Thrombotic microangiopathy in aHUS and beyond: clinical clues from complement genetics

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Abstract | Studies of complement genetics have changed the landscape of thrombotic microangiopathies (TMAs), particularly atypical haemolytic uraemic syndrome (aHUS). Knowledge of complement genetics paved the way for the design of the first specific treatment for aHUS, eculizumab, and is increasingly being used to aid decisions regarding discontinuation of anti-complement treatment in this setting. Complement genetic studies have also been used to investigate the pathogenic mechanisms that underlie other forms of HUS and provided evidence that contributed to the reclassification of pregnancy- and postpartum-associated HUS within the spectrum of complement-mediated aHUS. By contrast, complement genetics has not provided definite evidence of a link between constitutional complement dysregulation and secondary forms of HUS. Therefore, the available data do not support systematic testing of complement genes in patients with typical HUS or secondary HUS. The potential relevance of complement genetics for distinguishing the underlying mechanisms of malignant hypertension-associated TMA should be assessed with caution owing to the overlap between aHUS and other causes of malignant hypertension. In all cases, the interpretation of complement genetics results remains complex, as even complement-mediated aHUS is not a classical monogenic disease. Such interpretation requires the input of trained geneticists and experts who have a comprehensive view of complement biology.

The study of complement genetics has had a crucial role in improving understanding of the pathogenesis of primary atypical haemolytic uraemic syndrome (aHUS)[1]. The discovery in 1999 of abnormalities in the *CFH* gene, which encodes complement factor H (CFH), in patients with inherited or sporadic HUS[2] provided the first evidence of a contribution of dysregulation of the complement alternative pathway (CAP) to endothelial cell dysfunction and the formation of microvascular thrombi. Subsequently, loss-of-function variants in genes that encode two other complement regulators, membrane-cofactor protein (MCP; also known as CD46)[3–5] and complement Factor I (CFI)[6], as well as gain-of-function variants in genes that encode two main components of the alternative C3 convertase, complement C3 (REF.[7]) and complement factor B (CFB)[8], were also identified in patients with aHUS. These genetic associations have important clinical implications and their discovery led to targeted treatment of aHUS with C5 blockade[9,10]. The findings also sparked a renewed interest in the study of the role of complement in a wide variety of thrombotic microangiopathies (TMAs) beyond aHUS, such as pregnancy-associated TMA, secondary HUS and malignant hypertension-associated TMA.

HUS likely results from interactions between genetic susceptibility factors in and/or potentially outside the complement system and environmental factors (such as infections and injuries) that trigger complement activation and/or endothelial cell damage. One of the best examples of such a mechanism occurs in haematopoietic stem cell transplantation (HSCT)-associated TMA, in which endothelial injury owing to the transplantation procedure and associated complications frequently leads to the development of TMA in patients with genetic susceptibility factors, including complement dysregulation. The setting of HSCT provides a unique opportunity to dissect the underlying mechanisms, predict the occurrence and treat this severe form of TMA. However, the respective contributions of genetic susceptibility factors and environmental triggers varies between types of HUS and between patients. This variation might explain the occurrence of aHUS in the absence of complement gene variants.

Currently, three types of TMA raise some clinically challenging questions regarding a potential role of CAP

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Key points

- Knowledge of complement genetics has transformed the landscape of atypical haemolytic uraemic syndrome (aHUS) and other forms of HUS.
- To date, aHUS is the only form of HUS that has been clearly associated with genetic susceptibility factors related to complement regulation.
- Pregnancy- and postpartum-associated HUS is part of the spectrum of complement-mediated HUS.
- Secondary forms of HUS do not share genetic risk factors with aHUS.
- Malignant hypertension is highly prevalent in patients with aHUS; however, aHUS is a rare cause of malignant hypertension.
- Interpretation of complement genetics results requires comprehensive expertise in complement biology.

dysregulation in their pathogenesis, namely pregnancy- and postpartum-associated TMA, secondary HUS and malignant hypertension-associated TMA (FIG. 1). In this Review, we summarize the current knowledge of complement genetics in aHUS and discuss how complement studies affect the clinical management of patients with other types of TMA.

Rare complement variants in HUS

Screening for rare variants in complement genes usually involves next-generation sequencing and/or sanger sequencing of at least five complement genes, *CFH*, *C3*, *CFI*, *CFB* and *MCP*, as well as multiplex ligation-dependent probe amplification to identify potential *CFHR1*–CFH hybrid genes, which are caused by non-allelic homologous recombination[1,11–14]. To date, more than 500 variants in these five complement genes have been identified in patients with aHUS[15]. These genes encode proteins that have been implicated in both cell surface and fluid phase CAP regulation, with the exception of *MCP*, which is involved in cell surface CAP regulation only[16]. The results of studies that screened for rare complement gene variants in more than 3,000 patients worldwide have established that aHUS is a complement-mediated disease[15]. Notably, in all reported series approximately half of the detected variants were located in the *CFH* gene[15] (Supplementary Figure 1).

Genetic data are also now available for >140,000 individuals from diverse populations in the Genome Aggregation Database (gnomAD). These data indicate that rare variants in the five complement genes with minor allele frequencies (MAFs) of <1% and <0.1% are present in 12% and 3.7% of healthy individuals, respectively[17,18]. Thus, it is not the presence but the enrichment of rare variants in one or several complement genes in patients with HUS that suggests a role of CAP dysregulation in the pathogenesis of the disease[19].

The frequency of rare variants in *CFH*, *C3*, *CFI*, *FB* and *MCP* or *CFHR1–CFH* hybrid genes in cohorts of patients with aHUS varies from 26% to 62%, depending on whether patients with secondary forms of HUS, paediatric patients and patients with malignant hypertension were included[12–15,18,20]. In 2013, we reported the results of complement gene variant screening in 125 adult patients with aHUS in France[12]; 61% had at least one rare variant in one of the five aHUS susceptibility genes. This study excluded patients with secondary HUS (that is, HUS associated with drug exposure, autoimmune diseases, infections or solid organ transplantation) but included those with pregnancy- or postpartum-associated HUS (P-HUS). By contrast, a US series that used a broad definition of HUS based on TMA in the absence of Shiga toxin and complete ADAMTS13 deficiency (and thus included patients with secondary HUS) reported a lower frequency of rare variants (37 in 118 patients; 32%)[14]. Thus, the precise definition of the phenotype of patients with a type of HUS is of the utmost importance for the interpretation of the results of complement gene screening.

In contrast to aHUS without coexisting diseases, the frequency of rare complement variants is only moderately increased compared with healthy individuals in patients with Shiga toxin-producing *Escherichia coli*-associated HUS (STEC-HUS) (12 of 75 patients; 16%)[17] and in those with de novo TMA after kidney transplantation (6 of 24 patients, 25%)[21] and is not increased in patients with secondary HUS[22]. Of note, a high frequency of rare complement gene variants has been identified in patients with HSCT-associated TMA[23–25]. However, the majority of these variants have no documented pathogenic effect and their relevance for the disease phenotype remains to be fully established. A comparison of the frequencies of rare and pathogenic variants in the five major at-risk complement genes in healthy individuals from the 1000 Genomes Project[17,18,26] and in patients with various forms of HUS[12,17,18,22,27–30] or disorders with features of TMA[31–33] suggests that only aHUS is associated with clear genetic susceptibility factors related to complement regulation (FIG. 2). Thus, routine screening of complement genes is only recommended for patients with primary aHUS (TABLE 1).

Limitations of clinical translation. Translation of complement genetic findings into the clinical management of patients with HUS has limitations. First, complement activation, regardless of the presence or absence of genetic dysregulation, is not necessarily harmful in all settings. Moreover, such activation might be transient and spontaneously self-remitting.

Second, aHUS is not a monogenic disease and familial forms have been reported in less than 20% of patients[12,20]. Furthermore, the penetrance of the disease is highly variable, with healthy carriers of complement gene variants in the families of affected patients[34]. Thus, in contrast to the carriers of variants that are associated with monogenic diseases, not all carriers of complement gene variants will develop aHUS. The classical genetic approach, including family-based studies, has proved valuable for understanding the pathogenic mechanisms of HUS, as illustrated by the initial identification of *CFH* variants in patients with aHUS[2]. Moreover, a whole-exome sequencing approach is increasingly being used in patients with aHUS without identified complement gene variants, particularly in familial cases. However, to date, this strategy has only led to the identification of variants in *DGKE*, which encodes diacyl glycerol kinase epsilon (a protein that does not have a role in the complement system), in rare early-onset forms of HUS[35].

