ORIGINAL ARTICLE

Influence of the *NRGN* gene on intellectual ability in schizophrenia

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Genome-wide association studies have reported an association between schizophrenia and rs12807809 of the *neurogranin* (*NRGN*) gene. We have recently found that an rs12807809–rs12278912 haplotype of the gene is associated with schizophrenia in a Japanese population and that the *NRGN* expression of the high-risk TG haplotype is lower than that of the protective TA haplotype in immortalized lymphoblasts. In this study, we investigated the influences of *neurogranin* genotypes (rs12807809 and rs12278912), haplotypes and diplotypes and genetic variant–diagnosis interactions on intellectual ability in 414 Japanese patients with schizophrenia and healthy subjects. We detected possible effects of the genome-wide screen-supported rs12807809, haplotypes, diplotypes and their genetic variant–diagnosis interactions on intellectual abilities at the threshold level of $P < 0.05$. After applying Bonferroni correction for 13 genotype measures and setting P-values for significance ($P < 0.0039$; 0.05/13), three effects remained significant: the rs12807809–rs12278912 diplotype–diagnosis interactions on performance intelligence quotient (CG/CG: $P = 3.9 \times 10^{-13}$; TA/TA: $P = 1.1 \times 10^{-7}$) and TA/TA diplotype on performance intelligence quotient in patients with schizophrenia ($P = 8.2 \times 10^{-8}$) remained significant. The intellectual abilities of the high-risk TG/TG diplotype of the *neurogranin* gene were lower compared to those with the non-risk TA/TA diplotype. Our findings suggest that the genetic risk variant in the *neurogranin* gene may be related to reduced intellectual ability.

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INTRODUCTION

Schizophrenia is a common and complex psychiatric disease with strong genetic components. Schizophrenia has an estimated heritability of approximately 80%,[1,2] and many genes have been implicated in the pathogenesis of schizophrenia.[3]

Three genome-wide association studies (GWAS) and follow-up case–control studies have reported seven single-nucleotide polymorphisms (SNPs) in combined samples of 12 945 patients with schizophrenia and 34 591 controls of European Caucasian ethnic background.[4] Of the seven SNPs, only one SNP, rs12807809 of the *neurogranin* (*NRGN*) gene, was common in the HapMap Japanese samples in Tokyo as previous reported.[5] The frequency of the T allele of rs12807809 was higher in individuals with schizophrenia than in those without the disorder in both the original study (odds ratio = 1.15)[4] and recent follow-up study (odds ratio = 1.12).[6] We have recently found using a gene-based approach that the rs12807809–rs12278912 haplotype was the variant near the *NRGN* gene most strongly associated with schizophrenia in a Japanese

population.[5] Moreover, we have found that the *NRGN* expression of the high-risk TG haplotype of rs12807809–rs12278912 was significantly lower than the expression of the protective TA haplotype in immortalized lymphoblasts derived from the HapMap Japanese samples in Tokyo samples and our Japanese case–control samples.[5] The *NRGN* gene on chromosome 11q24.2 spans 7.3 kb of genomic DNA and contains four exons.[7] *NRGN* is the human homolog of the neuron-specific rat gene RC3/neurogranin. *NRGN* encodes a postsynaptic protein kinase substrate that binds to calmodulin (CaM) in the absence of calcium.[8] NRGN has an important role in the Ca^{2+}–CaM signaling pathway.[9] Ca^{2+} influx-induced oxidation of NRGN leads to postsynaptic activation of CaM-dependent protein kinase II by CaM, which is associated with strengthened *N*-methyl-D-aspartate receptor signaling.[10] Reduced function of *NRGN* is considered to mediate the effects of the *N*-methyl-D-aspartate hypofunction implicated in the pathophysiology of schizophrenia.

