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Original Article

The association between *5-HTTLPR/rs25531* polymorphisms and behavior problems in Mongolian children

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Abstract: To determine the association between *5-HTTLPR/rs25531* polymorphisms and behavior problems of Mongolian school-age children, 311 (female = 157, male = 154) healthy children aged 7 to 12 were investigated in Chifeng city of Inner Mongolia. Children's behavior problems and temperament types were evaluated by the Child Behavior Checklist (CBCL) and Behavior Style Questionnaire (BSQ) or Middle Childhood Temperament Questionnaire (MCTQ), respectively. The *5-HTTLPR/rs25531* genotypes were determined by mf-RFLP (multiplex fluorescence-restriction fragment length polymorphism) analysis. Association between the genotypes and behavior problem scores was analyzed using a generalized linear model (GLM). The results showed that: (1) no significant difference was found in genotypes based on age, gender, or prevalence of behavior problems in the Mongolian children; (2) the children with low-expression genotypes (L_GL_G, SL_G, or SS) had significantly higher scores in withdrawn, aggression, internalizing, externalizing, and total behavior problems than those with high-expression genotypes (L_AL_A, SL_A, or L_AL_G); (3) withdrawal behavior score was affected by the interaction of *5-HTTLPR/rs25531* genotypes with temperament types ($P < 0.01$). The children with passive temperament and L_AL_A, SL_A, or L_AL_G genotypes tended to suffer from withdrawal behavior; (4) there was a significant interaction between *5-HTTLPR/rs25531* genotypes and age in aggressive behavior score ($P < 0.01$). The children with L_GL_G, SL_G, or SS genotypes were more likely to suffer from aggressive problems in an older age. Overall, the present study suggests that *5-HTTLPR/rs25531* polymorphisms might contribute to certain behavior problems, and that the interaction between genotypes and major affected factors in behavior problems should be considered.

Keywords: Mongolian nationality, school-age children, behavior problems, gene polymorphism

Introduction

Behavior problems have been grouped into two categories, namely the internalizing and externalizing problems. The former consists of problems such as emotional reactivity, anxious or depressed mood, somatic complaints, and social withdrawal, whereas the latter includes problems such as experiencing conflict with other people, aggression, hyperactivity, attention problems, and the total problems that comprise both internalizing and externalizing problems [1, 2]. The behavior problems that have been ignored in early childhood may result in antisocial behavior and mental disorders in late childhood [3, 4]. The World Health Organization (WHO) reported that 10-20% of children and adolescents worldwide experience mental disorders, placing mental disorders among the leading causes of ill-health and disability (http://www.who.int/mental_health/management/en/). Data from the China National Children's Center (CNCC) recently showed that at least 30 million children and adolescents, under the age of 17, in China, exhibit a range of behavior problems. Hence, early detection, treatment, and prevention of such problems are important in the healthy upbringing of children.

Genetic studies have documented that genetic determinants contribute substantially to the variance in some behavior problems [5, 6]. The most frequently studied candidate gene for psychiatric disorder traits such as affective disorders, anxiety, depression, suicide, obsessive-compulsive disorders, and addiction is the serotonin transporter (*5-HTT*)-linked polymorphic region (*5-HTTLPR*) in the promoter of the *SLC6A4* gene, which plays an important role in serotonergic neurotransmission by facilitating the re-uptake of serotonin from the synaptic cleft [7]. The *5-HTTLPR* polymorphism comprises a 43-base pair insertion or deletion. The most common alleles are classified into two main groups, namely, the short (S) allele (14 repeats) and long (L) allele (16 repeats) [8], the L-allele having an mRNA expression approximately three-fold higher than the S-allele [9]. A previous study had shown that *5-HTT* contains an A/G single nucleotide polymorphism (SNP) within the encoding gene, rs25531, which lies within the L-allele of *5-HTTLPR* [10]. Combination of the two polymorphisms (*5-HTTLPR/rs25531*) appears as a triallelic locus, including three alleles L_A, L_G, and S, in which the L_G allele is functionally equivalent to the S allele; the rs25531 G-allele reduces the transcriptional efficiency of L-allele of *5-HTTLPR* to an extent comparable to that of S-allele [11]. Hence, there has been critical discussion regarding the inadequacy of the traditional dichotomous analysis of *5-HTTLPR*, in distinguishing between the L_A and L_G alleles [12], which may reduce its statistical power. So far, only a few studies have attempted to demonstrate the effect of triallelic polymorphism in behavior problems. Some studies have indicated that individuals who were homozygous for the low expressing *5-HTTLPR* alleles (S and L_G) experienced greater difficulty in disengaging their attention from sorrow, happiness, and fear stimuli than those with the high expressing *5-HTTLPR* homozygotes [13]. Xhosa adolescents exhibited the *5-HTTLPR/rs25531* L_G haplotype, associated with a reduction in anxiety sensitivity [14]. However, the triallelic (*5-HTTLPR/rs25531*) polymorphisms were not related to major depressive disorders in Chinese Han adolescents [15].

