Comprehensive Complement Analysis of Atypical Hemolytic Uremic Syndrome in Argentina: A Case Study

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Introduction

- Atypical hemolytic uremic syndrome (aHUS) is an ultra-rare complement-mediated disease.
- The characterization of aHUS includes thrombocytopenia, microangiopathic hemolytic anemia, and acute kidney injury.
- aHUS is often associated with genetic or acquired drivers involving complement dysregulation.
- Coupled comprehensive genetic testing and biomarker testing is important for diagnosis, genetic counseling and treatment.

Here we present a 27-year-old Argentinean female presenting with post-partum aHUS and her five unaffected family members as a case study.

Methods

- A custom targeted genomic enrichment and massively parallel sequencing (TGE+MPS) panel was used to screen 11 genes associated in complement-mediated diseases (*CFH*, *CFI*, *CD46*, *CFB*, *CFHR5*, *C3*, *THBD*, *DGKE*, *PLG*, *ADAMTS13* and *MMACHC*).
  - Filtering: QD≥5; Qvar≥50; MAF<1%; non-synonymous, indels and splice-site variants.
- Multiplex Ligation-Dependent Probe Amplification (MLPA) was used to determine copy-number variation (CNV) of the *CFH-CFHR5* genomic region.
- A battery of biomarker and complement function assays by ELISA, radial immunodiffusion, C3 deposition and Luminex Multiplex Assays were used to determine complement dysregulation.

AR-17-1 Clinical Background

- 27 yo Argentinean female
- Episode of thrombotic microangiopathy (TMA) 30 days post-partum
  - Arterial hypertension
  - Myocarditis
  - Bilateral pneumonia
- Hematological parameters:
  - Hemoglobin: 6.5 g/dL
  - Hematocrit: 19%
  - Platelet: 130 x 10^9/L
  - LDH: 1517 U/L
  - Serum Creatinine: 3.37 mg/dL
- Renal biopsy description:
  - Typical TMA
  - Moderate-severe acute tubular necrosis
  - IgA deposition
- Treatment:
  - Plasmapheresis x10
  - Dialysis 3x/week
  - Eculizumab (after recurrence)

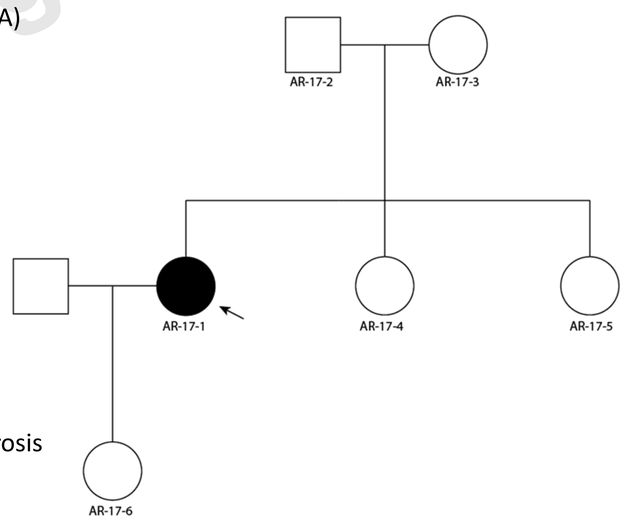

Figure 1: AR-17 family pedigree. Proband indicated by arrow.

Results

TGE+MPS Results

Table 1. TGE+MPS Results: Single nucleotide candidate variants for family AR-17 . A dash (-) indicates no data is available.

Gene	Nuc. Change	AA Change	Exon	MAF (%) 1KG	MAF (%) EVS	MAF (%) GnomAD	GERP	PhyloP	PP2	SIFT	Mutation Taster	LRT	CADD
CFH	NM_000186:c.575G>A	p.Cys192Tyr	5 (SCR3)	-	-	-	C	C	D	D	N	-	24.8
CFI	NM_000204:c.1189G>T	p.Val397Leu	11 (SP)	-	-	-	NC	NC	B	T	N	N	0.001
THBD	NM_000361:787C>G	p.Pro263Ala	1	-	-	0.001	C	NC	P	T	-	N	21.7

Segregation Analysis and MLPA

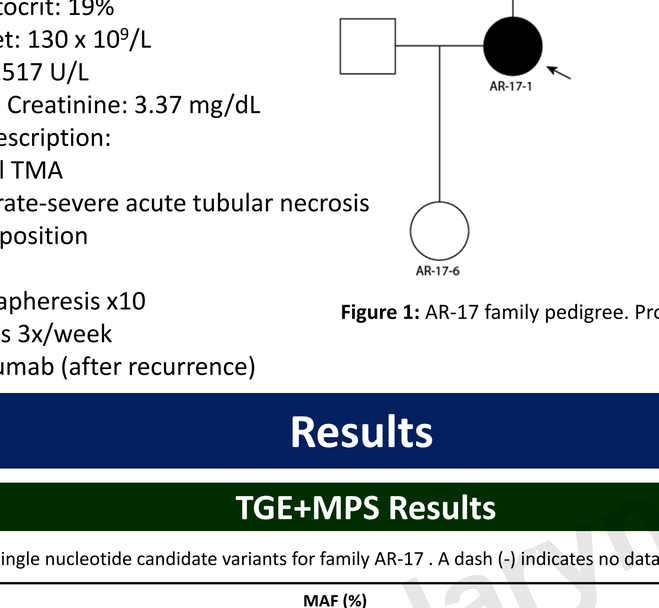

Figure 2: AR-17 family pedigree showing segregation of *CFH* p.Cys192Tyr, *CFI* p.Val397Leu and *THBD* p.Pro263Ala, MLPA data for the *CFH-CFHR5* genomic region, and segregation of *CFH* aHUS risk/protective haplotypes. Red alleles indicate a variation.

