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A puffy child – a rare case of steroid resistant nephrotic syndrome with ANLN mutation

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CASE REPORT

Recent advances in genomics have uncovered the molecular mechanisms involved in the broad spectrum of variation associated with steroid-resistant nephrotic syndrome. Over 50 monogenic causes of steroid-resistant nephrotic syndrome have been discovered; however, these genes are implicated in only a small proportion of cases. Using a combination of whole-exome sequencing and genome-wide linkage studies, a missense mutation in anillin (ANLN) has been identified as a cause of focal segmental glomerulosclerosis, a pattern of glomerular injury associated with steroid-resistant nephrotic syndrome. We report a case of 2-year-6-month-old male child, who presented with severe edema and oliguria for 6 weeks. He was found to be an early steroid non-responder,

hence renal biopsy and genetic testing were ordered. These findings were in favour of focal segmental glomerulosclerosis, a common cause of childhood steroid-resistant nephrotic syndrome. It is important to identify the causative agent to avoid unnecessary immunosuppressive therapy and its associated risks.

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INTRODUCTION

Nephrotic syndrome (NS) encompasses a heterogeneous group of disorders characterised by massive proteinuria, hypoalbuminemia and edema. The most common glomerular disease of childhood is NS, with an incidence of approximately 1–2 per 100,000 [1]. Nearly 85% of pediatric NS cases respond to steroids, with the remaining 15% being steroid-resistant [2]. Steroid-resistant nephrotic syndrome (SRNS) may be characterised further based on renal histopathology, with almost 20% showing focal segmental glomerulosclerosis (FSGS) [3]. Steroid resistance and persistent proteinuria are key determinants of impending risk for end-stage renal disease (ESRD) [4].

According to recent evidence, genetic etiology is found in nearly 30% of SRNS cases [1]. Anillin Actin Binding Protein (ANLN) is one of the monogenic mutations responsible for SRNS. Anillin plays a pivotal role in cellularisation and cytokinesis. Mutation in ANLN causes upregulation of PI3K/AKT/mTOR/p70S6K/Rac1 pathway, elucidating its importance in pathogenesis of podocyte dysfunction in FSGS. ANLN has also been recognized as a driver of cellular proliferation in various forms of human tumours [5]. Therefore, it is crucial to expand our understanding of the pathobiology involved in the disease to design personalised treatment strategies.

CLINICAL CASE DESCRIPTION

A 2-year-6-month-old male child, born to non-consanguineous parents, presented with persistent facial swelling, abdominal distention and reduced urine output for 6 weeks. He had recent onset breathing difficulty. He was a known case of NS being treated with prednisolone 2 mg/kg/day. His antenatal and postnatal history were uneventful. His nutrition and immunization status were appropriate for his age. He had nil significant family history.

On examination, the child was active and alert. Anthropometric examination recorded findings appropriate for his age. Examination of vitals revealed tachycardia (heart rate: 108/min), tachypnoea (respiratory rate: 27/min) and oxygen saturation of 93% in room air. He had elevated blood pressure of 130/90 mmHg (>95th percentile for his age and height) and pitting pedal edema.

Routine blood investigations, including renal and liver function tests were within normal limits. His baseline coagulation study and thyroid function test were normal. Urine analysis revealed nephrotic range proteinuria (4+ on dipstick and elevated protein creatinine ratio of 15.78) and microscopic hematuria. He also had hypoalbuminemia (1.4 g/dL) and hypercholesterolemia (1427 mg/dL). Ultrasound showed moderate pleural effusion, ascites and enlarged hyperechogenic kidneys. Suspecting SRNS, renal biopsy was performed, which revealed features of FSGS (Figure 1). In view of severe anasarca and respiratory distress, he was initiated on albumin infusion with 20% albumin, 1 g/kg followed by diuretics, furosemide 1mg/kg/dose. After 2 sessions of combined albumin and diuretic therapy, edema settled and albumin level increased to 2 g/dL. Genetic study using targeted exome sequencing, revealed heterozygous missense mutation in exon 14 of ANLN gene that results in amino acid substitution of

methionine for threonine at codon 821 (p.Thr 821Met;ENST00000265748.7). Currently, he is being treated with tacrolimus 0.1 mg/kg/day and enalapril 0.3 mg/kg/day, along with normal protein and no added salt diet. His parents have been counselled about the disease course, trial of immunosuppressive therapy and the need for renal transplantation in future.

