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NR3C1 variants and glucocorticoid response in childhood nephrotic syndrome in North India

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Abstract

Background Nephrotic syndrome (NS) is a common kidney disorder in children, characterized by significant proteinuria, hypoalbuminemia, and peripheral edema. While glucocorticoids (GCs) are the first-line treatment for pediatric nephrotic syndrome (NS), a subset of patients exhibit steroid resistance, leading to poor prognosis and a higher risk of long-term kidney damage. The glucocorticoid receptor gene (NR3C1) plays a pivotal role in mediating the effects of GCs, and its polymorphisms have been implicated in variable GC responses.

Methods This study investigates the association between *NR3C1* single nucleotide polymorphisms (SNPs) (rs6877893 and rs10482634) in 50 patients with steroid-sensitive nephrotic syndrome (SSNS) and 50 steroid-resistant nephrotic syndrome (SRNS) individuals by Kompetitive Allele Specific Polymerase Chain Reaction (KASP) assay and altered GC receptors (GRα and GRβ) expression by Real-Time PCR (RT-PCR). Adverse effect of steroid therapy was also assessed.

Results Genotyping revealed a significant association between GG genotype of SNP rs10482634 and steroid resistance, suggesting that it may contribute to the heterogeneity in GC response in this population. There is also a positive association of AA genotype of rs10482634 with short stature and AG genotype of rs10482634 with cushingoid habitus, as a side effect of steroid therapy. We have also found an enhanced expression of GRα in SSNS population. No significant association was found between the SNP rs6877893 and the response to steroid treatment in the study cohort. Our study revealed higher rates of drug-related complications in patients receiving larger cumulative doses of steroids.

Conclusion These findings highlight the importance of genetic screening of *NR3C1* SNPs in predicting steroid responsiveness and tailoring personalized therapeutic strategies in pediatric NS.

Clinical trial number not applicable.

Keywords Glucocorticoid receptor, GRα and GRβ, Nephrotic syndrome, NR3C1, Steroid-sensitive nephrotic syndrome, Steroid-resistant nephrotic syndrome

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Introduction

Nephrotic syndrome (NS) remains one of the most prevalent glomerular causes of end-stage renal disorders in children worldwide [1]. The Indian subcontinent has a higher incidence (~1:10000) of pediatric nephrotic syndrome (NS) among Asian countries [2]. Pediatric NS is ramified based on the responsiveness of the child to steroid therapy. Oral corticosteroids stabilize the podocyte skeleton in addition to their anti-inflammatory actions [3]. About 85–90% of patients achieve complete remission with steroids and are categorized as steroid-sensitive nephrotic syndrome (SSNS) [4]. Steroid-resistant cases demonstrate irreversible changes in podocyte morphology which could be attributed to genetic abnormalities or immune system dysregulation affecting the production and functionality of podocyte proteins [5, 6]. According to The International Study of Kidney Disease in Children (ISKDC), responsiveness to steroids and other immunosuppressive agents is the most crucial prognostic factor, regardless of biopsy findings.

Due to interindividual differences in the clinical course and adverse effects of glucocorticoids in children with NS, it is essential to identify specific markers to individualize the treatment. Pharmacogenetics plays an important role in this regard. One of the factors that could affect GC sensitivity is the glucocorticoid receptor (GR) gene *NR3C1* (nuclear receptor subfamily 3, group C, member 1), in particular, the variants of this gene. *NR3C1* encodes intracellular steroid receptors, which mediate molecular response to exogenous and endogenous glucocorticoids. It is expressed in all body cells and the tissue sensitivity to steroids depends on the receptor expression and function [7, 8].

Several studies suggest an association of GC sensitivity with genetic variations of NR3C1. According to Huizenga et al., a particular GR haplotype (a common *NR3C1* polymorphism) is associated with increased GC sensitivity [9, 10]. Wasilewska et al. found a temporary decrease in glucocorticoid receptors in monocytes and lymphocytes while on therapy with GCs in children with SSNS [11]. Some children with steroid-resistant nephrotic syndrome are 1–7 years old at the time of the first attack, which often makes pediatric nephrologists suspect that these children will experience remission with steroids. Some patients experience resistance to steroids despite experiencing their first attack in this age range. This makes it possible to suspect an abnormality in the steroid receptor.

