

Whole Exome Sequencing as a Diagnostic Tool of Primary Complement Component Deficiencies: A Multicenter Experience of Three Novel Mutations

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Abstract

Diagnosis of primary complement deficiencies requires a high index of suspicion. Thus, susceptible patients are often underdiagnosed and untreated. Here, we present a multi-center experience with three novel inborn errors of the classical complement system. This is a retrospective multicenter analysis of computerized medical records of children (> 18 years) admitted in the period between 2003 and 2018 at Shaare Zedek Medical Center in Jerusalem and Edmond and Lily Safra Children's Hospital, Tel-Hashomer Medical Center, in Ramat Gan, Israel. Patients were genetically diagnosed by a complimentary immune work-up. We identified 5 patients (3 males) from four different consanguineous families harboring three novel mutations in the complement components C6-C8. Genetic mutations were identified by whole exome sequencing. Clinical manifestations consisted of meningitis with or without meningococemia. The immune work-up demonstrated nearly absent levels of CH50, compatible with a complement pathway defect. The mean diagnosis delay was 10.56 (0–30) years. *Conclusion:* Invasive meningococcal infections may be life-threatening and cause severe neurological sequela. Awareness of risk factors for primary complement deficiencies, even at the first infectious episode, should facilitate prompt immune and genetic investigations, commencing diagnosis and proper treatment.

What Is Known

- Deficiencies in complement components increase susceptibility to invasive meningococcal infections.
- Recurrent meningococcal infections mandate a diagnostic work-up of the complement system.

What is new:

- Whole exome sequencing can be utilized for prompt genetic diagnosis of complement deficiencies.
- High rates of consanguinity, even in the presence of a single meningococcal infection, are indicative of a need for genetic and immune work-ups.

Introduction

The prevalence of primary complement component (C) deficiencies among patients with primary immune deficiencies (PIDs) is estimated to be 1–6%, though higher rates have also been reported [1]. Decreased levels of terminal components C5-C9 are known to increase susceptibility to infection with *Neisseria* spp., including *Neisseria meningitidis* [1]. Moreover, inherited disorders of regulators of the alternative pathway, such as properdin deficiency, are found in patients with recurrent meningococcal infections [1; 2].

Clinical investigation of patients with recurrent meningococcal infections usually warrants screening complement activity by measuring the 50% hemolytic complement activity of serum (CH50) and the alternative pathway hemolytic assay (AH50), followed by quantification of C1-C9. This facilitates the treatment of susceptible patients with prophylactic antibiotics and meningococcal vaccination, reducing the risk of future invasive meningococcal infections. However, the diagnosis of complement deficiencies requires a high index of suspicion, especially when treating patients with a single infectious episode [3]. Such patients are often underdiagnosed and untreated until the next episode of meningitis or meningococemia [4].

The introduction of next-generation sequencing has been a powerful tool in the diagnosis of inborn errors of immunity [5]. In addition, accumulating evidence suggests that whole exome sequencing (WES) is effective in promoting the diagnosis of complement deficiencies, such as C1Q deficiency, which induces the development of systemic lupus erythematosus (SLE) [6; 7].

Here, we present a multi-center experience of five patients with three novel inborn errors of the classical terminal complement system. We discuss the genetic and immune characteristics of these patients and review the corresponding literature.

Materials And Methods

Study design and population

This retrospective multicenter analysis was based on the computerized medical records of pediatric patients (> 18 years of age) admitted from 2003–2018 to Shaare Zedek and Tel-Hashomer Medical Centers in Israel. Patients were genetically diagnosed by complimentary immune work-up.

Immune work-up

The immune work-up consisted of screening the complement system by measuring CH50, followed by quantifying C1-C9. Asplenia was ruled out by abdominal ultrasonography.

Genetic work-up

High throughput sequencing for whole exome sequencing was performed on genomic DNA samples from the patient. Coding regions were enriched with a SureSelect Human All Exon V5 Kit (Agilent) and then sequenced as 100-bp paired-end runs on an Illumina HiSeq 2500 (Illumina Inc). We used the BWA mem algorithm (version 0.7.15)[8] for alignment of the sequence reads to the human reference genome (hg19 or Hg38). The HaplotypeCaller algorithm of GATK version 3.4 was applied for variant calling, as recommended in the best practice pipeline [9]. KGG-seq v.1.0 was used for annotation of identified variants [10] and in house scripts were applied for filtering, based on family pedigree and local dataset of variants detected in previous sequencing projects. All mutations of the complement genes were validated by dideoxy Sanger sequencing in the carriers and healthy control. Data were evaluated using Sequencer v5.0 software (Gene Codes Corporation).

