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# Complement factor H and susceptibility to major depressive disorder in Han Chinese

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**Background**
Accumulating evidence suggests that altered immunity contributes to the development of major depressive disorder (MDD).

**Aims**
To examine whether complement factor H (CFH), a regulator of activation of the alternative pathway of the complement cascade, confers susceptibility to MDD.

**Method**
Expression analyses were tested in 53 unmedicated people with MDD and 55 healthy controls. A two-stage genetic association analysis was performed in 3323 Han Chinese with or without MDD. Potential associations between *CFH* single nucleotide polymorphisms and age at MDD onset were evaluated.

**Results**
*CFH* levels were significantly lower in the MDD group at both protein and mRNA levels ($P=0.009$ and $P=0.014$ respectively). A regulatory variant in the *CFH* gene, rs1061170, showed statistically significant genotypic and allelic differences between the MDD and control groups (genotypic $P=0.0005$, allelic $P=0.0001$). Kaplan–Meier survival analysis showed that age at onset of MDD was significantly associated with the *C* allele of rs1061170 (log rank statistic $\chi^2=6.82$, $P=0.009$). The *C*-allele carriers had a younger age at onset of MDD (22.2 years, s.d. = 4.0) than those without the *C* allele (23.6 years, s.d. = 4.3).

**Conclusions**
*CFH* is likely to play an important role in the development of MDD. rs1061170 has an important effect on age at onset of MDD in Han Chinese and may therefore be related to early pathogenesis of MDD, although further study is needed.

**Declaration of interest**
None.



Major depressive disorder (MDD) is a common, chronic and complex mood disorder, with a high lifelong morbidity, a high suicide rate and heavy economic burdens. MDD's prevalence among Han Chinese populations is estimated to be in excess of 17%.[1] Given this relatively common level of occurrence, understanding the aetiology and pathogenesis of MDD is among the most urgent challenges in current psychiatric research. Growing evidence from family, twin and adoption studies clearly supports the involvement of a genetic component in the development of MDD, which is unsurprising given that this disorder is characterised by a high degree of heritability.[2] Like many complex and polygenic orders, however, many genes are likely involved in forming the risk factors for MDD, so elucidating its exact genetic mechanism has eluded current research efforts. Epidemiological data support the psychosomatic hypothesis that MDD is associated with an increased risk of type 2 diabetes, essential hypertension and atherosclerotic cardiovascular disease.[3–5] One emerging hypothesis for these associations is that chronic low-grade activation of inflammation and the immune system likely plays an active role in the pathophysiology of MDD.[6] Clinical observations indicated that elevated pro-inflammatory cytokines and other inflammation-related proteins are usually seen in plasma and cerebrospinal fluid in people with MDD.[7] Our recent study using whole-genome cRNA microarrays observed overexpressed genes in the immune pathway in patients with MDD who were drug-naive, which provided evidence supporting a potential role of immune dysfunction in the pathophysiology of MDD.[8]

Compared with healthy individuals, those with acute MDD exhibit significantly elevated levels of complement component 3 (C3) and 4 (C4).[9] Likewise, the complement system is known to play a critical role in innate and adaptive immune functions. Among the complement members, C3 and C4 contribute to nearly all physiological activities and activated pathways as key complement members and host defence proteins.[10] In the complement metabolism of C3 and C4, complement factor H (CFH) acts as a regulator of activation of the alternative pathway of the complement cascade.[11] Abnormalities in the structure or function of CFH can accordingly unbalance the normal homeostasis of the complement system, resulting in 'bystander' damage to healthy tissues.[12,13] At the molecular level, a recent two-stage genome-wide association study (GWAS) identified that genetic variation within the gene encoding CFH (*CFH*) influences the levels of C3 and C4 in Han Chinese.[14] The *CFH* gene is located on chromosome 1q31, a putative genetic linkage region to mood disorders.[15,16] Recent meta-analyses have demonstrated that the *CFH* gene is a major risk factor for the development of age-related macular degeneration (AMD),[17] which has a high comorbidity with MDD.[18,19] Accordingly, the above findings provided converging evidence that *CFH* may be a promising candidate gene associated with MDD. To shed light on the role of *CFH* and its potentially associative susceptibility to MDD, we first performed an expression study to examine the association between *CFH* expression and MDD. Taking *CFH*'s role in C3, C4 and alternative pathway complement activity into consideration,[11] we also investigated their expression in patients with MDD. Second, we sought to characterise the association between genetic variations within *CFH* and the risk of developing MDD within Han Chinese populations from Eastern China.

