

Full Length Research Paper

## Factor I mutation in Tunisian patient with atypical hemolytic uremic syndrome

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Atypical hemolytic uremic syndrome or the Shiga toxin-producing *Escherichia coli* (STEC) negative hemolytic uremic syndrome (HUS) is a rare disorder typically classified as familial or sporadic. Recent literature has suggested that approximately 50% of patients have mutations in factor H (CFH), factor I (CFI), or membrane cofactor protein (encoded by CD46). Importantly, results of renal transplantation in patients with mutations in either CFH or CFI are dismal, with recurrent disease leading to graft loss in the majority of cases. In this study, a case was described of a patient who developed atypical hemolytic uremic syndrome waiting for a kidney graft. A patient suffering from aHUS and his family was screened for CFI, CFH and membrane cofactor protein (MCP) mutations. The sequencing results of CFH, CFI, and CD46 genes revealed that the patient was heterozygous for a missense mutation, a substitution of a proline residue for a leucine residue at amino acid 64 in CFI. However, the molecular investigation for the family showed the presence of the same mutation in the *CFI* gene in two members. *In silico* study demonstrate a functional consequence of this abnormal protein. This study reemphasizes the importance of screening patients with atypical hemolytic uremic syndrome for mutations in the *CFI*, *CFH* and *MCP* genes before renal transplantation and shows the challenges in the management of these patients.

**Key words:** Hemolytic uremic syndrome (Ahus), complement proteins, factor I mutation, transplantation, *in silico*.

### INTRODUCTION

Hemolytic uremic syndrome (HUS) is characterized by the triad of thrombocytopenia, Coomb's test negative microangiopathic hemolytic anemia, and acute renal failure. In recent years, mutations in genes coding for regulators of the complement system, factor H (CFH), membrane cofactor protein (MCP, CD46), factor I (CFI),

C3 complement and Factor B (FB), have been associated with development of aHUS (Caprioli et al., 2006; Sanchez-Corral., 2004; Bresin et al., 2013). Mutations in the gene encoding thrombomodulin, a membrane-bound glycoprotein with anticoagulant properties that modulates complement activation on cell surfaces, have also been

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described in aHUS (Noris et al., 2010). In addition to inherited defects in CFH, acquired abnormalities affecting factor H function are also seen in the form of inhibitory autoantibodies. Factor H auto-antibodies are reported in 5 to 10% of aHUS patients.

CFI is an 88 kDa glycoprotein that circulates in plasma in the active form at a concentration of 35 mg/ml (Catterall et al., 1987). During post-translational modification, CFI is proteolytically cleaved into the heavy chain (50 kDa) and the light chain (38 kDa), which are covalently linked via a disulfide bond (Fearon, 1977). The heavy chain is composed of five domains: the FI membrane attack complex domain (FIMAC), the CD5-like domain, the low-density lipoprotein receptor 1 and 2 domains (LDLr1 and 2) and finally a region of no known homology (amino acids 277 to 295). The serine protease (SP) FI is special since it degrades C4b and C3b in the presence of specific cofactors like C4BP, FH, MCP or CR1. CFI is a unique protease since it has no natural inhibitors and works only together with its cofactors (Nilsson et al., 2010). The crystal structure of the complement regulatory enzyme human factor I was determined (Roversi et al., 2011).

Heterozygous mutations within the *CFI* gene have been identified in individuals with atypical hemolytic uremic syndrome (aHUS). Mutations consequence on the proteins and symptoms of aHUS is not clear but poor regulation of the alternative pathway of complement activation in the kidney has been suggested to be a causative factor (Noris et al., 2005). The majority of CFI mutations induce a lack of protein synthesis (Vyse et al., 2010), and only few mutations have been associated with a functional deficiency (Kavanagh et al., 2008).

In this study, we described one patient with severe aHUS with a heterozygous mutation in the *CFI* gene. The effect on CFI protein function was showed using *in silico* studies. In fact, it is important to understand how the complement system is regulated in this patient, especially with a view to developing therapeutic options.