China is a country with a very large population, composed of many nationalities. Each one has his/her own particular custom and cultural background, which influences the development of children's psychological behavior. Mongolian nationality is one of the main ethnic groups in Inner Mongolia. So far, most of the studies have been concerned with the common psychological problems of Mongolian children and adolescents, including depression, aggression, and internalizing problems. However, documents on the behavior problems of Mongolian children were mainly related to the study of environmental factors [16, 17]. There has been no study on the relationship of *5-HTTLPR/rs25531* polymorphisms with behavior problems of Mongolian school-age children. In order to provide a molecular foundation to the comprehensive understanding of behavior problems of Mongolian children, this study examined the influence of *5-HTTLPR/rs25531* polymorphisms on the behavior problems of healthy Mongolian school-age children.

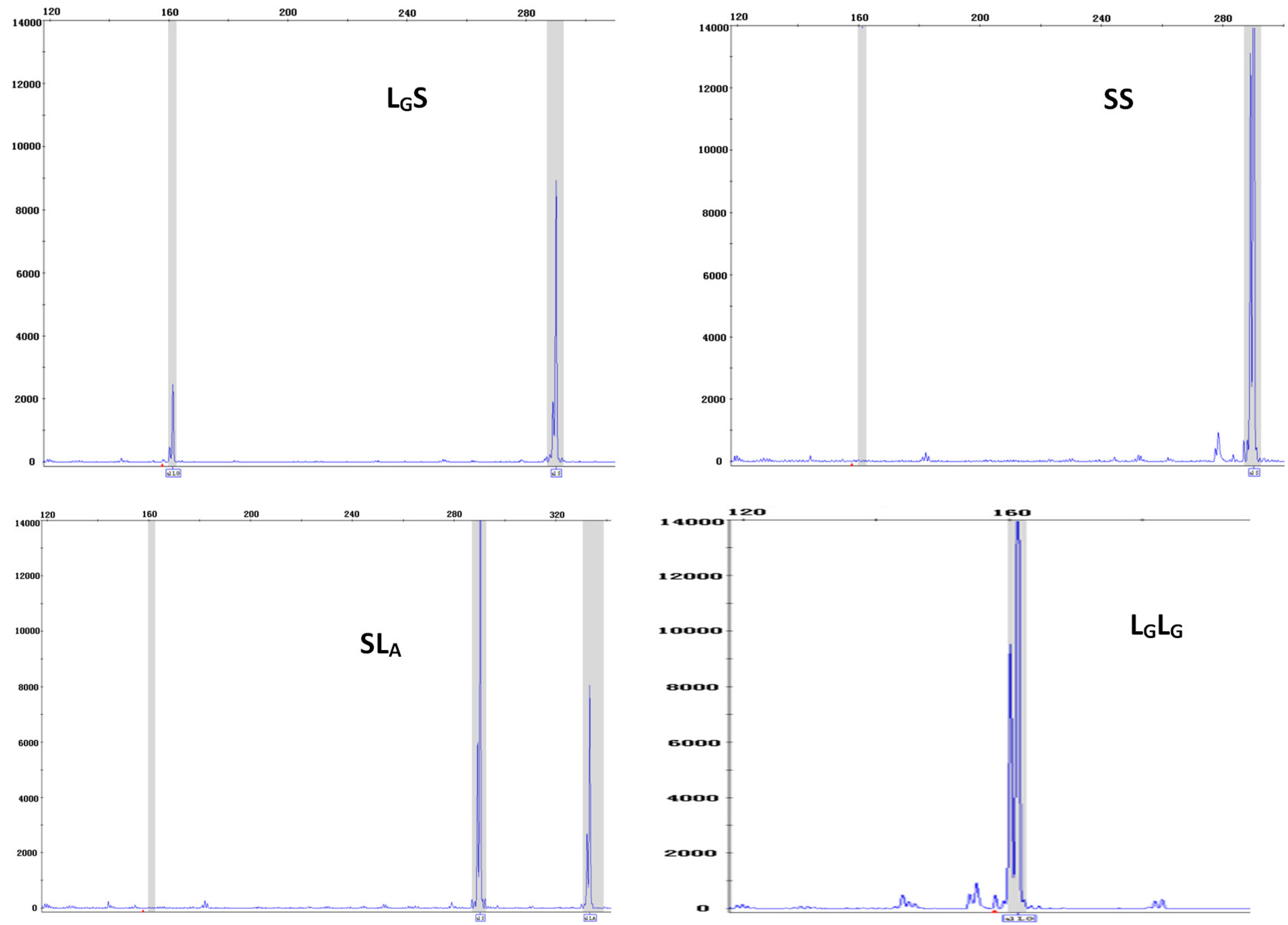
Materials and methods

Subjects and questionnaires

A total of 311 healthy Mongolian children aged 7-12 years (female = 157, male = 154; mean age ± SD = 9.23 ± 1.51 years) were recruited in Tianshan Mongolian Experimental Primary School, Chifeng, Inner Mongolia, China. The previous three generations of the immediate family were of Mongolian nationality in all cases.

