Copy Number Variations: A Missing Genetic Driver in C3G

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Introduction

- C3 glomerulopathy (C3G) encompasses a group of rare diseases including:
  - C3 glomerulonephritis (C3GN)
  - Dense Deposit Disease (DDD)
- The characterization of C3G includes deposition of complement activator C3 in the glomerulus in association with dysregulation of the alternative pathway of complement.
- Patients with C3G often have an identifiable driver of disease that is either genetic (~25%) and/or acquired in the form of autoantibodies (~50-80%).
- Targeted Genomic Enrichment and Massively Parallel Sequencing (TGE+MPS) is often used to identify single nucleotide variants while multiplex ligation-dependent probe amplification (MLPA) is used to identify copy number variations (CNVs) of the *CFH-CFHR5* genomic region.
- CNVs in other genes associated with C3G are not routinely screened for in diagnostic testing.

Here we present CNVs as a potential missing genetic driver of C3G and a detection methodology that is widely accessible for identifying CNVs in genes outside of the *CFH-CFHR5* genomic region.

Methods

- A custom TGE+MPS panel was used to screen 13 genes implicated in complement-mediated diseases including thrombotic microangiopathies and C3G for single nucleotide variants. Genes included:
  - *CFH*, *CFI*, *CD46*, *CFB*, *CFHR5*, *C3*, *THBD*, *DGKE*, *PLG*, *ADAMTS13*, *MMACHC*, *G6PD*, and *WT1*
- Sequencing was performed on the Illumina MiSeq and data was analyzed using a custom bioinformatic pipeline.
  - Filtering: QD≥5; Qvar≥50; MAF<1%; non-synonymous, indels and splice-site variants.
- Multiplex Ligation-Dependent Probe Amplification (MLPA) was used to identify CNVs in the *CFH-CFHR5* genomic region.
- Copy number detection utilized normalized read-depth data across a large pool of samples (n=88) using a sliding window approach.
- Potentially causative CNVs were confirmed using long-range PCR and gel electrophoresis.

Case Report

Clinical Information

- 19-year-old Asian female with C3GN and chronically low C3 protein levels diagnosed in April 2020
- No family history of renal disease
- Renal biopsy: 4/2020
- Hematuria present
- Hematological data:
  - HG/HCT: 12.1/35.3
  - Platelets: 246
  - sCr/BUN: 5.9/41
  - uProt/uCr: >7
  - C3: 59
  - C4: 32

TGE+MPS Results

Table 1. TGE+MPS Results: Single nucleotide candidate variants. A dash (-) indicates no data is available.

Gene	Nuc. Change	AA Change	Exon	MAF (%)			GERP	PhyloP	PP2	SIFT	Mutation Taster	LRT	CADD
				1KG	EVS	GnomAD							
No single nucleotide variants identified													

MLPA Results

Table 2. MLPA Results.

Region Analyzed	Allele 1	Allele 2	Results
CFH-CFHR5 genomic region	normal allele	normal allele	no deletions or duplications

Functional and Biomarker Panel Results

Table 3: Biomarker and functional assay results indicated mild complement dysregulation and renal insufficiency; however, no acquired drivers were identified.

Test	Reference	Results	Interpretation
CH50	>70 U Eq/mL	116 U	Normal
APFA	50-130%	87%	Normal
Hemolytic	**<3%**	**15.7%**	**1+**
FHAA	<200 AU	58	Normal
FBAA	<200 AU	121	Normal
Fluid Phase Activity	<7.5%	4.4%	Negative
C3Nef	<20%	18%	Negative
C5Nef	<20%	16%	Negative
C4Nef	<20%	8%	Negative
C3 Level	**0.9-1.8g/L**	**0.7**	**Low**
C3c Level	<1.5 mg/L	0.4	Normal
C4 Level	0.15-0.57 g/L	0.36	Normal
FB Level	22-50 mg/dL	32.1	Normal
Ba Level	**<1.2 mg/L**	**3.4**	**High**
Bb Level	<2.2 mg/L	1.0	Normal
C5 Level	13.5-27 mg/L	22.8	Normal
Properdin Level	10-33 mg/L	22.6	Normal
sC5b-9	<0.3 mg/L	0.16	Normal
FI Level	18-44 mg/L	29.8	Normal
FH Level	180-420 mg/L	241	Normal

