



GENETIC DRIVERS OF ATYPICAL HEMOLYTIC UREMIC SYNDROME AND C3 GLOMERULOPATHY

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GENETIC DRIVERS OF AHUS

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GENETIC ABNORMALITIES AND PREVALENCE

- Genetic variants in complement genes (CFH, CD46, CFI, C3, CFB, THBD and DGKE) have been identified in 50-60% of aHUS patients.
- Gene conversion and genomic rearrangements between CFH and CFHR1 or CFHR3 have been identified in 3-5% of aHUS patients.
- Polymorphism and risk haplotypes in CFH and CD46 predispose to aHUS
- FHR-1 deficiency, in most cases due to homozygous deletion of CFHR3-CFHR1 is strongly associated with development of anti-FH antibodies and aHUS.

GENETIC COMPLEXITY

- Disease shows incomplete penetrance (50%) in carriers of the mutations.
- Concurrence of multiple genetic factors has been reported: combined mutations and mutations plus risk/protection polymorphisms (FH and MCP haplotypes).
- Environmental factors act as disease triggers in most mutation carriers. Small gender differences (females > males)
- Sound genotype-phenotype correlations regarding disease progression and treatment responses.

FUNCTIONAL CONSEQUENCES

● Loss of Function

- CFH: mostly affecting the binding of FH to cells and surface bound C3b.
 - Both FH deficiencies (overlap C3G) and C-ter mutations (prototypical aHUS)
- CD46: quantitative MCP defect or decreased cofactor activity.
- CFI: low FI levels or decreased activity (overlap C3G).
- THBD: decreased FH binding, reduced TAFI activation (C3a/C5a; fibrinolysis)

● Gain of Function

- C3: decreased binding and inactivation mainly by FH and MCP.
- CFB: overactive C3 convertase resistant to decay.

● Polymorphisms determine expression levels and activity

PATHOGENETIC MECHANISMS (surface dysregulation + complement)

- Surface-restricted complement dysregulation leading to endothelial injury and loss of anti-thrombogenic properties.
- Complement regulation in fluid phase is generally preserved and serum complement C3 levels are normal in about 50% of patients.

Animal models replicate the consequences of the aHUS-associated FH mutations.

aHUS is C5 dependent

GENETIC DRIVERS OF C3G

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- PREVALENCE:

- Complement genetic variants have been identified in 30-40% of C3G patients.
- Presence of complement genetic variants overlaps that of C3Nef.
- Classic mutations, complex genomic rearrangements and risk polymorphisms identified.

- GENES INVOLVED:

- Genetic heterogeneity. Main genes involved are **CFH**, **CFHR1-5** and **C3**. Also *MCP*, *CFB*, *CFI* and *DGKE*.
- In general there are not genes that uniquely associate with C3G. However, mutations found in C3G patients are often exclusive of C3G (exceptions: FH, FI, other).
- Disease show high penetrance in carriers of mutations (CFHR5, males>>females)
- Concurrence with non-genetic risk factors has been reported (C3Nef, Infections).

- FUNCTIONAL CONSEQUENCES:

- C3 consumption

- Genetic variants associated with DDD usually cause complete C3 consumption.
- Genetic variants associated with C3GN have reduced impact on C3 levels.

- Complement regulation

- Most C3G-associated mutations impair FH function
 - Directly, like the FH deficiencies and dysfunctional N-terminal FH mutants,
 - or indirectly as it is the case of the C3 mutant resistant to FH (and CR1) or the *CFHRs* genetic variants, increasing expression levels or causing gain of function multimeric FHR complexes, both resulting in excessive FH/FHRs competition.

- PATHOGENIC MECHANISMS:

- Fluid phase dysregulation.

- In cases involving FH deficiencies (C3 mutations?) the pathogenic mechanism likely involves massive C3 activation in plasma with iC3b/C3dg fragments playing a relevant role.

- Surface dysregulation.

- In cases involving extra *CFHR* gene copies or gain-of-function *CFHR* gene rearrangements, an excessive FH/FHRs competition has been identified that may result in complement dysregulation on surfaces. C3 mutations (?)

Animal models replicate the consequences of the C3G-associated FH (and C3) mutations.

Why performing genetic analysis in aHUS and C3G?

What can we learn from genetics? Is genetic testing clinically relevant?

The more we know about the functional consequences of mutations the best we will understand the mechanisms underlying the pathology.

Finding a causative mutation provides understanding of the etiological factor, which reinforces diagnosis, may inform prognosis and assists therapeutic decisions.

Genetics is based on phenotypes – homogeneous clinical diagnosis is crucial

aHUS is mechanistically quite homogeneous. Sound data on genotype /phenotype correlations regarding prognosis and response to treatments.

C3G is complex and may be mechanistically heterogenous. Most data generated from quite unique familial cases. Understanding of C3G much behind aHUS

1. When should molecular diagnostics be done?

Criteria for accepting a positive diagnosis range from the most strict to the most lax.

Should genetic analysis condition clinical diagnosis and treatment?

Always / familial cases only?



2. How is molecular diagnostics evolving?

3. Which criteria should be used to define variants as pathogenic/likely pathogenic vs VUS (e.g., minor allele frequency (MAF) in general population, in silico prediction, functional insights, others)?

Sanger seq. / NGS / Both ?

Is DNA sequencing enough?

Do we need complementary analysis and knowledge?
(levels, functional, structural, genomic analysis, family data, etc)

How genetic variants in the screened genes are interpreted?
Should the expected functional abnormalities associated with the pathology be considered?



4. Disease penetrance: How do multiple genetic risk factors determine risk for disease?

Combined genetic risk factors, the rule or the exception?
When to stop searching for genetic drivers?

How do we incorporate the risk/protection component associated with polymorphisms in the genetic equation?

How we deal with risk polymorphisms in the absence of mutations?



5. What are the benefits of molecular diagnostics?

5a. How does identification of pathogenic/likely pathogenic variants inform prognosis and treatment response?

5b. How do genetic profiles help in resolving complement-related overlapping phenotypes?

5c. Can complement blockade treatment be stopped safely and if so, in which patients and when?

5d. What are the indications and utility of molecular diagnostics in living-related kidney donor transplantation

aHUS vs C3G

6. What does the failure to identify a genetic cause in aHUS and C3G patients mean?

Not finding a genetic cause generates uncertainties in the clinicians regarding diagnosis and treatment continuity.

How to deal with a negative genetic result in the context of a positive / negative response to treatment?

6a. Comprehensive analysis of known candidate genes?

6b. Search for additional genes?

6c. Non-complement genes?

6d. Improved clinical diagnosis?

7. What does the clinician expect from a genetic test report?

7a. What information should be included and how should this information be reported?

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8. Can we move towards more individualized management and treatment of aHUS and C3G patients based on genetic profiles?

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