Supplementary material

Case Reports of familial cases

Below we provide a brief description of clinical history and pedigrees (Supplementary Fig.1, panel A) for familial forms.

Family #130. The proband, patient F169, presented at the age of 9 months with anemia, thrombocytopenia, nephrotic range proteinuria and gross hematuria**.** A diagnosis of Stx-HUS with nephrotic syndrome was entertained due to positive stool test for *E. coli* strain O157:H7. He received blood transfusions, hemolysis and renal function improved although proteinuria remained in the nephrotic range. At 37 months of age he developed a relapse of aHUS with negative stools both for Shiga toxin-producing *E. coli* and for Shiga toxins. At the last follow-up he maintained a low-grade hemolysis while renal function and urinary protein excretion were normal. His maternal aunt, patient F582, developed aHUS 4 days after delivery of her third child. She was treated with intensive plasma exchange and corticosteroids. The hemolysis improved, but renal failure persisted. She received chronic hemodialysis for two years until a cadaver donor kidney transplant was performed. She was well at three years post-transplant. C3, C4, CFH and CFI levels, evaluated during remission, were within the normal ranges in both patients. Patients F169 and F582 carry a heterozygous G1194D mutation in *CFH* (SCR20) and a heterozygous F242C mutation in *MCP* (SCR4) [1].

Nineteen unaffected relatives were also screened for the above mutations: 6 subjects carry in heterozygosis the G1194D in *CFH*, 1 carries the F242C in *MCP*, while both mutations are present in 4 healthy subjects.

Of note, out of the 6 subjects with combined *CFH/MCP* mutations the two patients F582 and F169 and the healthy mother of patient F169 carry both the *CFH*-H3 and *MCPggaac* risk haplotypes, whereas the other three compound heterozygous subjects carry only the *MCPggaac* risk haplotype [2].

Family #024. The proband, patient F108, developed aHUS at the age of 3 years. Six years later he manifested a relapse with renal sequelae and developed ESRF at the age of 12 years. The older brother, patient F106, presented aHUS at the age of 8 years and 10 years later he manifested a relapse. Both episodes resolved without renal sequelae. Biochemical evaluations of the 2 patients during remission showed low C3 levels while C4, CFH and CFI were normal. A younger brother died at the age of 6 years after a very severe episode of aHUS.

Patients F106 and F108 carry a heterozygous R1210C mutation in *CFH* (SCR20) [3] inherited from the unaffected father and two mutations in *MCP* (C35Y, in SCR1, inherited from the mother and R59X, in SCR1, inherited from the father).

Family FRA13/FRA15. The proband, patient FRA13, developed aHUS at 47 years of age. She was treated with plasma obtaining hematological remission, but not complete recovery of renal function and within 3 years she reached ESRF. She then received a kidney allograft that at 6 year follow-up was still functioning.

A first cousin, patient FRA15, developed pregnancy-associated aHUS at the age of 29 years with ESRF as outcome. She underwent three kidney transplants, the first lost after 2 years for chronic rejection, the second within 1 year for aHUS recurrence and the third after 6 years for venous thrombosis.

Biochemical evaluations in both patients showed normal C3, C4, CFH and CFI levels. Genetic analysis in FRA13 revealed the presence of three heterozygous mutations: R1210C in the SCR20 of CFH, Y29X in the signal peptide of MCP, and P553S in the serine-protease domain of CFI. The first two mutations, R1210C in CFH and Y29X in MCP, were also found in the affected cousin, patient FRA15, and in the healthy father of FRA15.

Family#265. The proband, patient F870, is a 6-year-old Caucasian male who developed renal insufficiency, hemolytic anemia and thrombocytopenia without diarrhea, sepsis, or signs of infection at the age of 9 months. The child suffered many episodes of aHUS, each treated with plasma exchange. Despite initiation of a prophylactic regimen of plasma exchange, his renal

function declined significantly. At the age of 4 years he received a combined liver-kidney transplant (LKT) with preoperative plasma exchange and enoxaparin anticoagulation. Initial function of both grafts was excellent and maintained for nearly 2 years [4]. His maternal family history included three female cousins of the mother with ESRF secondary to aHUS: one (patient F868) received an isolated kidney transplantation that failed for disease recurrence, one is dialysis-dependent and the other one died due to complications of ESRF. The proband has two heterozygous mutations: the c.3572C>T change in *CFH*, that leads to the amino acid substitution S1191L in SCR20, and the c.1661A>T change in *CFI* that causes the E554V substitution in the serine-protease domain. The patient inherited from the healthy mother both mutations that are present also in the unaffected grandmother. By contrast the affected maternal cousin F868 carries only the *CFH* mutation. The same mutation is present in 4 unaffected relatives.

