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Significance of neurexin and neuroligin polymorphisms in regulating risk of Hirschsprung's disease

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ABSTRACT

By performing a basic case–control study among a Chinese population, the aims of this study were to explore if single nucleotide polymorphisms (SNPs) within neurexin and neuroligin were associated with susceptibility to Hirschsprung's disease (HD). Eleven SNPs within neurexin and neuroligin were selected in this basic case–control study, and this study recruited 210 children with HD and 187 healthy children. The t-test and χ^2 test were used to find the difference between case and control in their clinical variables. OR and 95% CI were used to assess the association between HD susceptibility and neurexin/neuroligin polymorphisms/haplotypes. Several SNPs were significantly associated with altered risk of HD in the Chinese Han population, including rs1421589 within *NRXN1*, rs11795613 and rs4844285 within *NLGN3*, as well as rs5961397, rs7157669 and rs724373 within *NLGX4X* (all $P < 0.05$). Further studies presented that the effects of rs1421589 within *NRXN1*, rs4844285 and rs11795613 within *NLGN3*, as well as rs5961397 within *NLGX4X* on HD phenotypes were also statistically significant (all $P < 0.05$). Conclusively, the polymorphisms and haplotypes situated within neurexin and neuroligin were markedly associated with the onset of HD, implying that mutations of neurexin and neuroligin might serve as the treatment target for HD for the Chinese children.

INTRODUCTION

Hirschsprung's disease (HD), a developmental disorder, was featured by congenital malformations in the gastrointestinal tract, and it could be commonly found among cases of pediatric surgery. The morbidity of HD (ie, 1/2000–1/5000) was ranked high at second of all disorders relevant to newborn congenital digestive tract malformation, and males possessed three-fold more susceptibility to HD than females.¹ The pathological changes of HD lied in the dysfunctional enteric nervous system (ENS), which was particularly manifested as shortage of intestinal ganglion cells within myenteric nerve plexus or intestinal submucosa of distal spastic colon and disorderly arranged nerve fibers. The above symptoms would render intestinal canal to take the spastic shape, making the fecal sediment difficult to empty the dung and facilitating compensatory dilation of proximal intestine.^{2,3}

Significance of this study

What is already known about this subject?

- The morbidity of Hirschsprung's disease (HD) was ranked high at second of all disorders relevant to newborn congenital digestive tract malformation.
- About 20% of patients with HD were accompanied with family heredity.
- The development of HD may be related with migration of neural crest cells in the embryonic period.

What are the new findings?

- The C allele of *NRXN1* rs1421589 acted as a protective element for HD risk.
- The mutant alleles of rs11795613 (A>G) and rs4844285 (G>A) within *NLGN3* were both associated with significantly lower susceptibility to HD than their wide alleles.
- The C allele of *NLGX4X* rs5961397 could reduce the risk of HD in comparison to the T allele.
- The subjects carrying mutant alleles of rs7157669 and rs724373 were more prone to suffer from HD than carriers of the corresponding wide alleles.

How might these results change the focus of research or clinical practice?

- The results strongly evidenced the hypothesis that the aberrant neuroligin and neurexin expressions caused by genetic mutations participated in the connections between intestinal neurons, leading to developmental immaturity of enteric nervous system and thus the presence of such diseases as HD.

Up to now, the familial incidence of HD was demonstrated to account for around 3.6%–7.8% of the overall incidence of HD, and about 20% of patients with HD were accompanied with family heredity.⁴ Thus, the consensus has been reached that one dominating etiologic factor of HD was genetic variation of ENS. For instance, single nucleotide polymorphisms (SNPs) within *RET* (ie, rs2435357 and rs2506030) and *SEMA3* (ie, rs12707682) could

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impart tremendous risk of HD development and disorders of phenotypic conditions among a Chinese population.⁵ A Chinese genome-wide association study disclosed that *NRG3* mattered a lot to the onset of HD,⁶ yet later a case-control study did not uncover the association of three *NRG3* SNPs with HD within a Chinese crowd.⁷ Nonetheless, it was documented that rs78356888 of *NRG1*, as well as rs2506030 and rs2435357 of *RET*, was significantly correlated with susceptibility to HD.⁷ Since the study results about the association of SNPs with HD risk were quite limited and were sometimes in dispute, finding novel target SNPs or conduction of replicated studies were critical to hindering the HD onset.

