An Insertion Variant in *CRH* Confers an Increased Risk of Central Serous Chorioretinopathy

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Purpose. To identify a novel corticotropin-releasing hormone (CRH) gene variant relevant in patients with central serous chorioretinopathy (CSC).

Methods. We performed a genetic study of CSC in families and sporadic cases with controls. Using whole-exome sequencing and linkage analysis, we identified a heterozygous insertion variant, Gln52insPro, in the CRH gene that cosegregated in two Chinese families with CSC. This variant was evaluated among an additional 1307 patients with CSC and 1438 ethnicity-matched control individuals from three independent Chinese cohorts.

Results. The CRH variant was strongly associated with CSC in these cohorts of Chinese patients ($P_{meta} = 1.24 \times 10^{-11}$; odds ratio, 3.01; 95% confidence interval, 2.15–4.21). The risk variant Gln52insPro decreased CRH gene expression.

Conclusions. Our results implicate the hypothalamic–pituitary–adrenal stress response system in the pathogenesis of CSC and provide a novel rationale for therapeutic intervention.

Keywords: central serous chorioretinopathy (CSC), corticotropin-releasing hormone (CRH), stress, choroidal vasculopathy, pathogenesis

Central serous chorioretinopathy (CSC), an eye disease that causes blurred and distorted central vision, is one of the most common vision-threatening retinopathies.[1,2] CSC is characterized by the accumulation of transparent fluid at the posterior pole of the fundus. Currently, the application of retinal imaging, particularly indocyanine green angiography, provides a better understanding of the pathophysiology of CSC: CSC is primarily an exudative choroidal vasculopathy. It is widely accepted that a congested choriocapillaris, dilated choroidal vessels,[3] and diffuse choroidal vasculature hyperpermeability increase the choroidal hydrostatic pressure, leading to a breakdown of the retinal pigment epithelium barrier with subsequent leakage of fluid from the choroid into the subretinal space.

CSC has race and sex predispositions. The incidence rate of CSC is 21 cases (27 for male and 15 for female individuals) per 100,000 person-years in the Chinese population[4] and is 5.78 (95% confidence interval [CI], 4.44–7.11; 9.9 in men, and 1.7 in women) per 100,000 in Caucasians in two large population-based studies.[5] However, the underlying reason for this race and sex predisposition, with a higher incidence in Chinese and male individuals and a lower occurrence in Caucasians and female individuals, is unknown.

The pathogenesis of CSC remains incompletely understood. Clinically, patients with CSC have frequently had a preceding stressful event.[2,6] CSC has also been associated with imbalanced corticosteroid levels. Increased levels of catecholamines have been associated with CSC. In animal studies, CSC can be induced by injections of norepinephrine and corticosteroids.[2] Genetic background may predispose to CSC.[7] Complement factor H,[8,9] complement component C4B,[10] cadherin 5,[11] the TNF receptor superfamily 10A[12] and GATA binding protein 5[13] are associated with CSC. However, none of these genetic studies used a large sample size or multicenter cohorts. Complement factor H can bind and interact with adrenomedullin, and the latter affects choroidal blood flow and increases microvascular permeability,[9] while cadherin 5 plays a role in endothelial cell biology by controlling the cohesion and organization of intercellular junctions.[11] The TNF receptor superfamily 10A is involved in angiogenic processes,[12] and GATA may affect the susceptibility to CSC through vascular endothelial dysfunction in the

choriocapillaris.[13] Most reported genes are associated with the complement system, intercellular junctions, or vascular permeability. Genes associated with stress or hormone levels are rarely reported and deserve further exploration. To date, no specific genotype reported to be associated with CSC can model or definitively recognize the cause of the disease.

Although several candidate genes have been reported to be associated with CSC in different populations, a large-scale Chinese cohort is still lacking. However, few CSC familial cases have been reported to date, and the inheritance mode of CSC remains unclear.[14] Therefore, a large-scale Chinese CSC genetic association study based on familial cases and sporadic patients is required Further investigation of the genetic background of CSC makes sense for a better understanding of the pathophysiology and development of novel therapeutic approaches.

