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ORIGINAL RESEARCH

Association study of *MIF* promoter polymorphisms with suicide completers in the Japanese population

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Background: Numerous studies suggest that inflammation plays a key role in suicidal behavior. Macrophage migration inhibitory factor (MIF), a proinflammatory cytokine, has received increasing attention in depression research. However, no study has investigated whether *MIF* has genetic involvement in completed suicide. In this study, we sought to explore the relationship between two functional polymorphisms on the *MIF* gene promoter (*MIF*-794CATT$_{5–8}$ microsatellite and *MIF*-173G/C single-nucleotide polymorphism [SNP]) and completed suicide by using one of the largest samples of suicide completers ever reported.

Methods: The subjects comprised 602 suicide completers and 728 healthy controls. We genotyped *MIF*-794CATT$_{5–8}$ microsatellite by polymerase chain reaction–based size discrimination assay and *MIF*-173G/C SNP by TaqMan® SNP genotyping assay. The allele-, genotype-, or haplotype-based association analyses between the suicide completers and the controls were carried out with the χ^2 test, the Cochran–Armitage trend test, or Fisher's exact test.

Results: Analyses of allele or genotype frequency distributions of the polymorphisms studied here did not reveal any significant differences between the suicide completers and the controls. Haplotype analysis also revealed no association with completed suicide.

Conclusion: To our knowledge, this is the first study that has examined the genetic association between MIF and completed suicide. Our results suggest that the effects of *MIF*-794CATT$_{5–8}$ microsatellite and *MIF*-173G/C SNP on the *MIF* gene promoter might not contribute to the genetic risk of completed suicide in the Japanese population.

Keywords: MIF, suicide, microsatellite, SNP, haplotype, promoter region

Introduction

Suicide is a significant public health problem worldwide, with ~1 million suicides each year. A review of the literature showed that >2% of the current traffic accidents were suicide attempts, and this phenomenon may be underreported.[1] The involvement of genetic factors in suicidal behavior is supported by family studies, twin and adoption studies, candidate gene analyses, and genome-wide association studies. Various studies have shown that numerous genes (eg, serotonin receptors and transporters, brain-derived neurotrophic factors [BDNF], or catechol-*O*-methyltransferase [COMT]) are linked to suicidal behavior, although conflicting reports exist.[2,3] In addition, recent studies reported aberrant cytokine levels in blood, cerebrospinal fluid, and postmortem brain samples from suicidal completers.[4] For instance, increased blood levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are associated with suicidal ideation and attempt.[5,6] Alternatively, mRNA and protein levels of IL-1β, IL-6, and TNF-α were increased postmortem in the prefrontal cortexes of teenaged suicide completers.[7]

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Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine involved in the regulation of innate and adaptive immunity.[8] MIF was originally identified as a product of T-lymphocytes on the basis of its ability to prevent random migration of macrophages.[9,10] MIF is secreted in response to inflammatory stimuli, including microbial products and glucocorticoids.[11,12] Upon release, MIF acts in an autocrine and paracrine manner to promote proinflammatory cytokine production, eg, IL-1β, IL-6, and TNF-α.[13,14] MIF has also been shown to facilitate DNA damage response and cell cycle regulation.[15] In addition, MIF production has been discovered in many other nonimmune cells ubiquitously, being highly expressed in the liver, kidneys, anterior pituitary, and brain.[8,16] It is reported that *MIF* expression in the brain was seen in neurons of the cortex, hypothalamus, hippocampus, cerebellum, and pons,[17] and in astrocytes and the subgranular zone of the hippocampus.[18] Increasing evidence suggests a role for MIF in depression.[19] Deletion of MIF resulted in increased depressive behaviors in rodent models.[18] In addition, several studies have explored MIF as a biomarker in major depressive disorder (MDD) and other mood disorders. Some studies have identified increased serum MIF both in patients with MDD and in healthy subjects with depressive symptoms.[20–22] Exhaustive serum proteomic profiling of MDD has identified MIF as one of the robust changed analytes in patients compared with controls.[23,24] Furthermore, it has been established that MIF has an intricate relationship with the hypothalamic–pituitary–adrenal axis. For instance, Edwards et al[25] found that elevated serum MIF was associated with depressive symptoms, reduced cortisol response to acute stress, and lower morning cortisol values. Alternatively, MIF was shown to specifically counteract the glucocorticoid-induced suppression of inflammatory cytokine secretion in activated macrophages (IL-1β, IL-6, and TNF-α).[12] The hypothalamic–pituitary–adrenal axis has also gained interest as a neurobiological factor related to suicide, on the basis of the findings of studies investigating dexamethasone suppression test (DST) response, since nearly a 10-fold higher risk of completed suicide was found in DST nonsuppressors than in DST suppressors in a depressed cohort.[2,26]

