Fig 3. Relationship between hemolytic activity and complement abnormalities in 45 patients with atypical hemolytic uremic syndrome (aHUS). The results of the hemolytic assay for 45 aHUS patients are shown according to the predisposing (bold type) or potentially predisposing mutations and/or the acquired abnormalities. The patients carrying two mutations were classified according to the predisposing mutation, and the other mutations were described alongside the predisposing mutations or black bars. Two patients (2V1 and H2) were categorized into the C3 group, although they had two predisposing mutations (C3-p.I1157T and THBD-p.D486Y). In two patients (2P1 and 3G1), the hemolysis induced by serum was represented by gray bars. The normal range of hemolysis (5.4±1.8%: mean ± standard deviation) obtained from 20 healthy individuals (10 males and 10 females) is shown at the bottom of Fig 3. The results of the restriction fragment length polymorphism (RFLP) analysis of C3-p.I1157T are summarized to the right of the hemolytic assay results. Regarding the RFLP analysis of C3-p.I1157T (c.3470T>C), the heterozygous mutation (T/C) showed two bands (278 bp, 314 bp) and the homozygous mutation (C/C) showed one band (314 bp). † H2 had a homozygous mutation in C3-p.I1157T.

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no appreciable hemolysis. The enhanced hemolysis detected in these four family members was corrected by the addition of purified CFH (Fig 6C). However, none of the family members had anti-CFH autoantibodies or CFH deficiency. These results suggest that the family of Patient

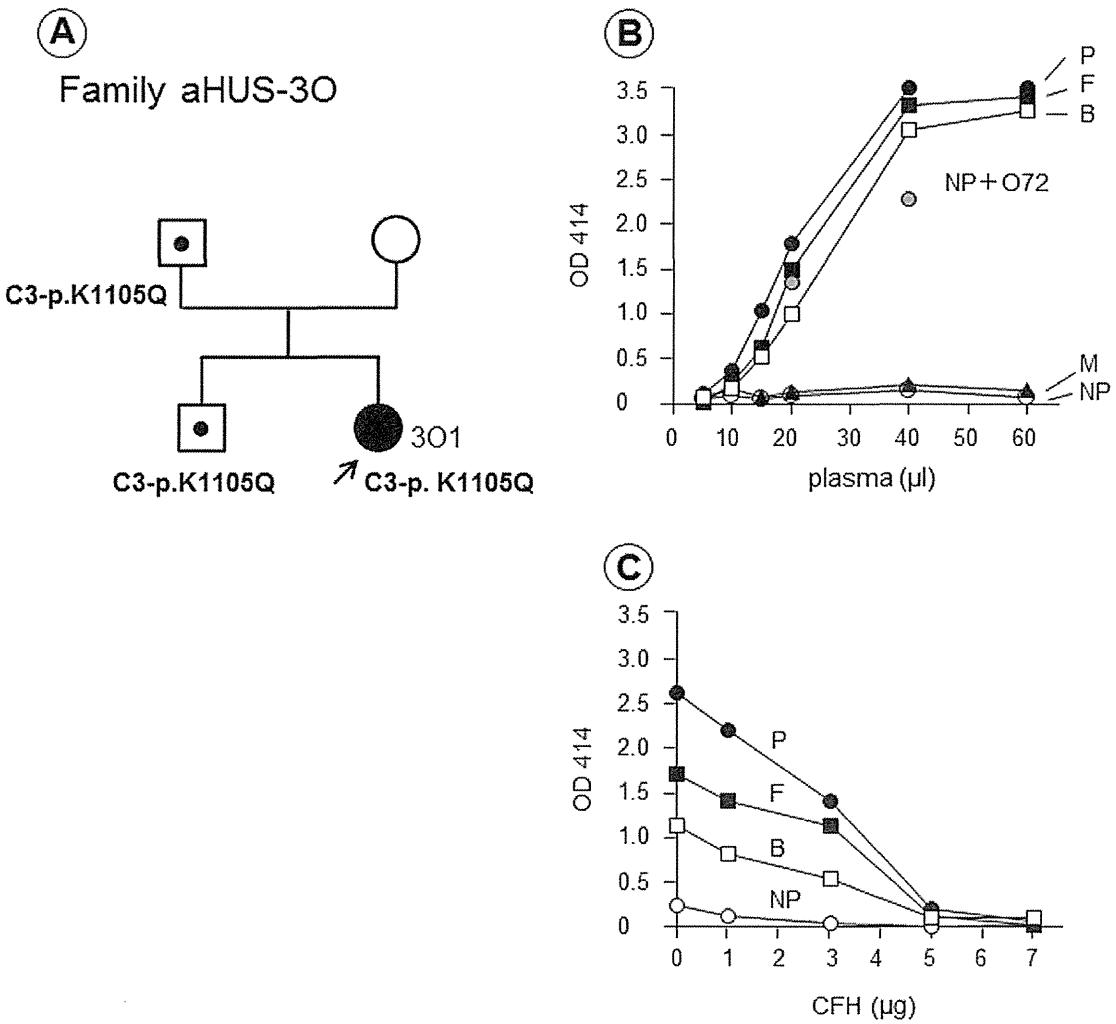

Fig 4. Family 3O with a C3-p.K1105Q mutation. (A) Patient 3O1 (female) developed atypical hemolytic uremic syndrome (aHUS) at the age of five months. (B) In the hemolytic assay, plasma samples from three family members (patient: P, father: F, and brother: B) induced strong hemolysis of sheep red blood cells (RBCs), but her mother (M) had no appreciable hemolysis, with results similar to normal plasma (NP). (C) Lysis of sheep RBCs detected in the three family members was corrected by the addition of complement factor H (CFH) in a dose-dependent manner. Genetic analysis showed that the patient and her father and brother, whose plasma clearly showed hemolysis, carried a potentially predisposing mutation p.K1105Q in C3, not in CFH.

doi:10.1371/journal.pone.0124655.g004

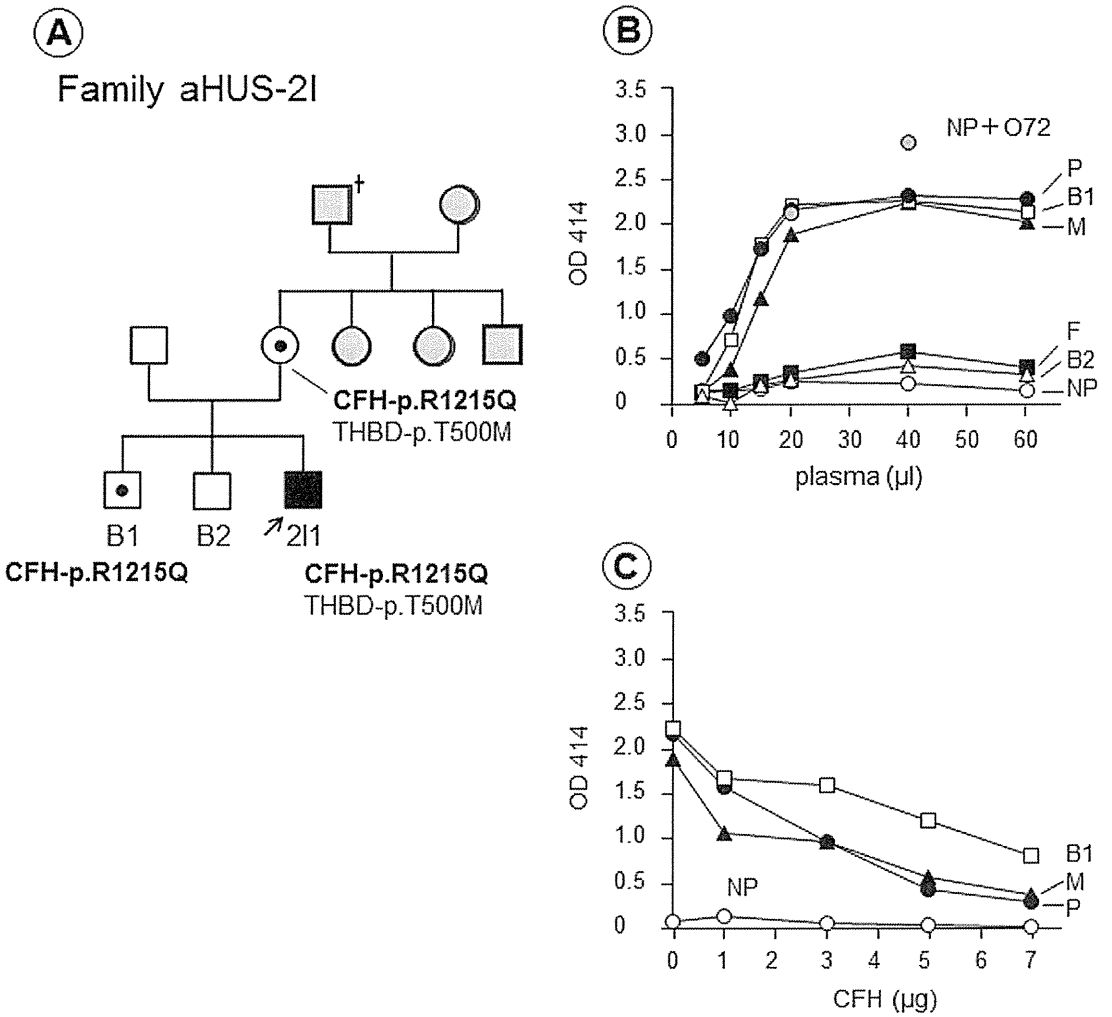

Fig 5. Family 2I with a CFH-p.R1215Q mutation. (A) Patient 2I1 (male) had episodes of atypical hemolytic uremic syndrome (aHUS) at the age of 28 and 29. Gray squares or circles indicate individuals who were not analyzed in this study. (B) The hemolytic assay showed that plasma from the patient (P), his mother (M), and one brother (B1) induced severe hemolysis, whereas samples from his father (F) and the other brother (B2) showed no appreciable hemolysis. (C) The hemolysis detected in the three family members was corrected by the addition of purified complement factor H (CFH). Genetic analysis showed that the patient, his mother, and brother 1 carried a CFH-p.R1215Q mutation, but his mother and brother 1 have never had the episodes of aHUS.

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2K1 might have a genetic abnormality involved in the complement regulation system, inherited from her father.

3. Mild hemolysis: 25–50%. Six patients (3M1, 2J2, 3B1, 3C1, 2W1, and 3E1) were in this category. Three patients had predisposing mutations. Patients 3M1 and 2J2 had a p.I1157T mutation in the TED of C3 (described below) and Patient 3C1 had a p.D486Y mutation in the serine-threonine-rich region of THBD. Furthermore, two other patients (3B1 and 2W1) had potentially predisposing mutations: p.A311V in the transmembrane region of MCP, and p. N331D in the von Willebrand type A domain of CFB, respectively. Only one patient (3E1) had no abnormalities in the six candidate genes and the CFHR1 and CFHR3 proteins based on Western blot.

