R1210C, A PREVALENT MUTATION IN COMPLEMENT FACTOR H ASSOCIATED WITH ATYPICAL HAEMOLYTIC UREMIC SYNDROME.

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Running title: Prevalent factor H aHUS mutation

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ABSTRACT

Mutations in the gene encoding complement factor H that alter the C3b/polyanions-binding site at the C-terminal region of the protein impair the capacity of factor H to protect host cells and are strongly associated with atypical haemolytic uremic syndrome (aHUS). Most of these aHUS-associated CFH mutations are ‘unique mutations’ normally found in only a single patient or family. One exception is the CFHR1210C mutation that has been found in several unrelated aHUS patients from distinct geographical origins. Here, 5 aHUS pedigrees and 7 individual aHUS patients (represented by 13 affected and 16 healthy CFHR1210C mutation carriers) were analyzed to identify potential correlations of the CFHR1210C mutation with particular clinical phenotypes and to characterize the origins of this mutation. Our results showed that the clinical phenotype of aHUS patients carrying the CFHR1210C mutation was heterogeneous. Interestingly, 12 out of 13 patients carry at least one additional genetic factor conferring predisposition to aHUS. These data are in accord with the low penetrance of aHUS (30%) in the CFHR1210C mutation carriers, indicating that the presence of other genetic or environmental risk factors significantly contribute to the manifestation and severity of aHUS in CFHR1210C mutation carriers. Genotype analysis of CFH and CFHR3 SNPs in the 12 unrelated carriers suggests that the CFHR1210C mutation has a single origin. In conclusion, CFHR1210C is a prevalent and prototypic aHUS mutation that may be present, as a rare polymorphism, in many human populations.
INTRODUCTION

HUS is characterized by thrombocytopenia, Coomb’s test negative microangiopathic haemolytic anemia and acute renal failure. The typical form of HUS follows a diarrhoeal prodrome and is associated with Shiga toxin-producing 0157:H7 E. coli infections. However, five to ten percent of HUS patients lack an association with infection. This atypical form of HUS (aHUS) occurs in both adults and in young children and has a poor prognosis. Recurrent episodes of aHUS are common with a mortality rate that approaches 30%. Endothelial cell injury appears to be the primary event in the pathogenesis of HUS. The endothelial damage triggers a cascade of events that result in the formation of platelet-fibrin hyaline microthrombi that occlude arterioles and capillaries. A hallmark of HUS is the presence of schistocytes (fragmented cells) that generate as the red blood cells traverse these partially occluded microvessels.

aHUS is associated with mutations or polymorphisms in the genes encoding the complement regulatory proteins factor H (CFH), membrane cofactor protein (MCP, CD46), and factor I (IF, CFI), and with mutations in the complement activating components factor B (BF, CFB) and C3. Importantly, mutations in CFH, MCP (CD46) and IF (CFI) are loss-of-function mutations while mutations in BF (CFB) are gain-of-function mutations. In addition, autoantibodies and deletion of the CFHR1-CFHR3 genes are associated with aHUS.

Missense mutations in the exons encoding the C-terminal region of factor H are the commonest genetic alteration among aHUS patients. Carriers of these CFH mutations express factor H molecules that present normal regulatory activity in plasma but a limited capacity to protect cells from complement lysis. The combination of both an active complement system in plasma and a defective protection of cellular surfaces is critical in the pathogenesis of aHUS. In a situation that triggers complement activation, such as during bacterial or viral infections, exposure to drugs that cause endothelial activation and damage or the presence of immune complexes,
deposition and amplification of C3b on the microvasculature cellular surfaces cannot be controlled and this results in tissue damage and destruction.

$CFH_{R1210C}$ is the prototypic aHUS-associated $CFH$ mutation. Functional analysis demonstrated that R1210C mutant factor H has normal cofactor activity but defective binding to C3b, heparin and endothelial cells. A unique feature of the R1210C factor H mutant is its capacity to form covalent heterodimers with serum albumin through the cysteine residue generated by the mutation. Interestingly, $CFH_{R1210C}$ is a common mutation that has been described in several unrelated aHUS patients from different countries.

