METHODS AND DATA SUPPLEMENT

Complement activity and complement regulatory gene mutations are associated with thrombosis in APS and CAPS

SUPPLEMENTAL METHODS

TARGETED SEQUENCING FOR COMPLEMENT REGULATORY GENES

Genomic DNA was quantitated using Qiagen Qubit fluorometric assay and 50ng of DNA was used as input for targeted sequencing with a custom Ampliseq panel (Illumina). Amplicons were designed to cover all exons of 15 genes with known function related to complement activation/regulation (*CFH*, *CFB*, *CFI*, CFD, CFP, *CFHR1*, *CFHR2, CFHR3*, *CFHR4, CFHR5*, *C3*, *CD46* (*MCP*), *THBD, CR1, DGKE*) with amplicon length between 100-350 base pairs (bp) (median 307 bp, mean 285 bp). Libraries were generated per manufacturer's protocol. Briefly, targets were amplified using a Veriti 96-well Thermal Cycler, followed by amplicon digestion, index ligation and purification. Amplicons were then amplified and purified, followed by quantification via Qubit fluorometric assay. Library quality was assessed using Agilent 2100 Bioanalyzer. Subsequently, libraries were normalized and pooled prior to sequencing via Illumina MiSeq using v3 (600-cycle) reagents performed by the Genetic Resources Core Facility at Johns Hopkins School of Medicine. MiSeq optimization and quality control was performed by the GRCF, and mean amplicon coverage for all samples was 640x. Individual amplicon coverage for a representative sample is shown in supplemental figure S2. Analysis of raw sequencing data (FASTQ) was performed using the DNA Amplicon pipeline (v2.1.1) via the Illumina BaseSpace platform. Alignment to (GRCh37/hg19) human genome reference was performed using the banded Smith-Waterman algorithm in the targeted regions. Variant calls were made using an

Illumina-developed germline variant caller and filtered using VariantStudio software (v3.0). Variants not passing Illumina's variant quality filters were excluded, followed by filtering using the following criteria to identify rare germline single nucleotide variants and indels: 1) depth greater than 50X; 2) non-synonymous coding region or splice variants; 3) variant allele frequency between 40% and 60%; 4) minor allele frequency less than 0.005 in any ethnic population in the genome aggregation database (gnomAD, total 141,456 individuals). Large deletions were determined by complete loss of signal for multiple consecutive amplicons (Figure S2). Homozygous deletion of *CFHR1* and *CFHR3*, reported to occur in approximately 2% of the population, was included in our analysis due to its association with CFH antibody formation and association with aHUS.[47]

Table S1. Demographic characteristics of study population

	SLE (N = 74)	**APS (N= 59)**	**CAPS (N = 8*)**
Age (years) , median (IQR)	48.5 (37.0, 58.0)	48 (41.5, 57.0)	47.5 (41, 64)
Female sex, n (%)	66 (89.2)	33 (55.9)	5 (50%)
aPL profile			
Single positive		22 (37.3%)	
Double positive		15 (25.4%)	
Triple positive		22 (37.3%)	

*N=8 (of 10) patients with available sera for complement assays

Table S2. Complement regulatory gene variants in patients with CAPS.

ID	Gene	Variant location/base change	Global MAF (GnomAD)	VAF (%)	Read Depth	dbSNP	Change	Consequence	RefSeq	SIFT	PolyPhen-2	PROVEAN
CAPS-3	THBD	chr20:23028640/G>A	0.001785473	43.9	155	rs1800579	p.P501L	Missense Variant	NP_000352.1	Damaging	Possibly Damaging	Deleterious
CAPS-5	CR1	chr1:207785099/G>T	0.001845663	46.9	260	rs202148801	p.V1675L	Missense Variant	NP_000564.2	Tolerated	Possibly Damaging	Neutral
CAPS-6	CFHR4	chr1:196879471/G>A	0.000110592	49	496	rs188298928	p.R287H	Missense Variant	NP_001188479.1	Tolerated	Probably Damaging	Neutral
CAPS-6	CR1	chr1:207757992/A>G	N/A	47.6	227	rs1257030512	c.3953-2A>G	Splice Acceptor Variant	NG_007481.1	N/A	N/A	N/A
CAPS-7	CFHR1 & CFHR3	chr1:196743970-196801301/del	N/A	100	0	N/A	CFHR3,1Δ	Transcript Ablation	NC_000001.10	N/A	N/A	N/A
CAPS-8	CR1	chr1:207782682/A>G	0.00031016	52.4	208	rs189863730	p.S1532G	Missense Variant	NP_000564.2	Tolerated	Probably Damaging	Neutral
CAPS-8	CFHR1 & CFHR3	chr1:196743970-196801301/del	N/A	100	0	N/A	CFHR3,1Δ	Transcript Ablation	NC_000001.10	N/A	N/A	N/A
CAPS-10	CFHR1 & CFHR3	chr1:196743970-196801301/del	N/A	100	0	N/A	CFHR3,1Δ	Transcript Ablation	NC_000001.10	N/A	N/A	N/A