Next-generation sequencing
A high-throughput methodology that enables rapid sequencing of the base pairs in DNA samples.

Sanger sequencing
This 'first-generation' DNA sequencing method is considered to be the gold standard for validating DNA sequences, including those that have been obtained using next-generation sequencing.

Multiplex ligation-dependent probe amplification
A multiplex assay to detect copy number variations of genomic DNA sequences.

Non-allelic homologous recombination
A molecular mechanism of exchange between two long segments of DNA (~300 bp or longer) that have very high sequence homology.

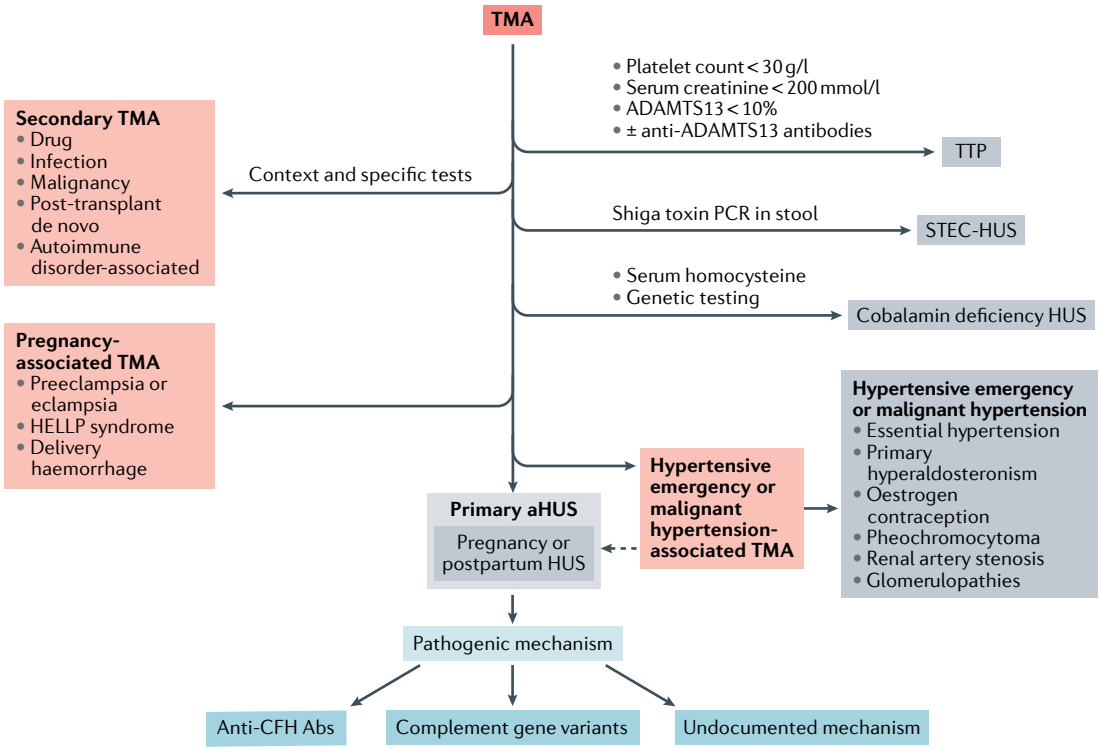

Fig. 1 | **Algorithm for the diagnosis of primary aHUS.** Thrombotic microangiopathy (TMA) arises from various pathogenic mechanisms that share a final common phenotype of endothelial cell injury leading to thrombosis. The initial diagnostic work-up of TMA is aimed at ruling in or out various causes, some of which require specific treatments (for example, plasma therapy with or without immunosuppressive therapies in ADAMTS13-deficiency-related TMA and hydroxocobalamin supplementation in cobalamin-deficiency-related TMA). Currently, complement-mediated atypical haemolytic uraemic syndrome (aHUS) is an exclusion diagnosis that is made once alternative diagnoses have been ruled out with a reasonable clinical probability. This step-by-step diagnostic work-up is more complex in certain clinical situations. For example, pregnancy and postpartum are high-risk periods for various types of TMA ranging from preeclampsia to aHUS, with potential overlap between these disorders (for example, aHUS can be complicated by preeclampsia). Malignant hypertension is another clinically challenging setting during which clinicians have to urgently rule in or out aHUS (an ultra-rare cause of malignant hypertension that requires specific treatment with complement blockade) from a list that includes much more frequent causes of TMA resulting from accelerated hypertension. TTP, thrombotic thrombocytopenic purpura; STEC-HUS, Shiga toxin-producing *E. coli*-associated haemolytic uraemic syndrome; HELLP, haemolysis, elevated liver enzymes and low platelet count; Anti-CFH Abs, anti-complement factor H antibodies.

Third, not all detected complement gene variants have clinically relevant consequences. For this reason, they are classified along a gradient ranging from those that almost certainly have a pathogenic role to those that are very likely benign[36]. This classification is largely based on the frequency of a variant in healthy individuals (BOX 1 and Supplementary Table 1). As a general rule, variants with a MAF <0.1% might be considered relevant for the pathogenesis of aHUS or other complement-mediated disorders[19]; 18 of 19 rare variants in the *CFH* gene with a MAF <0.1% that have been identified in patients with aHUS are classified as pathogenic based on the demonstration that they impair CFH regulatory activity[37]. However, this rule does not apply to rare variants in *CFB*; 5 of 11 rare variants in the *CFB* gene with MAF <0.1% that have been identified in patients with aHUS are not associated with a clear alteration in the function of the encoded protein[38]. Analysis of functional alterations in complement proteins takes into account the level of expression of the encoded protein (in plasma for CFH and CFI and at the granulocyte surface for CD46), the impact of the variant on the function of the encoded protein (assessed using in vitro assays[37,38] (Supplementary Table 2)) and prediction of the pathogenicity of a variant based on functional domains and in silico analyses.

Variants with a MAF >0.1% are unlikely to be pathogenic but are cautiously classified as pathogenic in rare instances when experimental proof of an associated functional defect is provided. Importantly, one cannot be absolutely certain that a pathogenic variant (defined based on genetic findings) or a dysfunctional variant (defined by complement experts based on functional assays and prediction of pathogenicity) is disease-causing. Enrichment of a variant in patients compared with healthy controls is therefore not sufficient to determine if a variant is a genetic susceptibility factor for HUS. For example, the rare *C3* variant Gln155 is detected at a similar frequency in patients with aHUS and healthy individuals[12] but is usually classified as a dysfunctional variant because it leads to resistance of C3 to proteolytic inactivation by CFH and CFI[39]. The lack

of demonstrated enrichment of this variant in patients with aHUS might simply be due to the limited number of cases that have been studied. Thus, until further data clearly define the clinical relevance of Gln155, this variant, which is not rare, should be cautiously considered to be a potential contributor to the pathogenesis of aHUS, which is a very rare disease. Similarly, one may postulate that in some instances, aHUS might be the consequence of the accumulation of common variants and/or polymorphisms within several complement genes and potentially even in genes outside the complement system, particularly those that are involved in vascular physiology and repair.

Limitations of variant classification. The current classification of complement gene variants has several limitations. Analyses of the potential enrichment of these variants in patients with aHUS are based on comparisons with genetic data from large population studies such as The 1000 Genomes Project[40]. These studies were designed to detect fairly frequent variants using techniques distinct from those that are routinely used in the clinic and are therefore not necessarily optimal tools to assess the frequency of rare variants that are of interest for aHUS. In addition, the frequency (and thus rarity) of variants might vary markedly between populations. For example, some hot-spots of pathogenic complement gene variants have been identified in restricted geographic areas such as Cyprus[41,42]. An increasing number of identified variants are novel and have not yet been functionally characterized[19].

To date, the issue of race has not been fully addressed in the field of complement genetics and no adequate strategy has been used to control for the racial diversity in studied populations. For example, the majority of patients with aHUS in European cohorts are white and do not necessarily share the same ancestry as individuals included in The 1000 Genomes Project. Importantly, data on the incidence of aHUS and of rare complement gene variants in Black individuals are still very limited and crucially lacking. This important knowledge gap might lead to the underdiagnosis or late diagnosis of aHUS in Black individuals and to uncertainties in the classification of complement gene variants in these patients. Thus, research into the incidence of rare complement gene variants in healthy Black people and in Black patients with complement-driven kidney diseases, including aHUS, are crucially needed to enable optimal management.