Here we have both examined whether there is a common clinical phenotype associated with $CFH_{R1210C}$ and analyzed the origins of this mutation. To this end we evaluated in 13 aHUS patients and 16 healthy carriers the clinical phenotypes, determined the $CFH$ genotypes associated with $CFH_{R1210C}$ and analyzed the concurrence of other known aHUS risk factors. These include mutations and polymorphisms in the $CFH$, $MCP$ ($CD46$) and $IF$ ($CFI$) genes, and genomic rearrangements in the $CFH$-$CFHR1$-$5$ genomic region.
RESULTS AND DISCUSSION

Our series of aHUS patients carrying the $CFH_{R1210C}$ mutation includes 13 affected individuals comprising 11 unrelated individuals (R016, S006, H29IV-1, D45348, D40308, S198, S197, S223, S196, FHUSA11 and 6089) and two brothers (F106 and F108). These patients were identified in routine $CFH$ mutation analyses of aHUS patients. They belong to 5 aHUS cohorts, including approximately 800 unrelated patients, from France, Germany, Italy, Spain and the UK (See Patients, Materials and Methods). The overall number of patients who carry the $CFH_{R1210C}$ mutation ranges from 1 to 4% in the different cohorts, which represents an estimable 5 to 15% of all the patients carrying mutations in $CFH$ and illustrates the high prevalence of the $CFH_{R1210C}$ mutation among atypical HUS patients.

Age at onset ranges from 6 months to 53 years, with 6 patients developing the disease under 13y old and 7 patients over 21y old. Eight patients developed end stage renal disease (ESRD) needing dialysis, following their initial presentation (R016, D45348, S198, S223 and FHUSA11) or following recurrent episodes (S006, S197 and F108). Three patients developed chronic renal insufficiency (CRI) needing dialysis following their initial presentation (S196 and D40308) or several recurrences (H29IV-1). In contrast, patient 6089 had a single episode and recovered renal function without sequelae and patient F106 had two episodes that also resolved without any renal sequelae. Time interval to reach ESRD or CRI from the initial episode ranged from 3mo (H29IV-1) to 15 years (R016 and F108). Four patients (S198, R016, D45348 and FHUSA11) have been transplanted and three of them had a recurrence in the allograft. Transplantation was successful without recurrence of the disease in patient S198 and also in patient D45348 when he was transplanted a second time. In agreement with previous reports $^{16}$ there is an elevated frequency of recurrences in the allograft among $CFH_{R1210C}$ mutation carriers. Patient D45348 was transplanted twice and illustrates the risks associated with living related donors who share aHUS mutations with the receptor. D45348 had a recurrence in the allograft from her mother, with whom she
shares two aHUS risk factors (the $CFH_{R1210C}$ mutation and the $MCP_{ggaac}$ haplotype, Figure 1). In a second unrelated cadaver renal transplant (unlikely carrying the $CFH_{R1210C}$ mutation) she has had no episodes of recurrent HUS and the graft is functioning well.

As a whole, data summarized in Table 2 illustrate significant heterogeneity in the clinical phenotype and severity of the disease in the 13 affected individuals. This does not support a correlation between clinical phenotype and the $CFH_{R1210C}$ mutation. Moreover, $CFH_{R1210C}$ mutation analysis in the relatives of patients R016, F106/F108, H29IV-1, D45348 and S006 demonstrated the presence of 16 asymptomatic $CFH_{R1210C}$ mutation carriers (Figure 1), suggesting that presence of other genetic or environmental risk factors contribute to the manifestation and severity of aHUS in $CFH_{R1210C}$ mutation carriers. Penetrance of the disease among $CFH_{R1210C}$ mutation carriers was estimated to be ~30%.