Methods

Patients

This study was approved by the ethics committee of Peking University People's Hospital and was conducted according to the Declaration of Helsinki principles. All subjects provided informed consent before participation in the study. The data for family A were collected from Mongolia: I-2 was 83 years of age, II-4 was 57 years, II-6 was 47 years, and II-7 was 45 years. The data for family B were collected from Zhejiang: II-1 was 65 years of age and II-2 was 60 years. The patients with CSC were recruited from the following centers: Peking University People's Hospital; Beijing Tongren Hospital; Beijing Friendship Hospital of Capital Medical University; Peking Union Medical College Hospital; West China Hospital of Sichuan University; First Affiliated Hospital of Inner Mongolia Medical University; Wuhan General Hospital of Guangzhou Military Region; First Affiliated Hospital of Harbin Medical University; Second Affiliated Hospital of Harbin Medical University; The Fourth People's Hospital of Shenyang; Tianjin Medical University Eye Hospital; the Second Hospital of Jilin University; Eye Hospital of Hebei Province; The First Affiliated Hospital of Xi'an Medical University; The Affiliated Eye Hospital of Nanjing Medical University; The First Affiliated Hospital of Chongqing Medical University; The First People's Hospital of Xuzhou; First Affiliated Hospital of Kunming Medical University; The First Affiliated Hospital of Zhengzhou University; Zhejiang Eye Hospital; Renmin Hospital of Wuhan University; and Affiliated Eye Hospital of Nanchang University.

For familial CSC, only a family with at least one first-degree relative diagnosed with CSC, except that the proband could be included. For sporadic cases, patients were confirmed to be diagnosed by a senior retinopathy specialist, and patients with choroidal neovascularization, AMD, or polypoidal choroidal vasculopathy, diabetic retinopathy, and a history of receiving exogenous corticosteroids that might affect the genetic analysis were excluded. Normal age-matched controls were recruited. To exclude the influence of anterior or posterior segment diseases, all controls were only diagnosed with cataracts, but no other eye disease.

All participants underwent a standard ophthalmic examination, including visual acuity measurement, slit-lamp biomicroscopy, and dilated fundus examination with a 90-diopter lens, all of which were performed by retinal specialists. All the subjects diagnosed with CSC underwent fundus fluorescein angiography, optic coherence tomography, and indocyanine green angiography. A total of 1307 patients with CSC and 1438 normal controls participated in this study (Supplementary Table S1).

Whole-Exome Sequencing

Exome sequencing was performed on the genomic DNA of the three samples at Zhongguancun Huakang Gene Institute (Beijing, China). Genomic DNA was captured using the SureSelect Human All Exon v5 kit (51 Mb; Agilent Technologies, Santa Clara, CA). We performed sequencing using the Illumina NextSeq500 platform with 110-bp paired-end reads for each captured library independently (Illumina, San Diego, CA). Each sample had an average coverage of 100-fold. The raw image files were processed using the Illumina Pipeline (version 1.3.4) for base calling with default parameters.

Genotyping

The initial Beijing cohort of 457 patients with CSC was genotyped, and allele frequencies were compared with 473 normal controls. The expanded sample for second stage genotyping of CRH c.152-154dupCGC included 850 patients (519 from North China and 331 from South China) and 965 normal controls (535 from North China and 430 from South China).

Sanger sequencing was performed on the genomic DNA extracted from CSC patient and normal control blood samples. We designed primers for the target sites and performed PCR amplification. Sequencing was independently performed for each PCR amplification on the ABI 3130 platform. Chromas software was used to analyze the sequencing results. The primers were as follows:

CRH-EX2-1F-AGGCAGTCCCGTAGGAAGAC;
CRH-EX2-1R-TTCCTGTTGCTGTGAGCTTG;
CRH-EX2-2F-CTGGGGAACCTCAACAAGAG;
CRH-EX2-2R-AAACACCTGGAAACGGAAAC.

