

ORIGINAL ARTICLE

COMBINED IMMUNE DEFICIENCY AND RENAL PHENOTYPE
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Background: Congenital Nephrotic Syndrome (CNS) is a paediatric kidney disease that is defined by massive protein loss in the urine, hypoalbuminemia, and hyperlipidemia. Mutations in *PLCE1* are associated with autosomal recessive form of nephrotic syndrome associated with elevated T-cells. **Methods:** A two-month-old female patient from a Pakistani family suffering from recurrent renal infections with fever and cough was investigated in this study. Laboratory tests including renal function test, lipid profile, lymphocyte subset analysis using flow cytometry, serum immunoglobulin level and blood complete picture were performed. After detailed clinical evaluation, whole blood samples were collected in EDTA tubes for genetic analysis. **Results:** Complete blood count (CBC) showed low haemoglobin levels and lymphocytosis. Flow cytometry revealed elevated CD4/CD8 T-cells. Low serum immunoglobulin levels were observed. Genetic analysis revealed a missense mutation [c.6790A>G; (p.Lys2264Glu)] in the gene *PLCE1*. **Conclusion:** The current study describes a novel homozygous genetic mutation in *PLCE1* gene. Clinical investigations revealed disease features partially fulfilling the criteria of inherited nephrotic syndrome.

Keywords: CRP, DNA Sequencing, Flow cytometry, Nephrotic Syndrome, *PLCE1* gene

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INTRODUCTION

Inherited Nephrotic Syndrome (INS) (OMIM 256300) is a rare paediatric disorder affecting glomerular function, characterized by massive proteinuria, hypoalbuminemia, and oedema that have impaired glomerular function.^{1–3} Among children, the idiopathic type of nephrotic syndrome is more common, and most cases respond to corticosteroids; hence, it is termed steroid-sensitive nephrotic syndrome (SSNS).⁴ Approximately 15–20% of these patients are steroid-resistant, referred to as steroid-resistant nephrotic syndrome (SRNS).^{5,6} Among SRNS patients, 50% of the cases worsen in renal function and eventually develop end-stage renal failure (ESRF) within 15 years of diagnosis.⁷

Mutations in genes encoding podocyte-associated proteins are pivotal in certain forms of nephrotic syndrome, particularly the SRNS. Genetic mutations approximately 30% of SRNS patients are significant contributors to the disease pathogenesis.⁷ Podocyte-related genes: Mutations in genes like nephrotic syndrome, type 1 (*NPHS1*), nephrotic syndrome, type 2 (*NPHS2*), Wilms tumour gene 1 (*WT1*), laminin subunit beta-2 (*LAMB2*), and phospholipase C epsilon 1 (*PLCE1*) are commonly implicated. These genetic anomalies compromise the structure and functions of podocytes and immunological mechanisms.⁸

Studies have identified immune dysregulation in idiopathic nephrotic syndrome patients, including altered T-cell function and cytokine profiles.⁹ The onset and progression of the nephrotic syndrome are linked to

immune system dysregulation such as T-cell response, altered B-cell activity, and cytokine imbalance. This interplay between genetic predisposition and immune-mediated injury underpins the complexity of nephrotic syndrome, necessitating comprehensive diagnostic and therapeutic approaches. The convergence of podocyte gene mutations and immune system dysfunction highlights the multifactorial nature of this condition.¹⁰

The aetiology of childhood NS remains unclear; however, knowledge of disease pathogenesis continues to expand. Next Generation Sequencing (NGS) has yielded pathogenic variants in >80 genes that are enriched in the podocyte and have been associated with SRNS in 10–30% of cases.^{11,12} Over the past decade, numerous genes associated with immune system have been discovered to play pivotal roles in the development of nephrotic syndrome. For example, *NPHS1* (Nephrin) and *NPHS2* (Podocin), primarily known as structural podocyte proteins, also mediate immune modulation within podocytes. *PLCE1* (Phospholipase C epsilon 1) contributes to podocyte differentiation and intracellular signalling pathways influencing cell growth and development. *CD2AP* (CD2-associated protein) is involved in T-cell activation and maintaining podocyte architecture, while *TNFRSF13B* (TACI) plays a regulatory role in B-cell function and humoral immunity.¹³

This study presents genetic analysis in a suspected nephrotic syndrome patient and reveals missense variant [c.6790A>G; (p.Lys2264Glu)] in *PLCE1* gene.