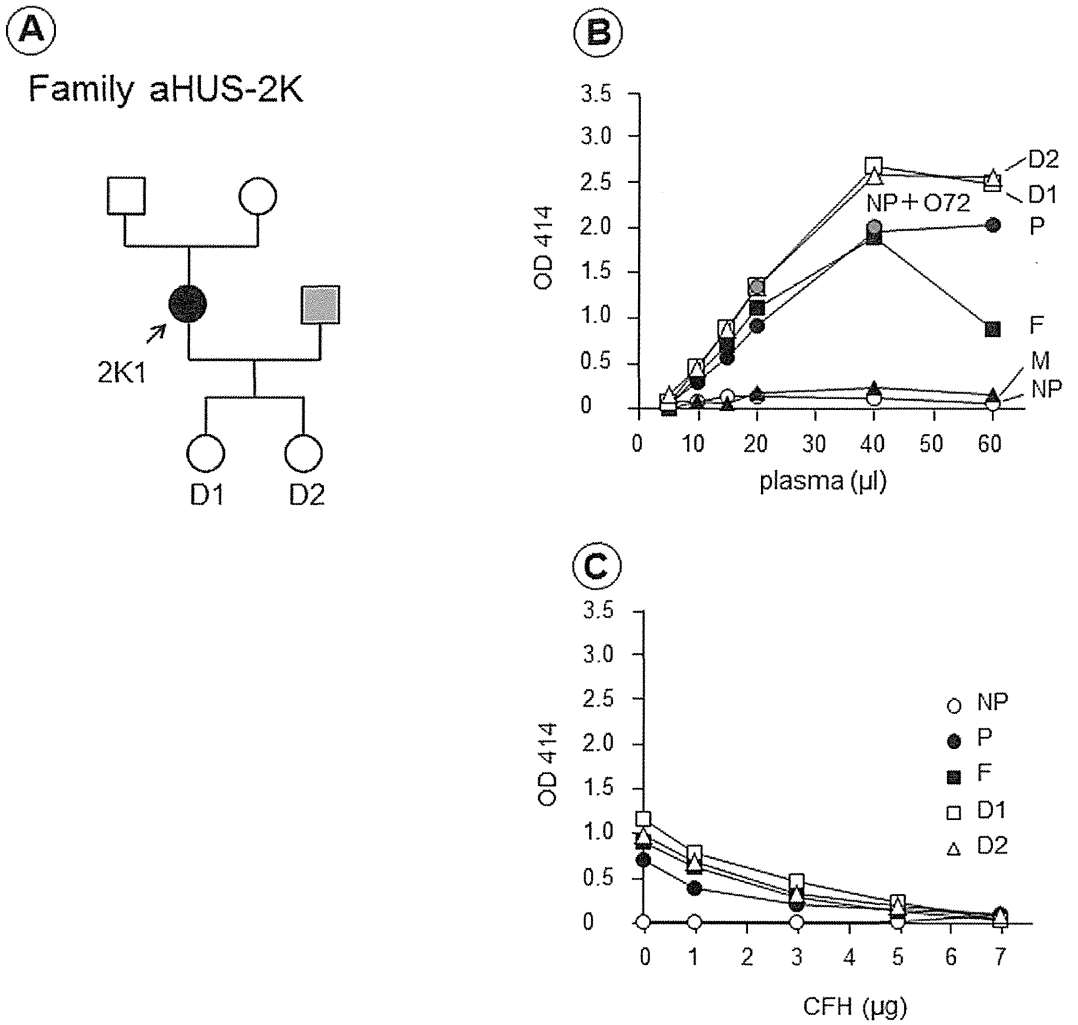

Fig 6. Family 2K with no predisposing or potentially predisposing mutations. (A) Patient 2K1 (female) developed atypical hemolytic uremic syndrome (aHUS) at the age of 35. Her husband (gray square) was not analyzed in this study. (B) The hemolytic assay showed that plasma from the patient (P), her father (F), and two daughters (D1 and D2) induced severe hemolysis, which was not observed in her mother (M). (C) The enhanced hemolysis detected in these four family members was corrected by the addition of purified complement factor H (CFH). However, no predisposing or potentially predisposing mutations were detected in Patient 2K1.

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4. No apparent hemolysis: <25%. There were 28 patients in this category, of whom 16 had C3 mutations. Surprisingly, among these 16 patients, 14 had the same predisposing mutation, p.I1157T in the TED of C3. The remaining two patients (2H1 and 2F1) had potentially predisposing mutations in C3, p.R425C and p.S562L, respectively. Two patients (2D1 and 2X1) had predisposing mutations in two transmembrane proteins. One had a p.Y189D mutation in the SCR3 domain of MCP, and the other had a p.D486Y mutation in THBD. Patient 3J1 had a potentially predisposing mutation, p.V231I in THBD. Curiously, Patient 3G1 having anti-CFH autoantibody, but without deletion in CFHR1 and CFHR3 proteins, was also categorized in this group. This finding was quite different from other patients having anti-CFH autoantibodies. Further, the antibody titer in 3G1 (5,995 AU/mL) determined by quantitative ELISA was higher than that of 2Y1 (360 AU/mL) and 2G1 (4,813 AU/mL) (S3 Table). Thus, the

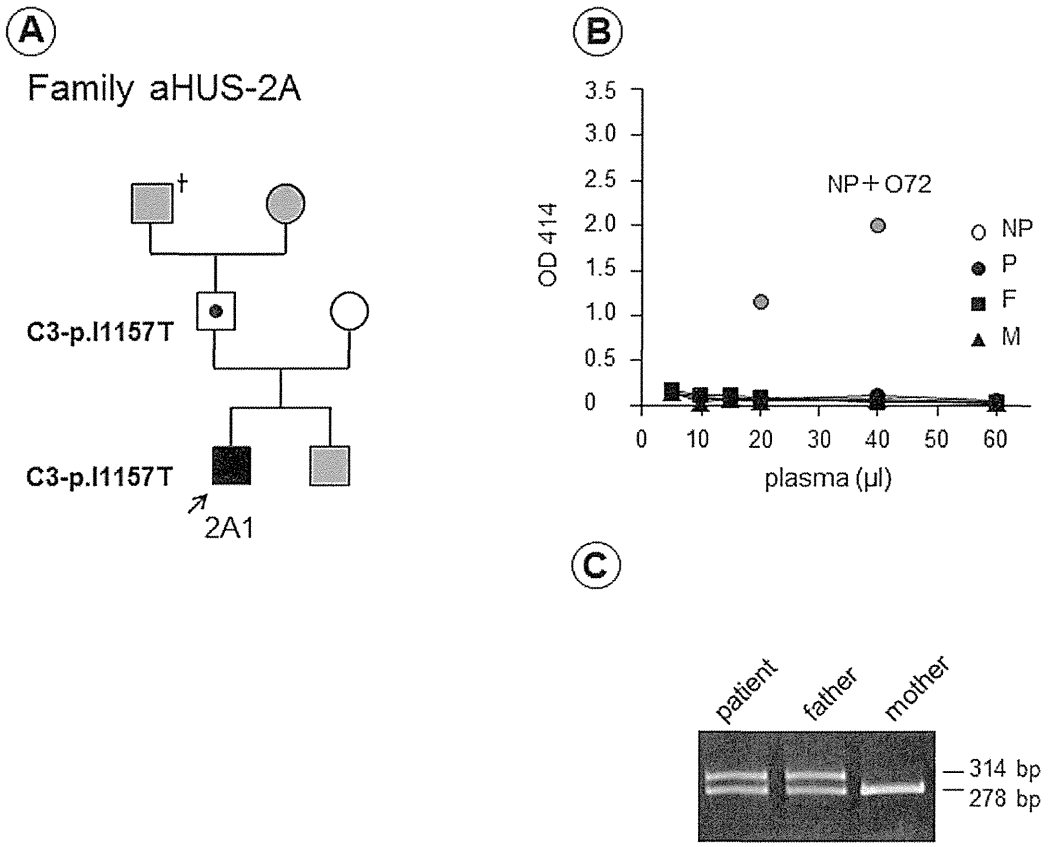

Fig 7. Family 2A with a C3-p.I1157T mutation. (A) Patient 2A1 (male) had six bouts of atypical hemolytic uremic syndrome (aHUS), at the age of 9, 15, 18, 22 and 29 (twice). Gray squares or circle indicate individuals who were not analyzed in this study. (B) The hemolytic assay showed that neither patient (P) nor his parents (father: F and mother: M) had appreciable hemolysis. However, Patient 2A1 and his father carried the same predisposing mutation p.I1157T in C3, confirmed by restriction fragment length polymorphism (RFLP) analysis (C) and direct DNA sequencing.

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discrepancy between the degrees of hemolysis and antibody titer might be attributable to the epitope specificity of anti-CFH autoantibodies. On the other hand, Patient 3G1 had low C3 level (30 mg/mL) (S1 Table), which might contribute to a low degree of hemolysis.

As a consequence, the most frequent genetic abnormality associated with congenital aHUS in Japan, p.I1157T in C3, was unable to be detected by the hemolytic assay. In Fig 7A, we showed the family of Patient 2A carrying a heterozygous C3-p.I1157T mutation as a representative example. Neither the patient nor his family members had enhanced hemolysis (Fig 7B), but the RFLP analysis of C3-p.I1157T clearly indicated that the patient and his asymptomatic father carried the same heterozygous predisposing mutation (Fig 7C), which was also confirmed by direct DNA sequencing. The results of the RFLP analysis in all 45 patients are shown alongside in the hemolytic assay results (Fig 3). These results clearly indicated whether the patient had a heterozygous or homozygous mutation in C3-p.I1157T or not, and 16 carriers were easily diagnosed by the RFLP analysis. Thus, in total a high frequency of a single C3 gene mutation (36%, 16/45) was identified in our aHUS cohort.

Most interestingly, a geographical distribution of gene mutations of aHUS patients showed that the patients carrying the C3-p.I1157T mutation were only found in an extremely restricted

area (Kansai district including Mie [31], Nara, Kyoto, and Osaka) and not found in other areas of West Japan (S1 Fig).

Discussion

Rapid differential diagnosis of TMA is needed in clinical practice to select the optimal therapeutic approach. Evaluations of ADAMTS13 activity and STEC infection are often necessary, both of them usually can be done within a few hours. Further, comprehensive genetic analysis has been proposed as being able to consistently diagnose aHUS in approximately 70% of patients, but it usually takes at least several weeks, resulting in almost no clinical utility during emergencies. Since a relatively high proportion (approximately 20–30%) of mutations occurs in CFH among Caucasian aHUS patients [5, 11], the usefulness of a qualitative hemolytic assay using sheep RBCs has been postulated [12, 13]. However, it appears that the hemolytic assay has not been commonly used in many laboratories. This may be due to unstable viability of sheep RBCs over long-term storage at 4°C, unreal 100% hemolysis of sheep RBCs exposed to distilled water, and the type of blood specimens used, such as serum, EDTA plasma, or citrated plasma. Here, we have shown that serum is not a good specimen for the hemolytic assay, and EDTA plasma cannot be used in the ADAMTS13 activity assay.

In this study, we have successfully generated a novel murine anti-CFH mAb O72, which induces severe hemolysis at 200 μg/ml (final concentration) when spiked into an assay mixture of normal human plasma and sheep RBCs. The mAb O72 epitope resides in SCR18, which could explain its ability to block CFH binding to the sialic acid-rich cell surface of sheep RBCs. Interestingly, the degree of hemolysis induced by normal plasma spiked with mAb O72 is almost the same as that mediated by plasma from a patient with a heterozygous CFH-p.R1215Q mutation. In both these plasma samples, enhanced hemolysis caused by the presence of non-functional CFH was corrected by the addition of purified CFH in a dose-dependent manner, and totally eliminated at high concentrations. By using the novel quantitative hemolytic assay, we have retrospectively analyzed 45 aHUS patients in our cohort. The aHUS is an extremely rare disease, and so far only one hundred patients have been diagnosed across Japan. We are expecting to find merely 10–15 new patients every year, and therefore the prospective analysis is difficult to perform at the moment. This is why we performed retrospective analysis in this study.

