Case Report

Novel Mutations in the *DGKE* Gene in Two Indian Patients with Early-onset Atypical Haemolytic Uraemic Syndrome

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Abstract

Atypical haemolytic uremic syndrome (aHUS) is a clinically and genetically heterogeneous condition caused by a complex interplay between genomic susceptibility factors and environmental influences. Pathogenic variants in the *DGKE* gene are recently identified in cases with infantile-onset autosomal recessive aHUS. The presence of low serum C3 levels, however, has rarely been described in cases of *DGKE*-associated aHUS. Molecular genetic testing was performed by a commercial next-generation sequencing (NGS) panel as well and by an in-house developed targeted NGS for *DGKE* gene. Copy number variations (CNVs) were computed from NGS data by calculating a normalised copy number ratio of aligned number of reads at targeted genomic regions against multiple reference regions of the same sample and multiple controls. We report here two such novel clinically relevant variants (c.727_730delTTGT and c.251_259delGCGCCTTC) in the *DGKE* gene, in two families of infantile aHUS with low serum C3 levels.

Keywords: *Atypical haemolytic uremic syndrome, DGKE, next-generation sequencing, novel mutations*

Introduction

Haemolytic uraemic syndrome (HUS) falls under thrombotic microangiopathies (TMA) characterised by a triad of thrombocytopenia, microangiopathic anaemia and acute kidney injury. Atypical HUS (aHUS) is a subset of HUS without coexisting/preceding infections or coexisting diseases. Compared to typical HUS, aHUS are associated with a poorer clinical outcome, have higher chances of end-stage renal disease (ESRD) and exhibit increased episodes of relapses. Most aHUS cases have a dysregulated complement pathway thought to result from a complex interaction of genetic and acquired factors.[1] Other non-complement-associated aHUS pathologies have also been identified; hence, newer terminologies like complement-HUS, diacylglycerol kinase ε (*DGKE*) mutation–HUS and cobalamin C (cblC) defect-HUS might be more appropriate.[2] Biallelic loss-of-function mutations (or pathogenic variants) in the *DGKE* gene was established as a cause of infantile-onset aHUS (in 27% cases) and young-onset nephrotic syndrome in 2013.[3] Here, we report two novel genomic variants in the *DGKE* gene in two unrelated Indian families. Both cases also exhibited low serum C3 levels; additionally, one patient exhibited high anti-factor H antibody levels.

Case Details

Case 1 was an 11-month-old boy, born in a third-degree consanguineous family presenting with anasarca and hypertension. The investigation results were suggestive of microangiopathic haemolytic anaemia, deranged renal function, high anti-factor H antibody titres (1210 AU/mL) and a C3 level of 40 mg/dL (Normal: 90–180 mg/dL). Daily plasma infusions (PIs) and immunosuppression with prednisolone and azathioprine were initiated. The patient underwent two sessions of acute peritoneal dialysis (PD), each lasting 72 h. After 2 weeks of treatment, his haematological parameters improved; however, his eGFR remained between 25 and 30 mL/min/1.73 m^2 (Normal eGFR = 48–117.2 mL/min/1.73 m^2).[4] The frequency of PIs was decreased to alternate days. Two weeks later, he became oliguric again and his renal function deteriorated. PD and daily PIs were re-initiated; however, the patient's parents decided to withdraw the patient from further management.

How to cite this article: Sharma J, Lobo V, Singhal J, Anand S, Kadam S, Ranade S, *et al*. Novel mutations in the *DGKE* gene in two Indian patients with early-onset atypical haemolytic uraemic syndrome. Indian J Nephrol 2021;31:182-6.

Received: 01-10-2019
Revised: 17-01-2020
Accepted: 16-02-2020
Published: 02-04-2021

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Access this article online

Website: www.indianjnephrol.org

DOI: 10.4103/ijn.IJN_336_19

Quick Response Code:

Case 2 was a 5-month old male, born in a nonconsanguineous family and was referred with a diagnosis of HUS and eGFR of 16 mL/min/1.73 m^2 (normal eGFR = 43.9–99.5 mL/min/1.73 m^2).[4] Anti-CFH antibody levels were normal (8.85 AU/mL) and had a C3 level of 46 mg/dL. Acute PD was initiated, and daily PIs were administered during the 1st week, followed by PIs on alternate days. Haematological and renal parameters improved (no features of haemolysis, eGFR = 56 mL/min/1.73 m^2) following 3 weeks of PIs. Four weeks later, his urine output decreased, and renal parameters worsened (eGFR = 34 mL/min/1.73 m^2). The parents withdrew the patient from further management.

