Characterization of a New DGKE Intronic Mutation in Genetically Unsolved Cases of Familial Atypical Hemolytic Uremic Syndrome

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Abstract

Background and objectives Genetic and acquired abnormalities causing dysregulation of the complement alternative pathway contribute to atypical hemolytic uremic syndrome (aHUS), a rare disorder characterized by thrombocytopenia, nonimmune microangiopathic hemolytic anemia, and acute kidney failure. However, in a substantial proportion of patients the disease-associated alterations are still unknown.

Design, setting, participants, & measurements Whole-exome and whole-genome sequencing were performed in two unrelated families with infantile recessive aHUS. Sequencing of cDNA from affected individuals was used to test for the presence of aberrant mRNA species. Expression of mutant diacylglycerol kinase epsilon (DGKE) protein was evaluated with western blotting.

Results Whole-exome sequencing analysis with conventional variant filtering parameters did not reveal any obvious candidate mutation in the first family. The report of aHUS-associated mutations in DGKE, encoding DGKE, led to re-examination of the noncoding DGKE variants obtained from next-generation sequencing, allowing identification of a novel intronic DGKE mutation (c.888+40A>G) that segregated with disease. Sequencing of cDNA from affected individuals revealed aberrant forms of DGKE mRNA predicted to cause profound abnormalities in the protein catalytic site. By whole-genome sequencing, the same mutation was found in compound heterozygosity with a second nonsense DGKE mutation in all affected siblings of another unrelated family. Homozygous and compound heterozygous patients presented similar clinical features, including aHUS presentation in the first year of life, multiple relapsing episodes, and proteinuria, which are prototypical of DGKE-associated aHUS.

Conclusions This is the first report of a mutation located beyond the exon-intron boundaries in aHUS. Intronic mutations such as these are underreported because conventional filtering parameters used to process next-generation sequencing data routinely exclude these regions from downstream analyses in both research and clinical settings. The results suggest that analysis of noncoding regions of aHUS-associated genes coupled with mRNA sequencing might provide a tool to explain genetically unsolved aHUS cases.


Introduction

Atypical hemolytic uremic syndrome (aHUS) is a rare disorder resulting in thrombocytopenia, nonimmune microangiopathic hemolytic anemia, and acute kidney failure (1). It has a poor prognosis with approximately 60% of patients progressing to ESRD and a mortality rate between 4% and 25% (1,2). Extensive studies showed that hyperactivation of the complement alternative pathway is the main pathogenetic effector mechanism leading to endothelial damage and microvascular thrombosis in most patients with aHUS (1,2). Genetic and autoimmune abnormalities affecting complement proteins (complement factor H [FH], factor H–related proteins [FH–], factor H [HI], factor B [FB], complement component 3 [C3], membrane cofactor protein [MCP], and thrombomodulin [THBD]) have been documented in nearly 60% of patients (1–10). These findings paved the way for complement-tailored treatments (1,11) that have led to impressive improvements in short- and long-term prognosis (12). However, the underlying cause remains elusive for a substantial proportion of patients. The advent of next-generation sequencing has resulted in progress toward filling these knowledge gaps, allowing for a rapid exome-/genome-wide search for pathogenic mutations (13). Recently, using whole-exome sequencing (WES), Lemaire and colleagues successfully identified recessive mutations in DGKE, encoding diacylglycerol kinase epsilon (DGKE), as a novel cause of aHUS (14). Patients showed a specific clinical
Materials and Methods

aHUS was diagnosed on the basis of microangiopathic hemolytic anemia and thrombocytopenia defined by hematocrit <30%, hemoglobin level <100 g/L, serum lactate dehydrogenase level >460 U/L, undetectable haptoglobin, fragmented erythrocytes in peripheral blood smear, and platelet count <150x10^9/L, associated with acute kidney failure.

Family 1 and 30 unrelated pediatric patients with aHUS were recruited from the International Registry of HUS/Thrombotic Thrombocytopenic Purpura. Patient II-1 from family 2 underwent clinical WGS as part of the Genomics Medicine Clinic, in collaboration with Children’s Hospital of Wisconsin and Froedtert Hospital (19). Twenty unrelated French patients with pediatric aHUS undergoing genetic screening in Paris were included in this study.

Peripheral blood samples were collected for DNA, RNA, and protein isolation. Samples from 89 Italian healthy persons were analyzed as controls.