Based on the quantitative hemolytic assay, 11 of 45 aHUS patients in Japan had more than 50% (moderate-to-severe) hemolysis, in whom the following gene mutations or abnormalities were identified: two patients (2I1 and X1) with CFH-p.R1215Q, one patient (2M1) with CFH-p.R1215G, five patients (3T1, W1, 2Y1, 2G1 and 2P1) with anti-CFH Ab, one patient (3O1) with C3-p.K1105Q, and two patients (3A1 and 2K1) without any gene mutations. Severe hemolysis was also identified in three asymptomatic family members (Patient 2I's mother and brother 1, and Patient X1's father) with a heterozygous CFH-p.R1215Q mutation. Presently, we are unable to address whether they are asymptomatic carriers or latent patients.

An interesting family (aHUS-2K) was identified by the hemolytic assay as shown in Fig 6. In this family, plasmas of the patient, her father, and two daughters caused severe hemolysis, while the mother's plasma did not cause hemolysis in this assay. However, predisposing mutations were not identified in Patient 2K1, and anti-CFH autoantibodies were negative in all members of this family tested. Roumenina et al. [13] reported that the presence of a hybrid CFH/CFHR1 gene caused appreciable hemolysis of sheep RBCs. Thus, this patient might have a hybrid CFH/CFHR1 gene, or hitherto unrecognized genetic defect, inherited from the paternal side. Notably, without this novel hemolytic assay, evidence of complement-associated

Table 1. Comparison of genetic or acquired abnormalities among Western countries and Japan.

	Frequency			
Genetic or acquired abnormalities	Noris et al (n = 273) Italy	Maga et al (n = 144) USA	Fremeaux-Bacchi et al (n = 214) France	Yoshida et al (n = 45) Japan
CFH	24%	27%	28%	7%
anti-CFH Ab	3%	ND	7%	13%
C3	4%	2%	8%	42%
MCP	7%	5%	9%	5%
THBD	5%	3%	0%	7%
CFB	0.4% (n = 1)	4%	2%	2%
CFI	4%	8%	8%	0%
unidentified	~30%	54%	34%	24%

The frequency of each genetic or acquired abnormality in our aHUS cohort in Japan was compared to that in three other cohorts (Italy [32], USA [33], France [34]). The data reported by Noris et al. included 47 secondary aHUS patients. Sixty-percent of aHUS patients in our cohort were enrolled from West Japan. In the data of Japan, both predisposing and potentially predisposing mutations were counted. Although two patients (2V1 and H2) had two predisposing mutations (C3-p.I1157T and THBD-p.D486Y), they were counted as C3 group.

CFH: complement factor H, Ab: antibody, C3: complement component C3, MCP: membrane cofactor protein, THBD: thrombomodulin, CFB: complement factor B, CFI: complement factor I, ND: no data

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aHUS in this patient would have been difficult to obtain. The C3-p.K1105Q mutation in Patient 3O1 is a unique exception, which will be discussed later.

The remaining 34 aHUS patients had less than 50% (almost none-to-mild) hemolytic reactions, in whom the following predisposing gene mutations were identified: 16 patients with C3-p.I1157T, two patients (3C1 and 2X1) with THBD-p.D.486Y, one patient (2D1) with MCP-p.Y189D and nine patients with no mutations and no CFH autoantibodies. Moreover, one patient (3G1) had anti-CFH autoantibodies.

This is the first report that a patient with the C3 mutation, C3-p.K1105Q shows severe hemolytic activity (Figs 3 and 4), because usefulness of the hemolytic assay has been mostly referred to functional defects of CFH in previous publications [12, 13]. The C3-p.K1105Q mutation is located in the TED of C3 as shown in S2A Fig. A structural model of the complex of C3d with CFH-SCR19-20 showed that the C3-p.K1105Q mutation was positioned at the contact interface between C3d and CFH-SCR19, suggesting the defect of C3d binding to CFH-SCR19 (S2C Fig). In contrast, the p.I1157T mutation identified in 16 aHUS patients with low (less than 50%) hemolytic activity was positioned at the contact interface between C3b and CFH-SCR4, suggesting the defect of C3b binding to CFH-SCR1-4 (S2B Fig). Based on these differences, we assume that C3-p.K1105Q mutant with defective binding to CFH-SCR19 could induce the severe hemolysis.

We also identified interesting patients of 3B1 with MCP mutation and 3C1 with THBD mutation with mild hemolysis (25–50%), because both of these transmembrane proteins do not directly affect *in vitro* hemolysis. Thus, presently the mechanism of mild hemolysis shown in these plasma samples left unaddressed.

We found that the frequency of C3 mutation in aHUS patients in Japan (42%, 19/45) was much higher than that in other aHUS cohorts in Western countries [32–34] (Table 1). Moreover, Schramm et al. recently reported the frequency of C3 mutation in the French, Italian, UK,

and USA aHUS cohorts to be 11.4%, 4.6%, 6.1% and 4.5%, respectively [35]. As shown in S1 Fig, the patients with C3-p.I1157T mutation were only found in the Kansai district, West Japan [31] and not found in other areas of Japan, suggesting a reflection of 'founder effect'. Now, we have started to perform a cohort study of aHUS in East Japan.

In summary, we report that 24% (11/45) of aHUS patients in Japan have moderate-to-severe (≥50%) hemolysis, whereas the remaining 76% (34/45) patients had mild or almost no hemolysis (<50%). In the former group, this was largely attributed to CFH abnormalities, whereas in the latter group, 16 patients carried C3-p.I1157T mutation, which was identified by a simple RFLP analysis. We assume that this novel quantitative hemolytic assay would be more useful for the diagnosis of aHUS in Caucasians, who have a higher proportion of CFH mutations.

Supporting Information

S1 Fig. Geographical distribution of patients with atypical hemolytic uremic syndrome (aHUS) in Japan. Each aHUS patient is described as a black or red circle. Sixteen patients with C3-p.I1157T mutation shown by red circles were found only in the Kansai district, including Mie, Nara, Kyoto, and Osaka prefectures. Interestingly, patients carrying C3-p.I1157T mutation were not found in other areas of West Japan, which may indicate a reflection of 'founder effect'.
(TIF)

S2 Fig. Locations of the C3-p.I1157T and C3-p.K1105Q mutations in the C3b-complement factor H (CFH)-SCR1-4 complex and the C3d-CFH-SCR19-20 complex. (A) A structural model of the complex of C3b and short consensus repeat (SCR) 1–4 of CFH (ID: 2WII). The α and β chains of C3b are shown in cyan and blue, respectively and the SCR1-4 domains are depicted with gray, red, orange, and magenta, respectively. The I1157 and K1105 residues shown by the grey-labeled side chains are located in the thioester-containing domain (TED) in C3b. **(B) Close-up view of the region around the contact interface between C3b and SCR1-4.** The p.I1157T mutation identified in 16 patients with low (less than 50%) hemolytic activity, but not the p.K1105Q mutation identified in a patient with high (100%) hemolytic activity, is positioned at the interface between C3b and SCR1-4 and would interfere the C3b binding to CFH-SCR1-4. **(C) A structural model of the complex of C3d and SCR19-20 of CFH (ID: 3OXU).** C3d, SCR19, and SCR20 are depicted with cyan, blue, and yellow, respectively. The p.K1105Q mutation, but not the p.I1157T mutation, is positioned at the interface between C3d and SCR19-20, and would interfere the C3b binding to CFH-SCR19-20. Diagram was generated with the PyMOL molecular visualization system.
(TIF)

S1 Table. The levels of C3 and complement factor H (CFH) protein in 43 of 45 patients with atypical hemolytic uremic syndrome (aHUS). C3 level was determined by immune-nephelometry (SRL, Inc., Japan), and CFH level was measured by Laurell's immunoelectrophoresis using rabbit anti-CFH serum prepared in our laboratory. The levels of C3 and CFH in 43 aHUS patients and the mean ± standard deviation of these patients and normal plasma from 20 healthy individuals were described. CFH: complement factor H, C3: complement component C3, NP: normal plasma, ND: not determined
(TIF)

S2 Table. Plasmids and primers for expression of recombinant complement factor H (CFH) and its domains. SCR: short consensus repeat, CFH: complement factor H, Pk tag: GKPIPNPLLGLDST sequence, aa: amino acids.
(TIF)

S3 Table. Characteristics of five of six patients with anti-complement factor H (CFH) autoantibodies according to ELISA. Determination of CFH autoantibody titer was performed by CFH-IgG ELISA kit (Abnova). Antibody titer was calculated according to the manufacturer's protocol by using standard curve. AU: arbitrary unit, ND: not determined.
(TIF)

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Author Contributions

Conceived and designed the experiments: TM MM TK YF. Performed the experiments: YY HSI YU YO. Analyzed the data: YY TM MM HSI YU YO TK YF. Contributed reagents/materials/analysis tools: TM MM TK YF. Wrote the paper: YY TM MM YO TK YF.

References

1. Moake JL. Thrombotic microangiopathies. N Engl J Med. 2002; 347: 589–600. doi: 10.1056/NEJMra020528 PMID: 12192020.
2. Coppo P, Veyradier A. Thrombotic microangiopathies: towards a pathophysiology-based classification. Cardiovasc Hematol Disord Drug Targets. 2009; 9: 36–50. doi: 10.2174/187152909787581318 PMID: 19275576.
3. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med. 1998; 339: 1585–1594. doi: 10.1056/NEJM199811263392203 PMID: 9828246.
4. Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature. 2001; 413: 488–494. doi: 10.1038/35097008 PMID: 11586351.
5. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009; 361: 1676–1687. doi: 10.1056/NEJMra0902814 PMID: 19846853.
6. Kavanagh D, Goodship T. Genetics and complement in atypical HUS. Pediatr Nephrol. 2010; 25: 2431–2442. doi: 10.1007/s00467-010-1555-5 PMID: 20526633.
7. Jozsi M, Licht C, Strobel S, Zipfel SL, Richter H, Heinen S, et al. Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. Blood. 2008; 111: 1512–1514. doi: 10.1182/blood-2007-09-109876 PMID: 18006700.
8. Dragon-Durey MA, Blanc C, Marliot F, Loirat C, Blouin J, Sautes-Fridman C, et al. The high frequency of complement factor H related CFHR1 gene deletion is restricted to specific subgroups of patients with atypical haemolytic uraemic syndrome. J Med Genet. 2009; 46: 447–450. doi: 10.1136/jmg.2008.064766 PMID: 19435718.
9. Moore I, Strain L, Pappworth I, Kavanagh D, Barlow PN, Herbert AP, et al. Association of factor H autoantibodies with deletions of CFHR1, CFHR3, CFHR4, and with mutations in CFH, CFI, CD46, and C3 in patients with atypical hemolytic uremic syndrome. Blood. 2010; 115: 379–387. doi: 10.1182/blood-2009-05-221549 PMID: 19861685.
10. Lemaire M, Fremeaux-Bacchi V, Schaefer F, Choi M, Tang WH, Le Quintrec M, et al. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. Nat Genet. 2013; 45: 531–536. doi: 10.1038/ng.2590 PMID: 23542698.
11. Loirat C, Fremeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011; 6: 60. doi: 10.1186/1750-1172-6-60 PMID: 21902819.
12. Sanchez-Corral P, Gonzalez-Rubio C, Rodriguez de Cordoba S, Lopez-Trascasa M. Functional analysis in serum from atypical Hemolytic Uremic Syndrome patients reveals impaired protection of host cells associated with mutations in factor H. Mol Immunol. 2004; 41: 81–84. doi: 10.1016/j.molimm.2004.01.003 PMID: 15140578.