Parents of all the participants filled out a set of questionnaires. Chinese version of the Child Behavior Checklist (CBCL), consisting of 113 items, was administered to determine the behavior problem scores and prevalence. Depending on how well each item describes the child, the respondent rates from 0 (it is not true at all/had no chance to observe) to 2 (it is completely true), considering the last two months. The Chinese version of CBCL scale has been confirmed with satisfactory reliability and validity [18].

The Chinese version of standardized children's temperament questionnaire for children between 3-7 years contained 100 items (Behavior Style Questionnaire, BSQ) and that for children between 8-12 years comprised 99 items (Temperament in Middle Childhood Questionnaire, TCMQ). This scale has been validated for test-retest reliability [19]. According to temperament dimensions, temperament types can be divided into two categories, i.e. active temperament types that include easy and inter-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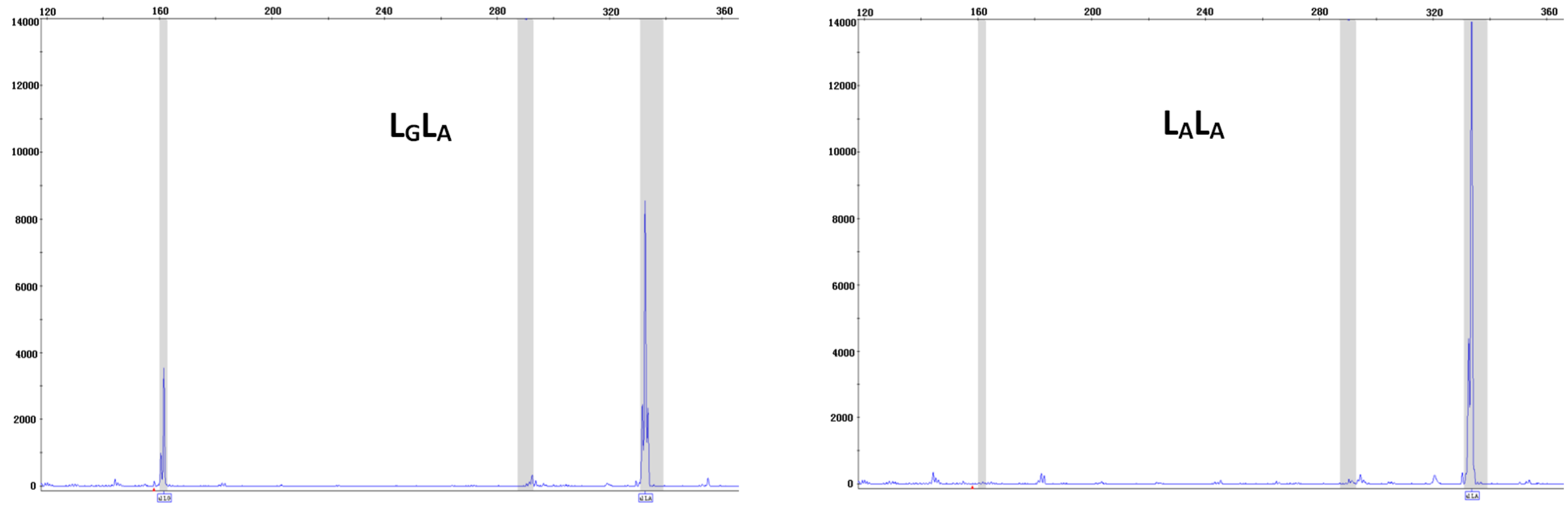

Figure 1. Genotyping results for rs25531 using mf-RFLP.

Table 1. Prevalence of behavior problems in Mongolian school-age children

Variable	Total		Male		Female		χ^2 (Fisher)	*P*
	n	%	n	%	n	%		
Anxiety and depression	43	13.83	17	11.04	26	16.56	1.989	0.158
Withdrawal behavior	27	8.68	7	4.55	20	12.74	6.583	0.010
Physical discomforts	5	1.61	3	1.95	2	1.27	(0.223)	0.682
Thought problem	61	19.61	29	18.83	32	20.38	0.119	0.731
Attention problem	10	3.22	2	1.30	8	5.10	(3.590)	0.104
Socialization problem	14	4.50	9	5.84	5	3.18	1.279	0.258
Rule-breaking behavior	9	2.89	5	3.25	4	2.55	(0.135)	0.714
Aggressive behavior	14	4.50	8	5.19	6	3.82	0.341	0.559
Internalizing problem	17	5.47	6	3.90	11	7.01	1.455	0.228
Externalizing problem	9	2.89	4	2.60	5	3.18	(0.095)	0.758
Total behavior problems	93	29.90	40	25.97	53	33.76	2.247	0.134

mediate low type, and passive temperament types that include difficult, intermediate high and slow to warm up type.