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## Method

### Participants

A total of 53 unmedicated patients with MDD and 55 age- and gender-matched healthy controls were recruited for the expression analyses. Demographic data on age, gender, smoking status, alcoholic misuse, duration of illness prior to admission, number of episodes and family history of mood disorders were collected. Assessments of the Hamilton Rating Scale for Depression (HRSD-17)[20] were conducted independently by two experienced psychiatrists (interrater reliability, kappa ($\kappa$) = 0.84).[21] Clinical data are provided in online Table DS1.

For the genetic association study, two independent Han Chinese sample sets from Eastern China were recruited. In sample-set 1, 1012 people with MDD (386 men and 626 women, age 33.9 years, s.d. = 13.1) were recruited from our previous clinical trials – the OPERATION (OPtimized trEatment stRAtegies for Treatment-resIstant depressiON) study and the CARE-SSD/MDD (Construct An Rough Evaluation index system for subsyndromal symptomatic depression and major depressive disorder) study.[22,23] Individuals who participated in the expression study were not included in the genetic association study. For the control group, 1196 gender- and age-matched healthy individuals (507 males and 689 females, age 34.3 years, s.d. = 12.9) were enrolled from the hospital staff and students of the Shanghai Jiao Tong University School of Medicine. They were interviewed by a specialised psychiatrist using the Structured Clinical Interview for DSM–IV–TR Axis I Disorders (SCID-P) for exclusion of psychiatric disorders.[24] Any individuals in the control group with any chronic physical disease were also excluded from our analysis, based on their recent annual physical examination records.

Individuals in sample-set 2 were composed of 542 people with MDD (195 men and 347 women, age 32.6 years, s.d. = 9.4) and 573 controls (214 men and 359 women, age 31.9 years, s.d. = 8.6). The patients were recruited from the Tongde Hospital of Zhejiang Province and Hangzhou Seventh People's Hospital. The controls were randomly selected from the general populations in Hangzhou. All participants were unrelated through at least three generations and had self-reported Han nationality. All patients were diagnosed with MDD according to DSM-IV[25] criteria. Standard diagnostic assessments were supplemented with clinical information obtained by a review of medical records and interviews with family informants. The age at onset of MDD was evaluated retrospectively by patient self-report and was defined as the age at which the first manifestation of MDD occurred.

The procedures were reviewed and approved by the institutional review boards of the Shanghai Mental Health Center and other participating institutions. This study was performed in accordance with the guidelines laid out in the Declaration of Helsinki as revised in 1989. All participants provided written informed consent before any study-related procedures were performed.

### Plasma collection and RNA preparation

On admission, 20 mL peripheral blood of fasting patients and healthy controls were collected between 07.00 and 09.00 h, to avoid potential diurnal influence. Plasma samples were separated from 10 mL peripheral blood and centrifuged at 3500 rpm at 4°C for 20 min. All plasma samples were then frozen to −80°C. Total RNA was extracted from 10 mL peripheral blood samples using the QIAamp RNA blood Mini Kit (Qiagen, Chatsworth, California, USA) and then treated with DNase (Qiagen). The complementary DNA (cDNA) was synthesised by incubating DNase-treated total RNA with omniscript reverse transcription reagents (Qiagen) and a random primer according to the manufacturer's instruction.

### Analysis of plasma levels

C3 and C4 were determined by laser nephelometry (Nefelometer, BN II System, Dade Behring, Liederbach, Germany). Alternative pathway complement activity was evaluated using the Wieslab complement alternative pathway assay kit (Wieslab AB, Lund, Sweden). CFH was measured using CFH IgG ELISA kit (KA 1477, Abnova, Heidelberg, Germany). Each plasma sample was measured in duplicate according to the manufacturer's protocols. Several sample measurements were replicated to confirm reproducibility of the assay and the interassay coefficient of variation was 3.87%. Researchers were masked to the diagnosis of all participants.