Anti-complement factor H antibody testing

To check the level of the anti-CFH antibodies, ELISA was performed with the Viditest anti-complement factor H kit (Vidia; ODZ-166) and standardised against the reference method at National Renal Complement Therapeutics Centre, Newcastle University. The normal range for our population was determined from healthy blood donors, school children and patients admitted with diseases other than HUS.

Molecular genetic testing

DNA was extracted from the peripheral blood sample using the DNeasy Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's protocol. For case 1, next-generation sequencing (NGS) was performed using the Trusight One Panel (Illumina; Cat. # 15046895) followed by analysis using an in-house developed bioinformatics pipeline. The *DGKE* gene is not included in this NGS panel. Therefore, a targeted amplicon-based NGS assay was developed to analyse the *DGKE* gene. Briefly, the assay involved an initial PCR in which the *DGKE* gene was amplified in three segments—exon 1, exons 2–6 and exons 7–11 using primers previously published in the literature.[5] Exon one, which is 916 bp in length, was amplified using a ramp-up PCR and Quantitect qPCR mastermix (Qiagen; Cat # 204543). Long-range PCRs were used to amplify exons 2–6 and exons 7–11 in separate tubes using Q5 Hotstart HiFi DNA polymerase (New England Biolabs (NEB); cat # M0493L), which are 5.4 kb and 6.7 kb in length, respectively. Furthermore, the PCR products were purified with ExoSap [Exonuclease I (NEB; M0293) and shrimp alkaline phosphatase (NEB; M0371)] to digest unused, single-stranded oligonucleotide primers and unincorporated nucleotides and quantified using a Qubit fluorometric assay (Life Technologies, CA, USA). These purified PCR products were pooled/normalised and then subjected to transposon-mediated adapter ligation (tagmentation) using the NexteraXT library preparation kit (Illumina; cat. # 15032355), followed by barcoding and adaptor ligation of the pooled amplicons with custom-synthesised oligonucleotides. The adaptor-ligated products were analysed for their quality on an Agilent Bioanalyzer and quantified accurately with an in-house qPCR and a Qubit fluorometric assay. The library was sequenced on an Illumina MiSeq sequencer using paired-end sequencing-by-synthesis chemistry. Sequencing for case 2 was performed using a 6640-gene (including the coding region of *DGKE* gene) clinical exome-capture assay at an external laboratory.

Bioinformatic analysis was performed using an in-house developed pipeline on both datasets including alignment of the FASTQ reads to a GRCh37/hg19 human reference genome assembly. For copy number variations (CNVs) determination**,** we developed an in-house pipeline from NGS data by calculating a normalised copy number ratio of aligned number of reads at targeted genomic regions against multiple reference regions of the same sample and multiple control samples. A ratio <0.65 was suggestive of a heterozygous deletion whereas a ratio >1.3 was suggestive of duplication. An absence of aligned reads was highly suggestive of a homozygous deletion.

In case 1, we found a novel 4-bp deletion [c. 727_730delTTGT] in *DGKE* exon 4, predicted to shift the protein reading frame and was reported as 'likely pathogenic' [Figure 1a]. Findings were confirmed by targeted capillary sequencing [Figure 1b] in the patient (in a homozygous state). In case 2, a 9-bp in-frame homozygous deletion [c. 251_259delGCGCCTTCT] was detected in *DGKE* exon 2 [Figure 1c]. This variant has not been previously reported in the literature. Bioinformatically, this variant is predicted to cause a 3-amino acid (A-F-C) deletion [Figure 1c]. *In silico* pathogenicity prediction algorithms including MutationTaster and LoFTool predicted this variant as disease-causing and possibly damaging, respectively. Based on the available evidence and clinical correlation, this variant was classified as a variant of unknown significance, possibly pathogenic.

No clinically relevant sequence variants were detected in the *C3*, *CFB*, *CFH*, *CFI*, *CD46* (*MCP*) and *THBD* genes. In both these cases, complete coding regions of these genes along with exon-intron boundaries were covered with >20X coverage. Additionally, clinically relevant CNVs were not detected in the *CFHR1*/*CFHR3* genes.

Discussion

We report two novel clinically relevant variants, in the coding region of the *DGKE* gene in two infantile aHUS cases with low serum C3 levels. This report expands the spectrum of *DGKE* gene variants and associated clinical phenotype. We also describe the development and validation of a custom amplicon-based targeted NGS assay for the *DGKE* gene.