To determine whether mutations or polymorphisms in other complement genes, previously associated with aHUS, influence the development and severity of aHUS in the $CFH_{R1210C}$ mutation carriers, we undertook mutation screening of $MCP$ ($CD46$) and $IF$ ($CFI$) and genotyped all samples for the $MCP_{ggaac}$, $CFH_{TGTGGT}$ and $ΔCFRH3-CFRH1$ polymorphisms. Figures 1 and 2 and Table 3 summarises these data. Two of our patients are also compound heterozygotes for mutations in $MCP$ (F106 and F108) and a third one carries a mutation in the second $CFH$ allele (H29IV-1) (Table 3).

Convalescent complement C3 levels are clearly decreased only in patients who also carry $MCP$ mutations (F106 and F108) or $CFH_{low}$ expression alleles (H29IV-1), suggesting a constitutive dysregulation of the alternative complement pathway in these patients (Table 2). Interestingly these three individuals and D45348, who is homozygous for $CFH_{R1210C}$, have an early onset of the disease. All but one of the 13 aHUS patients included in this report carry additional aHUS risk factors and, in general, these affected individuals tend to have more aHUS risk factors than their asymptomatic relatives who are $CFH_{R1210C}$ carriers (Figure 1). With this in mind it is interesting that
patient 6089, who does not carry additional known aHUS risk factors, had only a single aHUS episode and recovered renal function without sequelae (Table 2). In addition to CFH haplotypes that predispose to aHUS, we have recently reported the existence of CFH haplotypes that protect from aHUS (CFH-H2 in Table 1)\(^9\). Interestingly, some of the asymptomatic CFHR1210C carriers also have the aHUS low-risk CFHCATAAG haplotype (Figure 1).

Therefore, the data presented here are consistent with previous reports which show that affected individuals carry more than one aHUS risk factor\(^4,5,9\) and strongly support the concept that susceptibility to aHUS results from the additive effect of multiple genetic factors involving plasma and membrane-associated complement regulators. Within the families included in this report there are, however, relatives who apparently share identical genetic susceptibility profiles with the aHUS patients and have not developed the disease (Figure 1). As indicated before, this suggests that there are other, as yet unidentified, genetic or environmental risk factors contributing to manifestation of the disease.

Haplotype analysis using six CFH and two CFHR3 intragenic SNPs enable us to identify three extended CFH haplotypes segregating with CFHR1210C in the affected individuals (Table 3). In families 020, 024 and HUS29, CFHR1210C was associated with a rare CFH haplotype (CFHTGTAAT, H4b* in Table 4) that was absent in a control population of 317 unrelated individuals (Figure 1 and Table 1) and differs from haplotype H4b only at SNP 2808G>T. Interestingly, this unique CFH haplotype was also present in patients S198, S197, D40308, 6089 and FHUSA11, which suggest that CFHR1210C was also associated with the CFHTGTAAT haplotype in these individuals.

Family D4534 illustrates that the CFHCGTGGT haplotype is also associated with the CFHR1210C mutation (Figure 1). Finally, patient S223, who is a CFHCGTGGT homozygote, unambiguously associates the CFHTGTAAT haplotype with CFHR1210C (Figure 2). Haplotype CFHCGTGGT is also likely associated with the CFHR1210C in patients S006 and S196 (Figures 1 and 2). This conclusion is supported by the observation that patients
S006 and S196 lack one copy of the CFHR1 and CFHR3 genes which is associated with the CFH\textsubscript{CGTAAG} and CFH\textsubscript{TGTAAG} haplotypes (Martinez-Barricarte \textit{et al}, unpublished observations), making the most probable genotypes for S006 and S196, CFH\textsubscript{TGTGGT}/CFH\textsubscript{CGTAAG} and CFH\textsubscript{TGTGGT}/CFH\textsubscript{TGTAAG}, respectively.