Family GUI. The proband, patient HUS109, presented with HUS of unknown etiology at the age of 12 months after an episode of fever and vomits. She needed a transfusion of red blood cells and was also given plasma infusion. The hemolytic crises disappeared 4 days after diagnosis and dialysis was not necessary. Eighteen months later she had a second HUS episode with severe anemia and thrombocytopenia but normal renal function. She was treated with plasma infusion and recovered in two weeks. At the age of 5 years (October 2009) she suffered a third HUS episode. Eculizumab treatment was then initiated and it has been maintained since then. She currently shows chronic kidney disease stage 2 and hypertension and is under conservative treatment.

The father, patient HUS62, presented with acute renal insufficiency, Coombs' test negative, microangiopathic hemolytic anemia and thrombocytopenia at the age of 34 years and was diagnosed with HUS of unknown etiology. He required hemodialysis and was treated with immunosuppressant agents and steroids. He received 27 sessions of plasmapheresis with plasma infusion, however full recovery of renal function was not achieved. The patient remained stable under hemodialysis since December 2001. In May 2008 he received a kidney allograft that in February 2012 was still functioning.

Both patients had normal C3, C4 and CFH levels but low CFI levels (50% and 53% respectively). They carry a mutation in MCP (R103W in SCR2), and a mutation in CFI (N151S in SRCR domain). Moreover, patient HUS109 inherited from her healthy mother a deletion from base 800 to 820 of *MCP* that causes the loss of 7 amino acids (TIVCDSN) in SCR4.

Family #176. The proband, patient F1314, is a 2.5-year-old Caucasian female who presented at 6 months of age with hemolytic anemia, thrombocytopenia and oliguric acute renal failure. Familial history disclosed several paternal relatives with unclassified lethal renal diseases (not shown in the supplementary Fig. 1) and a 30-year-old alive cousin of the father (F617) with ESRF secondary to aHUS and a genetically proven *CFH* abnormality (*CFH/CFHR1* hybrid gene) [1]. Therefore, plasma exchange was immediately started. Renal replacement therapy was not necessary and after one week all blood parameters were within normal range. At the last follow-up, normotension and normal kidney function without proteinuria were observed. Genetic analysis confirmed the presence of the *CFH/CFHR1* hybrid gene in the proband and in her father; the same abnormality is present in a proband's older unaffected sister. The proband carries also the heterozygous c.1429+1G>C mutation in *CFI*, inherited from the healthy mother and present also in the unaffected younger sister.

Family FRE44. The proband, patient FRE44, is a 17 year-old boy who had six recurrences of aHUS. The first episode was at 3 years of age. He regained normal renal function after each episode. His father (FRE44F) had three episodes of aHUS at age 28, 41 and 42 years. He is now 44 years old with renal insufficiency (creatinine clearance of 24 ml/min). Patients FRE44 and FRE44F carry a mutation in *C3* (H1464D). Patient FRE44 also inherited from his mother a rare genetic variant of *MCP* (A353V) that has been associated with reduced complement regulatory activity [5].

Family RCO. This family was previously described [6]. The proband, patient HUS84, and patient HUS68 are first cousins with a history of recurrent aHUS that lead to ESRF. They had normal plasma levels of C3, C4 and CFH while CFI plasma levels were low/half normal. The patients carry

a heterozygous mutation in MCP (P165S in SCR3) and a c.1610insAT in *CFI* that generates a truncated protein **(**T538X). Three unaffected family members were found to carry both mutations, while 2 carry the *CFI* mutation and 2 the *MCP* mutation alone.

Family FRE60. The proband, patient FRE60, is a 11 year-old girl who had three recurrences of aHUS at age 2, 7 and 8 years. She regained normal renal function after each flare. C3, C4, CFH and CFI levels were normal. She inherited from her healthy mother the heterozygous P50A mutation in CFI and the heterozygous R103W MCP mutation from her affected father, who carries this mutation in homozygosity. The father manifested aHUS during childhood, he received plasma and recovered without sequelae.

Family HUS143. The proband, patient HUS143, developed pregnancy-associated aHUS at 27 years of age; she was treated with plasma obtaining partial remission. In 2009, a younger sister of the proband also developed aHUS.

