THE GENETICS OF COMPLEMENT DEFICIENCIES: A STUDY OF SIBLINGS

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LEARNING OBJECTIVES

Upon completion of this learning activity, participants should be able to:

- Objective 1: distinguish various presentations of early complement deficiencies.
- Objective 2: evaluate for early complement deficiency.
- Objective 3: recognize which patients should undergo genetic evaluation.

THE COMPLEMENT SYSTEM

Acts as part of the innate immune system to eliminate pathogens.

Decreased complement activity may occur due to inherited abnormalities affecting complement components or regulatory proteins or by acquired activation of complement through either of the three pathways (alternative, classical, lectin).

As such, there may be dysregulation of microorganism defense, self-tolerance, and inflammatory responses.

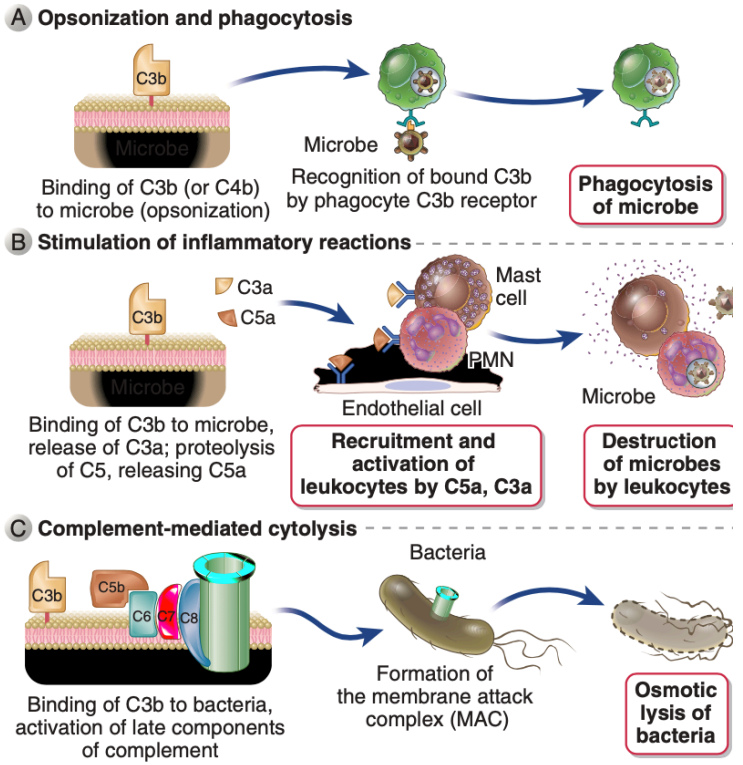


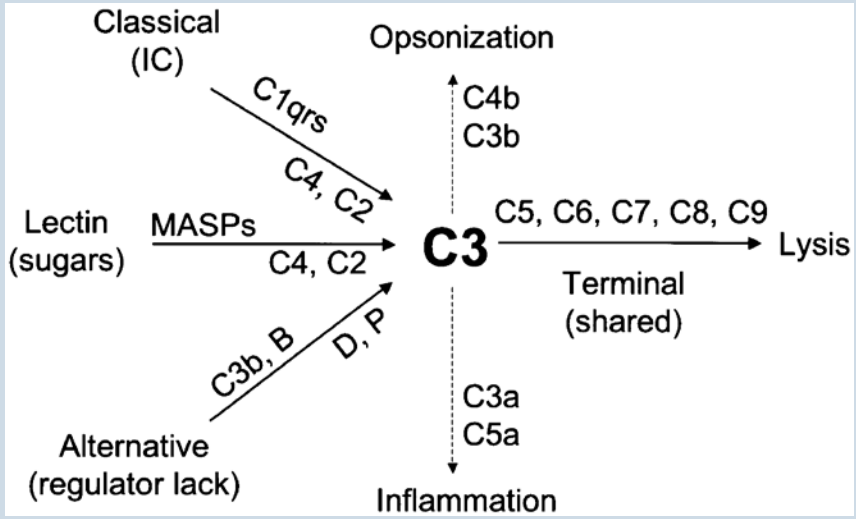
FIGURE 13.17 Functions of complement. The major functions of the complement system in h defense are shown. Cell-bound C3b is an opsonin that promotes phagocytosis of coated cells **(A)**; the p teolytic products C5a, C3a, and (to a lesser extent) C4a stimulate leukocyte recruitment and inflammat **(B)**; and the membrane attack complex (MAC) lyses cells **(C)**.