### Quantitative real-time polymerase chain reaction (qRT-PCR)

Relative *CFH* mRNA expression levels were assessed by qRT-PCR with commercially available TaqMan gene expression assays for the target gene *CFH* and glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*) as the reference gene (Applied Biosystems, California, USA). All experiments were conducted in optical 384-well reaction microtiter plates on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). PCR was performed in a total volume of 10 µL containing 1 × TaqMan Universal Master Mix with AmpErase UNG, 1 × Assay Mix (Applied Biosystems) and complementary DNA template at cycle conditions: 95°C for 15 min, followed by 40 cycles at 95°C for 15 s and 60°C for 60 s. All reactions were run in triplicate. In each sample, the expression of *CFH* was normalised to the expression of the reference gene *GAPDH*. Results were reported in fold change using $2^{-\Delta\Delta t}$.

### Single nucleotide polymorphisms (SNPs) selection

In our recent study,[26] we performed an extensive analysis for SNPs in *CFH* and selected a total of 11 SNPs with 80% coverage of the gene. We genotyped all these SNPs in this study, including nine tagging SNPs (rs800292, rs10801555, rs10922096, rrs10733086, rs10737680, rs11582939, s2019727, rs1410996, rs426736) from the 5′ to 3′ regions of *CFH* that were selected from phase 2 of the HapMap project (http://hapmap.ncbi.nlm.nih.gov/) using the Tagger algorithm with an $r^2$ cut-off of 0.8 (minor allele frequency (MAF) > 0.05), and two important functional variants rs1061170 (p.Y402H) and rs460184 (p.V1197A) that were previously reported to be associated with AMD and other human diseases.[27,28] Detailed information for these selected SNPs is shown in online Table DS2.

### Genotyping

Genomic DNA was isolated from whole blood using a Tiangen DNA isolation kit (Tiangen Biotech, Beijing, China). The 11 SNPs were detected using multiplex PCR and the SNaPshot assay, as described in our previous study.[26] In brief, multiplex PCR (mPCR) mL reaction solution containing 4–20 ng template DNA, 0.4 mM dNTPs, 0.2–0.5 µM of each primer (online Table DS3), 2.0 mM $MgCl_2$ and 1.0 U of Faststart Taq polymerase (Roche). The thermal amplification programme consisted of one denaturation cycle at 94°C for 3 min, 40 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C



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for 1 min and finally incubated at 4°C. The mPCR products were cleaned up by using 1.0 U of shrimp alkaline phosphatase (SAP) and 0.5 U of Exonuclease I (TaKaRa Biotechnology, Dalian, China) at 37°C for 40 min, followed by incubation at 90°C for 10 min to deactivate the enzyme. The single-base extension reaction was performed in a total volume of 10 μL reaction solution, which contains 4 μL of the above-treated PCR products, 5 μL SNaPshot Multiplex Ready Reaction Mix and 0.4–0.8 μM pooled SNP-specific extension primers (Table DS3) according to the protocol of the ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems). The thermal cycling programme for single-base extension contained 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 30 s. The SBE products were treated by SAP (1.0 U) at 37°C for 40 min, followed by a heat inactivation at 75°C for 20 min. We loaded 1–4 μL of products, 6–9 μL of Hi-Di formamide and 0.5 μL of GeneScan 120 LIZ size standard (Applied Biosystems) for capillary electrophoresis on ABI PRISM 3730xl DNA analyzer (Applied Biosystems). GeneMarker software[29] was used to read the genotyping results.

## Statistical analysis

Demographic data were analysed using chi-squared or *t*-test as appropriate. For expression analyses, ANCOVA was carried out with age, gender and smoking status as covariates controlled in the model, to minimise the potential effect of these factors on the expression levels of CFH, C3/C4 and alternative pathway activity. Hardy–Weinberg equilibrium, allele, genotype and haplotype comparisons between case and control groups for all SNPs were performed using SPSS 17.0 and/or PLINK software. Unconditional logistic regression models were used to obtain maximum likelihood estimates of the odds ratios (ORs) and their 95% confidence intervals. The pairwise linkage disequilibrium analysis for all pairs of SNPs was applied to detect the intermarker relationship in case–control samples. The linkage disequilibrium blocks were identified using the solid spine of linkage disequilibrium method, with extended spine if $D' > 0.5$ in Haploview (version 4.1). The association between age at onset and candidate polymorphism was evaluated using the Kaplan–Meier method and the log-rank test for analyses of survival. Power analysis was performed using Quanto 1.2.3 (http://biostats.usc.edu/software). To correct for multiple testing for each SNP using the Bonferroni method, correct *P*-values were set at a raw *P*-value multiplied by *k* (independent significance tests). Criterion for statistical significance was set at $\alpha = 0.05$ and all values were two-tailed.

