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Case Report

Novel LAGE3 Pathogenic Variants Combined with TRPC6 and NUP160 Variants in Galloway-Mowat Syndrome: A Case Report

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Keywords
Galloway-Mowat syndrome · Nephrotic syndrome · *LAGE3*

Abstract
Galloway-Mowat syndrome (GAMOS) is a rare autosomal recessive disorder characterized by early-onset nephrotic syndrome and microcephaly with brain anomalies in children. Researchers studying GAMOS reported the first pathogenic variant identified was the *WDR73* gene, and more recently, four new pathogenic genes, *OSGEP, LAGE3, TP53RK*, and *TPRKB*, have been identified. In the present study, we report a new mutation of c.290T>G (p.L97R) *LAGE3* in a 4-year-old boy with specific urological and nephrological complications. The patient presented with early-onset proteinuria, brain atrophy, delayed language and motor development, and axial hypotonia. This patient also had mutations in two other genes: *TRPC6* and *NUP160*, make the clinical presentation of this patient more diverse. Our novel findings add to the spectrum of pathogenic variants in the *LAGE3* gene. In addition, early genetic diagnosis of GAMOS is essential for genetic counseling and prenatal care.

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Introduction

Galloway-Mowat syndrome (GAMOS) is a rare autosomal recessive disorder characterized by early-onset hormone-resistant nephrotic syndrome, microcephaly, and brain abnormalities [1]. Renal alterations in GAMOS manifest in a variety of forms, ranging from isolated proteinuria to hormone-resistant nephropathy, end-stage renal disease, and multiple organ failure, leading to death [2]. Kidney biopsies also show various types of histological changes, including diffuse mesangial sclerosis, focal segmental glomerulosclerosis (FSGS), mesangial hyperplasia, increased mesangial matrix, and minimal change [3–5].

Genetic mutations are the main cause of GAMOS. A truncating variant in the *WDR73* gene is described as the first monogenic factor in GAMOS [6]. Data from Jinks et al. [6] demonstrate that in humans, *WDR73* interacts through mitotic microtubules to regulate cell cycle progression, proliferation, and survival in the brain and kidney. Recently, mutations in *OSGEP, TP53RK, TPRKB*, and *LAGE* have also been identified as a cause of GAMOS [1]. These genes encode 4 subunits of the highly conserved KEOPS complex. The KEOPS complex catalyzes a universal post-transcriptional modification of transfer RNA and plays an important role in gene transcription and genome maintenance, and also plays an important role in brain and renal development [7]. Clinically, patients with KEOPS complex mutations have primary microcephaly, developmental delay, predisposition to seizures, and early-onset nephrotic syndrome [1]. Inheritance is autosomal recessive or X-linked (*LAGE3*).

Here, we report a patient with GAMOS carrying the newly identified *LAGE3* pathogenic variants with microcephaly, severe developmental delay, and renal phenotype (large proteinuria), while normal glomerular filtration rate and serum albumin levels were maintained. Of note, this patient has never been diagnosed with nephrotic syndrome (average albumin 30 g/L).

Case Presentation

The child was hospitalized for pneumonia at the age of 1, with abnormal urinalysis. The urinalysis showed protein 3+, occult blood+, and serum albumin 27.2 g/L. The patient showed growth retardation and delayed development in the past. Until the age of 3, he went to the doctor due to growth and development problems. The Gesell Developmental Observation-Revised (GDO-R) assessment showed gross motor development was equivalent to 11.5 months old, fine motor development was equivalent to 21 months old, adaptability development was equivalent to 15 months, language development was equivalent to 12 months, social behavior development was equivalent to 19.5 months, and the overall evaluation was low intelligence. Physical examination of the patient on admission: all fingers are short and stubby. The forehead is narrow and slanted. The patient's cardiopulmonary examination is not special. He has weak muscle tone. Main laboratory results are shown on Table 1. The patient's creatinine was consistently normal. A dual-energy X-ray showed low bone mass (*Z*-score: −3.0) and an otoacoustic emission examination indicated that the patient had normal hearing at all evaluation rates (750–8,000 Hz). Brain MRI showed extensive symmetrical abnormal signaling in the white matter with brain atrophy (significant atrophy of the cerebellar hemispheres) (Fig. 1d). The electromyography (EMG) test showed myogenic damage. There were no obvious abnormalities seen in cardiac color on Doppler ultrasound or electrocardiogram. The patient had no sensorineural ataxia or tremor, and inherited metabolic disorders were excluded by laboratory tests.