Abbas AK, Lichtman AH, Pillai S, Baker DL. *Cellular and Molecular Immunology*. Tenth edition. Elsevier; 2022.

C1Q DEFICIENCY

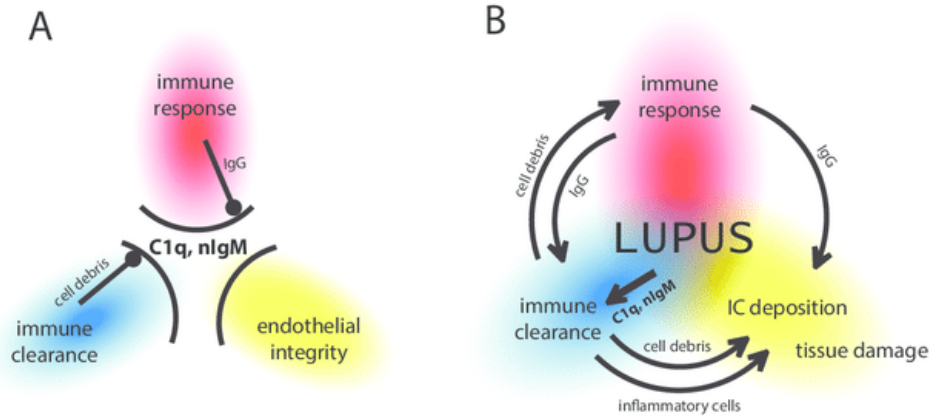
- May be due to decreased levels or functional deficiency.
- Most commonly these patients will develop SLE in early childhood and recurrent infections.
- Other common manifestations that may occur: Raynaud phenomenon, alopecia, Sjogren syndrome, Hyper IgM syndrome, and/or central nervous system involvement.

- Deficiency of other complement components – C1r/C1s, C2, C4 – are associated with SLE but C1q deficient patients have the most prevalent and severe autoimmune symptoms.