NRGN is abundantly expressed in the brain regions involved in cognitive functioning and especially enriched in CA1 pyramidal

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neurons in the hippocampus.[11] *NRGN* has been shown to have a role in dendritic spine formation, synaptic plasticity, long-term potentiation and spatial learning.[12,13] *Nrgn* knockout mice displayed deficits in spatial learning and anxiety-like tendencies, supporting a role for *nrgn* in the hippocampus-mediated interaction between stress and performance.[14] The SNP rs12807809 was associated with diminished hippocampal activation during a contextual fear conditioning task.[15] On the other hand, reduced NRGN immunoreactivity has been observed in Brodmann areas 9 and 32 of the prefrontal cortex in post-mortem brains from patients with schizophrenia.[16] We have found that carriers of the risk allele of rs12807809 (T) had a smaller gray matter volume in the left anterior cingulate cortex (Brodmann area 32) than carriers of the non-risk allele (C) in patients with schizophrenia.[17] It has been reported that rs12807809 is associated with differential neural activation in the anterior and posterior cingulate cortices during episodic memory encoding and retrieval tasks.[18] These results suggest that the *NRGN* gene is related to the function of the hippocampus and anterior cingulate and that dysfunction of the gene leads to neurocognitive deficits in patients with schizophrenia.

Intelligence quotient (IQ) is a standardized measure of human intellectual capacity that takes into account a wide range of cognitive skills.[19] The intellectual ability of patients with schizophrenia is lower than healthy subjects. Approximately 50% of patients with schizophrenia show cognitive deterioration, with an IQ decline of 10 points from the premorbid IQ.[20] The declined IQ in schizophrenia remains stable, although there is considerable interindividual variation in the degree of decline.[21] Intellectual dysfunction in unaffected relatives of schizophrenia patients is similar to but somewhat less pronounced than that in patients with schizophrenia.[22] The estimated heritability of IQ is high in the general population (69–85%) and individuals with familial schizophrenia (64–74%).[23,24] Schizophrenia and IQ are related and both highly heritable, but their genetic overlap is controversial.[25–27] It has been reported that there is a low phenotypic correlation between premorbid IQ and psychosis[25] vs the high correlation between postmorbid IQ and schizophrenia.[27] To a greater or lesser extent, some susceptibility genes for schizophrenia would mediate liability for the disorder at least partly by influencing intellectual abilities. Although two studies have investigated the association of the genome-wide screen-supported rs12807809 with IQ in three Caucasian populations,[28,29] these studies reported no association between rs12807809 and IQ in the populations. So far, however, no study has investigated the effect of the rs12807809 on IQ in a Japanese population. Moreover, although we have previously reported that the rs12807809–rs12278912 haplotype is associated with risk for schizophrenia and the *NRGN* expression,[5] no study has investigated the effects of *NRGN* genetic variants, including haplotypes and diplotypes, except for the rs12807809 on intellectual abilities. In this study, we used IQ to find downstream effects of these genetic variants (rs12807809 and rs12278912), the rs12807809–rs12278912 haplotype and rs12807809–rs12278912 diplotype of the *NRGN* gene in Japanese patients with schizophrenia and healthy volunteers.

MATERIALS AND METHODS
Subjects
This study was conducted with 157 patients with schizophrenia (52.2% males (82 males and 75 females); mean age ± s.d., 37.1 ± 12.4 years) and 257 healthy subjects (41.6% males (107 males and 150 females); mean age ± s.d., 37.5 ± 12.0 years). All subjects were biologically unrelated within the second-degree of relationship and of Japanese descent.[30,31] All subjects (100%) and the

majority of subjects (79.2%) in this study have been included the previous *NRGN* genetic association and imaging genetic studies, respectively.[5,17] Subjects were excluded if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headache, previous head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active cancer, cerebrovascular disease, epilepsy, seizures, substance-related disorders or mental retardation. Cases were recruited from the Osaka University Hospital. Each patient with schizophrenia had been diagnosed by at least two trained psychiatrists according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) based on the Structured Clinical Interview for DSM-IV (SCID). Controls were recruited through local advertisements at Osaka University. Psychiatrically, medically and neurologically healthy controls were evaluated using the non-patient version of the SCID to exclude individuals who had current or past contact with psychiatric services or received psychiatric medication. The mean age and sex ratio did not differ significantly between cases and controls ($P > 0.036$), whereas the years of education and estimated premorbid IQ were significantly lower in the patients with schizophrenia than in the controls ($P < 0.0039$) (Supplementary Table S1). When the three genotypes of either rs12807809 or rs12278912 were compared, we found no differences in demographic variables ($P > 0.020$) (Supplementary Table S1). Written informed consent was obtained from all subjects after the procedures had been fully explained. This study was conducted in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University.

