A Rare Intronic *CD46* Variant is Associated With Partial Exon 8 Skipping in C3 Glomerulonephritis

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Background

Complement is a cornerstone of the innate immune system. A well-regulated complement system is essential for pathogen clearance, the removal of damaged cells and cellular debris, and activation of the humoral immune response. Dysregulation of the complement system can lead to attacks on healthy tissues and drive complement-mediated diseases such as C3 glomerulopathy (C3G).

Membrane cofactor protein (MCP), encoded by *CD46*, is a key regulator of complement activation at cell surfaces. By serving as a cofactor for factor I (FI), MCP promotes the cleavage and inactivation of C3b and C4b to protect host tissues from complement-mediated injury. MCP is a transmembrane protein composed of four extracellular short consensus repeat (SCR) domains, an O-linked glycosylated serine/threonine-rich (ST) domain, a linker region, a transmembrane (TM) domain, and a cytoplasmic (Cyt) tail. In the kidney, MCP is expressed predominantly on podocytes and at lower levels on endothelial and mesangial cells.

CD46 deficiency is classically associated with atypical hemolytic uremic syndrome (aHUS). However, in a patient with C3 glomerulonephritis (C3GN), we identified the intron 8 variant *CD46* c.901+24T>G, classified as a variant of uncertain significance. We hypothesized that this variant alters mRNA splicing, resulting in defective *CD46* expression and/or function, glomerular complement dysregulation, and ultimately the development of C3GN.

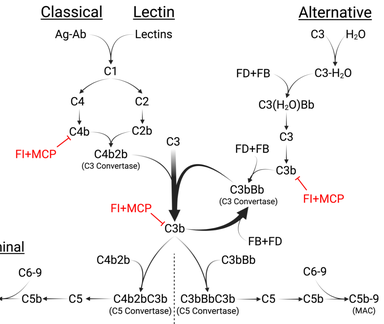

Figure 1. Complement Cascade. Complement is activated through the classical (CP), lectin (LP), and alternative pathways (AP). All three pathways converge on the cleavage of C3 into C3a and C3b. C3b can either enter the AP amplification loop or initiate the terminal pathway. MCP serves as a cofactor for FI-mediated cleavage and inactivation of C3b and C4b, thereby preventing continued complement activation.

Methods

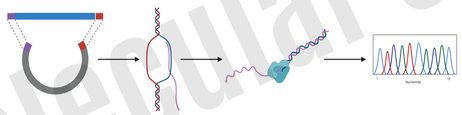

Figure 2. Minigene Experimental Design. The *CD46* variant, c.901+24T>G, was amplified and cloned into the pET01 plasmid by recombination. Following transfection into mammalian cells, RNA was isolated, reverse transcribed into cDNA, amplified, and sequenced to determine whether the variant affects mRNA splicing.

Results

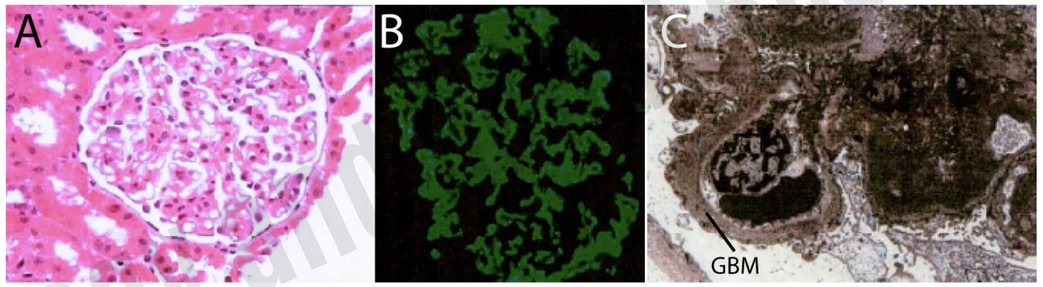

Figure 3. Patient Renal Biopsy. Renal biopsy findings are consistent with C3GN. (**A**) Light microscopy of the patient's glomerulus stained with hematoxylin and eosin (H&E) demonstrates mesangial cell proliferation and thickening of the glomerular basement membrane (GBM). (**B**) Immunofluorescence microscopy of the glomerulus shows dominant C3 deposition (green), with no evidence of immunoglobulin or C1 deposition. (**C**) Electron microscopy reveals thickening of the GBM, electron-dense deposits, and podocyte foot process distortion.

Table 1. Patient Information

Age	Sex	Diagnosis	C3Nef	C5Nef	C4Nef	FHAA (<200 AU)	FBAA (<200 AU)	APFA (50-130%)	CH50 (41-95 U/mL)	C3 (90-180 mg/dL)	C4 (15-47 mg/dL)	sMAC (<0.3 mg/L)
22	F	C3GN	Neg	Neg	Neg	Neg	Neg	50%	41	96	24	0.17

Table 2. Patient Variant Information

Gene	Genomic Position	Zygosity	Variant	Protein	MAF*	SpliceAI	Classification
CD46	chr1:g.207767847 T>G	Het	c.901+24T>G		4.45×10^{-6}	0.22	VUS
ADAMTS13	chr9:g.133454630 C>T	Het	c.3249+11C>T		4.30×10^{-5}	0	VUS/LB
C3	chr19:g.6714086 G>C	Het	c.683-4C>G		1.00×10^{-3}	0	VUS/LB
CFB	chr6:g.31950303 C>T	Het	c.1524C>T	p.His508His	2.38×10^{-3}	0	LB

*Variant annotations were retrieved from Franklin by QIAGEN using CRCh38/hg38.

