G6PD deficiency: a partner in crime in complement mediated diseases or an “innocent” bystander?

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Introduction

The thrombotic microangiopathies (TMAs) are clinical syndromes defined by thrombocytopenia, nonimmune microangiopathic hemolytic anemia (MAHA), and organ damage. The diagnosis implies a specific pathologic lesion characterized by abnormalities in the endothelium of capillaries and small arteries in association with microvascular thromboses. Defining the underlying pathogenic mechanism of TMAs remains challenging and is critical to determining the optimal therapeutic approach. Here we illustrate a group of patients clinically diagnosed with TMA who carry pathogenic variants in the *G6PD* gene.

Methods

- A custom targeted genomic enrichment and massively parallel sequencing panel was used to screen 12 genes implicated in complement-mediated diseases including thrombotic microangiopathies and C3 glomerulopathies for single nucleotide variants. Genes included:
  - *CFH*, *CFI*, *CD46*, *CFB*, *CFHR5*, *C3*, *THBD*, *DGKE*, *PLG*, *ADAMTS13, MMACHC,* and *G6PD*
- Sequencing was performed on the Illumina MiSeq and data were 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.

Patient Cohort

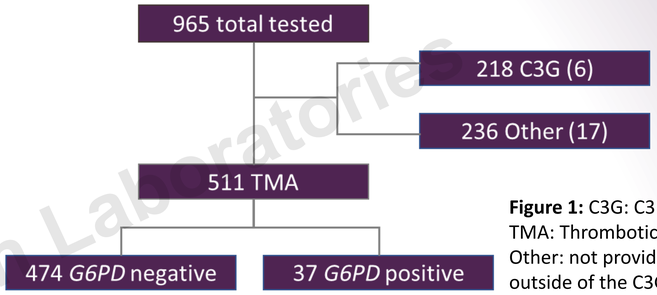

Figure 1: C3G: C3 Glomerulopathy; TMA: Thrombotic Microangiopathy; Other: not provided or a diagnosis outside of the C3G/TMA umbrellas

Results

	Ethnicity	Variant	Zygosity	G6PD class	MLPA	Hematuria	Schistocytes	Platelets (150-400K)	Bb (<2.2mg/L)	sC5b-9 (<0.3mg/L)	C3 level (90-180 mg/dL)	Eculizumab
1	AFR	A-	hemi	Class III	Δ *R3-R1* / WT	n/a	n/a	130	n/a	n/a	n/a	Ecu
3	AFR	A-	hemi	Class III	WT	n/a	Yes	22	n/a	n/a	n/a	Ecu
4	SAS	p.(Ser188Phe)	hemi	Class II Sassari	Δ *R3-R1* / WT	Yes	Yes	92	abnormal	normal	low	Ecu
5	Asian	p.(Arg454His)	hemi	Class II Andalus	Δ *R3-R1* / WT	Yes	Yes	112	abnormal	abnormal	normal	n/a
6	Asian	p.(Arg454His)	hemi	Class II Andalus	Δ *R3-R1* / WT	Yes	Yes	89	abnormal	abnormal	normal	n/a
7	AFR	A-	hemi	Class III	Δ *R3-R1* / WT	n/a	n/a	n/a	n/a	normal	n/a	Ecu
8	AFR	A-	hemi	Class III	WT	No	n/a	192	normal	normal	low	n/a
9	AFR	A-	hemi	Class III	Δ *R3-R1* / *R3-R1*	n/a	n/a	n/a	n/a	normal	n/a	n/a
10	AFR	A-	hmz	Class III	Δ *R3-R1* / WT	n/a	n/a	273	normal	normal	normal	n/a
11	AFR	A-	hmz	Class III	Δ *R3-R1* / WT	No	No	n/a	n/a	n/a	n/a	n/a
13	AFR	A-	het	Class III	Δ *R3-R1* / WT	n/a	n/a	n/a	normal	abnormal	normal	n/a
14	Asian	p.(Ala335Thr)	het	Class II Chatham	Δ *R3-R1* / WT	No	Yes	19	abnormal	abnormal	normal	n/a
18	AFR	A-	het	Class III	WT	Yes	Yes	256	abnormal	abnormal	normal	Ecu
19	AFR	A-	het	Class III	Δ *R3-R1* / *R3-R1*	Yes	Yes	426	n/a	n/a	n/a	n/a
23	AFR	A-	het	Class III	WT	No	No	253	normal	normal	normal	n/a
26	AMR	p.(Leu323Pro)	het	Class III Nefza	Δ *R3-R1* / WT	Yes	Yes	296	normal	normal	normal	Ecu
27	AFR	A-	het	Class III	Δ *R3-R1* / WT	n/a	n/a	n/a	normal	normal	normal	n/a
29	AFR	A-	het	Class III	Δ *R3-R1* / WT	n/a	n/a	462	n/a	n/a	n/a	Ecu
30	AFR	A-	het	Class III	Δ *R3-R1* / *R3-R1*	Yes	No	198	abnormal	abnormal	low	Ecu
36	AMR	A-	het	Class III	WT	No	Yes	232	n/a	n/a	n/a	n/a
37	AFR	A-	het	Class III	Δ *R3-R1* / *R3-R1*	Yes	Yes	57	n/a	n/a	low	Ecu

Table 1: Idiopathic TMAs with *G6PD* variants identified. AFR: African/African American; AMR: Latino/Admixed American; SAS: South Asian; Hmz: homozygous; Het: heterozygous; Hemi: hemizygous; WT: wildtype; A-: Class III *G6PD* allele including two variants, p.(Val68Met) and p.(Asn126Asp); Class II: G6PD activity <10% of normal, severe enzyme deficiency; Class III: 10-60% of normal, moderate to mild enzyme deficiency, intermittent acute hemolysis; n/a: not available.