Clinicians should be mindful that the current classification of complement gene variants is not optimal and relies at least in part on prediction rather than proof of pathogenicity. As the classification continuously evolves with the identification of new familial forms and the accumulation of enrichment and/or functional data, the categorization of a given variant may change over time and requires regular reassessment. Assays have now been developed that can assess the capacity of plasma or serum from patients with TMA to induce complement deposition (mostly C5b-9) on endothelial cells in vitro[43–45]. Use of these assays could potentially improve analyses of the pathogenicity of variants and help to achieve a more accurate classification. However, they have yielded discrepant results between laboratories depending on whether serum or plasma were used and in some instances have shown an unacceptably high variability of results within the same laboratory[44,46]. Such variability might result from the variable activation state of endothelial cells used in in vitro assays. These assays require duplication of results in different laboratories as well as standardization before their clinical usefulness and contribution to the classification of complement gene variants can be fully assessed. Translation of these assays into clinical practice should therefore be cautious and is currently premature.

Classification of complement gene variants is not only a semantic issue. Misinterpretation of complement genetics results might have important consequences for patients and their relatives. Determining whether a variant is pathogenic is of the utmost importance and directly affects the clinical management of patients with aHUS and potentially other forms of HUS. In aHUS, complement genetics is a valuable tool to provide proof of a link between complement dysregulation and the disease, to assess disease severity[20], and most importantly to predict the risk of recurrence after

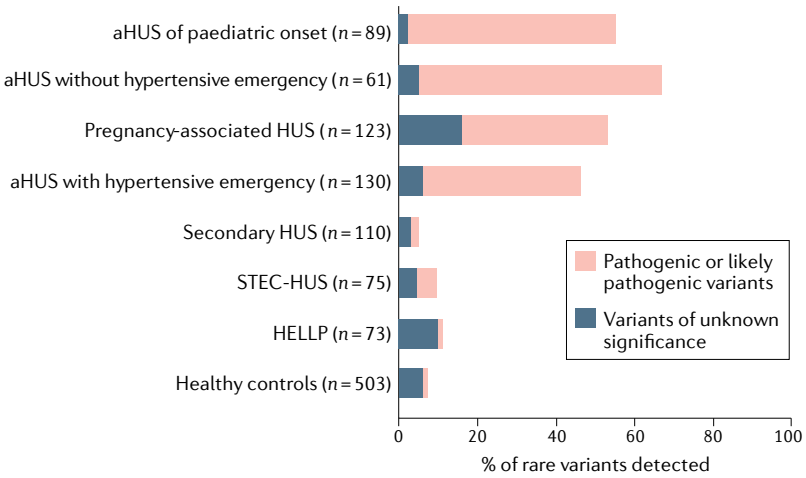

Fig. 2 | **Frequencies of rare variants in *CFH*, *CFI*, *MCP*, *C3*, *CFB* and *THMD* genes identified in TMA.** Pathogenic and likely pathogenic variants in complement genes are more frequent in atypical haemolytic uraemic syndrome (aHUS) of paediatric onset (data from the French HUS cohort[12]), aHUS of adult onset with or without hypertensive emergencies (data from the French HUS cohort[12,18]) and pregnancy-associated HUS (data from Bruel et al., Gaggl et al. and Huerta et al.[27–29]) but not more frequent in secondary HUS (data from the French HUS cohort[22]), in Shiga toxin-producing *E. coli*-associated HUS (STEC-HUS; data from the French HUS cohort[17]) or in HELLP (haemolysis, elevated liver enzymes and low platelet count) syndrome[31–33] than in cohorts of healthy individuals[12,18,26]. These data derive mainly from European cohorts that included mostly white European individuals. For classification of complement variants, we adapted criteria from the 2015 American College of Medical Genetics and Genomics consensus recommendations[36], which were used at the 2017 KDIGO Controversies Conference[83] and are summarized in BOX 1. Variants identified in *CFH*, *CFI* and *MCP* genes are classified as pathogenic if they fulfil the evidence criteria for both pathogenic very strong 1 (PVS1; that is null variants) and pathogenic strong 3 (PS3, that is variants that result in well-established in vitro or in vivo functional abnormalities) or likely pathogenic if they fulfil the criteria for both PVS1 and pathogenic moderate 2 (PM2, that is variants with a MAF <0.1%). Variants identified in *CFH*, *CFI*, *MCP*, *C3* and *CFB* are classified as likely pathogenic if they fulfil the criteria for PS3 and PM2. aHUS, atypical haemolytic uraemic syndrome; CFH, complement factor H; MCP, membrane-cofactor protein; CFI, complement factor I; CFB, complement factor B; TMA, thrombotic microangiopathy.

Table 1 | **Suggested indications for complement tests and gene screening in TMA**

Test	Indication	Notes
Measurement of C3, C4, FH, FI and CH50 and in plasma and CD46 expression on granulocytes	Primary aHUS	Clinical relevance in the initial work-up of other forms of TMA remains to be fully assessed
Anti-CFH antibodies	Primary aHUS	Valuable for the diagnosis of anti-CFH autoantibody-associated HUS, which potentially requires immunosuppressive treatment
Measurement of sC5b-9 in plasma	Primary aHUS	Potentially useful to aid decisions on whether to discontinue eculizumab
Complement deposition in vitro	TMA	Clinical relevance not yet clear
Complement gene testing (screening for variants and hybrid genes in *CFH*, *CFI*, *MCP*, *C3*, *CFB*, *THMD* and *DGKE* using next-generation sequencing and multiplex ligation-dependent probe amplification)[a]	Primary aHUS	Recommended for all patients. The results can potentially enable discontinuation of complement blockade, individualized prophylactic use of complement blockade in kidney graft recipients and retrospective confirmation of complement-mediated aHUS. Genetic results are not required for urgent diagnosis
	STEC-HUS	To be discussed on a case-by-case basis. Complement gene testing is indicated for children with severe forms leading to kidney failure within 3 years of diagnosis
	Post-transplant de novo TMA	To be discussed on a case-by-case basis following the exclusion of alternative causes of TMA in the kidney graft (such as drugs and humoral rejection)
	TTP	Not currently recommended
	Cobalamin deficiency HUS (resulting from mutations in *MMACHC*)	Not currently recommended
	Preeclampsia, eclampsia or HELLP syndrome	Not currently recommended
	Delivery haemorrhage	Not currently recommended
	Secondary TMA	Not currently recommended

HELLP, haemolysis, elevated liver enzymes and low platelet count; STEC-HUS, Shiga toxin-producing *E. coli*-associated HUS; CH50, total complement activity 50%; sC5b-9, soluble C5b-9; CFH, complement factor H; MCP, membrane-cofactor protein; CFI, complement factor I; CFB, complement factor B; THMD, thrombomodulin. [a]The classification of complement gene variants is not optimal and is continuously evolving, and the pathogenicity of a variant requires regular reassessment based on newly generated data compared with healthy individuals and/or the lack of clinical implications of genetic results.

kidney transplantation[47] and of disease relapse after discontinuation of anti-C5 (eculizumab) treatment[48–50]. In several countries, complement genetics are used to enable highly individualized prophylactic complement blockade in patients with aHUS who are undergoing kidney transplantation. This approach has contributed to a dramatic improvement in graft survival[51]. Similarly, retrospective data indicate that the presence or absence of a pathogenic complement gene variant is currently the most reliable predictive factor for relapse of aHUS after eculizumab discontinuation. The risk of relapse is <10% in patients with no detected complement gene variants and ranges from 30 to 60% in carriers of these variants[48–50]. A prospective study that assessed a strategy of eculizumab discontinuation based mainly on complement genetics found that such discontinuation is safe in patients with no detected complement gene variants[52]. The risk of aHUS relapse following eculizumab discontinuation in these patients was <5%. Such a strategy could potentially enable more cost-effective use of this efficient, innovative and expensive treatment.

Complement genetics beyond rare variants

Complement gene haplotypes, mainly homozygous *CFH-H3* and *MCP*ggaac (which are present in ~5% of heathy individuals), together with triggers such as infection or pregnancy, might also contribute to the development of aHUS[12,34]. These polymorphisms might at least partly explain the incomplete penetrance of the disease in carriers of pathogenic variants and potentially also the occurrence of aHUS in patients with no detectable variants. This latter hypothesis remains to be confirmed as the functional consequences of at-risk haplotypes in aHUS are not fully understood.

Genetic defects in *THBD*, which encodes thrombomodulin, a protein that interconnects the coagulation cascade and complement system, have been suggested to contribute to the pathogenesis of aHUS[53]. However, this hypothesis has now been challenged[11]. The reported variants in *THBD* are heterozygous missense variants that result in a loss of cofactor activity but not the complete loss of thrombomodulin secretion. Although rare variants in *THBD* have been reported in 41 patients

Box 1 | **Tools used for the classification of complement gene variants in aHUS**

Prevalence of the variant in healthy individuals*

Strong evidence of pathogenicity

- Variant occurs significantly more often in affected individuals than in healthy individuals.

