Comprehensive Analysis of Complement Genes in Patients with Atypical Hemolytic Uremic Syndrome

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Key Words

Atypical hemolytic uremic syndrome · Chinese ·
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Abstract

Background: Genetic defects in complement proteins reportedly contribute to the atypical hemolytic uremic syndrome (aHUS). Numerous genetic studies have been published in recent years, but limited data have been gathered from Asian countries. **Methods:** Genetic variants of 11 complement genes were analyzed in 23 Chinese patients with aHUS by high-throughput sequencing. The genotype–phenotype relationship in the Han population was evaluated and compared with the relationship that existed in other ethnicities. **Results:** We identified 20 causative mutations in complement genes, including 19 missense mutations and 1 splicing mutation. Six previously reported mutations, 6 mutations detected for the first time, and 8 rare polymorphisms were noted. Twelve out of 23 patients harbored complement mutations. Among the patients, one was a homozygote (Arg142Cys in CFHR3), and 4 carried combined mutations. Chinese patients have a similar prevalence of complement mutations as European, Japanese, and American patients. Complement factor H (CFH) mutations were common in aHUS in different ethnicities, but Chinese patients exhibited a higher percentage of complement factor B mutations than were found in European patients and a lower percentage of component 3 (C3) mutations than in Japanese

patients. Compared with non-carriers, the aHUS patients carrying mutations had reduced C3 levels. In particular, patients with CFH mutations had a worse renal function than those with membrane cofactor protein mutations, a higher level of serum creatinine at the disease onset and a higher percentage of renal insufficiency during follow-up. **Conclusions:** Because complement genetic dysfunction has clinical significance in aHUS, a comprehensive assessment of variants is necessary for the proper management of aHUS patients in China.

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Introduction

Atypical hemolytic uremic syndrome (aHUS) is a rare disease characterized by microangiopathic hemolysis, thrombocytopenia, and renal failure [1]. The condition has a poor prognosis. In total, 25% of patients die in the acute phase, and 50% of patients progress to end-stage renal disease (ESRD) [2]. This disease is reportedly associated with uncontrolled activation of the complement pathway [3–5]. Genetic mutations play a role in such activation, and more than half the number of patients with aHUS have causative mutations in complement genes [6]. Both loss-of-function mutations in regulators (comple-

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ment factor H (CFH), complement factor I (CFI), membrane cofactor protein (MCP) and thrombomodulin (THBD)) and gain-of-function mutations in key complement components (complement component 3 (C3) and complement factor B (CFB)) predispose individuals to aHUS [7]. The genotype–phenotype correlations of aHUS have clinical significance in predicting renal recovery and transplant outcomes [8].

In a recent large genetic screen of 794 aHUS patients, mutations in CFH, C3, CFI, CFB, or CD46 were identified in 41% of patients, and combinations of mutations were noted in 3% of patients [8]. Single nucleotide polymorphisms (SNPs), haplotypes, genetic intervals and fusions in complement genes are also suggested to be involved in the occurrence of aHUS [9]. Various additional genetic contributions to this disease have been reported [10]. These studies clearly implicate mutations in complement genes in the pathogenesis of aHUS. However, most studies are from Western countries and are focused on Caucasians. It is very important to validate these finding in Asia because the human genome has high ethnic variation. Moreover, physical differences and physiological differences are noted between people from Asia and people from other parts of the world [11]. There are even differences in features between people from northern and southern China. The distinct prevalence of chronic kidney diseases in China has also been noted [12].

We enrolled 23 sporadic patients with aHUS from Eastern China. Using targeted genomic enrichment and massively parallel sequencing (TGE + MPS), we conveniently screened the coding sequences and splice sites of 11 reported candidates of complement genes. We then filtered and prioritized variants based on frequency and functional effects. As expected, we identified novel deleterious variants in multiple complement genes. We also determined the genetic features in Chinese patients from different ethnicities.

Subjects and Methods

Subjects

Twenty-three Chinese patients with aHUS were recruited from the Renal Disease Biobank of the Research Institute of Nephrology, Jinling Hospital. All cases were hospitalized and underwent renal biopsy for diagnosis in this institute from 2000 to 2012. Diagnosis of aHUS was defined by the simultaneous occurrence of microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure without being associated with Shigatoxin [13, 14]. This study was performed with the written informed consent of all patients and family members, and the procedure was approved by the Ethics Committee of Nanjing University, School of Medicine.