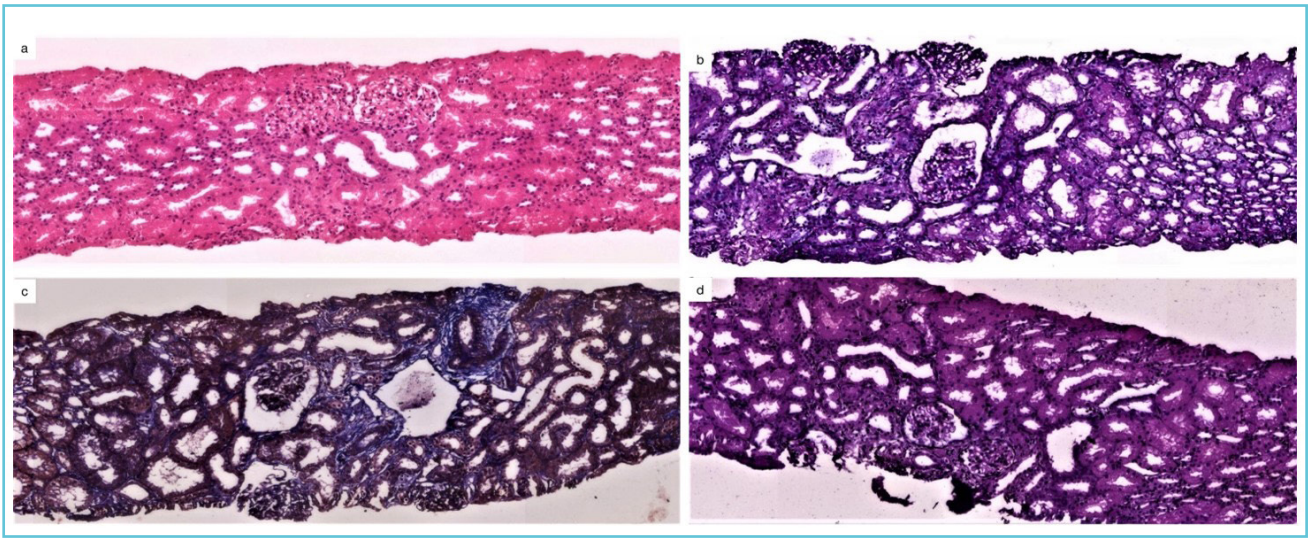
DISCUSSION

Nephrotic syndrome (NS) is a non-inflammatory disorder of glomerulus, characterised by increased glomerular leakage of proteins, predominantly albumin. It is denoted by a group of features including edema, proteinuria and hypoalbuminemia. Corticosteroids are the mainstay of therapy for NS; however, approximately 15% of patients fail to attain remission even after 4 to 6 weeks of daily prednisolone and are classified as steroid-resistant nephrotic syndrome [2].

Glomerular filtration barrier (GFB) is a multifaceted apparatus composed of specialized fenestrated endothelium, podocyte, slit diaphragm, and glomerular basement membrane (GBM). Podocytes are highly specialized epithelial cells, which play a vital role in structural and functional integrity of GFB. These podocytes, along with their interdigitating foot processes are connected together with the aid of slit diaphragm. This ultrastructure of GFB regulates the ultrafiltration of molecules, thereby preventing excretion of albumin and other large plasma proteins. Dysfunction of any component of the GFB, can result in severe proteinuria leading to NS [1,6].

