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ORIGINAL ARTICLE

Individual risk alleles of susceptibility to schizophrenia are associated with poor clinical and social outcomes

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Many patients with schizophrenia have poor clinical and social outcomes. Some risk alleles closely related to the onset of schizophrenia have been reported to be associated with their clinical phenotypes, but the direct relationship between genetic vulnerability to schizophrenia and clinical/social outcomes of schizophrenia, as evaluated by both practical clinical scales and 'real-world' function, has not been investigated. We evaluated the clinical and social outcomes of 455 Japanese patients with schizophrenia by severity of illness according to the Clinical Global Impression-Severity Scale (CGI-S) and social outcomes by social adjustment/maladjustment at 5 years after the first visit. We examined whether 46 single nucleotide polymorphisms (SNPs) selected from a Japanese genome-wide association study of susceptibility to schizophrenia were associated with clinical and social outcomes. We also investigated the polygenic risk scores of 46 SNPs. Allele-wise association analysis detected three SNPs, including rs2623659 in the CUB and Sushi multiple domains-1 (*CSMD1*) gene, associated with severity of illness at end point. The severity of illness at end point was associated with treatment response, but not with the severity of illness at baseline. Three SNPs, including rs2294424 in the C6orf105 gene, were associated with social outcomes. Point estimates of odds ratios showed positive relationships between polygenic risk scores and clinical/social outcomes; however, the results were not statistically significant. Because these results are exploratory, we need to replicate them with a larger sample in a future study.
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INTRODUCTION

Schizophrenia is one of the most severe psychiatric disorders, with a lifetime risk of about 1% of the population in all cultures and a refractory clinical course.[1] Approximately 30% of patients with schizophrenia fail to respond adequately to the usual antipsychotic medications and are classified as having treatment-resistant schizophrenia (TRS);[2] further, the rate of full recovery from schizophrenia is reported to be only 13.7%.[3] Owing to the poor clinical outcome, patients with schizophrenia typically have suffered severe social impairment in their 'real-world' lives.[4,5] For example, the employment rate of patients with mental illness (that is, schizophrenia, bipolar disorder, major depressive disorder or epilepsy) is extremely low (17.3%), whereas the general employment rate of the working-age population (15–64-year-old) in Japan is 72.3%. Further, the costs of unemployment in schizophrenia are higher, although the prevalence rate is lower than the corresponding rates of depression or anxiety in Japan.[6]

The heritability of schizophrenia is estimated to be over 80% by family, twin and adoption studies.[7,8] Many genetic studies, including genome-wide association studies (GWAS), on the onset of schizophrenia have been reported.[9–11] One single nucleotide polymorphism (SNP), rs1344706 of the zinc-finger protein 804A gene, is positively related to onset and also associated with the volume of the frontal lobes, severity of clinical symptoms and visual memory.[10,12,13] Rs12807809 of the neurogranin gene is positively related to onset and also associated with widespread cortical thinning and decreased intellectual ability.[14–16] These cognitive deficits or changes of brain structure in patients with schizophrenia may affect their social or clinical outcomes. Genes are associated with both the onset and clinical outcomes and observed in many diseases. For example, in Moyamoya disease and Kawasaki disease, one SNP closely related to the onset has been reported to be associated with clinical outcomes.[17,18] However, a direct relationship between genetic vulnerability to schizophrenia and clinical/social outcomes of schizophrenia as evaluated by both practical clinical scales and 'real-world' function has not been demonstrated. The identification of a genetic 'risk' factor associated with poor outcomes may induce patients with schizophrenia to seek early clinical/social interventions and intensive treatments. On the other hand, the identification of a genetic 'benefit' factor associated with good outcomes may predict a positive prognosis for patients with schizophrenia and allow proactive public participation.

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In the clinical course of schizophrenia, the first 5 years after the first episode of psychosis is called the critical period and it impacts the prognosis of patients with schizophrenia.[19] Clinical symptoms progress more rapidly in the early stage, and the risk of suicide is higher, especially in the first 5 years.[19] The symptoms usually stabilize ('plateau effect') 5 years after the first episode of psychosis[19] and may anticipate clinical/social outcomes in the future.