Clinical events preceding the acute aHUS episodes were recorded, and laboratory data were collected before treatments with immunosuppressive agents or plasma exchanges. Acute kidney injury (AKI) is defined as an increase in serum creatinine (SCr) of ≥ 0.3 mg/dl (26 μ mol/l) within 48 h or ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days, or urine volume of 0.5 ml/kg/h or less for 6 h. Glomerular filtration rate was estimated (eGFR) using the simplified Modification of Diet in Renal Disease formula [15]. All cases were regularly followed up at the out-clinic until December 1, 2014. During follow-up, ESRD was defined as eGFR < 15 ml/min/1.73 m² or requirement of dialysis. If a patient presented AKI, renal remission was defined as SCr < 1.6 mg/dl (141 μ mol/l).

DNA Extraction and Targeted Exon Sequencing

Genomic DNA was extracted from peripheral blood using the GentraPure gene kit (Qiagen Inc., Valencia, Calif., USA). RefSeq coding exons of the following genes were targeted with an extra 100 bases upstream and downstream: C3 (NM_000064), MCP (NM_002389), CFB (NM_001710), CFH (NM_000186), CFHR1 (NM_002113), CFHR2 (NM_005666), CFHR3 (NM_021023), CFHR4 (NM_006684), CFHR5 (NM_030787), CFI (NM_000204) and THBD (NM_000361). Detailed information for capture design, sequence capture, library preparation and Ion Torrent sequencing is available in the online supplementary methods (for all online suppl. material, see www.karger.com/doi/10.1159/000445127).

After sequencing, the mean reads generated per sample was 435,861, with quality control of 89–93%. The total number of bases per sample was approximately 60.9 Mbp, and the mean length of a read was 132 bp. The longest read was 370 bp.

Variant Calling and Mutation Polarization

The flowchart of variant calling and mutation polarization is presented in online supplementary figure S1. A total of 2,237 (of 2,609; 85.74%) variants passed quality control, and 895 had non-synonymous substitutions, frame shifts, splicing site changes, or indel variations. The called variants were further reviewed in databases and analyzed in 100 local healthy controls. Commons variants were excluded, which were defined as variants with a minor allele frequency (MAF) value of $> 3\%$ in the Asian population based on the 1000 Genomes Project (April 2012) or a frequency of $> 1\%$ in the local control cohort [16].

The polarized variants were confirmed by Sanger Sequencing and then considered causative mutations (online suppl. fig. S2). The variants were further divided into 3 subtypes: disease-related mutations, novel mutations and rare polymorphisms. Here, a disease-related mutation is a pathogenic mutation reportedly related to certain diseases. No novel mutations were found in the 1000 Genomes data, the National Center for Biotechnology Information dbSNP database, or the local cohort. A rare polymorphism was detected in $\leq 3\%$ of the Asian population based on the 1000 Genomes Project (April 2012) and in $\leq 1\%$ of the local control cohort.

In addition, the potential pathogenicity of these causative mutations was multiply predicted by 5 methods in silico, including SIFT (<http://sift.jcvi.org>), Align-GVGD (<http://agvgd.iarc.fr/>), PMut (<http://mmb.pcb.ub.es/PMut/>), SNAP (<https://roslab.org/services/snap/>), and PolyPhen2.0 (<http://genetics.bwh.harvard.edu/pph2/dbsearch.shtml>). A mutation was predicted to be pathogenic if it was positive in 3 or more of the methods.

Literature Review

Reported mutations associated with aHUS in complement genes were further reviewed. The PubMed database was searched for published cohort studies up to December 5, 2014 using the combination of the following key words: 'mutation', 'cohort', 'alternative', 'complement' and 'aHUS'. Relevant studies with cohorts screened for more than 5 complement genes in aHUS patients were enrolled. Finally, the 3 cohorts from Europe, America and Japan were reviewed (online suppl. table S1) and further compared with our Chinese cohort.