Alignment of the three extended CFH haplotypes associated with the CFH\textsubscript{R1210C} mutation illustrates that they are identical around the mutation site suggesting that the three alleles may have a common origin and have been generated by recombination from a single original CFH chromosome carrying the CFH\textsubscript{TGTAAT} haplotype (Table 4, Figure 3). Because the CFH\textsubscript{TGTAAT} haplotype is strongly associated with the CFH\textsubscript{R1210C} mutation and is extremely rare in Caucasians, we postulate that the CFH\textsubscript{R1210C} mutation originated in a different population, where the CFH\textsubscript{TGTAAT} haplotype is relatively frequent, and that was introduced in the European populations later on. However, we cannot rule out the possibility of a mutational event in this rare chromosome in Caucasians

In conclusion, this study shows that aHUS patients carrying CFH\textsubscript{R1210C} do not have a common clinical phenotype. This heterogeneity, the incomplete penetrance of the disease among the CFH\textsubscript{R1210C} mutation carriers and the identification of additional aHUS-associated risk factor in the affected individuals is a common feature seen in all aHUS patients. The relatively high prevalence of the CFH\textsubscript{R1210C} mutation among aHUS patients suggests that CFH\textsubscript{R1210C} is a rare functional polymorphism in the general population that specifically predisposes to development of aHUS.
PATIENTS, MATERIALS AND METHODS.

Patients.

Our study includes five aHUS pedigrees and seven individual aHUS patients carrying the \( CFH_{R1210C} \) mutation from Italy, U. K., Australia, Germany, France and U.S.A. These R1210C pedigrees and patients were identified in routine \( CFH \) mutation analysis of aHUS patients. aHUS was diagnosed because of the presence of one or more episodes of microangiopathic hemolytic anemia and thrombocytopenia defined on the basis of hematocrit (Ht)<30%, hemoglobin (Hb)<10mg/dl, serum lactate dehydrogenase (LDH)>460U/L, undetectable haptoglobin, fragmented erythrocytes in the peripheral blood smear, and platelet count <150,000/µl, associated with acute renal failure. Patients with Stx-HUS, defined as the presence of Shiga toxin in the stools (by the Vero cell assay) and/or of serum antibodies against Shiga toxin (by ELISA) and/or LPS (O157, O26, O103, O111 and O145, by ELISA) were excluded. None of the aHUS patients carrying the \( CFH_{R1210C} \) had thrombotic thrombocytopenic purpura-like manifestations.

**Family 024.** There are three affected individuals (F106, II-3 and F108, II-4) in this pedigree, two of whom are alive. Patients F106 and F108 first presented at the age of 8 and 3 years, respectively. The family history revealed that a younger brother died from HUS at the age of six and the father, who had hepatitis C, was noted on various occasions to have thrombocytopenia and anemia. In addition, microscopic proteinuria/haematuria and a slightly elevated creatinine (1.5 mg/dl) were also reported. A paternal uncle died at the age of 28 years from renal failure.

**Family 020.** This pedigree, included several asymptomatic carriers of the R1210C mutation, but only one affected individual (II-1, R016), who was first admitted to hospital at the age of 31. Fifteen years later, after a progressive deterioration in renal function, she started peritoneal dialysis. She received a cadaveric renal transplant that was unsusscesful due to a recurrence of HUS. No relevant family history was reported.
The same mutation has been found in the father, in four of five healthy siblings and in one of her three children.

**Family 069.** This pedigree includes a female (S006), who first presented at 30y old. After two recurrences she started chronic hemodialysis. No relevant clinical history was reported in her family. The same mutation has been found in the healthy sister.

**Family HUS29.** This pedigree has 4 generations of carriers and only one affected (H29IV-1) who first presented with HUS at 6 months of age. She was treated with peritoneal dialysis and fresh plasma infusions (20 ml/kg 3 times a week for two weeks) and completely recovered renal function. She had 3 recurrences in the following 3 months and is currently treated with peritoneal dialysis and plasma infusions every 6 weeks.

