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RESEARCH LETTER

Role of the I416L Variant of Complement Factor I in Thrombotic Microangiopathy Among Patients of African Ancestry

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INTRODUCTION

Hemolytic uremic syndromes (HUS) is a pathological spectrum characterized by thrombocytopenia, microangiopathic hemolytic anemia, and predominantly renal injury. HUS comprises a variety of diseases with distinct pathogenic mechanisms, including genetic causes, infections, medications, autoimmune conditions, transplantation, pregnancy, and hypertensive crises. Typically, patients are categorized as having atypical HUS (aHUS or primary HUS) when a genetic cause, whether related to complement regulation or not, is documented or in case anticomplement factor H autoantibodies are detected. Secondary forms of HUS lump together all other forms of the disease. However, this classification does not account for the growing recognition that 2 or more conditions may coalesce into triggering aHUS.[1] Concurrently, pathogenic variants in the alternative complement factors have been repeatedly detected in case series of secondary aHUS,[2] thereby blurring the boundary between primary and secondary aHUS. For instance, aHUS is acknowledged to predispose to hypertensive crisis and reciprocally, malignant hypertension may precipitate thrombotic microangiopathy (TMA) via endothelial shear stress. Lately, patients experiencing TMA as a complication of hypertensive crisis have been shown to exhibit a high prevalence of either pathogenic variations of complement genes or rare variants.[2] Such patients display a more severe renal prognosis and a higher posttransplant disease recurrence rate than patients without complement variants.[3,4]

The I416L variant of complement factor I (CFI) is specific to the population of African ancestry. It occurs on a single nucleotide at position c.1246 A>C, at the level of exon 1 and determines a missense mutation, whereby an isoleucine is replaced by a leucine in the serine protease domain and affects a key regulator of the alternative complement pathway. An *in vitro* model has predicted that the I416L mutants produce a low amount of *CFI* in the intracellular compartment leading to a quantitative deficiency of *CFI*.[5]

By running a query through the BIOMNIS whole-exome genetic databank we sought to identify and provide a phenotypic description of the patients carrying the I416L *CFI* variant.

METHODS

The purpose of the study was to describe the population of patients with *CFI* I416L mutation detected by genomic investigation using whole exome sequencing (WES).

All samples were sequenced at the BIOMNIS center (Lyon, France) using the Illumina platform and subsequently processed via the in-center bioinformatics pipeline. Patients were included if they were over 18 years old and the target mutation was found through the genomic platform during the period ranging from January 2011 to March 2022.

The BIOMNIS genetic databank compiles a total of 3122 WES nationwide (single unrelated patients) for renal (n = 1340) and nonrenal indications (n = 1782). Once patients were recognized to carry the I416 variant, deidentified clinical data was provided by the attending nephrologist.

Inclusion criteria included the presence of the I416L mutation of *CFI* in any adult patient. The retrospective analysis identified a population of 12 patients carrying the I416L variant (1 patient's data could not be collected). All these subjects were subjected to WES as part of a genetic screening for kidney disease of undetermined cause. In addition, 5 patients carrying the I416L mutation were found in the group that performed genetic testing for nonrenal indication.

RESULTS

Descriptive Data

Among 1782 WES performed for nonrenal indications, 5 unrelated patients harbored the I416L variant (Figure S1). Out of the 1340 WES performed for renal indications, we identified a population of 12 unrelated patients carrying the I416L *CFI* variant (Table 1). All patients were of African ancestry and were heterozygous for the variant, including 6 males and 6 women with median age of 32 years (range: 19–42 years). None of these patients were acknowledged to have aHUS prior WES.

