TITLE PAGE

ECULIZUMAB LONG-TERM THERAPY FOR PEDIATRIC RENAL TRANSPLANT IN aHUS WITH *CFH/CFHR1* HYBRID GENE.

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Running Title:

ECULIZUMAB THERAPY FOR PEDIATRIC RENAL TRANSPLANT

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Abberviations: aHUS, atypical hemolytic uremic syndrome; C3, complement component 3, C5, complement component 5; FH, complement factor H; FI, complement factor I; FB, complement factor B; MCP, membrane cofactor protein; CFHR1, CFH-related protein 1; SCR, short consensus repeat domain. TMA, thrombotic microangiopathy, BP, blood pressure.

ABSTRACT

Background Atypical hemolytic uremic syndrome (aHUS) is a form of TMA caused by dysregulation of the complement system. The outcome of kidney transplantation is poor due to aHUS recurrence and loss of the graft. Patients carrying CFH mutations or *CFH/CFHR1* hybrid genes present very high risk of recurrence despite preventive plasmapheresis. Evaluation of recent data suggests that prophylactic Eculizumab pretransplant is the prefered therapy if drug is available.

Case-diagnosis/treatment We report three year follow-up data in a 9-year-old boy with aHUS and successful renal transplant treated with prophylactic eculizumab without recurrence. He presented with aHUS at age 3, irreversible renal failure and uncontrolled severe hypertension with concentric left ventricular hypertrophy, recurrent acute pulmonary edema and congestive heart failure despite 5 hypotensive agents and bilaeral nephrectomy. Complement analysis demonstrated the presence in the patient of a *CFH/CFHR1* hybrid gene inherited from his mother and a SNP risk *CFH* haplotype inherited from his father. Kidney transplant was performed with prophylactic eculizumab and subsequent fortnightly administration. Three years post-transplant, graft function remains stable (serum creatinine 0.9 mg/dl), hypertension is controlled, no left ventricular hypertrophy, no opportunistic infections and negative clinical chemistry parameters for hemolysis.

Conclusion Eculizumab is a safe and effective therapy to prevent TMA recurrence and provides long-term graft function in aHUS with *CFH/CFHR1* hybrid gene.

Key words: Atypical hemolytic uremic syndrome, eculizumab, renal transplant recipient, *CFH/CFHR1* hybrid gene

INTRODUCTION

Atypical hemolytic uremic syndrome (aHUS) is a form of TMA caused by dysregulation of the complement system. Prognosis is poor and over half of patients progress to end-stage renal failure or die. Conventional renal transplant is not a safe treatment option due to the high risk of recurrence and loss of the graft (1). Patients on dialysis have poorer chances of survival and present cardiovascular risk secondary to hypertension. The efficacy of plasmapheresis is transient and the morbidity/mortality rates for combined liver-kidney transplant are high.

Eculizumab is a humanized anti-C5 monoclonal antibody that blocks activation of the terminal complement cascade. It has recently been approved for the treatment of aHUS and shown to be effective both in native and non-native kidney. 9 patients had received prophylactic eculizumab to prevent posttransplant aHUS recurrence (2).

We report three year follow-up data in a boy with aHUS and renal transplant treated with prophylactic eculizumab without recurrence.

CASE REPORT

Our 9-year-old patient presented at age 3 with oliguric renal failure, microangiopathic hemolytic anemia with schistocytes and thrombocytopenia. He had no diarrhea or neurological symptoms, stool cultures were negative. Hypertension, hematuria and nephrotic syndrome were present from onset (serum creatinine 2.57 mg/dl, creatinine clearance 20 ml/min/1.73 m^2, hemoglobin 8.8 g/dl, platelets 55,000/mm^3, LDH 2445

UI, undetectable haptoglobin, nephrotic proteinuria, albumin 2,7 g/dl). Hemodialysis was started on day 9 with serum creatinine of 5.8 mg/dl, thrombocytopenia (31,000/mm^3). Anemia (Hb 5.8 g/dl) persisted despite 5 transfusions of cell concentrate in the first month.