In this study, we investigated whether high-risk alleles discovered by GWAS of the onset of schizophrenia were also associated with poor clinical and social outcomes of patients with schizophrenia at 5 years after the first visit.

MATERIALS AND METHODS

Subjects

The subjects of this association study comprised 455 unrelated Japanese patients fulfilling the ICD-10 (International Classification of Disease, Version 10, WHO 1992) diagnostic criteria for schizophrenia (243 males and 212 females, mean age ± s.d. at the time of blood sampling 50.4 ± 12.6 years). Diagnosis of schizophrenia was performed by two trained psychiatrists (HU and MT) on the basis of all available information and clinical interviews. This study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. Written informed consent was obtained from all participants.

Assessment of clinical and social outcomes of patients with schizophrenia

To assess clinical outcomes in patients with schizophrenia, we rated the severity of illness according to the Clinical Global Impression-Severity (CGI-S) scale (1 point: normal, not at all ill to 7 points: the most extremely ill) 5 years after the first visit to the psychiatric hospital as the end point. For allele-wise analyses, we defined severe cases as CGI-S $\geqslant 4$ and non-severe cases as CGI-S <4 in accordance with the criteria of Kane *et al*.[20] To investigate whether the severity of illness 5 years after the first visit was related to the severity at the first visit as the baseline or to the treatment response, we examined the CGI-S score at the baseline and the response rate from the baseline: (CGI-S at first visit—CGI-S 5 years later)/(CGI-S at first visit) × 100 (%). We dichotomized the treatment response in patients with schizophrenia with a response rate $\leqslant 20\%$ as 'non-responders' and those with a response rate $>20\%$ as 'responders' because the median response rate of all patients was 20%.

To assess social outcomes in patients with schizophrenia, we defined social adjustment according to the socio-economic groups in the Japan Statistical Yearbook. 'Social adjustment' was defined as functioning as a member of society, including as employee, housewife and student. 'Social maladjustment' was defined as being an inpatient or unemployed except for housewives and students. An employee was defined as a person who is employed at an enterprise or office and receives wages, without regard to the kind of work, according to provisions of the Japanese Labor Standards Act. A housewife was defined to a person who fulfilled the central roles in keeping house, and a student was defined to a person who attended school beyond compulsory education. Patients outside the working age in Japan (15–64-year-old) at the end point were excluded from the social outcome analysis.

We also assumed the following variables as possible confounding factors for clinical and social outcomes in patients with schizophrenia: age at end point, sex, duration of untreated psychosis (DUP), age at onset, length of hospitalization and doses of antipsychotics (equivalent doses of chlorpromazine). Two trained psychiatrists (MT and SS) assessed all clinical and social outcomes based on a review of the medical records at 5 years after the first psychiatric visit in accordance with previous studies.[3,21] The assessments were made independently and the scores were averaged. The two raters were blind to each other's assessments and to the genetic information. Differences between outcome groups (severe/non-severe cases and patients with social adjustment/maladjustment) were tested by *t*-test (Student's *t*-test for homoscedastic variables and Welch's *t*-test for heteroscedastic variables).

SNP selection and SNP genotyping

Candidate SNPs were selected for this study as follows. First, we selected 91 SNPs from the 200 most significant markers in a Japanese GWAS of schizophrenia,[22] following the quality control criteria (that is, call rate $\geqslant 95\%$, autosomal chromosomes, Hardy–Weinberg equilibrium $\geqslant 0.0001$, and minor allele frequency $\geqslant 5\%$) and criteria used in a previous study.[22] Second, of these 91 SNPs, we identified 46 SNPs that had the same direction of risk for susceptibility to schizophrenia in both the original GWAS sample and the replication sample of a previous study.[22] For the 46 SNPs, we set reference alleles as having an odds ratio (OR) of >1 to define 'risk alleles' (that is, patients with schizophrenia had more risk alleles than the controls).

Peripheral blood was obtained from subjects and genomic DNA was extracted from peripheral leukocytes using a standard procedure. Genotyping was performed using the Sequenom iPLEX Gold System (Agena Bioscience, San Diego, CA, USA). Markers that could not be assayed on this platform were genotyped using a TaqMan assay (Applied Biosystems, Foster City, CA, USA). All genotyping was performed at the Department of Psychiatry, School of Medicine, Fujita Health University.