FIGURE S1

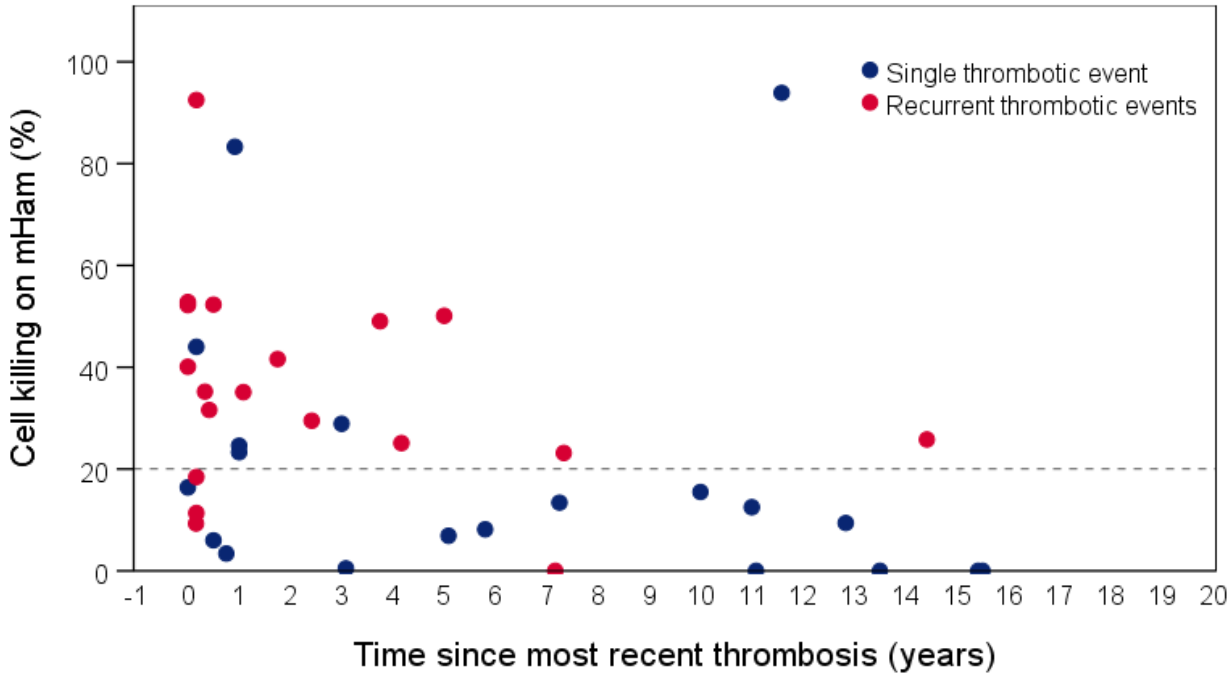

Figure S1. APS patient sera are more likely to demonstrate a positive mHam assay (complement dependent cell killing) close to the time of the thrombotic event. For patients with a known date of most recent thrombosis (N=41), the mHam was positive in 68.4% (13 of 19) of patients when thrombosis occurred within 1 prior to testing, compared with 31.8% (7 of 22) of patients with their most recent thrombotic event more than 1 year prior to testing (P=0.019). A positive mHam assay >1 year after thrombosis was associated with recurrent VTE [85.7% (6 of 7), P= 0.014] and triple positivity [71.4% (5 of 7), P= 0.04].