13. Roumenina LT, Roquigny R, Blanc C, Poulain N, Ngo S, Dragon-Durey MA, et al. Functional evaluation of factor H genetic and acquired abnormalities: application for atypical hemolytic uremic syndrome (aHUS). Methods Mol Biol. 2014; 1100: 237–247. doi: 10.1007/978-1-62703-724-2_19 PMID: 24218264.

14. Fujimura Y, Matsumoto M. Registry of 919 patients with thrombotic microangiopathies across Japan: database of Nara Medical University during 1998–2008. Intern Med. 2010; 49: 7–15. doi: 10.2169/internalmedicine.49.2706 PMID: 20045995.

15. Sawai T, Nangaku M, Ashida A, Fujimaru R, Hataya H, Hidaka Y, et al. Diagnostic criteria for atypical hemolytic uremic syndrome proposed by the Joint Committee of the Japanese Society of Nephrology and the Japan Pediatric Society. Clin Exp Nephrol. 2014; 18: 4–9. doi: 10.1007/s10157-013-0911-8 PMID: 24343712.

16. Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, et al. Age, gender, and body length effects on reference serum creatinine levels determined by an enzymatic method in Japanese children: a multicenter study. Clin Exp Nephrol. 2011; 15: 694–699. doi: 10.1007/s10157-011-0452-y PMID: 21505953.

17. Scully M, Goodship T. How I treat thrombotic thrombocytopenic purpura and atypical haemolytic uraemic syndrome. Br J Haematol. 2014; 164: 759–766. doi: 10.1111/bjh.12718 PMID: 24387053.

18. Campistol JM, Arias M, Ariceta G, Blasco M, Espinosa M, Grinyo JM, et al. An update for atypical haemolytic uraemic syndrome: diagnosis and treatment. A consensus document. Nefrologia. 2013; 33: 27–45. doi: 10.3265/Nefrologia.pre2012 PMID: 23364625.

19. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975; 256: 495–497. PMID: 1172191.

20. Mumberg D, Muller R, Funk M. Regulatable promoters of Saccharomyces cerevisiae: comparison of transcriptional activity and their use for heterologous expression. Nucleic Acids Res. 1994; 22: 5767–5768. doi: 10.1093/nar/22.25.5767 PMID: 7838736.

21. Li MZ, Elledge SJ. Harnessing homologous recombination in vitro to generate recombinant DNA via SLIC. Nat Methods. 2007; 4: 251–256. doi: 10.1038/nmeth1010 PMID: 17293868.

22. Ohtsuki K, Kasahara K, Shirahige K, Kokubo T. Genome-wide localization analysis of a complete set of Tafs reveals a specific effect of the taf1 mutation on Taf2 occupancy and provides indirect evidence for different TFIID conformations at different promoters. Nucleic Acids Res. 2010; 38: 1805–1820. doi: 10.1093/nar/gkp1172 PMID: 20026583.

23. Ohyama Y, Kasahara K, Kokubo T. Saccharomyces cerevisiae Ssd1p promotes CLN2 expression by binding to the 5'-untranslated region of CLN2 mRNA. Genes Cells. 2010; 15: 1169–1188. doi: 10.1111/j.1365-2443.2010.01452.x PMID: 20977549.

24. Fan X, Yoshida Y, Honda S, Matsumoto M, Sawada Y, Hattori M, et al. Analysis of genetic and predisposing factors in Japanese patients with atypical hemolytic uremic syndrome. Mol Immunol. 2013; 54: 238–246. doi: 10.1016/j.molimm.2012.12.006 PMID: 23314101.

25. Udagawa T, Motoyoshi Y, Matsumura Y, Takei A, Ariji S, Ito E, et al. Effect of eculizumab and recombinant human soluble thrombomodulin combination therapy in a 7-year-old girl with atypical hemolytic uremic syndrome due to anti-factor H autoantibodies. CEN Case Reports. 2014; 3: 110–117. doi: 10.1007/s13730-013-0097-7

26. Wu J, Wu YQ, Ricklin D, Janssen BJ, Lambris JD, Gros P. Structure of complement fragment C3b-factor H and implications for host protection by complement regulators. Nat Immunol. 2009; 10: 728–733. doi: 10.1038/ni.1755 PMID: 19503104.

27. Kajander T, Lehtinen MJ, Hyvarinen S, Bhattacharjee A, Leung E, Isenman DE, et al. Dual interaction of factor H with C3d and glycosaminoglycans in host-nonhost discrimination by complement. Proc Natl Acad Sci U S A. 2011; 108: 2897–2902. doi: 10.1073/pnas.1017087108 PMID: 21285368.

28. Jozsi M, Strobel S, Dahse HM, Liu WS, Hoyer PF, Oppermann M, et al. Anti factor H autoantibodies block C-terminal recognition function of factor H in hemolytic uremic syndrome. Blood. 2007; 110: 1516–1518. doi: 10.1182/blood-2007-02-071472 PMID: 17495132.

29. Strobel S, Hoyer PF, Mache CJ, Sulyok E, Liu WS, Richter H, et al. Functional analyses indicate a pathogenic role of factor H autoantibodies in atypical haemolytic uraemic syndrome. Nephrol Dial Transplant. 2010; 25: 136–144. doi: 10.1093/ndt/gfp388 PMID: 19666655.

30. Oka M, Ohtsuka Y, Inada Y, Sato T, Yoshida Y, Fujimura Y, et al. A case of DEAP-HUS with CFH autoantibodies and the homozygous deletion of the CFHR1 gene. Nihon Shoni Jinzobyo Gakkai Zasshi. 2013; 26: 285–291. http://dx.doi.org/10.3165/jjpn.26.285.

31. Matsumoto T, Fan X, Ishikawa E, Ito M, Amano K, Toyoda H, et al. Analysis of patients with atypical hemolytic uremic syndrome treated at the Mie University Hospital: concentration of C3 p.I1157T mutation. Int J Hematol. 2014; 100: 437–442. doi: 10.1007/s12185-014-1655-2 PMID: 25135378.

32. Noris M, Caprioli J, Bresin E, Mossali C, Pianetti G, Gamba S, et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Am Soc Nephrol. 2010; 5: 1844–59. doi: 10.2215/CJN.02210310 PMID: 20595690.

33. Maga TK, Nishimura CJ, Weaver AE, Frees KL, Smith RJ. Mutations in alternative pathway complement proteins in American patients with atypical hemolytic uremic syndrome. Hum Mutat. 2010; 31: E1445–60. doi: 10.1002/humu.21256 PMID: 20513133.

34. Fremeaux-Bacchi V, Fakhouri F, Garnier A, Bienaime F, Dragon-Durey MA, Ngo S, et al. Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide French series comparing children and adults. Clin J Am Soc Nephrol. 2013; 8: 554–62. doi: 10.2215/CJN.04760512 PMID: 23307876.

35. Schramm EC, Roumenina LT, Rybkine T, Chauvet S, Vieira-Martins P, Hue C, et al. Functional mapping of the interactions between complement C3 and regulatory proteins using atypical hemolytic uremic syndrome-associated mutations. Blood. 2015 (in press). doi: 10.1182/blood-2014-10-609073 PMID: 25608561.

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Familial C3 glomerulonephritis associated with mutations in the gene for complement factor B

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ABSTRACT

We report the first case of familial C3 glomerulonephritis (C3GN) associated with mutations in the gene for complement factor B (CFB). A 12-year-old girl was diagnosed with biopsy-proven C3GN. Her mother had a history of treatment for membranoproliferative glomerulonephritis, and her brother had hypocomplementemia without urinary abnormalities. DNA analysis revealed heterozygosity for CFB p.S367R in the patient, mother and brother. Evaluation of the structure–function relationship supports that this mutation has gain-of-function effects in CFB. The present case suggests that CFB has an important role in the etiology of C3GN and provides a new insight into anticomplement therapy approaches.

Keywords: C3 glomerulonephritis, complement alternative pathway, complement factor B, genetic mutation

BACKGROUND

C3 glomerulonephritis (C3GN) is a recently described disorder that results from dysregulation of the complement alternative pathway (AP) and is typically characterized by dominant C3 deposition with an absence or paucity of immunoglobulin deposition measured by immunofluorescence (IF) [1–6].

The continuous low-level activation of AP in plasma is tightly regulated by activating and regulatory complement proteins such as complement factor B (CFB), complement factor H (CFH), complement factor I (CFI) and membrane cofactor protein (MCP). CFB, a key molecule that activates the early stages of AP, is cleaved by complement factor D (CFD) into two fragments: Ba and Bb. Bb, a serine protease, then combines with C3b to generate C3 convertase, leading to the generation of a membrane attack complex. The causes of AP dysregulation in previous reported cases of C3GN include mutations in complement genes and autoantibodies that stabilize C3 convertase, or autoantibodies that affect pathway inhibition [4–6].

Here, we report a case of familial C3GN associated with mutations in the *CFB* genes. To the best of our knowledge, this is the first case report that demonstrates the involvement of CFB in the etiology of C3GN.

CASE

A 9-year-old girl was found to have proteinuria and hematuria during the Japanese school urinary screening system in May 2009. At the initial visit to a local hospital, her serum C3 level was low at 5 mg/dL (normal range, 65–135 mg/dL). In April 2012, at the age of 12 years, she developed massive proteinuria with gross hematuria and was referred to our hospital.

She had no symptoms at admission, and physical examination revealed no edema. Laboratory investigation on admission was as follows: serum total protein, 5.05 g/dL; serum albumin, 2.30 g/dL and serum creatinine, 0.43 mg/dL. By urinalysis, protein excretion was 2.1 g/day. Immunological evaluation was as follows: C3, 15 mg/dL; C4, 16 mg/dL (normal range, 13–35 mg/dL); hemolytic complement activity (CH50), 19 U/mL (normal range, 30–45 U/mL) and antinuclear antibody titer, <20-fold. Soluble C5b-9 was markedly elevated at 1.61 mg/L compared with normal healthy controls (0.87 ± 0.22 mg/L, $n = 5$). Levels of soluble C5b-9 were determined by using a BD OptEIA™ human C5b-9 ELISA Set (BD Biosciences, San Diego, CA).

A kidney biopsy was performed and light microscopy showed membranoproliferative glomerulonephritis (MPGN); global mesangial proliferation and segmental endocapillary proliferation with lobular formation and remarkable double

contour of glomerular basement membrane (Figure 1). IF analysis revealed dominant C3 deposition with an absence of immunoglobulin deposition, and electron microscopy demonstrated subendothelial, mesangial and intramembranous electron-dense deposits. She has been treated with drugs including steroid and cyclosporine for 2 years, and has now achieved partial remission with u-P/Cr of 0.2 g/g Cre and no hematuria despite persistent low C3 levels.

Regarding the family history, her mother was diagnosed with MPGN type I (IF findings were not available) in her teenage years, but she had discontinued periodic examination 18 years ago. Recent urinalysis of the mother showed moderate proteinuria with u-P/Cr of 0.5 g/g Cre without hematuria, and laboratory investigation showed hypocomplementemia (C3, 15 mg/dL) with normal kidney function. Her elder brother also had hypocomplementemia (C3, 24 mg/dL), but no urine abnormalities. Her father and younger sister had normal urinalysis and complement levels.