Moderate evidence of pathogenicity

- MAF <0.1%.

Prediction of the impact of the variant on protein structure or function

Very strong evidence of pathogenicity

- Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene in which loss-of-function is a known mechanism of disease (*CFH*, *CFI*, *MCP* or *DGKE*).

Strong evidence of pathogenicity

- The results of well-established in vitro or in vivo functional studies support a damaging effect on the gene or gene product.
  - The variant affects protein synthesis: assessment of protein expression or level in plasma (Factor H and I) or on granulocytes (CD46) and/or well demonstrated in vitro lack of synthesis (CFH (including missense variant implicating Cys), CFI, CD46).
  - The variant affects protein function: in vitro studies supportive of a damaging functional effect on the gene product (FH, FI, THMD, CD46) or an indirect or direct gain-of-function (C3, CFB).

Moderate evidence of pathogenicity

- A rare missense variant found in a disease-related functional domain of *CFH* (that is, in *CFH* SCR1-4 or *CFH* SCR19-20) or located at the surface of C3b close to the binding site of Factor H and/or MCP or in CFB near to the C3b binding site.

Supporting evidence of pathogenicity

- A variety of in silico tools that score the predicted deleteriousness of a variant (such as PolyPhen, SIFT, Mutation Taster and CADD) can aid in the interpretation of sequence variants but should be used carefully.

Variant segregation

Supporting evidence of pathogenicity

- Co-segregation of a variant and the disease within families (the proband and all of his or her affected relatives are expected to carry the same variant).

Important limitations

- Even strong evidence that a variant is deleterious does not equate to a disease-causing variant.
- In the absence of definite evidence or prediction of the impact of a variant on protein structure or function, all variants with MAF <0.1% will be classified as variants of uncertain significance.
- Variant classification is a dynamic process that evolves with the accumulation of segregation and functional data.

*Assessment of MAF is available in public databases such as gnomAD.
aHUS, atypical haemolytic uraemic syndrome; CFH, complement factor H; CFI, complement factor I; DGKE, diacyl-glycerol kinase epsilon; CFB, complement factor B; MAF, minor allele frequency; MCP, membrane-cofactor protein; SCR, short consensus repeats; CADD, combined annotation dependent depletion; SIFT, Sorting Intolerant from Tolerant.

Combined annotation dependent depletion (CADD). An in silico tool that is designed to predict the pathogenicity of variants. CADD scores are based on diverse genomic features derived from the surrounding sequence context, gene model annotations, evolutionary constraint, epigenetic measurements and functional predictions. In silico predictive scores should be used, at most, as supporting evidence of pathogenicity.

with aHUS[15], their frequency is not significantly higher in these patients than in the general population[11] and no *THBD* variants without coexisting complement gene variants were detected in a French cohort of 214 patients with aHUS[51]. Thus, the role of *THBD* in the pathogenesis of aHUS is questionable (Supplementary Figure 1).

Notably, complete deletion of *CFHR1* and *CFHR3* is not a rare complement gene variant and is not currently considered to be a risk factor for aHUS. The frequency of this complete deletion varies from 5 to 10% depending on the ethnicity of the screened population[54]. However, this variant is associated with the presence of anti-CFH antibodies, which are detected in 5–13% of patients with aHUS in European cohorts[13,55] and in up to 56% of patients with aHUS in India[56]. In all published studies, approximately 80% of patients with aHUS and anti-CFH antibodies had complete CFHR1 deficiency owing to homozygous *CFHR3-CFHR1* deletion or, more rarely, to compound heterozygous *CFHR3-CFHR1* and *CFHR1-CFHR4* deletions. The link between lack of CFHR1 and the development of anti-CFH antibodies has not yet been clarified. Nevertheless, complete CFHR1 deficiency frequently occurs in healthy individuals and has not been shown to affect CAP regulation so should not be considered a genetic risk factor for aHUS. Similarly, no association with an increased risk of aHUS has been shown for rare variants in some other complement genes, including *CFHR5* (which encodes complement factor H-related protein 5), *CD55* (which encodes complement decay-accelerating factor), *CD59* (which encodes CD59 glycoprotein) and *CR1* (which encodes complement receptor 1).

The potential clinical relevance of rare variants in genes such as *PLG* (which encodes plasminogen)[11], *INF2* (which encodes inverted formin 2)[57], *VTN* (which encodes vitronectin)[11] and *CLU* (which encodes clusterin)[58] warrants further assessment. The *INF2* variants segregated completely with HUS in families and functional data are available for at least one of these variants[57], but whether HUS was the primary event or a consequence of focal segmental glomerulosclerosis and nephrotic syndrome is unknown. Other forms of severe focal segmental glomerulosclerosis and profound nephrotic syndrome are not associated with HUS, which suggests that similar to *DGKE*[59] variants, *IFN2* variants might be implicated in the pathogenesis of both TMA and nephrotic syndrome. In the absence of segregation and functional data, variants identified in *VTN* or *PLG* should be reported with caution.

Genetics studies have also expanded the spectrum of pathogenic mechanisms leading to aHUS beyond the complement cascade. The best illustration is the identification by exome sequencing of rare recessive loss-of-function variants in the *DGKE* gene in children with very early onset aHUS[35]. DGKE is an enzyme with a key role in the regulation of endothelial cell activation[60] but no clear connection with the complement cascade.

In the subset of patients with aHUS who do not respond to complement inhibiting therapy, alternative diagnoses, including a deficiency in MMACHC, should be ruled out[61] (FIG. 1).

Complement and pregnancy-associated TMA

Pregnancy-associated and P-HUS was long considered to be a type of secondary HUS. The detection of rare variants in complement genes in a substantial proportion (40–56%) of patients with P-HUS[27,29], as well as other similarities between this type of HUS and atypical HUS (such as severity at presentation and kidney outcomes), led to the inclusion of P-HUS within the spectrum of complement-mediated HUS (FIG. 1). This reclassification has important clinical implications as patients with P-HUS can be treated with complement blockers. In published case reports and small series, eculizumab seems to be similarly efficacious in P-HUS and

in primary aHUS that is not related to pregnancy[27,29,62–67]. However, HUS is a rare cause of pregnancy or postpartum TMA, which is most frequently linked to preeclampsia, eclampsia or HELLP (haemolysis, elevated liver enzymes, and low platelets) syndrome[68] and to a lesser extent to postpartum haemorrhage[69]. Preeclampsia and HELLP syndrome have several clinical and biological similarities to P-HUS and some patients with HELLP syndrome show features of complement activation in their serum[70,71].

The available data indicate that constitutional CAP dysregulation is not a major risk factor for preeclampsia, eclampsia and HELLP syndrome. In a series from the USA, the incidence of complement gene variants in patients with preeclampsia was 12% (12 of 99 patients)[72]. This series included 7 individuals who were heterozygous for the pathogenic *MCP* variant p.Ala353Val, which is associated with reduced CAP control on the cell surface (MAF 7% versus 2% in healthy individuals). Similarly, among 73 patients with HELLP syndrome included in three small series[31–33], only 11 (15%) had identified rare variants (MAF <1%) in complement genes, a frequency that is similar to that in healthy individuals.

Studies in animal models and limited clinical data suggest that complement activation might occur in the settings of preeclampsia, eclampsia and HELLP syndrome[44,73,74]. However, the available biomarkers of complement activation (including serum C3, Ba/Bb and soluble C5b-9) cannot reliably determine if complement activation has a role in a subtype of TMA. C3 plasma levels are reduced in <30% of patients with acute complement-mediated aHUS[1] and soluble C5b-9 levels are elevated in only ~50% of patients with acute aHUS, do not correlate with disease activity and can remain at detectable levels even in those receiving eculizumab[43,75]. In a small series, sera from patients with preeclampsia, eclampsia or HELLP syndrome induced increased deposition of C5b-9 at the surface of endothelial cells in vitro compared with sera from healthy individuals[44]. However, complement deposition persisted up to 90 days after clinical remission of preeclampsia, eclampsia and HELLP syndrome, suggesting that this finding might not be clinically relevant. The fact that preeclampsia, eclampsia and HELLP syndrome occur in patients with aHUS[76] and paroxysmal nocturnal haemoglobinuria[77] despite treatment with eculizumab also suggests that complement activation does not have a pivotal role in the pathogenesis of these disorders.