Biochemical evaluations in the proband showed low C3 and CFH levels, but normal CFI levels. Genetic analysis revealed the presence of two heterozygous mutations: a frameshift in CFH (T30Nfs10X in the SCR1) and a substitution in MCP (I208Y in the SCR3), the latter identified also in the healthy child.

References

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Legend to Supplementary Figures

Supplementary Figure 1.

Pedigrees of patients with familial (panel A) and sporadic (panel B) aHUS. The genotyped affected subjects are evidenced by their specific code and highlighted with bold circles (females) or bold squares (males) (n=31; 4 with mutations in a single gene: FRE60F, with a homozygous mutation in *MCP,* #265F868, with a *CFH* mutation*,* #176F617, with a CFH/CFHR1 hybrid gene, and FRE44F with a C3 mutation; 27 with combined gene mutations). Diamond symbols are used when sex is unknown. The black arrows indicate the proband in each pedigree. The geographical origin of each pedigree is indicated at the upper left corner. Mutations in *CFH* are reported in red boxes, mutations in *MCP* are reported in yellow boxes, mutations in *CFI* are reported in green boxes, mutations in *C3* are reported in blue boxes, and the mutation in *CFB* is reported in grey box. Genotypes of SNPs targeting the *CFH*-H3 risk haplotype (rs3753394, c.1-332C>T and rs1065489, c.2808G>T, p.E936D) and of SNP targeting the *MCPggaac* risk haplotype in *MCP* (rs7144, c.*897T>C) are marked in red.

n.a.=DNA not available; n.m.=no mutation. * Pedigree included in the calculation of penetrance.

Supplementary Figure 2.

HUS penetrance in subjects carrying two gene mutations in different gene combinations.

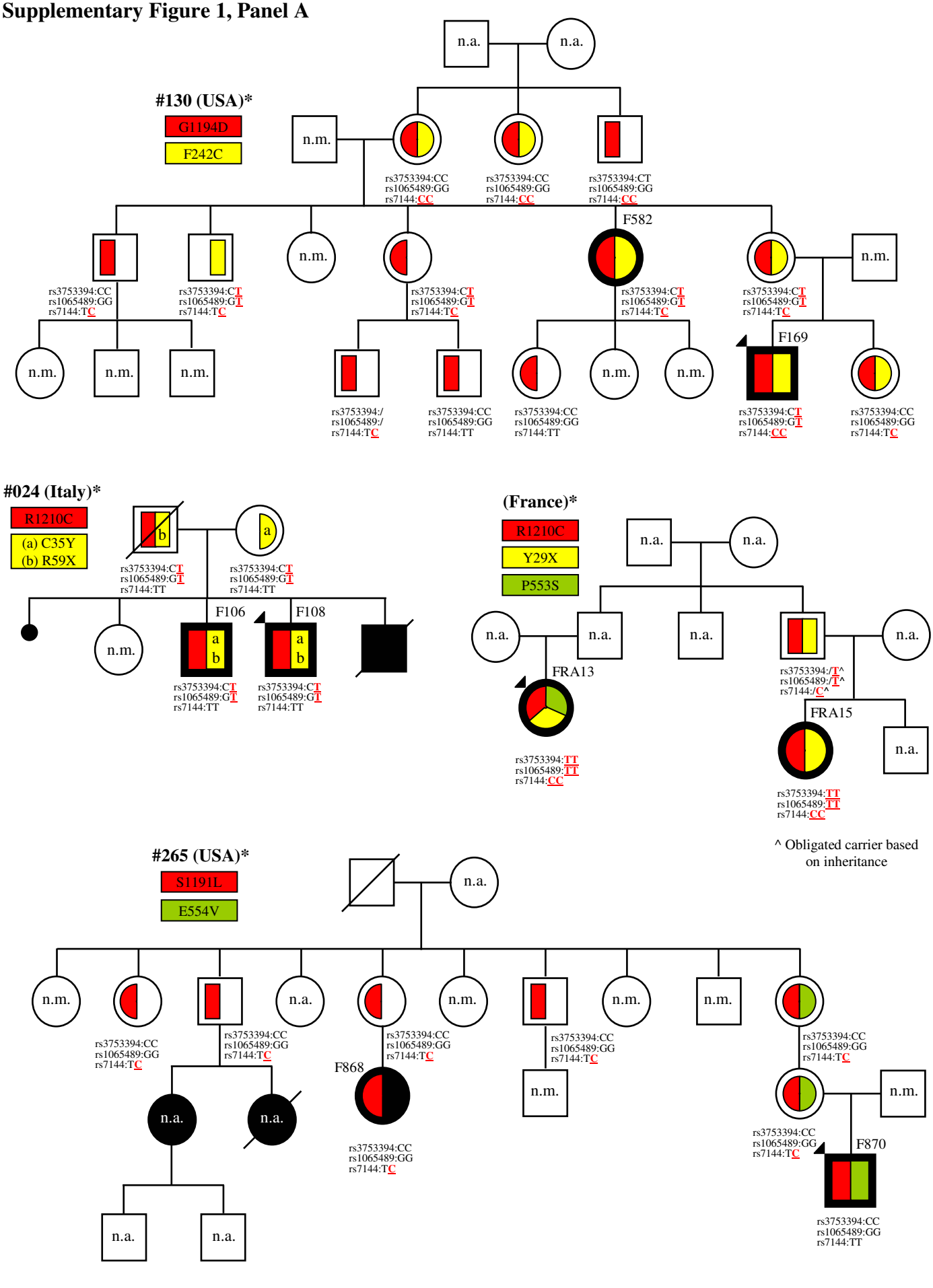
The percentage of affected subjects among carriers have been calculated considering only pedigrees in which at least two subjects have been screened. In the bottom of the X-axis, the number of subjects with two mutations in each gene combination have been reported.