The proband undergoes kidney biopsy under general anesthesia. The pathological results suggested that 3/20 glomeruli were focal segmental sclerosis. H&E staining of renal puncture tissue suggested FSGS (Fig. 1b). Under electron microscope, the thickness

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Table 1. Main laboratory examination indexes of the proband

	Index	Analytical finding	Reference range
Urinalysis	pH value	6.5	5.5–6.5
	Urine protein	3+	Negative
	Urinary occult blood	–	Negative
	The ratio of urine protein/creatinine 24-h urine protein	22.8	<0.03
	24-hour urine protein	4,756.1 mg	<150 mg/24 h
	Trace total protein in urine	18,372.1 mg/L	<100 mg/L
	α-microglobulin	15.46 mg/L	0–12 mg/L
	β2-microglobulin	1.27 mg/L	0–0.3 mg/L
	Urinary transferrin	>480 mg/L	0–2 mg/L
	Urinary retinal binding protein	2.0 mg/L	0–0.7 mg/L
	Urinary immune globin	476.13 mg/L	0–8 mg/L
Biochemical results	Albumin	29.3 g/L	35–50 g/L
	Urea nitrogen	3.88 mmol/L	2.8–7.6 mmol/L
	Creatinine	17 μmol/L	21–65 μmol
	Triglycerides	1.1 mmol/L	<1.7 mmol/L
	Cholesterol	5.16 mmol/L	3–5.7 mmol/L
	Uric acid	225 μmol/L	155–357 μmol/L

of the glomerular basement membrane was ~120–280 nm, and the foot processes were diffusely fused, with microvilli degeneration, and a small amount of electron dense deposits were seen in individual mesangial areas (Fig. 1c). The patient was diagnosed with FSGS.

In order to clarify the cause, after medical ethics review and the parents of the child signed an informed consent form, 2 mL of the peripheral blood samples of the child and the parents were collected for whole-exome genome sequencing. Whole-exome sequencing revealed a hemizygous mutation of c.290T>G (p.L97R) in the *LAGE3* gene. This mutation was not detected in the patient's father, but the patient's mother was a heterozygous carrier. At present, the professional version of HGMD data only includes 4 variants of the *LAGE3* gene, including one classic splice site (c.188+1G>A, c.317+4A>G), and two missense variant sites (c.316G>T, c.410T>C). The mutation site in our patient was close to site 316, and combined with the clinical manifestations of the patient, we speculated that the mutation was also pathogenic. The family members of the proband were verified by first-generation sequencing, and it was found that the mutation site was inherited from the mother, and the father was wild type. At the same time, mutations in the *TRPC6* gene (c.2206-6G>A), and the *NUP160* gene (c.562A>G) were detected. The patient's mother was also a heterozygote for the *TRPC6* variant (Fig. 1a).

Before we get the genetic sequencing results, methylprednisolone sodium succinate 10 mg b.i.d. was given for 6 days, and enalapril maleate 2.5 mg q.n. was given as a symptomatic treatment, but the proteinuria of the child did not improve, the drug was discontinued. Regular follow-up visits are currently in the outpatient clinic. Test and evaluate his