The study was approved by the Ethics Committee of the Azienda Sanitaria Locale, Bergamo, Italy, and the Institutional Review Boards at the Medical College of Wisconsin and Yale University School of Medicine. Informed consent was obtained from participants or by their parents according to the Declaration of Helsinki.

Detailed description of materials and methods are reported in the Supplemental Material.

Results

Identification of a Novel Homozygous Intronic DGKE Mutation in Family 1

We studied a consanguineous family (family 1, Figure 1A) from the North of Italy (South Tyrol) with two affected siblings (including a 13-year-old girl and 10-year-old boy at present) whose parents are healthy second cousins. aHUS was diagnosed at 10 months in the girl (no. 452) and at 5 months in the boy (no. 1200). Both siblings had thrombocytopenia, hemolytic anemia with schistocytes on blood smear, and renal impairment (Table 1). Mild proteinuria and hypertension were also documented. C3 levels were lower than normal, and C4 was normal. Complete remission was achieved for both patients with supportive therapy, which only included correction of anemia with packed erythrocytes and antihypertensive therapy. Both siblings had relapsing disease with one to three bouts a year, often in comitance with viral or bacterial infections (Supplemental Figure 1), without evidence of C3 consumption. During relapses, they manifested renal impairment (serum creatinine, no. 452: 1.4–1.7 mg/dl; no. 1200: 0.7–0.85 mg/dl), with hematuria and high-degree proteinuria (>2 g/24 h). After every relapse, renal and hematologic parameters returned to baseline with supportive therapy alone or with plasma infusion/exchange. From the age of 8 (no. 452) and 5 (no. 1200) years, the siblings received two plasma infusions a year as prophylaxis. Patient 1200 had a relapse at 7 years and 3 weeks after plasma infusion. The relapse was treated with plasma infusion with prompt recovery. Thereafter, no further relapse occurred in both children. At the last follow-up (age of no. 452, 13 years; age of no. 1200, 10 years), the siblings had mild persistent proteinuria with normal renal function (Table 1).

Sanger sequencing did not reveal any mutation in known aHUS-associated genes (CFH, MCP, CFI, CFB, C3, and THBD) or the CFH-H3 and MCPgamma risk haplotypes. Multiplex ligation-dependent probe amplification analysis showed the presence of two copies of CFHR1 and CFHR3 and excluded genomic CFH-CFHRS rearrangements. ELISA for anti-FH autoantibodies was negative. To identify the genetic basis of aHUS in this family, we performed WES coupled with homozygosity mapping (20).

WES performed on patient 452 showed a 72X mean coverage over the targeted exons, with 95.1% of targets covered at an average depth of 4X or higher. Variant detection identified 30,267 single nucleotide variants (SNVs) and 1137 insertions/deletions. After excluding variants with minor allele frequency (MAF) >1%, 4507 candidates remained. Among those, 143 (131 SNVs and 12 insertions/deletions) were homozygous protein-altering variants (nonsense, frameshift, or affecting canonical splice sites). We focused on identifying autozygous mutations because the affected siblings are from a consanguineous family and are likely to have two recessive disease alleles inherited from a common ancestor. Homozygosity mapping on the basis of WES data were used to identify all autozygous blocks, defined as runs of homozygosity of at least 1.5 Mb (20). This analysis yielded seven uninterrupted homozygous regions across the genome (Supplemental Table 1), which collectively spanned 77.6 Mb (approximately 2.7% of the genome), therefore confirming parental consanguinity at the level of second cousins (inbreeding coefficient, F=1/64). Within the homozygous blocks, we detected three missense variants in WDR6, UQCRCL1, and ZMYND10, whose segregation patterns were consistent with recessive inheritance (Supplemental Table 2). Only the WDR6 variant was not present in public databases (dbSNP137, 1000 Genomes Project, and National Heart, Lung, and Blood Institute Exome Variant Server). However, none of the three segregating
variants appeared to be convincingly causative for aHUS, and none were predicted to be damaging.

