLPR polymorphism on behavioral inhibition. \( F(2, 254) = 4.49, p < 0.01, \eta^2 = 0.03 \). A post hoc power analysis using the GPower computer program (Erdfelder, Faul, & Buchner, 1996) revealed that the power to detect an effect in the present condition was 0.69. There was no significant main effect of gender, \( F(1, 254) = 0.71, p > 0.05 \), or interaction between gender and 5-HTTLPR polymorphism, \( F(2, 254) = 0.54, p > 0.05 \). Follow-up analysis indicated that children with the s/s allele had significantly lower inhibition scores than children with the l/s allele (\( p < 0.01 \)). The difference between children with the s/s allele and children with the l/l allele was marginally significant (\( p < 0.067 \)). Children with the l/s allele and the l/l allele did not differ. The means and standard deviations of behavioral inhibition for the genotype groups are presented in Table 2, and the means and standard errors of behavioral inhibition for the groups are illustrated in Figure 1.

DISCUSSION
Over the past several decades, researchers have been interested in relations between gene polymorphisms of neurotransmitters and individual behaviors and characteristics. Since Lesch et al. (1996) reported the link between the 5-HTTLPR polymorphism and self-reported anxiety and neuroticism, a number of studies have been conducted to examine the associations between the 5-HTTLPR polymorphism and adults’ and children’s anxiety-related personality traits including shyness-inhibition in Western European and North American countries (e.g., Miller et al., 2013 for a recent review). The results of the present study concerning the relation between the 5-HTTLPR poly-
morphism and behavioral inhibition in Chinese children may help us understand the expression of the genotype in non-Western contexts.

Researchers have found higher frequencies of short 5-HTTLPR alleles in Chinese and Japanese people than in White people (Kumakiri et al., 1999; Nakamura et al., 1997; Tsai et al., 2002). We did not include a Western sample in this study for direct comparisons. However, our results were consistent with the findings in the literature. Specifically, whereas the percentage of infants in our sample with the l/s allele was similar to those in previous studies with Western samples (approx. 50%, e.g., Fox et al., 2005; Lakatos et al., 2003; Lesch et al., 1996; Whisman et al., 2011), the percentage of infants with the s/s allele in the Chinese sample (32%) was considerably higher than those in the Western samples (approx. 15–20%), and the percentage of infants with the l/l allele in the Chinese sample (16%) was considerably lower than those in the Western samples (approx. 30–35%).

In addition to the differences in the distribution of the genotype, the association between the 5-HTTLPR polymorphism and behavioral functioning in Chinese children was interesting. As indicated earlier, the general findings from most studies with Western individuals indicate that the short allele tends to be associated with higher levels of anxiety-related behaviors including inhibition (e.g., Battaglia et al., 2005; Lakatos et al., 2003; Lesch et al., 1996; Miller et al., 2013; Whisman et al., 2011). The results of the present study showed that the homozygous short 5-HTTLPR allele was associated with lower levels of observed behavioral inhibition and wariness in the stressful and challenging situations.

The association between the 5-HTTLPR polymorphism and behavioral inhibition may need to be understood from a contextual perspective because the association is likely to involve social processes, which determine, in part, the meaning of the behavior (Chen & French, 2008; Yoshikawa, Way, & Chen, 2012). It is possible that children with the s/s 5-HTTLPR allele, which conveys relatively decreased 5-HTT transcription, lower transporter levels, and reduced serotonin uptake with implications for the function of neural circuits regulated by serotonin (Fox et al., 2005; Hariri et al., 2002), are less able to respond readily to socialization practices and develop corresponding socially desirable behavior. Researchers have found that whereas childhood behavioral inhibition is viewed as incompetent and maladaptive in Western societies, it is considered an indication of social maturity and competence in China (e.g., Chen et al., 1998). Thus, whereas