## MATERIALS AND METHODS

Patient HUS161 was a 35-year-old man presenting typical signs of aHUS. He had two relatives with glomerulosclerosis, one of them being dead. Currently, the patient is undergoing chronic hemodialysis and waiting for a kidney graft (Leban et al., 2011). C3, C4, CFH and CFI proteins levels were measured by nephelometry and sensitive enzyme linked immunosorbent assay (ELISA) methods as described (Perez-Caballero et al., 2001; Gonzalez-Rubio et al., 2001). All protein levels were normal in the patient and all family members (Table 1). All exons of the *CFI*, *CFH*, *MCP* and *C3* genes were amplified by the polymerase chain reaction (PCR). PCR products were purified with alkaline phosphatase and exonuclease (Amersham, The Netherlands). Subsequently, a sequence reaction was performed using the ready reaction sequence mix (Applied Biosystems). After precipitation, the

fragments were sequenced by the ABI Prism 3130 Genetic Analyzer. Analysis of sequence of all exons encoding for *CFH*, *MCP*, *CFI*, *C3* and *FB* genes (Esparza-Gordillo et al., 2006; Rodriguez de Cordoba et al., 2008) were performed using Chromas 2. Fifty (50) healthy controls and his family were analyzed for the mutations. The PolyPhen server (<http://www.bork.embl-heidelberg.de/PolyPhen/>) was used to predict the possible impact of amino acid substitution observed in patient HUS161.

## RESULTS

Serum levels of C3, C4, CFH and CFI are shown in Table 1. All protein levels were normal in the patient and all family members. In this study we describe one patient with severe aHUS and a new heterozygous mutation in exon 2 (c.191C>T) (Figure 1) resulting in an amino acid substitution from proline to leucine acid at position 64 in the FIMAC domain of the CFI protein (Figure 2). The unaffected father and one brother of patient have the same heterozygous *CFI* mutation. The *CFI* mutation was not found in 50 healthy controls. In patient, we found a heterozygous risk polymorphism in *CFH*, -257C > T (promoter region) which has been described to predispose to aHUS (Caprioli et al., 2006). No *MCP*, *CFH*, *C3* and *FB* mutations were found in the patient.

## DISCUSSION

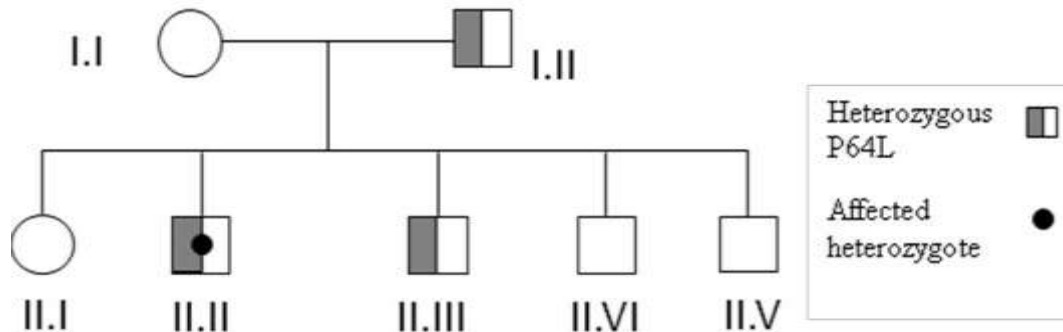
Atypical HUS is a disease that during the last years has been associated with impaired regulation of the alternative pathway of complement. In more than 50% of aHUS patients, one or several genetic abnormalities have been identified in complement inhibitors (Nilsson et al., 2010). A heterozygous mutation in CFI, a regulatory protein of the complement system, was detected in the studied patient, his father and his brother (Figures 1 and 2). This mutation was described by Maga et al. (2010) and resulted in an amino acid substitution from proline to leucine acid at position 64 in the FIMAC domain.