For the c.152-154dupCGC genotype, we PCR-amplified genomic DNA extracted from CSC patient and normal control blood samples. Oligonucleotide primers as above were used in PCR samples containing 9.5 µL of ddH_2O, 12.5 µL of 2 × Goldstar Taq mix, 2 µL of the primer pair (5 µm/µL), 1 µL of DNA. The DNA in the samples was denatured at 95°C for 10 minutes, followed by 34 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final incubation at 72°C for 5 minutes.

Data Analysis

All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). The frequency and susceptibilities of mutations between the CSC and normal cohorts were compared using chi-squared test or Fisher's exact test. All odds ratios (ORs) and corresponding 95% CIs were calculated using the corresponding χ^2 distribution test to estimate the risk size for the risk alleles. A two-sided P value of less than 0.05 was considered statistically significant.

Results

We encountered two CSC families (Figs. A, B) in our clinical work. To identify the pathogenic gene, we performed whole-exome sequencing and identified 1023 variants shared by affected individuals A-I:2, A-II:4, A-II:6, and A-II:7 in family A (Fig. A). After replication in family B, only one poten-

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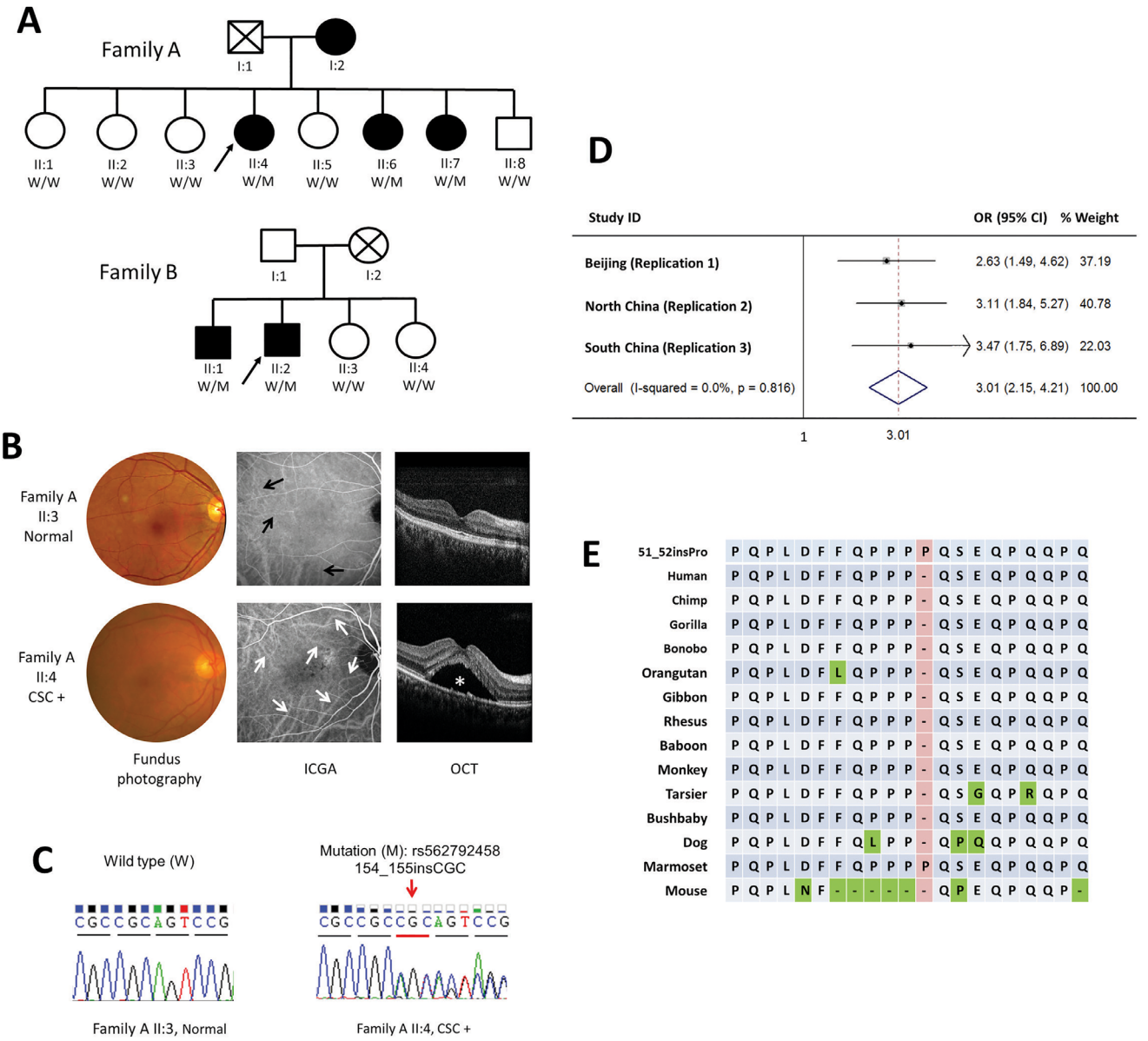