Ethical review of the study

This study was approved by the institutional review board committees of Shaare Zedek and Tel-Hashomer Medical Centers.

Results

Clinical characteristics

Patient characteristics are detailed in Table 1. We identified 5 patients (3 males and 2 females) from four different families (A-D). Consanguinity was noted in three of the families. Excluding P3, all patients were from a Jewish origin. Family history of recurrent meningococcal infections was notable in three patients (P1, P2, and P4). Representative pedigrees of families A and D are shown in Fig. 1A and B, respectively.

Table 1
Clinical characteristics of the patients

Patient	Family	Age (diagnosis/presentation; years)	Gender/ethnicity	Consanguinity	Family history of recurrent meningococcal infections	Complement component impaired	Genetic mutation	Clinical presentation
P1	A	14/ 14	F/ J	+	+	C6	c.C1786T; p.R596X exon 12, homozygous	Meningococemia, meningitis
P2		20.5/ 2.5	F/ J					Meningococemia, meningitis
P3	B	14.1/ 13.8	M/ A	-	-	C7	n.c.G1135C;p.G379R; exon 10, homozygous	Meningococemia, meningitis, right perichondritis, abducens palsy
P4	C	9.5/ 5	M/ J	+	+			Meningococemia, meningitis
P5	D	37/ 7	M/ J	+	-	C8b	c.361C > T, p.Arg121Ter, exon 3/12, homozygous	Recurrent meningococcal meningitis, recurrent sinopulmonary infections

F- female; M- male; A - Arab; J- Jew; C- complement component.

Mean age at presentation was 8.46 (2.5–14) years. Clinical manifestations in all patients consisted of meningococcal meningitis with or without meningococemia. Neurological complications included abducens nerve palsy (P3), white matter lesions of the pons (P1), and neurosensory hearing loss (P5). Mean delay between clinical presentation and diagnosis was 10.56 (0–30) years. Notably, P5 was diagnosed 30 years after his initial meningococcal infection.

Immune work-up

Asplenia was ruled out in all patients. The work-up of the complement pathway is presented in Table 2, demonstrating nearly absent levels of CH50, compatible with a classic complement pathway defect. Detailed measures of the complement pathway were available for four patients and yielded reduced levels of C6 (P1 and P2), C7 (P3 and P4), and C8 (P5).

Table 2
Complement component levels of the patients

Complement components	P1 (18 years)	P2 (24 years)	P3 (16 years)	P4 (9.5 years)	P5 (37 years)	Normal range
CH50 (U/mL)	3	2	0	0	0	60–144
C1q (mg/dL)	16	20	20	NA	14.8	12.6–20.8
C2 (mg/dL)	1.7	2.2	3.8	NA	2.4	1.8-3
C3 (mg/dL)	112	98.9	163	NA	123	90–180
C4 (mg/dL)	20.2	28.1	27.8	NA	18.8	10–40
C5 (mg/dL)	11	14	20	NA	> 20	12.4–19.2
C6 (mg/dL)	< 0.6	< 0.6	> 12	NA	11.3	8.0-13.2
C7 (mg/dL)	7.3	11	0	NA	6.5	5.9–9.5
C8 (mg/dL)	20	18	> 20	NA	6.2	12-18.4
C9 (mg/dL)	31	38	45	NA	> 50	23.6–45.8
Total (%)	Traces	Traces	NA	NA	Traces	40–100

NA- data is not available; CH50–50% hemolytic complement activity of serum; C- complement component; **In bold** – values above normal range; *Italics*- values below normal range.

Genetic work-up

WES revealed a novel homozygous mutation in *C6* (c.1786C > T; p.R596X,) in P1 and P2 (Fig. 2A). A mutation in *C7* (c.1135G > C; p.G379R) was identified in P3 and P4 (Fig. 2B). P5 had a novel homozygous mutation in *C8b* (c.361C > T; p.R121X, Fig. 2C). Interestingly, P5 also had a homozygous mutation in *CECAM16* (c.703C > T; p.R235C), which is related to hearing loss [11]. These variants were considered rare and pathogenic by different predictive software. Sanger sequencing confirmed the genetic findings with Mendelian inheritance yielding heterozygous carriers among first-degree relatives, including parents and siblings.

Treatment and outcome

All patients were prescribed extended meningococcal vaccines and prophylactic amoxicillin. Interestingly, prior to the diagnosis of her C6 deficiency, P2 received prophylactic amoxicillin due to rheumatic fever. This may account for her 18-year delay in diagnosis.