In this study, we also investigated the parental education background and occupation, average family income, delivery methods, and the number of children in a family. 6 participants were excluded based on genotyping, for a final sample size of 311 participants.

Genotyping

Buccal swabs were collected and the entire genomic DNA was extracted via the TIANamp Swab DNA Kit according to the manufacturer's instructions. The quality of DNA was assessed for each sample using agarose gel electrophoresis, while the quantity of DNA was determined by spectrophotometer (Quawell). The *5-HTTLPR/rs25531* genotypes were determined by mf-RFLP (multiplex fluorescence-restriction fragment length polymorphism) analysis. The forward 5'-CGGGATGCGGGGGA-ATACTGGT-3' and reverse primers 5'-TTGCC-GCTCTGAATGCCAGCAC-3' were used for genotyping the *5-HTTLPR* polymorphism. The PCR product was resolved on a 1.6% agarose gel in TBE solution and visualized by UV light. The 100-bp low ladder (Sangon Biotech, Shanghai, China) was used to determine the length of the fragments. To identify the A/G SNP rs25531 on L allele, the fragments were digested using restriction endonuclease MspI. Digestion products were analyzed using an ABI 3730XL DNA Analyzer (Applied Biosystems, Waltham, MA). Digested products that included a 163-bp fragment indicated an L_G allele, a 293-bp fragment indicated an S allele, and a 337-bp fragment indicated an L_A allele (**Figure 1**).

Statistical analysis

To check the normal distribution of behavior problem score, Kolmogorov-Smirnov test was performed. The nonparametric tests were used to examine the association of behavior problem scores with each of the following factors: gender, age, temperament type, and environmental factors. The main effect of *5-HTTLPR/rs25531* polymorphisms and the relevant above-mentioned factors along with the interaction between the genotypes and the relevant factors on behavior problem scores were used to perform the generalized linear model (GLM) analysis. In addition, the prevalence of behavior problems was compared using chi-square or Fisher's exact test. The statistical significance limit was set at $P = 0.05$. All statistical analyses were performed using SPSS version 20.

Results

Descriptive statistics

In this study, the total prevalence of behavior problems was 29.90%, among which the prevalence of thinking problem was the highest (19.61%), and that of physical complaints was the lowest (1.61%). Chi-square test or Fisher's exact test results showed that there was a significant gender difference across the prevalence of withdrawal behavior (**Table 1**). Kolmogorov-Smirnov test results showed that the behavior problem score was an abnormal distribution. Therefore, the nonparametric tests were used to examine the relationship between the behavior problem scores and relevant factors including gender, age, temperament types, and family environmental factors. As is well known, children entered puberty close to 10 years of age, with growth spurts and sexual development, resulting in a series of changes in

Table 2. Effect of age, temperament types, and parents' education on behavior problems scores of the children