SNP selection and genotyping
We selected rs12807809 and rs12278912 of the *NRGN* gene for genotyping as described in the introduction. The rs12807809 is located on 3457 bases upstream of the *NRGN* gene and the rs12278912 is located in intron 1 of the gene. The T/C polymorphism rs12807809 and G/A polymorphism rs12278912 have been described previously in the GWAS and our studies.[4,5,17] An rs12807809–rs12278912 haplotype is a combination of the two alleles at adjacent loci on 11q24.2 that are inherited together. An rs12807809–rs12278912 diplotype is a combination of the haplotypes for each individual. Venous blood was collected from the subjects, and genomic DNA was extracted from whole blood according to standard procedures. The SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Assay ID: rs12807809; C_32029000_20, rs12278912; C_32029002_10; Applied Biosystems, Foster City, CA, USA) as described previously.[32,33] Detailed information on the polymerase chain reaction conditions is available upon request. No deviation from the Hardy–Weinberg equilibrium was detected in the examined SNPs in the patients or controls ($P > 0.05$).

Measurement of intellectual abilities and assessment of current symptoms of schizophrenia
To assess intellectual abilities, we used the full-scale IQ, which is divided into performance IQ and verbal IQ, of the Japanese version of the Wechsler Adult Intelligence Scale-revised or third edition.[34] The subjects were assessed by trained clinical psychologists to obtain full-scale, performance and verbal IQ scores on the Wechsler Adult Intelligence Scale. Current symptoms of schizophrenia were evaluated using the positive and negative syndrome scale.[35]

Statistical analyses
Differences in clinical characteristics between patients and controls or between genotypes were analyzed using χ^2 tests for categorical variables and the Mann–Whitney *U*-test or Kruskal–Wallis test for continuous variables using PASW Statistics 18.0 software (SPSS Japan, Tokyo, Japan). Deviation from the Hardy–Weinberg equilibrium was tested separately in test cases and controls using χ^2 tests for goodness of fit using SNPAlyze V.5.1.1 Pro software (Dynacom, Yokohama, Japan).

The effects of diagnosis, *NRGN* genotype and their interaction on intellectual abilities were analyzed by two-way analyses of covariance. Diagnosis and genotype status were included in the analysis as independent variables. Full-scale, performance and verbal IQ scores were included as

dependent variables. As intellectual abilities may be influenced by sex and years of education, these variables were corrected for as covariates. We did not include age as a covariate because IQ scores were already corrected for age.

HPlus (http://qge.fhcrc.org/hplus) is a software application for estimating haplotype/diplotype frequencies, inferring individual haplotypes/diplotypes based on expectation-maximization and progressive ligation algorithms,[36] and assessing haplotypic/diplotypic associations with various phenotypes using linear regression. The minimum frequency for a haplotype or diplotype to be estimated for association was 1% of patients and controls. The differences in intellectual abilities between patients with schizophrenia and healthy subjects or among haplotypes or diplotypes were analyzed using logistic regression or linear regression with HPlus. We also examined interactions of haplotype–diagnosis or diplotype–diagnosis using linear regression. Each genotype was treated as the number of major alleles (0, 1 or 2) in the analysis. For joint haplotype analysis in HPlus, each haplotype or diplotype was tested against the reference haplotype or diplotype (the most frequent haplotype or diplotype). Sex (1: male; 2: female), years of education and diagnosis (0: controls; 1: patients) were corrected for in these analyses as covariates. We applied Bonferroni correction in the all statistical tests, based on the number of 13 genotype measures; SNPs (two), haplotypes (four haplotypes minus a reference haplotype) and diplotypes (nine diplotypes minus a reference diplotype). The significance level for all statistical tests was finally set at two-tailed $P < 0.0039$ (0.05/13).

RESULTS

Impact of genetic variants of the *NRGN* gene on intellectual abilities

First, we investigated the effects of diagnosis, *NRGN* genotype (genome-wide supported rs12807809 and rs12278912) and their interaction on full-scale IQ (Table 1). We found significant effects of diagnosis ($P < 2.95 \times 10^{-9}$) and a possible effect of genotype of rs12807809 ($P = 0.017$) on full-scale IQ. As expected, patients with schizophrenia showed significantly lower IQ than healthy subjects in all analyses of this study. There was no effect of diagnosis–genotype interactions or rs12278912 genotype on full-scale IQ ($P > 0.14$). Second, we investigated the effects of diagnosis and genotype and their interaction on performance and verbal IQ, respectively. We found significant effects of diagnosis ($P < 4.12 \times 10^{-6}$) and possible effects of rs12807809 genotype ($P = 0.017$) and diagnosis–rs12807809 interactions ($P = 0.040$) on performance IQ. Full-scale and performance IQ have a tendency to be lower in individuals with the major risk T allele of rs12807809 than those with the minor C allele. There was no effect of rs12278912 or diagnosis–rs12278912 interactions on performance IQ or genotype or diagnosis–genotype interaction on verbal IQ ($P > 0.058$).