Proline usually imposes greater conformational constraints on the polypeptide backbone than other amino acids. It makes a positive contribution to protein stability through entropic effects, especially in regions where it can be tolerated, such as in loops and turns (Nilsson et al., 2010). In the current situation, while this mutation could slightly destabilize the domain, it could not be structurally tolerated at this position. In fact, the CFI concentration in the serum of our patient was found to be normal. This indicates that the heterozygous mutation P64L in CFI does not significantly influence the CFI serum concentration. Previous researches have showed that mutations in CFI, especially in the FIMAC domain induce reduced cleavage of fluid-phase C4b and C3b (Nilsson et al., 2010; Maga et al., 2010). These studies

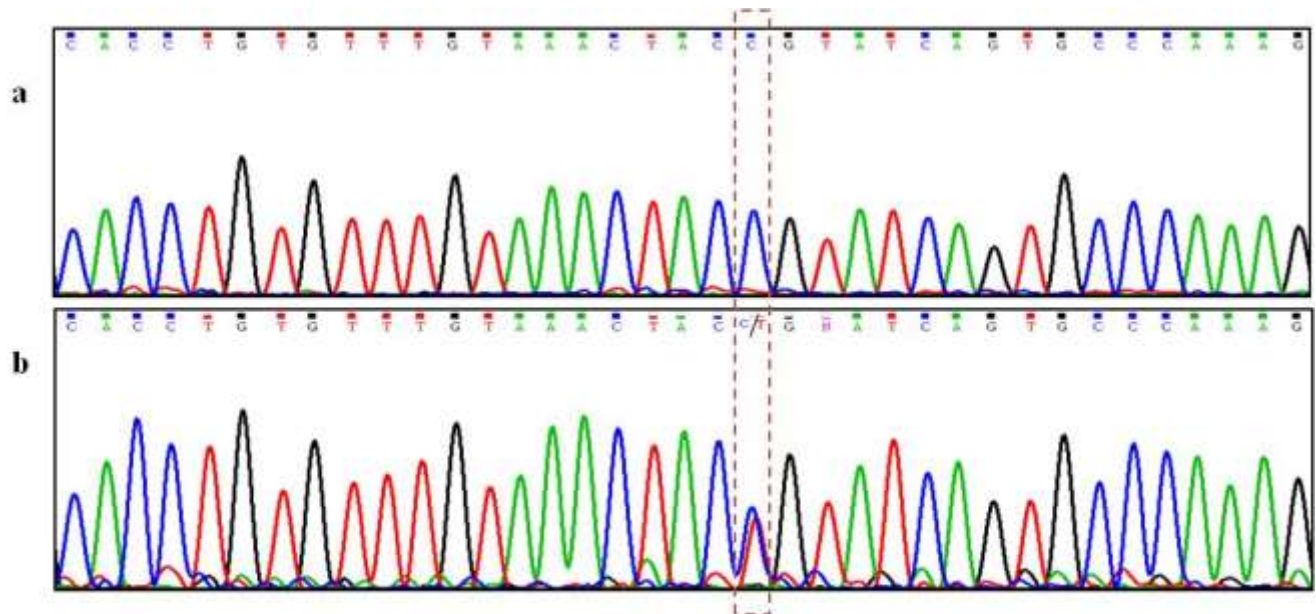
**Table 1.** Complement data in patient with aHUS.

	C3 mg/dl	C4 mg/dl	CFH mg/dl	CFI%	AP	CH50	RVA	MCP%
<b>Patient</b>	97	29.9	32.5	100	normale	normale	normale	121

Normal values: C3 (77 to 135 mg/dl), C4 (14 to 47 mg/dl); CFH (12 to 56 mg/dl); CFI (71 to 115%); MCP (91 to 109%).



**Figure 1.** Pedigree of the patient family.



**Figure 2.** (a) Wild-type DNA sequence. (b) Sequence of patient HUS161. A single base pair change c.191C>T in exon 2 of *CFI* gene.

described that eight patients have presented a mutation in *CFI* gene and have progressed to end stage renal disease (ESRD); one of them showed a P64L mutation in *CFI* gene.