CAP dysregulation and secondary HUS

Secondary HUS are a heterogeneous group of disorders associated with drug administration, infections, autoimmune diseases and malignancies[22] (FIG. 1). They are more frequently encountered in clinical practice than primary aHUS[68] but their heterogeneity is a limitation for a global comprehensive analysis. Secondary HUS raises two clinically relevant questions, particularly in the context of the availability of eculizumab. The first is whether secondary forms of HUS are linked to a constitutional or acquired CAP dysregulation. The second is whether complement blockade is beneficial in patients with secondary HUS.

Two series provided some evidence to answer these questions. The first from Spain[78] included 29 patients, 52% of whom had drug-induced HUS. All patients were treated with eculizumab and 22 had complement gene analysis. The second, larger series from France[22] included 110 patients with secondary HUS, which was drug-induced in 29% and associated with autoimmune diseases in 24%. All patients had complement assays including gene analysis and 39 (35%) received eculizumab. In both studies, the incidence of rare variants in complement gene was very low: 6.8% (2 patients) in the Spanish series[78] and 5.4% (6 patients) in the French series[22]. Importantly, these frequencies did not significantly differ from those in two cohorts of healthy individuals. Similarly, only 2 patients (9%) in the Spanish series and 1 patient (<1%) in the French series had low-titre anti-CFH autoantibodies with questionable pathogenicity. Thus, secondary HUS are not driven by constitutional or acquired CAP dysregulation, a finding that might explain the <1% risk of relapse of secondary HUS once the underlying condition has been treated or the associated drug has been withdrawn[22,78].

Even in the absence of constitutional or acquired CAP dysregulation, the possibility that complement activation may occur as a 'second-hit' event in some forms of secondary HUS cannot be excluded. Such activation might contribute to the persistence of TMA initially triggered by the underlying condition or drug in the absence of complement-induced endothelial cell damage. The two available studies do not provide a definite answer as to whether such complement activation might occur. Markers of complement activation during the acute phase of secondary HUS were not assessed in the Spanish study. In the French study, C3 serum levels were reduced in only 15% of patients (8% had reduced C3 with normal C4) and other markers were not assessed.

As discussed above, the currently available biomarkers of complement activation lack sensitivity and specificity. Thus, their utility in determining whether complement activation is involved in secondary HUS is limited. Therapeutic complement blockade might provide indirect evidence for or against clinically significant complement activation in patients with secondary HUS. However, the two available series provide discrepant results regarding the outcomes of eculizumab treatment in these patients. In the Spanish series, 20 of 29 (68%) patients with severe HUS (requiring dialysis in 14 patients), had a rapid resolution of TMA and 15 (51%) had a ≥50% decrease in serum creatinine levels after initiation of eculizumab[78]. However, 50% of patients had tacrolimus-associated TMA, a form of HUS that resolves with drug withdrawal in a substantial number of cases. In addition, eculizumab-treated patients were not compared with a control group who had not received a complement blocker. In the French series, the kidney outcome was similarly bad in eculizumab-treated patients ($n = 38$) and those not treated with eculizumab ($n = 72$), with kidney failure occurring in 36% and 38% and chronic kidney disease stage 3–4 in 51% and 33% of patients, respectively[22]. However, the eculizumab-treated patients had more severe disease in terms of dialysis requirement and neurological involvement than those who were not treated with eculizumab.

Thus, in contrast to primary aHUS for which the benefit of eculizumab was evident in small prospective trials[9,10] and retrospective series[79], to date no hard evidence exists of a benefit of eculizumab in secondary HUS. Planned or ongoing prospective trials assessing the impact of complement blockade in secondary HUS are unlikely to provide definitive answers owing to the heterogeneity of secondary HUS and the confounding effect of treatment of the underlying condition. The use of eculizumab in patients with secondary HUS remains empirical and varies between countries as well as between institutions within the same country. A reasonable approach might be to restrict the use of eculizumab to patients with severe kidney and/or extra-kidney manifestations and those who do not achieve haematological remission after withdrawal of the associated drug or treatment of the underlying condition and/or plasma exchange.

Malignant hypertension and aHUS

The link between malignant hypertension and aHUS is the subject of an ongoing debate[18,30,80] that focuses on how to distinguish aHUS from malignant hypertension of other causes and how to determine whether TMA complicating malignant hypertension that is not related to aHUS is linked, at least in part, to constitutional dysregulation of the CAP. Complement genetics may provide some helpful clues to inform the discussion.

Clarification of semantics regarding malignant hypertension and HUS is crucial. Malignant hypertension was originally defined by the association of severe hypertension (>200/120 mmHg) with advanced bilateral retinopathy (haemorrhages, cotton wool spots or papilloedema) without any reference to other acute organ damage. By contrast, the concept of hypertensive emergencies emphasizes multi-organ damage. A 2019 position paper from the European Society of Cardiology[81] defines hypertensive emergencies as situations where very high blood pressure values are associated with acute hypertension-induced organ damage requiring immediate blood pressure control. The terms hypertensive emergency and malignant hypertension are sometimes used interchangeably, including in clinical series of patients with TMA and/or aHUS[18,30,82].

Similarly, different classifications include distinct definitions of aHUS. Some researchers restrict the use of aHUS to primary complement-mediated HUS[1], whereas others such as ourselves refer to secondary aHUS[83] (for example, drug, infection or malignancy-associated aHUS). Hypertension-associated TMA is sometimes included in the spectrum of secondary HUS[1], but this inclusion might be misleading because malignant hypertension might complicate the course of several types of HUS, including primary complement-mediated aHUS. A definition of aHUS based on complement genetics is not appropriate because 40–60% of patients with clinically diagnosed aHUS do not carry complement gene variants. The terms complement-mediated aHUS or complement blockade-responsive aHUS might be more relevant but are clearly not optimal. To date, primary aHUS remains an exclusion diagnosis owing to the lack of a specific positive test (FIG. 1).

The complexity of the issue of malignant hypertension and aHUS derives from the clinical overlap between these two conditions. On the one hand, malignant hypertension frequently complicates the course of primary aHUS. In two 2019 series[18,30], severe hypertension (>200/120 mmHg) was present in 53% (28 of 55 patients in the Spanish registry)[30] and 55% (76 of 137 patients in the French registry)[18] of patients with aHUS. These studies have raised awareness that around 50% (14 of 28 and 39 of 75, respectively) of patients with aHUS and malignant hypertension carry rare pathogenic variants in complement genes (MAF <0.1%). Most interestingly, 85% of the identified variants are pathogenic with specific functional alteration of the proteins. In these series[18,30], patients with and without malignant hypertension did not differ in their clinical severity of aHUS at presentation (that is, requirement for dialysis and extra-kidney manifestations), frequencies of complement gene variants, responses to treatment (including with eculizumab) or kidney outcomes. Thus, malignant hypertension does not seem to increase the severity of aHUS. However, the French study identified a subset of patients without malignant hypertension who had no detected complement gene variants and a favourable kidney outcome with or without eculizumab treatment[18]. This finding suggests that the presence of complement gene variants is associated with the severity of aHUS.

On the other hand, aHUS is a classical cause of malignant hypertension, but only a small minority of cases of malignant hypertension (<3%) can be attributed to aHUS[84]. Consequently, patients who present with malignant hypertension have a low clinical probability of having aHUS. Nevertheless, diagnosis of aHUS in this setting is of the utmost importance to enable initiation of specific therapeutic complement blockade.

Reports from a series of patients with TMA and severe hypertension (in which the terms hypertension-associated TMA, hypertensive emergency and TMA have been used in alternation by the researchers) have added some complexity to the discussion[45,80,82]. The most recent report, published in 2020 (REF.[82]), included 26 patients, most of whom were of European descent (85%), with hypertensive emergency and TMA confirmed by kidney biopsy. These patients presented with severe disease (neurological manifestations were noted in 23% and dialysis was required in 65%) but profound systemic haemolysis was absent in 73%. Rare complement gene variants were present in 8 patients (31%). Sera from 17 of the 25 patients who were tested induced increased deposition of C5b-9 on endothelial cells in an in vitro assay compared with pooled normal human sera, regardless of the presence or absence of complement gene variants. Moreover, 61% of patients progressed to kidney failure within 3 months of diagnosis, 5 of 7 patients responded at least partially to eculizumab and 4 kidney transplant recipients experienced TMA recurrence in seven renal grafts.