Continuing on dialysis for 4 years the patient had two further hemolytic crises requiring transfusion and also experienced persistent headaches, abdominal pain, paresthesia and general malaise. The clinical course was dominated by uncontrolled hypertension with concentric left ventricular hypertrophy despite daily hemodialysis and 5 hypotensive agents. He suffered three hypertensive crises with acute pulmonary edema and congestive heart failure. In view of life-threatening hypertension, bilateral nephrectomy was decided three months after initial onset. Renal pathology showed cortical glomerular cystic transformation and severe arteriosclerosis. However, severe hypertension continued to be poorly-controlled. He had two further hypertensive crises, the second being 2.5 years after onset and involving hypertonic seizure and cardiorespiratory arrest.

COMPLEMENT ASSESSMENT

Complement analysis in the patient and their relatives illustrated normal levels of C3, C4, FI, FB and FH. Assays to identify C3Nef or anti-FH autoantibodies were negative. Levels of MCP on the surface of peripheral blood lymphocytes were also normal. Functional analysis of FH, using a sheep red cell hemolytic assay (3), demonstrated an abnormal FH activity in the patient and in three of his relatives (Table 1), which suggested that the patient and his relatives carry a functional alteration in the C-terminal

region of FH. Importantly, this type of functional alteration in FH is prototypical of aHUS (4).

Sequencing analyses identified that the patient and the relatives with functional alteration in FH carry two genetic variations in the *CFH* gene (c.2669G>T, p.Ser890Ile and c.3019G>T, p.Val1007Leu) in heterozygozygosis (Suppl. Figure 1). In addition, multiplex ligation-dependent probe amplification (MLPA) identified in these individuals a genetic rearrangement in the *CFH-CFHR* region resulting in a *CFH/CFHR1* hybrid gene similar to that previously found in other aHUS patients (Table 1; Supp. Figure 1) (5). The product of this hybrid gene is a mutant FH protein in which the C-terminal region is substituted for the C-terminal region of FHR1, introducing two amino acid changes, Ser1191Leu and Val1197Ala. Interestingly, the Ser890Ile and Val1007Leu genetic variations and the hybrid gene segregate together in the same *CFH* allele. In summary, the FH protein encoded by this *CFH/CFHR1* gene presents four amino acid differences compared to the FH reference protein (Supp. Figure 1). No mutations were found in *CFI, MCP, C3, CFB* and *THBD.*

KIDNEY TRANSPLANTATION UNDER ECULIZUMAB

The patient's condition on hemodialysis continued to be life-threatening. Published results showing effective inhibition of complement mediated TMA by eculizumab in aHUS (6,7). In 2010, four years after onset, deceased donor kidney transplant was proposed with pre-emptive eculizumab treatment. Informed consent was obtained from the family for treatment under terms of compassionate use.

Prior to transplant he was appropriately immunized against *haemophilus influenzae, pneumococcus,* viral hepatitis B, varicella and tetravalent meningococcal vaccine. Anti-HLA antibodies were negative. Kidney transplant was scheduled with induction with basiliximab, prednisone, mycophenolate mofetil and tacrolimus, plus prophylactic eculizumab. The regimen consisted of administration of the first dose of eculizumab

600 mg 6 hours pre-transplant, then within 24 hours, then 4 weekly doses and subsequently, doses of 600 mg in fortnightly cycles.

Kidney transplant was performed from deceased donor with 3 compatibilities. There was immediate diuresis on unclamping. Serum creatinine decreased from 9 to 0.7 mg/dl over the initial few weeks post-transplant. There were no side effects after administration of eculizumab. He was started on tacrolimus on day +2 , the steady decrease in creatinine continued. He received prophylaxis with valganciclovir, co-trimoxazole and phenoxybenzylpenicillin (ongoing).