All but 2 patients (P5 and P11) had a history of chronic kidney disease when WES was performed, and 5 of them were kidney transplant recipients. Four patients disclosed genetic risk factor for kidney disease including APOL-1 pathogenic variants (G1 and/or G2) and URAT1-related pathogenic variant (P1, P2, P11, and P5, respectively). All patients had a history of early-onset (median age of onset 33 years) and uncontrolled hypertension (range 19–38 years). Seven patients had at least 1 additional risk factor for renal disease. One patient (P12) was discovered to have chronic kidney disease and hypertension in the course of preoperative management for kidney cancer. The I416L mutation has a higher prevalence in the population with nephropathy than in the control population (allele frequency 0.0052 vs. 0.0014).

Main Results

Five patients (42%) experienced HUS (P1, P2, P6, P7, and P10); 4 during bouts of hypertensive crisis, with concurrent biological TMA. P1 exhibited signs of TMA even on renal biopsy with superimposed signs of malignant nephrosclerosis (Table 2). In the fifth case (P6), signs of TMA occurred in kidney transplantation biopsy (1 year after transplantation) under tacrolimus therapy. In all cases of HUS ensuing renal injury required dialysis treatment. In 8 cases, patients developed end-stage chronic kidney disease.

Patients were initially categorized as having secondary HUS related to hypertension (P1, P2, P7, and P10) or tacrolimus therapy (P6). None of the patients received anticomplement C5 inhibitor therapy (eculizumab). None of the patients presented with recurrence of TMA during

Table 1. Patient characteristics at the time of genetic exam

Patient	Sex	Age	Ethnicity	Kidney disease	Physician's final diagnosis	HTN (Age of onset)	Other renal risk factors	Genetic risk factors	*CFI* pathogenic variant
P1	M	39	African	CKD stage IV	Malignant nephrosclerosis	Yes (33 y)	no	APOL1 G1/G2	I416L Het
P2	M	47	African	CKD stage V/HD	Nephrosclerosis	Yes (37 y)	Obesity DM Type 2	APOL1 G1/G1	I416L Het
P3	F	43	African	CKD stage V/KT	Undetermined nephropathy	Yes (38 y)	no	no	I416L Het
P4	M	38	African	CKD stage III	Undetermined nephropathy	Yes (31 y)	UC Obesity	no	I416L Het
P5	F	22	African	Preeclampsia	Preeclampsia	Yes (21 y)	no	URAT1	I416L Het
P6	F	48	African	CKD stage V/KT	Undetermined nephropathy	Yes (33 y)	Anorexia	no	I416L Het
P7	M	21	African	CKD stage V/KT	Undetermined nephropathy	Yes (19)	Chronic HBV	no	I416L Het
P8	M	46	African	CKD stage V	Undetermined nephropathy	Yes (35)	CAD DM Type 2	no	I416L Het
P9	F	31	African	CKD stage V/KT	Undetermined/ nephropathy	Yes (29)	CAM with cardiac transplantation	no	I416L Het
P10	M	50	African	CKD stage V/KT	Undetermined nephropathy/ nephrosclerosis	Yes (33)	no	no	I416L Het
P11	F	42	African	Preeclampsia	Preeclampsia	Yes (42)	no	APOL1 G1/G1	I416L Het
P12	F	61	African	CKD stage IV	Tubulointerstitial nephritis	Yes (38)	Nephrectomy Recurrent urinary tract infections	no	I416L Het

CAD, chronic coronary artery disease; CAM, cardiomyopathy with normal coronary artery; *CFI*, complement factor I; CKD, chronic kidney disease; DM, diabetes mellitus; G6PDD, glucose-6-phosphate dehydrogenase deficiency; HBV, hepatitis B virus; HD, hemodialysis; Het, heterozygous; HTNA, hypertension; KT, kidney transplant; UC, ulcerative colitis.