Table 1 Bio-demographic variables for subjects with clinical and social outcomes

		Severity of illness			*Social outcome*		
	All patients	*Severe cases (CGI-S ⩾ 4)*	*Non-severe cases (CGI-S < 4)*		*Patients with social maladjustment*	*Patients with social adjustment*	
Variables	(N = *455*)	(N = *282*)	(N = *173*)	P-*value*	(N = *331*)	(N = *124*)	P-*value*
Age at end point (years)	31.7 ± 9.7	30.8 ± 9.2	33.2 ± 10.2	**0.011**	31.1 ± 9.3	33.3 ± 10.4	**0.032**
Sex (male/female)	243/212	158/124	85/88	0.192	183/148	60/64	0.148
DUP (years)	1.2 ± 3.7	1.3 ± 3.9	1.1 ± 3.4	0.638	1.3 ± 3.9	0.5 ± 0.5	0.243
Age at onset (years)	25.4 ± 8.7	24.5 ± 8.1	27.0 ± 9.4	**0.003**	24.7 ± 8.4	27.4 ± 9.4	**0.005**
Length of hospitalization (per 30 days)	25.7 ± 21.9	34.8 ± 21.3	11.1 ± 13.2	**<0.001**	32.0 ± 21.8	8.9 ± 10.3	**<0.001**
Doses of antipsychotics (per 100 mg)	4.5 ± 5.2	5.3 ± 5.9	3.3 ± 3.6	**<0.001**	5.0 ± 5.7	3.1 ± 3.3	**<0.001**

Abbreviations: CGI-S, Clinical Global Impression-Severity scale; DUP, Duration of untreated psychosis.
Mean ± s.d. are shown. *P*-values <0.05 are boldface and underlined.
Age at end point, sex, DUP and age at onset were tested by Student's *t*-test.
Length of hospitalization and dosage of antipsychotics were tested by Welch's *t*-test.

Allele-wise association analysis

For the 455 patients with schizophrenia, we investigated whether higher risk allele frequencies for 46 SNPs were associated with poor outcomes (severe cases or social maladjustment) by χ^2 tests (one-tailed). These statistical analyses were performed by using the software SNPAlyze (Dynacom Co., Chiba, Japan). For the *post hoc* power analysis calculations, the G* power program was used.[23] In this program, we used effect size calculated by Cramer's V in most significant SNPs and an α error was defined as 0.05. For all analyses, statistical significance was defined as $P<0.05$. Because we considered this study exploratory, we did not correct for multiple testing.

Polygenic risk score analysis

For calculation of polygenic risk scores, we counted the average score of risk alleles in all 46 SNPs by the additive model in each case. For example, because the T allele of rs2623659 was reported to be a risk for schizophrenia, the T/T genotype scored 2 points, C/T genotype scored 1 point and C/C genotype scored 0 points. The total score of the 46 SNPs was divided by the number of genotyped SNPs in each case. Cases with extremely few genotyped SNPs (0 or 1 SNP) were excluded from the polygenic risk score analysis. We did not give weight to effect size in the calculation of polygenic risk scores because the ORs of the 46 SNPs showed almost equivalent impacts on the susceptibility to schizophrenia in previous GWAS.[22]

To evaluate the relationships between polygenic risk scores and clinical outcome, we used a logistic regression analysis. In model 1, we calculated crude ORs or β coefficients. In model 2, we included patient characteristics (age at end point and sex). In model 3, we included clinical characteristics (age at onset, length of hospitalization and doses of antipsychotics) in addition. DUP was omitted from adjusted variables because DUP could be directly calculated from age at end point and age at onset (age at end point = age at the onset +DUP+5 years). We conducted all analyses using SPSS Statistic software version 19.0 (SPSS Japan Inc., Tokyo, Japan).

RESULTS

Differences in bio-demographic variables in clinical and social outcomes

The bio-demographic variables in each outcome of patients with schizophrenia are shown in Table 1. Patients with poor outcomes (severe cases and social maladjustment) had variables that were reasonably considered to be associated with the severity and refractoriness of schizophrenia (for example, younger onset, longer hospitalization and increased doses of antipsychotics). There was no difference in DUP in any outcome.

