Brief report

Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency

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Atypical hemolytic uremic syndrome (aHUS) is a severe renal disease that is associated with defective complement regulation caused by multiple factors. We previously described the deficiency of factor H–related proteins CFHR1 and CFHR3 as predisposing factor for aHUS. Here we identify in an extended cohort of 147 aHUS patients that 16 juvenile individuals (ie, 11%) who either lacked the CFHR1/CFHR3 completely (n = 14) or showed extremely low CFHR1/CFHR3 plasma levels (n = 2) are positive for factor H (CFH) autoantibodies. The binding epitopes of all 16 analyzed autoantibodies were localized to the C-terminal recognition region of factor H, which represents a hot spot for aHUS mutations. Thus we define a novel subgroup of aHUS, termed DEAP HUS (deficiency of CFHR proteins and CFH autoantibody positive) that is characterized by a combination of genetic and acquired factors. Screening for both factors is obviously relevant for HUS patients as reduction of CFH autoantibody levels represents a therapeutic option. (Blood. 2008;111:1512-1514)
Frequency of CFH autoantibodies

In a cohort of 147 aHUS patients, we identify 16 children (ie, 11%) as positive for CFH autoantibodies by ELISA (Table 1). CFH autoantibodies were completely absent in a control group of 100 healthy individuals, thus indicating that CFH autoantibodies are associated with aHUS. Similar to the young age of the patients of the Jena cohort, the 8 previously identified CFH autoantibody–positive HUS patients (5-17 years) were also juvenile, suggesting related mechanisms for autoantibody induction.

Further analyses of the CFH autoantibody–positive group revealed by Western blotting that the patients either showed the complete absence of CFHR1 and CFHR3 in plasma (14 patients) or displayed low, barely detectable levels of CFHR1 and CFHR3 (Table 1 and data not shown). The strong correlation between the occurrence of CFH autoantibodies and absence or reduction of CFHR1/CFHR3 in plasma suggests that this deficiency represents a risk factor for CFH autoantibody formation. The mechanism involved in how a deficiency of these plasma proteins leads to the generation of CFH autoantibodies is currently unknown and requires further investigations. The 22 CFHR1/CFHR3-deficient patients of the Jena cohort include 16 CFH autoantibody–positive and 6 patients who have no autoantibodies to CFH. The frequency of the deficient group without CFH autoantibodies is 4% in this cohort and thus slightly higher than in the Jena and Newcastle control groups (2% each) or in the Iowa, Columbia, and Finnish AMD study cohorts (2.7%, 3.0%, and 2.5%, respectively). Concordance of 2 risk factors in development of aHUS has been reported for combined mutations in either the CF1 and the MCP genes or for various CFH haplotypes.

Here we report a new combination of 2 disease-associated conditions in predominantly juvenile aHUS patients, namely the presence of CFH autoantibodies and absence of CFHR1/CFHR3 in plasma.

Family studies

Family studies were performed to analyze how autoantibodies to CFH or CFHR1/CFHR3 deficiency influences or predisposes to the disease. Three CFH autoantibody–positive, CFHR1- and CFHR3-deficient patients and their family members were assayed for both parameters (Figure 1). In family A, the patient (AII1) (Figure 1A) was positive for CFH autoantibodies (Figure 1D) and CFHR1 and CFHR3 proteins were absent in his plasma (Figure 1B lane 2). The mother (AI2, Figure 1B lane 6) showed lower plasma levels of CFHR1 and CFHR3 proteins, indicating heterozygous deficiency. The other family members lacked CFHR1 and CFHR3 proteins, which corresponds to homozygous deficiency. Genetic analyses confirmed homozygous CFHR1 and CFHR3 deficiency for the patient (AII1), the healthy brother (AII2), the healthy sister (AII3), and the healthy father (AI1). The CFH gene was intact in all family members (data not shown). A similar scenario was observed for families B and C. In family B the patient, but no other relative, was positive for CFH autoantibodies (Figure 1D). CFHR1 and CFHR3 proteins were absent in the plasma of the patient (BI1) and the unaffected healthy sister (BI2; Figure 1B lanes 8,9), but were detected in sera of the healthy mother and the father (Figure 1B lanes 10,11). Genetic analyses confirmed that the patient and his sister were homozygous for the CFHR1/CFHR3 gene deletion. Similarly, in family C the

Table 1. Frequency of CFH autoantibodies with CFHR1 and CFHR3 deficiency in the Jena cohort

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<th>CFH autoantibodies, no. (%)</th>
<th>CFHR1/CFHR3 deficiency, no. (%)</th>
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<tbody>
<tr>
<td>aHUS patients</td>
<td>147 (11)</td>
<td>22 (15)</td>
</tr>
<tr>
<td>Controls</td>
<td>100 (0)</td>
<td>2 (2)</td>
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The 16 patients who developed CFH autoantibodies either lack CFHR1/CFHR3 completely in plasma (n = 14) or show extremely low levels of the 2 CFHR proteins (n = 2) as determined by Western blotting. The CFHR1- and CFHR3-deficient group includes the 16 patients of the CFH autoantibody–positive group and 6 deficient patients who have no autoantibodies to CFH. No CFH autoantibodies were detected in the control group representing 100 healthy individuals. The mean absorbance of all 100 control probes was OD 0.17 (± 0.1). The highest value determined for one sample of the control group was 0.35 OD; therefore, the cutoff for false positive was set to 0.35 OD.
In summary, we identify a new subgroup of aHUS patients who are deficient for CFHR1 and CFHR3 in plasma and positive for CFH autoantibodies. This deficiency may favor development of specific autoantibodies that bind to the recognition region of CFH and likely block cell binding. It remains to be shown if disease progression of this new subgroup differs from that of other HUS patients (eg, patients with CFHR1/CFHR3 deficiency and the absence of CFH autoantibodies or patients with CFH mutations).

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Authorship

Contribution: M.J., C.L., P.F.Z., and C.S. designed the research and wrote the paper; S.S., S.L.H.Z., H.R., and S.H. performed experiments and analyzed data; C.L. provided patient specimen.

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References

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