Impact of rs12807809–rs12278912 *NRGN* haplotype on intellectual abilities

Based on the effect of genotype, we next investigated the effects of diagnosis, rs12807809–rs12278912 haplotype and their interaction on full-scale IQ and performance IQ (Table 2). These intellectual scores were lower in patients with schizophrenia than healthy subjects ($z > -11.2$, $P < 3.3 \times 10^{-14}$). We found a subtle effect of diagnosis–CA haplotype interactions on performance IQ ($z = 2.3$, $P = 0.022$). When we further explored the effect of haplotype on performance IQ separately in patients with schizophrenia and healthy subjects, there was a subtle effect of the CA haplotype on performance IQ in patients with schizophrenia ($z = 2.2$, $P = 0.026$). There was no effect of the CA haplotype in healthy subjects ($z = -1.2$, $P = 0.21$). Although there was a subtle association, the performance IQ of the high-risk TG haplotype (reference haplotype) of rs12807809–rs12278912 has a tendency to be lower than that of the CA haplotype in patients with schizophrenia. There was no effect of other haplotypes or diagnosis–haplotype interactions on performance IQ or any haplotype or diagnosis–haplotype interactions on full-scale IQ ($P > 0.11$).

Impact of rs12807809–rs12278912 *NRGN* diplotype on performance IQ

Based on the effect of haplotype, we further investigated the effects of diagnosis, rs12807809–rs12278912 diplotype and their interaction on performance IQ (Table 3). As described above, the intellectual scores were lower in patients with schizophrenia than healthy subjects. We found two significant diagnosis–diplotype interactions (CG/CG: $P = 3.9 \times 10^{-13}$; TA/TA: $P = 1.1 \times 10^{-7}$) and four possible effects of diagnosis–diplotype interactions (CA/TG: $P = 0.022$; TA/CG: $P = 0.022$) and diplotypes (CA/TA: $P = 0.048$; TA/TA: $P = 0.039$) on performance IQ. Other diplotypes and interactions had no effect on performance IQ ($P > 0.11$). Because we found two significant diagnosis–diplotype interactions on performance IQ, we separately examined the effect of diplotype on performance IQ in patients with schizophrenia and healthy subjects. There was a diplotype with significant effect on performance IQ in patients with schizophrenia (TA/TA: $P = 8.2 \times 10^{-8}$), whereas there were five diplotypes with possible effects on performance IQ in patients with schizophrenia (CA/TG: $P = 0.035$; TA/CG: $P = 0.035$; CA/CG: $P = 0.013$) and in healthy controls (CA/TA: $P = 0.044$; TA/TA: $P = 0.036$). The performance IQ of the TG/TG diplotype group was significantly lower than that of the TA/TA diplotype group of patients with schizophrenia.

