Complement Factor I deficiency: A novel homozygous CFI gene mutation

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Abstract
Complement Factor I deficiency is a very rare autosomal recessive disease, with only 38 cases reported in the literature. It is caused by a homozygous mutation in the CFI gene (complement Factor I), which encodes for the Factor I protein, an important regulator of the complement system. Clinically, complement Factor I deficiency presents various symptoms in several organs. We report the case of a child with a history of seizures and unexplained recurrent fever. Whole exome sequencing revealed a novel homozygous missense CFI gene mutation (V270F) of unknown significance. Using multiple bioinformatics tools, we suggest the probable pathogenicity of this mutation. This analysis will help us manage precisely his case, preventing infections and the development of kidney failure, a possible and fatal consequence of complement Factor I deficiency. This study emphasizes the importance of molecular analysis in the diagnosis of rare and atypical diseases and in the establishment of appropriate and effective care.

Keywords
Complement Factor I, hereditary complement deficiency diseases, whole exome sequencing, case report

Date received: 20 March 2022; accepted: 23 May 2022

Introduction
The complement system is an essential component of the innate immune system. Besides plasma and membrane proteins, complement components play a crucial role in the first defense against pathogens and cellular debris elimination.1,2

The Factor I (FI) complement is a serine protease and one of the major fluid-phase regulators of the complement system. Its major role is to cleave C3b and C4b in the presence of other cofactors such as factor H (FH), C4b-binding protein (C4BP), complement receptor 1 (CR1), or membrane cofactor protein (CD46).3 This will prevent the formation of C3 (C3bBb) and C5 (C3bC3bBb) convertase enzymes and the alternative pathway inhibition. The FI deficiency or malfunction results in dysregulation of homeostasis.4 Consequently, inappropriate generation of C3 convertase enzyme will excessively degrade C3, leading to recurrent infections, glomerulonephritis, and autoimmune diseases.5

The CFI gene encodes the FI protein. Mutations in this gene can lead to various diseases with different modes of inheritance. Indeed, homozygous deleterious mutations in this gene cause the complement FI deficiency (OMIM 610984), a rare autosomal recessive disease responsible for primary immunodeficiency. Approximately 38 cases have been recorded in the literature to date.6

Case report
The case is a 9-year-old boy, born to healthy second-degree consanguineous parents. He has two siblings: one is 23 years old and the other is 20 years old with no family history of any significant illness. The proband has a history of chronic cephalgia and two episodes of seizures. The first seizure occurred at 6 years old. It was a unilateral myoclonic seizure, associated with temporary amnesia. Later that same year, he developed apyretic status epilepticus. The seizures were resolved after sodium valproate administration. Currently, he

Here, we report the first Moroccan case carrying a homozygous mutation in the CFI gene associated with complement FI deficiency syndrome.

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presents recurrent unexplained fever episodes with loss of consciousness, decreased ability to concentrate, memory trouble, polyuria, and glossitis.

Magnetic resonance imaging (MRI) showed extensive areas of diffuse leukopathy developed mainly and bilaterally in the posterior parietal area with slight atrophy of the corpus callosum, vermilion, and subcortical areas in places. The electroencephalogram (EEG) recorded a tracing of diffuse brain damage. C3 fraction analysis showed a low serum level: 0.6 g/L (reference: 0.8–1.93 g/L), and CH50 level was normal: 43.3 U/mL (reference: <31.5 U/mL). Echocardiography, blood, urine, and hormonal screenings were normal. Similarly, the analysis of amino acids in blood or urine using chromatography was normal.

**Whole exome sequencing**

Whole exome sequencing (WES) analysis was performed on the child. The human coding exome and mitochondrial genome were sequenced targeting 98% of the coding RefSeq from the human genome build GRCh37/hg19 and completed by a paired-end sequencing with at least 20× coverage depth for 98% of the targeted areas.

**Bioinformatics analysis**

Polyphen-2 (genetics.bwh.harvard.edu/pph2), Mutation Taster (https://www.mutationtaster.org), and Scale-Invariant Feature Transform (SIFT) (https://sift.bii.a-star.edu.sg) tools were used to predict the effect of the mutation. Moreover, the three-dimensional (3D) structural analysis of the protein was depicted by SWISS-MODEL (http://swissmodel.expasy.org/). We used I-TASSER tool to analyze the effect of the mutation on secondary structure and solvent accessibility.

**Results**

The WES revealed a novel homozygous missense variant (c.808G>T, p.Val270Phe) of uncertain significance (according to the American College of Medical Genetics and Genomics (ACMG) guideline) in the CFI gene (GenBank: NM_001318057.1) associated with an autosomal recessive complement FI deficiency. In addition, two other heterozygous frameshift mutations involved in recessive autosomal diseases have been identified: the c.5313del (p.Le1772Phefs*15) mutation in HYDIN gene (GenBank: NM_001270974.2) responsible for ciliary dyskinesia and the c.1643_1644del (p.Val548Alafs*25) mutation in XPC gene associated with xeroderma pigmentosum (GenBank: NM_004628.4).