**Family D4534.** This consanguineous pedigree is remarkable comprising two healthy R1210C carrier parents and one affected R1210C homozygote daughter (D45348) who presented with HUS at the age of 1 year. She received a live related transplant from her mother at the age of 4 years. This was complicated by five episodes of recurrent HUS, each of which was treated with plasma exchange but the graft lasted only 2 years and 3 months. She then returned to haemodialysis. She received a cadaver renal transplant at the age of 10. There have been no episodes of recurrent HUS and the graft is functioning well.

**Patient D4038.** She presented at the age of 40 with post partum HUS. She did not recover renal function and remains dialysis dependent.

**Patient S198** is a male who first presented at the age of 34. He did not recover renal function and started hemodialysis. He received a cadaveric renal transplant. Initial immunosuppression was with steroids, cyclosporine and mycophenolate mofetil. The outcome of the transplant has been good with no episodes of recurrent disease. No relevant clinical history was reported in his family.
*Patient S197* is a female who first presented at 6 months of age. After several recurrences over the next three years she developed end stage renal failure. She is currently on dialysis. There is no relevant family history.

*Patient S223* is a male who had his first episode of HUS at the age of 43. He did not recover renal function and is currently on peritoneal dialysis. There is no relevant family history.

*Patient S196* is a female who had his first episode of HUS at the age of 53. Her renal function did not recover. She is currently on chronic hemodialysis.

*Patient FHUSA11.* She was referred at the age of 21 with HUS. She did not recover renal function and started hemodialysis. At age 23, she received a cadaveric renal transplant. She lost the allograft within 6 days to recurrent HUS.

*Patient 6089* is a male who developed HUS at 13 years. HUS diagnosis was confirmed by renal biopsy. His condition resolved spontaneously without either dialysis or plasma therapy. Eighteen months later the only detectable abnormality was microscopic proteinuria.

**CFH and MCP genotyping**

A set of six SNPs, representing a minimal informative set for genetic variations within *CFH* (c.-331C>T, c.184G>A, c.1204C>T, c.2016A>G, IVS15-543C>T and c.2808G>T), and two additional SNPs in *CFHR3* (rs370789 and rs389897) were genotyped on genomic DNA using Taqman probes (Applied Biosystems) and real time PCR equipment (IQ5, Applied Biosystems), according to the manufacturer’s specifications, or by automatic DNA sequencing of PCR derived amplicons in a sequencer (ABI 3730; Applied Biosystems) using dye terminator cycle sequencing kit (Applied Biosystems). *CFH* haplotypes in the pedigrees were determined by segregation analysis. *CFH* haplotypes in individual aHUS patients were assigned on the basis of the *CFH* haplotype frequencies in a sample of 317 Spanish controls (Table
1). The aHUS-associated \textit{MCPggaac} haplotype was genotyped as described previously\textsuperscript{4}.

\textbf{CFHR3 genotyping}

Two SNPs in the intron 3 of \textit{CFHR3} (rs370789 IVS3-1106G>A and rs389897 IVS3-1046T>A) were genotyped on genomic DNA by automatic DNA sequencing of PCR derived amplicons in a sequencer (ABI 3730; Applied Biosystems) using dye terminator cycle sequencing kit (Applied Biosystems). Copy number variation of \textit{CFHR1} and \textit{CFHR3} was analyzed by MLPA (Multiplex Ligation-Dependent Probe Amplification) as previously described\textsuperscript{26}.

\textbf{Statement of IRB approval}

All participating centers have IRB approval for the studies included in this report.
ACKNOWLEDGEMENTS

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STATEMENT OF FINANCIAL SUPPORT

Authors declare that no financial conflict of interest exists.
REFERENCES


Syndrome Cluster in Exons 1820, a Domain Important for Host Cell Recognition


FOOTNOTES FOR THE FIGURES

FIGURE 1. aHUS pedigrees carrying $CFH_{R1210C}$. Patient code and age of onset are indicated. The six SNPs that compose the $CFH$ haplotypes are represented in columns. $CFH$ haplotypes that carry the R1210C mutation are squared and shaded. The protective $CFH$-H2 haplotype is squared and blank. A code for all symbols is included in the figure.