Meanwhile, the article by Lemaire and colleagues (14), documenting the association of DGKE mutations and aHUS, was published. Similarities concerning disease inheritance mode (recessive), age of onset (<1 year), and clinical phenotype (recurrent disease) among our patients and the ones described by Lemaire et al. (14) led us to reanalyze in detail WES data around the DGKE locus. We found a homozygous stretch, including eight SNVs spanning the DGKE locus (Supplemental Table 3), whose length (approximately 1.4 Mb), however, was just under the 1.5 Mb cutoff used. Among those SNVs, seven had MAF ≤1%; the eighth was a novel homozygous intronic variant (NM_003647.2: c.888+40A>G, Figure 2A), which had been filtered out by computational pipeline parameters. This variant is located in intron 5 and is absent from public databases. Sanger sequencing confirmed that the c.888+40A>G variant segregates with disease, with a recessive pattern: both affected siblings are homozygous, and both healthy parents are heterozygous (Figure 2A). In addition, the c.888+40A>G mutation was not found in 178 chromosomes from adult Italian controls.

Analyses with GenScan software (21) predicted that in the mutant sequence the probability of the correct splicing of exon 5 decreases from 0.999 to 0.118 versus the wild-type sequence (Figure 2B). The presence of a longer exon 5 (exon 5*, Figure 2B) was predicted with a probability of 0.882. Additional analyses with Human Splicing Finder software (22) predict that this mutation creates a gt cryptic splicing donor site within intron 5, with a score higher than the canonical donor site (Figure 2A). This change introduces new exonic splicing enhancer motifs for SF2/ASF, SRp40, and SF2/ASF (IgM-BRCA1) that are predicted to alter the normal behavior of the splicing regulatory proteins that process DGKE pre-mRNA molecules (Supplemental Figure 2).

Characterization of the Effects of c.888+40A>G on DGKE Transcript and Protein

DGKE is expressed in peripheral blood leukocytes (23). To assess the effects of the c.888+40A>G mutation at a transcriptional level, we obtained peripheral blood samples from the siblings and parents from family 1 and from a healthy unrelated Italian control. RNA analysis was performed by RT-PCR using primers spanning exons 4–6 of the DGKE transcript. Electrophoresis of the amplification products showed that the wild-type amplicon (273bp) found in the control was absent in both affected children (Figure 3A). Instead, their cDNA exhibited three additional bands with molecular weights higher than that of the wild-type. The band corresponding to an approximately 300 bp amplicon (mutant isoform 1) was more prominent than the other two, measuring approximately 400 and 650 bp, respectively (mutant isoforms 2 and 3, Figure 3A). Parental samples revealed the wild-type and mutant amplicons.

The nucleotide sequences of each isoform were obtained after extraction of the bands from the agarose gel. Mutant isoform 1 (312 bp) results from the retention of 39 intronic nucleotides that immediately follow exon 5 (c.888_889ins39, Figure 3B). This confirms in silico prediction that the neo-splice donor sites created by the mutation is used instead of the weak canonical splice donor site of exon 5. Isoform 1 transcript is predicted to yield a DGKE protein 13 amino acids longer than the wild-type (p.Lys296_Gly297ins13, Figure 4). Isoform 2 (407 bp) differs from isoform 1 because it also includes a 95-bp-long pseudoexon (c.888+278–372) within intron 5 (Figure 3B) because of the recognition of cryptic splice acceptor and donor sites at positions 276–277 and 373–374 bp, respectively, downstream of exon 5 (Supplemental Table 4). The resultant aberrant mRNA (c.888_889ins134) is predicted to yield a truncated DGKE protein because of a frameshift (p.Gly297Valfs*88, Figure 4). This transcript is, however, likely to be degraded by

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**Figure 1.** Pedigrees of family 1 (A) and family 2 (B) carrying DGKE mutations. Square symbols represent male family members, and circles represent female family members. Consanguineous unions are represented by double horizontal lines. Black-filled symbols represent affected individuals, and white symbols represent unaffected individuals. Slashes represent deceased individuals. Black-filled triangles represent the DGKE c.888+40A>G mutation. Empty triangles represent the DGKE c.966G>A mutation. wt, wild-type allele.
Table 1. Clinical characteristics of patients with DGKE mutations

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Family 1</th>
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<th>Family 2</th>
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<tr>
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<td>Patient 452</td>
<td>Patient 1200</td>
<td>Patient II-1</td>
<td>Patient II-2</td>
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<td>—</td>
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<td>—</td>
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<td>+ a</td>
<td>Negative</td>
<td>+ a</td>
<td>Negative</td>
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</tbody>
</table>