# Results

## Expression analyses

We observed significant increases in C3 and C4 expression levels (2735.3 (s.d. = 964.8) pg/mL *v.* 2225.1 (s.d. = 726.3) pg/mL, $P = 0.004$ and 1619.3 (s.d. = 754.3) pg/mL *v.* 1352.5 (s.d. = 503.4) pg/mL, $P = 0.016$ respectively) and a marginally increase in alternative pathway complement activity (82.1% (s.d. = 20.3) *v.* 73.9% (s.d. = 21.8), $P = 0.078$) in the individuals with MDD compared with the healthy controls (online Table DS4). CFH levels were significantly lower in the participants with MDD at both protein and mRNA levels (415.9 (s.d. = 157.6) AU/mL *v.* 485.0 (s.d. = 112.5) AU/mL, $P = 0.009$ and 2.75 (s.d. = 1.12) *v.* 3.37 (s.d. = 1.19), $P = 0.014$ respectively, Fig. 1), and their statistical power was 49.0% and 69.5% respectively. The qRT-PCR experiment showed that there was no significant difference in *GAPDH* mRNA expression between the MDD and control groups (online Fig. DS1).

## Genetic association study

To investigate the genetic association of *CFH* with MDD, a two-stage genetic association analysis was performed in 3323 Han Chinese with or without MDD. In the stage 1 study, we detected allele and genotype frequencies of the 11 SNPs within *CFH* in 1012 individuals in the MDD group and 1196 individuals in the control group in sample-set 1. No deviation from the Hardy–Weinberg equilibrium was observed in the genotype distribution of any of the SNPs studied, except for rs460184 and we excluded this SNP from the following study. The genotype and allele frequencies of these *CFH* SNPs in sample-set 1 are listed in online Table DS5. The allelic and genotypic frequencies of rs1061170 showed statistically significant differences between the MDD and control groups (genotypic $P = 0.004$; allelic $P = 0.001$, OR = 1.50, 95% CI 1.17–1.92), when the unconditional logistic regression model was adjusted for age and gender. The association of rs1061170 with MDD could be validated in the stage 2 sample (genotypic $P = 0.08$; allelic $P = 0.03$, OR = 1.70, 95% CI 1.19–2.42), albeit the sample size was smaller than in the stage 1 sample. When the two independent samples were combined (Table 1), rs1061170 showed an even stronger association, which survived correction for multiple testing (genotypic $P = 0.0005$; allelic $P = 0.0001$, OR = 1.56, 95% CI 1.28–1.91). On the basis of the genotype data, the statistical power of the rs1061170 polymorphism was 97%

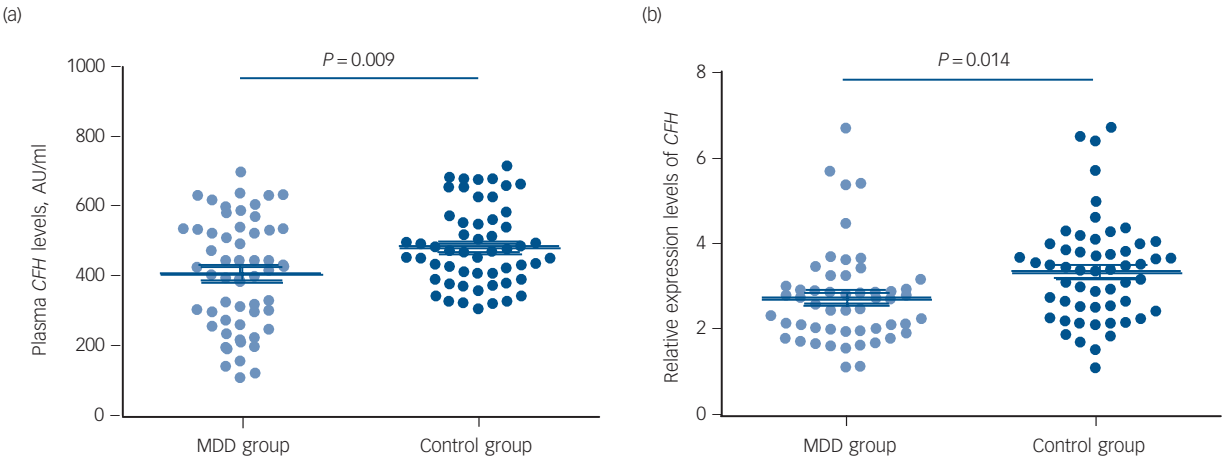


**Fig. 1** Expression levels of *CFH* protein and mRNA in peripheral blood in unmedicated patients with major depressive disorder (MDD, $n = 53$) and healthy controls ($n = 55$).