From these findings, we hypothesized that MIF is involved in the genetic pathophysiology of suicide. The *MIF* gene promoter contains two functional variations: *MIF*-794CATT$_{5–8}$ repeat (rs5844572) and *MIF*-173G/C single-nucleotide polymorphism (SNP) (rs755622), which have been well documented to affect the gene expression and protein levels of MIF.[27,28] Indeed, many studies have examined the association between these two polymorphisms and various physical diseases.[29–34] However, no previous study has investigated the role of both these polymorphisms in suicidal behavior. Hence, we investigated the association of the two functional polymorphisms on the *MIF* promoter in suicide using the large sample size of 602 suicide completers and 728 controls.

Materials and methods

Subjects

This study was approved by the Ethical Committee for Genetic Studies of Kobe University Graduate School of Medicine. The study population consisted of 602 suicide completers (408 males: mean age ± standard deviation (SD), 51.0±17.4 years; 194 females: 50.7±18.8 years) and 728 unrelated healthy volunteers (340 males: mean age ± SD, 53.3±18.8 years; 388 females: 54.5±18.3 years). All subjects were ethnically Japanese.

Autopsies on 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 examination and the police investigation as required by Japanese law. The methods of suicide were neck hanging (399), jumping from heights (100), gas suffocation (29), drowning (16), jumping in front of a vehicle (9), drug overdose (8), self-inflicted penetrating wounds (8), self-burning (3), taking poison (2), other methods (10), and unknown (18). We subdivided suicide completers into violent suicide (neck 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 Dumais et al.[35] The healthy controls were recruited with consent after the purpose and procedures of the study were explained. The psychiatric assessment of each healthy participant was conducted as previously described.[36,37] Briefly, none of them manifested psychiatric problems in unstructured interviews using unnamed symptom checklists based on the *Diagnostic and Statistical Manual of Mental Disorders 4th edition* criteria by two psychiatrists. Demographics of suicide completers and healthy controls are shown in Table 1. Informed consent was obtained from all of the participants and from the families of the subjects used for postmortem blood experiments.

Blood and DNA sampling

Peripheral blood was obtained from all suicide completers and controls. Blood samples were stored at −80°C before use. DNA was extracted using the QIAamp DNA Blood Midi Kit (Qiagen Inc., Valencia, CA, USA). Each DNA sample was quantified and qualified using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Table 1 Demographics of suicide completers and healthy controls

Subjects	Suicide (n=602)	Control (n=728)
Mean age ± SD	50.9±17.9	54.0±18.5
n (male:female)	408:194	340:388
Suicide methods		
Neck hanging	399	
Jumping from heights	100	
Gas suffocation	29	
Drowning	16	
Jumping in front of vehicles	9	
Drug overdosing	8	
Self-inflicted penetrating wounds	8	
Self-burning	3	
Taking poison	2	
Other	10	
Unknown	18	

Abbreviation: SD, standard deviation.