FIGURE 2. Individual aHUS patients carrying $CFH_{R1210C}$. Most likely $CFH$ haplotypes indicated. Patient code and onset age are shown. The $CFH$ haplotype that carries $CFH_{R1210C}$ is shaded. Code for symbols is as in Figure 1.

FIGURE 3. Model for the origin of the three $CFH$ haplotypes associated with $CFH_{R1210C}$. This model shows two putative recombination events that may have generated the three $CFH$ haplotypes found associated with $CFH_{R1210C}$ in our patients. H1 and H3 are relatively frequent $CFH$ haplotypes in the general population (Table 1).
<table>
<thead>
<tr>
<th>CFH haplotypes(^1)</th>
<th>CFH SNPs</th>
<th>Haplotype frequency(^2) (\text{n}=317)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-331C&gt;T</td>
<td>184G&gt;A</td>
</tr>
<tr>
<td>H1</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>H2</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>H3</td>
<td>T</td>
<td>G</td>
</tr>
<tr>
<td>H4a</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>H4b</td>
<td>T</td>
<td>G</td>
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<td>H5</td>
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<td>G</td>
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<td>C</td>
<td>G</td>
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</tr>
<tr>
<td>H10</td>
<td>T</td>
<td>G</td>
</tr>
<tr>
<td>H11</td>
<td>C</td>
<td>A</td>
</tr>
</tbody>
</table>

1) *CFH-H2* and *CFH-H3* haplotypes are associated with decreased and increased risk to aHUS respectively. The *CFH-H4a* and *CFH-H4b* haplotypes are both associated with the deletion of the *CFHR1* and *CFHR3* genes.

2) Frequencies of the *CFH* haplotypes in the Spanish control population were not different from those reported for other Caucasians populations.\(^{10}\)
Table 2. Clinical data in aHUS patients carrying the $CFH_{R1210C}$ mutation

<table>
<thead>
<tr>
<th>ID</th>
<th>Gender</th>
<th>Biopsy</th>
<th>Onset</th>
<th>Triggering factor</th>
<th>Time interval to ESRD/CRI</th>
<th>Renal status 1</th>
<th>Recurrences (number)</th>
<th>Renal transplant</th>
<th>Recurrence after transplant</th>
</tr>
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<tbody>
<tr>
<td>S198</td>
<td>M</td>
<td>-</td>
<td>34y</td>
<td>Vomiting</td>
<td>During 1st episode</td>
<td>ESRD</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>S197</td>
<td>F</td>
<td>Yes</td>
<td>6mo</td>
<td>?</td>
<td>3y</td>
<td>ESRD</td>
<td>Yes (several)</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>S196</td>
<td>F</td>
<td>Yes</td>
<td>53y</td>
<td>Treatment RA 3</td>
<td>During 1st episode</td>
<td>CRI</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>S223</td>
<td>M</td>
<td>-</td>
<td>43y</td>
<td>?</td>
<td>During 1st episode</td>
<td>ESRD</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>F106</td>
<td>M</td>
<td>-</td>
<td>8y</td>
<td>Bronchopneumonia</td>
<td>-</td>
<td>Normal</td>
<td>Yes (1)</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>F108</td>
<td>M</td>
<td>Yes</td>
<td>3y</td>
<td>Diarrhoea/Vomiting</td>
<td>15y</td>
<td>ESRD</td>
<td>Yes (1)</td>
<td>No</td>
<td>-</td>
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<tr>
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<td>F</td>
<td>Yes</td>
<td>30y</td>
<td>Flu-like episode</td>
<td>4mo</td>
<td>ESRD</td>
<td>Yes (1)</td>
<td>No</td>
<td>-</td>
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<tr>
<td>R016</td>
<td>F</td>
<td>Yes</td>
<td>31y</td>
<td>Vomiting</td>
<td>15y</td>
<td>ESRD</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>D40308</td>
<td>F</td>
<td>-</td>
<td>40y</td>
<td>Postpartum</td>
<td>During 1st episode</td>
<td>CRI</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>D45348</td>
<td>F</td>
<td>-</td>
<td>1y</td>
<td>?</td>
<td>During 1st episode</td>
<td>ESRD</td>
<td>No</td>
<td>Twice</td>
<td>1st Yes/ 2nd No</td>
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<tr>
<td>6089</td>
<td>M</td>
<td>Yes</td>
<td>13y</td>
<td>Infection</td>
<td>-</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
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<tr>
<td>FHUSA11</td>
<td>F</td>
<td>-</td>
<td>21y</td>
<td>?</td>
<td>During 1st episode</td>
<td>ESRD</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>H29IV-1</td>
<td>F</td>
<td>-</td>
<td>6mo</td>
<td>?</td>
<td>3mo</td>
<td>CRI</td>
<td>Yes (3)</td>
<td>No</td>
<td>-</td>
</tr>
</tbody>
</table>