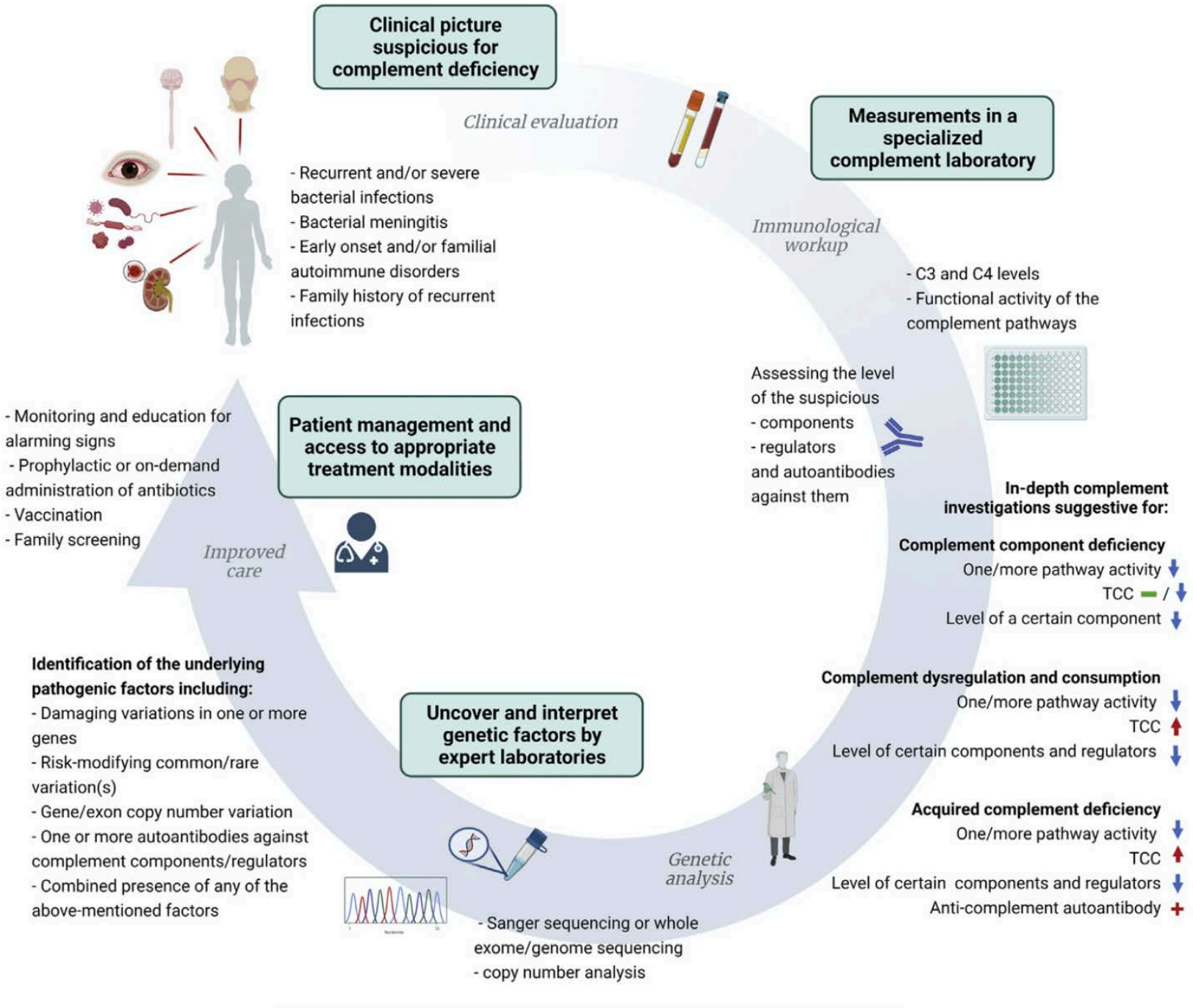
Protein	Genetics	Infection susceptibility (including typical microorganisms)	Other disease associations
C1q	Autosomal recessive; most frequent mutation is g.2687C>T of C1qA, leading to a stop codon	Sepsis, meningitis, pneumonia, *Streptococcus (S.) pneumoniae, Neisseria (N.) meningitis*	SLE/SLE-like disease (~90%)
C1r/C1s	Autosomal recessive	Sepsis, meningitis, pneumonia due to encapsulated bacteria	SLE/SLE-like disease (~60%)
C4	Autosomal recessive	Sepsis, meningitis, pneumonia due to encapsulated bacteria	SLE/SLE-like disease (~80%), glomerulonephritis
C2	Autosomal recessive >90% caused by 28bp deletion in C2 gene	Sepsis, pneumonia, meningitis, osteitis *S. pneumoniae, Staphylococcus (S.) aureus, N. meningitidis*	SLE or other rheumatic disease (~40%) Cardiovascular disease
C3	Autosomal recessive	Respiratory tract infections, meningitis *N. meningitidis, S. pneumoniae, Haemophilus (H.) influenzae, S. pyogenes, S. aureus*	Immune-complex disease; glomerulonephritis, vasculitis

C1Q DEFICIENCY AND SLE



Prechl J, Czirják L. The endothelial deprotection hypothesis for lupus pathogenesis: the dual role of C1q as a mediator of clearance and regulator of endothelial permeability. *F1000Res*. 2015;4:24.