Allele-wise association analysis in clinical and social outcomes

Genotypic distributions of all SNPs were in Hardy–Weinberg equilibrium. Risk allele frequencies, *P*-values, OR and 95% confidence interval for each outcome for all 46 SNPs are shown in Supplementary Table 1. In the analysis of severity of illness at the end point, severe cases had higher risk allele frequencies than non-severe cases in three SNPs: rs8116303 ($\chi^2=8.37$, df = 1 and $P=0.002$), rs3129601 ($\chi^2=6.84$, df = 1 and $P=0.004$), and rs2623659 ($\chi^2=5.19$, df = 1 and $P=0.011$; Table 2A). Of the three SNPs associated with clinical outcomes, treatment non-responders had higher risks of carrying these

Table 2 Associations between risk alleles of susceptibility to schizophrenia in GWAS and clinical or social outcomes in patients with schizophrenia

A: Allele-wise analyses of severity of illness at end point

SNPs	*chr*	*BP*	*Closest gene*	*R*[a]	*NR*[b]	*RAF_S*[c]	*RAF_NS*[d]	P-*value*	*OR (95% CI)*
rs8116303	20	37914989		A	G	16.26	9.00	**0.002**	1.96 (1.24–3.12)
rs3129601	13	21460993		A	G	21.76	14.24	**0.004**	1.68 (1.14–2.47)
rs2623659	8	3623779	*CSMD1*	T	C	52.04	43.71	**0.011**	1.40 (1.05–1.86)

B: Allele-wise analyses of clinical factors influencing severity of illness at end point

	Treatment response				*Severity of illness at the baseline*			
SNPs	*RAF_NR*[e]	*RAF_R*[f]	P-*value*	*OR (95% CI)*	*RAF_S*[c]	*RAF_NS*[d]	P-*value*	*OR (95% CI)*
rs8116303	21.52	15.13	**0.011**	1.56 (1.06–2.30)	13.96	6.52	0.076	2.33 (0.71–7.63)
rs3129601	51.23	44.74	**0.037**	1.30 (0.97–1.73)	18.99	19.57	0.538	0.96 (0.45–2.04)
rs2623659	15.15	10.93	**0.050**	1.44 (0.93–2.24)	49.33	39.13	0.090	1.51 (0.82–2.78)

C: Allele-wise analyses of social outcome

SNPs	*chr*	*BP*	*Closest gene*	*R*[a]	*NR*[b]	*RAF_SM*[g]	*RAF_SA*[h]	P-*value*	*OR (95% CI)*
rs2294424	6	11860537	*C6orf105*	A	G	66.12	55.36	**0.002**	1.57 (1.15–2.16)
rs6550146	3	32679747		A	G	43.38	34.43	**0.012**	1.46 (1.05–2.03)
rs8116303	20	37914989		A	G	14.93	9.91	**0.032**	1.6 (0.97–2.63)

Abbreviations: BP, base position; chr, chromosome; CI, confidence interval; GWAS, genome-wide association study; NR, non-risk allele; OR, odds ratio; R, risk allele; RAF, risk allele frequency; SNPs, Single nucleotide polymorphisms.
P-value < 0.05 are boldface and underlined.
[a]Risk allele on GWAS (ref. 22).
[b]Non-risk allele on GWAS (ref. 22).
[c]Risk allele frequencies of severe cases.
[d]Risk allele frequencies of non-severe cases.
[e]Risk allele frequencies of non-responders.
[f]Risk allele frequencies of responders.
[g]Risk allele frequencies of patients with social maladjustment.
[h]Risk allele frequencies of patients with social adjustment.