Routine Diagnostic Conclusion:

Routine diagnostic testing including TGE+MPS screening for C3G associated genes, MLPA for CNV screening of the *CFH-CFHR5* genomic region, and biomarker and functional assays did not reveal any identifiable driver of disease.

Copy Number Variation Analysis

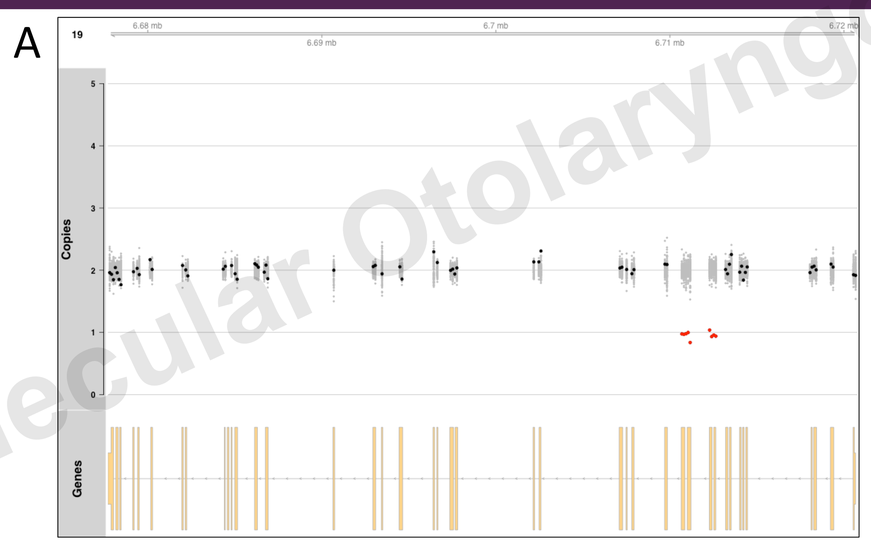

Figure 1: A) Normalized read-depth data of the gene *C3* in 88 patients with C3G. A decrease in read-depth can be seen in exons 10-13 (MG3-MG4 domain) for the patient of interest (red dots). This variant is predicted to result in a frameshift and stop codon, predicted to result in haploinsufficiency and explaining the patient's chronically low C3 levels in the absence of severe complement dysregulation.
Yellow bars: represents exons within the gene *C3*; grey dots: normalized batch read depth data; black dots: case report read depth data; red dots: case report abnormal read depth when compared to the sample cohort indicating presence of a deletion.

B) Gel electrophoresis confirming the presence of a heterozygous multi-exon deletion in *C3*. Primers were designed surrounding the predicted deletion location in the gene *C3*. Two dominate bands were identified, representing the wild type allele (top band) and the truncated allele (bottom band) (Δ ~2500 base pairs).
L: Ladder; C: Control; P: Patient; B: Blank.

Discussion

- Patients with C3G and other complement-mediated renal diseases are not routinely screened for CNVs outside the *CFH-CFHR5* genomic region.
- In a batch of 88 patients, a heterozygous multi-exon deletion in *C3*, predicted to result in a null allele and haploinsufficiency, was identified in a 19-year-old female with C3G and chronically low C3 levels.
- Low C3 levels are often attributed to consumptive activation of the alternative pathway, however, as in this case, could instead be attributed to haploinsufficiency of C3.

Based on these results, potentially consequential copy number variants could be present in >1% of the C3G patient population, however, the functional implications of these CNVs in C3G and other complement-mediated diseases should be investigated further.

References

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