Table 1 Impact of genetic variants in the *NRGN* gene on intellectual function

	Schizophrenia (N = 157)			Control (N = 257)			P-values (F_{2406}-values)		
Variables	*M/M*	*M/m*	*m/m*	*M/M*	*M/m*	*m/m*	*Diagnosis*	*Genotype*	*Interaction*
rs12807809[a]	T/T (N = 91)	T/C (N = 62)	C/C (N = 4)	T/T (N = 142)	T/C (N = 94)	C/C (N = 21)			
Full-scale IQ	84.2 ± 17.7	88.2 ± 18.2	101.5 ± 10.7	108.5 ± 11.9	109.7 ± 13.0	111.0 ± 8.2	**2.95×10^{-9} (36.8)**	0.017 (4.1)	0.14 (2.0)
Performance IQ	80.1 ± 16.3	85.2 ± 18.1	99.3 ± 10.8	107.6 ± 11.4	107.3 ± 13.0	108.7 ± 11.4	**8.11×10^{-11} (44.6)**	0.017 (4.1)	0.040 (3.3)
Verbal IQ	90.3 ± 17.5	92.3 ± 17.7	102.8 ± 8.2	107.7 ± 13.1	109.9 ± 13.3	112.8 ± 11.7	**4.12×10^{-6} (21.8)**	0.052 (3.0)	0.28 (1.3)
rs12278912	G/G (N = 107)	G/A (N = 45)	A/A (N = 5)	G/G (N = 157)	G/A (N = 87)	A/A (N = 13)			
Full-scale IQ	85.2 ± 18.3	88.6 ± 17.1	87.4 ± 19.5	109.7 ± 11.6	109.0 ± 12.7	102.5 ± 11.9	**6.16×10^{-12} (50.2)**	0.37 (1.0)	0.23 (1.5)
Performance IQ	81.4 ± 17.4	84.9 ± 16.7	88.0 ± 20.2	108.4 ± 11.4	107.4 ± 12.9	99.1 ± 10.2	**2.32×10^{-12} (52.4)**	0.55 (0.6)	0.058 (2.9)
Verbal IQ	90.7 ± 18.0	93.2 ± 16.1	88.6 ± 21.8	109.1 ± 12.6	109.2 ± 14.0	104.5 ± 13.3	**2.25×10^{-8} (32.5)**	0.31 (1.2)	0.67 (0.4)

Abbreviations: IQ, intellectual quotient; M, major allele; m, minor allele; NRGN, neurogranin; SNP, single-nucleotide polymorphism.
Means ± s.d. are shown. To control for confounding factors, the effects of diagnosis, *NRGN* genotype and their interaction on IQ were analyzed by two-way analyses of covariance, with sex and years of education as covariates. Significant P-values (P < 0.0039) are shown in boldface and underlined.
[a]The genome-wide supported SNP for schizophrenia.[4]

Table 2 Association between rs12807809 and rs12278912 haplotype and performance IQ

					P-values (z-scores)	
Haplotypes	Frequency (%)	Coefficient	s.e.	CI	Haplotype effect	DH interaction
Total subjects (N = 414)						
TG	61.5	0 (ref.)	—	—	—	
CG	18.3	0.93	1.49	(−1.99 to 3.84)	0.53 (0.6)	0.59 (0.5)
TA	13.7	−2.16	1.36	(−4.82 to 0.50)	0.11 (−1.6)	0.46 (0.7)
CA	6.6	−2.66	2.08	(−6.73 to 1.41)	0.20 (−1.3)	0.022 (2.3)
Schizophrenia (N = 157)						
TG	66.2	0 (ref.)	—	—	—	
CG	16.3	3.51	3.37	(−3.09 to 10.11)	0.30 (1.0)	
TA	11.5	1.20	2.96	(−4.60 to 7.01)	0.69 (0.4)	
CA	6.0	9.81	4.40	(1.19 to 18.43)	0.026 (2.2)	
Healthy control (N = 257)						
TG	58.5	0 (ref.)	—	—	—	
CG	19.5	0.92	1.59	(−2.19 to 4.02)	0.56 (0.6)	
TA	15.0	−2.14	1.49	(−5.05 to 0.78)	0.15 (−1.4)	
CA	7.0	−2.90	2.33	(−7.47 to 1.67)	0.21 (−1.2)	

Abbreviations: CI, confidence interval; DH interaction, diagnosis–haplotype interaction; IQ, intelligence quotient; s.e., standard error.
Joint Association Analysis (the reference haplotype is the most frequent TG haplotype). For the joint haplotype test, several haplotypes were tested against the reference TG haplotype using linear regression analysis. There was a significant effect of diagnosis ($z = −11.2$, $P = 1.1 \times 10^{-14}$). There was no significant P-value ($P < 0.0039$).

DISCUSSION

In this study, we investigated the impacts of *NRGN* SNPs, haplotypes and diplotypes and genetic variant–diagnosis interactions on intellectual abilities that are known to be impaired in schizophrenia, in 157 patients with schizophrenia and 257 healthy subjects. After correction for multiple tests, we have provided evidence for the rs12807809–rs12278912 diplotype–diagnosis interactions. There was a significant effect of the diplotype of the *NRGN* gene on performance IQ in patients with schizophrenia but not in healthy subjects. The risk variant of the *NRGN* gene was associated with low intellectual ability.

