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MICRO REPORT

Association study of a single nucleotide polymorphism in the hypoxia response element of the macrophage migration inhibitory factor gene promoter with suicide completers in the Japanese population

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Abstract

Background: More than 800000 people die by suicide annually. The heritability of suicide is 30%–50%. We focused on the hypoxia response element (HRE), which promotes the expression of macrophage migration inhibitory factor (MIF) via the hypoxia-inducible factor (HIF) pathway, important in neurogenesis and neuroprotection. We examined a genetic polymorphism of rs17004038, a single-nucleotide polymorphism (SNP), in suicide completers and controls.

Methods: The study population included 1336 suicide completers and 814 unrelated healthy controls. All participants were Japanese. We obtained peripheral blood, extracted DNA, and genotyped the patients for SNP rs17004038 (C > A).

Results: No significant differences were observed between the two groups in either the allele or genotype analyses. Subgroup analyses by sex, age (<40 or ≥40), and suicide method (violent or nonviolent suicide) were performed with similar results.

Conclusion: No association was observed between SNP rs17004038 and suicide completion. Although it is challenging to collect a large number of samples from suicide completers, further MIF-related genetic studies, including those of rs17004038, are necessary with larger sample sizes.

KEYWORDS
genetics: Human, hypoxia inducible factor, macrophage migration inhibitory factor, single nucleotide polymorphism, suicide: basic/clinical

1 | INTRODUCTION

More than 800 000 people die by suicide annually.[1] Many studies, including genome-wide association studies (GWAS), have investigated the association between genetic factors and suicide. If one sibling committed suicide, the risk of suicide in the remaining siblings increased among females and males (odds ratio: 3 and 2, respectively).[2] The heritability of suicide is 30%–50%.[3] Recent studies have reported that genes or proteins relevant to the immune system are altered in samples such as brain tissue of suicide completers.[4]

Macrophage migration inhibitory factor (MIF) is a multifunctional cytokine that regulates innate and adaptive immunity. MIF plays a crucial role in neurogenesis and neuroprotection.[5–7] It facilitates DNA damage responses and cell cycle regulation.[8] MIF is expressed in neurones and astrocytes of various brain regions.[5,6] Increasing evidence suggests that MIF plays a role in psychiatric disorders. Edwards et al.[9] reported high serum MIF is associated with depressive symptoms and reduced cortisol response to acute stress. Conboy et al.[6] showed deletion of the *MIF* gene increased the depressive behavior of the rodent. Our previous studies suggested that MIF is upregulated by antipsychotic drugs,[10] and we speculate MIF function as physiological protective factors against psychiatric disorders.

This suggests that functions of MIF are important beyond disease boundaries. Functional variants in the *MIF* gene promoter, *MIF*-794CATT$_{5-8}$ repeat (rs5844572) and *MIF*-173G/C single-nucleotide polymorphism (SNP) (rs755622), affect *MIF* gene expression and protein levels.[11,12] Our previous study showed no significant association between completed suicide and these polymorphisms,[13] but one study demonstrated a significant association between attempted suicide and *MIF*-173G/C SNP.[14]

Recent studies have shown that MIF expression is facilitated via the hypoxia-inducible factor (HIF) pathway under hypoxic conditions, and that hypoxia-induced MIF expression depends on the hypoxia response element (HRE) in the 5′-UTR of the *MIF* gene. The SNP rs17004038, which is located in the functional HRE, prevents hypoxia-induced MIF expression.[15,16] We previously reported that the SNP rs17004038 was associated with schizophrenia and disrupted hypoxia-induced MIF expression via the HIF pathway in primary cultured astrocytes derived from the neonatal mouse forebrain.[17] We described the possibility that hypoxia increases the risk of schizophrenia by inhibiting MIF-mediated hippocampal development. Perinatal hypoxia is a risk for schizophrenia.[18]

Suicide rates in patients with schizophrenia are approximately 10%,[19] which is much higher than that in the general population. Furthermore, *MIF* is involved in neurogenesis in the hippocampus[6] and postmortem hippocampal tissue from suicide deaths has been shown to decrease neurotrophic/growth factors.[20] Therefore, we hypothesized that there are associations between suicide completion and the SNP rs17004038 in the HRE of the *MIF* promoter. Our previous study of MIF in patients with schizophrenia has failed to obtain information from each patient on suicidal risk, including suicidal ideation.[17] Docherty et al.[21] conducted meta-analysis integrating GWAS for patients with suicidal behaviors, identifying 12 risk loci. However, most studies did not target suicide deaths. To reduce the number of suicidal completers, we urgently need indicators to identify patients at risk. We investigated the association between the SNP rs17004038 and suicide by comparing patients with suicidal completers and controls, at least on the largest scale in East Asia.