DNA ANALYSIS

DNA analysis was performed under written informed consent and approval of the ethics committee of Nara Prefectural Medical University, Nara, Japan, and the National Cerebral and Cardiovascular Center, Osaka, Japan. All exons of genes that encode the molecules regulating AP, *C3*, *CFB*, *CFH*, *CFI*, *MCP* and *THBD* were analyzed by direct sequencing of amplified genomic DNA obtained from a whole blood sample of the patient [7]. DNA analysis demonstrated heterozygous p.S367R in the *CFB* gene, p.R201S in the *CFI* gene and p.V916I in the *C3* gene (Figure 2A). polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the family members revealed CFB p.S367R in the patient's mother and elder brother, CFI p.R201S in her mother and younger sister and C3 p.V916I in her father (Figure 2B).

DISCUSSION

Here, we describe two cases of C3GN and one case of hypocomplementemia without urine abnormalities that occurred within a family, in which CFB p.S367R was considered to contribute to the dysregulation of AP.

It is highly likely that p.S367R causes a gain of function in CFB through a structure–function relationship. The p.S367R is located in a von Willebrand factor type A (vWfA) domain of the catalytic subunit Bb of CFB. *In vitro* experimental data demonstrated a strong association between mutations in the vWfA domain and gain of function in CFB through the promotion of high-affinity C3 binding [9]. In atypical hemolytic uremic syndrome, which also results from the dysregulation of AP, a recent report of functional analysis of *CFB* mutations revealed that six genetic changes which were concluded to have relevance to disease were all located in the vWfA domain of CFB, suggesting that this domain plays an important role in CFB function [8]. Additionally, structural evaluation demonstrates that p.S367R is located close to p.K323E/Q, one of the mutations that causes CFB gain of function as demonstrated by surface plasmon resonance analysis (Figure 2C) [8]. The p.K323E/Q showed increased resistance to inactivation of C3 convertase by CFH, and a similar functional consequence is considered for p.S367R [8].

In this study, CFB p.S367R was present concurrent with two other genetic changes, CFI p.R201S and C3 p.V916I, in the patient and in various combinations in family members; however, we speculate that these additional two genetic changes are not associated with potential disease relevance. First, the mutations of p.R201S and p.V916I had no impact on the phenotype of her sister and father, respectively. Second, although p.R201S was detected in the patient and her mother, both of whom had C3GN, p.R201S is a polymorphic allele found in Far East populations, with a frequency of about 0.03 in Japan [10]. However, functional assays of mutant CFB, CFI

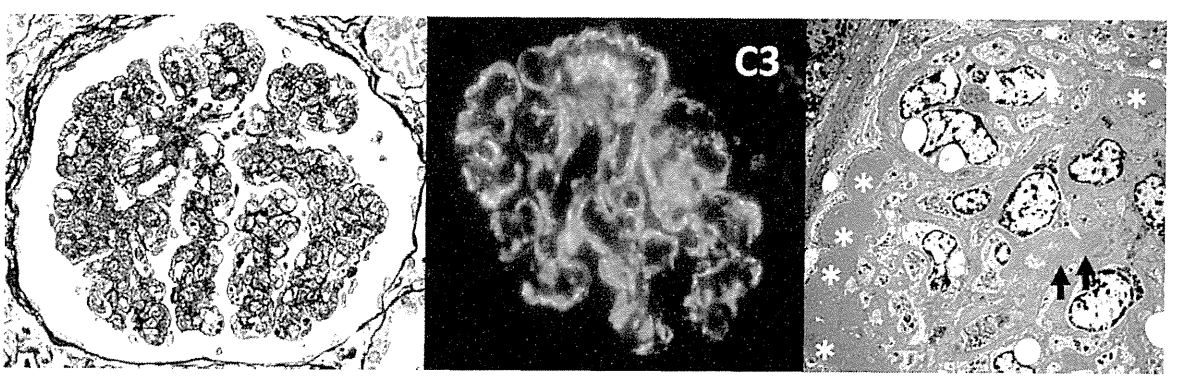

FIGURE 1: Histological findings of the patient. Light microscopy shows global mesangial proliferation and segmental endocapillary proliferation with lobular formation and remarkable double contour of glomerular basement (PAM, magnification ×400; left panel). IF shows dominant C3 deposition in the mesangium and along the capillary walls with negative immunoglobulin deposition (middle panel). Electron microscopy shows remarkable subendothelial electron-dense deposits (asterisk) and mesangial electron-dense deposits (black arrows). Mesangial interposition (white arrows), double contour of glomerular basement membrane and endocapillary hypercellularity are observed (right panel). These findings indicate C3GN rather than dense-deposit disease, because electron-dense deposits were mainly observed not within the glomerular basement membrane but in the subendothelial and mesangial areas, while ribbons of electron-dense transformation of glomerular basement membranes are not found.

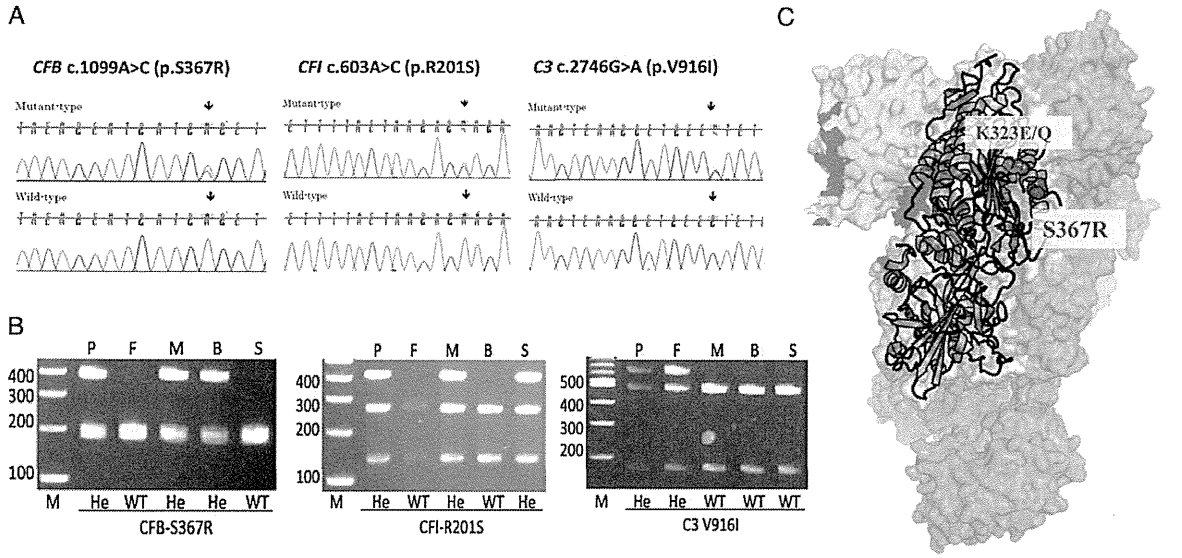

FIGURE 2: DNA analysis of the patient and family members and structural evaluation of CFB S367R. (**A**) Direct sequencing of patient genomic DNA shows heterozygous p.S367R (c.1099 A>C) in exon 8 of the *CFB* gene, p.R201S (c.603 A>C) in exon 4 of the *CFI* gene and p.V916I (c.2746 G>A) in exon 21 of the *C3* gene. The Met encoded by the translation initiation site (start codon) is numbered as residue 1. Upper row: mutant type, lower row: wild type. (**B**) PCR-RFLP analysis of family members shows CFB p.S367R in the mother and elder brother, CFI p.R201S in the mother and younger sister and C3 p.V916I in the father. P, patient; F, father; M, mother; B, elder brother; S, younger sister; WT, wild type; He, heterozygous. (**C**) Visualization of the complex of CFB, CFD and C3b. The figure was prepared using PyMOL (www.pymol.org). Each molecule is represented as follows: CFB, gray; CFD, yellow; C3b, cyan and p.S367R, red sphere. p.S367R is not located attached to CFD or C3b but rather on the surface of CFB and close to p.K323E/Q (blue sphere), of which the gain of function of CFB was proven by surface plasmon resonance analysis in a previous report [8]. p.K323E/Q was described as p.K298E/Q in the original article, with the notation using the nomenclature system of mature proteins.

and C3 to assess their involvement in the activation of AP are required to prove a detailed etiological mechanism.

Some limitations exist in this study. C3NeF and other autoantibodies were not investigated; these autoantibodies may also be associated with our cases, concomitant with *CFB* mutations. Although low C3 levels and elevated soluble C5b-9 levels indicate continuous activity of the AP, detailed complement investigations (e.g. the measurement of Ba, Bb, C3a, C3d and C5a) are required to clarify the complement activation mechanisms more precisely.

In conclusion, this study suggests that CFB has a critical role in AP in the pathogenesis of C3GN and expands our understanding of the genetic factors conferring predisposition to C3GN and supports the development of anticomplement therapies, including those targeting CFB activation.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST STATEMENT

None declared.

REFERENCES

1. Fakhouri F, Frémeaux-Bacchi V, Noël L-H *et al.* C3 glomerulopathy: a new classification. Nat Rev Nephrol 2010; 6: 494–499
2. Sethi S, Fervenza FC, Zhang Y *et al.* Proliferative glomerulonephritis secondary to dysfunction of the alternative pathway of complement. Clin J Am Soc Nephrol 2011; 6: 1009–1017
3. Bomback AS, Appel GB. Pathogenesis of the C3 glomerulopathies and reclassification of MPGN. Nat Rev Nephrol 2012; 8: 634–642
4. Barbour TD, Pickering MC, Cook HT. Recent insights into C3 glomerulopathy. Nephrol Dial Transplant 2013; 28: 1685–1693
5. Sethi S, Nester CM, Smith RJH. Membranoproliferative glomerulonephritis and C3 glomerulopathy: resolving the confusion. Kidney Int 2012; 81: 434–441
6. Pickering MC, D'Agati VD, Nester CM *et al.* C3 glomerulopathy: consensus report. Kidney Int 2013; 84: 1079–1089
7. Fan X, Yoshida Y, Honda S *et al.* Analysis of genetic and predisposing factors in Japanese patients with atypical hemolytic uremic syndrome. Mol Immunol 2013; 54: 238–246
8. Marinozzi MC, Vergoz L, Rybkine T *et al.* Complement factor B mutations in atypical hemolytic uremic syndrome—disease-relevant or benign? J Am Soc Nephrol 2014; 25: 2053–2065
9. Hourcade DE, Mitchell LM, Oglesby TJ. Mutations of the type A domain of complement factor B that promote high-affinity C3b-binding. J Immunol 1999; 162: 2906–2911
10. Yuasa I, Nakagawa M, Umetsu K *et al.* Molecular basis of complement factor I (CFI) polymorphism: one of two polymorphic suballeles responsible for CFI A is Japanese-specific. J Hum Genet 2008; 53: 1016–1021

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Comment on de Vries et al, page 3949