To examine the association between the genome-wide screen-supported rs12807809 of the *NRGN* gene and intellectual ability (verbal, performance and full-scale IQ), this study was conducted on a Japanese population of patients with schizophrenia and healthy subjects. Thus far, two studies have investigated the association of the SNP with intellectual ability and reported no association between the rs12807809 and any IQ. Donohoe *et al.*[28] investigated the association between the SNP and general cognitive ability (verbal, performance and full-scale IQ) in 393 Irish patients with schizophrenia or schizoaffective disorder and 157 controls, and follow-up samples of 240 German patients and 1344 healthy participants. Krug *et al.*[29] investigated the association between the SNP and verbal IQ using 521 healthy subjects with Western and Middle European descent. We also did not find a significant association between the SNP and intellectual abilities in Japanese subjects, consistent with the previous studies in the different Caucasian populations.[28,29] These findings suggest that the *NRGN* polymorphism may not have a major role in the intellectual abilities.

This report is the first investigation of the association of haplotypes and diplotypes of the *NRGN* gene with intellectual abilities in patients with schizophrenia and healthy subjects. We have determined that the frequencies of the major TG and TA haplotypes of rs12807809–rs12278912 were higher and lower, respectively, in patients with schizophrenia compared with healthy controls.[5] In addition, we have found that *NRGN* gene expression of the high-risk TG haplotype was

significantly lower than that of the protective TA haplotype in lymphoblasts. According to these findings, we hypothesized that the IQ of the high-risk TG haplotype group would be lower than that of the protective TA group. However, this prediction was not confirmed in this study, suggesting that the haplotype of the *NRGN* gene may not have a major role in contributing to the intellectual impairments. Instead, we determined that the performance IQ of the major TG/TG diplotype was lower than that of the TA/TA diplotype. When we examined the exploratory association between *NRGN* diplotype and schizophrenia using a Japanese sample (2019 schizophrenia patients and 2574 controls) included in previous study,[5] the ratio of the frequency of the TA/TA diplotype vs the major TG/TG diplotype of rs12807809–rs12278912 in patients (0.020/0.388) was lower compared with controls (0.028/0.349, odds ratio = 0.65, $P = 0.036$). These findings suggest that the performance IQ of subjects with the high-risk TG/TG diplotype was lower compared with the protective TA/TA diplotype in schizophrenia. Expression assay of haplotypes in our previous study[5] was performed using lymphoblasts but not brain tissues. Although the sample sizes of the associated diplotypes are small and limited, further research investigating an association between the diplotype and RNA expression data derived from lymphoblastic cell lines and several tissues including brain is required to enhance our findings.

There are several limitations to interpreting our results. Whether this study has adequate statistical power to detect genetic effects is important. Power calculation was performed using the G*Power 3 program.[37] In the power calculation, our sample size had power > 80% to detect an effect size index f of > 0.152 among genotype groups with $\alpha = 0.05$. Effect sizes are typically categorized as small ($f = 0.10$), medium ($f = 0.25$) or large ($f = 0.40$). A false-negative association could not be excluded in our study because the effect size index f for genotype on intellectual function was 0.143. Because a number of statistical analyses (64 tests), including the effects of diagnosis, genetic variants of genotype (two SNPs), haplotypes (four haplotypes) and diplotypes (nine diplotypes) and their interaction on

