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Molecular Case Studies

RAPID COMMUNICATION

Rapid genome sequencing identifies novel variants in complement factor I

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Abstract Complement factor I deficiency (CFID; OMIM #610984) is a rare immunodeficiency caused by deficiencies in the serine protease complement factor I (CFI). CFID is characterized by predisposition to severe pneumococcal infection, often in infancy. We report a previously healthy adolescent male who presented with respiratory failure secondary to pneumococcal pneumonia and severe systemic inflammatory response. Rapid genome sequencing (rGS) identified compound heterozygous variants in *CFI* in the proband, with a novel maternally inherited likely pathogenic variant, a single nucleotide deletion resulting in premature stop (c.1646del; p.Asn549ThrfsTer25) and a paternally inherited novel likely pathogenic deletion (Chr 4:110685580–110692197del).

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Ontology terms: atelectasis; cough; elevated C-reactive protein level; elevated serum transaminases during infections; exudative pleural effusion; hyponatremia; lymphopenia; myocarditis; nausea; normocytic anemia; respiratory failure; ST segment elevation; sinus tachycardia; tension-type headache; unexplained fevers

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CASE PRESENTATION

A previously healthy 13-yr-old male presented to an outside hospital in Mexico with respiratory distress reporting 1 wk of fever and emesis and 4 d of cough and shortness of breath. Upon arrival to the presenting hospital, he was noted to be hypoxemic and tachypneic requiring initiation of high-flow nasal cannula. He was transferred to a tertiary children's hospital for further management.

On admission to the pediatric intensive care unit the patient was in respiratory failure and shock necessitating bilevel positive airway pressure (BIPAP) and epinephrine for cardiorespiratory support. A chest radiograph was notable for moderate bilateral pleural effusions. Electrocardiogram showed diffuse ST-segment elevation concerning for possible myocarditis. Echocardiogram demonstrated a mildly reduced ejection fraction of 51% (ref 56%–78%). Initial laboratory studies were significant for mild elevation in B-natriuretic peptide 135 pg/mL (ref 0–100 pg/mL) with a normal troponin. Complete blood count was notable for mild anemia, hemoglobin 11.7 (ref 12.5–16.1 g/dL), and leukocytosis with neutrophilic predominance, white blood cell counts 28.5 TH/μL (ref 4.0 to 15.5 TH/μL) and 42% bands (ref 0%–10%). There was marked elevation of C-reactive protein 46.2 mg/dL (ref 0–0.99 mg/dL), D-dimer 1.52 μg/mL (ref <0.4 μg/mL), and ferritin 1932 ng/mL (ref 6–70 ng/mL) indicative of a

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hyperinflammatory state. Complete metabolic panel was notable for elevated transaminases with AST 584 units/L (ref 15–40 units/L) and ALT 185 units/L (ref 5–52 units/L), hyponatremia 128 mmol/L (ref 133–143 mmol/L), and acute kidney injury with BUN of 84 mg/dL (ref 7–18 mg/dL) and creatinine of 1.3 (ref 0.4–1.00 mg/dL). A respiratory multiplex PCR panel (ePLEX, Genmark Diagnostics) from the nasopharynx was positive for respiratory syncytial virus. SARS-Cov-2 nucleocapsid immunoglobulin G (IgG) antibody was positive, indicative of prior COVID-19 infection. Complement levels were obtained and C3 level was undetectably low, with C4 at 11 mg/dL (ref 21–51 mg/dL). The differential diagnosis was therefore broad, and he was treated with broad-spectrum antibiotics (ceftaroline and azithromycin) for potential hyperferritinemic sepsis, pulse-dose steroids (1 g daily) for possible systemic lupus erythematosus, and intravenous immunoglobulin G (IVIG 2 g/kg) and anakinra (100 mg BID) for presumed multisystem inflammatory syndrome in children (MIS-C) secondary to prior SARS-CoV-2 infection.