Statistical Analyses

Data were analyzed using SPSS 19.0. The t test or the Mann-Whitney test was used to compare means. Data are displayed as the mean $\pm$ SD or medians and interquartile ranges. Chi-square tests were performed for qualitative data, and the data were expressed as percentages (%) in a group. The renal survival rate was analyzed with Kaplan-Meier analysis. p values <0.05 were considered significant.

Results

All study participants were of the Han descent in China. The mean age of our patients with aHUS was 35.8 ± 13.6 (range 13.0–60.5). The study included 10 men and 13 women (table 1). All patients presented with acute renal failure and microangiopathic hemolytic anemia, and 69.6% had thrombocytopenia. The typical lesions in the kidney were platelet-fibrin rich microthrombi causing small vessel thrombosis, schistocytosis, and thrombotic microangiopathy (online suppl. fig. S3). Strong C3 staining was observed in the mesangial area of the glomeruli. No family history of aHUS or kidney disease was reported. With a mean follow-up of 8.3 months, 11 (47.8%) patients progressed to ESRD.

Mutation Identification

After 11 complement genes were screened in these 23 patients, a total of 19 nonsynonymous variants and 1 splicing variant were identified in these aHUS patients (online suppl. fig. S1). These mutations were located in 9 genes with an average 1.82 (range 0–4) mutations per gene (fig. 1a). Four mutations were identified in C3 at macroglobulin domain 1–3, a domain for binding to complement regulator factors (fig. 1b). Complement genes often contain short consensus repeats (SCRs) [1, 17, 18]. Three mutations in MCP were located at the N-terminus and in SCR2/SCR3 (fig. 1c). Three mutations in CFB were located at its SCR1 and serine protease (SP) site in the C-terminus (fig. 1d). The CFH gene and the genes encoding the 5 CFHR proteins reside in the centromere, a 355-kb segment on chromosome 1q32, and

Table 1. The demonstration and clinical profile of patients with aHUS

Characteristic	aHUS
Patients, n	23
Male, n (%)	10 (43.5)
Age, years	35.9 (23.0–41.3)
AKI, n (%)	23 (100)
Anemia, n (%)	23 (100)
Thrombocytopenia, n (%)	16 (69.6)
Hypertension, n (%)	16 (69.6)
Family history, n (%)	0
Urine protein, g/24 h	0.87 (0.52–2.79)
Erythrocyturia, $\times 10^4$ /ml	15.5 (2.5–165)
SCr, $\mu\text{mol/l}$	770.6 $\pm$ 479.3
eGFR, ml/min/1.73 m ²	11.64 $\pm$ 9.98
Complement C3 level, g/l	0.73 $\pm$ 0.19
Follow-up duration, months	8.3 (2.2–23.3)
Renal survival rate, n (%)	12 (52.2)

they were clustered as CFH family genes [19, 20]. A total of 8 mutations were identified in the CFH family genes, and the mutations were mainly located in the domains for binding to C3 and heparin-like oligosaccharides (fig. 1e).

Among the 20 causative mutations, 6 were disease-related mutations, 6 were novel as aHUS mutations, and 8 were rare polymorphisms (table 2). Four disease-related mutations (p.Thr162Arg in C3, p.Lys533Arg in CFB, p.Leu1189Phe in CFH and p.Arg1215Gly in CFH) were causally related to aHUS [8, 21–23], and 2 (p.Pro314Leu and p.Arg102Gly in C3) were reportedly associated with age-related macular degeneration (AMD) [24, 25]. AMD is heritable and also strongly associated with complement dysfunction [26]. Eight rare polymorphisms had an average MAF of 0.0078 ± 0.0100 (range 0.0034–0.0288) in 2,500 subjects from 1000 genomes (<http://www.ncbi.nlm.nih.gov/projects/SNP>). Their percentage in our control cohort was 0.00375 ± 0.0044 (range 0.00–0.01). Six novel mutations were not reported and not detected in the control cohort, either. In addition, 9 (45%) of 20 mutations were predicted to be deleterious, including 4 (66.7%; of 6) disease-related, 2 (33.3%; of 6) novel and 3 (37.5%; of 8) rare variants.