- Lack of C1q is thought to result in an impaired capacity for removal of apoptotic cells and immune complexes, which may contribute to SLE development.
- Deficiency of this complement component is associated with more severe autoimmune symptoms


Clinical picture suspicious for complement deficiency
Clinical evaluation
- Recurrent and/or severe bacterial infections
- Bacterial meningitis
- Early onset and/or familial autoimmune disorders
- Family history of recurrent infections
Measurements in a specialized complement laboratory
Immunological workup
- C3 and C4 levels
- Functional activity of the complement pathways
Assessing the level of the suspicious
- components
- regulators
and autoantibodies against them
In-depth complement investigations suggestive for:
Complement component deficiency
One/more pathway activity
TCC
Level of a certain component
Complement dysregulation and consumption
One/more pathway activity
TCC
Level of certain components and regulators
Acquired complement deficiency
One/more pathway activity
TCC
Level of certain components and regulators
Anti-complement autoantibody
Genetic analysis
- Sanger sequencing or whole exome/genome sequencing
- copy number analysis
Uncover and interpret genetic factors by expert laboratories
Identification of the underlying pathogenic factors including:
- Damaging variations in one or more genes
- Risk-modifying common/rare variation(s)
- Gene/exon copy number variation
- One or more autoantibodies against complement components/regulators
- Combined presence of any of the above-mentioned factors
Patient management and access to appropriate treatment modalities
Improved care
- Monitoring and education for alarming signs
- Prophylactic or on-demand administration of antibiotics
- Vaccination
- Family screening

SIBLINGS' PRESENTATION

Each sibling was diagnosed with systemic lupus erythematosus (SLE) at ages 6 and 4

- Older sibling (age 6)
  - Major manifestations: diffuse proliferative glomerulonephritis and leukocytoclastic vasculitis confirmed on respective biopsies (kidney, skin)
- Younger sibling (age 4)
  - Major manifestations: pericarditis, refractory arthritis

Rheumatologic diagnostic work-up for both siblings was positive as follows:

- Positive findings: antinuclear antibody, anti-double-stranded DNA, anti-smith and ribonucleoprotein antibodies, and erythrocyte sedimentation rates