We can suggest that the mutation altered the function of the CFI protein. This deduction is supported by the

results previously described by Nilsson et al. (2010) who reported impaired function of several mutations in the FIMAC domain towards degradation of both C4b and C3b especially the mutation R62A. The R62A localization is very near from our mutation P64L (two amino acids). This study analyzed binding of the FI mutant to C3b. R62A

was the only mutant in the FIMAC domain, which still showed detectable but decreased activity compared to wild type. However, the mutant protein showed substantially impaired ability to degrade C3b deposited on cell surfaces. To study the mutation structure and/or function effect on protein, the PolyPhen server was used to predict the possible impact of amino acid substitution observed in patient HUS161.

PolyPhen predicts the p.P64L mutation to be probably damaging with a percentage of 99.9% (sensitivity: 0.11; specificity: 99%). This result can be explained by the presence of the proline at position 64 that can perturb interdomain contacts or form new interactions with a FI ligand when C3b is part of a deposited C3-convertase. The mutation P64L reduced function in degrading C4b and C3b in solution and only when C4BP and CFH were used as cofactors. This result confirms that the mutation P64L perturbs the functional regulation of the CFI protein and consequently the alternative complement pathway. Further *in vitro* studies remain necessary to confirm our finding.

Many studies found significant associations between several known variants in CFH in aHUS patients (Dragon-Durey, 2004; Fremeaux-Bacchi et al., 2005; Pickering et al., 2007). Four of the SNPs genotyped in that study were included in our analysis (-25C > T, c.184G > A, c.1204C > T, c.2016A > G and c.2808G > T). In patient, we found a heterozygous risk polymorphism in CFH, -257C > T (pro-moter region) which has been described to predispose to aHUS (Caprioli et al., 2003). The polymorphisms identification in the patient family is under study to explain the non appearance of the disease especially the father showing the P64L mutation in *CFI* gene.

In general, renal transplantation is prone to failure in patients with a heterozygous mutation in CFI. Considering mutations in CFI, one of the patients reported by Fremeaux-Bacchi et al. (2005) had a recurrence of HUS in two transplants. The first patient of Kavanagh et al. (2008) had initially an excellent renal function after transplantation, but a recurrence and deterioration of the function occurred after 2 months. The second patient had a recurrence of HUS, which was noted 20 months after the transplantation. The patient with CFI or CFH mutations have much worse prognoses since the CFI and CFH proteins are mainly produced in the liver. There have been some successful combined renal and liver transplantation where the patients with a *CFH* mutation received extensive plasma therapy before, during and after the operation and as consequences do not show any evidence of disease in the renal graft (Jalanko et al., 2008).

The patient HUS161 progressed to end-stage renal disease and required renal transplantation. The renal graft is not recommended by the family because of the

presence of the P64L mutation in two members. After the identification of the molecular abnormality in the *CFI* gene, the therapeutic treatment of the patient was directed to a plasmapheresis. Indeed, the renal transplantation was contraindicated and the combined renal and liver transplantations are not available in Tunisia. Genetic analyses of complement proteins are not yet possible on the research and clinical levels in Tunisia. These studies merit to be developed in Tunisian research laboratories in order to decrease the percentage of the recurrence of aHUS in the Tunisian patients. Also, it is important to assess the functional impact of mutations/polymorphisms identified in aHUS patients because this knowledge can affect the mode of treatment and that which we have found in the current study.

This study has important clinical implications. It is important in patients who are being considered for transplantation that it be known whether they have a *CFH*, *CFI*, or *MCP* mutation so that they can be informed appropriately of the risks for recurrence. We would not recommend live related transplantation in our patient who is known to have either a *CFI* mutation unless the donor has been screened for the same mutation. The description of mutations in *CFI*, *CFH*, and *MCP* genes has established that atypical HUS is a disease of complement dysregulation. Understanding the molecular mechanisms that are responsible for this disease allows us now to examine the potential of complement inhibition as a means of therapy.

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