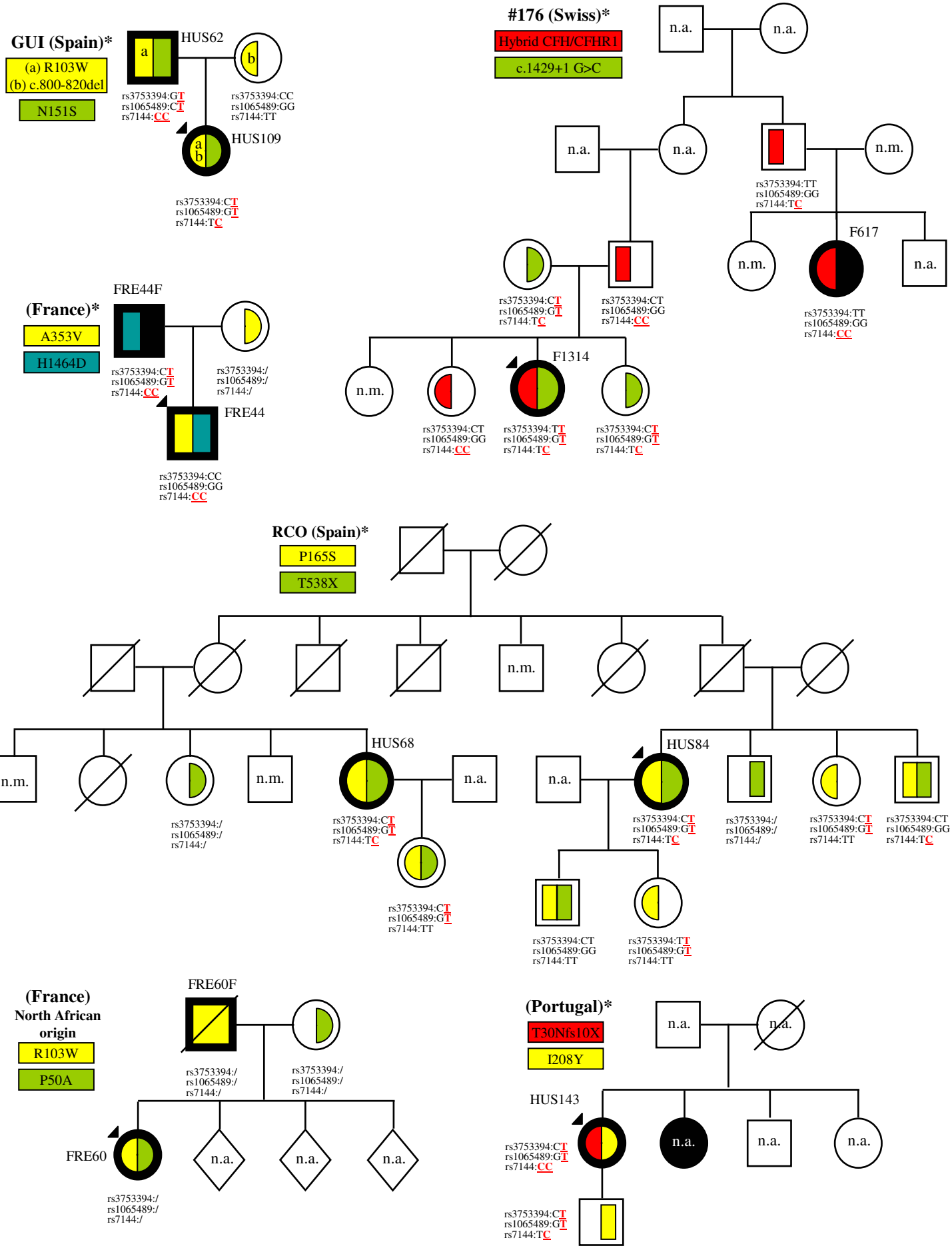
Supplementary Table 1. Characteristics of patients with combined mutations receiving isolated kidney transplants.