CLINICAL COURSE

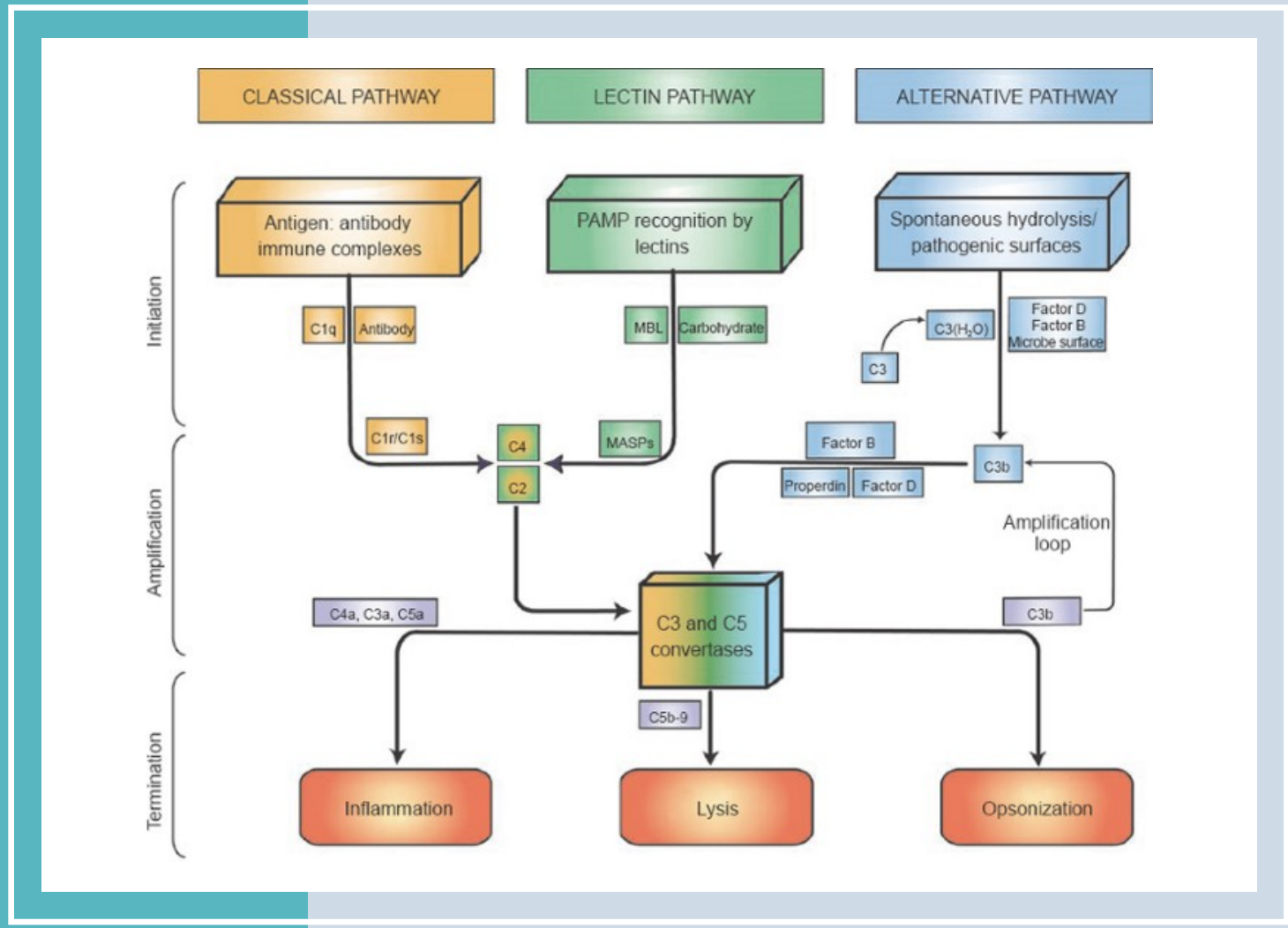
- Older sibling
  - Developed hypertension secondary to chronic steroid use and lupus nephritis
    - On lisinopril
  - Completed cyclophosphamide regimen
  - Currently on mycophenolate, hydroxyquinone, and tapering steroids (currently at 6mg daily)
  - Will intermittently receive IVIG for hypogammaglobulinemia

- Younger sibling:
  - Received rituximab previously for persistent arthritis
  - Developed intermittent arthralgias, mycophenolate was changed to methotrexate
  - Developed myositis with elevated muscle enzyme levels
  - Tapering steroids, currently at 3mg daily, and on hydroxychloroquine
  - Received IVIG for 2 months in early 2022

IMMUNODEFICIENCY EVALUATION

Initial screening:

- Absent total hemolytic complement (CH50)
- Normal C3, C4 levels



Complement Assay	Result (Older sibling)	Result (Younger sibling)	Interpretation	Reference Range with units
Alternative pathway (AH50), serum	137		Normal	77-159 units/ml
Classical pathway activity (CH50), serum	0		LOW	176-382 units/ml
C1q function, serum	0	0	LOW	2515-9414 units/ml
C1 function, serum	0	0	LOW	116373-264072 units/ml
C1q level, plasma	82	134	Borderline LOW/HIGH	83-125 mcg/ml
C1q autoantibody test, serum	0	0	Normal	0-7.0 (% of STD)
C2 function, serum	22795	32150	Normal	15354-46242 units/ml
C4 function, serum	5859419	13225843	Normal	400000-43000000 units/ml

Interpretation of lab results for Complement deficiency

CH50 = 0 or very low AH50 is OK	→	Missing C1q, C1r, C1s, C2 or C4
AH50 = 0 or very low CH50 is OK	→	Missing B or D (very rare), or Properdin
AH50 and CH50 = 0 or very low	→	Missing C3, C5, C6, C7, C8, or C9
Late components low, especially C3, AH50 and CH50 low	→	Missing factor H or factor I

Complement activation is indicated if more than one component is low or if decrease in CH50 and/or AH50 is sporadic. Activation can be verified by measuring complement split products (C3a, C4a, Bb, C5a, SC5b-9).