Table 3 Association between rs12807809–rs12278912 diplotype and performance IQ

					P-values (z-scores)	
Diplotypes	Frequency (%)	Coefficient	s.e.	CI	Diplotype effect	DD Interaction
Total subjects (N = 414)						
TG/TG	37.9	0 (ref.)	—	—	—	—
CG/TG	22.7	2.52	1.99	(−1.38 to 6.41)	0.21 (1.3)	0.80 (−0.3)
TA/TG	16.4	0.08	2.05	(−3.94 to 4.10)	0.97 (<0.1)	0.88 (−0.2)
CA/TG	7.9	−1.45	2.32	(−5.99 to 3.09)	0.53 (−0.6)	0.022 (2.3)
TA/CG	4.9	−1.45	2.32	(−5.99 to 3.09)	0.53 (−0.6)	0.022 (2.3)
CG/CG	3.1	−1.14	4.26	(−9.50 to 7.21)	0.79 (−0.3)	**3.9×10^{-13} (7.3)**
CA/CG	2.7	5.01	3.11	(−1.09 to 11.11)	0.11 (1.6)	0.11 (1.6)
CA/TA	2.2	−10.93	5.52	(−21.74 to 0.12)	0.048 (−2.0)	0.32 (1.0)
TA/TA	1.9	−5.39	2.61	(−10.50 to 0.28)	0.039 (−2.1)	**1.1×10^{-7} (5.3)**
CA/CA	0.2	—	—	—	—	
Schizophrenia (N = 157)						
TG/TG	42.0	0 (ref.)	—	—	—	
CG/TG	25.5	0.73	3.33	(−5.80 to 7.25)	0.83 (0.2)	
TA/TG	14.6	−1.06	3.53	(−7.98 to 5.86)	0.76 (−0.3)	
CA/TG	8.2	8.61	4.08	(0.61 to 16.61)	0.035 (2.1)	
TA/CG	3.9	8.61	4.08	(0.61 to 16.61)	0.035 (2.1)	
CG/CG	0.6	—	—	—	—	
CA/CG	1.9	14.01	5.61	(3.02 to 25.00)	0.013 (2.5)	
CA/TA	1.9	1.70	12.49	(−22.78 to 26.18)	0.89 (0.1)	
TA/TA	1.3	11.64	2.17	(7.39 to 15.90)	**8.2×10^{-8} (5.4)**	
CA/CA	0	—	—	—	—	
Healthy control (N = 257)						
TG/TG	35.4	0 (ref.)	—	—	—	
CG/TG	21.0	2.33	1.99	(−1.57 to 6.23)	0.24 (1.2)	
TA/TG	17.5	−0.05	2.06	(−4.09 to 4.00)	0.98 (<0.1)	
CA/TG	7.7	−1.57	2.35	(−6.18 to 3.04)	0.50 (−0.7)	
TA/CG	5.5	−1.57	2.35	(−6.18 to 3.04)	0.50 (−0.7)	
CG/CG	4.7	−1.17	4.11	(−9.22 to 6.89)	0.78 (−0.3)	
CA/CG	3.1	4.85	3.14	(−1.30 to 11.01)	0.12 (1.5)	
CA/TA	2.3	−10.97	5.44	(−21.63 to 0.31)	0.044 (−0.2)	
TA/TA	2.3	−5.51	2.63	(−10.66 to 0.36)	0.036 (−2.1)	
CA/CA	0.4	—	—	—	—	

Abbreviations: CI, confidence interval; DD interaction, diagnosis–diplotype interaction; IQ, intelligence quotient; s.e., standard error.
The minimum frequency for a diplotype to be estimated for association was 1% of patients or controls. Joint Association Analysis (the reference diplotype is the most frequent TG/TG diplotype).
For the joint diplotype test, several diplotypes were tested against the reference TG diplotype using linear regression analysis. There was a significant effect of diagnosis ($z= -11.2$, $P=1.1 \times 10^{-14}$). Significant *P*-values ($P<0.0039$) are shown in boldface and underlined.

intellectual abilities (three IQs), were performed, a correction for multiple testing should be considered. However, a consensus on how to correct such multiple testing has not been reached in this research field. Because all tests including genetic variants (genotypes, haplotypes and diplotypes) and each IQ (full-scale, performance and verbal) were not independent and several hypotheses were included, we applied the Bonferroni correction for all statistical tests based on the numbers of 13 genotype measures (genotypes, haplotypes and diplotypes). Because of applying the methods of correcting such multiple testing, we cannot exclude the possibility of false-positive results. As there was no evidence for a specific dominant or recessive model (homozygous allele carriers vs homozygous carriers of other one or two alleles; comparison of two genotype groups) of genetic variants in *NRGN*, our analysis was based on the comparison of three genotype groups. The reason why the diplotype but not the SNPs or the haplotype of the *NRGN* gene was associated with IQ is unclear. Because our results were based on a relatively small

number of individuals with the TA/TA diplotype of rs12807809–rs12278912, a future replication study using larger sample sizes is needed to confirm our findings.

In this study, we found an effect of *NRGN* genetic variant on intellectual ability. Our results support an association between the *NRGN* gene and schizophrenia and a hypothesis that the *NRGN* gene may mediate the risk associated with schizophrenia via intellectual dysfunction.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)