	Age groups		Z	Temperament types		Z	Father's education		Z	Mother's education		Z
	7-10 y	11-12 y		Active	Passive		A	B		A	B	
AD	2.00 (1.00-5.00)	5.00 (3.00-9.00)	2.696$^{\#}$	3.00 (1.00-5.00)	3.00 (1.00-6.00)	0.643	0.00 (0.00-2.25)	3.00 (1.00-6.00)	1.903$^{\#}$	0.00 (0.00-2.00)	3.00 (1.00-6.00)	2.051$^{\#}$
WB	2.00 (1.00-4.00)	3.00 (2.00-5.00)	2.096$^{\#}$	2.00 (1.00-4.00)	3.00 (1.00-5.00)	1.672*	1.00 (0.00-2.25)	2.00 (1.00-4.00)	1.104	1.00 (0.00-2.00)	2.00 (1.00-4.00)	1.370*
PD	0.00 (0.00-1.00)	1.00 (0.00-4.00)	1.940$^{\#}$	0.00 (0.00-2.00)	0.00 (0.00-2.00)	0.478	0.00 (0.00-1.00)	0.00 (0.00-2.00)	0.666	0.00 (0.00-1.00)	0.00 (0.00-2.00)	0.928
TP	0.00 (0.00-2.00)	2.00 (0.00-3.00)	1.737$^{\#}$	0.00 (0.00-2.00)	2.00 (0.00-3.00)	1.773$^{\#}$	0.00 (0.00-1.25)	1.00 (0.00-2.00)	0.728	0.00 (0.00-1.50)	1.00 (0.00-2.00)	0.963
AP	2.00 (0.00-4.00)	3.00 (1.00-5.75)	1.279	2.00 (0.00-4.00)	3.00 (2.00-6.00)	1.607*	1.00 (0.00-2.50)	2.00 (0.00-4.25)	1.446*	1.00 (0.00-2.00)	2.00 (0.00-4.25)	1.328
SP	2.00 (1.00-3.00)	2.00 (1.00-4.00)	1.067	2.00 (1.00-3.00)	2.00 (1.00-4.50)	0.909	2.00 (0.00-2.00)	2.00 (1.00-4.00)	0.973	2.00 (0.00-2.00)	2.00 (1.00-4.00)	1.192
RB	1.00 (0.00-2.00)	2.00 (1.00-3.00)	2.066$^{\#}$	1.00 (0.00-2.00)	2.00 (0.00-3.00)	0.964	1.00 (0.00-3.00)	1.00 (0.00-2.00)	0.437	1.00 (0.00-2.50)	1.00 (0.00-2.00)	0.736
AB	4.00 (1.00-7.00)	5.00 (2.00-10.00)	1.820$^{\#}$	4.00 (1.00-7.00)	6.00 (2.00-10.00)	1.130	3.00 (0.00-6.00)	4.00 (1.00-8.25)	0.916	3.00 (0.00-6.00)	4.00 (1.00-9.00)	1.093
IP	5.00 (2.00-9.00)	10.00 (5.00-15.75)	2.366$^{\#}$	6.00 (3.00-10.00)	7.00 (3.00-15.50)	1.056	3.00 (0.00-5.25)	6.50 (3.00-12.00)	1.544*	3.00 (0.00-5.00)	7.00 (3.00-12.00)	1.819$^{\#}$
EP	5.00 (2.00-9.00)	6.00 (4.00-13.00)	1.487*	5.00 (2.00-9.00)	7.00 (3.00-12.50)	1.185	4.00 (0.00-6.00)	5.00 (2.00-11.00)	0.957	4.00 (0.00-6.00)	5.00 (2.00-11.00)	1.001
TP	20.00 (11.00-37.00)	30.00 (18.50-51.50)	1.961$^{\#}$	22.00 (12.00-38.00)	30.00 (16.50-46.50)	1.295	16.00 (5.00-22.25)	24.00 (11.00-39.00)	1.472*	16.00 (5.00-22.50)	24.00 (11.00-39.00)	1.623$^{\#}$

Note: $^{*}P < 0.05$, $^{\#}P < 0.01$; A: University or more, B: college or less.

Table 3. Demographic data of the study subjects according to *5-HTTLPR/rs25531* genotypes

	Genotypes (n, %)		Statistical test
	$L_AL_A/SL_A/L_AL_G$	$L_GL_G/SL_G/SS$	
Total sample			
n	82	229	
Sex, female (%)	22.29%	77.70%	χ^2 = 2.710, *P* = 0.10
Age (year)	9.11 ± 1.56	9.27 ± 1.49	t = -0.823, *P* = 0.408
Female participants			
n	35	122	
Age (year)	9.00 ± 1.53	9.17 ± 1.47	t = -0.605, *P* = 0.546
Male participants			
n	47	107	
Age (year)	9.21 ± 1.72	9.36 ± 1.66	t = -0.486, *P* = 0.628

Note: Continuous variables are reported as mean ± standard deviation; categorical variables are listed as a column-wise percentage.

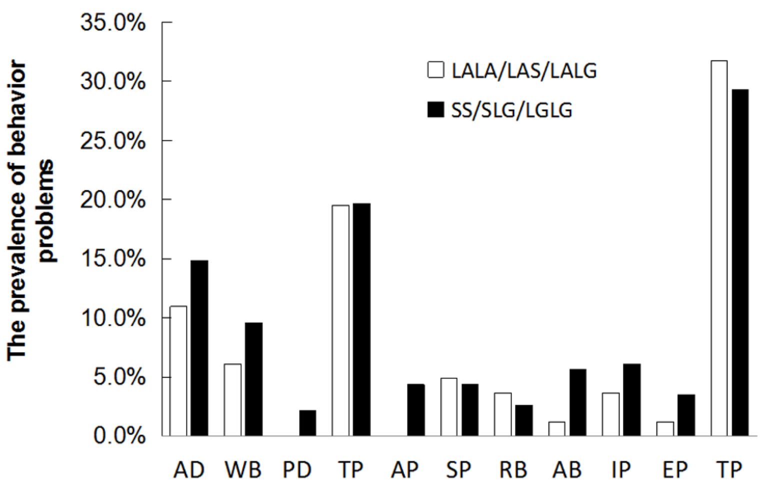

Figure 2. Prevalence of behavior problems in different genotypes of Mongolian school-age children. AD: Anxiety and depression. WB: Withdrawal behavior. PD: Physical discomforts. TP: Thought problem. AP: Attention problem. SP: Socialization problem. RB: Rule-breaking behavior. AB: Aggressive behavior. IP: Internalizing problem. EP: Externalizing problem. TP: Total behavior problems.