Mutation Prevalence

In this study, 12 (52.2%) of 23 cases in Chinese patients with aHUS were associated with complement mutations (fig. 2a). Eight (34.8%) patients carried one complement mutation, and 4 (17.3%) patients carried combined muta-

Table 2. Causative mutations identified in aHUS patients

Nucleotide	Gene	Genotype	Patient	AA change	dbSNP	MAF	Location	Prediction
<i>Reported disease-related variants</i>								
c.941C>T	C3	Heterozygous	ADB81	Pro314Leu	rs1047286	0.0699	MG3	1
c.304C>G	C3	Heterozygous	ADB81	Arg102Gly	rs2230199	0.0873	MG1	1
c.485C>G	C3	Heterozygous	ADB69	Thr162Arg	NA	NA	MG2	1
c.1598A>G	CFB	Heterozygous	ADB81	Lys533Arg	rs149101394	0.0192	SP	0
c.3565C>T	CFH	Heterozygous	ADB71	Leu1189Phe	NA	NA	SCR20	0
c.3643C>G	CFH	Heterozygous	ADB67	Arg1215Gly	NA	NA	SCR20	1
<i>Rare polymorphisms</i>								
c.1028G>A	C3	Heterozygous	ADB81	Arg343His	rs373511900	NA	MG3	0
c.221G>A	CFB	Heterozygous	ADB74	Arg74His	rs117314762	0.0010	SCR1	1
c.2509G>A	CFH	Heterozygous	ADB82	Val837Ile	rs55807605	0.0034	SCR16	0
c.424C>T	CFHR3	Homozygous	ADB82	Arg142Cys	rs61737525	0.0288	Unstructured region	1
c.434G>A	CFHR5	Heterozygous	ADB82	Gly145Glu	rs57960694	0.0107	Unstructured region	0
c.508G>A	CFHR5	Heterozygous	ADB67	Val170Met	rs201073457	0.0012	SCR3	1
c.293C>T	MCP	Heterozygous	ADB72	Thr98Ile	rs116800126	0.0010	SCR2	0
c.38C>T	MCP	Heterozygous	ADB88 ADB73	Ser13Phe	rs138843816	0.0086	N-terminal	0
<i>Rare polymorphisms identified in this study</i>								
c.2008A>G	CFB	Heterozygous	ADB84	Lys670Glu	NA	NA	SP	0
c.242C>T	CFHR5	Heterozygous	ADB83	Pro81Leu	NA	NA	SCR1	1
c.335A>T	CFHR2	Heterozygous	ADB82	Ile112Phe	NA	NA	SCR1	0
c.574G>C	MCP	Heterozygous	ADB78	Asp192His	NA	NA	SCR3	0
c.1336G>A	THBD	Heterozygous	ADB78	Glu446Lys	NA	NA	EGFD5	0
c.904+1G>A	CFI	Heterozygous	ADB78	Splicing	NA	NA	NA	1

The MAF from 1000 genomes is referred in <http://www.ncbi.nlm.nih.gov/projects/SNP>.
EGFD = Epidermal growth factor domain; NA = not available.

Clinical Findings

aHUS patients were divided into two groups, mutation carriers and non-carriers, based on the presence or absence of complement mutations (table 4). Compared with non-carriers, patients carrying mutations presented with a significantly lower level of C3 (0.65 vs. 0.81 g/l, $p = 0.04$). Patients carrying mutations also had relatively young onset ages, a high percentage of thrombocytopenia, increased SCr, and decreased levels of platelet and hemoglobin. However, these characters did not reach statistical significance between 2 groups.

All patients with aHUS presented with AKI at onset, and approximately half the number of patients experienced remission after treatment (table 4). The rate of remission was similar between patients with and without mutations ($p = 0.4$). During follow-up, the carriers seemed to have better prognosis than non-carriers (fig. 3a). However, this value did not reach statistical significance, partly given the small size of our cohort.