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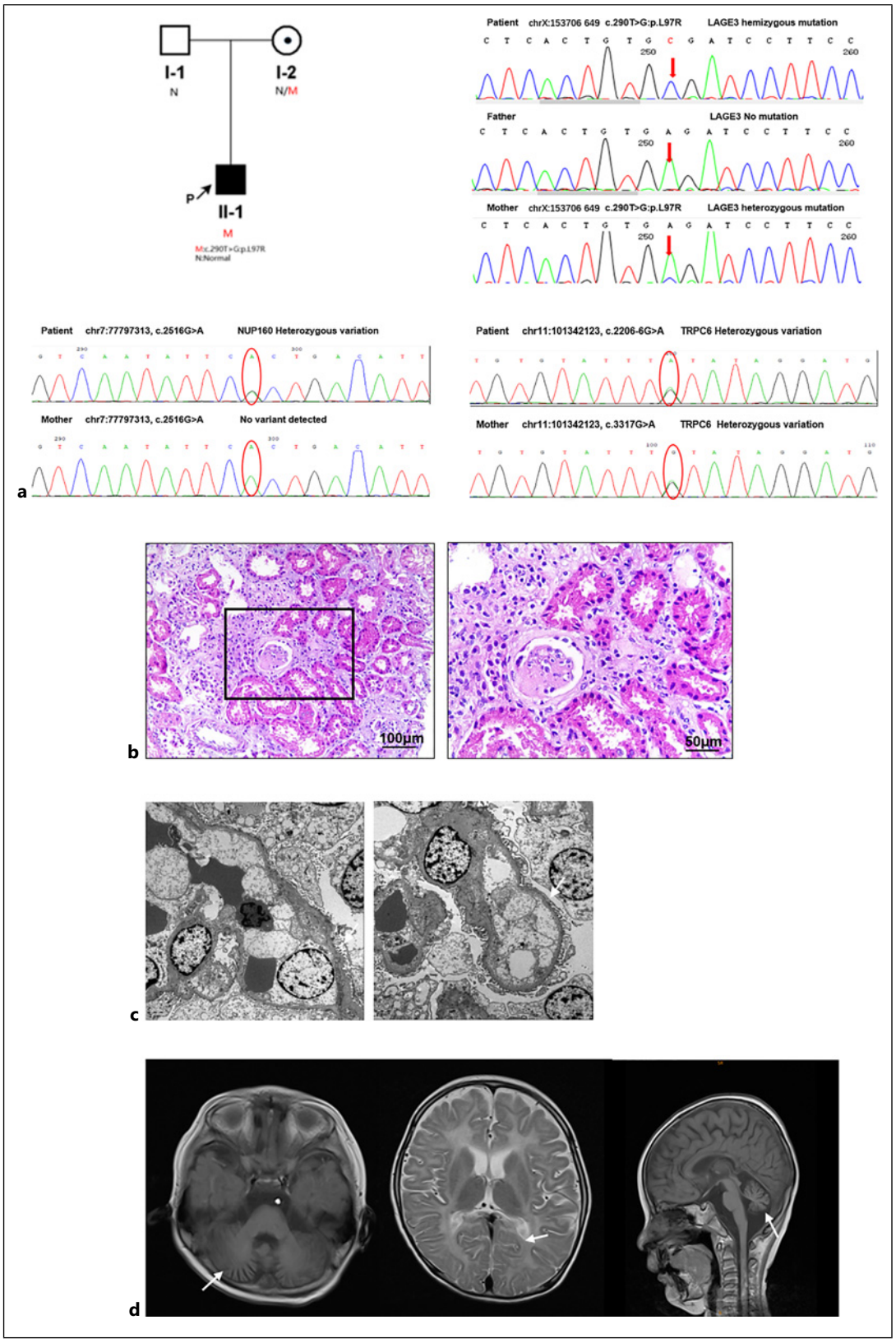

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urine output, blood pressure, and monitor urine routine, urine protein quantitative, kidney function, and other related indicators. The child was then admitted to the rehabilitation department and started formal rehabilitation treatment.

Discussion and Conclusions

Galloway-Mowat syndrome (GAMOS) was first reported in 1968, describing a pair of siblings suffering from the primary nephrotic syndrome-hiatal hernia-microcephaly triad [8]. Recent studies have revealed the important role of gene mutation in the pathogenesis of GAMOS. In 2014, Colin et al. first reported that the loss of *WDR73* (WD repeat domain 73) expression can lead to abnormal nuclei of glomerular podocytes, changes in microtubule networks, and cell viability [9]. Braun et al. [1] found that the subunits coded by the four genes *LAGE3/OSGEP/TP53RK/TPRKB* constitute a highly conserved kinase-endopeptidase and other proteins of small size complex (KEOPS), which is one of the key factors in the pathogenesis of GAMOS. Phenotypically, the patients with KEOPS subunit mutations primarily had microcephaly, developmental delay, predisposition to seizures, and early-onset nephrotic syndrome. Most affected patients die in early childhood [1]. There have been 3 cases of *LAGE3* gene mutation reported so far: c188+1G>A, c.316G>T (p.val106Phe), c410T>C (p.Phe137Ser), and all have the phenotypes mentioned above [9]. CRISPR/Cas9 *LAGE3* knockout mouse embryos had significantly reduced cortical length, cortical-midbrain length, and cortical width, reproducing the human microcephaly phenotype [9].

It is well known that nephrotic syndrome is an important factor in the diagnosis of GAMOS. However, the patient in this study had persistent macroalbuminuria without serum albumin below 25 g/L, which did not meet the diagnostic criteria for nephrotic syndrome. This suggests that proteinuria may not always amount to the nephrotic range, which illustrates various manifestations of *LAGE3* gene mutations. The OSGEP mutation reported by Tao et al. is also an isolated case of proteinuria that did not meet the diagnostic criteria for nephrotic syndrome [10]. The mutation found in our patient was in the *LAGE3* gene, and may be the first patient not diagnosed with nephrotic syndrome due to a LAGE3 mutation.