Mutations in genes encoding podocyte-associated structural proteins have been demonstrated in approximately 30% of childhood SRNS. ANLN, a multi-domain protein, found to

Figure 1 **Histopathological findings of renal biopsy showing features of focal segmental glomerulosclerosis**



a) Two Glomeruli showing mesangial hypercellularity and segmental capillary lumen obliteration and sclerosis (H&E; x 400).

b) Periodic Acid Schiff stain highlighting the sclerosed glomeruli and focal tubular atrophy (PAS x 400).

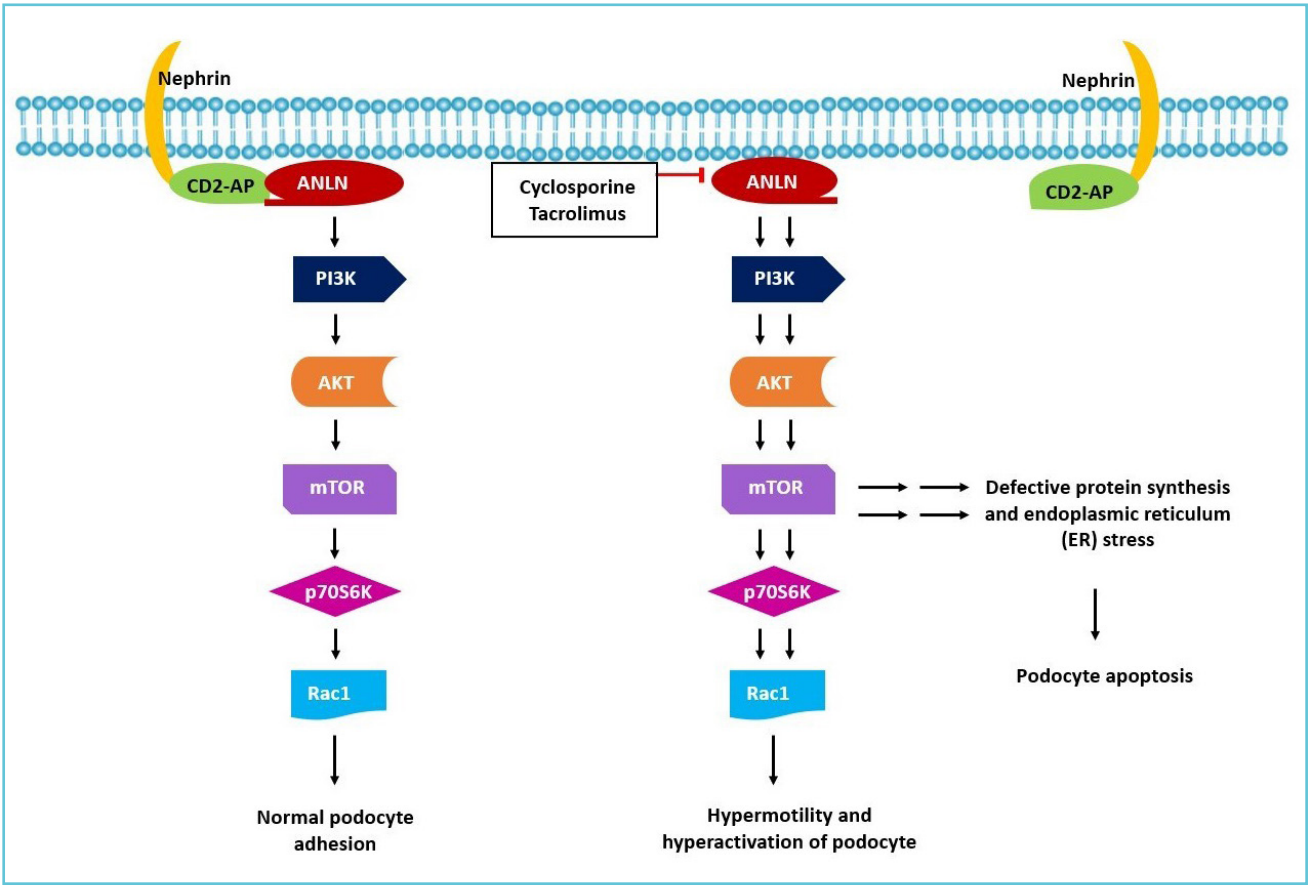
c) Masson Trichrome stain showing mesangial hypercellularity, tubular cyst formation and medial wall hypertrophy of the blood vessels confirming the vascular changes secondary to hypertension (MT x 400).