Five (62.5%, of 8) patients with a single mutation exhibited the gradual recovery of renal function and they remained stable during follow-up. However, for those carrying combined mutations, only one (25.0%, of 4) patient

Table 3. Gene profile of Chinese aHUS patients compared with those of Caucasians and Japanese cohorts

Genes screened	CFH, C3, CFB, CFI and MCP			CFH, C3, CFB, CFI, MCP and THBD			CFH, C3, CFB, CFI, MCP, THBD and CFHR5		
	Chinese	European [8]	p value	Chinese	Japanese [11]	p value	Chinese	American [18]	p value
Cohort number	23	795		23	10		23	144	
Mutation prevalence, n (%)	11 (47.8)	323 (40.6)	0.49	11 (47.8)	8 (80.0)	0.13	12 (52.2)	66 (45.8)	0.57
Individual gene, n (%)									
CFH	3 (13.0)	158 (19.9)	0.60	3 (13.0)	2 (20.0)	0.63	3 (13.0)	39 (27.1)	0.20
C3	2 (8.70)	45 (5.66)	0.39	2 (8.70)	5 (50.0)	0.02	2 (8.70)	3 (2.09)	0.14
CFB	3 (13.04)	9 (1.13)	0.01	3 (13.0)	0 (0.00)	0.54	3 (13.04)	6 (4.17)	0.11
CFI	1 (4.35)	46 (5.79)	1.00	1 (4.35)	0 (0.00)	1.00	1 (4.35)	12 (8.33)	1.00
MCP	4 (17.39)	65 (8.18)	0.12	4 (17.4)	2 (20.0)	1.00	4 (17.39)	7 (4.86)	0.04
THBD	–	–	–	1 (4.35)	1 (10.0)	0.52	1 (4.35)	4 (2.78)	0.53
CFHR5	–	–	–	–	–	–	3 (13.04)	4 (2.78)	0.06
Combined mutation prevalence, n (%)	2 (8.70)	27 (3.40)	0.19	2 (8.70)	0 (0.00)	1.00	4 (17.39)	8 (5.56)	0.06

Fig. 2. Distribution of different numbers of causative mutations identified in the aHUS patients of this study. **a** Eleven (47.8%) patients did not carry the gene mutations of complement alternative pathway, 8 (34.8%) patients carried single mutation, 1 patient car-

ried 2 mutations, another patients carried 3 mutations and the last 2 patients carried 4 combined mutations, respectively. A detailed description of the combined variants in 4 patients is given in **(b)**.

showed remission with immunosuppressive treatment. Two patients even required permanent renal replacement therapy. Two patients carried the same mutation (p.Ser13Phe in MCP), but they had different outcomes.

In addition, patients with CFH mutations and MCP mutations were further compared in table 4. Patients with CFH mutations exhibited increased levels compared to those with MCP mutations at onset ($1,465 \pm 220$ vs. 822 ± 246 $\mu\text{mol/l}$, $p = 0.01$). Two patients with CFH mutations suffered from anuria, and urine protein could not be compared between the 2 groups. After follow-up at 90 months, all patients with CFH mutation developed ESRD, but only 1 (25%) patient with an MCP mutation developed this condition. This difference was not statistically significant ($p = 0.17$; fig. 3b).

Discussion

In this study, 23 sporadic Chinese patients with aHUS had 11 complement genes systematically screened with efficient TGE + MPS. After polarization and validation, we identified 20 causative mutations in 52.2% of patients. In total, 30% of the mutations were reportedly causative, indicating that these mutations are pathogenic for aHUS. The remaining 14 mutations, including 8 rare polymorphisms and 6 novel mutations, were considered to be causative based on the findings of the prediction programs, a literature review and prevalence of the mutations in population databases and a control cohort.

Approximately half of the Chinese patients with aHUS carried complement mutations, and a similar prevalence

Table 4. Clinical profile of aHUS patients with different genetic characteristics in alternative complement genes