Genotyping for the *MIF*-794CATT$_{5–8}$ repeat polymorphism (rs5844572)

Genomic DNA (100 ng) was subjected to polymerase chain reaction (PCR) for one cycle at 95°C for 10 minutes, followed by 40 cycles at 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds and final extension at 72°C for 7 minutes. The forward primer was 5′-TTG-CAC-CTA-TCA-GAG-ACC-3′ labeled with a carboxyfluorescein fluorescent dye, and the reverse primer was 5′-TCC-ACT-AAT-GGT-AAA-CTC-G-3′. The 25 µL PCR reaction volume contained 100 ng of genomic DNA, 12.5 µL of AmpliTaq Gold 360 Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) and 1.0 µL of forward and reverse primer. The amplification included an initial holding at 95°C for 10 minutes, followed by a three-step PCR program: 95°C for 30 seconds, 55°C for 30 seconds, and 40 cycles at 72°C for 30 seconds, and finally, 72°C for 7 minutes. The PCR product was appropriately diluted and supplemented with GeneScan 600 size standard (Applied Biosystems, Foster City, CA, USA). The mixture was then separated on a 50-cm polyacrylamide gel at 15,000 V for 45 minutes on an ABI 3130 capillary sequencer and analyzed with GeneMapper® 4.0 software (Applied Biosystems). The PCR product sizes were 207 bp, 211 bp, and 215 bp in length, and these corresponded to 5-, 6-, and 7-CATT repeats, respectively. 8-CATT repeats were not observed in this study.

Genotyping for the *MIF*-173G/C SNP (rs755622)

We selected the predesigned TaqMan SNP genotyping assays from the Applied Biosystems database (http://www.appliedbiosystems.com) for the *MIF*-173G/C SNP (rs755622). Genotyping was performed with a 7500 Real-Time PCR System (Applied Biosystems) according to the manufacturer's protocol.

Statistics

We used the Haploview version 4.2 software program (http://www.broad.mit.edu/mpg/haploview) to determine the Hardy–Weinberg equilibrium and allelic or haplotype frequencies for each of the *MIF* promoter polymorphisms.[38] The allele-, genotype-, or haplotype-based association analyses between the suicide and control groups were carried out with the χ^2 test, Cochran–Armitage trend test, or Fisher's exact test, as appropriate, using Haploview software, R version 3.1.0 (The R Foundation for Statistical Computing, 2014, Vienna, Austria) and Excel 2010 (Microsoft, Redmond, WA, USA) with the Statcel3 add-on (OMS, Saitama, Japan). The power analysis was performed with the PS v2.1.3.1 program.[39] Statistical significance for the two *MIF* polymorphisms study was defined as two-tailed $P<0.05/2=0.025$.

Results

MIF-794CATT$_{5–8}$ repeat and *MIF*-173G/C SNP analyses

No deviation from Hardy–Weinberg equilibrium was seen in suicide completers and healthy controls for both polymorphisms. The *MIF*-794CATT$_{5–8}$ repeat genotype and allele frequencies are shown in Table 2. Neither the genotype distribution nor the allelic frequency of the polymorphism was significantly associated with completed suicide. The *MIF*-173G/C SNP genotype and allele frequencies in suicide completers and healthy controls are shown in Table 3. Neither the genotype distribution nor the allelic frequency of the SNP was significantly associated with completed suicide. Subgroup analyses based on sex difference or methods of suicide (violent or nonviolent) also yielded no significant findings (Tables S1–S5).

MIF promoter haplotype analyses

Linkage disequilibrium between each CATT repeat and the −173C was measured as follows: CATT$_5$ and −173C: D' =0.495, r^2=0.043; CATT$_6$ and −173C: D' =0.871, r^2=0.170; CATT$_7$ and −173C: D' =0.984, r^2=0.678. The CATT$_7$ and −173C polymorphisms were observed in strong linkage disequilibrium in our samples. We then examined whether the different haplotypes of the *MIF*-794CATT$_{5–8}$ repeat and the *MIF*-173G/C SNP would show different distribution patterns in suicide completers compared with controls. The reconstructed frequencies of each possible haplotype based on the observed genotype data are shown

Table 2 Association of *MIF*-794CATT$_{5-8}$ repeat with suicide completers and controls