In the first week post-transplant, the patient developed a surgical complication consisting of punctiform intestinal perforation without TMA signs resolved by simple suturing. One month after surgery, a bladder fistula was resolved with a double J stent. He developed *Klebsiella* pyelonephritis related to the catheter which was resolved without complications. There were no clinical or laboratory signs showing complement activity during the episodes of infection, thus requiring no modification of the eculizumab treatment regimen.

Three years post-transplant, graft function continues stable, serum creatinine 0.9 mg/dl, no proteinuria, Hb 11.9 g/dl, normal platelets, hypertension controlled with calcium channel blocker and beta blocker without ventricular hypertrophy, no cytotoxic antibodies, negative BK viruria, no CMV infection and negative clinical chemistry parameters for hemolysis. He has remained free of any further evidence of TMA with fortnightly administration as an outpatient of eculizumab, the current dose of 900 mg

adjusted to body weight. His growth is normal, as with his activities and performance at school, with full social and familial integration.

DISCUSSION

Our experience supports the utility and safety of eculizumab for the prevention of aHUS recurrence post-transplant in patients carrying a *CFH/CFHR1* hybrid gene. There are reports of 9 patients with aHUS who have been treated with eculizumab prior to renal transplant in the literature, four cases with prophylactic plasma therapy preceded eculizumab and five with eculizumab alone (2). Two patients had a *CFH/CFHR1* hybrid gene (8,9). Our patient´s outcome is favorable and renal function normal 36 months after kidney transplantation without recurrence. He remains free of any further clinical TMA manifestations taking eculizumab alone.

The genetic analysis allow us to conclude that the *CFH/CFHR1* hybrid gene is the underlying genetic defect responsible of aHUS in our patient. The FH/FHR1 hybrid protein encoded by this gene is unable to provide appropriate regulation of the C3 convertase, exposing platelets and endothelium to the uncontrolled activation of the complement pathway that characterises aHUS. The *CFH/CFHR1*hybrid gene in our patient carry two additional nucleotide changes that are irrelevant. In fact, in a separate study, we have recently shown that Ser890Ile and Val1007Leu genetic variations are polymorphisms with no functional consequences (10). The *CFH/CFHR1* hybrid gene was inherited from the mother and likely originated earlier in that lineage, since is it also found in one of the patient's aunts. The *CFH/CFHR1* hybrid gene is necessary but not sufficient to develop aHUS, as is clearly illustrated by the fact that three other relatives carrying it have not developed aHUS. Previously, it has been shown that the

incomplete penetrance of the disease in carriers of mutations associated with aHUS can be explained by the concurrence of additional genetic or environmental factors (11, 12). Analysis of the levels of expression of the normal and mutant *CFH* alleles in the carriers of *CFH/CFHR1* hybrid gene in the pedigree of the patient (Supp. Figure 1) suggests that different levels of FH produced the normal *CFH* allele in these individuals is a plausible explanation for the incomplete penetrance of the disease in this pedigree. In addition, our patient carries the *CFH* SNP haplotype associated with increased risk for aHUS, inherited from his father (Table 1).

The prognosis for aHUS in patients with complement abnormalities is very poor as a result of the progression to renal failure, the TMA in other organs, the ineffectiveness of the conventional treatments and the high risk of post-transplant recurrence with loss of the graft. TMA occurs systemically in up to 48% of patients and in 38% of cases is associated with macro- and microvascular thrombosis. Hence the survival rate of < 40% in dialysis patients. The risk of recurrence is higher for carriers of mutations in *CFH, CFB, C3* and *CFH/CFHR1* hybrid gen. The therapeutic response to plasma therapy in both native kidney and renal graft is poor and transient.

Identification of the genetic defect in our patient and the high associated risk of morbidity/mortality ruled out conventional renal transplant. The discouraging results reported with combined liver-kidney transplant led us to also rule out that option, due to the complexity of the procedure with particularly high risk of uncontrolled ischemia-reperfusion-induced complement activation despite extensive plasmatherapy, early graft failure and serious infection.