Despite immunomodulatory therapies, the patient had persistent respiratory distress. Computed tomography (CT) of the chest demonstrated left hydropneumothorax and bilateral large pleural effusions without evidence of parenchymal lung disease. He underwent multiple tube thoracostomies as well as video-assisted thoracoscopic surgery (VATS) for hydropneumothorax. Fluid analysis and cytology were consistent with empyema and pleural biopsy demonstrated pleuritis. His blood and pleural cultures remained negative, but plasma microbial cell-free DNA next-generation sequencing (mcfDNA-NGS, Karius, Inc.) demonstrated 80,000 mpm (molecules per million) of *Streptococcus pneumoniae*, presumed to be the cause of his pleural disease. Next-generation metagenomic sequencing (Explify, IDbyDNA) was obtained from the pleural fluid and confirmed the presence of *Streptococcus pneumoniae* DNA.

The patient later endorsed a protracted history of frequent "allergies and sinus problems," manifesting as intermittent periods of congestion and fatigue, with no previous hospitalizations or serious infections. There was no family history of recurrent sinopulmonary infections, otitis media, cutaneous infections, or gastrointestinal infections, and the patient had no previous hospitalizations. Given the severe presentation of *Streptococcus pneumoniae*, rapid genome sequencing (rGS) was obtained in addition to a full immune evaluation. Immune evaluation was notable for complement studies demonstrating low CH50 (24 units/mL; ref 31–60 units/mL) and AH50 (<10%; ref >46%), and persistently undetectable C3 and low C4. These findings are indicative of low classical and alternative complement pathway activity. Evaluation for functional asplenia was negative.

The patient completed 4 wk of ceftaroline for pneumococcal pneumonia, with an oxygen requirement for 32 d and tube thoracostomies removed on hospital day (HD) 34. He was discharged home on HD 46 with penicillin for encapsulated bacteria prophylaxis and daily colchicine for ongoing inflammation and pleural effusions.

TECHNICAL ANALYSIS AND METHODS

Parental consent was obtained for genome sequencing. Solo rGS was performed at Rady Children's Institute for Genomic Medicine as described (Kingsmore et al. 2019). Following DNA isolation from whole blood, sequencing libraries were generated using the Illumina PCR-Free Tagmentation kit (Illumina) according to the manufacturer's instructions. Paired-end sequencing was performed on a NovaSeq 6000 instrument and S1 flow cell (Illumina). Read alignment to the reference human genome assembly GRCh37 and single-nucleotide variant (SNV)/insertion–deletion (indel) calling was performed using the DRAGEN Bio-IT Platform (Illumina). Copy-number variant (CNV) calling was performed using CNVnator and Manta (Kingsmore et al. 2019). SNVs/indels and CNVs were annotated and analyzed using Fabric Enterprise version 6.5.2 (Fabric Genomics). Human Phenotype Ontology (HPO) terms

used during GS analysis included respiratory failure (HP:0002878), fever (HP:0001945), headache (HP:0002315), cough (HP:0012735), nausea and vomiting (HP:0002017), tachycardia (HP:0001649), pericarditis (HP:0001701), ST segment elevation (HP:0012251), myocarditis (HP:0012819), cardiogenic shock (HP:0030149), atelectasis (HP:0100750), tachypnea (HP:0002789), pleural effusion (HP:0002202), lymphopenia (HP:0001888), normocytic anemia (HP:0001897), leukocytosis (HP:0001974), hyponatremia (HP:0002902), elevated hepatic transaminase (HP:0002910), elevated c-reactive protein level (HP:0011227), hyperfibrinogenemia (HP:0011899), and acute kidney injury (HP:0001919). SNVs/indels were filtered to retain variants with allelic balance between 0.3 and 0.7 and allele frequency <0.5% in Genome Aggregation Database (gnomAD), prioritized by Phevor Gene Rank (Kingsmore et al. 2019), and classified according to ACMG and AMP standards and guidelines (Richards et al. 2015). CNVs were filtered to retain variants within coding exons or near genes shown to have an established gene–disease association and classified according to ACMG standards and guidelines (Kearney et al. 2011). Variants of interest were orthogonally confirmed in the proband, mother, and father by Sanger sequencing and MLPA.