GWA study for ADAMTS13 activity

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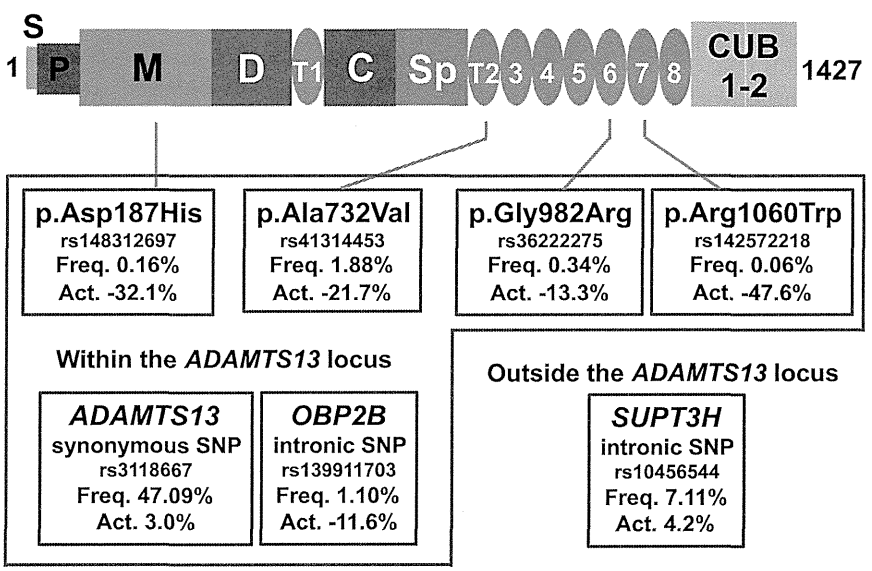
In this issue of *Blood*, de Vries et al report the contribution of genetic variants to plasma ADAMTS13 (disintegrin and metalloproteinase with thrombospondin motifs 13) activity with a hypothesis-free genome-wide association (GWA) approach using the Rotterdam Study, a large population-based cohort study.[1]

ADAMTS13 is a plasma metalloprotease that specifically cleaves the plasma adhesive protein von Willebrand factor (VWF). VWF is a large plasma glycoprotein and mediates platelet adhesion at sites of vascular injury. VWF is synthesized in an ultralarge multimeric form that has a very strong platelet aggregation activity. VWF-induced platelet aggregation depends on its multimeric size, which is controlled by ADAMTS13.[2] ADAMTS13 has a discrete domain structure, and the gene consists of 29 exons located on chromosome 9. Severe deficiency of ADAMTS13 activity due to *ADAMTS13* variants or acquired autoantibodies that inhibit ADAMTS13 activity leads to thrombotic thrombocytopenic purpura (TTP), a disease caused by platelet aggregation by ultralarge VWF multimers. Rare causative loss-of-function genetic variants have been identified in patients with congenital TTP.[3] In addition to TTP, ADAMTS13 may contribute to other thrombotic disorders. Low plasma levels of ADAMTS13 are associated with an increased risk for myocardial infarction and stroke. In addition, low plasma level is observed in severe sepsis, disseminated intravascular coagulation, and complicated malarial infection.[4] These findings suggest a contribution of VWF-dependent platelet aggregation in some thrombotic disease states. If so, the identification of genetic and acquired factors that affect plasma ADAMTS13 activity is very important.



ADAMTS13 domain structure and functional genetic variants. Variants boxed with green are located within the *ADAMTS13* locus. Variants boxed with red are significantly associated common variants among genome-wide SNPs. Variants boxed with blue are significantly associated rare variants among exome-wide SNPs. Act., percentage of increased or decreased plasma ADAMTS13 activity; C, Cys-rich domain; CUB, complement C1r/C1s; D, disintegrin-like domain; Freq., allele frequency; M, metalloprotease domain; P, propeptide; S, signal peptide sequence; Sp, spacer domain; T, thrombospondin type 1 repeat.

So far, rare variants of the *ADAMTS13* gene causing TTP have been identified. In addition, a few common genetic variants with modest effects on ADAMTS13 are reported. However, it is not clear whether the genetic variants exhibit strong associations at the locus. Furthermore, genetic variations outside the *ADAMTS13* locus remain unknown.

In this issue, de Vries et al adopted a systematic hypothesis-free GWA study approach to identify genetic variants that affect plasma ADAMTS13 activity by using a large, prospective, population-based cohort study, the Rotterdam Study.[1] ADAMTS13 activity was measured using a fluorogenic peptidyl substrate (fluorescence resonance energy transfer substrate [FRETS]-VWF73)[5] in >6000 individuals. Genome-wide single nucleotide polymorphisms (SNPs) for common genetic variants and exome-wide SNPs for rare genetic variants were genotyped. After careful examination of these genotyped data, de Vries et al identified p.Ala732Val (rs41314453) in *ADAMTS13* as the strongest genetic determinant of ADAMTS13 activity; the minor allele was associated with a decrease of >20% (see figure). Furthermore, they identified independent associations with a common variant in *SUPT3H* outside the *ADAMTS13* locus and 5 genetic variants at the *ADAMTS13* locus. The variant p.Ala732Val in *ADAMTS13* explained 3.6% to 6.5% of the variance in ADAMTS13 activity, which was comparable to the variance explained by age (3.9%-6.5%) or by sex (4.5%-6.7%). The 4 independently significant common SNPs (boxed with red in the figure) explained 5.8% to 8.2% of the variance.

The genetic variants influencing plasma ADAMTS13 activity have been mostly restricted to the *ADAMTS13* locus (see figure). The only exception was *SUPT3H* outside the *ADAMTS13* locus, and its effect was relatively small. This restriction is in sharp contrast to genetic factors influencing VWF. Genome-wide analysis for plasma VWF levels showed that multiple loci are involved.[6] Various steps, including VWF synthesis, packaging into Weibel-Palade bodies, secretion, and removal from the circulation, are involved in determining VWF levels,[7] and genetic variants of proteins involved in these steps could affect plasma VWF levels. ADAMTS13 activity may be

regulated in a much simpler manner than VWF.

Because rare genetic variants have generally emerged relatively recently, they show greater geographic clustering than common variants. A previous study done in the Japanese population showed that p.Pro475Ser in the *ADAMTS13* gene, restricted to the East Asian population and having an allele frequency of 5%, was associated with 14% decreased activity.[8] In Northern and Central European countries, the E1382Rfs*6 mutation due to the 4143insA mutation is frequent among patients with TTP.[9] The present study did not identify these variants, probably because all the participants were from a small geographic area.

As for the relation to congenital TTP or other thrombotic disorders, 2 rare genetic variants, p.Asp187His and p.Arg1060Trp, have previously been reported in patients with TTP. The present study revealed that their allele frequencies are 0.16% and 0.06%, respectively, indicating that a substantial number of individuals carry these variants in a homozygous or compound heterozygous state and have a genetic risk for TTP. Furthermore, it may be important to prospectively follow these variant heterozygous carriers to determine an increased risk for myocardial infarction or stroke. Thus, the results obtained from the GWA study for ADAMTS13 activity have a big impact for not only TTP but also other thrombotic disorders.

Conflict-of-interest disclosure: T.M. is employed by the National Cerebral and Cardiovascular Center, which has an awarded patent on the use of the reagent FRETS-VWF73. ■

REFERENCES

1. de Vries PS, Boender J, Sonneveld MAH, et al. Genetic variants in the *ADAMTS13* and *SUPT3H* genes are associated with ADAMTS13 activity. *Blood.* 2015;125(25):3949-3955.

2. Crawley JT, de Groot R, Xiang Y, Luken BM, Lane DA. Unraveling the scissile bond: how ADAMTS13 recognizes and cleaves von Willebrand factor. *Blood.* 2011; 118(12):3212-3221.

3. Lotta LA, Garagiola I, Palla R, Cairo A, Peyvandi F. ADAMTS13 mutations and polymorphisms in congenital thrombotic thrombocytopenic purpura. *Hum Mutat.* 2010; 31(1):11-19.

4. Schwameis M, Schörgenhofer C, Assinger A, Steiner M, Jilma B. VWF excess and ADAMTS13 deficiency: a unifying pathomechanism linking inflammation to thrombosis in DIC, malaria, and TTP. *Thromb Haemost.* 2015;113(4):708-718.

5. Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br J Haematol.* 2005;129(1):93-100.

6. Smith NL, Chen MH, Dehghan A, et al; Wellcome Trust Case Control Consortium. Novel associations of multiple genetic loci with plasma levels of factor VII, factor VIII, and von Willebrand factor: The CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium. *Circulation.* 2010;121(12): 1382-1392.

7. Lenting PJ, Christophe OD, Denis CV. von Willebrand factor biosynthesis, secretion, and clearance: connecting the far ends. *Blood.* 2015;125(13):2019-2028.

8. Kokame K, Kokubo Y, Miyata T. Polymorphisms and mutations of ADAMTS13 in the Japanese population and estimation of the number of patients with Upshaw-Schulman syndrome. *J Thromb Haemost.* 2011;9(8): 1654-1656.

9. Schneppenheim R, Kremer Hovinga JA, Becker T, et al. A common origin of the 4143insA ADAMTS13 mutation. *Thromb Haemost.* 2006;96(1):3-6.

Atypical haemolytic uraemic syndrome in a Japanese patient with *DGKE* genetic mutations

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Dear Sirs,

Atypical haemolytic uraemic syndrome (aHUS) is characterised by thrombosis in the microvasculature and is caused by dysregulation of the complement alternative pathway via mutations or autoantibodies. Recessive mutations in the diacylglycerol kinase ε gene (*DGKE*) were recently identified in aHUS patients under two years old (1, 2) as well as in patients with membranoproliferative glomerulonephritis, membranoproliferative-like glomerular microangiopathy, or thrombotic microangiopathy (TMA) (3, 4). A clinical feature of patients with homozygous or compound heterozygous *DGKE* mutations is initial acute kidney injury, typically in children less than one year old. The aHUS caused by *DGKE* mutations is independent of complement dysregulation (5) and the exact mechanism is not known. Loss of DGKE expression in endothelial cells showed a proinflammatory and prothrombotic phenotype, with increased expressions of ICAM-1 and tissue factor (6).

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To obtain additional clinical information for aHUS patients with *DGKE* mutations, we performed a genetic analysis of *DGKE* in Japanese patients with an aHUS onset in the first two years of life. Japan's Nara Medical University has functioned as a TMA referral centre since 1988 (7), and has collected 1,122 Japanese TMA patients until the end of 2013. The database includes 77 patients with aHUS, which is defined by acute renal failure, thrombocytopaenia, and microangiopathic haemolytic anaemia, with no severe ADAMTS13 activity deficiency or Shiga toxin-producing *Escherichia coli* infection. Patients with organ or haematopoietic stem cell transplantation were excluded from aHUS. Some of genetic analyses of aHUS have been previously reported (8, 9). From the database, we selected 14 aHUS patients with a disease onset in the first two years of life. Direct sequencing of the polymerase amplification reaction products was performed using the 3730xl DNA Analyzer (Applied Biosystems Japan, Tokyo, Japan) (8). The study protocol was approved by the Ethical Committee of the National Cerebral and Cardiovascular Center, the Hiroshima Prefectural Hospital, and Nara Medical University, and written informed consents for genetic analysis were obtained.

Among the 14 selected patients, we identified one patient who had a splice site mutation c.1213–2A>G derived from his father and a frameshift mutation c.71delT encoded with p.Leu24Cysfs*145 derived from his mother (▶Figure 1). Both mutations are likely deleterious for the DGKE function and pathogenic loss-of-function mutations. Neither genetic mutation was found in the NCBI database. A DNA sequence analysis in six complement-related genes (*CFH, C3, MCP, CFI, CFB* and *THBD*) showed that the patient had the missense polymorphisms *CFH*: p.Val62Ile, p.Glu936Asp, *CFB*: p.Leu9His, p.Arg32Gln, and *THBD*: p.Ala473Val, but did not have mutations predisposing for aHUS. The patient did not have autoantibodies against complement factor H.