Figure. CRH variant in two families and 1307 sporadic patients with CSC. (**A**) Pedigree of two CSC families. All the family members from family A and family B were evaluated by whole-exome sequencing, and the 154_155insCGC variant in the CRH gene was segregated in all the affected family A and family B members. The arrow pointing to the filled symbol indicates the index proband patient; the crossed lines indicate deceased subjects. W, wild-type allele; M, mutant allele. (**B**) Color fundus photography, indocyanine green angiography (ICGA) and optical coherence tomography (OCT) of the patient (family A, II:4) and normal family member (family A, II:3). The black arrows indicate normal choroid vessels, and the white arrows point to dilated choroid vessels. *Subretinal fluids. (**C**) The heterozygous mutation 154_155insCGC, rs562792458, p. Pro51_Gln52insPro (M) was found in all patients with CSC in family A and family B. (**D**) The OR plot shows the allelic effects of the 154_155insCGC allele of rs562792458 in 1307 sporadic patients and 1438 controls in 3 independent cohorts. The error bars represent the 95% CIs of the ORs. (**E**) A homolog study showed that the CRH gene is relatively conserved in most animals.

tial susceptibility variant was identified, residing in the corticotropin-releasing hormone (CRH) gene (rs562792458, NM_000756.3: c.154_155insCGC; p.Pro51_Gln52insPro). All the affected relatives carried the variant in a heterozygous state, whereas the variant was absent from all unaffected family A and family B members.

To confirm the association between CRH and CSC, we investigated this variant in sporadic patients with CSC. First, we assessed rs562792458 in 457 patients with CSC and 473 age- and sex-matched normal controls (Beijing Cohort). We noted significant evidence of rs562792458 of the gene for CRH ($P_{\text{replication for Beijing}} = 0.001$). Furthermore, we performed replication genotyping in two independent cohorts, the North China cohort (519 cases and 535 controls; $P_{\text{replication for North China}} = 2.12 \times 10^{-4}$) and South China cohorts (331 cases and 430 controls; $P_{\text{replication for South China}} = 1.00 \times 10^{-5}$) (Table, Supplementary Table S1, and Supplementary Fig. S1). We observed supportive evidence for replication in these three CSC cohorts and yielded a genome-wide significant observation when all CSC cohorts were meta-analyzed ($P_{\text{meta}} = 1.24 \times 10^{-11}$; OR, 3.01; 95% CI, 2.15–4.21) (Fig. D). No heterogeneity upon meta-analysis of all three cohorts was found (heterogeneity $\chi^2 = 0.41$; $P = 0.816$; I^2 index $< 0.01\%$). A homologous study of rs562792458 showed that the CRH gene is relatively conserved among most vertebrates (Fig. E).