behavior. Therefore, in this study, the children were divided into a younger group (7-10 y) and an older group (11-12 y). Results of the non-parametric tests are shown in **Table 2**. Age was found to significantly affect anxiety and depression, withdrawal behavior, physical discomforts, thought problem, rule-breaking behavior, aggressive behavior, internalizing problem, externalizing problem, and total behavior problems ($P < 0.05$). Temperament types significantly influenced withdrawal behavior, thought problem, and attention problem ($P < 0.05$). Anxiety and depression, internalizing problem, and total behavior problems were significantly influenced by parents' education ($P < 0.05$); attention problem was only affected by the father's education while withdrawal behavior was only affected by the mother's education ($P < 0.05$).

The association of 5-HTTLPR/rs25531 genotypes with behavior problems

Testing for Hardy-Weinberg equilibrium (HWE) did not yield significant deviations from the expected distribution of *5-HTTLPR/rs25531* genotypes (χ^2 = 2.793, P = 0.425). According to literatures on different mRNA expression level of *5-HTTLPR* in vitro [20, 21], the *5-HTTLPR/rs25531* genotypes in this paper have been grouped into two levels for analysis, one containing a high-expression group (L_AL_A, SL_A, or L_AL_G = 82) and another containing a low-expression group (L_GL_G, SL_G, or SS = 229). The result showed no significant age or gender difference across the genotypes ($P > 0.05$) (**Table 3**).

Genotypes did not reflect the difference in prevalence of each behavior problem using χ^2-test ($P > 0.05$), but the prevalence of most behavior problems was lower in children with L_AL_A, SL_A, or L_AL_G genotypes than in those with L_GL_G, SL_G, or SS genotypes (**Figure 2**).

The association between genotypes and behavior problem score was examined using GLM analysis, in which the behavior problem score was taken as dependent variable and genotypes were considered as the independent variable. The results showed that children with L_GL_G, SL_G, or SS genotypes had higher scores of withdrawn, aggressive, internalizing, external-

Table 4. Correlation analysis between *5-HTTLPR/rs25531* genotypes and the behavior problems scores of Mongolian school-age children

Variables	Genotypes	$\overline{X}$ ± SD	B	S.E	95% Wald CI		χ^2	*P*
					Lower	Upper		
Withdrawn	Intercept		2.873	0.066	2.744	3.003	1.89E+03	0.000
	$L_AL_A/SL_A/L_AL_G$	2.62 ± 2.16	-0.251	0.129	-0.504	0.001	3.816	0.051
	$L_GL_G/SL_G/SS$	2.87 ± 2.58	0[a]					
Aggression	Intercept		5.183	0.066	5.054	5.313	6.15E+03	0.000
	$L_AL_A/SL_A/L_AL_G$	4.91 ± 4.29	-0.269	0.129	-0.521	-0.017	4.362	0.037
	$L_GL_G/SL_G/SS$	5.18 ± 4.86	0[a]					
Internalizing	Intercept		7.917	0.066	7.788	8.047	14.354E+03	0.000
	$L_AL_A/SL_A/L_AL_G$	7.51 ± 5.66	-0.405	0.129	0.657	0.153	9.896	0.002
	$L_GL_G/SL_G/SS$	7.92 ± 7.08	0[a]					
Externalizing	Intercept		6.856	0.066	6.726	6.985	10.764E+03	0.000
	$L_AL_A/SL_A/L_AL_G$	6.60 ± 5.84	-0.258	0.129	-0.511	-0.006	4.030	0.045
	$L_GL_G/SL_G/SS$	6.86 ± 6.23	0[a]					
Total problems	Intercept		27.742	0.066	27.613	27.872	17.625E+03	0.000
	$L_AL_A/SL_A/L_AL_G$	27.28 ± 18.48	-0.462	0.129	-0.714	-0.210	12.880	0.000
	$L_GL_G/SL_G/SS$	27.74 ± 20.36	0[a]					

Note: a, set to zero, with the parameter as the standard.