Another aHUS patient who presented aHUS at eight months of age had a missense mutation, p.Ile195Met, in the *DGKE* gene. *In silico* analyses for functional prediction of the missense mutation suggested that the p.Ile195Met mutation in *DGKE* was "tolerated" by the SIFT algorithm (10) and "benign" by the Polyphen-2 algorithm (11). To date, all of the aHUS patients with *DGKE* mutations are in the homozygous or compound heterozygous state, and parents with heterozygous *DGKE* mutation had no clinical abnormalities (5). We thus did not regard a *DGKE* p.Ile195Met mutation as a disease-causing mutation. The remaining 12 patients did not carry the nonsynonymous *DGKE* mutations.

We previously reported the clinical phenotypes of the former patient with compound heterozygous *DGKE* mutations (12). He was a male baby who developed plasmapheresis-resistant aHUS at four months of age and showed extremely severe hypertension. His C3 level was undetectable (<20 mg/dl), and he had high lactate dehydrogenase and creatinine levels. He received repeated plasma infusions and nine sessions of plasmapheresis. However, no treatment was effective for his haemolysis and renal failure. His severe hypertension did not initially respond to fluid removal by haemodiafiltration and was also refractory to treatment with a large intravenous dose of nicardipine chloride, peroral enarapril, and losartan. Finally, treatment with the complement C5 blockage drug eculizumab every three weeks for 17 months resulted in the control of severe hypertension and the cessation of peritoneal dialysis (12). After the administration of eculizumab, the platelet counts and C3 level increased and the lactate dehydrogenase levels decreased.

The number of aHUS patients with *DGKE* mutations who were treated with eculizumab at the acute phase and as maintenance therapy is limited. At the acute phase, one patient showed a negative

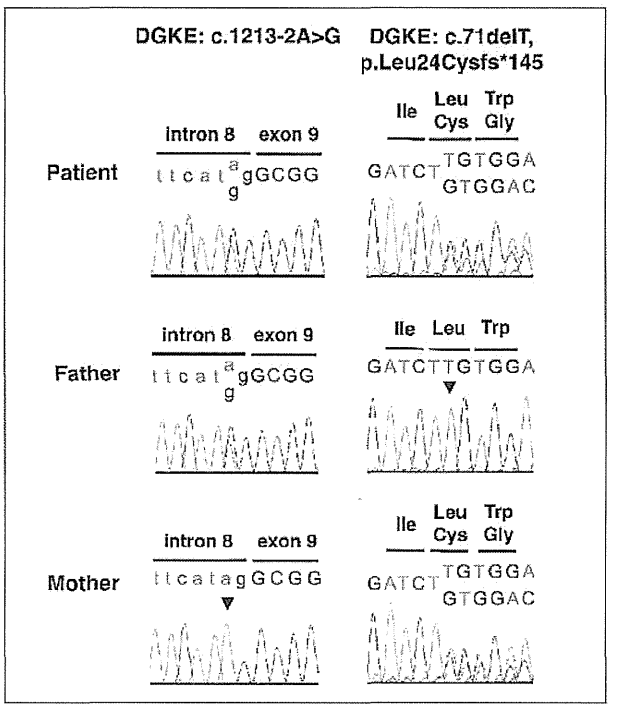

Figure 1: Chromatograms of two mutations in the *DGKE* gene in an affected patient and his parents. Small letters and capital letters are intron and exon, respectively. The A of the ATG translation initiation start site was designated as position +1, and the initial Met was denoted as +1. The c.1213–2A>G mutation disturbs the invariant splicing consensus sequence AG at the end of the intron. The c71delT mutation produces the frameshift, leading to the stop codon downstream at 145 amino acid residues.

response and another showed a positive response (1, 2). Lemaire et al. reported seven aHUS patients with *DGKE* mutations treated with eculizumab (1). None of the patients showed an abnormality in the complement system. In that study, one patient with *DGKE* mutations had aHUS recurrences even after eculizumab treatment. The genetic study of a Spanish aHUS registry reported an aHUS patient with concurrent *DGKE* and *C3* mutations who was treated with eculizumab (2). After presenting with aHUS at eight months of age, she had several aHUS recurrences, and biweekly plasma infusions were effective in normalising blood parameters; subsequent eculizumab treatment resolved the infection-associated edemas that were typical in this patient. Sanchez Chinchilla et al. suggested that the association of *DGKE* mutations concomitant with a *C3* gene mutation in this particular patient possibly contributed to more severe disease with chronic activation of TMA and a positive response to eculizumab treatment.

We report here a patient with *DGKE* mutations who presented plasmapheresis-resistant aHUS and severe hypertension in the first year of life. We did not identify mutations predisposing for aHUS in 6 complement genes in the patient, however he was successfully treated with eculizumab. The treatment strategy for aHUS patients with *DGKE* mutations is not yet settled (1, 2, 5). Further studies are needed to identify the appropriate therapeutic strategies for aHUS patients with *DGKE* mutations.

Conflicts of interest

T. Miyata has received lecturing fees from Bayer, Daiichi Sankyo, Boehringer Ingelheim, Shino-Test, Kyowa Kirin and Bristol-Myers. T. Ohta has received lecturing fees from Asahikasei Pharma, Pfaizer, Alexion Pharma, Daiichi Sankyo, Kyowa Kirin and Kyorin. Y. Fujimura is a recipient of research grant from Alexion Pharmaceuticals. None of the other authors declares a conflict of interest.

References

1. Lemaire M, Fremeaux-Bacchi V, Schaefer F, et al. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. Nat Genet 2013; 45: 531–536.
2. Sanchez Chinchilla D, Pinto S, Hoppe B, et al. Complement mutations in diacylglycerol kinase-epsilon-associated atypical hemolytic uremic syndrome. Clin J Am Soc Nephrol 2014; 9: 1611–1619.
3. Ozaltin F, Li B, Rauhauser A, et al. DGKE variants cause a glomerular microangiopathy that mimics membranoproliferative GN. J Am Soc Nephrol 2013; 24: 377–384.
4. Westland R, Bodria M, Carrea A, et al. Phenotypic expansion of DGKE-associated diseases. J Am Soc Nephrol 2014; 25: 1408–1414.
5. George JN, Nester CM. Syndromes of thrombotic microangiopathy. N Engl J Med 2014; 371: 654–666.
6. Bruneau S, Neel M, Roumenina LT, et al. Loss of DGKε induces endothelial cell activation and death independently of complement activation. Blood 2015; 125: 1038–1046.
7. Fujimura Y, Matsumoto M, Isonishi A, et al. Natural history of Upshaw-Schulman syndrome based on ADAMTS13 gene analysis in Japan. J Thromb Haemost 2011; 9 (Suppl 1): 283–301.
8. Fan X, Yoshida Y, Honda S, et al. Analysis of genetic and predisposing factors in Japanese patients with atypical hemolytic uremic syndrome. Mol Immunol 2013; 54: 238–246.
9. Matsumoto T, Fan X, Ishikawa E, et al. Analysis of patients with atypical hemolytic uremic syndrome treated at the Mie University Hospital: concentration of C3 p.I1157T mutation. Int J Hematol 2014; 100: 437–442.
10. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 2009; 4: 1073–1081.
11. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010; 7: 248–249.
12. Ohta T, Urayama K, Tada Y, et al. Eculizumab in the treatment of atypical hemolytic uremic syndrome in an infant leads to cessation of peritoneal dialysis and improvement of severe hypertension. Pediatr Nephrol 2015; 30: 603–608.

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Case Report

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A Unique Case Involving a Female Patient with Upshaw-Schulman Syndrome: Low Titers of Antibodies against ADAMTS13 prior to Pregnancy Disappeared after Successful Delivery

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Keywords
Upshaw-Schulman syndrome · Pregnancy · ADAMTS13 antibody · *ADAMTS13* gene mutation · Fresh frozen plasma

Summary
Background: Upshaw-Schulman syndrome (USS) is usually suspected based on severe deficiency of ADAMTS13 activity without ADAMTS13 antibody, but the definitive diagnosis is made by *ADAMTS13* gene analysis. We present a unique case of USS with low titers of ADAMTS13 antibodies before pregnancy. Interestingly, titers of ADAMTS13 antibodies decreased to almost undetectable levels after delivery. **Case Report**: In patient LL4, the diagnosis of USS was confirmed at age 27 by *ADAMTS13* gene analysis. She became pregnant at age 30. During the pregnancy, she received regular fresh frozen plasma (FFP) infusion. Plasma von Willebrand factor levels increase as pregnancy progresses. To prevent platelet thrombi, much more ADAMTS13 supplementation is necessary during late gestation in patients with USS. Therefore, we shortened the interval between and increased the volume of FFP infusions as pregnancy progressed. At 39 weeks, she delivered a healthy baby girl. Before pregnancy, she had low titers of both neutralizing and binding anti-ADAMTS13 antibodies. Despite frequent FFP infusions, titers of the antibodies did not increase, but rather decreased to almost undetectable levels during pregnancy. **Conclusion**: Both the neutralizing and binding antibodies against ADAMTS13 decreased to almost undetectable levels after delivery in this patient, which can be caused by an immunological reset.

Introduction

Upshaw-Schulman syndrome (USS) is caused by a deficiency of ADAMTS13 activity due to a mutation in its gene [1]. ADAMTS13 specifically cleaves unusually large von Willebrand factor (VWF) multimers (UL-VWFMs) released from vascular endothelial cells. When ADAMTS13 activity is deficient, UL-VWFMs are not cleaved, which induces platelet thrombi formation in the microcirculation under high shear stress. Deficiency of ADAMTS13 activity is also caused by autoantibodies against ADAMTS13 in patients with acquired thrombotic thrombocytopenic purpura (TTP) [2]. There are two types of ADAMTS13 autoantibodies. One type acts as an inhibitor of ADAMTS13 function, and the other type binds to ADAMTS13, accelerating its clearance from the circulation. USS is usually suspected to be based on severe deficiency of ADAMTS13 activity without the presence of autoantibodies, but the definitive diagnosis is usually made by *ADAMTS13* gene analysis.

USS patients often experience episodes of severe neonatal jaundice with a negative Coombs test requiring an exchange blood transfusion as well as repeated episodes of thrombocytopenia and

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Table 1. Plasma levels of anti-ADAMTS13 autoantibodies

Age, years	Gestational weeks	FFP infusion	ADAMTS13 activity, %	ADAMTS13 inhibitor, BU/ml	ADAMTS13 IgG type antibody, units/ml	Clinical status
21	*	–	<0.5	1.4	42.9	TTP bout
22	*	–	<0.5	1.7	35.0	remission
27	*	–	3.7	0.8	48.9	remission
27	*	–	1.9	1.6	33.3	remission
30	8	–	<0.5	<0.5	28.2	pregnancy
30	10	+	6.5	0.5	34.4	pregnancy
30	11	+	4.5	0.5	31.2	pregnancy
30	13	+	3.4	0.5	19.9	pregnancy
30	15	+	3.3	0.8	30.2	pregnancy
30	20	+	3.2	0.9	23.7	pregnancy
30	24	+	2.9	<0.5	21.6	pregnancy
30	29	+	2.3	0.6	13.9	pregnancy
30	33	+	3	<0.5	19.7	pregnancy
30	38	+	2.9	<0.5	14.9	pregnancy
30	39	+	6.9	0.5	16.1	pregnancy
30	*	–	1.9	0.6	13.9	1 month after delivery
32	*	–	5.2	<0.5	15.4	1.5 years after delivery
32	*	–	1.8	<0.5	9.8	2 years after delivery

microangiopathic hemolytic anemia in childhood that are reversible by infusions of fresh frozen plasma (FFP) (early-onset phenotype) [3]. On the other hand, patients with the 'late-onset phenotype' are diagnosed with USS in adulthood, usually during episodes of infectious disease or pregnancy [3]. Moatti-Cohen et al. [4] reported that the rate of USS is much higher in pregnancy-onset TTP patients than in all adulthood-onset TTP patients.