METHODOLOGY

The project was presented and received formal approval from ethical research board of the HBS Medical College, Islamabad. Written informed consent was obtained from all male and female participants before the start of the study. The mother of the patient voluntarily provided related clinical and family histories and consented to genetic analysis, permitted to publish clinical and family histories, and genetic findings. The study adhered to the principles of the Declaration of Helsinki and relevant national and institutional regulations regarding human genetic research.

A-2-months old female patient from a highly consanguine family was enrolled in this study. She was brought to emergency with complaints of fever, cough and a single episode of fit and, was admitted to the Department of Paediatrics, Aga Khan University Hospital, Karachi, Pakistan. Detailed clinical and family history was acquired from the mother of the patient. Complete blood count (CBC) was performed, and serum immunoglobulins were estimated. According to mother in two months this was second time she suffered from fever and acute cough. According to mother, family had a history of two early deaths of a male and a female child with similar clinical manifestations. To rule out the involvement of underlining aetiology related to immune system physician also ordered flow cytometry to evaluate lymphocyte subset levels. Complete blood count, Renal Function Test, Erythrocyte Sedimentation Rate, serum immunoglobulin levels, and using flow cytometry, lymphocyte subset analysis were performed in the Armed Forces Institute of Pathology, Rawalpindi, Pakistan.

Five mL whole blood samples from the patient and the parents were collected in EDTA tubes. Whole-exome sequencing (WES) was performed on genomic DNA using the SureSelect Human All Exon kit (Agilent) and sequenced on the Illumina HiSeq 2500 platform (Macrogen, Seoul, South Korea), generating 100 bp paired-end reads. Libraries were prepared with the Illumina Paired-End Sample Prep Kit VI. Reads were aligned to the GRCh38 reference genome using BWA-MEM algorithm.

RESULTS

A two-months-old female child weighing 5 Kg was enrolled from Attock City, Punjab, Pakistan. Family history revealed that two males (III-2 and III-3) and one female (III-4) patients had died (Figure-1A). The onset of the disease was at age 2 months to 1.5 years in all sufferers. At the time of admission our indexed patient had fever of 102 °F and acute cough.

Blood CBC revealed low haemoglobin levels (8.1 g/dL) and neutrophilia (42.9%). Low serum Immunoglobulin's were observed (IgA<0.15 g/L, IgM,

0.30 g/L and, IgG, 2.26 g/L). Details of Immunoglobulin with reference ranges are shown in Table-1. C-reactive proteins and renal function tests values were within the reference range.

Flow cytometry subset mediated lymphocyte subset analysis revealed increased absolute counts of CD3+ total T-Lymphocytes and CD19 total B-lymphocytes. CD56+ Natural Killer (NK) cells were moderately low. Both subsets of T-lymphocytes (CD4+ and CD8+) were elevated. The report suggested a case of Chronic Granulomatous Disease (CGD). (Table-2).

Genetic analysis revealed multiple homozygous and heterozygous variants. Two interesting variants were identified in *VISTA* and *PLCE1*. The interesting variants related to CGD were checked for their segregation in the family. A missense variant [c.6790A>G; (p.Lys2264Glu)] was identified in *PLCE1* gene (phospholipase C epsilon 1). The mutation was homozygous in the patient while heterozygous in the parents and one of the healthy siblings confirmed its segregation with the disease in the family (Figure-1).