FIGURE S2

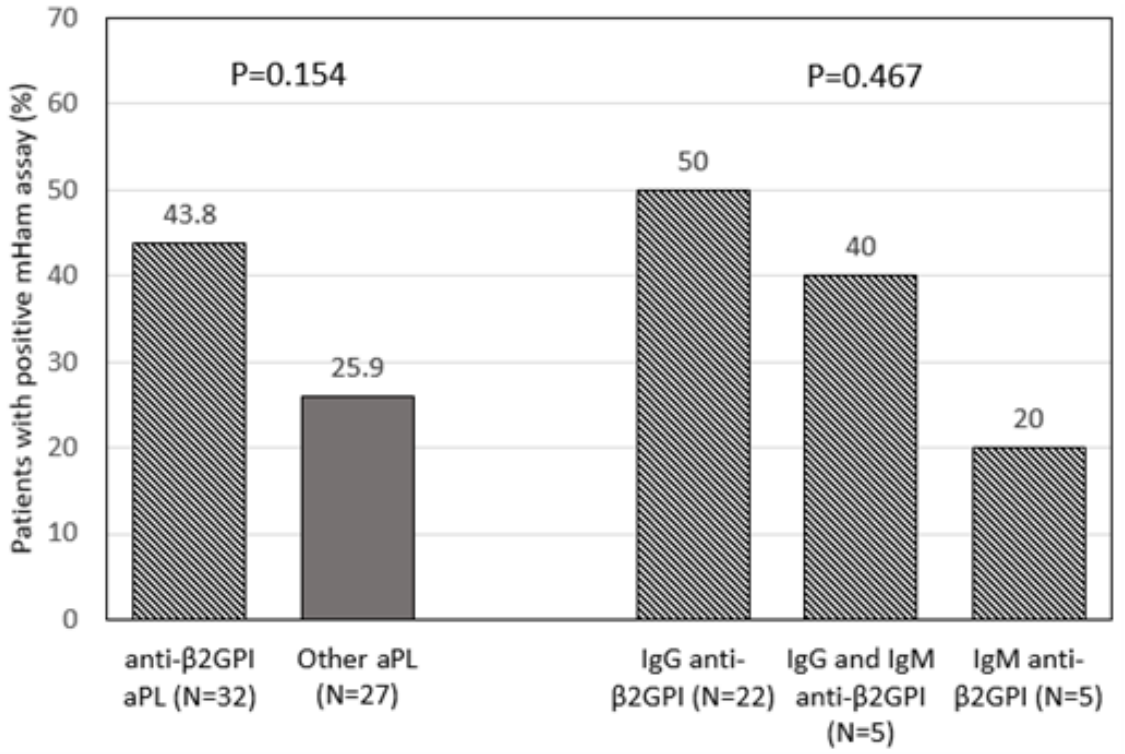

Figure S2. Rates of modified Ham positivity categorized by aPL profile. The mHam test was positive in 43.8% (14 of 32) patients with positive anti-β2GPI antibodies compared with 25.9% (7 of 27) of patients who had only aPL other than anti-β2GPI antibodies (P=0.154). Among patient with anti-β2GPI antibodies), The mHam assay was positive in 50% (11 of 22) with anti-β2GPI IgG, 20% (1 of 5) with anti-β2GPI IgM, and 40% (2 of 5) with anti-β2GPI IgG and IgM (P=0.300).

FIGURE S3

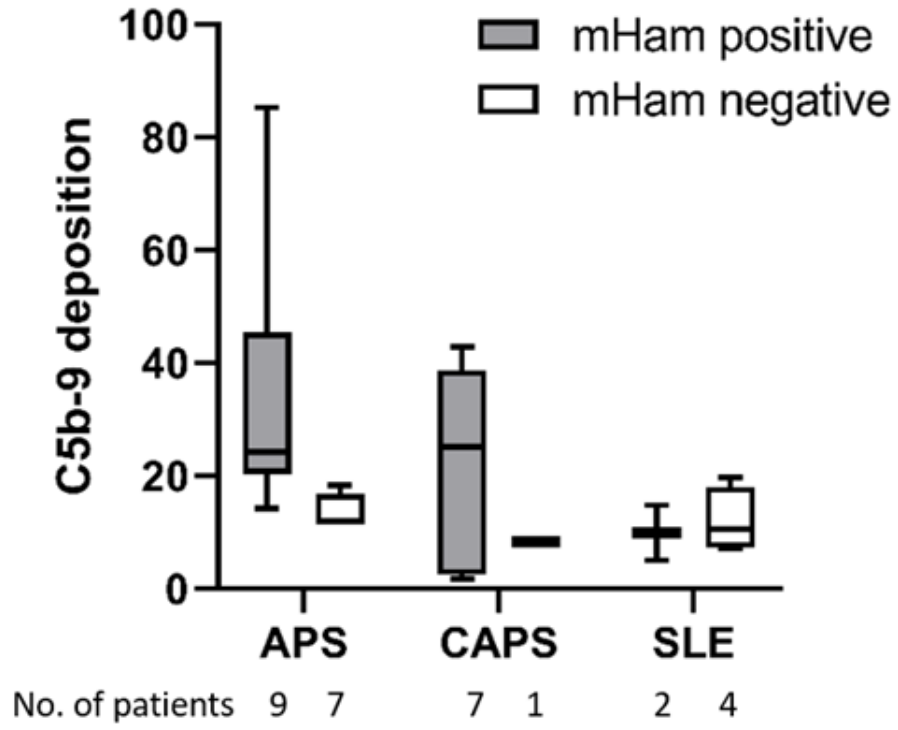

Figure S3. Distribution of C5b-9 deposition for patients with APS, CAPS and SLE categorized by mHam results.