Of note, the achievements related with the development of central nervous system were vital to the exploration with respect to development of ENS, for that ENS and central nervous system were both originating from embryonic neural crest.⁸ Since production of synapse symbolized complete development of central nervous system, the sensory and motor functions of ENS should also be guaranteed by the appropriate synaptic actions. It has been verified that neurexin family and neuroligin family were located within presynaptic membranes or across the subsynaptic membranes.^{9,10} Furthermore, neurexin might interact with neuroligin to mediate synaptic junctions within the central nervous system via affecting scaffolding protein.¹¹⁻¹³ Hence, it was hypothesized that mutants of neurexin and neuroligin SNPs were highly potential factors accounting for ENS-relevant disorders, such as HD.

Accordingly, the current study was aimed to explore whether SNPs within neurexin and neuroligin were associated with susceptibility to HD.

METHODS

Subjects

Totally 210 children with HD were retrospectively recruited from Zhoukou Central Hospital from March 2011 to February 2016. They all experienced the transanal pull-through operation for the first time. According to the diagnostic criteria for HD declared by the fourth international conference on HD and related neural crest diseases,¹⁴ the children with HD were preoperatively managed with barium enema examination, rectum mucosa biopsy, H&E staining and acetylcholinesterase staining. Consecutive sections were performed for each sample, and HD was confirmed when hardly any neurons were observed and obvious hyperplasia of nerve plexus were found within submucosa. Since there existed a cell area featured by physiological hyporesponsiveness and aganglionosis beyond the normal rectal line, biopsies for HD diagnosis were obtained based on age: (1) 2 cm beyond the line for newborns; (2) 2.5 cm beyond the line for the children aged <1 year; (3) 3 cm beyond the line for the children aged 1–3 years; (4) 3.5 cm beyond the line for the children aged >4 years. In addition, HD was divided into the short-segment HD, the long-segment HD and the total-segment HD. In particular, the lesions of short-segment HD were limited to be within distal sigmoid, rectal, as well as the border between distal sigmoid and rectal; the lesions of long-segment HD covered proximal sigmoid, ascending colon and even a wider range; the lesions of total-segment HD ran through rectum, total

colon and terminal ileum (<30 cm far from ileocecal valve). Besides, 187 normal children receiving the physical examination in our hospital were included in the control group, and they all had no malformations relevant to digestive tract and neural crest. Parents of all subjects have signed informed consents.

Selection of SNPs

The investigated SNPs were partly selected with aid of the bioinformation database (<http://www.ncbi.nlm.nih.gov/SNP/>). Specifically, with the SNP browser software, tag SNPs were chosen if their r^2 was more than 0.8 and their minor allelic frequency was at least 5%, in accordance with the pairwise r^2 method. Other SNPs were deemed as eligible based on related documentations.^{15,16} Finally, SNPs within *NRXN1* (ie, rs1363032 and rs1424589), *NRXN3* (ie, rs11624704, rs7157669 and rs724373), *NLGN1* (rs13074723 and rs1488547), *NLGN3* (rs11795613 and rs4844285) and *NLGN4* (rs6529901 and rs5961379) were chosen and arranged for the following studies.

Genotyping

Peripheral blood (volume: 2 mL) was extracted from HD and healthy children and 3.8% sodium citrate was used for anticoagulation. The Qiagen DNA extraction kit was utilized to extract the DNA, and specific procedures were carried out strictly in accordance with the manufacturers' protocols. The primers were designed based on the primer 5 software and were synthesized with assistance of Shanghai Invitrogen Biotechnology Co (Supplementary table 1). All the primers were all purified via polyacrylamide gel electrophoresis and were then recycled using C18 column.