VARIANT CLASSIFICATION

This patient was identified to be compound heterozygous for a maternally inherited, likely pathogenic novel frameshift variant (c.1646del; p.Asn549ThrfsTer25) and a paternally inherited, likely pathogenic novel deletion of ~6.32 kb located at 4q25 (110685880–110692197 × 1) in the *CFI* gene (Fig. 1). Mutations in the *CFI* gene have been implicated in complement factor I deficiency.

The maternally inherited frameshift variant was found in the last exon of *CFI* and is therefore predicted to escape nonsense mediated mRNA decay. This variant has not been reported or functionally characterized, however frameshift variants located downstream from this variant have been reported as disease causing variants (PSV1_moderate) (Seddon et al. 2013; El Sissy et al. 2019). Loss-of-function variants in *CFI* have been reported in affected individuals (Alba-Domínguez et al. 2012; Shields et al. 2019; Naesens et al. 2021). The c.1646del (p.Asn549ThrfsTer25) variant was absent from the gnomAD population database and presumed to be rare (PM2_supporting). The result was confirmed by orthogonal testing analysis of parental samples that demonstrated the mother is heterozygous and the father is negative for this variant. This variant was detected in *trans* with the paternally inherited exon 2 deletion (PM3). Based on the available evidence (PSV1_moderate, PM2_supporting, PM3, PP4), the c.1646del (p.Asn549ThrfsTer25) variant was classified as likely pathogenic (Table 1).

The paternally inherited deletion encompassed exon 2 of 13 in the *CFI* gene (Fig. 1). Partial deletions of the *CFI* gene, including deletions encompassing exon 2, have been reported in individuals with CFI deficiency (PSV1) (MIM: #610984; Alba-Domínguez et al. 2012; Naesens et al. 2021). This variant has not been observed at a significant frequency in the Database of Genomic Variants or gnomAD SVs and thus is presumed to be rare (PM2_supporting). This result was confirmed by orthogonal testing. Parental samples showed the mother was negative and the father was heterozygous for this variant. Based on the available evidence (PVS1, PM2_supporting, PP4), this deletion was classified as likely pathogenic (Table 1).

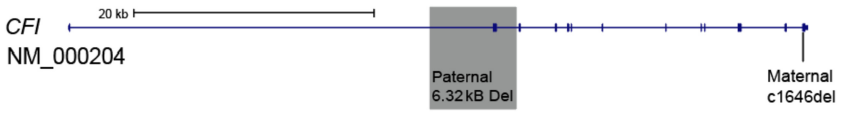

Figure 1. Integrative Genomics Viewer (IGV) depiction of variants on *CFI* gene.

Table 1. Characteristics of the variants leading to a diagnosis of complement factor I deficiency (CFID)

Gene	Genomic location	HGVS cDNA	HGVS protein	Zygosity	Parent of origin	Variant classification
CFI	4:110662154 (GRCh37)	NM_000204.4 c.1646del	NP_000195.2 p.Asn549ThrfsTer25	Heterozygous	Maternal	Likely pathogenic
CFI	4:110685880-110692197 (GRCh37)	NM_000204.4 c.58-4217_329-34del	NP_000195.2-p?	Heterozygous	Paternal	Likely pathogenic