	Mutation carriers (n = 12)	Mutation non-carriers (n = 11)	p value	CFH mutation carriers (n = 3)	MCP mutation carriers (n = 4)	p value
Age, years	32.0 (19.3–41.2)	39.1 (30.2–50.7)	0.36	33.9 (30.0–60.5)	19.4 (13.7–36.4)	0.16
Male, n (%)	6 (50.0)	6 (54.5)	0.83	2 (66.7)	3 (75.0)	–
Clinical diagnosis, n (%)						
Hypertension	8 (66.6)	8 (72.7)	1.00	3 (100.0)	2 (50.0)	–
AKI	12 (100.0)	11 (100.0)	–	3 (100.0)	4 (100.0)	–
Anaemia	12 (100.0)	11 (100.0)	–	3 (100.0)	4 (100.0)	–
Thrombocytopenia	10 (83.3)	6 (54.5)	0.19	3 (100.0)	3 (75.0)	–
Laboratory profiles						
Urine protein, g/24 h	1.13 (0.45–3.30)	0.85 (0.59–6.11)	1.00	0.72 [#]	1.83 (0.61–5.31)	–
Erythrocyturia, $\times 10^4$ /ml	33.5 (1.5–465.0)	12.0 (3.3–82.5)	0.57	160.0 (17.0–560.0)	7.5 (1.5–138.0)	0.16
SCr, μ mol/l	921 $\pm$ 493	606 $\pm$ 425	0.12	1,465$\pm$220	822$\pm$246	0.01
eGFR, ml/min/1.73 m ²	8.28 $\pm$ 6.51	15.31 $\pm$ 12.01	0.09	2.93 $\pm$ 0.22	8.09 $\pm$ 3.94	0.08
Hemoglobin, g/l	62.6 $\pm$ 15.0	75.4 $\pm$ 22.1	0.12	64.3 $\pm$ 18.1	56.8 $\pm$ 13.5	0.55
Platelets, $\times 10^9$ /l	73.1 $\pm$ 48.7	120.5 $\pm$ 98.3	0.15	70.7 $\pm$ 15.4	76.5 $\pm$ 71.6	0.90
Complement C3 level, g/l	0.65$\pm$0.12	0.81$\pm$0.22	0.04	0.69 $\pm$ 0.10	0.61 $\pm$ 0.16	0.49
After 90-month follow-up						
eGFR, ml/min/1.73 m ²	52.41 $\pm$ 52.35	34.29 $\pm$ 38.26	0.36	4.91 $\pm$ 1.87	57.68 $\pm$ 65.12	0.23
Remission rate, %	50 (6/12)	27 (3/11)	0.40	0 (0/3)	50 (2/4)	–
Renal survival rate, %	58 (7/12)	45 (5/11)	0.54	0 (0/3)	75 (3/4)	–

[#] Two CFH mutation carriers were progressing to complete anuria at onset.

Fig. 3. Association of carried mutations and renal survival. **a** Patients with aHUS were divided into 2 groups by mutation carriers or non-carriers and the renal survival of 2 groups followed up 90

months was not significantly different ($p = 0.19$). **b** No statistical difference was observed between CFH mutation carriers and MCP mutation carriers ($p = 0.17$).

was observed in different ethnic groups. Although the screened genes were different and the cohort sizes were not comparable among reported cohorts, these primary data still confirmed that inherited defects play a pivotal role in the pathogenesis of this disease. One-third of patients with aHUS carried combined mutations and one

Chinese patient even had 4 mutations in different CFH family genes. This observation implies that the synergistic effects of multiple mutations contribute to aHUS. We proposed that screening of more associated genes be done in order to enable better patient care. High-throughput sequencing is an approach that is increasingly used to an-

alyze related genes in the field of genetic studies. However, we did not identify gene insertions or deletions, which were occasionally reported in previous studies using multiplex ligation-dependent probe amplification [1, 9].

Considering the mutation percentages in individual complement gene, the mutations were mainly identified in the C3, MCP, CFB and CFH families. This issue appeared universal and was also reported among the Americans, Europeans and Japanese [8, 11, 18]. Only a few mutations were noted in CFI and THBD [27, 28], and no causative CFHR1/CFHR4 mutations were detected in Chinese patients. However, the percentages of CFI mutations were inconsistent. It was suggested that 4–10% of aHUS patients from Western populations carried CFI mutations [1], and 4.3% of our Chinese patient carried these mutations. However, Le Quintrec et al. reported that 16% of aHUS from a French registry carried CFI mutations [29].

C3 plays a major role in the complement system. C3 contains 8 macroglobulin domains (MG1–8) to form α - and β -chains (fig. 2a). Mutations in C3 reportedly account for the increased complement activation on platelets and glomerular endothelium [30, 31] and the etiology of 2–10% of the aHUS patients [1]. In this study, Chinese aHUS patients carried 4 different missense mutations in C3. These mutations were clustered in the MG1–3 domain, which is located in the β -chains. This domain is not the classic active domain; it is the domain that binds to complement regulator factors [32]. The p.Pro314Leu and p.Arg102Gly mutations have previously been reported in a Dutch bacterial meningitis cohort and in Icelanders [33], and the mutations are known to enhance complement activation by reducing the binding of C3b and CFH.