Then PCR-restriction fragment length polymorphism (RFLP) was implemented to genotype the SNPs. The PCR reaction system (50 μ L) mainly included double distilled water (32.5 μ L), 10 $\times$ buffer (5.0 μ L), 25 mmol/L magnesium chloride (4.0 μ L), 10 mmol/L deoxyribonucleotide triphosphate (4.0 μ L), 20 μ mol/L sense primer (1.0 μ L), 20 μ mol/L antisense primer (1.0 μ L), 5 U/ μ L Taq DNA polymerase and 100 ng DNA template (2.0 μ L). Moreover, the PCR reaction was proceeded according to the following process: (1) predenaturation at 95°C for 5 min; (2) 35 cycles of 95°C for 40 s, 60°C for 30 s and 72°C for 30 s and (3) extension at 72°C for 10 min and (4) conservation at 4°C.

Operation

The operation methods were selected in line with the age of children and the length of pathological intestinal tract. For the children aged between 6 months and 1 year, they would be treated with the transanal endorectal pull-through operation if they were diagnosed as short-segment HD. The children diagnosed as long-segment HD were alternatively treated with transabdominal heart anastomosis. As for the children with total colonic aganglionosis, they were first managed with colostomy and were then treated with Martin surgery or ileoanal anastomosis when they were around 1 year old. The children aged more than 1 year experienced the improved Duhamel surgery or transabdominal heart anastomosis.

Table 1 Baseline characteristics of the children with Hirschsprung's disease (case group) and healthy children (control group)

Clinical features	Case group	Control group	t-Test	P value
Number	210	187	–	–
Age (month)	7.30±1.18	7.35±1.08	0.44	0.661
Weight (kg)	7.15±0.46	8.42±0.53	1.8	0.073
Height (cm)	61.78±2.04	70.53±1.96	1.24	0.216
Pathological features				
Short segment type	69	–	–	–
Long segment type	101	–	–	–
Total type	40	–	–	–

Evaluation of HD phenotypes

The biopsies of all HD children were retrieved. In line with the range of the affected bowels, the samples were classified as short segment type, long segment type and total type (ie, the most serious type).¹⁷

Statistical analysis

The measurement data were exhibited in the form of mean±SD, and the enumeration data were presented as percentages (%). Besides, χ^2 test was conducted to compare the enumeration data, and t-test or Mann-Whitney test was utilized to analyze the measurement data. Furthermore, the Hardy-Weinberg equilibrium law was applied to examine whether the gene and genotype frequencies were balanced among the study samples. The linkage disequilibrium (LD) among neurexin/neurologin SNPs was fitted based on Shesis software, and the haplotypes of the SNPs were also analyzed with the Shesis software.¹⁸ In addition, the correlations between potential SNPs/haplotypes and risk/phenotype of HD were shown in the form of ORs and 95% CIs. It would be deemed as statistically significant when P was less than 0.05. All the statistical analyses were handled with SPSS V.13.0 software.

RESULTS

Baseline characteristics of the subjects

There showed hardly any significant distinctions between the HD group and healthy controls in the aspects of mean age, mean weight and mean height ($P>0.05$) (table 1). Moreover, the HD children altogether possessed 69 (32.86%) short segment type, 101 (40.09%) long segment type and 40 (19.05%) total type.

Correlation between SNPs/haplotypes within neurexin/neurologin and susceptibility to HD

Regarding the studied population, the LD of the selected neurexin/neurologin SNPs was shown as figure 1. The C allele of *NRXN1* rs1421589 acted as a protective element for HD risk when compared with T allele (OR 0.59, 95% CI 0.44 to 0.81, $P=0.001$) (table 2). Similarly, the mutant alleles of rs11795613 (A>G) and rs4844285 (G>A) within *NLGN3* were both associated with significantly lower susceptibility to HD than their wide alleles (OR 0.39,

95% CI 0.29 to 0.51, $P<0.001$; OR 0.55, 95% CI 0.41 to 0.73, $P<0.001$). Moreover, the C allele of *NLGN4X* rs5961397 could reduce the risk of HD in comparison to the T allele (OR 0.51, 95% CI 0.38 to 0.68, $P<0.001$). In contrast, the subjects carrying mutant alleles of rs7157669 and rs724373 were more prone to suffer from HD than carriers of the corresponding wide alleles (A vs C: OR 1.44, 95% CI 1.03 to 2.02, $P<0.034$; C vs T: OR 2.66, 95% CI: 1.88 to 3.78, $P<0.001$).

