Proteomic Analysis of Autosomal Dominant Neovascular Inflammatory Vitreoretinopathy

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Introduction and Methods

Objective: To characterize vitreous protein expression in autosomal dominant inflammatory vitreoretinopathy (ADNIV), a rare, progressive inflammatory intraocular disease caused by mutations in the CAPN5 gene

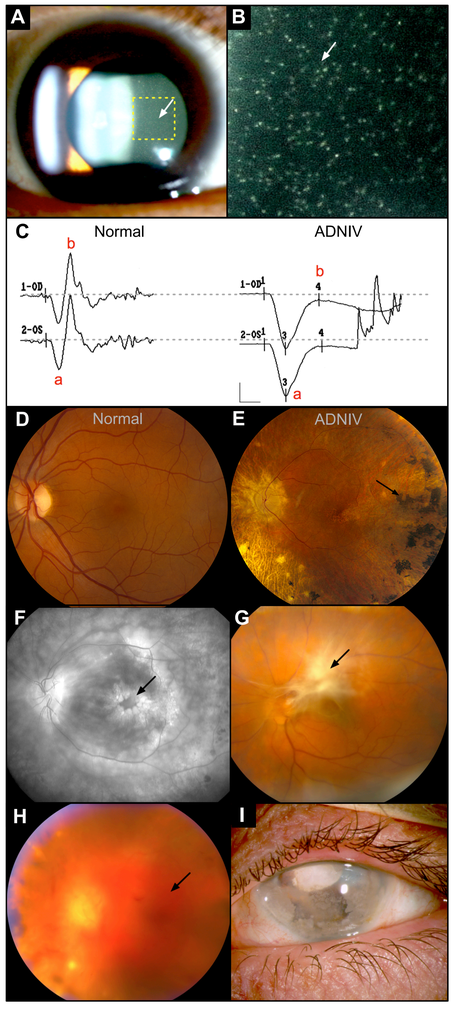

Figure 1. Clinical ADNIV phenotype: (A-B) Clusters of autoimmune reactive leukocytes in the vitreous cavity. **(C)** Electroretinography reveals early synaptic signaling defects in ADNIV patients, detected as loss of the b-wave. **(D)** Fundus image of the normal retina. **(E)** Fundus image of ADNIV retina showing pigmentary degeneration (arrow). **(F)** Fluorescein angiography reveals cystoid macular edema at the fovea, a consequence of intraocular inflammation. **(G)** Intraocular fibrosis and pre-retinal scar tissue formation (arrow). **(H)** Vitreous hemorrhage (arrow) caused by retinal neovascularization. **(I)** Phthisis bulbi and involution of eye tissues at end-stage ADNIV disease. Images courtesy of Mahajan, et al (2012).

Methods: Vitreous biopsies from 17 ADNIV patients were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Protein expression was analyzed by 1-way ANOVA, hierarchical clustering, pathway representation, and network analysis.