COMPLEMENT COMPONENT TESTING

- Undetectable C1 and C1q function
- Borderline low C1q level
- Negative C1q autoantibody testing

= FUNCTIONAL C1Q DEFICIENCY

RESULT: NO PATHOGENIC VARIANTS IDENTIFIED

Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
C1QC	c.164G>A (p.Gly55Glu)	homozygous	Uncertain Significance
C1S	c.514G>A (p.Gly172Arg)	heterozygous	Uncertain Significance

About this test

This diagnostic test evaluates 22 gene(s) for variants (genetic changes) that are associated with genetic disorders. Diagnostic genetic testing, when combined with family history and other medical results, may provide information to clarify individual risk, support a clinical diagnosis, and assist with the development of a personalized treatment and management strategy.

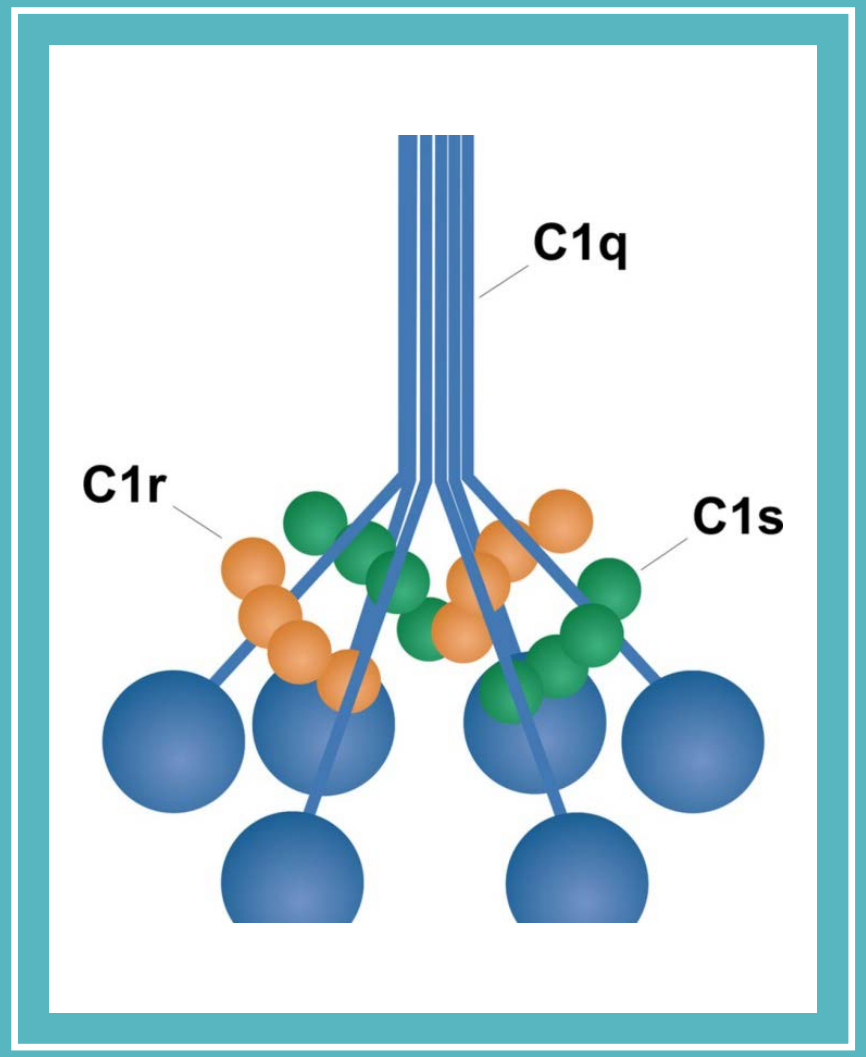
Variant details

C1QC, Exon 2, c.164G>A (p.Gly55Glu), homozygous, Uncertain Significance

- This sequence change replaces glycine, which is neutral and non-polar, with glutamic acid, which is acidic and polar, at codon 55 of the C1QC protein (p.Gly55Glu).
- This variant is not present in population databases (gnomAD no frequency).
- This variant has not been reported in the literature in individuals affected with C1QC-related conditions.
- Algorithms developed to predict the effect of missense changes on protein structure and function are either unavailable or do not agree on the potential impact of this missense change (SIFT: "Deleterious"; PolyPhen-2: "Probably Damaging"; Align-GVGD: "Class C0").
- In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance.

C1S, Exon 5, c.514G>A (p.Gly172Arg), heterozygous, Uncertain Significance

- This sequence change replaces glycine, which is neutral and non-polar, with arginine, which is basic and polar, at codon 172 of the C1S protein (p.Gly172Arg).
- This variant is present in population databases (rs375308014, gnomAD 0.01%).
- This variant has not been reported in the literature in individuals affected with C1S-related conditions.