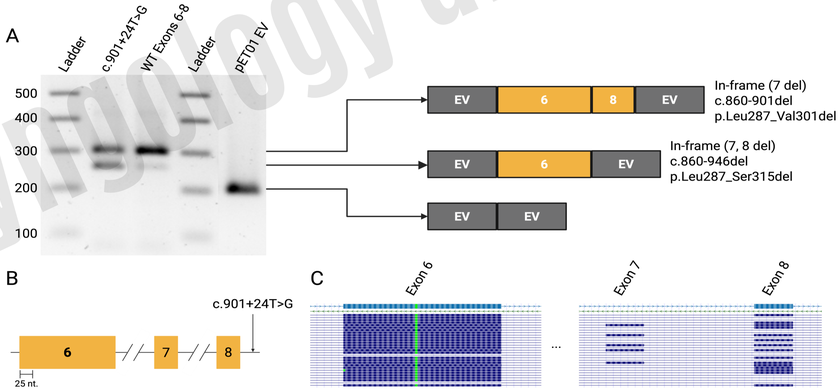

Figure 4. *CD46* c.901+24T>G induces partial exon 8 skipping. (**A**) RT-PCR results. Compared with the wild-type insert (WT Exons 6-8), the variant causes exon 8 skipping, resulting in the in-frame deletion of L287-S315. pET01 empty vector (EV) serves as the negative control. (**B**) Minigene construct and variant location. The minigene insert contains exons 6, 7, and 8, and the *CD46* c.901+24T>G variant is located in intron 8. (**C**) *CD46* transcripts. Exon 8 encodes the most heavily O-linked glycosylated portion of the ST domain, and exon 8–containing *CD46* transcripts (~95%) are enriched in the kidney and salivary glands, whereas exon 8–excluded transcripts (~5%) are more prevalent in the brain. The importance of O-linked glycosylation within this region is highlighted in malignancies, where exon 7 inclusion is promoted to increase glycosylation and likely to enhance resistance to complement-mediated damage (1).

Discussion

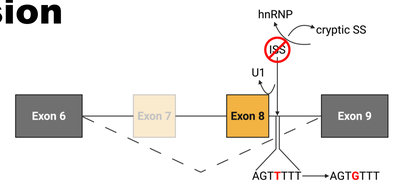

Figure 5. c.901+24T>G Proposed Pathogenic Mechanism. c.901+24T>G likely destroys an intronic splicing silencer (ISS) and simultaneously creates a cryptic splice site (SS) within intron 8. The destruction of the ISS and subsequent creation of the cryptic SS may reduce recognition of the native SS for exon 8, resulting in partial in-frame deletion of exon 8 from the final transcripts. hnRNP: heterogeneous nuclear ribonucleoprotein.

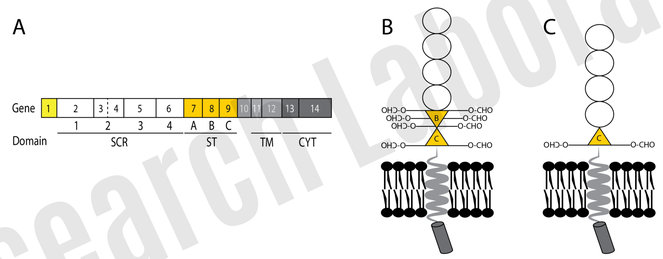

Figure 6. Effect of Exon 8 Skipping. (**A**) *CD46* gene showing the exons that encode the MCP domains. Exon 1 (yellow) encodes the signal peptide. (**B**) WT MCP. (**C**) Effect of exon 8 deletion, resulting in loss of the heavily O-linked glycosylated portion of the ST domain.

Conclusions

This study elucidates the pathogenic potential of an intronic *CD46* variant, c.901+24T>G, which causes partial skipping of exon 8 and removes part of the highly O-linked glycosylated ST domain from the mature transcript. Previous studies have shown that loss of the ST domain is associated with complement overactivation (1,2). Although MCP lacking the ST domain retains the ability to bind C3b and C4b, its FI cofactor activity is markedly reduced (1). Furthermore, the ST domain appears to serve as a critical spacer between the cell membrane and the SCR domains, as its deletion diminishes MCP function. Consistent with this role, removal of the ST domain from other membrane-bound complement regulators, including decay-accelerating factor, results in complete loss of regulatory activity (1). Together, these findings suggest that ST-domain loss may promote glomerular complement dysregulation and contribute to C3GN. However, whether this *CD46* variant directly drives disease or acts as a predisposing risk factor remains unclear.

Overall, this work highlights the importance of identifying splice-altering intronic variants alongside complement biomarker testing.

Acknowledgements

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Images were created using BioRender.com and Adobe Illustrator

References

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