Normal values are as follows: serum creatinine: children <1–5 years, 0.3–0.5 mg/dl; children 5–10 years, 0.5–0.8 mg/dl; children >10 years, 0.5–1.2 mg/dl. Platelet count: 150–400 × 10⁹/L. Hemoglobin: children 5 months to 1 year, 10.8–12.5 g/dl; children 1–5 years, 11.7–13.7 g/dl; children 5–10 years, 12–14.4 g/dl; female >10 years, 12–16 g/dl; male >10 years 14–18 g/dl. LDH: children <1–5 years, 125–206 IU/L; children 5–10 years, 104–201 IU/L; children 10–15 years, 90–199 IU/L. C3: 90–180 mg/dl. C4: 10–40 mg/dl. Proteinuria in children <0.2 mg/mg of protein/creatinine ratio in spot urine or <0.2 g/24 h proteinuria or negative dipstick. Nephrotic range proteinuria is defined as protein/creatinine ratio >2 mg/mg or proteinuria >3.5 g/24 h. SCr, serum creatinine; LDH, lactate dehydrogenase; C3, serum complement C3 levels; C4, serum complement C4 levels; Prot, proteinuria; Creat, creatinine; +, mild level; ++, moderate level; ++++, severe level.

a Dipstick was used for this evaluation.

b Strongly positive result.
nonsense-mediated mRNA decay, therefore explaining the lower intensity of the corresponding band versus the isoform 1 band. Isoform 3 (645 bp) results from skipping both the canonical and neo-splice donor sites: the splicing machinery only recognizes the cryptic splice donor site that defined the pseudoexon of isoform 2 (Figure 3B) (24). Notably, this site has the highest consensus value for all splice donor sites within intron 5 predicted by the Human Splicing Finder software (Supplemental Table 4). Isoform 3 is characterized by in-frame retention of 372 intronic nucleotides (c.888_889ins372, Figure 3B), which would result in a protein with 124 additional amino acids (p.Lys296_Gly297ins124, Figure 4A). These results suggest that in the presence of the c.888+40A>G mutation, the weak canonical splice donor site of exon 5 is ignored by the spliceosome, resulting in the production of at least three aberrant transcripts. Western blot of blood leukocyte lysates documented that isoform 1 is expressed at the protein level, whereas no signal was found for isoform 3 (Figure 4B).

Exon 5 encodes part of the catalytic domain of DGKE (Figure 4A) (23). Structural tridimensional modeling suggests that the 13 amino acid insertion in exon 5 is expected to result in structural changes that may affect DGKE kinase activity (Supplemental Figure 3 and Supplemental Material).

Identification of a Second Family with the DGKE c.888+40A>G Mutation

A second unrelated kindred (family 2, Figure 1B) with the same intronic mutation was identified independently on the basis of WGS performed on patient II-1. Despite excellent coverage (96% of the reference genome at an average depth of 33X), the initial data assessment did not reveal a clear molecular diagnosis (19). A previously described (14) heterozygous DGKE nonsense mutation, c.966G>A (p.Trp322*), was found in patient II-1 by WGS and was subsequently confirmed by Sanger in all relatives except the mother (Supplemental Figure 4A). A more in-depth analysis of all potential maternal-in-origin DGKE variants was therefore performed. No other coding, nonsynonymous variants were identified, and there was no structural variation within or near DGKE. Out of 52 intronic DGKE variants, only eight were rare (MAF, 1%, Supplemental Figure 4B). All were sequenced in the parents to find those originating from the maternal lineage. Genotyping of the three affected (nos. II-1, II-2, and II-4, Figure 1B) and one unaffected sibling (no. II-3) for the two maternal-specific variants (Supplemental Table 5) revealed that only the affected individuals harbored both noncoding variants in compound heterozygosity with the c.966G>A mutation (Supplemental Figure 4C). The first variant (c.888+40A>G) appeared more promising and was the same found in homozygosity in family 1. The other (rs145743671) has an MAF of 0.55% in control individuals and is 0.700 bp away from exons 9 and 10.