After investigating the combined role of the significant SNPs mentioned above in facilitating HD risk (table 3), it was observed that haplotypes CT of *NRXN1*, haplotypes AAC, ACT and CAC of *NRXN3*, haplotypes AG and GA of *NLGN1*, as well as haplotypes AGCT, AGTT and GACC of *NLGN3* and *NLGN4X*, respectively, served as the hazard parameter for susceptibility to HD (OR 1.74, 95% CI 1.25 to 2.42, $P=0.001$; OR 1.97, 95% CI 1.47 to 2.63, $P<0.001$; OR 2.05, 95% CI 1.26 to 3.31, $P=0.003$; OR 2.22, 95% CI 1.41 to 3.50, $P<0.001$; OR 2.97, 95% CI 2.20 to 3.99, $P<0.001$; OR 2.37, 95% CI 1.74 to 3.22, $P<0.001$; OR 6.34, 95% CI 3.53 to 11.40, $P<0.001$; OR 10.02, 95% CI 6.00 to 16.75, $P<0.001$; OR 3.40, 95% CI 2.31 to

Figure 1 The linkage disequilibrium of single nucleotide polymorphism within (A) *NRXN1* (ie, rs1363032 (chromosome 2: 49993858) and rs1421589 (chromosome 2: 50063015)), (B) *NRXN3* (ie, rs11624704 (chromosome 14: 78319734), rs7157669 (chromosome 14: 78471334) and rs724373 (chromosome 14: 78476555)), (C) *NLGN1* (ie rs13074723 (chromosome 3: 173804307) and rs1488547 (chromosome 3: 173807978)) and (D) *NLGN3* (ie rs11795613 (chromosome X: 71147478) and rs4844285 (chromosome X: 71150394) and *NLGN4X* (ie rs6529901 (chromosome X: 5937979) and rs5961397 (chromosome X: 6004425)).

Table 2 Correlation between SNPs within neurexin/neuroligin and susceptibility to HD

Gene	Rs number	Allele change	Group	Allele		HWE	Allelic model			Dominant model			Recessive model		
				W	M		OR	95%CI	P value*	OR	95%CI	P value*	OR	95%CI	P value*
NRXN1	rs1363032	T>C	Case	297	123	0.91	1.03	0.76 to 1.41	0.876	1.01	0.68 to 1.50	1.000	1.14	0.56 to 2.31	0.858
			Control	267	107										
	rs1421589	T>C	Case	318	102	0.10	0.59	0.44 to 0.81	0.001	0.6	0.40 to 0.89	0.012	0.37	0.19 to 0.75	0.005
			Control	243	131										
NRXN3	rs11624704	A>C	Case	349	71	0.05	1.23	0.84 to 1.81	0.328	1.17	0.75 to 1.83	0.568	1.7	0.66 to 4.35	0.359
			Control	321	53										
	rs7157669	C>A	Case	82	338	0.59	1.44	1.03 to 2.02	0.034	1.46	0.65 to 3.31	0.411	1.56	1.04 to 2.35	0.039
			Control	97	277										
	rs724373	T>C	Case	60	360	0.91	2.66	1.88 to 3.78	<0.001	2.69	1.14 to 6.34	0.025	3.27	2.14 to 5.01	<0.001
			Control	115	259										
NLGN1	rs13074723	G>A	Case	197	223	0.96	1.04	0.79 to 1.37	0.831	1.06	0.66 to 1.71	0.811	1.04	0.67 to 1.62	0.911
			Control	179	195										
	rs1488547	A>G	Case	179	241	0.72	1.26	0.95 to 1.67	0.116	1.43	0.88 to 2.33	0.174	1.3	0.85 to 2.01	0.231
			Control	181	193										
NLGN3	rs11795613	A>G	Case	279	141	0.98	0.39	0.29 to 0.51	<0.001	0.28	0.18 to 0.45	<0.001	0.29	0.17 to 0.48	<0.001
			Control	162	212										
	rs4844285	G>A	Case	233	187	0.97	0.55	0.41 to 0.73	<0.001	0.44	0.27 to 0.72	0.001	0.46	0.29 to 0.72	0.001
			Control	152	222										
NLGN4X	rs6529901	C>T	Case	273	147	1.00	1.2	0.89 to 1.61	0.257	1.23	0.83 to 1.84	0.313	1.33	0.70 to 2.51	0.426
			Control	258	116										
	rs5961397	T>C	Case	292	128	1.00	0.51	0.38 to 0.68	<0.001	0.43	0.28 to 0.65	<0.001	0.39	0.22 to 0.69	0.001
			Control	201	173										