These reports have sparked increased interest in hypertension emergency or malignant hypertension and complement biology and genetics. However, use of the term hypertension-associated TMA might be

Box 2 | **Features that suggest essential hypertension rather than aHUS is the primary cause of malignant hypertension complicated by TMA***

Male gender
Around 62–71% of patients with malignant hypertension due predominantly or exclusively to essential hypertension are male[84,88–91].

Age >45 years
The mean age of patients with malignant hypertension due predominantly or exclusively to essential hypertension is frequently ≥45 years[81,84,86,90].

History of hypertension and/or antihypertensive treatment cessation
Around 37–73% of patients with malignant hypertension due predominantly or exclusively to essential hypertension have a history of hypertension[87,89,90]. A history of chronic hypertension is rare (16%) in patients with malignant hypertension subsequently diagnosed with primary aHUS[18].

Rapid (<72 h) resolution of haematological TMA after strict hypertension control
Such resolution is not suggestive of aHUS.

Absence of glomerular thrombi in kidney biopsy
Kidney biopsy samples from 100 patients aged <50 years with severe hypertension, kidney failure and TMA who did not have pathogenic or likely pathogenic variants in complement or coagulation genes did not show evidence of glomerular thrombi[91].

Left ventricular hypertrophy
Around 55–90% patients with malignant hypertension due predominantly or exclusively to essential hypertension have left ventricular hypertrophy[86,87,89,90].

No requirement for dialysis
In a series of 168 consecutive patients with malignant hypertension that was attributed to essential hypertension in 74% of cases, less than 1% of patients required dialysis[84]. In this series only 15% of patients presented with TMA.

*Provided that common causes of secondary hypertension other than aHUS have been ruled out.

misleading. One could argue that most if not all of the patients in the series could be classified as having complement-mediated atypical HUS with severe hypertension. Indeed, as already stated, severe or malignant hypertension is highly prevalent in the setting of aHUS[18,30]. Furthermore, the absence of haematological features of TMA at referral does not rule out a diagnosis of complement-mediated aHUS; up to 15% of patients with acute phase aHUS have a normal platelet count[12] and patients with aHUS can undergo haematological remission (either spontaneously or following plasma exchanges) despite persistent severe impairment of kidney function. In the first report on this series, 12 of 17 patients had already reached kidney failure by the time of their referral to a tertiary centre. Thus, the diagnosis of aHUS might have been substantially delayed in a sizeable proportion of patients, which might explain the discrepancy between the absence of haematological TMA and severe kidney impairment, and the presence of chronic TMA and hypertensive vasculopathy in kidney biopsy samples. The abnormal complement findings, including the high prevalence of complement gene variants, would be less surprising if patients with hypertension-associated TMA were clinically reclassified as having primary aHUS with severe or malignant hypertension.

However, these observations raise two important questions. The first relates to the implication that complement dysregulation and thus complement-mediated endothelial cell injury might occur in TMA associated with hypertensive emergency or malignant hypertension in the absence of aHUS. This case scenario includes severe or malignant hypertension with TMA complicating the course of essential hypertension or secondary causes of hypertension, particularly glomerulopathies (FIG. 1). To date, no proof exists of a clinically relevant contribution of complement dysregulation to the pathogenesis of hypertension-associated TMA in patients who do not have primary aHUS. Screening for complement gene variants has not been performed in large cohorts of patients with TMA associated with severe hypertension of known or presumed cause (essential hypertension or secondary hypertension in patients without aHUS). This limitation is mainly due to the fairly rare occurrence of TMA in the setting of hypertensive emergency or malignant hypertension; in two large series, TMA complicated the course of malignant hypertension in only 5–15% of patients[30,84].

In a series of 6 patients with TMA and malignant hypertension, 2 patients with IgA nephropathy (IgAN) carried pathogenic variants in *CFH* and *CFI* genes[30]. However, an association between IgAN and malignant hypertension-associated TMA has long been acknowledged. In a retrospective series of 128 patients with IgAN, 68 (53%) had acute and/or chronic arteriolar and arterial TMA in their kidney biopsy samples, 26% of these patients had malignant hypertension and only 12% had haematological TMA[85]. However, none of the 11 patients with IgAN-associated TMA who were tested had complement gene variants.

The second question is how to distinguish aHUS from other causes of malignant hypertension. The difficulty stems in part from the absence of a reliable positive diagnostic for aHUS. Thus, ruling aHUS in or out in the setting of malignant hypertension is highly challenging. Clinicians can rely on some clues but no definite hard evidence. In the setting of TMA and malignant hypertension, some features or combinations of features such as demographic factors, history of chronic hypertension and complications, severity of renal impairment, rapidity of haematological TMA remission after hypertension control and kidney biopsy findings might suggest essential hypertension rather than aHUS (BOX 2). Evidence for a role of these features derives from clinical practice and series that included patients with malignant hypertension that was due exclusively or predominantly to essential hypertension, a minority of whom developed TMA[84,86–90]. These findings clearly require further validation in cohorts of patients with malignant hypertension and TMA that is ultimately attributed to essential hypertension rather than aHUS. Importantly, ethnicity criteria should be used with extreme caution in the setting of malignant hypertension. Owing to the lack of published data on the incidence of complement-mediated aHUS and complement gene variants in black individuals, malignant hypertension in these patients might be wrongly attributed to severe essential hypertension, leading to under-diagnosis or late diagnosis of aHUS and precluding or delaying the use of efficacious C5 blockade.

Kidney pathological features are rarely used as criteria for distinguishing the various causes of hypertension-associated TMA because kidney biopsies are not frequently performed in patients with malignant hypertension and TMA. However, some pathological

features may prove helpful. In a series of 100 patients aged <50 years who had severe hypertension, kidney failure and typical features of TMA (that is, arteriolar oedema, mucoid intimal hyperplasia, mesangiolysis or necrotizing vasculitis) but no glomerular involvement (particularly no glomerular thrombi) in kidney biopsy samples, none had a pathogenic or likely pathogenic variant in a wide range of complement and coagulation genes[91]. Thus, the absence of glomerular TMA might help to exclude complement-mediated aHUS in patients with severe hypertension whose kidney biopsy samples show other features of TMA.

In vitro assays of complement deposition on endothelial cells are a promising tool for identifying cases of complement-mediated aHUS among patients presenting with malignant hypertension[45]. However, their implementation in routine clinical practice would be premature. In some situations, clinicians still face the dilemma of whether or not to start anti-complement therapy in a patient who presents with TMA and malignant hypertension. The persistence or recurrence of haematological features of TMA despite blood pressure control is clearly a reason to start anti-complement therapy once causes of secondary hypertension have been excluded. A familial history of aHUS is also a reason to start anti-complement therapy. In all other cases, clinicians can only rely on their clinical experience and potential clues that have not yet been validated (BOX 2).

Conclusions

The study of complement genetics has clearly changed the landscape of TMAs. Knowledge of the role of complement genetics has enabled breakthroughs in diagnosis and, most importantly, in the treatment of aHUS, with the development of eculizumab and subsequently a wide range of complement modulators targeting the alternative, lectin or final common pathways, some of which are currently being tested in patients. Complement genetics has also led to the reclassification of P-HUS within the spectrum of complement-mediated HUS, resulting in improved management of women with this severe disorder. By contrast, the study of complement genetics has not dramatically changed our understanding of other forms of HUS, most notably secondary HUS.

However, the increasingly important role of complement genetics in the clinical management of patients with aHUS has also underlined its limitations. Although important clinical decisions such as whether to discontinue eculizumab in these patients or to start prophylactic complement blockade in those undergoing kidney transplantation are now based on complement genetics, the question of how to determine whether a variant is pathogenic (and thus clinically relevant) is not clearly settled, and a genetically pathogenic or dysfunctional variant is not necessarily disease causing. The current classification of complement gene variants is not optimal and should be considered a work in progress. The pathogenicity and clinical relevance of individual variants should therefore be regularly re-evaluated. Most importantly, data generated through complement gene screening require careful assessment by geneticists and experts in the classification of complement variants who have a comprehensive view of the complement work-up, of which genetics is only a part.