The efficacy of eculizumab in blocking complement activity and preserving renal function in native-kidney aHUS presented a promising therapeutic option for our patient. Treatment was indicated under the terms of compassionate use; it was in fact subsequently approved for the treatment of aHUS by the FDA in September 2011 and in Europe in November 2011. Our treatment strategy consisted of administering pre-transplant dose immediately before surgery and 24 hours after to block the uncontrolled complement activation induced by the ischemia/reperfusion. Subsequently, it was given weekly for 4 weeks and then at 10-14-day intervals. The patient has remained free of clinical manifestations of TMA on this therapy.

A recently-published study reported on 9 patients with aHUS treated pre-transplant with eculizumab, 4 of them on combined treatment with plasmapheresis, and 13 cases treated for recurrent HUS post-transplant (2). The same strategy as described in our case was used in 5 of the 9 patients treated pre-transplant. All the patients who received prophylactic treatment remain free of recurrence and the patients treated for relapses achieved remission after a median period of 8 months. At over 3 years since transplant, this patient has the longest follow-up period to be published so far.

In aHUS patients drug administration intervals longer than two weeks have been associated with early relapse and loss of graft refractory to eculizumab and plasmapheresis (1). Eculizumab blocks the last phase in the pathogenesis of aHUS: the formation of membrane attack complex. Therefore, in high risk mutations the treatment is lifelong since the optimal duration of eculizumab treatment has not yet been properly addressed (2,13). Close monitoring is vital for early detection of insufficient complement inhibition since in some patients the recommended empirical doses may be

too low, particularly in the course of respiratory infections, CMV, EBV and polyomavirus. This highlights the importance of monitoring for viral replication in these patients. The classic markers of extravascular hemolysis do not provide early warning of recurrence. Early markers must therefore be developed which are accessible in clinical practice, such as a C5-dependent functional test or the terminal complex test, for patient follow-up. Undetectable total hemolytic complement (CH50) activity suggests blockage of circulating C5. However, the detection of TMA in a renal biopsy in the absence of CH50 in two published cases with post-transplant aHUS suggests that tests are not yet sensitive enough for routine monitoring.

In our experience, eculizumab is well tolerated and has kept the patient free of renal and systemic TMA over three years of continuous treatment, with no infectious complications on prophylaxis with oral penicillin. Graft function remains stable, BP is well controlled and he has not developed opportunistic infections. He continues on fortnightly eculizumab therapy administered as an outpatient, allowing him to attend school and enjoy full social and familial relationships.

This case adds to the evidence on the efficacy of eculizumab prior to kidney transplantation in preventing progression of aHUS post-transplant and in the long term without the need for plasmapheresis. The development of early markers sensitive to disease activity would make it possible in the future to individualize the dose and frequency of administration, minimizing the risk of relapse.

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Disclosures

SRdeC has received fees from Alexion Pharmaceuticals for invited lectures. This interaction has not influenced the results and interpretations in this manuscript.

Tables legends

Table 1. Complement and Genetic data in patient H142 and relatives

Supplementary figure legends

Supp. Figure 1. Summary of the genetic data in the patient (H142) and their relatives.

A) The H142 pedigree. The members of the pedigree are identified, and for the affected individual H142 and the other carriers of the *CFH/CFHR1* hybrid gene, the levels of total plasma FH and of the normal FH (402Y) are indicated. Notice that H142 also inherits an aHUS risk allele from his father.

B) Summary of the MLPA data. Graphs illustrate the number of copies of different exons in the *CFH-CFHR1* region. Notice that levels of CFH exon 23, but not CFHR1 exon 6, are decreased in the patient and three of their relatives indicating they are carriers of a *CFH/CFHR1* hybrid gene.

C) Diagram of the FH molecule resulting from the *CFH/CHFR1* hybrid gene. The circles indicate each of the 20 SCR domain units that compose FH. The locations of the four amino acid differences found are indicated. Notice that the two changes in SCRs 15 and 16 are rare polymorphisms with no functional relevance (Tortajada et al. Kidney International, 2012).