MCP, a membrane-bound complement regulator highly expressed on most cell surfaces, acts as a cofactor of the CFI-mediated degradation of C3b and C4b. The 4 extracellular SCRs are the binding sites for C3b (fig. 2c), and 3 mutations identified in this study were found at those sites. Approximately 10–15% of the aHUS patients reportedly carried MCP mutations, and SCR-3 was the most affected domain previously reported [34]. The p.Thr98Ile and p.Ser13Phe mutations have been detected in Caucasian and Japanese patients [11], leading to a quantitative defect in a secreted, nonfunctional protein [35].

Mutations in CFB, which resulted in chronic alternative-pathway activation, accounted for the etiology in 1–2% of the aHUS patients [3]. CFB is composed of 3 complement control protein domains (CCP1–3), a von Willebrand type A domain, and an SP domain (fig. 2d).

In this study, one mutation was located in the CCP domains, and 2 mutations were found in the SP domain. Gain-of-function mutations and formation of the C3bB proenzyme have been reported in CFB [27]. The p.Lys533Arg and p.Arg74His mutations have previously been reported in French patients [21], and these mutations caused excess C3b affinity and hyperactive C3 convertase as well as enhanced C3b formation [36].

CFH, a principal regulator of the complement system, is composed of 20 SCR domains (fig. 2e). Deficiency of CFH is commonly associated with aHUS. Greater than 70% aHUS cases were detected with CFH mutations or anti-CFH autoantibodies [37, 38]. Missense mutations in the CFH family genes were most frequently identified in CFHR5, and mutations in CFHR1–4 have rarely been reported [39, 40]. Eight mutations of CFH family were identified in Chinese aHUS patients. Most CFH mutations are located at the C terminal SCR15–SCR20, especially SCR20 [34], and 2 mutations were detected in this study. These sites primarily mediate surface binding and target recognition. The other mutations were at SCR1–SCR4, where the CFH family helps the binding of C3 and heparin-like oligosaccharides.

The phenotype–genotype correlation of aHUS often has clinical significance. Age-of-onset and disease severity correlate with mutation type as well as response to plasma exchange therapy and outcomes following renal transplantation. In this study, complement mutations were associated with decreased levels of plasma C3. The complement mutations were also associated with renal survival, but it is difficult to draw a conclusion based on a limited number of patients. In addition, the activation of the complement alternative pathway is tightly regulated by a number of circulating and cell-bound complement regulatory proteins. C3b, CFB, CFD and properdin stabilize and activate the C3 convertase, whereas CFH, CFHR1–5, CFI, MCP and THBD inhibit or regulate the complement pathway in the fluid-phase or on the surface of host cells. A dynamic balance between complement activation and inactivation exists. Genetic mutations primarily lead to the dysfunction of target genes, and complement mutations in different genes play diverse role in aHUS. In the study, CFH mutation carriers exhibited a trend of early loss of the kidney, whereas patients with MCP mutations remained alive and dialysis-free in a long-term follow-up. Patients with CFH mutations were also reported with a bad prognosis [41]. Thus, understanding the functions of the mutations in certain genes and the whole complement system is critical for clinical evaluation not just for screening. In addition, genetic de-

fect often increases the susceptibility and further infection or other injury will lead to the development of aHUS. This is a well-known theory of disease, 'second attack', which was first proposed with regard to cancers. In this study, we observed that 10 patients had fever in the disease history. More environmental factors evaluated would enhance understanding of the mechanism and show therapeutic insight for clinicians.

In summary, the prevalence of genetic variation was evaluated in Chinese aHUS patients. Understanding the genetic background in China would facilitate the clinical management of aHUS patients in this region. However, our study only involved 23 patients. The small sample size might lead to analysis bias and reduce the significance of findings. The published cohort from Japan, the only oth-

er study published from Asia, enrolled only 10 patients. More studies are needed to understand this issue in Asia, including genetic profiles and genotype-phenotype associations.

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Disclosure Statement

None declared.

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