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1. Fakhouri, F., Zuber, J., Fremeaux-Bacchi, V. & Loirat, C. Haemolytic uraemic syndrome. *Lancet* **217**, 681–696 (2017).
2. Warwicker, P. et al. Familial relapsing haemolytic uraemic syndrome and complement factor H deficiency. *Nephrol. Dial. Transpl.* **14**, 1229–1233 (1999).
3. Richards, A. et al. Mutations in human complement regulator, membrane cofactor protein (CD46), predispose to development of familial hemolytic uremic syndrome. *Proc. Natl Acad. Sci. USA* **100**, 12966–12971 (2003).
4. Noris, M. et al. Familial haemolytic uraemic syndrome and an MCP mutation. *Lancet* **362**, 1542–1547 (2003).
5. Fremeaux-Bacchi, V. et al. The development of atypical haemolytic-uraemic syndrome is influenced by susceptibility factors in factor H and membrane cofactor protein: evidence from two independent cohorts. *J. Med. Genet.* **42**, 852–856 (2005).
6. Fremeaux-Bacchi, V. et al. Complement factor I: a susceptibility gene for atypical haemolytic uraemic syndrome. *J. Med. Genet.* **41**, e84 (2004).
7. Fremeaux-Bacchi, V. et al. Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. *Blood* **112**, 4948–4952 (2008).
8. Goicoechea de Jorge, E. et al. Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. *Proc. Natl Acad. Sci. USA* **104**, 240–245 (2007).
9. Fakhouri, F. et al. Terminal complement inhibitor eculizumab in adult patients with atypical hemolytic uremic syndrome: a single-arm, open-label trial. *Am. J. Kidney Dis.* **2016**, 84–93 (2016).
10. Legendre, C. M. et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N. Engl. J. Med.* **368**, 2169–2181 (2013).
11. Bu, F. et al. Comprehensive genetic analysis of complement and coagulation genes in atypical hemolytic uremic syndrome. *J. Am. Soc. Nephrol.* **25**, 55–64 (2014).
12. Fremeaux-Bacchi, V. et al. Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide French series comparing children and adults. *Clin. J. Am. Soc. Nephrol.* **8**, 554–562 (2013).
13. Noris, M. et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin. J. Am. Soc. Nephrol.* **5**, 1844–1859 (2010).
14. Bu, F. et al. High-throughput genetic testing for thrombotic microangiopathies and C3 glomerulopathies. *J. Am. Soc. Nephrol.* **27**, 1245–1253 (2016).
15. Osborne, A. J. et al. Statistical validation of rare complement variants provides insights into the molecular basis of atypical hemolytic uremic syndrome and C3 glomerulopathy. *J. Immunol.* **200**, 2464–2478 (2018).
16. Merle, N. S., Church, S. E., Fremeaux-Bacchi, V. & Roumenina, L. T. Complement system Part I — molecular mechanisms of activation and regulation. *Front. Immunol.* **6**, 262 (2015).
17. Fremeaux-Bacchi, V. et al. Complement gene variants and shiga toxin-producing escherichia coli-associated hemolytic uremic syndrome: retrospective genetic and clinical study. *Clin. J. Am. Soc. Nephrol.* **14**, 364–377 (2019).
18. El Karoui, K. et al. Impact of hypertensive emergency and complement rare variants on presentation and outcome of atypical hemolytic uremic syndrome. *Haematologica* **104**, 12 (2019).
19. Bu, F. et al. Genetic analysis of 400 patients refines understanding and implicates a new gene in atypical hemolytic uremic syndrome. *J. Am. Soc. Nephrol.* **29**, 2809–2819 (2018).
20. Schaefer, F. et al. Clinical and genetic predictors of atypical hemolytic uremic syndrome phenotype and outcome. *Kidney Int.* **94**, 408–418 (2018).
21. Le Quintrec, M. et al. Complement mutation-associated de novo thrombotic microangiopathy following kidney transplantation. *Am. J. Transpl.* **8**, 1694–1701 (2008).
22. Le Clech, A. et al. Atypical and secondary hemolytic uremic syndromes have a distinct presentation and no common genetic risk factors. *Kidney Int.* **95**, 1443–1452 (2019).
23. Jodele, S. et al. The genetic fingerprint of susceptibility for transplant-associated thrombotic microangiopathy. *Blood* **127**, 989–996 (2016).
24. Ardissino, G. et al. Acquired complement regulatory gene mutations and hematopoietic stem cell transplant-related thrombotic microangiopathy. *Biol. Blood Marrow Transpl.* **23**, 1580–1582 (2017).
25. Gavriilaki, E. et al. Pretransplant genetic susceptibility: clinical relevance in transplant-associated thrombotic microangiopathy. *Thromb. Haemost.* **120**, 638–646 (2020).
26. Auton, A. et al. A global reference for human genetic variation. *Nature.* **526**, 68–74 (2015).
27. Bruel, A. et al. Hemolytic uremic syndrome in pregnancy and postpartum. *Clin. J. Am. Soc. Nephrol.* **12**, 1237–1247 (2017).
28. Gaggl, M. et al. Maternal and fetal outcomes of pregnancies in women with atypical hemolytic uremic syndrome. *J. Am. Soc. Nephrol.* **29**, 1020–1029 (2018).
29. Huerta, A. et al. A retrospective study of pregnancy-associated atypical hemolytic uremic syndrome. *Kidney Int.* **93**, 450–459 (2018).
30. Cavero, T. et al. Severe and malignant hypertension are common in primary atypical hemolytic uremic syndrome. *Kidney Int.* **96**, 995–1004 (2019).
31. Vaught, A. J. et al. Germline mutations in the alternative pathway of complement predispose to HELLP syndrome. *JCI Insight* **3**, e99128 (2018).
32. Fakhouri, F. et al. Factor H, membrane cofactor protein, and factor I mutations in patients with hemolysis, elevated liver enzymes, and low platelet count syndrome. *Blood* **112**, 4542–4545 (2008).
33. Crovetto, F. et al. The genetics of the alternative pathway of complement in the pathogenesis of HELLP syndrome. *J. Matern. Fetal Neonatal Med.* **25**, 2322–2325 (2012).