The three affected siblings, born to an outbred union, were diagnosed with aHUS in early infancy. In individual II-1, aHUS was diagnosed at 9 months. He presented with thrombocytopenia, Coombs’ negative hemolytic anemia with
schistocytes, and mild renal impairment (Table 1). Marked hematuria and proteinuria were also documented. He had four relapses in the following year and a fifth relapse at 4 years (Supplemental Figure 4D, Supplemental Table 6). During the second and third relapses he had urine protein/creatinine ratios of 46 and 19 mg/mg, respectively (normal, 0.2 mg/mg). Treatment was supportive for all episodes, and peritoneal dialysis was only prescribed for the last one. At last follow-up (age 13 years), renal function, BP, and complement activity (CH50 and AH50) were normal, but hematuria and proteinuria were persistent (Table 1). Individuals II-2 and II-4 had aHUS 7 and 5 months, respectively (Table 1), associated with acute renal failure. C3 and C4 levels, measured in individual II-4 during the acute phase, were normal. Individual II-2 had two relapses both within 1 year of diagnosis, whereas II-4 had three relapses, all before 4 years of age (Supplemental Figure 4D, Supplemental Table 6). Both patients required peritoneal dialysis only during the first episode. Nephrotic-range proteinuria was documented during relapses in individual II-4 (urine protein/creatinine ratio 21.2–34.4) and at remission in individual II-2 (urine protein/creatinine ratio 2.6–8.8). Renal function, BP, CH50, and AH50 were normal for both patients at last follow-up, at ages 10 and 4 years, respectively (Table 1). Both parents and a 6-year-old sister are unaffected. The father is of Northern European descent, and the mother is half-German, half-Native American (Cherokee).

**Figure 3.** DGKE c.888+40A>G mutation causes an aberrant splicing of exon 5. (A) Agarose gel of the RT-PCR on cDNA obtained from peripheral blood leukocytes of homozygous mutated patients from family 1 (patients 452 and 1200), their heterozygous healthy parents (nos. 1417 and 1421), and a homozygous wild-type (wt) healthy control (CTR). Amplification products were obtained using primers (shown as red arrows in panel B) spanning DGKE exons 4–6. Only one band of 273 bp (wt isoform) was obtained from the healthy control individual, whereas a strong band (mutant isoform 1, approximately 310 bp long) and 2 weak bands of higher molecular weight (mutant isoforms 2 and 3, approximately 400 and 650 bp long, respectively) than the wt amplicon were present in the cDNA of both patients. The heterozygous healthy parents showed both the wt and the aberrant longer amplicons. (B) Diagrams of the normal (wt) and aberrant (mutant isoform 1 [Iso#1], mutant isoform 2 [Iso#2], mutant isoform 3 [Iso#3]) splicing of exon 5. The mutant Iso#1 is characterized by the retention of 39 nucleotides of intron 5. The mutant Iso#2 differs from Iso#1 for the further inclusion of a 95-bp-long pseudoexon from intron 5. The mutant Iso#3 is characterized by the retention of 372 nucleotides. The position of the c.888+40A>G is marked by a broken vertical red line.

**DGKE c.888+40A>G is a Founder Mutation that is Rare in aHUS**

Because two unrelated families of European descent have the exact same novel intronic variant, we reasoned that this may be caused by a rare founder mutation. We cross-referenced the list of the homozygous variants around DGKE locus in the WES data for individual 452 (Supplemental Table 3) and found that all were heterozygous in the WGS data for patient II-1. This concordance suggests a common origin for the c.888+40A>G mutation in the two families. Sequencing done to assess the frequency of the c.888+40A>G allele in 50 patients with pediatric-onset aHUS without a molecular diagnosis did not identify a single carrier.

**Discussion**

We report on the identification of a novel intronic DGKE mutation, c.888+40A>G, that interferes with normal mRNA splicing in two unrelated multiplex kindreds with phenotypes consistent with DGKE-associated aHUS. The pathogenicity of c.888+40A>G is supported by several lines of evidence. First, this mutation cosegregates with disease following a recessive pattern in both families: it is homozygous for family 1 and compound heterozygous for family 2 in concert with a known nonsense DGKE mutation (14). Second, the probability of having by chance two unrelated kindreds with phenotype concordant with that of DGKE-associated
aHUS (14,17) and the same novel intronic mutation is very low. Third, we demonstrate that this intronic mutation acts as a neo-splice site that not only results in the transcription of mutant mRNA isoforms predicted to be deleterious to DGKE catalytic activity, but also completely abrogates wild-type DGKE mRNA transcription.

Finding multiple patients from unrelated kindreds to have the exact same mutation is an unusual situation that is explained either by a mutational hotspot or by remote common ancestry. Our data support the notion that the pattern observed is caused by shared ancestry because all homozygous SNVs surrounding DGKE in family 1 are heterozygous in family 2.

Serum C3 levels of the two affected siblings from family 1 were slightly depressed at disease onset, but they were always normal when measured during disease relapse. They do not carry mutations in any of the known complement aHUS-associated genes. It remains unclear whether aHUS-associated DGKE deficiency may directly initiate complement activation or whether these patients carry another genetic abnormality in a modifier gene that affects complement biology.