**Discussion**

A complete FI deficiency disrupts the immune system, causing a heterogeneous phenotype. The classical manifestation is an increased vulnerability to infections with encapsulated bacteria that can range from simple infections to more serious ones, such as meningitis or sepsis. However, other non-infectious symptoms such as nephrological (glomerulonephritis), dermatological, neurological, and auto-immunological manifestations (e.g. systemic lupus erythematosus (SLE)) have been observed. Moreover, heterozygous mutations in the CFI gene generate an incomplete FI deficiency and can manifest as an atypical hemolytic uremic syndrome (OMIM 612923) or age-related macular degeneration (OMIM 615439).

The CFI gene (OMIM 217030) is located on chromosome 4q25 and encodes the FI complement. Structurally, this protein contains two chains linked together by a disulfide bond. The heavy chain is non-catalytic and contains several regions, namely, the N-terminal domain, the FI membrane attack complex (FIMAC) domain, the scavenger receptor cysteine-rich (SRCR) domain, two class low-density lipoprotein receptor domains (LDLR1 and LDLR2), and a C-terminal domain (Figure 1(a)). A chymotrypsin-like serine protease (SP) domain is found in the catalytic light chain. The heavy chain inhibits the FI activity. Once the FI gets in contact with the complex substrate-cofactor, the heavy–light chain interface will be disturbed and the FI will cleave the substrate. The catalytic domain contains three amino acids (His-362, Asp-411, and Ser-507) essential for the cleavage. An abnormality in the cleavage function will lead to C3 degradation. Our patient presents a low C3 serum concentration, which suggests the probable involvement of the V270F variant in his phenotype. Moreover, the CH50 serum level was normal as already reported in several FI deficiency patients.

The c.808G>T mutation (p.Val270Phe) has occurred within exon 6 and thereby in the conserved LDLr2 domain of the protein (Figure 1). Using in silico tools, this mutation is probably damaging according to PolyPhen2 with a score of 0.999 and disease-causing according to Mutation Taster. Nevertheless, the SIFT algorithm predicted that the substitution is tolerated with a score of 0.06.

Using I-TASSER, we found that the amino acid had minimal changes, but he kept its solvent accessibility exposed with a value of 2 and its secondary structure as coiled. Nevertheless, the Val270Phe caused changes in secondary structure and solvent accessibility, which indicates the probable damaging effect of the mutation (Table 1). The most probable damaging change is in residue 279, which is a ligand-binding residue site.

Several reports have described mutations in the same protein domain. Nilsson et al. have identified a compound heterozygous mutation (Q232K and S250L) in the LDLr2 domain, resulting in a low level of FI which could not effectively degrade C4b, probably responsible for SLE syndrome. The same author has reported later that mutation in LDLr2 such as V252A and I267A caused an increased degradation and severely impaired function. Moreover, Nita
et al.\textsuperscript{11} have reported the case of a female patient who carries a homozygous D289V mutation in LDLr2 responsible for recurrent upper and lower respiratory system infections, vasculitis, and arthralgia.

In regard to \textit{XPC} and \textit{HYDIN} genes, heterozygous mutations were not described to be associated with a defect in the central nervous system.

**Conclusion**

In conclusion, the WES analysis identified a novel c.808G>T (p.Val270Phe) homozygous mutation in the \textit{CFI} gene in a Moroccan patient. Regarding the in silico analysis results, the recurrent infections and the neurological manifestations reported by our patients, as well as the low C3 serum concentration, this mutation could be responsible for the present phenotype. Thus, rigorous surveillance is necessary and kidney analysis must be performed. Genetic counseling regarding the \textit{CFI} mutation has been conducted after family consent in order to inform and eventually prevent future manifestations of related diseases in the descendants. Moreover, because of the high incidence of consanguineous marriage in the Moroccan population, the family consented for a genetic counseling concerning the additional pathogenic \textit{XPC} and \textit{HYDIN} mutations to prevent the occurrence of related diseases.

**Figure 1.** Structure of the Factor I complement with the position of the Val270Phe mutation in the protein. (a) Two-dimensional structure highlighting the position of the mutation in the LDLR2 domain. (b) Three-dimensional structure of the mutated protein p.V270F (red arrow) using SWISS-MODEL.
Acknowledgements

The authors gratefully acknowledge the patient’s parents for their cooperation and permission to use their data.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Our institution does not require ethical approval for reporting individual cases or case series.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Informed consent

Written informed consent was obtained from the legally authorized representative of the patient for his anonymized information to be published in this article.

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Table 1. I-TASSER tool prediction in the mutated Factor I protein.

| Residue number | SS in wild protein | SS in the mutated Val270Phe protein | SA in wild protein | SA in the mutated Val270Phe protein |
|----------------|--------------------|-------------------------------------|-------------------|-------------------------------------|
| 17, 69, 94, 213, 218, 231, 277, 279, 309 | C | C | B | E |
| 28, 39, 84, 158, 171, 226, 251, 268, 269, 280, 284, 313 | C | C | E | B |
| 25, 26 | C | H | E | E |
| 30, 31, 32 | H | C | E | E |
| 53 | S | C | E | E |
| 54 | S | C | E | B |
| 61 | S | C | B | B |
| 130 | S | C | E | E |
| 237, 238 | H | C | E | E |
| 239 | H | C | B | B |
| 245 | S | C | E | E |
| 246 | S | C | B | B |
| 305, 307 | S | C | E | E |
| 306 | S | C | B | B |

SS: Predicted secondary structure: C—random coil; H—alpha-helix; S—beta-strand; SA: Predicted solvent accessibility at 25% cutoff: E—exposed; B—buried.