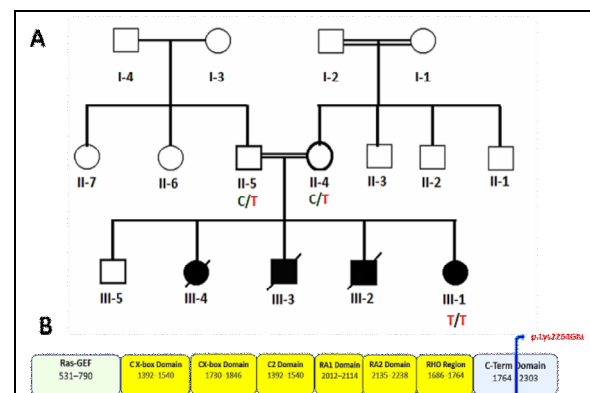


Figure-1: Family pedigree and protein structure

A. Squares represent males while circles represent females. Filled circle and squares represent patients. Double lines indicate consanguine marriages. Diagonal lines on circles and squares indicate deceased individual. 'C' below squares and circles (wild type) is abbreviation of cytosine, and mutated 'T' indicates thymine. The indexed female patient III-1 is homozygous for the mutated Thiamine. **B.** Protein structure of Phospholipase C epsilon 1 shows N-terminal RAS-GEF domain, CX-Box domain, CX domain, C2 domain, RA1 domain, RA2 domain, RHO domain, and a C-terminal domain along with their respective amino acids. Arrow head in C-terminal domain indicates point of mutation.

Table-1: CBC and serum immunoglobulin levels

Test	Results	Reference Range
Haemoglobin	8.1 g/dL	9.4–13 g/dL
Haematocrit	25%	28.0–42.0%
Red Blood Cells (RBC)	2.50×10 ¹² /L	3.1–4.3×10 ¹² /L
White Blood Cells	20.5×10 ⁹ /L	5–15×10 ⁹ /L
Lymphocytes	45.0%	67–80%
Eosinophils	0.4%	2–6%
Platelet Count	576×10 ⁹ /L	210–650
Serum Immunoglobulin Levels		
Immunoglobulin A (IgA)	<0.15 g/L	0.4–3.5 g/L
Immunoglobulin M (IgM)	0.30 g/L	0.5–3.0 g/L
Immunoglobulin G (IgG)	2.26 g/L	6.5–16 g/L

Table-2: Lymphocyte subset analysis

Test	Result	Reference Range
CD3+total T-Lymphocyte	4019	2800–3500
CD4+T-helper Lymphocytes	3535	800–1100
CD19+Total B-lymphocytes	2196	1000–1700

DISCUSSION

The *PLCE1* (OMIM, 608414) gene is localized on chromosome 10q23.33. It encodes the enzyme (Phospholipase C epsilon 1) a 230 kDa protein. The enzyme plays a crucial role in intracellular signalling by hydrolyzing phosphatidylinositol-4,5-bisphosphate (PIP₂) into two critical second messengers: inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG).^{14,15} It is a multi-domain protein comprising of 6 important domains including a Ras Guanine Nucleotide Exchange Factor (Ras-GEF) domain, a Phosphoinositide Phospholipase CX-box domain, a Phosphoinositide Phospholipase C Y-box domain, a C2 domain, a C-terminus Ras-binding (RA) domains, and an intracellular RHO activation region.¹⁶

The protein *PLCE1* plays a crucial role signal transduction processes by hydrolyzing PIP₂ intracellular signalling which lead various cellular responses, including cell proliferation and differentiation specially in nephrons. Through Ras Activation it activates MAP kinase pathway.^{17,18} Patients with mutated *PLCE1* gene may suffer from Nephrotic Syndrome Type 3, cancers or high level of circulatory T-cells.^{11,18–20}

We identified a missense mutation [c.6790A>G; (p.Lys2264Glu)] in RA2 (Ras-Associating 2) domain in C-terminal regulatory region of the protein. The C-terminal region is less characterized compared to its well-defined other protein domains. It may play a role in maintaining the structural integrity of the protein.^{21,22}

Though we did not have any findings related to nephrotic syndrome, we observed elevated levels of circulatory T-cell (CD4+/CD8+) and hypoinmunoglobulinemia which is well documented in earlier studies.^{17–23} As for as mutated *PLCE1* mediated nephrotic syndrome is concerned it is hypothesized that onset of disease may occur at a later age.

CONCLUSION

This study highlights a novel missense mutation [c.6790A>G; (p.Lys2264Glu)] in the C-terminal, Ras-Associating (RA2) domain of the *PLCE1* gene, a region not fully characterized yet. The immunological findings, including elevated circulatory T-cell populations and hypoinmunoglobulinemia, correlate with previously reported immune dysregulation linked to *PLCE1* mutations. It can be hypothesized that similar renal manifestations may emerge in our patient at a later stage of the disease. *PLCE1* variants may contribute to immune phenotypes independent of, or preceding, renal involvement. Further functional studies are warranted to

clarify the role of the RA2 domain in T-cell regulation and to explore the potential age-related onset of nephrotic features in such cases.

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AAA: Data Collection and Review of literature

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