Table 3 Impacts of polygenic risk scores on clinical and social outcomes adjusted by bio-demographic variables

	Model 1		*Model 2*		*Model 3*	
Variables	*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*
A: Logistic regression analysis for severity of illness						
Polygenic risk score	1.86 (0.38 – 9.17)	0.44	1.62 (0.32 – 8.07)	0.56	2.69 (0.30 – 24.15)	0.38
Patient characteristics						
Age at end point (years)			0.98 (0.96 – 1.00)	0.06	0.98 (0.90 – 1.08)	0.73
Sex						
Female			1.00		1.00	
Male			1.25 (0.83 – 1.88)	0.29	1.12 (0.65 – 1.95)	0.69
Clinical characteristics						
Age at onset (years)					0.98 (0.89 – 1.07)	0.60
Length of hospitalization (per 30 days)					1.08 (1.07 – 1.10)	<0.001
Doses of antipsychotics (per 100 mg)					1.17 (1.09 – 1.26)	<0.001
B: Logistic regression analysis for social maladjustment						
Polygenic risk score	2.22 (0.39 – 12.49)	0.37	1.90 (0.33 – 10.86)	0.47	3.32 (0.35 – 31.96)	0.30
Patient characteristics						
Age at end point (years)			0.98 (0.96 – 1.00)	0.07	1.02 (0.94 – 1.12)	0.50
Sex						
Female			1.00		1.00	
Male			1.30 (0.84 – 2.04)	0.24	1.11 (0.63 – 1.95)	0.73
Clinical characteristics						
Age at onset (years)					0.94 (0.85 – 1.03)	0.19
Length of hospitalization (per 30 days)					1.06 (1.07 – 1.11)	<0.001
Doses of antipsychotics (per 100 mg)					1.14 (1.06 – 1.23)	0.001

Abbreviations: CI, confidence interval; OR, odds ratio.

alleles than responders (Table 2B). However, the severity of illness at the baseline was not different (Table 2B).

In the analysis of social outcome, patients with social maladjustment had higher risk allele frequencies than patients with social adjustment in three SNPs: rs2294424 ($\chi^2 = 7.92$, df = 1 and $P = 0.002$), rs6550146 ($\chi^2 = 5.05$, df = 1 and $P = 0.012$) and rs8116303 ($\chi^2 = 3.42$, df = 1 and $P = 0.032$) (Table 2C). In the power analysis, we found that our sample size had >0.80 power to detect an effect size (Cramer's $V = 0.093$) for a SNP (rs816303 in Table 2A) in allele-wise association analysis.

Polygenic risk score analysis

The impacts of polygenic risk scores on severity of illness and social outcome are shown in Table 3. Point estimates of ORs showed positive relationships between polygenic risk scores and clinical/social outcomes; however, the results were not statistically significant.

To confirm the effects of specific SNPs associated with clinical or social outcome in separate analyses, we created risk scores consisting of only those three SNPs. We found that the risk scores were significantly associated with poor clinical and social outcomes in regression analyses even after adjustment by bio-demographic variables (Supplementary Table 2).

DISCUSSION

This is the first report of a genetic investigation of the genetic vulnerability to schizophrenia that was also directly related to clinical/social outcomes of schizophrenia as evaluated by both practical clinical scales and 'real-world' function. These results may be interesting because clinical and social outcomes, which are supposed to be affected by environmental factors,[24] are defined by genetic factors related to the onset of schizophrenia. We detected the association of three SNPs with severity of illness and three SNPs with social outcome. Furthermore, the three SNPs associated with severity of illness were also associated with treatment response. Because both severity of illness and treatment response are important factors in the definition of TRS,[25] these SNPs may be related to the development of TRS. Because all the current antipsychotic drugs were developed on the basis of dopamine antagonism, TRS is not necessarily related to the molecular function of cognitive declines.

Rs2623659 is an intron SNP located in the CUB and Sushi multiple domains-1 (*CSMD1*) gene on chromosome 8p23.2. CSMD1 is a complement control-related protein.[26] *In situ* hybridization and neuron immunolabeling showed that CSMD1 is synthesized in the developing central nervous system.[26] In neuropsychological behavior phenotypes, CSMD1 knockout mice showed behaviors suggestive of blunted emotional responses, anxiety and depression.[27] *CSMD1* is related to neurodevelopmental disorders, including schizophrenia, epilepsy and speech delay.[28–30] The other SNPs in *CSMD1* (rs10503253, rs7017888 and rs7011965) were listed as carrying risks for susceptibility to schizophrenia in a recent GWAS targeting a Caucasian population.[30–32] In addition, rs10503253 was reported to be associated with cognitive disability in both patients with schizophrenia and healthy participants.[33,34] Taken together with our findings, we speculate that *CSMD1* has a role not only in the susceptibility to schizophrenia but also in cognitive disability or clinical outcomes of patients with schizophrenia. Rs2294424 was mapped onto the *C6orf105* gene. Two previous Japanese GWAS analyses gave nominal significance to *C6orf105*.[22,35] Though *C6orf105* has been reported to be