### Table 1. Genotype Distribution of 5-HTTLPR Polymorphisms

<table>
<thead>
<tr>
<th>5-HTTLPR Polymorphism</th>
<th>Boys</th>
<th>Girls</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>s/s</td>
<td>40</td>
<td>45</td>
<td>85</td>
<td>32.3</td>
</tr>
<tr>
<td>l/s</td>
<td>71</td>
<td>63</td>
<td>135</td>
<td>51.3</td>
</tr>
<tr>
<td>l/l</td>
<td>23</td>
<td>20</td>
<td>43</td>
<td>16.3</td>
</tr>
</tbody>
</table>

### Table 2. Means and Standard Deviations of Behavioral Inhibition for 5-HTTLPR Genotype Groups

<table>
<thead>
<tr>
<th>Genotype Group</th>
<th>Mean</th>
<th>SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>s/s</td>
<td>-.52</td>
<td>1.67</td>
<td>85</td>
</tr>
<tr>
<td>l/s</td>
<td>.25</td>
<td>2.26</td>
<td>135</td>
</tr>
<tr>
<td>l/l</td>
<td>.19</td>
<td>2.12</td>
<td>43</td>
</tr>
</tbody>
</table>

**FIGURE 1** Means and standard errors (error bars) of behavioral inhibition scores for 5-HTTLPR genotype groups.
their Western counterparts often receive negative social evaluations and responses and experience adjustment problems, inhibited Chinese children appear to obtain social support and approval during socialization and function well in various domains (Chen et al., 2009; Rubin et al., 2009). As such, children with the s/s allele may display less socially valued inhibited behavior in China. Regardless of the underlying processes, our results suggest that the link between the 5-HTTLPR polymorphism and children’s specific behavior may be moderated by the particular context.

There were several major strengths in the present study including a relatively large random sample in a non-Western society and the use of an observational method in assessing behavioral inhibition. The study provided valuable information about the role of social and cultural context in shaping the association between the 5-HTTLPR polymorphism and children’s behaviors, which constituted a significant contribution to the literature. Nevertheless, some limitations in the study should be noted. First, as indicated earlier, the present study was conducted in a middle-size city in China that is currently experiencing rapid social change but has fallen behind major cities such as Shanghai and Beijing in social and economic development. Research conducted in Shanghai and Beijing (e.g., Chen, Cen, Li, & He, 2005) has showed that as new values such as social assertiveness and competitiveness are more appreciated and emphasized as a result of societal changes, shy, inhibited, and restrained behavior may be viewed as less adaptive and desirable. It will be interesting to study how macro-level social changes affect the relation between the 5-HTTLPR polymorphism and children’s behavioral inhibition.

Second, we collected information on parental education and income in this study and controlled them in the analyses. It will be important to collect more comprehensive demographic data such as parental mental health in future research.

The present study was the first to investigate genetic effects on behavioral inhibition in non-Western children. Our results clearly need to be replicated in the future. Moreover, researchers have reported that the 5-HTTLPR polymorphism interact with environmental factors such as the child’s early experience of maltreatment and stressful life events in predicting developmental outcomes in Western samples (e.g., Caspi et al., 2003b; Uber & McGuffin, 2008). As the next step, it will be important to explore the joint or interactive contributions of the 5-HTTLPR gene and socialization experience to child development in Chinese and other societies.

In addition, although most studies adopted biallelic functional classification of 5-HTTLPR, Hu et al. (2006) showed that the 5-HTTLPR was functionally triallelic, with short (S), long-A (L_A), and long-G (L_G) alleles. Only the L_A showed high 5-HTT mRNA levels, and the L_G and S alleles apparently had comparable low levels of gene expression. Thus, triallelic classifications of 5-HTTLPR polymorphism may be more accurate when investigating the relation between 5-HTTLPR polymorphism and behaviors, and future research needs to further distinguish L_A and L_G. It is also possible that gene polymorphisms related to other neurotransmitters, such as MAOA, DRD2, and DRD4 polymorphisms, are involved in affecting behavioral inhibition. For example, the MAOA polymorphism plays an important role in regulating the release and clearance of 5-HT, and MAOA activity is related to amygdala reactivity (Buckholtz & Meyer-Lindenberg, 2008). Thus, continued exploration of children’s behaviors from different perspectives is critical to achieving a further understanding of human adaptive and maladaptive development.

NOTE

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