FIGURE S4

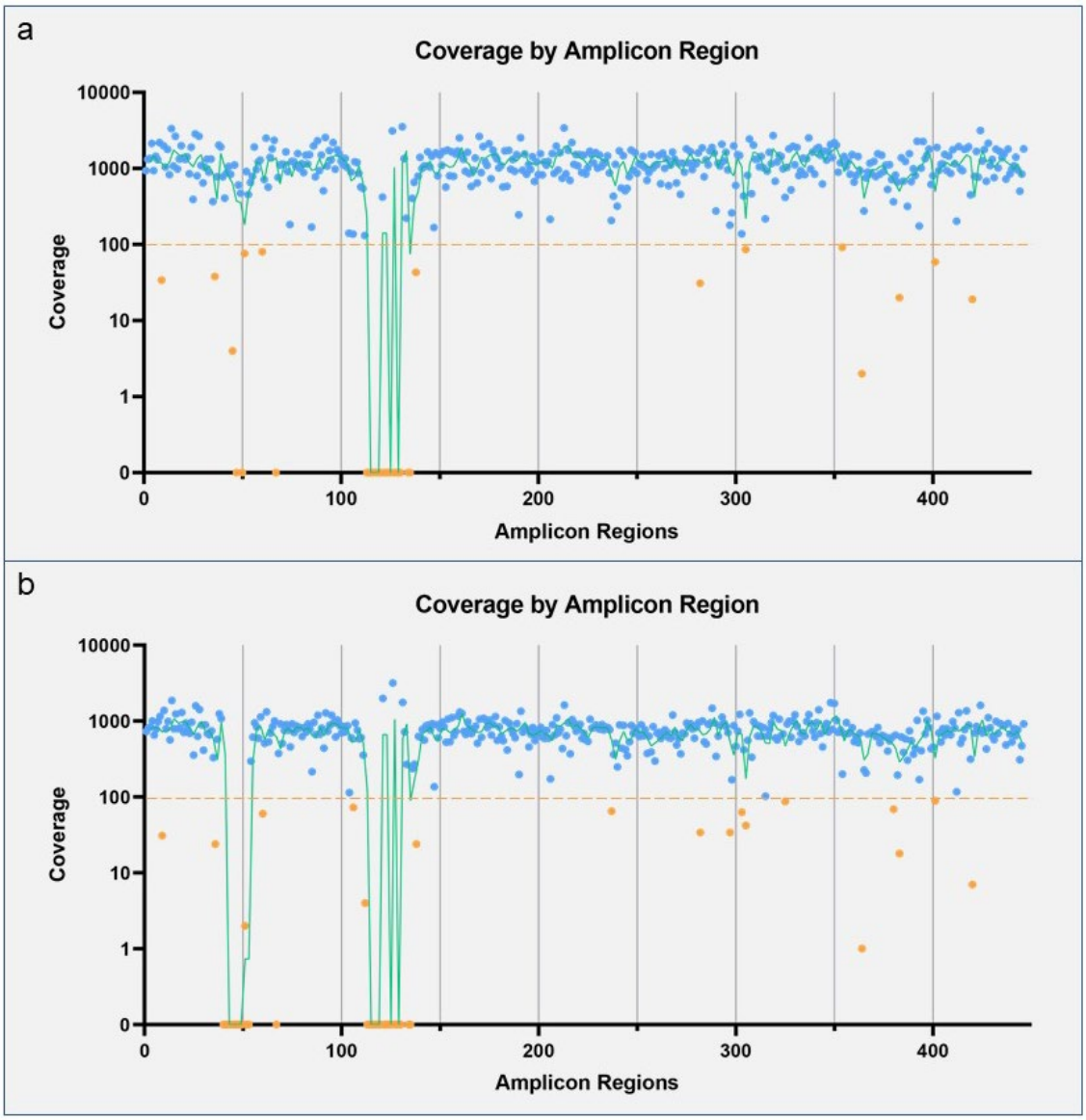

Figure S2. Target sequencing amplicon coverage. Coverage per amplicon is displayed for two representative samples. Each circle represents a distinct amplicon, with the threshold of 100x depth distinguishing blue and orange circles. Coverage for the majority of amplicons was greater than 1000x, however multiple contiguous amplicons corresponding to the low copy repeat regions of the *CR1* gene performed poorly in all samples (seen in panel a and b). Homozygous deletion of *CFHR1* and *CFHR3* was detected via a second region of zero depth observed in panel b.