The presence of biallelic pathogenic/likely-pathogenic variants in the *DGKE* was first reported by Lemaire *et al*.

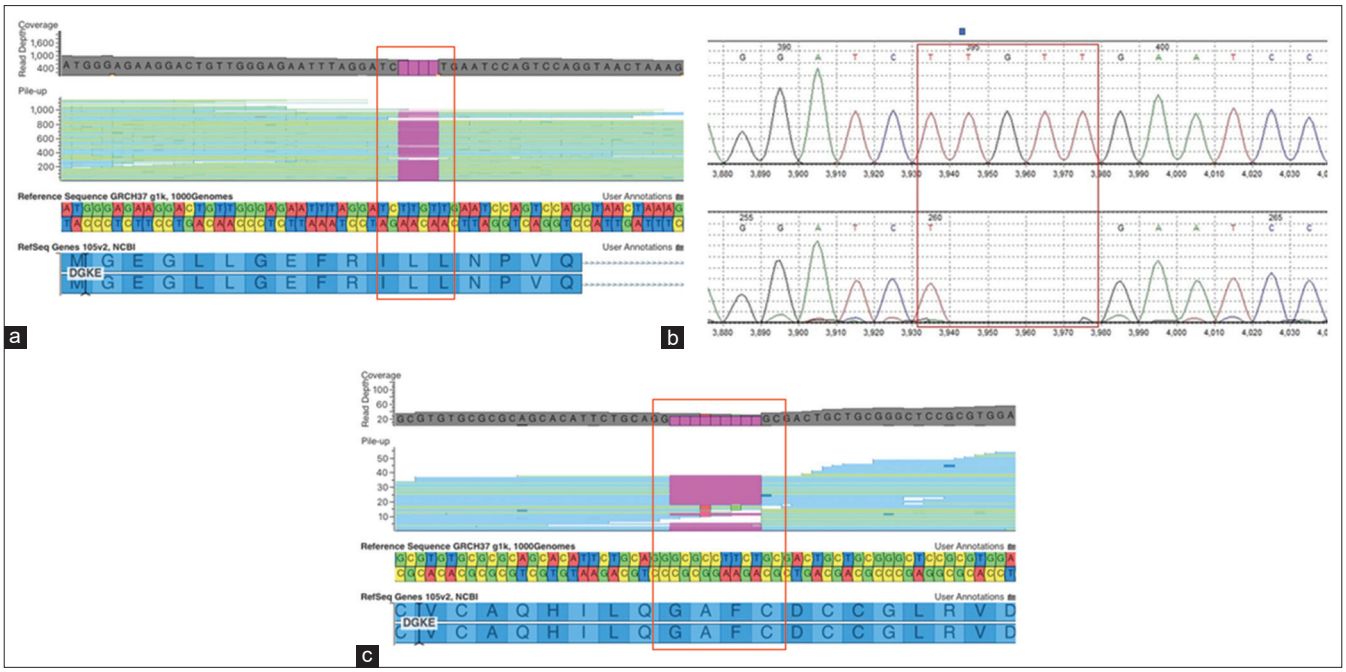

Figure 1: (a) Visualisation of the next generation sequencing (NGS) data for case 1 aligned to the reference genome shows a homozygous deletion of four bases (in pink) in *DGKE* exon 4 resulting in a frameshift that is predicted to be pathogenic. (b) Snapshot of Mutation Surveyer software (SoftGenetics. Pennsylvania, USA) highlighting the homozygous deletion in case 1. (c) NGS data for case 2 aligned to the reference genome shows homozygous deletion of nine bases (pink) in *DGKE* exon 2 resulting in a deletion of three amino acids (A-F-C), which was predicted to be variant of unknown significance possibly pathogenic