- ClinVar contains an entry for this variant (Variation ID: 625898).
- Algorithms developed to predict the effect of missense changes on protein structure and function (SIFT, PolyPhen-2, Align-GVGD) all suggest that this variant is likely to be disruptive.
- In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance.



GENETIC EVALUATION

- Given the siblings' similar presentations, sequence analysis identified a homozygous Gly55Glu mutation in the C1q gene and a heterozygous Gly172Arg mutation in the C1s gene for each sibling
- At this time, both are characterized as variants of uncertain significance (VUS)
- Findings and presentation for each sibling is most consistent with the homozygous mutation as a likely pathogenic variant state and the heterozygous mutation as a likely carrier state

Table 1 Mutations reported causing C1q deficiency

C1q chain	*Mutation*[a]	*Systematic names*	*Old nomenclature*[b]	*Total number of families described*	*Origin of families*
C1qA	**g.6149delG**	**Glu53fs**	Glu12fs	1	**Sweden** (this paper)
	g.7235C>T	**Gln64X**	**Gln42X**		
C1qA	**g.7667C>T**	**Gln208X**	**Gln186X**	8	**Iraq** (this paper) Turkey[25–29] Slovak Republic[26] Cyprus[27]
C1qA	**g.7693G>A**	**Trp216X**	Trp194X	1	**Sudan** (this paper)
C1qB	g.11393G>A	Gly42Asp	Gly15Asp	1	Morocco[22]
C1qB	g.12965C>T	Arg 177X	Arg150X	1	Mexico[20]
C1qB	**g.13166G>A**	**Gly244Arg**	Gly217Arg	1	**Inuit**[32]
C1qC	g.5499G>A	Gly34Arg	Gly6Arg	5	Germany[25] India[34] Saudi Arabia[21] Caucasian[23] Arabian[24]
C1qC	g.5564delG	Gly55fsX83		1	Pakistan[35]
C1qC	**g.8626C>T**	**Arg69X**	**Arg41X**	2	**Kosova** (this paper) Yugoslavia[34]
C1qC	g.8633delC	Gln71fsX137	Gln43fs→108X	1	England[34]
C1qC	**g.8647G>A**	**Gly76Arg**	**Gly48Arg**	1	**Turkey**[33]

[a]According to NCBI reference sequence NG_007282 (*C1qA*), NG_007283 (*C1qB*) and NG_007565 (*C1QC*).
[b]Codon numbers according to original publications or Sellar *et al*.[4]
Mutations and origin of patients identified in our laboratory are in bold.