Table 2. History of patientswith *CFI* I416L variant at initial presentation

Patient	Known CKD (age)	Occurrence of TMA	Age at presentation	Concurrent trigger or feature	Associated organ damage	Biopsy results	Glomerulosclerosis	IFTA	Vascular lesions	IF	ESKD after the episode[a]	Relapse[b]	Duration of follow-up
P1	No	Yes	36	Malignant hypertension	AKI	TMA associated with nephrosclerosis	50%	50%	Severe	None	Yes	No	6 years
P2	CKD stage III (43)	Yes	45	Malignant hypertension	AKI	ND	-	-	-	-	Yes	No	7 years
P3	CKD stage V (37)	No	-	-	No	ND	-	-	-	-	-	-	6 years
P4	No	No	-	-	No	Fibrous endarteritis Intratubular hemoglobin casts	8%	5%	Moderate	Mesangial C3+	-	-	5 years
P5	No	Preeclampsia HELLP syndrome	19	Pregnancy	No	ND	-	-	-	-	No	No	2 years
P6	CKD stage IV (33)	Yes	41	CNI therapy with Tacrolimus	AKI	TMA lesions transplanted kidney	57%	70%	Severe	Arteriolar C3 +	Yes	No	15 years
P7	No	Yes	19	Malignant hypertension	AKI	Extensive fibrous lesions without deposits	84%	80%	Severe	Arteriolar C3 +	Yes	No	2 years
P8	CKD stage IV (35)	No	-	-	No	ND	-	-	-	-	-	-	9 years
P9	CKD stage V (28)	Preeclampsia	28	-	AKI Heart failure	ND	-	-	-	-	Yes	-	3 years
P10	No	Yes	40	Malignant hypertension	AKI	ND	-	-	-	-	Yes	No	11 years
P11	No	Preeclampsia	42	Pregnancy	No	ND	-	-	-	-	No	No	1 years
P12	Yes (38)	No	-	-	-	-	-	-	-	-	-	-	13 years

AKI, acute kidney injury; CKD, chronic kidney disease; CNI, calcineurin inhibitor therapy; ESKD, end- stage kidney disease; HELLP, hemolysis, elevated liver enzymes, and low platelet count; IFTA, interstitial fibrosis and tubular atrophy; ND, not determined; TMA, thrombotic microangiopathy.

[a]None of the patients received anti-C5 antibody therapy either after initial manifestation of TMA or as prevention of transplantation.

[b]Relapses of TMA on native or transplanted kidney.

Vascular lesions are classified as minimal, moderate, or severe; IF, Deposits in immunofluorescence.

medical follow-up (mean duration 8 years). Out of 6 female patients, 3 (P5, P9, and P11) manifested hypertensive crisis in the setting of preeclampsia during each of their first pregnancy. In the first case (P5), preeclampsia occurred with intravascular hemolysis, thrombocytopenia, and increased liver indices (HELLP syndrome). In P9, preeclampsia occurred in the third trimester of a second pregnancy resulting in end-stage kidney disease. In the third case (P11), the patient presented with proteinuria in nephrotic range but with normal kidney function. In the case of P5, proteinuria and kidney function resolved after delivery. Subsequent follow-up with P11 revealed persistent proteinuria (2.5 g/g) with normal kidney function.

DISCUSSION

Key Results

These results epitomize both the promises and challenges of whole genome sequencing in patients with kidney diseases of undetermined cause. Streamlined genotyping of patients may detect pathogenic variants in patients otherwise deemed unlikely to carry the disease based on purely clinical features.[6] In this case, the results suggest that aHUS may go unrecognized or mimic vascular nephropathy as was the case for 5 of the patients, because none of them were suspected to have aHUS, and in some cases despite extensive investigations and a protracted follow-up.[7] Malignant hypertension complicated by TMA has recently been contemplated as a promising application for anti-C5 treatment, irrespective of genetic background.[2,8] Genetic evidence for aHUS would provide decisive support for this class of therapy. In fact, none of the patients herein received anti-C5 treatment in the face of a severe course of kidney disease and a potential risk of posttransplantation recurrence.