in two unrelated families, each with two affected children with infantile-onset aHUS and unaffected parents.[3] None of them had evidence of complement system activation and did not harbour mutations in any of the complement genes, suggesting that mutations in the *DGKE* gene cause a thrombotic phenotype independent of complement activation. The same group sequenced the *DGKE* gene in 47 unrelated paediatric aHUS without the presence of anti-factor H antibodies and found mutations in the *DGKE* gene in nine children (19.1%).[5] These children had a relapsing course of aHUS before 5 years of age and progressed to chronic kidney disease (CKD) by the second decade of life. Two of them were on anti-complement therapy (eculizumab and fresh frozen plasma infusions respectively). Ozaltin *et al*. identified mutations in the *DGKE* gene in three unrelated Turkish families as the cause of glomerular microangiopathy with histologic signs of membranoproliferative glomerulonephritis (MPGN) and endothelial dysfunction.[6] No complement data were reported in this study. The clinical phenotype of *DGKE*-associated diseases was expanded to hypocomplementemic aHUS by Westland *et al*.[7] They described two siblings with infantile HUS, one with low serum C3 at presentation and the other at 5 months after onset. Both siblings were managed with PIs at gradually increasing intervals. Capillary sequencing confirmed the presence of homozygous p.K101X mutation in the *DGKE* gene in both cases. These children did not exhibit the presence of anti-factor H antibodies or mutations in the complement genes. In the literature, only nine cases of infancy/early childhood aHUS with *DGKE* gene mutations have been reported who did not have anti-factor H antibodies but had evidence of complement activation on a presentation or on follow-up [Table 1]. These children were managed with various combinations of PIs, plasma exchange and eculizumab along with other supportive therapy. In the reported cases, the patients were treated with plasma infusions rather than exchanges. This was done keeping in mind view of the very small size of the infants, which increased the risk of adverse events and the technical difficulty in performing the procedure. Chinchilla *et al*.[8] reported two children with *DGKE* gene mutations and low C3 levels; one child harboured an additional mutation in the *C3* gene and a risk haplotype in the *MCP* gene in a homozygous state and the other child harboured a heterozygous *THBD* gene mutation and a high-risk haplotype in the *CFH* gene, thereby explaining the low C3 levels in both of them. None of the patients in our study harboured any clinically relevant variants in complement pathway genes. It remains unclear whether aHUS-associated DGKE deficiency directly initiates complement activation or whether these patients harbour other genetic abnormalities in modifier genes that affect the complement cascade.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Table 1: Patients with *DGKE* mutation and evidence of complement activation

Study	Westland *et al.*[7]		Azukaitis *et al.*[9]		Chinchilla *et al.*[8]		Mele *et al.*[10]		Miyata and Ohta[11]
Patient ID	**Ind 58**	**Ind 59**	**ID: 5.1**	**ID: 6.1**	**HUS 40**	**HUS 272**	**Pt 452**	**Pt 1200**	
Gender	Girl	Boy	Boy	Girl	Girl	Girl	Girl	Boy	Boy
Age at presentations	10 months	10 months	7 months	2 years	7 months	8 months	10 months	5 months	4 months
Age at last follow up	10 months	3.4 years	1.5 years	2 years and 10 months	17 years	4 years	13 years	10 years	18 months
C3 (mg/dL) at presentation	66 (N: 84-192)	86 (N: 90-200)	56 (N: 90-200)	Normal	NA	NA	70 (N: 90-180)	81 (N: 90-180)	<20 mg/dL
C3 (mg/dL) (at last follow-up)	86 (N: 84-192)	78	Normal	Low	87.8 (N: 90-200)	86.9 (N: 90-200)	147	151	104
Mutation in *DGKE*	Homozygous p.K101X	Homozygous p.K101X	p.IVS11+2/p.IVS11+2	p.I187Ffs*6/p.W322*	p.Trp322* p.Pro498Arg	p.Gln248His p.Gly484Glyfs*10	c. 888 + 40A>G	c. 888 + 40A>G	c. 1213-2A > G p.Leu24Cysfs*145
Complement mutation	None	None	None	None	THBD; c. 1456G.T; p.D486Y	C3; c. 784G.T; p.G262W	None	None	None
Relapses till last follow-up	None	None	1	None	3	≥3	11	8	None
Treatment	Daily PI[a] for 2 weeks followed by monthly for 1 year and then 3 monthly. It was followed by ACEI[b] and ARB[c] and 3 monthly PI	PI	PD[d]× 2 weeks and PE[e]- 3 sessions→ eculizumab followed by eculizumab infusions every 3 weeks	PI→PE→ eculizumab for 2 months→HD[f] for 1 month Followed by ACEI	PI followed by ACEI inhibitors	PI for 4 months followed by eculizumab	Supportive therapy Initially→PI twice per year	Supportive therapy Initially→PI twice per year	PI→PE Plasmapheresis resistant eculizumab

[a]PI: plasma infusions; [b]ACEI: angiotensin-converting-enzyme inhibitors; [c]ARB: angiotensin II receptor blockers; [d]PD: peritoneal dialysis; [e]PE: plasma exchange; [f]HD: haemodialysis

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