1) HUS diagnosis confirmed by renal biopsy
2) ESRD, End Stage Renal Disease; CRI, Chronic Renal Insufficiency;
3) RA, Reumatoid Arthritis.
Table 3. Complement and genetic data in aHUS patients carrying the $CFH_{R1210C}$ mutation

<table>
<thead>
<tr>
<th>ID</th>
<th>C3 levels (80-177mg/dL)</th>
<th>Additional aHUS risk factors$^†$</th>
<th>Mutations</th>
<th>$MCP_{ggaac}$</th>
<th>$CFH_{TGTGGT}$</th>
<th>$\Delta_{CFHR3-CFHR1}$</th>
</tr>
</thead>
<tbody>
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<td>S198</td>
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<td>H29IV-1</td>
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1) Mutations and polymorphisms in $CFH$ were considered only if they were carried in the $CFH$ allele without the R1210C mutation. $CFH_{low}$ is a low expression $CFH$ allele that produces 20% of the normal factor H amount (SRdeC, unpublished observation)
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<th>Patient</th>
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<th>CFHR3 SNPs</th>
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<td>T</td>
<td>G</td>
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1) H4b*, rare CFH haplotype, closely related to H4b
**Figure 1**

- **Family 020**
  - I
  - II
  - III
  - IV
  - Code for aHUS risk factors
  - Code for CFH SNPs
    - CFH<sub>-331 C/T</sub>
    - MCP<sub><u>-184 G/A</u></sub>
    - MCP<sub><u>+1204 T/C</u></sub>
    - MCP<sub><u>+2016 A/G</u></sub>
    - CFH<sub><u>low</u></sub>
    - ΔCFHR3-CFHR1
    - CFH<sub>R1210C (healthy)</sub>
    - CFH<sub>R1210C (aHUS)</sub>

- **Family HUS29**
  - I
  - II
  - III
  - IV

- **Family D4534**
  - I
  - II
  - III
  - IV

- **Family 024**
  - I
  - II
  - III
  - IV

- **Family 069**
  - I
  - II

---

**Code for aHUS risk factors**

- CFH<sub>-331 C/T</sub>
- MCP<sub><u>-184 G/A</u></sub>
- MCP<sub><u>+1204 T/C</u></sub>
- MCP<sub><u>+2016 A/G</u></sub>
- CFH<sub><u>low</u></sub>
- ΔCFHR3-CFHR1
- CFH<sub>R1210C (healthy)</sub>
- CFH<sub>R1210C (aHUS)</sub>
Figure 2
Figure 3

Original haplotype

\[ \text{T G T A A T} \star \text{G T} \]

\[ \text{CFH} \quad \text{CFHR3} \]

Recombination with H3 haplotype?

\[ \text{T G T A A T} \times \star \text{G T} \]

\[ \text{T G T G G T} \]

\[ \text{T G T G G T} \star \text{G T} \]

Recombination with H1 haplotype?

\[ \text{T G T G G T} \times \star \text{G T} \]

\[ \text{C G C A G G} \]

\[ \text{C G T G G T} \star \text{G T} \]