Post-Liver Transplant C3G with Highly Elevated Factor H Autoantibodies

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Introduction

Factor H (FH) is the key negative regulator of the alternative pathway (AP), both in the circulation and on the cell surface. Autoantibodies to FH (FHAAs) can be found in patients with complement-mediated rare renal diseases like atypical hemolytic uremic syndrome (aHUS) and C3 Glomerulopathy (C3G).

The presence of FHAAs is often associated with homozygous deletion of the *CFHR1* gene. Due to the presence of highly homologous sequences in this genomic region, the deletion of both copies of the *CFHR3–CFHR1* genes is a very common structural copy number variation in the human genome.

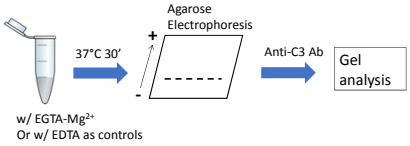
We present a unique case wherein a post-liver transplant recipient developed C3G secondary to highly elevated FHAAs that developed as a consequence of acquired deficiency of FHR1.

Patient

A 30-year-old male underwent a liver transplant at age 20 for auto-immune hepatitis. A few years later, he was diagnosed with declining renal function heralded by non-nephrotic proteinuria and micro-hematuria. A renal biopsy was performed and on immunofluorescence showed strong mesangial and segmental capillary C3 deposition (3+) in the absence of other immunoreactivity. On electron microscopy, electron-dense paramesangial and sub-epithelial deposits were seen and a diagnosis of C3 glomerulonephritis (C3GN; a sub-type of C3G) was made. A complement evaluation was performed.

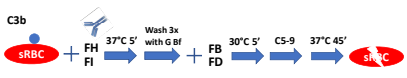
Methods

1. Complement biomarkers were quantified using a customized panel that employs a battery of techniques such as ELISAs, RIDs and hemolytic assays.
2. Autoantibodies to FH, FB, FI, CR1, C3 convertase (C3Nefs) and C5 convertase (C5Nefs) were measured by ELISAs or cell based methods.
3. Total IgG was isolated using a Melon Gel. IgG3 and IgG1/2/4 fractions were separated using Protein A. IgG1/2/4-λ was further absorbed using PureProteome Lambda Ig Binder Magnetic Beads.
4. Completed assays included:
   a) A fluid-phase C3 convertase assay (FPC3CA)



   b) A cell-based C3 convertase activity assay

   c) A cell-based cofactor activity assay



Results

1) Acquired Deficiency of FHR1

Multiplex ligation-dependent probe amplification (MLPA) detected a heterozygous deletion of *CFHR1-CFHR4* in the recipient (blood DNA) but homozygous deletions of both copies of *CFHR3- CFHR1* in the donor (liver DNA).

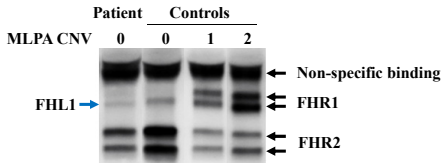


Figure 1. FHR1 expression in patient and controls. Western blotting shows no circulating FHR1 in the patient. FHR1 is produced by the liver, implying that the recipient has acquired FHR1 deficiency. A weak FHL1 band is seen. FHL1 is an alternately spliced FH version, which contains first 7 SCRs.

2) Complement Dysregulation

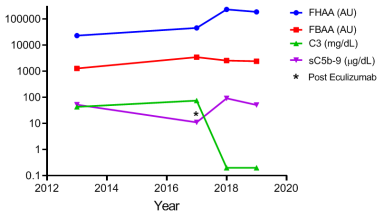


Figure 2. Autoantibodies associated with fluid-phase complement dysregulation. The patient was positive for both FHAAs and FBAAs 5 years post liver transplantation. He was negative for other autoantibodies (including C3Nefs and C5Nefs). Titers of FHAAs gradually increased and were associated with a decrease of serum C3 levels and an increase in soluble (C5b-9) (normal reference values for FHAA and FBAA: <200AU; C3: 90-180 mg/dL; sC5b-9: <30 μg/dL).

3) IgG Subclasses

Table 1. Isotypic ELISA

	IgG1	IgG2	IgG3	IgG4	K	λ
FHAA	+++				+++	
FBAA			++			++

FHAA is an IgG1κ and FBAA is an IgG3λ.

4) Epitope Mapping

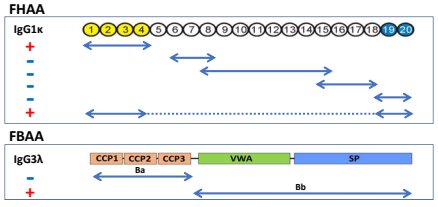


Figure 3. Epitope mapping. FHAA binds to the N-terminus (SCRs 1-4) of FH, while FBAA targets the Bb portion of FB.

5) AP Dysregulation in the Fluid Phase

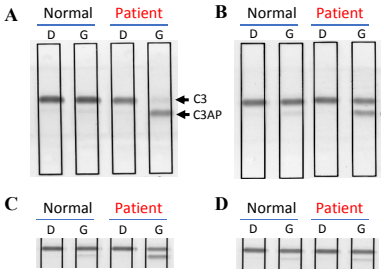


Figure 4. AP dysregulation assayed by FPC3CA. A) Incubation of pooled normal human serum with patient serum generated large amounts of C3 activation products (C3AP) under Mg^{2+}-EGTA conditions (lane G), but not under EDTA conditions (lane D). Such activity was not present in the normal serum; B, C and D) Repeated FPC3CA using purified IgG, IgG1/2/4 fraction and IgG3 fraction, respectively. These experiments indicate that FHAAs (IgG1κ) but not FBAAs (IgG3λ) activate the AP.

6) FHAA and Decay Accelerating Activity

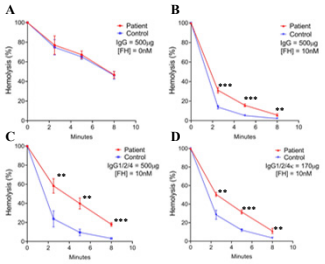


Figure 5. FHAAs impair the DAA of the FH. A) Patient total IgG does not affect the half-life of C3 convertase. B) Patient total IgG does impair FH DAA C3 convertase. C and D) IgG1/2/4 and its κ fraction (containing FHAAs) prevent DAA. (**: p<0.01; ***: p<0.001)

7) FHAA and Cofactor Activity

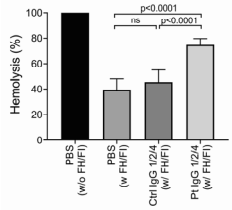


Figure 6. FHAAs impair CA of FH. C3b-coated sheep erythrocytes were incubated with FH and FI in the presence or absence of autoantibody. After washes, residual C3b was titrated out by adding FB and FD. Hemolysis was introduced by adding rat-serum in EDTA buffer. This result shows that FHAAs impair cofactor activity of FH (ns: not significant).

Conclusion

Here we present a case of acquired FHR1deficiency that led to the generation of high levels of FHAAs. Functional analyses show that these FHAAs impair the DAA and CA of FH. FBAAs do not stabilize C3 convertase on the cell surface. The role of FBAAs in the disease in this case is unclear.

This case highlights a causal relationship between deficiency of FHR1 and FHAA. Importantly, when FHR1 is absent, auto-antigenicity to FH can be induced even after maturation of the immune system.

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