Table 5. Effect of the interaction of *5-HTTLPR/rs25531* genotypes and environmental factors on behavior problems of Mongolian school-age children

Variables			B	S.E	95% Wald CI		χ^2	*P*
					Lower	Upper		
Aggression	*5-HTTLPR/rs25531*	Intercept	7.000	0.135	6.736	7.264	2.695E+03	0.000
		$L_AL_A/SL_A/L_AL_G$	-2.647	0.278	-3.191	-2.103	90.993	0.000
		$L_GL_G/SL_G/SS$	0[a]					
	Age	Younger age group	-2.391	0.155	-2.694	-2.088	2.389E+02	0.000
		Older age group	0[a]					
	Interaction	Genotype*Age groups	3.099	0.313	2.485	3.713	97.886	0.000
		Genotype*Age groups	0[a]					
Withdrawn	*5-HTTLPR/rs25531*	Intercept	3.184	0.162	2.866	3.502	3.853E+02	0.000
		$L_AL_A/SL_A/L_AL_G$	0.974	0.281	0.425	1.524	12.009	0.001
		$L_GL_G/SL_G/SS$	0[a]					
	Temperament	Active	-0.336	0.178	-0.685	0.014	3.550	0.060
		Passive	0[a]					
	Interaction	Genotype*Temperament	-1.629	0.317	-2.250	-1.007	26.402	0.000
		Genotype*Temperament	0[a]					

Note: a, set to zero, with the parameter as the standard.

izing, and total behavior problems than those with L_AL_A, SL_A, or L_AL_G genotypes ($P \leq 0.05$). The association between *5-HTTLPR/rs25531* genotypes and the behavior problem scores of Mongolian school-age children are depicted in **Table 4**.

Effect of G × E interaction on behavior problem scores

Based on the above results, effect of the interaction of genetic factor (G) and environment factors (E) on the behavior problem scores

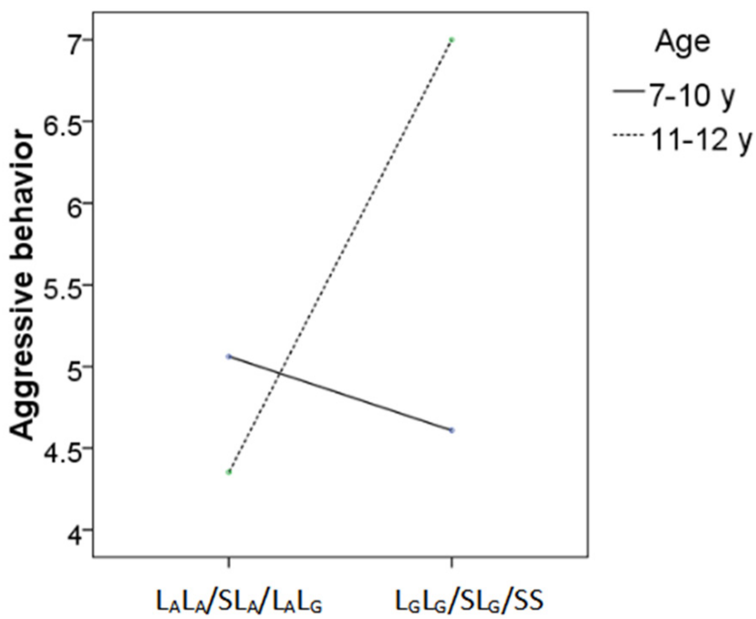

Figure 3. Effect of interaction of *5-HTTLPR/rs25531* genotypes and age on aggressive behavior score.

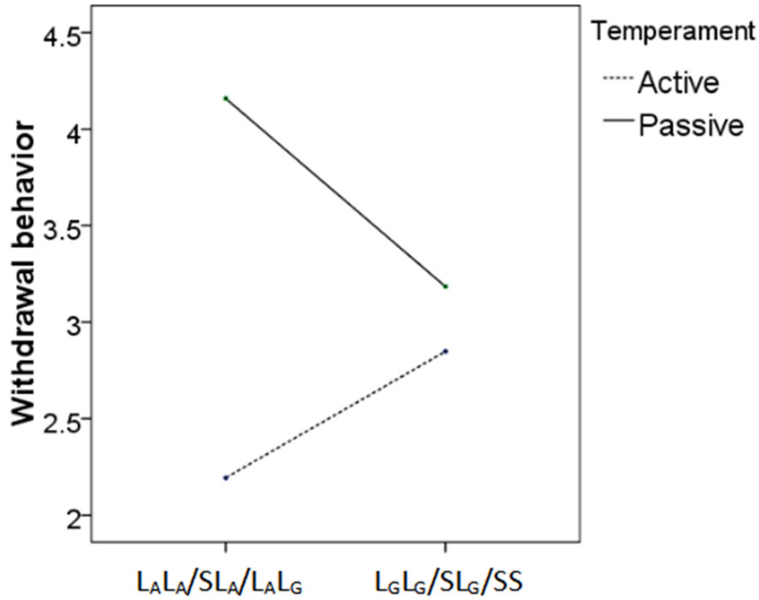

Figure 4. Effect of interaction of *5-HTTLPR/rs25531* genotypes and temperament on withdrawal behavior score.

were analyzed using GLM analysis, in which each behavior problem score was taken as the dependent variable, and *5-HTTLPR/rs25531* genotypes (genetic factor, G) were considered as the independent variables, each influenced environment factors (E), namely, age, temperament types, and parents' education. The results showed that aggressive behavior score was influenced by the *5-HTTLPR/rs25531* genotypes ($P < 0.05$), age ($P < 0.05$), and the interaction of *5-HTTLPR/rs25531* genotypes with age ($P < 0.05$). Moreover, the main effects of *5-HTTLPR/rs25531* genotypes ($P < 0.05$) and temperament types were on withdrawal behavior score, as well as on the interaction of *5-HTTLPR/rs25531* genotypes with temperament types ($P < 0.05$) (**Table 5**).

