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Relation between latrophilin 3 (LPHN3) gene polymorphism (rs2345039) and attention deficit hyperactivity disorder in children

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Abstract

Background: The most prevalent psychological disorder in children is attention deficit hyperactivity disorder (ADHD). Latrophilin 3 (LPHN3) is a G protein-coupled receptor family member. It is brain specific and related to attention deficit hyperactivity disorder (ADHD) genetic susceptibility. This study aimed to assess the association of LPHN3 gene with ADHD and its types.

Methods: The subjects were 2 groups: group I, thirty patients with ADHD, and group II, thirty healthy individuals as a control group. The process of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to establish the genetic association of ADHD with the polymorphic gene LPHN3 (rs2345039).

Results: The ADHD group included 20 male and 10 females; the mean age was 9.8±1.8 years. LPHN3 rs2345039 polymorphism genotypes distribution showed a statistical significant difference between ADHD and controls ($P=0.01$). In the ADHD group, individuals with CG genotype were 5.8-folds to have ADHD than CC individuals. Also, those with GG genotype were about six times more likely to have ADHD than CC ones. All of these relationships were significant statistically ($P=0.024$ and $=0.018$, respectively). Individuals carrying the G allele were 2.6 times more likely to develop ADHD than those carrying the C allele (OR = 2.6, 95% CI = 1.3–5.6, P value = 0.01).

Conclusion: Our results demonstrate an association between latrophilin 3 (LPHN3) gene rs2345039 and ADHD. Moreover, LPHN3 polymorphisms tend to have a key role in triggering the condition and exacerbating its severity.

Keywords: Latrophilin 3, LPHN3, Gene, Children, ADHD

Background

One of the neurodevelopmental disorders is attention deficit hyperactivity disorder (ADHD). It is characterized by age-inappropriate and persistent patterns of hyperactivity, inattention, and impulsivity. There are three types of ADHD according to the Diagnostic and Statistical Manual, Fifth Edition, of the American Psychiatric Association: inattentiveness, hyperactivity-impulsivity, and a mixture of both of them [1]. Attention deficit hyperactivity disorder is the most often diagnosed mental disease in children (ADHD). Children with ADHD may be impulsive and energetic, or they may have difficulty focusing. These habits have an effect on both school and home life. ADHD is more frequent in boys. It is usually starts in a child's early school years, when he or she begins to have difficulty paying attention [2]. The estimated prevalence for school-aged children and adolescents ranges from 5.3 to 7.1% [3].

Attention deficit hyperactivity disorder is a complex, heterogeneous disorder, yet uncertain in etiology. While environment performs a key role, twin, family, and adoption studies indicate that the genetic risk factors are a major component, indicating an approximate heritability

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of around 70–80 % [4]. Many molecular genetic susceptibility studies for ADHD were performed with many approaches used. Candidate gene studies focused mainly on monoamine system genes [5]. These studies found many variants, usually of minimal impact, that do not clarify the high ADHD heritability [6].

The frontal and prefrontal lobes are considered to be the main brain regions involved; the parietal lobe and cerebellum may also be involved. Children with ADHD who conducted response inhibition tasks had different stimulation in fronto-striatal areas than healthy controls, according to one functional MRI study. A 2010 study found that fronto-striatal dysfunction plays a part in the etiology of ADHD [7].

While ADHD has been linked to structural and functional changes in the fronto-striatal circuitry, recent research has found changes just outside of that area, mainly in the cerebellum and parietal lobes [8].

While symptoms and behavioral criteria are used to diagnose ADHD, neuropsychological assessments, such as continuous performance tests, are often used throughout the evaluation. Conner's Continuous Performance Test (CPT) is one such test, but views vary about how effective it is in evaluating patients with ADHD symptoms who present for assessment [9].

The quest for candidate genes linked to ADHD susceptibility and medication response continues to be a hot topic of research. The latrophilin 3 gene has recently been related to ADHD susceptibility (LPHN3) [10].

Latrophilin 3 (LPHN3) is a G protein-coupled receptor family member; it is brain-specific and related to attention deficit hyperactivity disorder (ADHD) genetic susceptibility. The LPHN subfamily of G-protein coupled receptors (GPCRs) plays a role in the regulation of neurotransmitter exocytosis and synaptic activity in the brain [11]. The current study aimed for evaluating the association of LPHN3 gene with ADHD and its types.

Methods

This case-control study was conducted to assess the association of LPHN3 gene polymorphism (rs2345039) with ADHD and its types. All patients were selected from outpatient clinic of pediatric and neuropsychiatric department at the Benha University and the pediatric department of Menoufia University.