Results

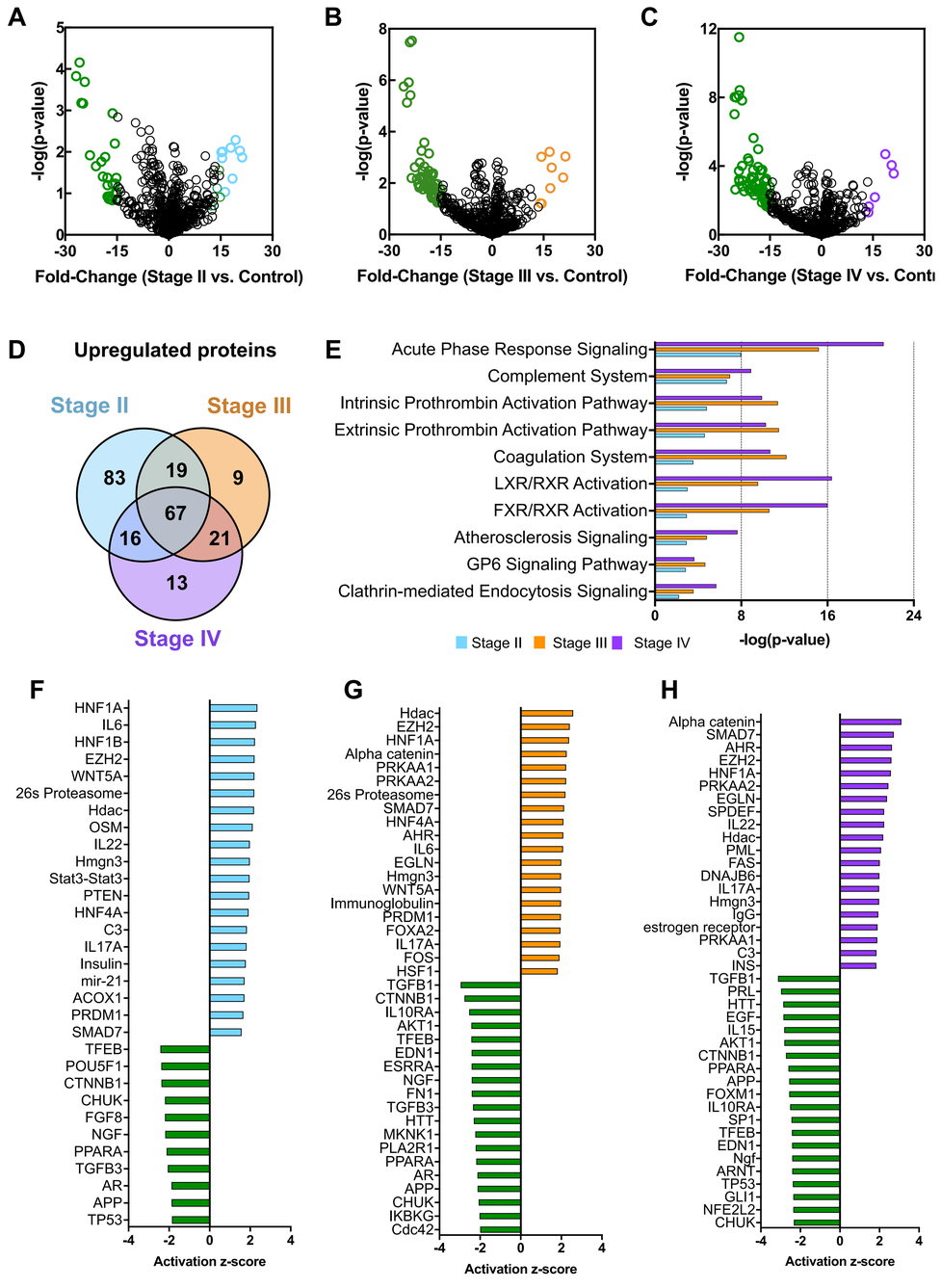

Figure 2 Pathway representation and upstream regulators in ADNIV vitreous: (A-C) Differentially-expressed proteins from Stage II-IV ADNIV compared to controls.Proteins with log2 fold-changes greater than 15 (upregulated) are colored cyan (Stage II), orange (Stage III), and purple (Stage IV) while proteins with log 2 fold-changes lower than -15 are represented in green (downregulated). **(D)** Comparative analysis of upregulated proteins using Venn diagrams. A total of 96 upregulated proteins are shared among the three stages compared to controls ($p < 0.05$). **(E)** Top ten pathways represented in Stage II-IV ADNIV. Pathways are ranked by their -log (p-value) obtained from the right-tailed Fisher's Exact Test. **(F-H)** Upstream regulators predicted based on proteins that were differentially-expressed in Stage II-IV ADNIV vs. controls ($p<0.01$). Upstream regulators are ranked by their activation z-score.

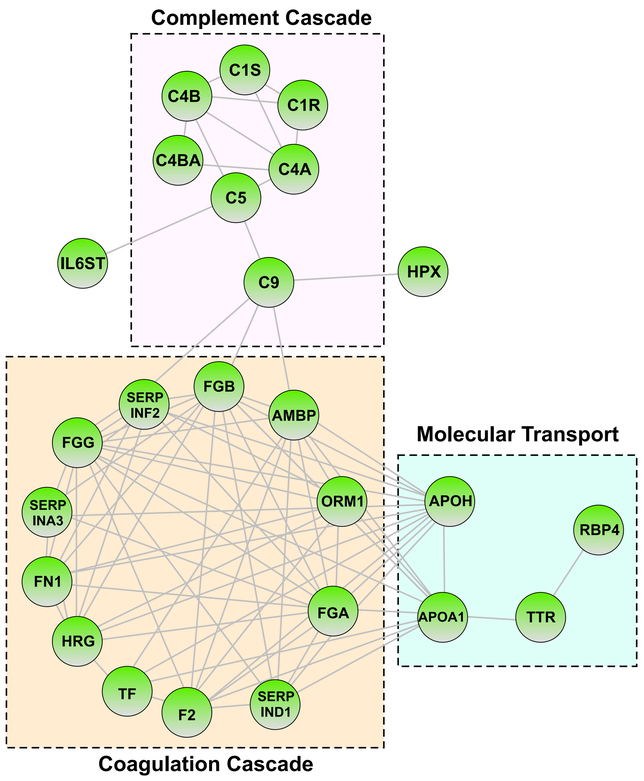

Figure 3. Acute phase response network: The acute phase response was the most-significantly represented pathway common to all ADNIV stages. There were 25 acute phase response proteins elevated in ADNIV. Results are displayed as a protein interaction network with proteins (nodes) represented as circles and connected by lines representing predicted or experimentally-confirmed interactions (edges). Nodes are highlighted by their respective molecular pathway or function: complement cascade, coagulation cascade, and molecular transport.

Conclusion

These proteins may represent key regulators of inflammation that contribute to pathogenesis in ADNIV uveitis. Further examination of these proteins may give insight disease mechanisms and possible therapeutic targets. For instance, our analysis pointed to elevated proteins that may be targeted by available drugs, including SOD, IL-12, IL-22, IL-17A, and C5. These elevated proteins may serve as biomarkers that can aid in the design of clinical trials that intervene at appropriate times.