(a) Protein expression; (b) mRNA expression. *CFH* mRNA was normalised to that of *GAPDH*. Scatter plots for the comparison between the MDD and control groups. Horizontal lines represent the mean *CFH* expression levels.



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**Table 1** Association between rs1061170 and major depresive disorder (MDD) in total samples

| Test, sample | Genotype, *n* (%) | | | | | Allele, *n* (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *C/C* | *C/T* | *T/T* | $P^a$ | $P^b$ | *C* | *T* | $P^a$ | $P^b$ | OR (95%CI) |
| Stage 1 | | | | | | | | | | |
| MDD group | 6 (0.6) | 139 (13.7) | 867 (85.7) | 0.004 | 0.04 | 151 (7.5) | 1873 (92.5) | 0.001 | 0.01 | 1.50 (1.17–1.92) |
| Control group | 2 (0.2) | 118 (9.9) | 1076 (90.0) | | | 122 (5.1) | 2270 (94.9) | | | |
| Stage 2 | | | | | | | | | | |
| MDD group | 2 (0.4) | 80 (14.8) | 460 (84.9) | 0.008 | 0.08 | 84 (7.7) | 1000 (92.3) | 0.003 | 0.03 | 1.70 (1.19–2.42) |
| Control group | 0 (0.0) | 54 (9.4) | 519 (90.6) | | | 54 (4.7) | 1092 (95.3) | | | |
| Combined | | | | | | | | | | |
| MDD group | 8 (0.5) | 219 (14.1) | 1327 (85.4) | 0.00005 | 0.0005 | 235 (7.6) | 2873 (92.4) | 0.00001 | 0.0001 | 1.56 (1.28–1.91) |
| Control group | 2 (0.1) | 172 (9.7) | 1595 (90.2) | | | 176 (5.0) | 3362 (95.0) | | | |

a. Raw *P*-values.
b. *P*-values adjusted for Bonferroni correction.

($\alpha = 0.05$) for our samples under the assumption of a moderate effect size and an additive model.

The estimation of linkage disequilibrium for all pairs of SNP markers showed three linkage disequilibrium blocks (online Fig. DS2). The overall haplotype covering these SNPs showed positive associations with MDD (global $P = 1.1 \times 10^6$). Sliding window analysis showed that haplotypes covering any SNP presented some significant associations with MDD, and the block 1 region around variant rs1061170 showed a similar trend of association under 2 SNP and 3 SNP windows (online Table DS6).

## Effect of rs1061170 on CFH mRNA expression level

To investigate the effect of MDD-related variant rs1061170 on *CFH* expression level, we performed expression Quantitative Trait Loci (eQTL) analysis using the brain eQTL database (http://caprica.genetics.kcl.ac.uk/BRAINEAC/), a large exon-specific eQTL dataset covering ten human brain regions.[30] For the effect of rs1061170 on CFH serum level, we used an earlier GWAS of plasma CFH levels.[31] As shown in Fig. 2, the risk allele *C* of rs1061170 (402H) was associated with a lower CFH level in the inferior olivary nucleus and occipital cortex. In addition, the GWAS data showed that the rs1061170 risk allele was significantly associated with a lower CFH plasma level.[31] As our results showed a significant decrease in the CFH serum level in the MDD group, this suggests that the risk allele of rs1061170 may lead to lower CFH levels, which may confer susceptibility to MDD.