Code	Date of Tx	Mutations	Genetic screening pre-Tx	Plasma prophylaxis	Calcineurin inhibitors	Eculizumab treatment	Outcome at 3 years
#130F582	2004	CFH/MCP	no	no	n.a.	no	good
FRA15	*1st* (1990)	CFH/MCP	no	no	yes (CsA)	no	Lost (CR)
	2nd (1996)		no	no	yes (Tac)	no	Lost (Rec)
	3rd (2005)		no	no	yes (Tac)	no	good
HUS186	2008	CFH/MCP	no	no	yes (Tac)	no	Lost (Rec)
FRE06	1998	CFH/CFI	no	no	yes (CsA)	no	good
HUS207	2002	CFH/CFI	no	no	yes (Tac)	no	Lost (Rec)
GUIHUS62	2008	MCP/CFI	yes	no	no	no	good
FRA106	*1st* (1989)	MCP/CFI	no	no	n.a.	no	Lost (Rec)
	2nd (1993)		no	no	n.a.	no	Lost (Rec)
	3rd (2006)		no	no	yes (Tac)	no	Lost (I)
HUS167	2008	MCP/CFI	yes	yes	no	no	good
S657	2007	MCP/CFI	yes	yes	yes (CsA, Tac)	no	good
FRA13	2002	CFH/MCP/CFI	no	no	no	no	good

Graft lost for disease recurrence, rejection or other causes.
Calcineurin inhibitors: Cyclosporine (CsA), Tacrolimus (Tac).
n.a.: not available information on whether or not the patient got the drug, CR: chronic rejection, Rec: recurrence, I: viral infection