Polymorphism	Suicide (n=602)		Control (n=728)		*P*-value[a]	Global *P*-value[b]	OR (95% CI)	Power
	N	Frequency	N	Frequency				
***MIF*-794CATT$_{5-8}$**								
Genotype								
55	86	0.143	105	0.145	0.943	0.409	0.99 (0.73–1.35)	0.050
56	231	0.384	251	0.345	0.141		1.18 (0.95–1.48)	0.313
57	77	0.128	95	0.130	0.889		0.98 (0.71–1.35)	0.051
66	111	0.184	158	0.217	0.140		0.82 (0.62–1.07)	0.319
67	83	0.138	93	0.128	0.587		1.09 (0.79–1.50)	0.084
77	14	0.023	26	0.036	0.185		0.64 (0.33–1.24)	0.277
Allele								
CATT$_5$	480	0.399	556	0.382	0.376	0.645	1.07 (0.92–1.25)	0.146
CATT$_6$	536	0.445	660	0.453	0.675		0.97 (0.83–1.13)	0.070
CATT$_7$	188	0.156	240	0.165	0.544		0.94 (0.76–1.15)	0.096

Notes: [a]Genotypic *P*-values and allelic *P*-values were tested with the χ^2 test. [b]Global *P*-values were calculated by Fisher's exact test.
Abbreviations: MIF, macrophage migration inhibitory factor; OR, odds ratio; CI, confidence interval.

in Table 4. There were six haplotypes in this study, none of which showed different distributions between suicide completers and controls. Subgroup analyses based on sex difference or methods of suicide (violent or nonviolent) also showed no significance (Tables S1–S5).

Discussion

To our knowledge, this is the first study to investigate the association between suicide and two functional polymorphisms in the *MIF* gene promoter, which are known to affect the gene expression and protein levels of MIF. We also conducted subgroup analysis to investigate sex differences because males tend toward a higher suicide rate than females do among all age groups worldwide.[40] Furthermore, we determined differences between violent and nonviolent suicides in this study because lethality associated with violent methods is considerably higher than that associated with nonviolent methods.[41] Moreover, some previous studies showed differences between subjects with violent vs nonviolent methods of suicide in other polymorphisms (for the *COMT* and *BDNF* genes) related to completed suicide.[42,43] As a result, no significant differences were found in any of the analyses, which indicated that *MIF*-794CATT$_{5-8}$ repeat microsatellite and *MIF*-173G/C SNP are not associated with an increased risk of suicide in the Japanese population. Recent meta-analyses with MDD cases also failed to show the genetic involvement of *MIF*.[44,45] Therefore, *MIF* might not be primarily involved in genetic susceptibility to suicide (and depression).

The limitations of the present study should be considered. First, the current sample provides low powers based on the observed frequencies of two polymorphisms. The number of subjects in the association study may not have been large enough to detect a significant difference, although our sample size of suicide completers was one of the largest ever reported. In fact, most previous candidate gene analyses and genome-wide association studies for completed suicide could not overcome the statistical limitation of small samples[2,3,46] because it is extremely difficult to obtain tissue samples from suicide completers. Second, our study included only the

Table 3 Association of *MIF*-173 SNP with suicide completers and controls

Polymorphism	Suicide (n=602)		Control (n=728)		*P*-value[a]	Global *P*-value[b]	OR (95% CI)	Power
	N	Frequency	N	Frequency				
***MIF*-173G/C**								
Genotype								
GG	373	0.620	451	0.619	0.997	0.714	1.00 (0.83–1.21)	0.050
GC	203	0.337	237	0.326	0.653		1.05 (0.84–1.33)	0.071
CC	26	0.043	40	0.055	0.326		0.78 (0.47–1.29)	0.168
Allele								
G	949	0.788	1,139	0.782	0.711		0.97 (0.80–1.16)	0.066
C	255	0.212	317	0.218				

Notes: [a]Genotypic *P*-values and allelic *P*-values were tested with the χ^2 test. [b]Global *P*-value was tested with the Cochran–Armitage test for trend.
Abbreviations: MIF, macrophage migration inhibitory factor; OR, odds ratio; CI, confidence interval.