## CFH and age at onset in the MDD group

The aforementioned results indicated a significant association between the rs1061170 polymorphism and MDD. We further opted to evaluate the role of rs1061170 in the age at onset of MDD given evidence of an effect of this SNP on gene function. The data for age at onset were captured in 467 patients with MDD from the OPERATION study. Kaplan–Meier survival analysis (Fig. 3) showed that the age at onset in MDD was significantly associated with the *C* allele of rs1061170 (log-rank statistic $\chi^2 = 6.82$, $P = 0.009$, after adjusted for gender). The *C*-allele

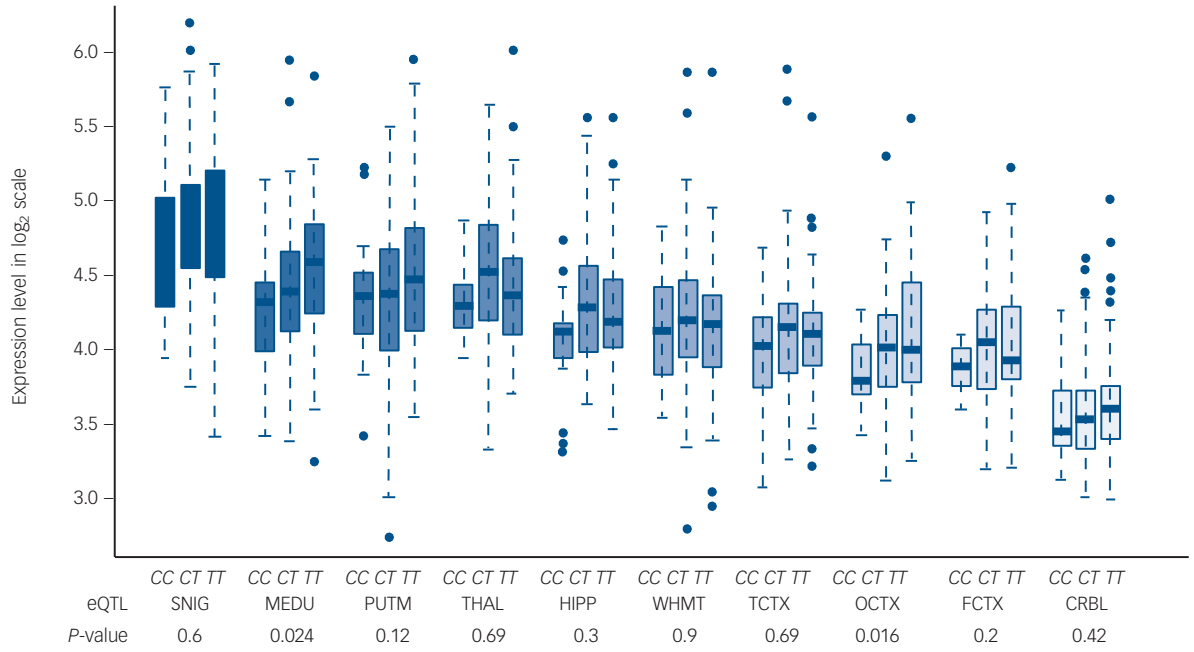


**Fig. 2** Association of rs1061170 with *CFH* mRNA expression level in ten brain regions (t2373336: at transcript-level).

Positive associations were observed for two brain regions (inferior olivary nucleus and occipital cortex). The *P*-value was 0.049 for the expression profile across all the tissues. Genotype counts: *CC* 17, *CT* 67, *TT* 50; allele frequency *C* 37.7%, *T* 62.3%. Whiskers represent standard error. Dashed lines represent mean expression level. eQTL, expression quantitative trait loci; SNIG, substantia nigra; MEDU, the inferior olivary nucleus (sub-dissected from the medulla); PUTM, putamen (at the level of the anterior commissure); THAL, thalamus (at the level of the lateral geniculate nucleus); HIPP, hippocampus; WHMT, intralobular white matter; TCTX, temporal cortex; OCTX, occipital cortex; FCTX, frontal cortex; CRBL, cerebellar cortex.



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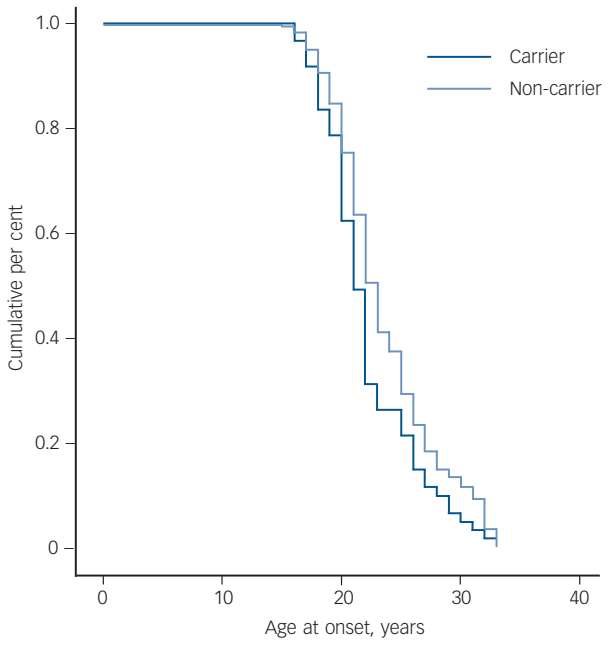


**Fig. 3** Correlation between age at onset and the *CFH* rs1061170 polymorphism in the major depressive disorder (MDD) group.

