Functional characterization of 105 Factor H variants associated with atypical HUS: lessons for variant classification

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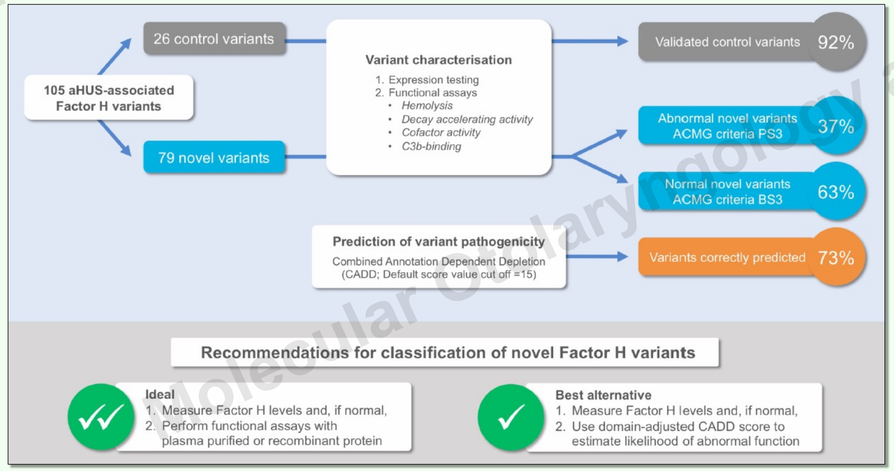
VISUAL ABSTRACT



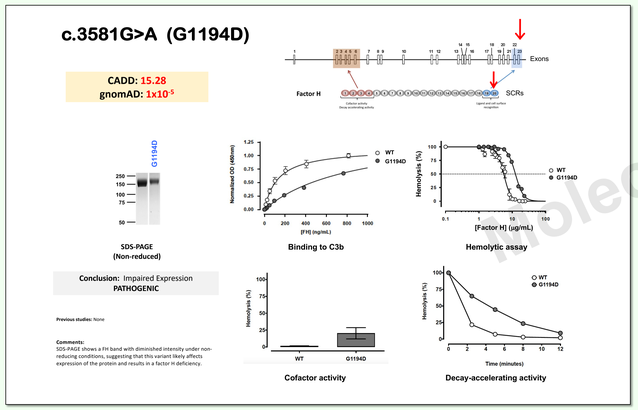
BACKGROUND

Testing for genetic variants in complement genes is becoming increasingly recognized as standard of care in the diagnosis and treatment of aHUS. Not only does the identification of a pathogenic, or likely pathogenic, variant help to confirm diagnosis, but it can also guide short- and long-term patient management. Variant classification, however, is not trivial and very often it is a barrier to the optimal medical use of genetic information. In this study, we address the dearth of functional studies for aHUS-associated genetic variants in the CFH gene by expressing and functionally characterizing 105 missense variants in the FH protein, with the goal of improving variant classification and thereby patient care.

MATERIALS AND METHODS

Recombinant FH variants were generated in Expi293. Expression, concentration and integrity of the protein was analyzed by SDS-PAGE and ELISA. Functional analysis were performed to determine their C3b-binding capacity (ELISA), overall regulatory activity (GP hemolysis assay), FI-cofactor activity (sheep hemolysis assay) and decay accelerating activity (sheep hemolysis assay). Twenty-six FH variants that were characterized previously were used to validate our functional assays. Variant pathogenicity was computationally predicted using Combined Annotation Dependent Depletion (CADD) version 1.6. gnomAD was used as the reference control database. All data generated for each FH variant was collected in an individual datasheet.