Interpretation

It may be questioned whether the I416L *CFI* variant constitutes an additional risk factor for renal disease rather than the expression of a genuine monogenic Mendelian disease. From this standpoint, additional triggers acting as second "hits" such as hypertension, pregnancy, or genetic and nongenetic renal risk factors may collude in precipitating kidney disease and aggravating its course. Accordingly, 9 patients were found to display other risk factors for kidney disease progression ($n = 7$) or concurrent pathogenic variants ($n = 4$). If proven valid, this concept would represent a significant shift away from our current conception of genetics pertaining to aHUS.[1] Based on readily available records, the I416L *CFI* variant had hitherto been categorized as either benign, likely benign, or of uncertain significance in numerous population-based registries.[9] These seemingly conflicting classifications are the product of the various criteria involved in variant grading and disparate results across different databases and ongoing reassessment.[S1] *In silico* prediction models and functional assays provided support for its pathogenicity. As of now, only a handful of reports have reported the I416L *CFI* variant. In 1 case it was associated with a homozygous CFHR1/CFHR3 deletion, which has also been tied to aHUS, yet again entertaining the concept of multiple aHUS risk factors.[5] In 1 series, the variant was detected in a patient whose family history was remarkable for vascular nephropathy.[S2] The I416L *CFI* variant was also detected in a cohort of patients with a history prominent for malignant hypertension.[S3] Another approach is to assess whether a particular variant is more prevalent compared to the general population.[S4] Herein, the frequency of the putative variant in the African population (2.3%, $n = 293/12{,}500$)[S5] was found to be close to that of our study group defined as patients of African ancestry (3.3%, $n = 9/266$, $P = 0.3$ with Fisher test), arguing against its pathogenicity (Supplementary Figure S2). Cosegregation is often contemplated as a means to ascertain the pathogenicity of a gene or variant. In our series, none of the patients had relatives available to undertake cosegregation and the meaningfulness of this strategy is further hindered by the variable penetrance of the disease.

Limitations

The better part of genetic data related to aHUS stems from studies conducted in Europe and North America. Because such data may be insufficiently enriched with patients of African ancestry and/or ethnic-related variables are not identified due to regulatory policies, these registries may not faithfully reflect the genetic profile of aHUS patients of this ethnic background.

In the same vein, the phenotypic expression of *CFI*-related pathogenic variants has been shown to hinge on the patient's ethnic background.[S6] This may account for some of the unusual traits of the clinical course of patients, namely the absence of recurrence including in high-risk conditions such as kidney transplantation ($n = 5$) and the absence of documented TMA in several instances ($n = 7$). Taken together, these data suggest that we may need to redefine the expected phenotypic expression of aHUS according to ethnicity and envision it as a protean disease with various courses. I416L *CFI* may act as a "disease modifier" as opposed to the more traditional driving genes within the scope of Mendelian complement-related disease.[S7] It further calls into question whether criteria developed to standardize the diagnosis of Mendelian diseases are applicable to aHUS or whether a new set of criteria should be elaborated.[S8]

Generalizability

At any rate, whole exome or genome sequencing does not systematically yield definitive, unambiguous conclusions but should rather be viewed as an integral component part of an ongoing multistep process necessitating repeated assessment. Future studies are warranted to specify the role of I416L *CFI* as a disease modifier or a full-fledged actor of C-HUS, in addition to recently recognized risk factors specific to patients of African ancestry.[S9,S10] To move forward, clinicians are in dire need of larger data collections more attuned to the ethnic diversity of patient population and robust functional assays.[S11] Given the therapeutic implications at hand, the stakes are potentially momentous.

DISCLOSURE

CR declares lecture fees with Alexion Pharma France and travel grants with Sanofi. LM declares lecture fees with Travere Pharmaceutical and Sanofi Pharma, travels grant with Sanofi France Pharma. All the other authors declared no competing interests.

None of the results presented in this paper have been published previously in whole or part.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Selection of participants.

Figure S2. Prevalence of the *CFI* I416L variant in the different study populations and other genomic databanks.

Supplementary Reference.

STROBE Statement.

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