Kaplan–Meier survival curves, describing lifetime distributions of age at onset in the patients affected by MDD (cumulative proportion of those remaining well until the first disease episode), as a function of *CFH* rs1061170 polymorphism allele (log-rank statistic $\chi^2 = 6.82$, $P = 0.009$). The dark blue line represents the *C*-allele carriers ($n = 61$) and light blue line the non-*C*-allele carriers ($n = 406$).

carriers had a younger age at onset of MDD (22.2 years, s.d. = 4.0) than those without the *C* allele (23.6 years, s.d. = 4.3).

## Discussion

In line with previous reports,[9] our results showed significant changes of alternative pathway complement activity, C3 and C4 in people with MDD. This suggested that complement dysfunctions may be implicated in the aetiology of MDD. At the protein and mRNA levels, *CFH* expressions were downregulated in these patients, implying that *CFH* possibly regulates the complement system and confers susceptibility to MDD. Therefore, we investigated whether genetic variations in the *CFH* gene are associated with MDD in Han Chinese populations. One SNP, rs1061170, was found to have a significant association with MDD. Given that MDD is a clinical syndrome notable for the heterogeneity of its clinical presentation and genetics, many genes may likely alter susceptibility to specific clinical traits rather than its diagnosis.[32] On that supposition, we further tested whether the rs1061170 polymorphism may influence the age at onset of MDD, because age at onset is considered a key indicator for a more heritable form of MDD, is crucial in delineating disorder subtypes and may greatly help in the identification of genetic vulnerability or susceptibility for MDD.[33] Intriguingly, we found significantly younger median ages at onset among the individuals with the *C* allele than those without the *C* allele, suggesting an important role of the rs1061170 polymorphism in the age at onset of MDD. To the best of our knowledge, this is the first study to provide evidence for the genetic association between *CFH* and age at onset of MDD.

The rs1061170 polymorphism is a non-synonymous SNP and is of particular interest because it is located within the region of short consensus repeat (SCR) domains 7 binding heparin and C-reactive protein.[34] The base transition of thymine to cytosine occurs in the exon 9 of the gene and leads to a tyrosine–histidine substitution in the protein.[35] Previous studies demonstrated that this variant exerts allelic differences on the binding affinity to C-reactive proteins, with the risk allele showing reduced affinity.[36] In doing so, this could influence complement activation, host immune status and the inflammation process, and hence account for approximately 17% of AMD liability.[37] Moreover, our findings showed that rs1061170 genotypes may affect the expression level of CFH not only in blood but also in brain, such as the occipital cortex. The rs1061170 polymorphism is therefore considered a functional SNP in *CFH*. Although neuroimaging evidence has arisen for the involvement of the occipital cortex in the aetiology of MDD,[38] the exact role of the rs1061170 polymorphism in the brain region is currently unknown. This needs to be clarified in further investigations.

The observed earlier age at onset in people with MDD carrying the *CFH* risk allele is consistent with recently published studies showing that mutations in this gene can lead to an earlier age at onset of immune-related disorders, such as AMD,[37] systemic lupus erythematosus[39] and haemolytic uraemic syndrome.[40] These observations imply that a compromised CFH function may lead to an earlier age at onset of immune-related disorders. CFH is a member of the regulators of complement activation family and is a complement control protein, which regulates the complement-mediated immune system involved in microbial defence, immune complex processing and programmed cell death.[41] It has been reported that early-onset MDD is associated with indices for immune dysfunction, including reduced natural killer cell activity and cell number, suggesting that immunological alternations may be more sensitive to detection in early-onset MDD.[42] Meanwhile, evidence is accumulating from ascendant and descendant family studies that early-onset MDD may be associated with greater familial risk to relatives, and therefore early-onset MDD could be an important variable in identifying some forms of MDD that are more genetically homogeneous or may carry some degree of relative increased genetic loading or phenotypic expression.[43] Considering the importance of age at onset in the immunology and genetics underlying the pathophysiological mechanism of MDD, we assume that patients with MDD with an early age at onset may be a valuable sample to clarify the contribution of the *CFH* gene to MDD incidence. However, this assumption needs to be tested by future studies.