The mean current patient age is 24 (16–37) years. Only one patient (P5) has demonstrated residual neurological impairment in the form of neurosensory hearing loss, which may have complicated his primary genetic hearing loss defect.

Discussion

In this study, we described our experience with primary complement component deficiencies. Using WES, we identified three novel mutations in *C6*, *C7* and *C8b* and confirmed the diagnosis by quantifying CH50 and different components of the classical pathway. Two patients (P1 and P2) with meningococemia were found to have a novel genetic mutation causing C6 deficiency. C6 interacts with C5b, C7, C8, and C9 to form the membrane attack complex, which catalyzes cell lysis [12]. Terminal complement component deficiencies (C5-C9) are associated with an up to 10,000-fold increased risk of invasive meningococcal infection [4]. High prevalence of C6 deficiency has mainly been reported among the African-American population [12], though it is also seen in other ethnicities, such as Spanish [13], Irish [14], and South-African [14].

Our study found a significant delay in diagnosis from the initial meningococcal infection. Diagnosis of C6 and C8b deficiencies in P2 and P5 was delayed by 18 and 30 years, respectively. Interestingly, prophylactic amoxicillin given to P2 due to rheumatic fever may also have a role in the delayed diagnosis due to lack of meningococcal infections while P2 was on prophylactic treatment. This further demonstrates the need for a high index of suspicion in patients with a single meningococcal infection episode.

Our report corresponds with other studies. In one study of 22 adult patients who previously experienced a single event of meningococcal meningitis, 2 were diagnosed with C6 and C7 deficiencies [3]. In South Africa, 12.8% of patients with invasive meningococcal infections have been found to have C5 and C6 deficiencies [15].

However, screening all children presenting with a single invasive meningococcal disease for complement deficiencies is costly and has not been routinely implemented, therefore investigation is directed by diagnostic clues. The presence of consanguinity, a family history of meningococcal infections, and recurrent episodes of invasive meningococcal infection are obvious "red flags" that indicate a need for investigation. Grumach et al. detailed other signs of complement deficiency, including meningococcal meningitis in a patient > 5 years old; meningococcal infections with less common serotypes; the presence of autoimmune disease, such as SLE; angioedema without urticaria; other recurrent bacterial infections; and renal and ophthalmic involvement in complement regulatory protein deficiencies, including factor H deficiency in hemolytic uremic syndrome [1].

The patients in our cohort presented with a single meningococcal meningitis event with or without meningococemia. The family history and consanguinity in families A, C, and D were indicators to start immune and genetic work-up. Furthermore, identification of meningococcal serotype Y in P1 and P2 was also a clue, as the most common serotypes reported in Israel are B and C (76.9% and 10.9%, respectively)[16].

Use of CH50 screening complemented by WES enhanced the diagnosis of some of our patients. We suggest implementing this methodology in patients with a single invasive meningococcal infection as a "red flag" for primary inborn errors of immunity to reduce the diagnostic delay. This will facilitate better patient care, decrease rates of neurological complications and promote early genetic counselling of siblings.

Our study has several limitations, particularly its size and retrospective design. Prospective large cohort studies investigating complement deficiencies using WES and CH50 screening in patients with a single meningococcal infection are needed.

In conclusion, management of a patient with a first invasive meningococcal infection requires awareness of complement component deficiencies. Upon clinical signs, prompt immune and genetic investigations should be recommended, commencing diagnosis and proper treatment.

List Of Abbreviations

AH50 - Alternative pathway hemolytic assay

C - Complement component

CH50 - 50% hemolytic complement activity of serum

PID - Primary immune deficiency

SLE - Systemic lupus erythematosus

WES - Whole exome sequencing

Declarations

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Availability of data and material: non-applicable.

Code availability: non-applicable.

Authors' contributions: OS- study design, data collection and analysis, and writing of the manuscript; AJS- genetic analysis and figure formation; SF- data collection; TS- manuscript revisions; OM- medical care of the patients and manuscript revision; OB- genetic analysis; RS and YT- immune consultation and manuscript revisions; OT- study design and supervision, medical care of the patients, manuscript revisions.

Ethics approval: This study was approved by the institutional review board committees of Shaare Zedek and Tel-Hashomer Medical Centers.

Consent to participate: non-applicable.

Consent for publication: non-applicable.

Clinical trials registration: non-applicable.