2 | METHODS

2.1 | Study samples

This study was approved by the Ethics Committee for Genetic Studies of Kobe University Graduate School of Medicine and was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants and the families of those used for postmortem blood analysis. The suicide group included individuals who committed suicide between June 1996 and September 2022. The demographic data for the suicide group and healthy controls are shown in Table 1, and the detailed subgroup data are shown in Table S1. The study population included 1336 suicide completers (879 males and 457 females) and 814 unrelated healthy volunteers (387 males and 427 females). All participants were ethnically Japanese.

Autopsies of the suicide victims were conducted at the Department of Legal Medicine, Kobe University Graduate School of Medicine. The definition of suicide was based on the results of medicolegal examinations and police investigations, as defined by Japanese law. The suicide methods are shown in Table 1. Some had used more than one method. We sub-grouped the methods into violent (hanging, jumping from heights, gas suffocation, drowning, jumping in front of a vehicle, self-inflicted penetrating wounds, and self-burning) and nonviolent suicide (taking poison and drug overdose) according to the method of Dumais et al.[22] If there was more than one method used, and any one of them was violent, it was considered a violent suicide.

Healthy controls were recruited from the staff of the participating hospitals after the study purpose and procedures had been explained. None of them manifested psychiatric problems during unstructured interviews with two psychiatrists using unnamed symptom checklists based on the Diagnostic and Statistical Manual of Mental Disorders 4th or 5th edition criteria. All showed good social and occupational skills and reported no history of psychiatric disorders.

2.2 | Blood and DNA sampling

Peripheral blood samples were obtained from all suicide completers and controls. Blood samples were stored at −80°C before analysis. DNA was extracted using a QIAamp DNA Blood Midi Kit (Qiagen, Valencia, CA, USA). We quantified and qualified each DNA sample using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

TABLE 1 Demographic data.

	Suicide	Control	*p*-Value
Number	1336	814	
Female (%)	34.2 (457/1336)	52.5 (427/814)	**1.06×10^{-16}***
Age median (IQR)	53 (39, 67)	54 (37, 67)	0.828**
Suicide methods	**Number**		
Hanging	859		
Jumping from heights	202		
Gas suffocation	62		
Drowning	34		
Self-inflicted Penetrating wounds	21		
Jumping in front of a vehicle	20		
Drug overdose	13		
Self-burning	9		
Taking poison	3		
Other methods	21		
Unknown	100		

Note: Significance was set at $p < 0.05$; significant differences are shown in bold.
Abbreviation: IQR, interquartile range.
*The *p*-value was calculated using Fisher's exact test.
**The *p*-value was calculated using a Mann–Whitney *U* test.

2.3 | Genotyping of SNP rs17004038 in the HRE of the *MIF* gene

Genotyping was performed as previously described.[10,17] The *MIF* gene is located on chromosome 22q11.23 (GenBank accession No. NM_002415). We analyzed the SNP rs17004038 (C > A), which is located in the HRE of the *MIF* promoter.[16] We obtained the predesigned TaqMan SNP genotyping assay for SNP rs17004038 from the Applied Biosystems database and genotyped the participants using a 7500 Real-Time PCR System (Thermo Fisher Scientific) in accordance with the manufacturer's protocol.

2.4 | Statistical analysis

Statistical analyses were performed using R version 4.2.1 (R Development Core Team, Vienna, Austria), EZR version 1.55 (Jichi Medical University, Saitama, Japan), and Haploview version 4.1 (Broad Institute, Cambridge, MA, USA). For demographic data, continuous variables were compared with the Mann–Whitney *U* test and categorical variables were compared with Fisher's exact test. Dummy variables (0 or 1) were used as required. We performed χ^2 tests for allele analyses and Cochrane–Armitage tests for genotyping analyses. In comparative analyses, significance was set at $p < 0.05$.

3 | RESULTS

We observed no deviations from Hardy–Weinberg equilibrium for polymorphisms in suicide completers and healthy controls ($p = 0.1991$). There was no significant difference in age; however, there was a significant difference in sex ratio (Table S1). We observe no significant association between either the genotype distribution ($p = 0.6333$) or allelic frequency ($p = 0.6316$) of rs17004038 and completed suicide. Subgroup analyses based on sex (males: $p = 0.4616$, 0.4654; females: $p = 0.8732$, 0.8704), age (<40: $p = 0.1260$, 0.1299; ≥40: $p = 0.6244$, 0.6204), and suicide method (violent suicide: $p = 0.5287$, 0.5260; non-violent suicide: $p = 0.4808$, 0.4848) also yielded no significant differences (Table 2).