### Limitations

One strength of this study was the use of a clinically well-defined and characterised sample of people with MDD and without any other comorbid psychiatric or physical conditions. When interpreting the results of our study, however, we would be remiss in not noting some limitations. First, the control group in sample-set 1 were recruited from hospital staff and students of the Shanghai Jiao Tong University School of Medicine in Shanghai, even though they were psychiatrically screened for mental disorder, the samples may not be representative of the general population. Second, although we have examined two independent samples in this study (including 3323 individuals), the sample size was still comparatively modest. This modest sample size precludes us from making any definitive statements on the associations between *CFH* and MDD in Han Chinese. Accordingly, our findings should be considered only preliminary, requiring further investigations to validate them in independent populations and more fully explain any potential relationship or lack thereof. Third, cross-sectional association analyses always have the potential for population


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stratification. Although the participants were all of Han Chinese origin and collected from Eastern China, we could not completely exclude the possibility of a population structure effect in our sample. Finally, the occurrence of MDD – being a polygenic disorder – is widely known to depend on the interaction of multiple factors.[32] Usually no single gene is responsible for this disorder, and the methods used in individual studies may have limited power to detect what may be a potentially small effect.

## Implications

In conclusion, our study provides first evidence that *CFH* is likely to play an important role in the development of MDD. Among its SNPs, the well-known AMD risk SNP rs1061170, a regulatory variant in *CFH*, has an important effect on age at onset of MDD, and therefore may be related to an early pathogenesis of MDD. Our findings may represent a significant genetic clue to the pathogenesis of MDD. Further genetic studies with large-scale early-onset MDD samples are required to clarify the exact role of *CFH* in the development of MDD.

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EXTRA CONTENT ONLINE

psychiatry in history

## An 18th-century view of demonomania. 2: Vampirism – explanation

**Fiona Subotsky**

Martinus Martini considers that a supernatural explanation is an easy way out for complex phenomena, and develops more naturalistic explanations. First, the symptoms of a vampire attack are 'sudden awakening, anxiety, oppression of the chest, difficulty breathing, a sensation of suffocation, and terror . . . Shortly afterwards they breathe their last.' He considers this very similar to a nightmare except for the fatal result, and wonders why people imagine that they have been attacked by the dead. His explanation is that:

> When a rumour of vampire attack spreads to the crowd . . . the terrifying idea occupies people's minds the whole day, because they fear immediate death . . . And when such a highly fixed idea is manifest in sleep, a situation in which imagination alone thrives, all senses are silent and are inactive, and all movements of the body are suspended with the exception of the vital powers, so it must be as vivid as that which is produced by sensations. This agreed, it is not inappropriate for people to say that they have seen this or that dead person and to have been oppressed by them, while at the same time pressure of the chest and constriction of the throat by muscles contracted in spasm take hold.

Next has to be considered why people die. The presence of acute disease is likely, and added to this are the effects of fear:

> a man suddenly with less will, a painful sensation as if the chest is compressed, palpitation of the heart, great difficulty in respiration and the whole body is convulsed and grows pale because of spasms of the muscles. In the greatest terror all these things are increased.

Then there is the question of why the bodies do not decompose: Martini points out that the environment of the grave affects the rate of decomposition, which is likely to be slowed if the surroundings are dry, cold or nitrous. Decomposition is more likely if the body itself is moist rather than dry: 'And so the bodies of scurvy sufferers decay sooner than those with typhoid, and those who have suffered with putrid fever [typhus], than those who have died from the emaciation of old age'.

Why do the hair and nails grow?

> Many doctors are united in the opinion that this is either a fallacy of the senses, because . . . as the smallest vessels of the superficial body collapse, the flesh shrinks . . . and so the roots of nails and hair become more prominent and look longer. Or, such dead are buried with longer hair, about which at the time nobody was concerned.

I have abbreviated Martini's comments, but, as he says: 'Let this brief contemplation suffice for the more noble mind, so that it may condemn the superstition about Vampires as chimeras'.

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