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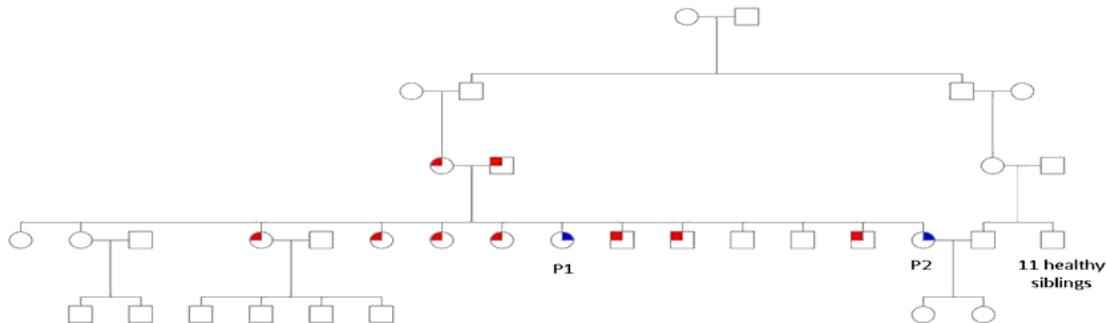
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Figures

A

Heterozygous
 Homozygous to C6 mutation



B

Heterozygous
 Homozygous to C8b mutation

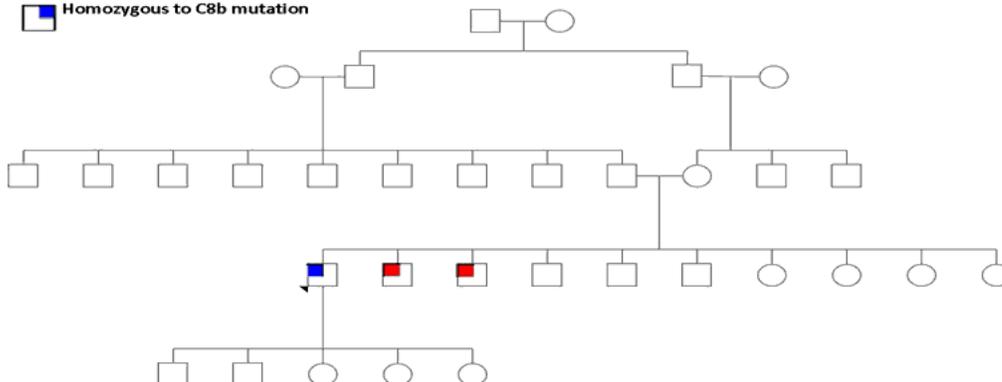


Figure 1

(A, B) Pedigrees of families A (P1, 2) and D (P5), respectively. Notable are consanguinity and heterozygous carriers.

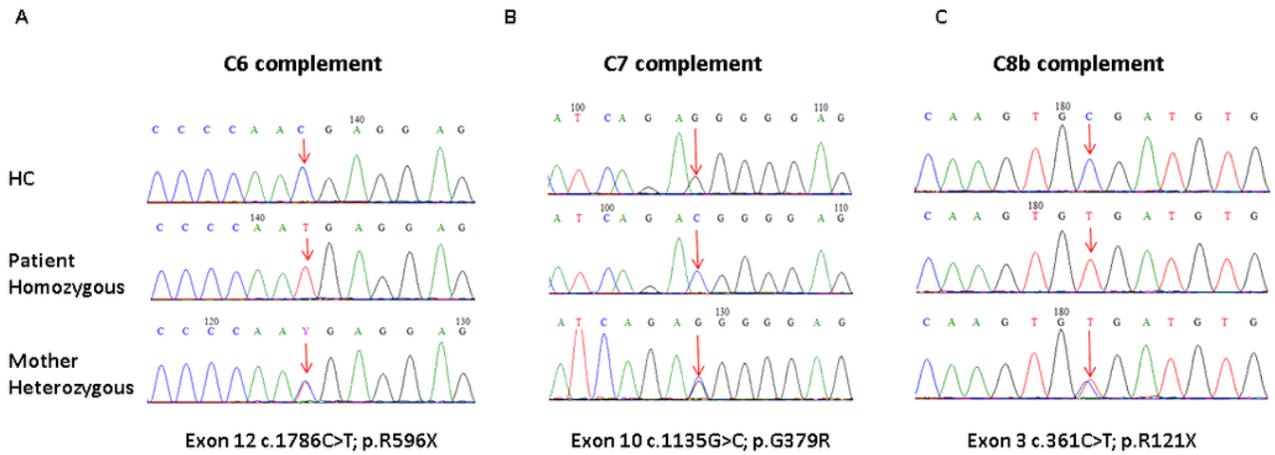


Figure 2

(A, B, C) Chromatograms of P1, P4 and P5 demonstrative of novel mutations in the complement components C6, C7 and C8b, respectively. HC- Healthy control.