Table 4 Association of haplotypes of MIF promoter polymorphisms with suicide completers and controls

Haplotype	Suicide (n=602)		Control (n=728)		*P*-value[a]	Global *P*-value[b]	OR (95% CI)	Power
	N	Frequency	N	Frequency				
Haplotype								
MIFCATT$_5$-173G	427	0.355	496	0.341	0.442	0.631	1.06 (0.90–1.25)	0.118
MIFCATT$_6$-173G	522	0.433	641	0.440	0.716		0.97 (0.83–1.13)	0.065
MIFCATT$_7$-173G	4	0.003	1	0.001	0.129		4.14 (0.54–31.7)	0.229
MIFCATT$_5$-173C	53	0.044	60	0.041	0.746		1.06 (0.73–1.55)	0.068
MIFCATT$_6$-173C	14	0.012	19	0.013	0.802		0.92 (0.46–1.84)	0.055
MIFCATT$_7$-173C	184	0.153	239	0.164	0.426		0.92 (0.75–1.13)	0.096

Notes: [a]*P*-values were tested with the χ^2 test. [b]Global *P*-value was calculated by Fisher's exact test or Cochran–Armitage test for trend, as appropriate.
Abbreviations: MIF, macrophage migration inhibitory factor; OR, odds ratio; CI, confidence interval.

Japanese population. The distribution of *MIF* polymorphisms determined by us was similar to that determined in the previous study for Japanese population by Llamas-Covarrubias et al,[47] but not for Caucasian by Swanberg et al.[48] Given that the *MIF* polymorphisms studied here differ among Caucasians, Africans, and Asians,[49] our results might not be extrapolated to other populations. In addition, *MIF* protein levels in the brains of individuals displaying suicidal behavior should be further investigated to clarify the associations between MIF and suicide other than genetic susceptibility.

Conclusion

In conclusion, our results suggest that *MIF*-794CATT$_{5-8}$ microsatellite and *MIF*-173G/C SNP microsatellite might not contribute to the genetic risk of completed suicide in the Japanese population.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Association of MIF promoter polymorphisms with suicide completers and controls for males

Polymorphism	Male suicide (n=408)		Male control (n=340)		*P*-value[a]	Global *P*-value[b]	OR (95% CI)	Power
	N	Frequency	N	Frequency				
***MIF*-794CATT$_{5-8}$**								
Genotype								
55	56	0.137	49	0.144	0.788	0.584	0.945 (0.625–1.429)	0.059
56	159	0.390	119	0.350	0.263		1.186 (0.880–1.599)	0.193
57	53	0.130	44	0.129	0.984		1.004 (0.654–1.542)	0.050
66	77	0.189	77	0.226	0.204		0.795 (0.557–1.133)	0.256
67	53	0.130	38	0.112	0.450		1.187 (0.761–1.850)	0.108
77	10	0.025	13	0.038	0.279		0.632 (0.274–1.460)	0.194
Allele								
CATT$_5$	324	0.397	261	0.384	0.601	0.875	1.057 (0.858–1.303)	0.080
CATT$_6$	366	0.449	311	0.457	0.733		0.965 (0.787–1.184)	0.061
CATT$_7$	126	0.154	108	0.159	0.815		0.967 (0.731–1.280)	0.058
***MIF*-173G/C**								
Genotype								
GG	252	0.618	218	0.641	0.507	0.753	0.904 (0.671–1.218)	0.100
GC	136	0.333	102	0.300	0.330		1.167 (0.856–1.591)	0.154
CC	20	0.049	20	0.059	0.553		0.825 (0.436–1.560)	0.097
Allele								
G	640	0.784	538	0.791	0.747		0.960 (0.748–1.231)	0.062
C	176	0.216	142	0.209				
Haplotype								
MIFCATT$_5$-173G	283	0.347	234.1	0.344	0.920	0.769	1.011 (0.816–1.253)	0.052
MIFCATT$_6$-173G	357.4	0.438	300.6	0.442	0.875		0.984 (0.801–1.207)	0.053
MIFCATT$_7$-173G	2.1	0.003	0.1	0.000	0.208		17.54 (0.031–9.989)	0.000
MIFCATT$_5$-173C	41	0.050	26.9	0.040	0.320		1.284 (0.781–2.112)	0.132
MIFCATT$_6$-173C	8.6	0.011	10.4	0.015	0.413		0.686 (0.276–1.702)	0.111
MIFCATT$_7$-173C	123.9	0.152	107.9	0.159	0.715		0.949 (0.717–1.257)	0.067