The effect of G×E interaction on behavior problem scores is also shown in **Figures 3** and **4**. According to **Figure 3**, the children with L_GL_G, SL_G, or SS genotypes in the older age group had a significantly higher aggressive behavior score than those in the younger age group. Therefore, the younger age group might have a protective factor that prevents aggressive behavior in children with the L_GL_G, SL_G, or SS genotypes.

As in **Figure 4**, there was a relatively small difference in withdrawal behavior score of children carrying L_GL_G, SL_G, or SS genotypes across the temperament types, but children with L_AL_A, SL_A, or L_AL_G genotypes and passive temperament had a significantly higher withdrawal behavior score than those with active temperament.

Discussion

With the recent process of rural urbanization, most Mongolian school-age children, from village or pastoral area, are being sent to school in the cities, either living in school or lodged with relatives, resulting in parents spending less time with them, and eventually developing a strained relationship between the parents and children. Psychological and behavior problems of those children might not be guided and treated immediately. In this study, the total prevalence of behavior problems among Mongolian school-age children was found to be 29.90%, which is higher than in the children of either Han, Zhuang, or Yao nationality in 2001 [22]. In addition, our study demonstrated a significant influence of the educational level of parents on some behavior problem scores ($P < 0.05$). With the rapid development of information technology, parents with relatively low education level are facing greater challenges in guiding their children. In short, the prevalence of behavior problems among children and adolescents will be on the rise and need to be attended more seriously.

Earlier studies showed that the SS genotype was present in 22% of Caucasians and in 60% of Asians, and the LL genotype was present in 29-43% of Caucasians, but only in 1-13% of East Asians [23, 24]. The results of genotype frequency (SS: 54.34%, LL: 6.43%) in our study were similar to those in other studies on Asians at home and abroad.

The results of GLM analysis in our study indicated that children with L_GL_G, SL_G, or SS genotypes had a significantly higher withdrawn, aggressive, internalizing, externalizing, and total behavior problem scores than children with L_AL_A, SL_A, or L_AL_G genotypes. Based on the available literature, the *5-HTTLPR/rs25531* polymorphisms have been found to modulate attention to threat [26], anxiety, negative effect, nutritional status in childhood, and suicidal ideation [26-28]. Additionally, the SS and SL_G genotypes seem to be associated with higher neuroticism [27] and more serious symptoms in patients who suffer from anxiety or depression [28]. These results, together with the present study, indicate that SS, SL_G, or L_GL_G genotypes may be the risk factors that contribute to the development of behavioral problems among Mongolian school-age children.

A previous study had indicated that verbal and relational aggression increased consistently from childhood, and became more hostile in late childhood [29]. Moreover, aggressive syndrome in childhood appears to be a stable characteristic that persists into adulthood [5]. A recent study showed that a reduction in 5-HT activity can be associated with aggressive behavior, particularly with impulsive aggression [30]. In our study, children with L_GL_G, SL_G, or SS genotypes in the older age group had a significantly higher aggressive behavior score than those in the younger group (**Figure 3**), suggesting that the individuals carrying the low expression genotypes (L_GL_G, SL_G, or SS) were more likely to have aggressive behavior at an older age. Therefore, children with L_GL_G, SL_G, or SS genotypes need to be given more attention and protection in early childhood.

As seen in the interaction curves (**Figure 4**), we concluded that carriers of L_AL_A, SL_A, or L_AL_G genotypes with passive temperament had a significantly higher withdrawal behavior score than those with active temperament. The main reason could be that the children with passive temperament were unable to adapt to the changes in new environment and hence suffered from emotional instability. For example, passive temperament was indirectly associated with overt antisocial behavior and substance abuse via poor social decisions [31]. Previous studies have shown that mild forms of anxiety were related to a child's temperament and emotion regulation [32]. Taken together, temperament may be one of the important factors that influence the development of psychological behavior.

In conclusion, the present study suggests that the *5-HTTLPR/rs25531* polymorphisms may be a factor that contributes to several behavior problems. Nonetheless, a specific gene cannot completely reflect the complexity of gene-environment interactions. Therefore, future research should consider gene-gene-environment interactions to gain a more comprehensive understanding of the mechanism and cause of behavior problems in Mongolian school-age children.

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Disclosure of conflict of interest

None.

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