MUTATIONS ASSOCIATED WITH C1Q DEFICIENCY

- C1q gene mutations are inherited in an autosomal recessive manner

NEXT STEPS

Initiating process for intravenous administration of fresh frozen plasma

May consider allogeneic hematopoietic stem cell transplantation in the future

Table 1 Clinical information about reported cases and current patients with SLE and C1q deficiency, treated with FFP

Reference	*Case number*	*Gender Origin*	*SLE onset (age, years)*	*Positive ACR*	*RI*	*Previous treatments*	*Outcome*	*FFP onset (age, years)*	*Duration of FFP treatment*	*Interval of FFP infusions which held remission*	*Outcome*
Berkel et al.[12]	1	M Turkish	3	DR, OUs, R, H, I: anti-Sm	Meningitis, otitis, sepsis	Not specified	Unimproved skin	10	3 days/ 3 sessions PE		Improved skin and fever; death 10 days after cessation of PE
Kirschfink et al.[13]	1	F German	6	MR, DR, Ph, OUs, A, S, N, H, I: anti-dsDNA, anti-Sm, ANA	No	Chlorambucil, cyclophosphamide, yclosporine, high-dose immunoglobulins, prednisolone	Unresponsive (last attack)	28	Only once		Severe immediate allergic reaction, death
Topaloğlu et al.[14]	1	F Turkish	3.5	DR, Ph, OUs, A, R, I: anti-ds DNA, ANA	No	Prednisolone	Responsive	5.5	Unknown	Unknown	Unknown
Mehta et al.[10]	1	F Pakistani	6	MR, DR, Ph, OUs, N, I: anti-Sm, ANA	Herpes zoster, bacterial and viral infection	Prednisolone, AZA,	Unresponsive	15	10 years	Every four weeks	Remission
Topaloglu et al.[11]	1	F Turkish	6	MR, Ph, OUs, A, I: ANA	Yes, not specified	Steroids, AZA, HQ	Unresponsive	15	3 years	Every three weeks	Remission
Higuchi et al.[15]	1	F Japanese	4	MR, DR, OUs, A, ANA	No	Prednisolone, mizoribine	Unresponsive	5	Unknown	Weekly	Remission
Current report	1	M Turkish	4.5	MR, DR, Ph, OUs, I: anti-Sm, anti-smRNP, ANA	No	Prednisolone, AZA, HQ	Unresponsive	5	5 years	Every three weeks	Remission
Current report	2	M Turkish	0.5	MR, DR, Ph, OUs, A, H, I: anti-Sm, ANA	No	Prednisolone, Mtx, HQ	Unresponsive	4.5	3.5 years	Weekly	Remission
Current report	3	F Turkish	3	MR, DR, Ph, OUs, A, I: anti-Ro, ANA	No	Prednisolone, Mtx, HQ	Unresponsive	13	3.5 years	Weekly	Remission

SLE: systemic lupus erythematosus; ACR, American College of Rheumatology; RI: recurrent infection; FFP: fresh frozen plasma; M: male; F: female; DR: discoid rash; OU: oral ulcer; R: renal involvement; H: hematologic disorder; I: immunologic disorder; PE: ; MR: malar rash; Ph: photosensitivity; A: arthritis; S: serositis; N: neurological disorder; AZA; azathioprine; HQ: hydroxychloroquine; Mtx: methotrexate; PE: plasma exchange

FUTURE OF GENETICS IN COMPLEMENT DEFICIENCIES

- The presentation of these brothers highlights the importance of genetic testing when there is high suspicion for monogenic disease based on:
  - Family history
  - Unusual disease presentation
- Early diagnosis of complement deficiency helps characterize not only the prognosis and therapy guidance, but possible manifestations that may occur.
    - This information may suggest which disease manifestations to monitor and to potentially mitigate for.
  - As seen in our case with a novel mutation not discussed previously elsewhere, genetic testing may allow for a more comprehensive compilation of causative mutations associated with early complement deficiencies as well as elucidation of possible complications that may be associated with specific mutations.
    - May lead to expedited diagnosis in the future as more mutations are compiled.

TAKE HOME MESSAGE

Genetic testing in unusual patient presentations, in this case early complement deficiencies, may help provide the most accurate clinical diagnosis that may then lead to the most precise treatment management.

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Have A Question?