Despite the advances in understanding aHUS genetic causes, approximately 40% of patients still do not carry mutations in CFH, MCP, CH, CFB, C3, THBD, and DGKE or deletions/rearrangements in CFH-CFHRs region (2,3,5,7,25). To our knowledge, this is the first report describing an intronic mutation located beyond exon/intron boundaries in DGKE and in general in aHUS-associated genes. Known intronic mutations far from exon/intron boundaries and causing human disorders are rare (26–28). However, these mutations may be underreported because they are not routinely investigated by standard analysis with Sanger sequencing, which is usually restricted to exons and canonical splice

**Figure 4.** Sequence changes identified in the three mutant DGKE isoforms. (A) Schematic representation of the domains identified in DGKE protein (from SMART, http://smart.embl-heidelberg.de) is illustrated (upper part of panel A) to show the relative position of the changes. In the lower part of panel A we present the sequences of bases and translated amino acids of wt DGKE protein to allow for comparison with those of the three mutant DGKE forms caused by the c.888+40A>G. The subscripts below the amino acids located at the beginning and the end of each exon are used to reveal their position relative to the wt protein. The sequence of exons 4, 5, and 6 are labeled in orange, green, and gray fonts, respectively. The red font is used to show the sequences that are unique to the three mutant DGKE proteins. (B) Protein blot of total proteins extracted from peripheral blood leukocytes. Lane 2: leukocytes from a healthy control; lane 3: leukocytes from patient 1200; lane 4: leukocytes from his mother (no. 1421) probed with an anti-DGKE antibody against the 2–51 N-terminal amino acids. Lane 1 contains total proteins extracted from healthy human platelets as additional control. Expected band size: DGKE wt, 64 kD; DGKE isoform 1, 65.5 kD; DGKE isoform 3, 78 kD (http://www.expasy.org/). A band corresponding to DGKE iso#1 is present in the sample from patient 1200, indicating that this mutant is expressed. The mutant iso#1 and wt DGKE (of control samples) had a very similar migration pattern when subjected to gel electrophoresis because of the very small difference in the predicted molecular weights. No band corresponding to iso#3 was detected, indicating that this isoform is produced in a very small amount, this isoform is unstable and rapidly degraded in the cells, or its conformation negatively affects the integrity of the targeted DGKE epitope. The blot were probed with an actin antibody as control for loading. DGKE, diacylglycerol kinase epsilon; wt, wild-type; C1, DGKE kinase C conserved region 1 (C1) domain; DAGKc, diacylglycerol kinase catalytic domain; DAGKa, diacylglycerol kinase accessory domain; LC, low-complexity region; TR, transmembrane region; iso#1, isoform 1; iso#2, isoform 2; iso#3, isoform 3.
slices. Also, in WES, common criteria for selection of variants restrict the analysis to coding regions, including the 2–4 bp immediately adjacent to the exons. The two families reported here suggest that a number of unexplained patients with aHUS may be accounted for by intronic mutations that cause aberrant splicing of known aHUS-associated gene transcripts.

In recent years, WES and WGS have allowed the discovery of a growing numbers of disease-associated genes (29); however, most variants in noncoding regions will be missed by WES. This shortcoming could be overcome by WGS; however, because of the large number of variants encountered, the choice regarding the ones deserving follow-up is a very challenging task. This is demonstrated by the fact that WGS failed to reveal a clear molecular diagnosis in family 2 because intronic mutations were initially filtered out.

Another striking feature is the diverse transcriptional effects of the mutant neo-splice site when it occurs in the context of a weak canonical splice donor and many strong cryptic donor and acceptor sites. The same phenomenon may occur in other DGKE introns, and perhaps in introns of other known aHUS-associated genes. It would be very interesting to determine if one could effectively prioritize at-risk introns via genome-wide identification of all weak donor and/or acceptor splice sites.

Because the DGKE c.888+40A>G mutation was shown to be recurrent among patients with early-onset aHUS, diagnostically and research laboratories should consider the inclusion of intron 5 in the routine genetic screening of DGKE. Sequencing of intronic regions of aHUS-associated genes followed by analysis of mRNAs sequence could provide a valuable, still underexplored tool to resolve patients with unknown genetic defects. Transcriptome sequencing (30) could soon represent an alternative valuable strategy to directly detect mutations, including variants that affect splicing.

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Disclosures

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