Supplementary Figure 1, Panel A




GUI (Spain)*
(a) R103W
(b) c.800-820del
N151S
HUS62
a
b
rs3753394:GT
rs1065489:CT
rs7144:CC
rs3753394:CC
rs1065489:GG
rs7144:TT
HUS109
a
b
rs3753394:CT
rs1065489:GT
rs7144:TC
#176 (Swiss)*
Hybrid CFH/CFHR1
c.1429+1 G>C
n.a.
n.a.
n.a.
n.a.
n.m.
rs3753394:TT
rs1065489:GG
rs7144:TC
F617
n.m.
n.a.
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rs1065489:GG
rs7144:CC
rs3753394:CT
rs1065489:GT
rs7144:TC
rs3753394:CT
rs1065489:GG
rs7144:CC
F1314
n.m.
rs3753394:CT
rs1065489:GG
rs7144:CC
rs3753394:TT
rs1065489:GT
rs7144:TC
rs3753394:CT
rs1065489:GT
rs7144:TC
FRE44F
(France)*
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H1464D
rs3753394:CT
rs1065489:GT
rs7144:CC
rs3753394:/
rs1065489:/
rs7144:/
FRE44
rs3753394:CC
rs1065489:GG
rs7144:CC
RCO (Spain)*
P165S
T538X
n.m.
n.m.
n.m.
HUS68
n.a.
rs3753394:/
rs1065489:/
rs7144:/
rs3753394:CT
rs1065489:GT
rs7144:TC
rs3753394:CT
rs1065489:GT
rs7144:TT
n.a.
HUS84
rs3753394:CT
rs1065489:GT
rs7144:TC
rs3753394:/
rs1065489:/
rs7144:/
rs3753394:CT
rs1065489:GT
rs7144:TT
rs3753394:CT
rs1065489:GG
rs7144:TC
rs3753394:CT
rs1065489:GG
rs7144:TT
rs3753394:TT
rs1065489:GT
rs7144:TT
(France)
North African origin
R103W
P50A
FRE60F
rs3753394:/
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rs7144:/
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rs1065489:/
rs7144:/
FRE60
n.a.
n.a.
n.a.
rs3753394:/
rs1065489:/
rs7144:/
(Portugal)*
T30Nfs10X
I208Y
n.a.
n.a.
HUS143
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rs1065489:GT
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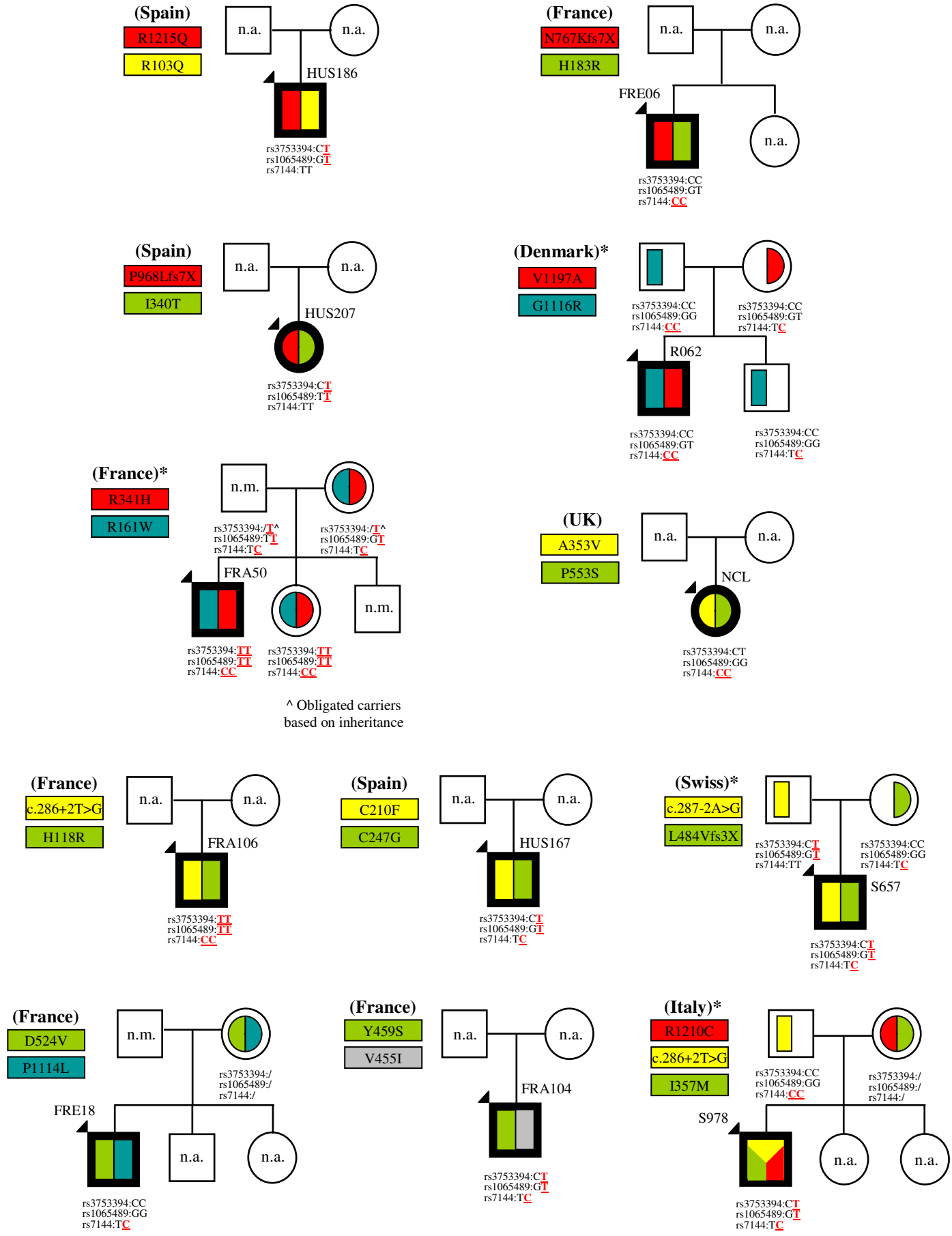
rs7144:TC

Supplementary Figure 1, Panel B



Supplementary Figure 2.