DISCUSSION

Complement Factor I deficiency (CFID) is ultra-rare, with less than 50 affected individuals reported in the literature. This primary immunodeficiency is associated with increased susceptibility to infection and affected individuals typically present with severe infections early in life while the adaptive immune system is still maturing (Vyse et al. 1996; Mollah and Tam 2021). Those with complement deficiency are also prone to inflammation including systemic inflammatory and acute CNS inflammatory disease, as well as immune complex deposition leading to autoimmune disease, vasculitis, and thrombotic disease (Sadallah et al. 1999; Caprioli et al. 2006; Servais et al. 2007; Broderick et al. 2013; El Sissy et al. 2019; Mollah and Tam 2021). CFID is usually hereditary and frequently inherited in an autosomal recessive pattern (Vyse et al. 1996; Mollah and Tam 2021). This diagnosis should be suspected in patients with history of recurrent infection with encapsulated organisms or those with recurrent sinopulmonary infections. Testing for complement deficiency includes tests of the classical complement pathway (CH50), the alternate pathway (AH50), and the mannose-binding lectin (MBL) pathway (MBL by ELISA) (Nilsson et al. 2009; Mollah and Tam 2021).

The individual described here was found to be a compound heterozygote with two novel variants in the *CFI* gene. One variant predicted a frameshift and premature stop codon, and the other was a large coding region deletion, which were associated with low levels of CFI. The *CFI* gene encodes complement factor I, a serine proteinase in the complement pathway required for cleavage and inactivation of C4b and C3b (Catterall et al. 1987). The absence of this regulator causes permanent activation of the alternative complement pathway ultimately leading to depletion of C3 and factor B (Grumach et al. 2006). Pathogenic variations in the *CFI* gene are associated with complement factor I deficiency, which is inherited in an autosomal recessive pattern (CFID) (MIM: #610984), autosomal dominant inheritance of susceptibility to atypical hemolytic uremic syndrome (aHUS) 3 (Fremeaux-Bacchi et al. 2004; Caprioli et al. 2006; Noris et al. 2007) and susceptibility to age-related macular degeneration-13 (ARMD13) (Pras et al. 2015). The relevant phenotypic features of CFID noted in the present patient are summarized in Table 2. Each parent was a heterozygous carrier of a likely pathogenic variant, and they appear to be unaffected with no evidence of an immunologic disorder, aHUS, or glomerulopathy.

Partial deletions of the *CFI* gene have been reported in patients with CFID, which is characterized by increased susceptibility to recurrent bacterial infections such as pneumococcal pneumonia and decreased levels of complement system components including CFI, CFB, and C3 (Grumach et al. 2006; Alba-Dominguez et al. 2012; El Sissy et al. 2019; Shields et al. 2019; Naesens et al. 2021). During this patient's hospitalization, complement biomarker profiling at the University of Iowa showed absent AH50 activity (ref 50%–130%) with normal CH50 [53 units/mL (ref 42–91 units/mL)], low C3 [46 mg/dL (ref 90–180 mg/dL)], normal C4 [40 mg/dL (ref 15–47 mg/dL)], low factor B [4.2 mg/dL (ref 22–50 mg/dL)], low properdin [3.2 mg/L (ref 10–33 mg/L)], low factor H [103 mg/L (ref 180–420 mg/L)],

Table 2. Phenotypic complement factor I deficiency (CFID) features

Complement factor I deficiency	Proband (II-1)	Relevance/age of onset
Recurrent sinusitis	Yes/suspected	Family unsure of specific age of onset
Recurrent otitis media	No	
Vasculitis	No	
Recurrent respiratory infections	Yes/suspected	Family unsure of specific age of onset
Renal insufficiency	No	
Glomerulonephritis	No	
Pyelonephritis	No	
Recurrent urinary tract infections	No	
Arthritis	No	
Recurrent skin infections	No	
Recurrent meningitis	No	
Decreased serum complement factor I	Yes	Confirmed at 13 yr of age during hospitalization
Activation of the alternative complement pathway and depletion of complement components	Yes	
Decreased serum complement factor B	Yes	
Decreased serum complement factor H	Yes	
Recurrent pyogenic infections	No	First confirmed pyogenic infection at 13 yr of age during hospitalization
Increased susceptibility to *Neisseria meningitidis* infections	Yes	
Increased susceptibility to *Streptococcus pneumoniae* infections	Yes	
Increased susceptibility to *Haemophilus influenzae* infections	Yes	
Onset in childhood	Yes	