Notes: [a]*P*-values were tested with the χ^2 test. [b]Global *P*-value was calculated by Fisher's exact test or Cochran–Armitage test for trend, as appropriate.
Abbreviations: MIF, macrophage migration inhibitory factor; OR, odds ratio; CI, confidence interval.

Table S2 Association of MIF promoter polymorphisms with suicide completers and controls for females

Polymorphism	Female suicide (n=194)		Female control (n=388)		*P*-value[a]	Global *P*-value[b]	OR (95% CI)	Power
	N	Frequency	N	Frequency				
***MIF*-794CATT$_{5-8}$**								
Genotype								
55	30	0.155	56	0.144	0.741	0.850	1.084 (0.670–1.755)	0.066
56	72	0.371	132	0.340	0.461		1.145 (0.799–1.639)	0.114
57	24	0.124	51	0.131	0.793		0.933 (0.555–1.568)	0.055
66	34	0.175	81	0.209	0.339		0.805 (0.517–1.255)	0.162
67	30	0.155	55	0.142	0.678		1.108 (0.684–1.794)	0.072
77	4	0.021	13	0.034	0.384		0.607 (0.195–1.888)	0.119
Allele								
CATT$_5$	156	0.402	295	0.380	0.470	0.762	1.096 (0.854–1.407)	0.112
CATT$_6$	170	0.438	349	0.450	0.708		0.954 (0.746–1.219)	0.067
CATT$_7$	62	0.160	132	0.170	0.656		0.928 (0.667–1.290)	0.070
***MIF*-173G/C**								
Genotype								
GG	121	0.624	233	0.601	0.589	0.390	1.103 (0.774–1.572)	0.082
GC	67	0.345	135	0.348	0.951		0.989 (0.688–1.420)	0.050
CC	6	0.031	20	0.052	0.256		0.587 (0.232–1.487)	0.196

(*Continued*)

Table S2 (*Continued*)

Polymorphism	Female suicide (n=194)		Female control (n=388)		*P*-value[a]	Global *P*-value[b]	OR (95% CI)	Power
	N	Frequency	N	Frequency				
Allele								
G	309	0.796	601	0.774	0.394		1.139 (0.845–1.536)	0.089
C	79	0.204	175	0.226				
Haplotype								
MIFCATT$_5$-173G	143.7	0.370	262.6	0.338	0.280	0.519	1.150 (0.892–1.483)	0.187
MIFCATT$_6$-173G	164.3	0.423	340.6	0.439	0.615		0.939 (0.734–1.201)	0.081
MIFCATT$_7$-173G	2.1	0.005	1.1	0.001	0.233		3.834 (0.380–38.62)	0.071
MIFCATT$_5$-173C	12.3	0.032	32.4	0.042	0.398		0.751 (0.385–1.465)	0.124
MIFCATT$_6$-173C	5.7	0.015	8.4	0.011	0.564		1.362 (0.467–3.975)	0.092
MIFCATT$_7$-173C	59.9	0.154	130.9	0.169	0.538		0.900 (0.645–1.256)	0.098

Notes: [a]*P*-values were tested with the χ^2 test. [b]Global *P*-value was calculated by Fisher's exact test or Cochran–Armitage test for trend, as appropriate.
Abbreviations: MIF, macrophage migration inhibitory factor; OR, odds ratio; CI, confidence interval.