All participants' parents gave written informed consent. All patients had a thorough history taking and clinical assessment. Each individual had a venous blood sample (2 ml) taken and collected into a sterile ethylenediaminetetraacetate "EDTA" (vacutainer) tube for DNA extraction. DNA was isolated from fresh samples and maintained at −20 °C until the time of the experiment. A real-time polymerase chain reaction (rt PCR) approach was used to determine the LPHN3 gene polymorphism (rs2345039).

We have two groups: group I, which included 30 patients who were diagnosed by Conners Assessment [12] as the ADHD group, and group II, which included 30 apparently healthy individuals age and sex matched as a control group.

Inclusion criteria

The age range is from 7 to 12 years. All patients were diagnosed as ADHD by Conners "continuous performance test" [12].

Exclusion criteria

Patients with other psychological problems were excluded from the study as well as patients with mental or significant medical comorbidities.

Statistical analysis

The SPSS version 16 software (SPSS Inc., Chicago, ILL Company) was used for data analysis. Categorical data were summarized as numbers and percentages, chi-square (χ_2) or Fisher's exact tests were used to analyze them, and odds ratios (OR) and the corresponding 95% CI were calculated when appropriate. Numerical data were tested for normality using the Shapiro-Wilks test. Normally distributed variables were summarized as means ± standard deviations and analyzed using independent t test for two independent groups, while non-parametric data were presented as medians and ranges, and Kruskal-Wallis (KW) test was used for the analysis of 3 independent groups. The determinants of ADHD were identified using binary logistic regression analysis. Significance was defined as a P value less than 0.05.

Genotype distributions follow the Hardy-Weinberg equilibrium in the studied groups (data not shown). Simple Hardy-Weinberg Calculator-Court Lab was used for calculation (Washington State University College of Veterinary Medicine, Pullman, WA, USA). Hardy-Weinberg equilibrium was calculated based on OEGE—Online Encyclopedia for Genetic Epidemiology studies [13].

Results

This study included 34 males and 26 females, which were in 2 groups: group I, which included 30 patients (20 males and 10 females) with ADHD, and their mean age was 9.8 ± 1.8 years, and group II, which included 30 apparently healthy age- and gender-matched individuals (14 males and 16 females) as a control group, and their mean age was 9.1 ± 1.4 years (Table 1). The individuals with CG genotype were 5.8-folds to have ADHD than CC individuals. Also, those with GG

Table 1 Demographics of the studied groups

Variable			ADHD group ($n=30$)		Control group ($n=30$)		Test of significance	*P*
Personal history	**Age (years)**	**Mean ± SD**	9.8 ± 1.8		9.1 ± 1.4		**St."t" = 1.58**	**0.119 (NS)**
		Range	7–12		7–12			
			No.	**%**	**No.**	**%**	**χ^2/**	***P***
	Sex	**Male**	20	66.7	14	46.7	**2.44**	**0.118 (NS)**
		Female	10	33.3	16	53.3		
	Birth order	**Median (range)**	1.0 (1–3)		2.0 (1–4)		**2.44**	**0.015 (S)**
	Residence	**Urban**	19	63.3	18	60.0	**0.07**	**0.79 (NS)**
		Rural	11	36.7	12	40.0		
Perinatal history	**Pregnancy complications**	**No**	13	43.3	29	96.7	**20.3**	**<0.001 (HS)**
		Yes	17	56.7	1	3.3		
	Birth complications	**No**	17	56.7	29	96.7	**13.4**	**<0.001 (HS)**
		Yes	13	43.3	1	3.3		
	Postnatal complications	**No**	18	60.0	27	90.0	**7.2**	**0.007 (S)**
		Yes	12	40.0	3	10.0		
Developmental history		**Normal**	18	60.0	27	90.0	**7.2**	**0.007 (S)**
		Abnormal	12	40.0	3	10.0		
Family history	**Consanguinity**	**No**	10	33.3	29	96.7	**26.4**	**<0.001 (HS)**
		Yes	20	66.7	1	3.3		
	Marital status	**Married**	25	83.3	28	93.3	**FET = 2.06**	**0.51 (NS)**
		Divorced	3	10.0	2	6.7		
		Widowed	2	6.7	0	0.0		

FET Fisher's exact test, *St."t"* Student *t*-test, χ^2 chi-square test, *S* significant, *NS* non-significant, *HS* high significance

genotype were about six times more likely to have ADHD than CC ones. Each of these correlations was statistically significant ($P=0.024$ and $P=0.018$, respectively). Individuals holding the G allele had a 2.6-fold increased risk of developing ADHD compared to those carrying the C allele (OR = 2.6, 95% CI = 1.3–5.6), a statistically significant difference ($P=0.01$) (Table 2). There were no statistically significant differences in the onset age or type of ADHD between the study groups (Table 3). There was a statistical difference between types of gene polymorphism and severity of the disease (Table 4 and Fig. 1).