d) Jones Methenamine Silver also highlights the segmental glomerular sclerosis (JMS x 400).

have autosomal dominant inheritance is typically associated with adult-onset FSGS [1]. The presenting child has early-onset SRNS associated with monogenic mutation in ANLN gene. In silico analysis, predictions of ANLN variant were found to be damaging by SIFT (Sorting Intolerant From Tolerant), Polyphen-2 (Polymorphism Phenotyping V-2) and LRT (Likelihood Ratio Test).

Anillin, coded by ANLN gene is one of the podocyte-associated protein responsible for maintaining the integrity of podocyte actin cytoskeleton. Studies performed in vitro using immortalized human podocytes and in animal models suggest that loss-of-function mutation in ANLN disrupts podocyte cytoskeletal dynamics and promotes podocyte apoptosis through hyperactivation of PI3K/AKT/mTOR/p70S6K/Rac1 pathway. In contrast with intact anillin, the mutant displays a significantly reduced binding affinity to the slit diaphragm-related protein, CD2-associated protein (Figure 2). These findings form the basis for pathogenesis of FSGS, a clinicopathological entity characterized by NS,

Figure 2 **Flowchart depicting the activation of PI3K/AKT/mTOR/p70S6K/Rac1 pathway by ANLN**



Flowchart depicting:

(i) Normal activation of PI3K/AKT/mTOR/p70S6K/Rac1 pathway by intact ANLN (Left)

(ii) Hyperactivation of PI3K/AKT/mTOR/p70S6K/Rac1 pathway by mutant ANLN resulting in defective protein synthesis and podocyte apoptosis, causing FSGS (Right), and

(iii) Site of action of calcineurin inhibitors (cyclosporine and tacrolimus).

with segmental sclerosis of glomeruli and effacement of podocyte foot processes. In addition, ANLN is upregulated in diverse site-specific human tumours including brain, lung, breast, renal, ovarian, endometrial, liver, pancreas, colorectal and bone marrow cancers [7].

A study published in Journal of American Society of Nephrology, involving 250 families with FSGS revealed ANLN mutation in a family of 26 members. After performing whole-genome sequencing, 9 members were found to have ANLN mutation. One of the family members had childhood-onset FSGS, five members progressed to ESRD and received renal transplantation but none of them had post-transplant disease recurrence [7].

The hallmark of NS is massive urinary loss of proteins, especially albumin. Other features include generalized edema, hyperlipidemia and hypoalbuminemia. Less frequent symptoms include hypertension, hematuria and oliguria. In addition, children with NS are prone to infections, acute kidney injury and thromboembolic events [4].

The diagnostic criteria of NS include massive proteinuria (3+ or 4+ in dipstick or >40 mg/m^2/hour), hypoalbuminemia (albumin <3 g/dL) and generalised edema. Renal biopsy has authentically been utilised as a main diagnostic and prognostic indicator for children with SRNS. In cases of early-onset SRNS, genetic testing has obviated the need for renal biopsy and serves as a less invasive diagnostic modality. Identification of mutation is important for genetic counselling and possible antenatal screening for future pregnancies, prediction of prognosis and post-transplant disease recurrence, and surveillance of other extra-renal phenotypes [2,8]. In addition, further discovery of novel genes will improve our understanding about the pathogenesis of SRNS and allow for a pragmatic approach to therapy.