The list of clinical features is based on the OMIM clinical synopsis related to *CFI* gene (#610984; complement factor I deficiency).

and undetectable factor I (ref 18–44 mg/L). There were also highly elevated levels of complement activation products, such as Ba [3.4 mg/L (ref <1.2 mg/L)], Bb [7.5 mg/L (ref <2.2 mg/L)] and soluble C5b-9 [1.1 mg/L (ref <0.3 mg/L)]. Together, these results are consistent with massive complement activation and consumption via the alternative pathway due to ongoing complement activity by C3 convertase that is not properly regulated. This biomarker profile is consistent with the findings documented in the CFID literature. Interestingly, this patient did not have a history of recurrent pyogenic infections before the present admission, which deviates from existing clinical reports of CFID (Vyse et al. 1996; Sadallah et al. 1999; Baracho et al. 2003; Grumach et al. 2006). Grumach et al. (2006) studied a family of 19 individuals with CFID (16 with partial CFID and three with complete CFID), those with complete CFID had undetectable CFI activity and recurrent infections throughout life. Only two family members with partial CFID did not have any history of infections.

Treatment for individuals with complement deficiency is supportive and preventive. Individuals require immunological monitoring. Patients should be educated regarding the signs and symptoms of bacterial infection and are recommended to receive all routine

vaccinations per the CDC schedule and the Infectious Disease Society of America (IDSA) (Rubin et al. 2014). This patient's vaccine history was verified, and he was boosted with Prevnar 13, meningococcal serogroup B, meningococcal conjugate vaccine (MENACWY), with plans to receive the Pneumovax 23 8 wk from discharge as recommended by the IDSA for patients with complement deficiency (Rubin et al. 2014). Currently, it is recommended that those with primary immunodeficiencies, including complement deficiency, receive prophylactic antibiotic therapy to protect against encapsulated organisms (Kuruvilla and de la Morena 2013). The patient was discharged on penicillin 250 mg twice daily (as used for functional asplenic patients) with a recommendation to be followed regularly by pediatric infectious disease specialists (Lee 2020).

This case demonstrates the clinical utility of rGS in assisting with identification of a rare diagnosis with novel variants due to rapid turnaround time. Exon deletions may be detected more effectively by GS than by gene panels. Establishing a molecular diagnosis in such cases allows for expanded phenotypic assessment, such as this case with minimal previous infectious history. Given the infrequency of this genetic diagnosis, it led to obtainment of CFI functional testing that would not otherwise have been obtained so rapidly. Altogether this led to early antibiotic prophylaxis against future infection.

SUMMARY

We describe two novel variants in the serum proteinase *CFI* (c.1646del; p.Asn549ThrfsTer25 and Chr 4:110685580-110692197 del) predicting loss of function and a diagnosis of complement factor I deficiency. Given the patient's age and lack of prior hospitalizations or severe infections, immunodeficiency was not immediately considered. A timely etiologic diagnosis was made by rGS and facilitated follow-up and initiation of prophylactic antibiotics.

ADDITIONAL INFORMATION

Data Deposition and Access

These variants were deposited into ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) under accession number SCV002578245.

Ethics Statement

Informed and signed consent forms were obtained for all sequenced individuals in this study. This project is approved by the Institutional Review Board of the University of California at San Diego and has received nonsignificant risk status in a pre-Investigational Device Exemption submission to the Food and Drug Administration.

Competing Interest Statement

The authors have declared no competing interest.

Referees

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Author Contributions

J.V. and K.M.R. contributed equally. J.V., K.M.R., and N.G.C. contributed to manuscript preparation. N.G.C. supervised the study. K.E., A.H., and D.D. contributed to variant classification. Y.Z. and R.J.H.S. contributed to complement biomarker profiling. M.D., E.M.A., R.S., B.G., H.M.W., and H.M.H. contributed to the editing and review of this article.

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