Unlike other case reports, our patient also has a mutation of the *TRPC6* gene (c.2206-6G>A.TRPC6). Pathways involving this mutated gene have been implicated in the pathogenesis of kidney diseases, especially in familial nephrosis [11]. *TRPC6* contributes to certain glomerular lesions as well as tubulointerstitial fibrosis [11–14]. More recent evidence has suggested that dysregulation of wild-type *TRPC6* channels cause acquired glomerular diseases, and in vitro models in which podocytes are exposed to serum or plasma samples from patients with recurrent FSGS, this dysregulation is a factor implicated in the pathogenesis of primary FSGS [12, 15, 16]. To date, we have observed a glomeruloprotective effect with *TRPC6* inactivation in 3 different chronic disease models (chronic PAN nephrosis, anti-GBM autoimmune glomerulonephritis, and aging). It is possible that having this *TRPC6* variant contributed to this patient not developing nephrotic syndrome. Agents that block or suppress *TRPC6* may also be effective in other genetic forms of FSGS [17]. Based on the actions of putative serum "permeability factors" on *TRPC6* channels in podocytes, including samples

Fig. 1. a Pedigree of a patient with a likely pathogenic homozygous LAGE3 variant, c.290T>G (p.L97R), while his mother is a healthy heterozygous carrier, and his father has no mutation. Our patient also has a mutation of the *TRPC6* gene (c.2206-6G>A), and the *NUP160* gene (c.562A>G). **b** H&E staining of renal puncture tissue suggests focal segmental glomerulosclerosis. **c** Transmission electron microscopy (TEM) showing podocyte foot process effacement. **d** MRI showing polymicrogyria and diffuse cerebellar atrophy.

taken from patients, it is possible that *TRPC6* inhibitors could be efficacious in people with primary FSGS [12, 16], and knockout studies in rats support the concept of *TRPC6* inhibition in adaptive forms of FSGS, commonly seen in patients with severe uncontrolled hypertension, reduced renal mass, or a markedly reduced number of functional nephrons [13]. So we hypothesized that mutations in *TPRC6* had a saving effect on FSGS in this patient.

The patient also has the *NUP160* gene c.562A>G mutation. Vasu et al. [18] confirmed nucleoporins *Nup160, Nup133, Nup107,* and *Nup96* exist as a complex in Xenopus egg extracts and in assembled pores, now termed the *Nup160* complex. The nuclear pore complexes are macromolecular assemblies that play roles in nucleocytoplasmic transport in both directions, and in the regulation of transcription and chromatin organization [19]. *NUP107* and *NUP133* are interacting subunits of the nuclear pore complex in the nuclear envelope during interphase, and these proteins are also involved in centrosome positioning and spindle assembly during mitosis. Recently, biallelic mutations in *NUP107* were identified in steroid-resistant nephrotic syndrome, 8,9 XX gonadal dysgenesis, 10 and GAMOS [20–22]. Of note, 4 families with a homozygous *NUP107* mutation (c.303G>A, p.Met101Ile) leading to exon 4 skipping were found to have GAMOS-like features such as microcephaly, intellectual disability, and steroid-resistant nephrotic syndrome [22, 23]. Fujita et al. [24] identify a homozygous *NUP133* mutation in a previously described consanguineous GAMO affected family [25]. In vitro and in vivo functional analyses of the mutation will be displayed, supporting the hypothesis that the *NUP133* mutation causes GAMOS [24]. Based on the above evidence, it is reasonable to speculate boldly that *NUP160* mutations may cause GAMOS.

In conclusion, we reviewed the case of a 4-year-old child with GAMOS who presented with symptoms such as proteinuria, delayed language development, and delayed motor development, corresponding to clinical phenotypes such as massive proteinuria, cerebellar hypoplasia, global developmental delay, and facial deformity. Distinctive from previous reports, the patient had 2 mutations in *TRPC6* (c.2206-6G>A) and *NUP160* (c.562A>G), being the first time this mutation site has been discovered. This case study will provide important guidance for the future clinical diagnosis and identification of the disease. Nephrological and urological complications of LAGE gene mutations can be challenging and will require a multidisciplinary approach. The CARE Checklist has been completed by the authors for this case report, attached as online supplementary material (for all online suppl. material, see https://doi.org/10.1159/000533580).

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Statement of Ethics

This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine (No. 2020047), Hangzhou, China. Written informed consent was obtained from the parent/legal guardian of the patient for publication of the details of their medical case and any accompanying images. We have received written informed consent for the publication of these details and any accompanying images.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

L.H., X.Z., Y.Z., Y.W., and J.M. participated in the acquisition of clinical data. Y.Z. and J.M. performed the mitochondrial DNA sequencing. L.H. wrote the manuscript and J.M. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author.

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