*The P value has been adjusted.

The bold values indicated significant results. HD, Hirschsprung's disease; HWE, Hardy-Weinberg equilibrium; M, mutant allele; SNP, single nucleotide polymorphism; W, wild allele.

Table 3 Correlation between haplotypes within neurexin/neuroigin and susceptibility to HD

Gene	Haplotype	Case (frequency)	Control (frequency)	χ^2	P value*	OR	95% CI
<i>NRXN1</i>	CT	123 (0.293)	72 (0.193)	10.75	0.001	1.74	1.25 to 2.42
	TC	102 (0.243)	96 (0.257)	0.20	0.653	0.93	0.67 to 1.28
	TT	195 (0.464)	171 (0.457)	0.04	0.842	1.03	0.78 to 1.36
<i>NRXN3</i>	AAC	267 (0.636)	165 (0.441)	21.26	<0.001	1.97	1.47 to 2.63
	ACC	22 (0.052)	58 (0.156)	26.51	<0.001	0.28	0.17 to 0.46
	ACT	60 (0.143)	26 (0.071)	8.76	0.003	2.05	1.26 to 3.31
	CAC	71 (0.169)	29 (0.079)	12.17	<0.001	2.22	1.41 to 3.50
<i>NLGN1</i>	AG	223 (0.531)	103 (0.276)	53.01	<0.001	2.97	2.20 to 3.99
	GA	179 (0.426)	89 (0.239)	31.04	<0.001	2.37	1.74 to 3.22
	GG	18 (0.043)	90 (0.24)	65.44	<0.001	0.14	0.08 to 0.24
<i>NLGN3</i> and <i>NLGX4X</i>	AACT	46 (0.11)	30 (0.08)	1.35	0.245	1.33	0.82 to 2.16
	AATT	0 (0)	17 (0.046)	19.58	<0.001	0.00	0.00 to 0.01
	AGCT	86 (0.205)	14 (0.037)	47.18	<0.001	6.34	3.53 to 11.40
	AGTT	147 (0.35)	18 (0.049)	102.81	<0.001	10.02	6.00 to 16.75
	GACC	128 (0.305)	41 (0.109)	41.10	<0.001	3.40	2.31 to 5.01
	GACT	13 (0.031)	42 (0.112)	21.95	<0.001	0.24	0.13 to 0.46

*The P value has been adjusted.

The bold values indicated significant results. HD, Hirschsprung's disease.

5.01, $P<0.001$). On the contrary, carriers with haplotypes ACC of *NRXN3*, haplotype GG of *NLGN1* and haplotypes AATT or GACT of *NLGN3* and *NLGX4X* possessed lower possibility of attacking HD (OR 0.28, 95% CI 0.17 to 0.46, $P<0.001$; OR 0.14, 95% CI 0.08 to 0.24, $P<0.001$; OR 0.00, 95% CI 0.00 to 0.01, $P<0.001$; OR 0.24, 95% CI 0.13 to 0.46, $P<0.001$).

Correlation between SNPs within neurexin/neuroigin and HD phenotypes

With respect to *NRXN3* rs724373 (table 4), the distribution of its genotypes (ie, TT, TC and CC) within the long-segment HD population and the total-segment HD population, respectively, differed from the short-segment HD ones ($P=0.024$ and $P=0.045$). The rs11795613 was also significantly linked with altered phenotypes of HD (long-segment HD vs short-segment HD: $P=0.003$; total-segment HD vs short-segment HD: $P=0.004$). Ultimately, the genotyping frequencies of *NLGN3* rs4844285 (G>A) was prominently discrepant between the total-segment HD versus short-segment HD groups ($P=0.014$), and *NLGX4X* rs5961397 (T>C) also displayed different genotyping frequencies among the long-segment HD group, when compared with the short-segment HD group ($P=0.001$).