	Ethnicity	Variant	Zygosity	G6PD class	Complement Variants	Path	MLPA	Hematuria	Schistocytes	Platelets (150-400K)	Bb (<2.2mg/L)	sC5b-9 (<0.3mg/L)	C3 level (90-180 mg/dL)	Eculizumab
2	AFR	A-	hemi	Class III	FHAA		Δ *R3-R1* / *R3-R1*	n/a	Yes	n/a	n/a	n/a	n/a	n/a
12	AFR	A-	hmz	Class III	*CFH* p.(Cys385Gly) *C3* p.(Gln1396Glu) *CFB* p.(Glu326Asp) *MMACHC* p.(Ile145Leu)	LP VUS VUS VUS	Δ *R3-R1* / WT	Yes	Yes	39	n/a	n/a	n/a	n/a
15	AFR	A-	het	Class III	*C3* p.(Ile1445Asn)	LP	WT	No	Yes	n/a	normal	normal	low	Ecu
16	SAS	p.(Ser188Phe)	het	Class II Sassari	*DGKE* p.(Lys109Glu) (hmz)	P	WT	Yes	n/a	n/a	normal	normal	normal	Ecu
17	AMR	p.(Arg454Cys)	het	Class II Union	*CFHR5* p.(Cys140Phe)	LP	Δ *R1-R4* / WT	Yes	No	260	n/a	n/a	n/a	Ecu
20	AFR	A-	het	Class III	*CFB* p.(Glu326Asp)	VUS	Δ *R3-R1* / *R3-R1*	No	No	217	normal	normal	normal	Ecu
21	AFR	A-	het	Class III	*C3* p.(Ile1157Asn)	LP	Δ *R3-R1* / *R3-R1*	n/a	n/a	n/a	abnormal	abnormal	normal	n/a
22	AFR	A-	het	Class III	*CFB* p.(Lys348Met)	VUS	WT	Yes	No	84	n/a	n/a	n/a	n/a
24	AFR	A-	het	Class III	*CFB* p.(Ile469Met)	VUS	WT	Yes	Yes	121	normal	abnormal	normal	n/a
25	Asian	p.(Val321Met)	het	Class II Viangchan	*CFH* p.(Pro682Ser) *CD46* p.(Asp192His)	VUS VUS	WT	Yes	n/a	35	abnormal	abnormal	low	n/a
28	AMR	p.(Leu323Pro)	het	Class III Nefza	*PLG* p.(Met482Thr)	VUS	WT	n/a	Yes	218	normal	normal	normal	n/a
31	Asian	p.(Leu128Pro)	het	Class II Vanua Lava	*THBD* p.(Glu560Gln)	VUS	WT	No	Yes	74	normal	normal	normal	Ecu
32	AFR	A-	het	Class III	*CFI* p.(Glu548Gln)	VUS	Δ *R3-R1* / *R3-R1*	No	No	114	normal	normal	normal	Ecu
33	AFR	A-	het	Class III	*CFH* p.(Glu625Ter)	P	Δ *R3-R1* / WT	n/a	Yes	70	n/a	n/a	n/a	Ecu
34	AFR	A-	het	Class III	*CD46* c.476-6_476-5 insdelG	VUS	WT	No	Yes	114	normal	abnormal	low	Ecu
35	AFR	A-	het	Class III	*PLG* p.(Asp258Asn)	VUS	Δ *R3-R1* / *R1-R4*	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Table 2: Complement-Mediated TMA with *G6PD* variants also identified. AFR: African/African American; AMR: Latino/Admixed American; SAS: South Asian; Hmz: homozygous; Het: heterozygous; Hemi: hemizygous; WT: wildtype; A-: Class III *G6PD* allele including two variants, p.(Val68Met) and p.(Asn126Asp); Class II: G6PD activity <10% of normal, severe enzyme deficiency; Class III: 10-60% of normal, moderate to mild enzyme deficiency, intermittent acute hemolysis; Path: Interpretation of complement variants; P: pathogenic; LP likely pathogenic; VUS: variant of unknown significance; n/a: not available.

Discussion

- 7% of patients with a TMA diagnosis have an identified potentially consequential *G6PD* variant
  - Class II *G6PD* variants seem to closely mimic a TMA presentation
  - This high incidence highlights the importance of testing G6PD activity and monitoring the use therapeutics
  - Ethnicity and sex (heterozygous females) may have an impact on G6PD activity and should be taken into consideration at the onset of disease
- **G6PD deficiency should be included in the differential diagnosis of complement-mediated renal diseases**

Pitfalls and Next Steps

- The relationship between G6PD deficiency and complement activation remains unclear
  - Accurate and available clinical information are lacking
  - Efforts should be made to thoroughly assess clinical parameters of complement activity at disease onset to determine whether a relationship exists
- Acute phase evaluation is necessary to compare G6PD deficiency and patients with complement variants and G6PD deficiency
- The high incidence of G6PD deficiency in TMA patients (7%) as compared to C3G patients (2%) warrants further investigation.

References

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