associated with the regulation of androgen-enhanced expression,[36] coronary artery disease[37] and non-syndromic oral clefts,[38] the exact function of *C6orf105* in psychiatric diseases is still unknown. The functions of the other three SNPs have not been revealed.

The polygenic risk score analysis among the 46 SNPs did not reveal a polygenic effect of risk alleles on poor clinical and social outcomes of patients with schizophrenia. This result may be due to insufficient numbers of risk alleles for the calculation of scores or an inadequate sample size of patients with schizophrenia. Several studies have reported polygenic effects on the clinical phenotypes or outcomes of schizophrenia. Negative and disorganized symptoms were reported to be correlated with polygenic risk scores generated using a case-control GWAS of schizophrenia examining 76 114 SNPs (*P*-value cut-off of 0.5) in 2454 subjects.[39] Patients with TRS demonstrated higher polygenic risk scores than patients without TRS, and the study examined 58 663 SNPs (*P*-value cut-off of 0.5) in 804 subjects.[40] Further evaluations using more risk alleles due to a less stringent *P*-value cut-off and larger sample size may be required.

In our study, DUP was not associated with poor clinical and social outcomes. A recent study demonstrated that DUP did not necessarily predict poor clinical and social outcomes in patients with schizophrenia.[41] On the other hand, many studies showed that a longer DUP was associated with poorer outcomes (for example, poorer response to antipsychotic treatment and worse social function).[42,43] The interpretation of our result may therefore need caution.

Our results have several limitations. First, we defined the statistical significance as $P<0.05$. If the Bonferroni correction is applied, the significant threshold of the alpha may be 5.43×10^{-4} for multiple tests of 46 SNPs and two subgroups ($\alpha=0.05/46\times2$) and the significance of our results may be negative. In this study, we could not provide the replication samples. Because these results are exploratory, we need to replicate them with a larger sample in a future study. The second limitation is our method of SNP selection and our sample size. Until December 2014, there were three GWAS reports about schizophrenia in Japanese populations.[22,35,44] The most significant SNPs in these studies (200 SNPs reported by Ikeda *et al.*, 1472 SNPs reported by Yamada *et al.* and 1536 tagSNPs reported by Shibata *et al.*) did not overlap. Another study using the most significant SNPs reported by Yamada and Shibata will be necessary to detect further genetic factors that contribute to the clinical and social outcomes of patients with schizophrenia. In addition, the other multi-stage GWAS of schizophrenia with a large sample size (up to 36 989 cases and 113 075 controls, including Japanese samples) revealed 108 schizophrenia-associated SNPs, which include rs10503253 in *CSMD1*.[11] In the future, the association between patient outcomes and these SNPs should be investigated with a larger sample. Because clinical and social outcomes of patients with schizophrenia are affected by non-genetic and environmental factors, the effects of SNPs may be restricted. The third limitation is the assessment of clinical outcomes of patients with schizophrenia. We used only the CGI scale. To assess the severity of schizophrenia in patients, more complex scales such as the Positive and Negative Syndrome Scale may be desirable. In addition, we did not examine medications administered except for antipsychotics. The fourth limitation is the assessment of social outcomes of patients with schizophrenia. In this study, we estimated social dysfunction by social adjustment, but we did not estimate other aspects of social dysfunction, such as those revealed by the Quality-of-Life Scale, Social Functioning Scale or Schizophrenia Outcomes Functioning Interview. In the future, we should estimate various aspects of social dysfunction in patients with schizophrenia.

In conclusion, we suggest that several individual risk alleles of susceptibility to schizophrenia were associated with poor clinical and social outcomes but that the polygenic score showed only a tendency. Further genetic studies of clinical and social outcomes of patients with schizophrenia will be required with a larger sample or distinct populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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