Table. Association Results of rs562792458 Across Cohorts in Genotype Frequencies, ORs

Stage	Cohorts	Geno Cases Heterozygous	Geno Controls Heterozygous	MAF Cases	MAF Controls	OR	*P* Value
Replication		154_155ins CGC/WT	154_155ins CGC/WT				
1	Beijing China	43/414	18/455	0.0470	0.0190	2.625 (1.491–4.624)	0.001
2	North China	56/463	20/515	0.0539	0.0186	3.114 (1.841–5.269)	2.12×10^{-4}
3	South China	30/301	12/418	0.0453	0.0140	3.472 (1.749–6.892)	1.00×10^{-5}
Total						3.011 (2.153–4.213)	1.24×10^{-11}

MAF, minor allele frequency.

Discussion

CSC is one of the most common vision-threatening retinopathies characterized by the accumulation of transparent fluid at the posterior pole of the fundus.[2] The application of retinal imaging provides a better understanding of the pathophysiology of CSC, but the pathogenesis of CSC remains incompletely understood. Genetic background may predispose to CSC, but lacks studies with a large sample size, multicenter cohorts, or further gene knockout animal investigation. In the present study, we identified one potential susceptibility variant in CRH for CSC.

The rs562792458 variant in CRH increasing the risk of CSC may depend on two mechanisms. First, CRH protects against stress injury. A recent study found that CRH potentiates tau phosphorylation in the brain by acute stress.[15] Another study found that CRH has neuroprotective activity against oxidative cell death.[16] Transgenic overexpression of CRH protects against neurodegeneration under acute excitotoxic stress.[17] CRH deficiency decreases this protection and leads to CSC. Second, animal experiments found that CRH-deficient mice have higher inflammatory cytokine levels, particularly IL-6[18] and TNF-α,[19] under stress. Both TNFα and IL-6 lead to hyperpermeability, and choroidal hyperpermeability is a key step in the pathogenesis of CSC.

Our findings can partially explain the race and sex predisposition. CRH is not a highly polymorphic gene. The minor allele frequency of rs562792458 is between 0.01 and 0.02 in the Chinese population, although this variant has not been identified in American, European, or African populations (according to the 1000 Genomes project; http://www.1000genomes.org/) (Supplementary Fig. S2). In our study, the minor allele frequency ranged from 0.014 to 0.019 in the control Chinese population. Because CRH is a susceptibility gene for CSC, a higher frequency of this variant in Chinese individuals may lead to a higher incidence than in American individuals. Furthermore, our findings indicate that CRH deficiency leads to CSC. The neuropeptide CRH plays a central role in the hypothalamic–pituitary–adrenal stress response system.[20–22] Previous studies have reported that CRH-deficient female mice conserved a reduced but significantly greater hypothalamic–pituitary–adrenal axis response to stress than male mice, suggesting that males are more sensitive to CRH deficiency.[23] In our study, the rs562792458 variant in CRH occurred approximately four times more frequently in male patients with CSC than in female patients with CSC, suggesting that the rs562792458 variant is much more frequent in male individuals and may be associated with susceptibility to CSC in male individuals. This finding may help to explain why CSC is more common in male than in female individuals.

Our study has limitations that should be mentioned. First, the relatively small sample size may decrease the strength of our results, and further replication in a larger study sample is required to confirm our preliminary results. Second, only Chinese cohorts were included in our present study. CSC is considered to have a race predisposition, and the genetic association between CRH and CSC should be further evaluated in cohorts with different ethnicities. Finally, functional verification is necessary to explore the genotype-phenotype correlations and should be reported in subsequent studies.

Together, these findings support a key role for CRH in CSC susceptibility and identify a potential new pathway for CSC pathogenesis.

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