DISCUSSION

ENS was composed of myenteric plexus and submucous plexus, which interacted with each other to regulate the motor and secretion function of the intestinal tract.¹⁹ In-depth studies also indicated that ENS was crucial to the development of the diseases relevant to the intestinal dynamics and gastrointestinal functions. The normal functioning of enteric nerves was dependent on the integral connection of intestinal nerve cells, intestinal muscle cells and epithelial cells. It was demonstrated that genetic mutations could induce disordered gastrointestinal dynamics, by way of rendering loss of interneuron functions and

disconnecting between neurons and their effectors.²⁰ It was the synaptic connection that prompted intestinal tubes to perform segmental motion, reflection secretion and neural immune responses.²¹ Hence, it was hypothesized that the genetic mutations that modified the synaptic function were involved in the development and maturation of ENS.

Neurexin and neuroigin have been verified to mediate the connection and differentiation of synapses through glycosylation, oligomerization and calcium binding (figure 2).^{10 22} It has been confirmed in a glycosylation experiment that the O-bound and N-bound saccharides could regulate neuroigin that was rich in Ser-Thr domains,²³ and the ChE-like domain (CLD) structural domain residues of neuroigin-1 might also affect the interaction between neuroigin and neurexin.^{22 24} Of note, the mutations of *EDNRB* could contribute to less inflow of calcium ions, and the attenuated binding of Ca^{2+} -dependent neuroigin and neurexin, which finally hindered the regular shaping of synapses and also ENS. In the meantime, mutations of *neuroigin* and *neurexin* would also aggravate this condition via damaging the aggregation of postsynaptic proteins and neuroigins.²²

Virtually, certain SNPs appeared to decisively modify the functions of *neuroigin* and *neurexin*. For instance, a haplotype composed of six *NLGN4X* SNPs, namely, rs663857 (G), rs3810687 (G), rs3810688 (T), rs1882260 (T), rs3810686 (C) and rs5916269 (G), was regarded as a hazard for autism risk among an Italian population.²⁵ Additional SNPs have also been affirmed with their impacts on the central nervous system-induced disorders, including rs3747333 or rs3747334 of *NLGN4X* and rs2303298 of *NRXN-1*.^{26–28} Accordingly, in our study, it was well founded that the mutated SNPs of *NRXN1* (ie, rs1421859), *NRXN3* (ie, rs7157669 and rs724373), *NLGN3* (ie, rs11795613 and rs4844285) and *NLGX4X* (ie, rs5961397) were involved in the etiology of ENS immaturation-caused HD. Nevertheless, the SNPs considered as crucial to HD were, in some degree, distinct from ones that exhibited significance

Table 4 Correlation between SNPs within neurexin/neurologin and HD phenotypes

Gene	rs number	Allele change	Genotype	SS type	LS type	Total type	SS type versus LS type		SS type versus total type	
							χ^2	P value*	χ^2	P value*
NRXN1	rs1363032	T>C	TT	40	47	19	1.12	0.291	2.83	0.093
			TC	24	48	13				
			CC	5	6	8				
	rs1421589	T>C	TT	36	65	20	2.35	0.126	0.04	0.846
			TC	28	31	17				
			CC	5	5	3				
NRXN3	rs11624704	A>C	AA	46	78	28	2.42	0.12	0	0.964
			AC	18	19	8				
			CC	5	4	4				
	rs7157669	C>A	CC	4	4	3	0.03	0.859	2.39	0.122
			CA	17	27	16				
			AA	48	70	21				
	rs724373	T>C	TT	3	2	3	5.08	0.024	4.03	0.045
			TC	5	30	9				
			CC	61	69	28				
NLGN1	rs13074723	G>A	GG	12	26	8	1	0.318	2.57	0.109
			GA	47	41	17				
			AA	10	34	15				
	rs1488547	A>G	AA	13	19	6	2.67	0.102	0.91	0.341
			AG	25	55	23				
			GG	31	27	11				
NLGN3	rs11795613	A>G	AA	22	46	26	8.68	0.003	8.14	0.004
			AG	32	50	9				
			GG	15	5	5				
	rs4844285	G>A	GG	16	29	20	0.69	0.407	6.02	0.014
			GA	37	52	14				
			AA	16	20	6				
NLGX4X	rs6529901	C>T	CC	37	38	14	1.93	0.165	3.62	0.057
			CT	24	53	18				
			TT	8	10	8				
	rs5961397	T>C	TT	23	56	23	10.22	0.001	2	0.158
			TC	37	41	10				
			CC	9	4	7				