34. Arjona, E., Huerta, A., Goicoechea de Jorge, E. & Rodriguez de Cordoba, S. Familial risk of developing atypical hemolytic-uremic syndrome. *Blood.* **136**, 1558–1561 (2020).
35. Lemaire, M. et al. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. *Nat. Genet.* **45**, 531–536 (2013).
36. Richards, S. et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **17**, 405–424 (2015).
37. Merinero, H. M., Garcia, S. P., Garcia-Fernandez, J., Arjona, E., Tortajada, A. & Rodriguez de Cordoba, S. Complete functional characterization of disease-associated genetic variants in the complement factor H gene. *Kidney Int.* **93**, 470–481 (2017).
38. Marinozzi, M. C. et al. Complement factor B mutations in atypical hemolytic uremic syndrome-disease-relevant or benign? *J. Am. Soc. Nephrol.* **25**, 2053–2065 (2014).
39. Seddon, J. M. et al. Rare variants in CFI, C3 and C9 are associated with high risk of advanced age-related macular degeneration. *Nat. Genet.* **45**, 1366–1370 (2013).
40. Genomes Project, C. et al. A global reference for human genetic variation. *Nature* **526**, 68–74 (2015).
41. Roumenina, L. T. et al. A prevalent C3 mutation in aHUS patients causes a direct C3 convertase gain of function. *Blood* **119**, 4182–4191 (2012).
42. Gale, D. P. et al. Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis. *Lancet* **376**, 794–801 (2010).
43. Noris, M. et al. Dynamics of complement activation in aHUS and how to monitor eculizumab therapy. *Blood* **124**, 1715–1726 (2014).
44. Palomo, M. et al. Complement activation and thrombotic microangiopathies. *Clin. J. Am. Soc. Nephrol.* **14**, 1719–1732 (2019).
45. Timmermans, S. et al. C5b9 formation on endothelial cells reflects complement defects among patients with renal thrombotic microangiopathy and severe hypertension. *J. Am. Soc. Nephrol.* **29**, 2234–2243 (2018).
46. Galbusera, M. et al. An Ex vivo test of complement activation on endothelium for individualized eculizumab therapy in hemolytic uremic syndrome. *Am. J. Kidney Dis.* **74**, 56–72 (2019).
47. Zuber, J. et al. Targeted strategies in the prevention and management of atypical HUS recurrence after kidney transplantation. *Transpl. Rev.* **27**, 117–125 (2013).
48. Ardissino, G. et al. Discontinuation of eculizumab treatment in atypical hemolytic uremic syndrome: an update. *Am. J. Kidney Dis.* **66**, 172–173 (2015).
49. Fakhouri, F. et al. Pathogenic variants in complement genes and risk of atypical hemolytic uremic syndrome relapse after eculizumab discontinuation. *Clin. J. Am. Soc. Nephrol.* **12**, 50–59 (2017).
50. Wetzels, J. F. & van de Kar, N. C. Discontinuation of eculizumab maintenance treatment for atypical hemolytic uremic syndrome. *Am. J. Kidney Dis.* **65**, 342 (2015).
51. Zuber, J. et al. Use of highly individualized complement blockade has revolutionized clinical outcomes after kidney transplantation and renal epidemiology of atypical hemolytic uremic syndrome. *J. Am. Soc. Nephrol.* **30**, 2449–2463 (2019).
52. Fakhouri, F. et al. Eculizumab discontinuation in children and adults with atypical haemolytic uremic syndrome: a prospective multicentric study. *Blood* https://doi.org/10.1182/blood.2020009280 (2020).
53. Delvaeye, M. et al. Thrombomodulin mutations in atypical hemolytic-uremic syndrome. *N. Engl. J. Med.* **361**, 345–357 (2009).
54. Holmes, L. V. et al. Determining the population frequency of the CFHR3/CFHR1 deletion at 1q32. *PLoS One* **8**, e60352 (2013).
55. Dragon-Durey, M. A. et al. Clinical features of anti-factor H autoantibody-associated hemolytic uremic syndrome. *J. Am. Soc. Nephrol.* **21**, 2180–2187 (2010).
56. Puraswani, M. et al. Clinical and immunological profile of anti-factor h antibody associated atypical hemolytic uremic syndrome: a nationwide database. *Front. Immunol.* **10**, 1282 (2019).
57. Challis, R. C. et al. Thrombotic microangiopathy in inverted formin 2-mediated renal disease. *J. Am. Soc. Nephrol.* **28**, 1084–1091 (2017).
58. Stahl, A. L. et al. A novel mutation in the complement regulator clusterin in recurrent hemolytic uremic syndrome. *Mol. Immunol.* **46**, 2236–2243 (2009).
59. Azukaitis, K. et al. The phenotypic spectrum of nephropathies associated with mutations in diacylglycerol kinase epsilon. *J. Am. Soc. Nephrol.* **28**, 3066–3075 (2017).
60. Bruneau, S. et al. Loss of DGKepsilon induces endothelial cell activation and death independently of complement activation. *Blood* **125**, 1038–1046 (2015).
61. Grange, S. et al. Adult-onset renal thrombotic microangiopathy and pulmonary arterial hypertension in cobalamin C deficiency. *Lancet* **386**, 1011–1012 (2015).
62. Canigral, C. et al. Eculizumab for the treatment of pregnancy-related atypical hemolytic uremic syndrome. *Ann. Hematol.* **93**, 1421–1422 (2013).
63. Chua, J., Paizis, K., He, S. Z. & Mount, P. Suspected atypical haemolytic uraemic syndrome in two post-partum patients with foetal-death in utero responding to eculizumab. *Nephrology* **22**, 18–22 (2017).
64. De Sousa Amorim, E., Blasco, M., Quintana, L., Sole, M., de Cordoba, S. R. & Campistol, J. M. Eculizumab in pregnancy-associated atypical hemolytic uremic syndrome: insights for optimizing management. *J. Nephrol.* **28**, 641–645 (2015).
65. Delmas, Y., Bordes, C., Loirat, C., Fremeaux-Bacchi, V. & Combe, C. Post-partum atypical haemolytic-uraemic syndrome treated with eculizumab: terminal complement activity assessment in clinical practice. *Clin. Kidney J.* **6**, 243–244 (2013).
66. Kourouklaris, A. et al. Postpartum thrombotic microangiopathy revealed as atypical hemolytic uremic syndrome successfully treated with eculizumab: a case report. *J. Med. Case Rep.* **8**, 307 (2014).
67. Zschiedrich, S., Prager, E. P. & Kuehn, E. W. Successful treatment of the postpartum atypical hemolytic uremic syndrome with eculizumab. *Ann. Intern. Med.* **159**, 76 (2013).
68. Bayer, G. et al. Etiology and outcomes of thrombotic microangiopathies. *Clin. J. Am. Soc. Nephrol.* **14**, 557–566 (2019).
69. Frimat, M. et al. Renal cortical necrosis in postpartum hemorrhage: a case series. *Am J Kidney Dis.* **68**, 50–57 (2016).
70. Haeger, M. et al. Increased release of tumor necrosis factor-alpha and interleukin-6 in women with the syndrome of hemolysis, elevated liver enzymes, and low platelet count. *Acta Obstet. Gynecol. Scand.* **75**, 695–701 (1996).
71. Vaught, A. J. et al. Direct evidence of complement activation in HELLP syndrome: A link to atypical hemolytic uremic syndrome. *Exp. Hematol.* **44**, 390–398 (2016).
72. Salmon, J. E. et al. Mutations in complement regulatory proteins predispose to preeclampsia: a genetic analysis of the PROMISSE cohort. *PLoS Med.* **8**, e1001013 (2011).
73. Penning, M. et al. Classical complement pathway activation in the kidneys of women with preeclampsia. *Hypertension* **66**, 117–125 (2015).
74. Yonekura Collier, A. R. et al. Placental sFLT1 is associated with complement activation and syncytiotrophoblast damage in preeclampsia. *Hypertens. Pregnancy* **38**, 193–199 (2019).
75. Bu, F. et al. Soluble c5b-9 as a biomarker for complement activation in atypical hemolytic uremic syndrome. *Am. J. Kidney Dis.* **65**, 968–969 (2015).
76. Servais, A. et al. Atypical haemolytic uraemic syndrome and pregnancy: outcome with ongoing eculizumab. *Nephrol. Dial. Transpl.* **31**, 2122–2130 (2016).
77. Kelly, R. J. et al. Eculizumab in pregnant patients with paroxysmal nocturnal hemoglobinuria. *N. Engl. J. Med.* **373**, 1032–1039 (2015).
78. Cavero, T. et al. Eculizumab in secondary atypical haemolytic uraemic syndrome. *Nephrol. Dial. Transpl.* **32**, 466–474 (2017).
79. Fakhouri, F. et al. Insights from the use in clinical practice of eculizumab in adult patients with atypical hemolytic uremic syndrome affecting the native kidneys: an analysis of 19 cases. *Am. J. Kidney Dis.* **63**, 40–48 (2014).
80. Timmermans, S. et al. Patients with hypertension-associated thrombotic microangiopathy may present with complement abnormalities. *Kidney Int.* **91**, 1420–1425 (2017).
81. van den Born, B. H. et al. ESC Council on hypertension position document on the management of hypertensive emergencies. *Eur. Heart J. Cardiovasc. Pharmacother.* **5**, 37–46 (2019).
82. Timmermans, S. et al. Diagnostic and risk factors for complement defects in hypertensive emergency and thrombotic microangiopathy. *Hypertension* **75**, 422–430 (2020).
83. Goodship, T. H. et al. Atypical hemolytic uremic syndrome and C3 glomerulopathy: conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference. *Kidney Int.* **91**, 539–551 (2017).
84. Rubin, S. et al. Malignant hypertension: diagnosis, treatment and prognosis with experience from the Bordeaux cohort. *J. Hypertens.* **37**, 316–324 (2019).
85. El Karoui, K. et al. A clinicopathologic study of thrombotic microangiopathy in IgA nephropathy. *J. Am. Soc. Nephrol.* **23**, 137–148 (2012).
86. Lane, D. A., Lip, G. Y. & Beevers, D. G. Improving survival of malignant hypertension patients over 40 years. *Am. J. Hypertens.* **22**, 1199–1204 (2009).
87. Lip, G. Y., Beevers, M. & Beevers, D. G. Complications and survival of 315 patients with malignant-phase hypertension. *J. Hypertens.* **13**, 915–924 (1995).
88. Polgreen, L. A., Suneja, M., Tang, F., Carter, B. L. & Polgreen, P. M. Increasing trend in admissions for malignant hypertension and hypertensive encephalopathy in the United States. *Hypertension* **65**, 1002–1007 (2015).
89. van den Born, B. J., Koopmans, R. P., Groeneveld, J. O. & van Montfrans, G. A. Ethnic disparities in the incidence, presentation and complications of malignant hypertension. *J. Hypertens.* **24**, 2299–2304 (2006).
90. Shantsila, A., Shantsila, E., Beevers, D. G. & Lip, G. Y. H. Predictors of 5-year outcomes in malignant phase hypertension: the West Birmingham Malignant Hypertension Registry. *J. Hypertens.* **35**, 2310–2314 (2017).
91. Larsen, C. P., Wilson, J. D., Best-Rocha, A., Beggs, M. L. & Hennigar, R. A. Genetic testing of complement and coagulation pathways in patients with severe hypertension and renal microangiopathy. *Mod. Pathol.* **31**, 488–494 (2018).

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