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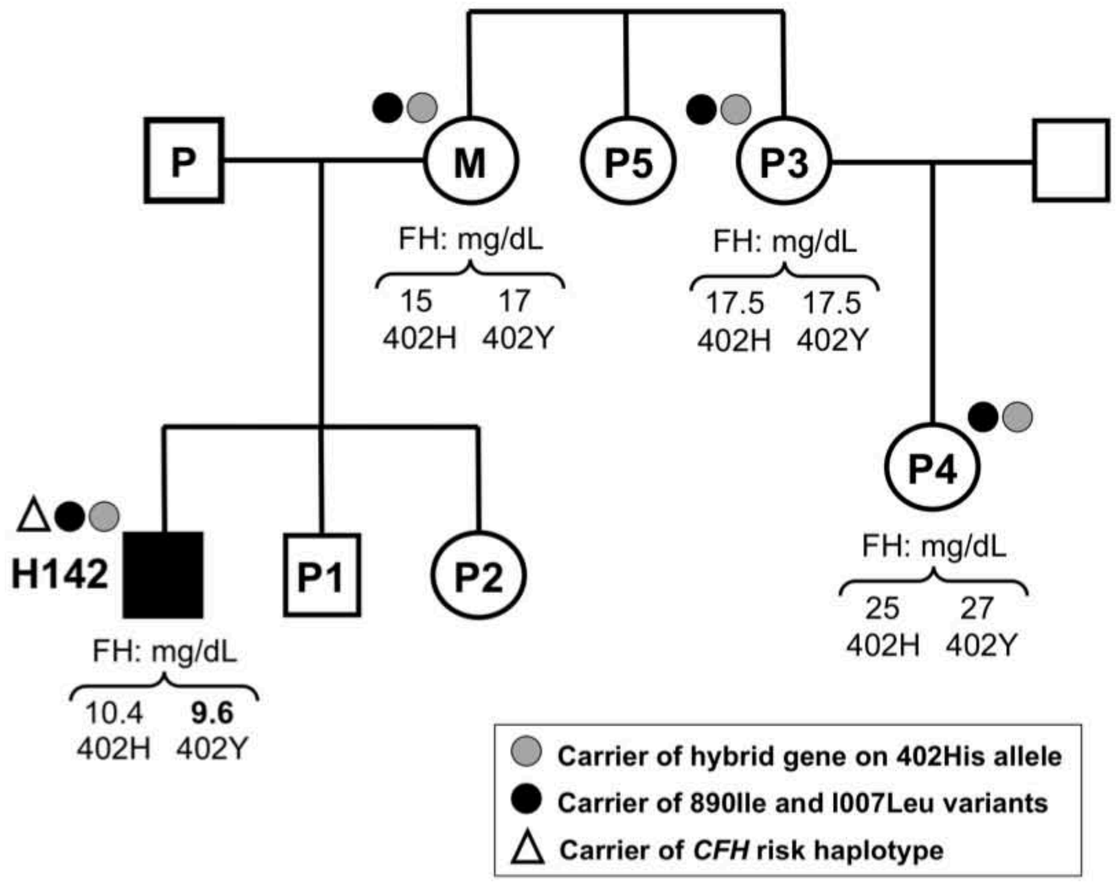
Table 1. Complement data	ID	C3 levels (80-177mg/dL)	C4 levels (14-47mg/dL)	fI levels (% of control)	fB antigenic (7-28 mg/dL)	fB hemolytic (% of control)	fH levels^ (12-56mg/dL)	fH hemolytic (% of lysis)	Anti-fH
H142	**Index case (*)**	94	24	>100	11	100	20 [9.6]	**100**	No
H142P	Healthy	117	30	>100	13	100	32	Neg.	No
H142M	**Healthy (*)**	100	32	>100	15	100	32 [17]	**40**	No
H142P1	Healthy	86	21	>100	8	100	39	Neg.	No
H142P2	Healthy	109	23	>100	11	100	33	Neg.	No
H142P3	**Healthy (*)**	133	32	>100	11	100	35 [17.5]	**64**	No
H142P4	**Healthy (*)**	116	14	-	9	100	52 [27]	**38**	No
H142P5	Healthy	119	23	-	18	100	-	Neg.	No