Steroid-resistant nephrotic syndrome is a highly heterogeneous disease, with over 60 known disease-causing genes. Using gene panel analysis, all protein-coding exons of multiple genes can be tested simultaneously using high-throughput polymerase chain reaction amplification and sequencing techniques. While, whole exome sequencing allows screening of all protein-coding regions of genes in a genome, targeted next generation sequencing uses predesigned gene panels containing important genes or gene regions associated with a disease or phenotype such as SRNS, thereby decreasing costs and increasing efficiency. Even though high-throughput sequencing technologies have revolutionized the identification of mutations responsible for a diverse set of genetic disorders, the identification of causal mutations continue to remain ambiguous in a significant proportion of patients. This could be partially due to pathogenic variants being located in non-coding regions (introns), which are largely missed by targeted exome sequencing. With the advent of whole-genome sequencing, detection of non-coding variations has become possible [7,9]. In our patient, extended gene panel analysis suggested ANLN mutation as the underlying cause for SRNS with no other possible disease-causing mutations being identified.

The fundamental aim of treatment in children with SRNS is to improve the prognosis, thereby ameliorating progression to ESRD. Angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARBs) can be employed to reduce proteinuria and lower blood pressure. The anti-proteinuric effect of ACEI/ARBs is predominantly due to post-glomerular vasodilation and pre-glomerular vasoconstriction, resulting in reduced filtration pressure, thereby lowering the leakage of proteins through GBM [10]. Statins can be used for management of hyperlipidemia in children with NS. The presenting child is being managed with

dietary modifications, since the use of statins is not warranted in children less than 8 years of age [11]. Parents have been counselled about lipid apheresis as an alternative treatment option, if lipid levels do not normalise with dietary modification and other supportive measures.

In children with SRNS, the treating nephrologist may choose to use non-glucocorticoid agents to induce complete or partial remission. There have been in vitro studies proving the potential benefits of calcineurin inhibitors in treatment of FSGS with ANLN mutation (Figure 2) [5]. The presenting child has been started on tacrolimus and will be continuously monitored until remission. The therapy will be stopped if he does not attain partial or complete remission within 6 months.

Albumin infusion along with diuretics, is considered as a therapy in cases of refractory edema [12,13]. The rationale behind albumin infusion relies upon "underfill" theory, i.e., hypoalbuminemia (reduced oncotic pressure) decreases intravascular-to-interstitial albumin gradient creating a surge in the movement of fluid from intravascular compartment into interstitial space. The infused albumin normalises the oncotic pressure and as a result, edema fluid is drawn-back into circulation, from where it is excreted by the kidneys with the help of diuretics.

According to PodoNet registry, 3 out of 4 children with monogenic SRNS progress to ESRD, requiring dialysis or renal transplantation. Fortunately, several studies have accentuated a negligible risk of post-transplant disease recurrence in patients with genetic forms of SRNS, compared to a whopping 30% in children with non-genetic forms of SRNS [14].

With ever-increasing number of genes involved in SRNS, the need for understanding the molecular mechanisms and genotype-phenotype correlations is inevitable. Given the heterogeneity of the disease, taking up next generation sequencing-based "bench to bedside" translational approaches would bring about revolutionary outcomes in its diagnosis and management.

LEARNING POINTS

1. Genetic testing should be performed in all children non-responsive to prednisolone therapy to help the treating clinician to provide personalised treatment options, possibly avoiding unnecessary immunosuppressive therapy.
2. Parents of children with SRNS should be counselled about the disease course and need for renal transplantation in future.
3. Children having monogenic forms of SRNS may be predisposed to certain other syndromes or site-specific tumours. Therefore, the treating pediatrician should be vigilant in monitoring these children for early disease detection.

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Ethical approval

All procedures were performed in accordance with the ethical standards of the institutional research committee at which the study was conducted and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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