Example of the datasheets generated for each FH variant



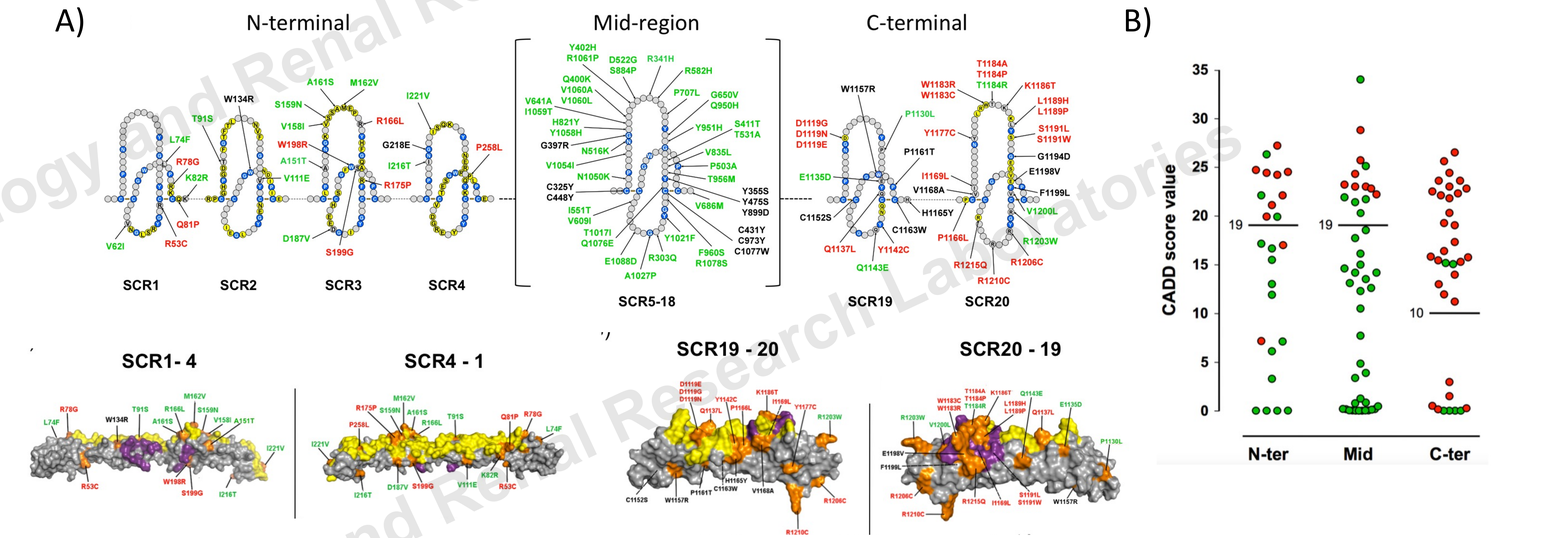
RESULTS

1 63% of the 79 uncharacterized FH variants do not show evidence of pathogenicity, with functional data in all assays indistinguishable from FH wild-type protein. FH pathogenic variants are not evenly distributed in the FH molecule. As expected, aHUS-associated pathogenic FH variants cluster in the FH C-terminal and are mainly located on the surface interacting with the TED domain of C3b and in the sialic acid binding site. Conversely, most benign variants cluster in the mid-region.

2 We identify important limitations in applying prediction algorithms to FH variants; 26% were classified incorrectly, mainly affecting residues in functionally relevant regions. A differential adjustment of the prediction algorithms to accommodate the peculiarities of the distinct FH regions improves overall predictions to 85%.

3 Rarity in control databases is not informative for variant classification. While pathogenic variants are more likely to be ultra-rare (MAF<0.01%) than benign variants, 25% of the benign variants are absent in gnomAD.

4 Overall, carriers of pathogenic FH variants presented more severe phenotypes than those carrying FH variants classified as benign. Average onset age for carriers of pathogenic variants was 17.6 years, compared to 29 years for carriers of benign variants. The likelihood of complete remission also skewed to favor carriers of benign variants. Carriers of benign variants have also less recurrences (24% vs 71% for benign vs pathogenic variants) and were more likely to segregate other genetic risk factors (49% vs 33% for benign vs pathogenic variants).



A) Top: Distribution of tested variants in the FH amino acid sequence. Amino acids corresponding to the SCR consensus sequence are depicted in black, those functionally relevant in yellow, and all other amino acids in gray. FH variant classification codes: green, benign; black, non-expressed; red, functionally impaired. Bottom: Mapping of tested variants in the FH N-terminal and C-terminal crystal structure. Depicted in yellow are the interacting surfaces with C3b (N-terminal) and C3d (C-terminal). The FI-interacting surface in the N-terminal is shown in purple. The sialic acid binding site in the C-terminal is also depicted in purple. The position of all variants is indicated in orange. FH variant classification codes according to functional assay results: green, benign; black, non-expressed; red, functionally impaired.
B) Distribution of FH classified experimentally as pathogenic (red dots) or benign (green dots) according to their CADD score values. Horizontal lines indicate the selected cut-offs for each FH region.

KEY POINTS

1. **Functional analysis, combined with level measurements, remains the gold standard to provide an accurate classification of FH variants.**
2. **The study reveals important limitations of routinely used variant classification methods.**
3. **Adapting prediction algorithms to FH domains helps to improve variant classification, while rarity in control databases can be misleading.**

LINKS

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Supplemental material for this publication includes individual datasheets with the complete functional characterization of the 105 *CFH* variants.
https://ars.els-cdn.com/content/image/1-s2.0-S0006497121012921-mmc1.pdf

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