Genetic data		***CFH***				***CFHRs***	***MCP***		**Mutations other genes**			
	ID	**Muta-tions**	**Hybrid gene**	**Risk haplotype**	***S890I* and *V1007L* polymorphisms**	**Del(R3-R1)**	**Muta-tions**	**Risk haplotype**	***CFI***	***CFB***	***C3***	***THBD***
H142	Index case	No	Yes	Yes (Het)	Yes	No	No	No	No	No	No	No
H142P	Healthy	-	No	Yes (Hom)	No	No	-	No	-	-	-	-
H142M	Healthy	-	Yes	No	Yes	No	-	Yes (Het)	-	-	-	-
H142P1	Healthy	-	No	Yes (Het)	No	No	-	Yes (Het)	-	-	-	-
H142P2	Healthy	-	No	Yes (Het)	No	No	-	No	-	-	-	-
H142P3	Healthy	-	Yes	No	Yes	No	-	Yes (Het)	-	-	-	-
H142P4	Healthy	-	Yes	-	Yes	No	-	No	-	-	-	-
H142P5	Healthy	-	No	No	No	No	-	Yes (Hom)	-	-	-	-

CFH risk haplotype: rs3753394 (c.-331T); rs800292 (c.184G); rs1061170 (c.1204T); rs3753396 (c.2016G); rs1065489 (c.2808T) ;MCP risk haplotype: rs2796267 (c.-652G); rs2796268 (c.-366AG); rs1962149 (c.989-78A); rs7144 (*897C). (*) These individuals carry the CFH::CFHR1 hybrid gene (Venables et al. PLoS Med 2006)(^) Levels of normal factor H are shown between brackets(-), Not done

A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M
P5
P3
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P4
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25 27
402H 402Y
H142
P1
P2
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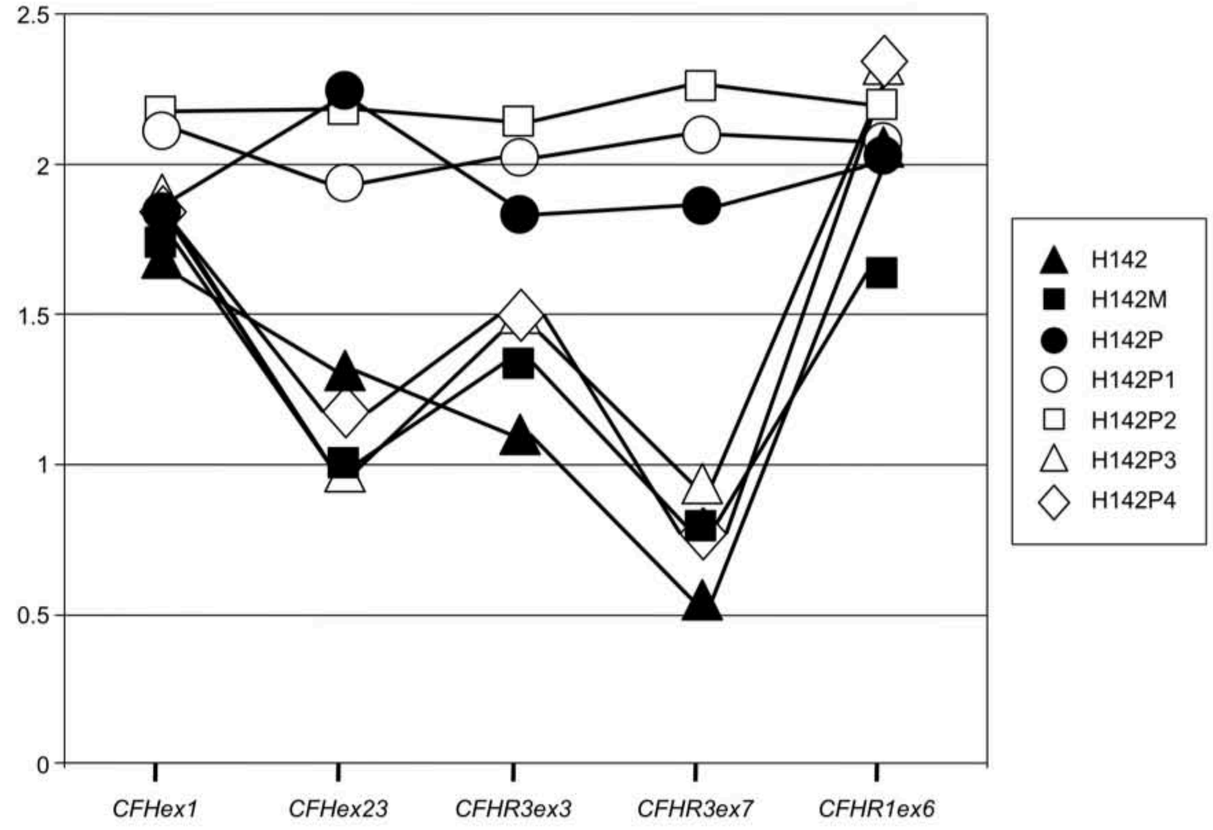
Carrier of 890Ile and I007Leu variants
Carrier of CFH risk haplotype