Table S3 Association of MIF promoter polymorphisms with violent suicide completers and controls

Polymorphism	Violent suicide (n=568)		Control (n=728)		*P*-value[a]	Global *P*-value[b]	OR (95% CI)	Power
	N	Frequency	N	Frequency				
***MIF*-794CATT$_{5–8}$**								
Genotype								
55	82	0.144	105	0.144	0.995	0.313	1.001 (0.733–1.368)	0.050
56	220	0.387	251	0.345	0.114		1.201 (0.957–1.509)	0.329
57	70	0.123	95	0.130	0.697		0.937 (0.673–1.303)	0.066
66	105	0.185	158	0.217	0.153		0.818 (0.621–1.078)	0.309
67	79	0.139	93	0.128	0.551		1.103 (0.799–1.522)	0.088
77	12	0.021	26	0.036	0.122		0.583 (0.291–1.165)	0.387
Allele								
CATT$_5$	454	0.400	556	0.382	0.357	0.554	1.078 (0.919–1.263)	0.152
CATT$_6$	509	0.448	660	0.453	0.791		0.979 (0.838–1.144)	0.057
CATT$_7$	173	0.152	240	0.165	0.387		0.910 (0.736–1.126)	0.148
***MIF*-173G/C**								
Genotype								
GG	355	0.625	451	0.620	0.840	0.542	1.024 (0.816–1.284)	0.054
GC	190	0.335	237	0.326	0.734		1.041 (0.825–1.315)	0.063
CC	23	0.040	40	0.055	0.230		0.726 (0.429–1.227)	0.250
Allele								
G	900	0.792	1,139	0.782	0.539		1.061 (0.878–1.283)	0.094
C	236	0.208	317	0.218				
Haplotype								
MIFCATT$_5$-173G	406.8	0.358	496.7	0.341	0.369	0.547	1.077 (0.916–1.268)	0.145
MIFCATT$_6$-173G	494.8	0.436	640.9	0.440	0.812		0.981 (0.839–1.148)	0.055
MIFCATT$_7$-173G	4.1	0.004	1.2	0.001	0.113		4.391 (0.573–33.63)	0.057
MIFCATT$_5$-173C	47.2	0.042	59.3	0.041	0.915		1.021 (0.691–1.509)	0.052
MIFCATT$_6$-173C	14.2	0.013	19.1	0.013	0.900		0.952 (0.477–1.901)	0.050
MIFCATT$_7$-173C	168.9	0.149	238.8	0.164	0.286		0.890 (0.718–1.103)	0.184

Notes: [a]*P*-values were tested with the χ^2 test. [b]Global *P*-value was calculated by Fisher's exact test or Cochran–Armitage test for trend, as appropriate.
Abbreviations: MIF, macrophage migration inhibitory factor; OR, odds ratio; CI, confidence interval.

Table S4 Association of MIF promoter polymorphisms with nonviolent suicide completers and controls

Polymorphism	Nonviolent suicide (n=10)		Control (n=728)		*P*-value[a]	Global *P*-value[b]	OR (95% CI)	Power
	N	Frequency	N	Frequency				
***MIF*-794CATT$_{5-8}$**								
Genotype								
55	1	0.100	105	0.144	0.692	0.890	0.659 (0.083–5.258)	0.037
56	5	0.500	251	0.345	0.306		1.900 (0.545–6.626)	0.187
57	1	0.100	95	0.130	0.776		0.740 (0.093–5.910)	0.036
66	1	0.100	158	0.217	0.371		0.401 (0.050–3.188)	0.073
67	2	0.200	93	0.128	0.498		1.707 (0.357–8.161)	0.152
77	0	0.000	26	0.036	0.543		–	–
Allele								
CATT$_5$	8	0.400	556	0.382	0.868	1.000	1.079 (0.438–2.656)	0.055
CATT$_6$	9	0.450	660	0.453	0.977		0.987 (0.406–2.396)	0.050
CATT$_7$	3	0.150	240	0.165	0.859		0.894 (0.260–3.075)	0.045
***MIF*-173G/C**								
Genotype								
GG	6	0.600	451	0.620	0.840	0.852	0.921 (0.258–3.294)	0.054
GC	4	0.400	237	0.326	0.874		1.381 (0.386–4.941)	0.089
CC	0	0.000	40	0.055	0.953		–	–
Allele								
G	16	0.800	1,139	0.782	0.935		1.113 (0.370–3.353)	0.047
C	4	0.200	317	0.218				
Haplotype								
MIFCATT$_5$-173G	6.8	0.340	496.6	0.341	0.980	0.971	0.995 (0.392–2.526)	0.050
MIFCATT$_6$-173G	8.9	0.445	641.2	0.440	0.965		1.019 (0.419–2.476)	0.050
MIFCATT$_7$-173G	0.0	0.000	1.0	0.001	0.907		–	–
MIFCATT$_5$-173C	1.2	0.060	59.4	0.041	0.642		1.501 (0.233–9.675)	0.120
MIFCATT$_6$-173C	0.1	0.005	18.8	0.013	0.744		0.384 (0.001–195.1)	0.004
MIFCATT$_7$-173C	3.0	0.150	239.0	0.164	0.863		0.899 (0.261–3.090)	0.045