*The P value has been adjusted.

The bold values indicated significant results. HD, Hirschsprung's disease; LS, long segment; SNP, single nucleotide polymorphism; SS, short segment.

in encouraging the development of other diseases. This phenomenon could be attributed to that SNPs that interfered with the occurrence of different diseases were also disparate, for that they might participate in different molecular mechanisms when diverse diseases were considered.

Similarly, diverse SNPs of the genes that impacted the functioning of nervous system have been confirmed to be associated with incremental susceptibility to HD. For instance, recent studies documented the association of SNPs within catechol-O-methyltransferase (COMT) (ie, rs6267) and armadillo repeat gene deleted in velocardiofacial syndrome (ARVCF) (ie, rs80068543) with HD.²⁹ The COMT was involved with canalization of brain catecholamine neurotransmitters and COMT interacted with ARVCF to modify the development of neuronal system.³⁰ Furthermore, as high as 50% of familial HD cases were discovered with missing of RET functional mutations, and it was demonstrated that the intron 1 of RET gene shared rs2435357 with a critical transcription factor of ENS.^{31 32} In addition, γ -aminobutyric acid

A receptor gamma 2 (GABRG2) mediated the neuron-inhibition process of the mammalian central nervous system,³³ and its SNPs (ie, rs209350 and rs169793) exhibited significantly distinct frequencies between HD subjects and controls.³⁴ Finally, RELN was correlated with migration and positioning of neuronal cells, and its tag SNPs also displayed marked association with HD risk, including rs802788, rs6977616 and rs56345626.^{34 35} It was further deeply stressed that whether the nervous system could work properly was vital to regulating the risk of HD.

To sum up, this hypothesis-generating study was the first one to investigate the correlation between SNPs relevant to *NLGN* or *NRXN* and risk of HD. Nonetheless, the selected SNPs might not have the largest representation of *NLGN* and *NRXN* functioning among all the genetic mutations, and more meaningful SNPs could be missed. Moreover, the research subjects were merely a Chinese Han population recruited from the hospital, so a large-scale prospective study targeting the entire population were demanded

Figure 2 The mechanism underlying the effects of neurexin and neuroligin on synapse-related information transfer. As an action potential is initiated (①), the Ca^{2+} -channel of the presynaptic terminal would be open (②), which triggers inflowing of Ca^{2+} . Subsequently, the synaptic vesicles within the presynaptic cell would be fused with the presynaptic plasma membrane (③), and neurotransmitters are released from vesicles into the synaptic cleft (④). Ultimately, the neurotransmitters would contact with the postsynaptic receptors, through which the process of information transfer is accomplished (⑤).

to replicate the study results. In addition, the limited size and confined ethnicities of the included children less able to be generalized to other populations, and the smaller population managed for the haplotype analysis made the results less convincing and less recommended for clinical application. Furthermore, thorough analyses regarding the interaction of exposure factors and SNPs on HD risk should also be completed in future. Finally, how the relationship between the SNPs and pathogenesis of HD was established remained unknown. Hence, *in vivo* and *in vitro* studies should be carried out to explore the potential mechanisms.

In conclusion, certain SNPs/haplotypes within *NLGN* or *NRXN* were believed as the biomarkers for risk of HD, though more diverse studies were in need to confirm the study result.

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