4 | DISCUSSION

To our knowledge, this is the first study to investigate the association between suicide and the SNP rs17004038 in the HRE of the *MIF* gene promoter. We also conducted a subgroup analysis of sex because males tend to have a higher suicide rate than females among all generations worldwide.[23] However, no significant differences were observed in each sex. Furthermore, we conducted subgroup analysis separating suicide completers to violent or non-violent suicides because mortality associated with violent methods is reported to be considerably higher than that associated with nonviolent methods.[24] This study finds no association between suicide and SNP rs17004038 in the HRE of the *MIF* promoter in any analyses.

This study had several limitations. First, the current sample provided low power based on the observed frequencies of the polymorphisms (Table 2). Despite our sample size of suicide completers, which is one of the largest reported worldwide, the number of participants in this association study may not have been sufficiently large to detect a significant difference. Most previous candidate gene analyses and GWAS for completed suicides could not overcome the statistical

TABLE 2 Distribution of rs17004038 in suicide completers and controls in this study.

		Genotype				Allele					
	N	CC	CA	AA	*p**	C	A	MAF	*p***	Odds ratio (95% CI)	Power
Overall Suicide	1336	1293	42	1	0.6333	2628	44	0.0165	0.6316	0.8918 (0.5584–1.4244)	0.0496
Non-violent suicide	13	13	0	0	0.4808	26	0	0.0000	0.4848	0.9889 (0.0589–16.6013)	9.04×10^{-24}
Violent suicide	1202	1165	36	1	0.5287	2366	38	0.0158	0.5260	0.8555 (0.4535–1.6137)	0.0878
Control	814	784	30	0		1598	30	0.0184			
Female											
Suicide	457	441	15	1	0.8732	897	17	0.0186	0.8704	1.0601 (0.5261–2.1361)	0.0363
Control	427	412	15	0		839	15	0.0176			
Male											
Suicide	879	852	27	0	0.4616	1731	27	0.0154	0.4654	0.7893 (0.4175–1.4922)	0.0936
Control	387	372	15	0		759	15	0.0194			
Age < 40											
Suicide	342	332	10	0	0.1260	674	10	0.0146	0.1299	0.5307 (0.2308–1.2205)	0.291
Control	239	226	13	0		465	13	0.0272			
Age ≥ 40											
Suicide	994	961	32	1	0.6244	1954	34	0.0171	0.6204	1.1597 (0.6449–2.0853)	0.0767
Control	575	558	17	0		1133	17	0.0148			

Abbreviations: CI, confidence interval; MAF, minor allele frequency.
*The *p*-value was calculated using the Cochrane–Armitage Test.
**The *p*-value was calculated using a χ^2 test.

limitation of sample sizes because it is extremely difficult to obtain tissue samples from suicide completers.[25,26] Second, it would be difficult to use only a single candidate gene approach to identify susceptibility genes for a complex phenotype such as suicide, and a combined analysis of multiple genes or loci, including the risk loci of suicidal attempters identified by Docherty et al.,[21] would be required. Third, our study only included Japanese participants. Our results may therefore not be generalizable to other population groups. Fourth, MIF protein levels in the brains of suicidal completers should be further investigated to clarify the association between *MIF* and suicide.

5 | CONCLUSIONS

Given the neuronal importance of the *MIF* gene and the reported association of SNP in HRE of *MIF* with schizophrenia, this study investigated the association between SNP in HRE of *MIF* and suicide completers but found no significant differences between suicide completers and healthy controls. However, we will continue to examine the association between various polymorphisms, including rs17004038, and suicide, using a dataset with a larger sample size.

AUTHOR CONTRIBUTIONS

Conceptualisation: Satoshi Okazaki and Akitoyo Hishimoto. Data curation: Toshiyuki Shirai, Takaki Tanifuji. Formal Analysis: Toshiyuki Shirai. Funding Acquisition: Satoshi Okazaki, Ikuo Otsuka and Akitoyo Hishimoto. Investigation: Toshiyuki Shirai. Project administration: Satoshi Okazaki. Resources: Toshiyuki Shirai, Satoshi Okazaki, Takaki Tanifuji, Ikuo Otsuka, Masao Miyachi, Shohei Okada, Ryota Shindo, Tadsu Horai, Kentaro Mouri, Motonori Takahashi, Takeshi Kondo, and Yasuhiro Ueno. Supervision: Akitoyo Hishimoto. Writing the original draft: Toshiyuki Shirai. Writing, review, and editing: Satoshi Okazaki and Akitoyo Hishimoto.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available within the article and its supplementary materials (Data S1).

ETHICS STATEMENT

Approval of the Research Protocol by an Institutional Review Board: The study design and related procedures were performed in accordance with the Declaration of Helsinki. This study was approved by the Ethical Committee for Genetic Studies of Kobe University Graduate School of Medicine (Approval No. 180240).

Informed Consent: Informed consent was obtained from all living participants and the families of the suicide victims.
Registry and the Registration No. of the Study: N/A.
Animal Studies: N/A.

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