Notes: [a]*P*-values were tested with the χ^2 test. [b]Global *P*-value was calculated by Fisher's exact test or Cochran–Armitage test for trend, as appropriate.
Abbreviations: MIF, macrophage migration inhibitory factor; OR, odds ratio; CI, confidence interval.

Table S5 Association of MIF promoter polymorphisms with violent and nonviolent suicide completers

Polymorphism	Violent suicide (n=568)		Nonviolent suicide (n=10)		*P*-value[a]	Global *P*-value[b]	OR (95% CI)	Power
	N	Frequency	N	Frequency				
***MIF*-794CATT$_{5-8}$**								
Genotype								
55	82	0.144	1	0.100	0.692	0.940	1.519 (0.190–12.15)	0.032
56	220	0.387	5	0.500	0.469		0.632 (0.181–2.209)	0.134
57	70	0.123	1	0.100	0.824		1.265 (0.158–10.13)	0.036
66	105	0.185	1	0.100	0.492		2.041 (0.256–16.29)	0.031
67	79	0.139	2	0.200	0.582		0.646 (0.135–3.099)	0.139
77	12	0.021	0	0.000	0.642		–	
Allele								
CATT$_5$	454	0.400	8	0.400	0.998	1.000	0.999 (0.405–2.462)	0.050
CATT$_6$	509	0.448	9	0.450	0.986		0.992 (0.408–2.413)	0.050
CATT$_7$	173	0.152	3	0.150	0.978		1.018 (0.295–3.511)	0.049

(*Continued*)

Table S5 (*Continued*)

Polymorphism	Violent suicide (n=568)		Nonviolent suicide (n=10)		*P*-value[a]	Global *P*-value[b]	OR (95% CI)	Power
	N	Frequency	N	Frequency				
***MIF*-173G/C**								
Genotype								
GG	355	0.625	6	0.600	0.871	0.932	1.111 (0.310–3.982)	0.056
GC	190	0.335	4	0.400	0.664		0.754 (0.210–2.704)	0.085
CC	23	0.040	0	0.000	0.516		–	–
Allele								
G	900	0.792	16	0.800	0.933		0.953 (0.316–2.878)	0.048
C	236	0.208	4	0.200				
Haplotype								
$MIFCATT_5$-173G	406.0	0.357	6.8	0.340	0.858	0.973	1.080 (0.425–2.745)	0.050
$MIFCATT_6$-173G	494.6	0.435	8.9	0.445	0.932		0.962 (0.395–2.341)	0.051
$MIFCATT_7$-173G	4.3	0.004	0.0	0.000	0.800		–	–
$MIFCATT_5$-173C	48.0	0.042	1.2	0.060	0.666		0.691 (0.107–4.475)	0.121
$MIFCATT_6$-173C	14.4	0.013	0.1	0.005	0.760		2.555 (0.005–1.304)	0.003
$MIFCATT_7$-173C	168.7	0.149	3.0	0.150	0.988		0.988 (0.287–3.409)	0.051

Notes: [a]*P*-values were tested with the χ^2 test. [b]Global *P*-value was calculated by Fisher's exact test or Cochran–Armitage test for trend, as appropriate.
Abbreviations: MIF, macrophage migration inhibitory factor; OR, odds ratio; CI, confidence interval.