We previously described 43 USS patients in Japan up to the end of March 2011 [3]. Among them, 9 patients developed bouts of TTP and were correctly diagnosed with USS in association with pregnancy [5]. These pregnancies often result in premature delivery or fetal loss. Recent papers have reported successful delivery with FFP infusion therapy in patients with USS diagnosed prior to pregnancy [6, 7]. However, a detailed therapeutic protocol including FFP infusions for pregnant women with USS has not yet been established.

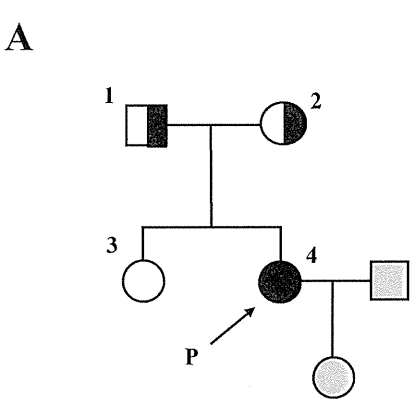
Here, we report a USS patient with low titers of neutralizing (inhibitory) and non-neutralizing (binding) antibodies against ADAMTS13 who successfully underwent delivery with the use of gradually increasing FFP infusions as the pregnancy progressed. The intervals between and volumes of FFP infused were determined by close monitoring of levels of ADAMTS13 activity and its inhibitor.

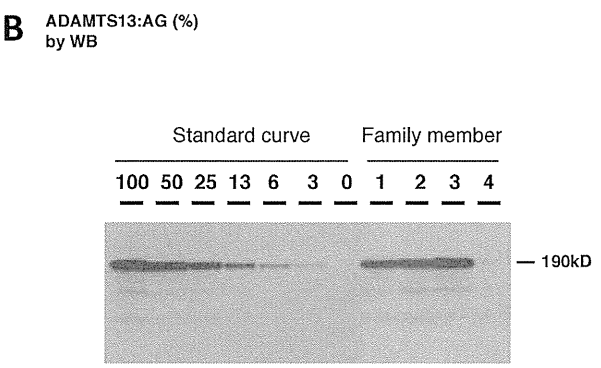
Material and Methods

Until 2005, ADAMTS13 activity was analyzed by a VWF multimer assay with a detection limit of 3% of normal controls [2, 8]. Since 2005, a highly sensitive chromogenic ADAMTS13-act-ELISA [9] with a detection limit of 0.5% of normal was developed and replaced the VWF multimer assay. Thus, we re-examined ADAMTS13 activity in stored plasma samples using this act-ELISA and reported the results by the act-ELISA in this study. Plasma ADAMTS13 inhibitor titers were also re-examined using the chromogenic ADAMTS13-act-ELISA in heat-inactivated plasma at 56 °C for 30 min. One Bethesda unit (BU) of inhibitor was defined as the amount of inhibitor that reduces ADAMTS13 activity to 50% of control [10]. ADAMTS13 inhibitor titers were defined as: <0.5 BU/ml (negative), 0.5–1.0 BU/ml (marginal), and ≥1.0 BU/ml (positive). Plasma levels of ADAMTS13 antigen were determined using a quantitative sandwich ELISA assay [11]. Plasma ADAMTS13 antigen was also analyzed by quantitative and qualitative western blotting (WB) under reducing conditions [12]. Densitometric analysis of ADAMTS13 antigen was performed for the 190 kDa band using NIH imageJ (developed by the National Institutes of Health, *http://rsb.info.gov/nih-image/*). Plasma anti-ADAMTS13 IgG antibody titers (binding antibody) were determined by TECHNOZYM® ADAMTS-13 INH (Technoclone, Vienna, Austria) according to the manufacturer's instructions. In this assay, plasma IgG levels less than 12 units/ml were defined as negative, 12–15 units/ml were considered borderline, and levels greater than 15 units/ml were defined as positive. *ADAMTS13* gene analyses [13] were performed with the permission of the Ethics Committees. The pathogenicity of missense mutations was analyzed in silico using PolyPhen-2 (*http://genetics.bwh.harvard.edu/pph2/*) to predict the functional significance of missense mutations. Written informed consent for *ADAMTS13* gene analysis was obtained from the patient and her family.

Case Report

Proband LL4 is a female born in 1981. Her parents and elder sister are apparently healthy. She did not have any episodes of severe neonatal jaundice requiring exchange blood transfusion. At 14 years of age, she developed thrombocytopenia and acute renal failure requiring hemodialysis during an upper respiratory tract infection. She had similar episodes during upper respiratory tract infections at the ages of 15, 16, 17, and 20 years. These bouts were ameliorated by FFP infusion. At 21 years of age, she was admitted to a local hospital complaining of diarrhea and high-grade fever. She was diagnosed with TTP based on the pentad of hemolytic anemia, thrombocytopenia, acute renal failure, fever, and mild neurological symptoms. Her condition improved with FFP administration. Soon after this episode, she got married. When the patient was 27 years old, detailed investigation including *ADAMTS13* gene analysis was performed in all members of her family. At 28 years of age, she underwent an elective termination at 6 weeks of gestation after the risk of developing TTP was taken into consideration. She has never received prophylactic FFP infusions without the presence of thrombocytopenia.





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	ADAMTS13:AC (%)	ADAMTS13:AG (%)		*ADAMTS13 gene*					
	ELISA	ELISA	WB	Thr339	Cys438	Gln448	Pro475	Pro618	Gly909
-1	27	23	37	T/T	C/S	Q/Q	P/P	P/P	G/G
-2	34	50	51	T/R	C/C	Q/E	P/S	P/A	G/R
-3	57	77	97	T/T	C/C	Q/Q	P/S	P/P	G/G
-4	3.7	1.2	<3	T/R	C/S	Q/E	P/P	P/A	G/R

Fig. 1. Pedigree and ADAMTS13 analysis of USS-LL4 and her family. **A** The propositus (denoted as P), USS-LL4, is the second child of nonconsanguineous parents. Squares and circles indicate males and females, respectively, and shaded symbols represent individuals who were not examined. Half-black symbols indicate asymptomatic carriers. **B** WB analyses of plasma ADAMTS13 antigen (AG) in the patient's family members are shown. **C** ADAMTS13 activity (AC) was determined using activity ELISA, and ADAMTS13 AG levels were measured using ELISA and WB. Results are shown as percentages of normal values. Identified mutations in ADAMTS13 are depicted using one-letter amino acid abbreviations.

ADAMTS13 Activity, Antibody, and Antigen Analysis

Plasmas obtained at 21 and 22 years of age showed severely decreased ADAMTS13 activity (<0.5 % of normal) and low titers of ADAMTS13 inhibitor (1.4 and 1.7 BU/ml, respectively) (table 1). In addition, ADAMTS13 binding IgG antibodies were found in both samples. These results indicated that this patient might have acquired TTP or USS with the presence of ADAMTS13 inhibitor. As shown in figure 1C, plasma ADAMTS13 antigen levels as analyzed by ELISA were 1.2% of normal values in the patient at 27 years of age. Further, plasma levels of ADAMTS13 antigen analyzed by WB were <3% of normal values in the patient.

ADAMTS13 Gene Analysis

We found 6 missense mutations (p.T339R, p.C438S, P.Q448E, p.P475S, p.P618A, and p.G909R) in this family (fig. 1C). Of these, p.T339R, p.Q448E, p. P475S, and p.P618S have been previously reported as single nucleotide polymorphisms (SNPs) in the Japanese population [14]. This patient had two mutations (p.C438S and p.G909R) that appear to be disease-causing mutations that have never been previously reported. We analyzed these two mutations using PolyPhen-2 to predict their effects on ADAMTS13. Both mutations were predicted to be 'probably damaging.' Thus, the patient was a compound heterozygote for two mutations in the *ADAMTS13* gene: p.C438S (c.1313G>C, exon 12) was inherited from her father and p.G909R (c.2725 G>A, exon 21) was inherited from her mother.

Clinical Course in Pregnancy

Although the patient had low levels of ADAMTS13 inhibitor, we diagnosed this patient with USS based on the results of the genetic analysis. Taking into account the risk of TTP, she chose elective abortion for her first pregnancy at 28 years of age. However, when she became pregnant again at the age of 30, she strongly hoped to have a child. After thorough discussions between the hematologists and obstetricians, we decided to continue the pregnancy with close monitoring of her condition and her fetus.

The patient's plasma ADAMTS13 activity was under 0.5% and ADAMTS13 inhibitor was negative at 8 weeks of gestation without FFP infusion. Starting at 9 weeks of gestation, 4 units of FFP were infused (480 ml / 92 kg body weight = 5.2 ml/kg). Between 11 and 17 weeks of gestation, the patient received 6 units of FFP (97 kg, 7.4 ml/kg) biweekly. In this period, ADAMTS13 activity was 3–4% of normal just before FFP infusion. At 17 weeks, she had fever with an upper respiratory infection. Her platelet count suddenly decreased to 141 × 10^9/l. Therefore, she received 6 units of FFP on the next day. Subsequently, 6 units of FFP were infused weekly, with plasma levels of ADAMTS13 activity measured before and after FFP infusion. After 32 weeks of gestation, the volume of FFP infusion increased to 8 units (103 kg, 9.3 ml/kg) per week. In addition to FFP infusion, she took low-dose aspirin (100 mg/day) between 9 and 34 weeks of gestation.

As shown in figure 2, this regimen maintained her platelet count over 200 × 10^9/l. Plasma levels of ADAMTS13 before FFP infusion were 3–5% of normal, and levels after FFP infusion were approximately 10%. The maximum level of ADAMTS13 inhibitor was 0.9 BU/ml at 20 weeks of gestation. Until 29 weeks of gestation, the levels of inhibitor were relatively high. However, after 30 weeks of gestation, inhibitor levels over 0.5 BU/ml were not observed except at 35 weeks (0.6 BU/ml). After delivery, ADAMTS13 inhibitor levels over 0.5 BU/ml were not detected. Moreover, levels of ADAMTS13 binding antibodies before pregnancy were over 30 units/ml (table 1). These levels gradually decreased as the pregnancy progressed, similar to levels of ADAMTS13 inhibitor. At 39 weeks of gestation, she gave birth to a healthy baby girl by cesarean section. She received 8 units of FFP on the day of surgery and 6 units on postoperative days 1, 3, and 5. Prophylactic FFP infusion was then stopped. After delivery, plasma levels of ADAMTS13 activity were maintained between 1.8 and 5.2%, and both ADAMTS13 inhibitor titers and IgG antibodies were almost undetectable on three different occasions without FFP infusion (table 1).

The birth weight of her baby was 3,474 g. External malformations were not found. The ADAMTS13 activity of the umbilical cord was 35.5%, and the level of inhibitor was 0.7 BU/ml. Pathological examination of the placenta revealed only mild infarcts in the periphery and at the insertion of the umbilical cord.