B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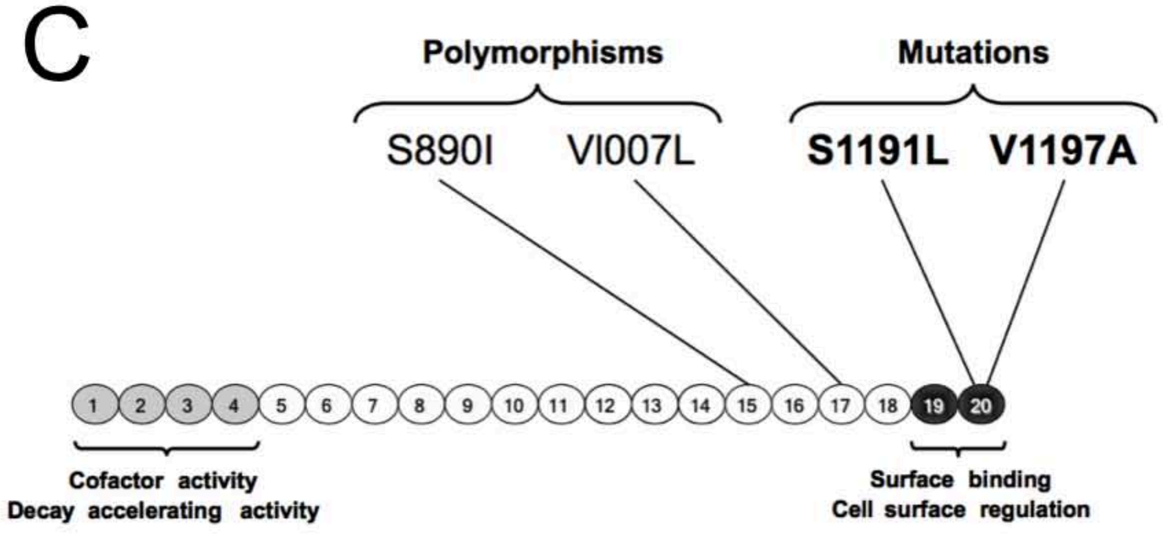
Polymorphisms
Mutations
S890I
VI007L
S1191L
V1197A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Cofactor activity
Decay accelerating activity
Surface binding
Cell surface regulation