Discussion

Latrophilin 3 is G-protein coupled receptor (GPCR) LPHN subfamily member, known as GPCRs. These receptors have complex and large N-terminal fragment which contains various motifs involved in cell adhesion. LPHN3 has a role in neurotransmitter and hormone exocytosis regulation. At synapses, highly enriched small G proteins play a crucial role in intracellular signaling [14].

We identified a statistically significant difference in the genotype distribution of the LPHN3 rs2345039 polymorphism between ADHD and controls ($P=0.01$) throughout our analysis. Individuals with the CG

Table 2 Comparing the studied groups regarding gene polymorphism

Variable		ADHD group ($n=30$)		Control group ($n=30$)		OR (95%CI)	*P*
		No.	**%**	**No.**	**%**		
LPHN3 gene polymorphism	**CC**	3	10.0	12	40.0	**Ref.**	
	CG	13	43.3	9	30.0	**5.8 (1.2–25.5)**	**0.024 (S)**
	GG	14	46.7	9	30.0	**6.2 (1.3–28.4)**	**0.018 (S)**
Allele	**C**	19	31.7	33	55.0	**2.6 (1.3–5.6)**	**0.01 (S)**
	G	41	68.3	27	45.0		

R Reference genotype or allele, *OR* Odd ratio, *CI* Confidence interval

Table 3 Disease characters according to gene polymorphism

Variable		CC ($n=3$)		CG ($n=13$)		GG ($n=14$)		FET	*P*
		No.	%	No.	%	No.	%		
Age of onset (y)	**Median (range)**	4.0 (4–5)		5.0 (3–6)		5.0 (2–6)		**KW = 1.15**	**0.56 (NS)**
Type of ADHD	**Hyperactive**	2	66.7	1	7.7	3	21.4	**8.1**	**0.053 (NS)**
	Inattentive	1	33.3	1	7.7	3	21.4		
	Combined	0	0.0	11	84.6	8	57.1		

FET Fisher's exact test, *KW* Kruskal-Wallis test, *NS* Non-significant

Table 4 Association between severity and polymorphism

			LPHN3 gene polymorphism			Total	FET (*P*)
			CC	CG	GG		
Severity	**Mild**	**Count**	3	4	2	9	8.95 (0.034, S)
		%	100.0%	30.8%	14.3%	30.0%	
	Moderate	**Count**	0	7	5	12	
		%	0.0%	53.8%	35.7%	40.0%	
	Severe	**Count**	0	2	7	9	
		%	0.0%	15.4%	50.0%	30.0%	
Total		**Count**	3	13	14	30	
		%	100.0%	100.0%	100.0%	100.0%	

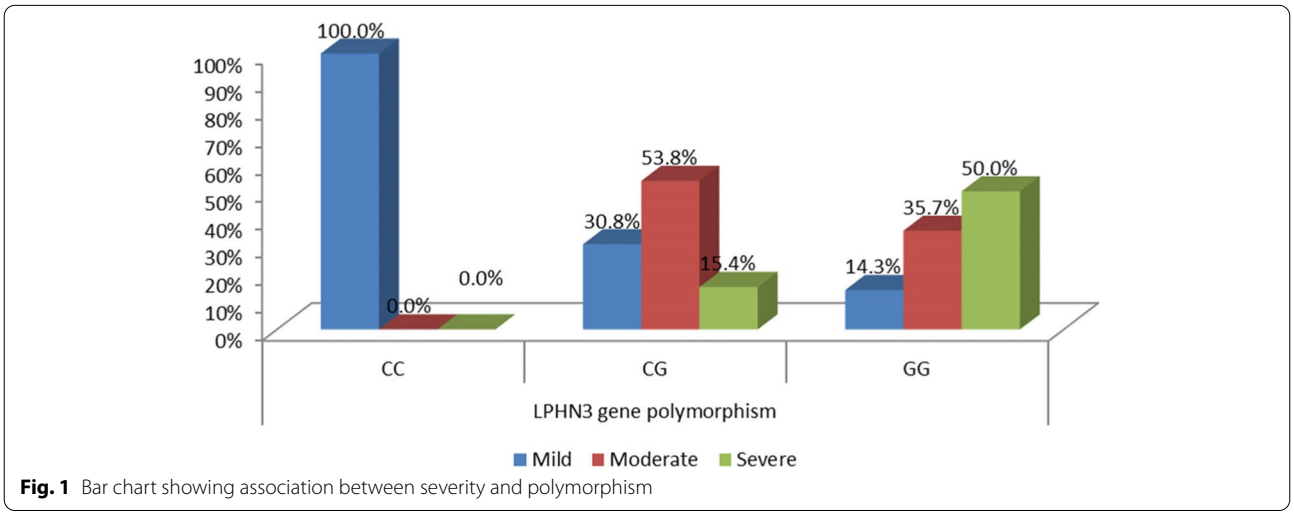

Fig. 1 Bar chart showing association between severity and polymorphism

genotype had a 5.8-fold increased risk of having ADHD compared to those with the CC genotype. Additionally, people with the GG genotype had a nearly sixfold increased risk of developing ADHD compared to those with the CC genotype. Each of these correlations was statistically significant ($P=0.024$ and $P=0.018$, respectively). Individuals with the G allele had a 2.6-fold increased risk of developing ADHD compared to those with the C allele (OR = 2.6, 95% CI = 1.3–5.6), a statistically significant difference ($P=0.01$) (Table 2).