Functional Results

Table 3: Biomarker and functional assay results for AR-17-1 (proband , post-eculizumab), AR-17-3 (mom) and AR-17-5 (sibling). FH protein level is low to low-normal in persons carrying the p.Cys192Tyr in the *CFH* gene (AR-17-1 and AR-17-3) while FI protein levels are low to low-normal in persons carrying the p.Val397Leu (AR-17-1 and AR-17-5).

Test	Reference	AR-17-1 (Proband)	AR-17-3 (Mom)	AR-17-5 (Sibling)
CH50	> 70 U Eq/mL	0	161	214
APFA	50-130%	1	92	73
Hemolytic	< 3%	1.3	1.2	1.1
FHAA	< 200 AU	< 50	< 50	< 50
C3 level	0.9-1.8 g/L	0.72	1.3	1.7
C4 level	0.15-0.57 g/L	0.40	0.40	0.31
FB level	22-50 mg/dL	37	43.7	60.8
Bb level	< 2.2 mg/L	3.47	2.04	0.98
Ba level	<1.2 mg/L	2.76	0.94	0.62
C5 level	10-21 mg/dL	37	17.3	24.5
sC5b-9	< 0.3 mg/L	0.41	0.35	0.32
FI level	17-39 mg/L	20.2	32.1	19.2
FH level	180-420 mg/L	168.4	189.2	343.1

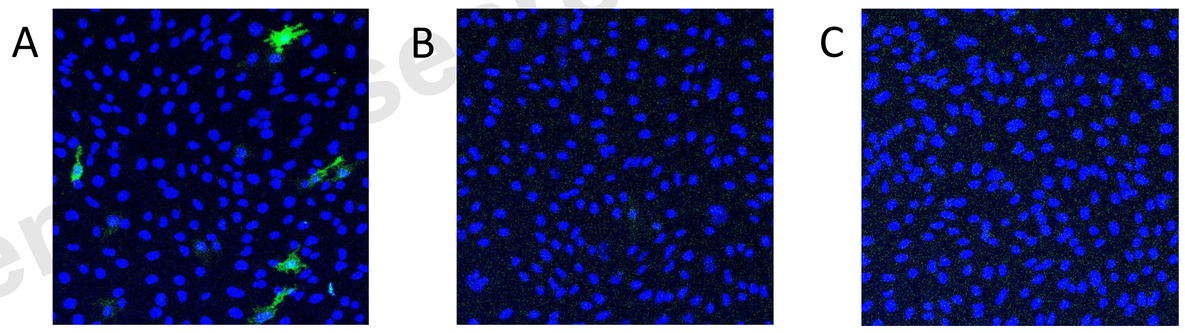

Figure 3: C3 Deposition Assay results for **A)** AR-17-1 (proband), **B)** AR-17-3 (mom) and **C)** AR-17-5 (sibling). Blue florescence indicates nuclei of cells and green florescence indicates C3 deposits. The antibody was a gift from Dr. Ronald P. Taylor (University of Virgina).

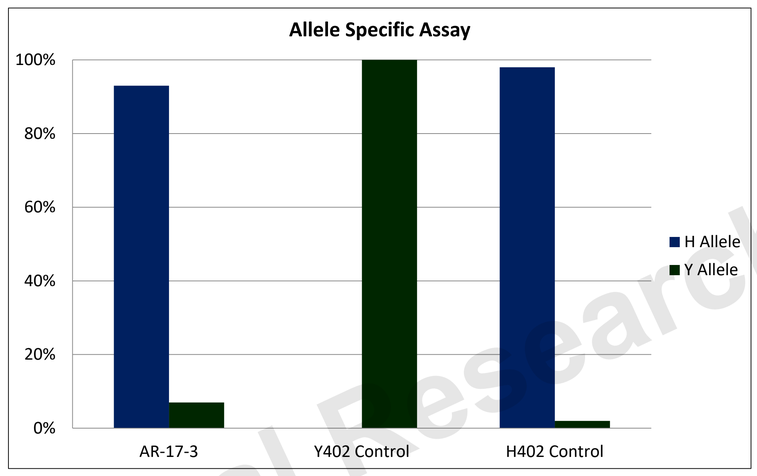

Figure 4: Allele-Specific Assay for heterozygous *CFH* H402Y in AR-17-3. Results show the Y allele carrying the p.Cys192 to be null at 7% expression. FH402 antibodies used for this assay were a gift from Dr. Paul Morgan (Cardiff University).

Conclusions

- The AR-17 family segregates cysteine change that results in a null allele of the gene *CFH* and leads to complement dysregulation resulting in the pregnancy-triggered aHUS in AR-17-1.
- Additionally, functional assays showed low-normal Factor I protein levels in persons carrying the *CFI* variant suggesting a hypomorphic allele, which may contribute to the proband's complement dysregulation as a risk variant.
- Functional assays showed C3 consumption and deposition, accumulation of breakdown products, and little to no expression of the Y *CFH* allele in AR-17-1, validating the pathogenicity of p.Cys192Tyr.
- This case shows the complexities and challenges in understanding the many facets associated with aHUS.
- Genetic testing can inform biomarker assays and complement function and vice versa to give insight into the disease course and progression.
- Diagnostic tools associated with genetic screening, biomarker assays, and complement function testing are needed to provide patients with the best clinical care, genetic counseling and treatment.

Integrated genetic testing and functional assays are vital to understanding aHUS and related TMAs.

References

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