Acosta et al. [15] discovered a substantial difference in the LPHN3 rs2345039 polymorphism allele distribution between ADHD and controls. Additionally, Bruxel et al. [10] established a statistically significant

connection between LPHN3 SNPs and the chance of developing ADHD in an adolescent population.

Zaslan and his colleagues [16] looked at the genotype distribution of CDH13 polymorphism rs6565113 and rs11150556 polymorphisms and LPHN3 polymorphisms in children and adolescents with ADHD and healthy, age- and sex-matched controls. They found that children and adolescents with ADHD had higher levels of these polymorphisms than healthy, matched controls, but not as high as those with normal, healthy genes. LPHN3 polymorphism rs68588066 and rs2345039 genotype and allele frequencies were compared to the genotype and allele frequencies of healthy people. There were no statistically significant differences between people with ADHD and healthy people ($P > 0.05$). However, there was a big difference in the genotype distribution of the LPHN3 rs6551665 and rs1947274 polymorphisms between people with ADHD and people who didn't have ADHD. GG genotypes rs6551665 and rs1947274 of the LPHN3 gene were found to be linked to an increased risk of ADHD, with the link being stronger in men.

In this study, all variables found to be significantly related to ADHD were entered in the binary logistic regression model to detect the predictors of ADHD. It was found that G allele, consanguinity, pregnancy, and birth complications are the significant predictors of ADHD ($P = 0.013$, $P = 0.016$, $P = 0.011$, $P = 0.0$ 14, respectively).

Choudhry et al. [17] agreed with us and reported a highly significant association of the four LPHN3 tag SNPs (rs1947274, rs6551665, rs2345039, rs6858066) with pregnancy-related mother tension. Subgroup analysis of mothers who were subjected to minimal stress during pregnancy revealed substantial links with ADHD, ADHD-related behavioral, and cognitive aspects, as well as treatment response.

In contrast to us, Acosta et al. [15] reported that LPHN3 variant rs2345039 predisposes to the development of a refined ADHD phenotype characterized by persistence of ADHD combined subtype symptoms into adolescence. To replicate the association of LPHN3 with ADHD, Ribases et al. [18] did a case–control study in which they genotyped 334 people with ADHD and 334 people who did not have ADHD for 43 single nucleotide polymorphisms (SNPs) in the LPNH3 gene. A study of both single and multiple markers also found a link between LPHN3 and mixed-form ADHD. The odds ratio (OR) was 2.25 (1.52–3.34) and $P = 0.005$; the df was 1.

There was a statistical difference between types of gene polymorphism regarding disease severity ($P = 0.034$). In the CC group, all cases (3 children) had a mild disease. In the CG group, 4 children (30.8%) had mild disease, 7 children (53.8%) had moderate disease, and 2 children (15.4%) had severe disease. In the GG group, 2 children (14.7%) had mild disease, 5 children (35.7%) had moderate disease, and 7 children (50%) had severe disease.

Similarly, Acosta et al. [15] determined that LPHN3 is not only valid but also correlates with a more severe type of ADHD. Additionally, Ribases et al. [18] revealed that LPHN3 was involved in the development of mixed type ADHD, particularly the severe form of the disorder.

Conclusion

Our results demonstrate an association between latrophilin 3 (LPHN3) gene rs2345039 and ADHD. Moreover, LPHN3 polymorphisms tend to play a role in triggering the condition and exacerbating its severity.

Abbreviations

LPHN3: Latrophilin 3; ADHD: Attention deficit hyperactivity disorder; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; CPT: Conner's Continuous Performance Test; GPCRs: G protein-coupled receptors; EDTA: Ethylenediaminetetraacetate; rt PCR: Real-time polymerase chain reaction.

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Authors' contributions

All authors contributed to the study conception and design. The initial plan and study design were made by A E, DS, and SD. Genetic testing was done by EB. Material preparation, data collection, and analysis were performed by AE, DS, and SD. The first draft of the manuscript was written by AE and SD. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The authors declare the availability of data used in the study.

Declarations

Ethics approval and consent to participate

The authors declare review and approval of the study by the research ethical committees in the Faculty of Medicine, Benha and Menoufia Universities. Freely given, informed, written consent to participate in the study was obtained from participants parents. Participants were informed about objectives of the study. Committee's reference number: not applicable.

Consent for publication

Not applicable.

Competing interests

None

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