Genetic mechanisms alter GR function at different levels. A single gene undergoes alternating splicing to produce the two isoforms of human GR namely GRα & GRβ. GRα consists of 777 amino acids, binds hormones, and activates genes reacting to glucocorticoids. In contrast to this, GRβ does not bind to glucocorticoids. Although GRβ's exact role is unknown, it may act as a negative regulator of GRα and facilitate glucocorticoid resistance. Thus, assessment of GR receptor single nucleotide polymorphisms (SNPs) and isoform distribution can aid in assessing the possibility of steroid resistance before initiating therapy with steroids in nephrotic syndrome.

Despite extensive investigations, the precise pathophysiology of pediatric NS is still unknown. Identification of the genetic or causal factors that influence GC response and the ensuing tailored therapy would enhance the therapeutic approach and limit the intake of medications, reducing drug toxicity and medical expenses. Hence, we proposed that *NR3C1* SNPs (rs6877893 and rs10482634) might have a role in steroid resistance in pediatric patients.

Materials and methods

Study setting and design

This was an observational prospective case-control study carried out in a tertiary care hospital. Subjects were recruited from January 2023 to July 2024. Sample size was calculated assuming a minor allele frequency of 41% in cases and 15% in controls with a power of 80% and a significance level of 5%. A total of 50 patients with steroid-sensitive nephrotic syndrome (SSNS) and 50 patients with steroid-resistant nephrotic syndrome (SRNS) were recruited. The study protocol was approved by the Institutional Ethics Committee (Ref No: IEC-INT/2022/MD-817).

Participants: inclusion and exclusion criteria

All children diagnosed with nephrotic syndrome from 1 to 12 years of age, restricted to 9 states across the northwestern sector of the Indian subcontinent were enrolled, to reduce the genetic variations that might arise due to differences in ethnicity. Infants, those with dysmorphisms, intellectual disability, and other associated major system involvement were excluded from the study to rule out the rare possibility of monogenic SRNS. Those who had gone into remission with 6 weeks of steroid therapy as per standard definition, were under the SSNS group.

SSNS group included those with the first episode, infrequent (<4 episodes/yr) /frequent (>4 episodes/yr or >2 episodes/yr) relapsers, and steroid dependant nephrotic syndrome (2 consecutive relapses within 14 days of stopping therapy or while on alternate day therapy). While those who did not attain remission with 6 weeks of full-dose steroid therapy at a dose of 2 mg/kg (or 60 mg/m2) per day, were under the SRNS group. This included patients with initial resistance (no response to steroids since the first episode) as well as those who attained resistance at a later stage. However, this classification was considered cross-sectionally at the time of enrolment, as both initial and late resistance with steroid therapy were classified under SRNS and hence the possibility of SSNS

developing resistance at a later stage could not be ruled out. Both new and follow-up cases, some of whom were referred from other hospitals, were enrolled. As samples were drawn at the time of enrolment, they were at different phases of treatment with steroids and other immunomodulators and were followed up longitudinally for further response to treatment and the associated adverse effects.

Data collection

Informed consent was taken from the patient's parents/guardians. Clinical details include the demographic details and details pertaining to the study such as age of onset, presentation at onset, response to steroid therapy, and the complications they had presented with. The clinical data, including histological findings of kidney biopsy, biochemical parameters such as degree of albuminuria, kidney function tests, and other relevant details, were taken from the patient's medical history. In addition to these, data regarding the complications associated with the disease as well as the various drug therapies used in the management of nephrotic syndrome, particularly steroids, were studied in detail. Details regarding the responsiveness of the patient to alternative therapy have also been documented as some immunomodulators are known to alter the steroid responsiveness of the patient.

Sample collection

Two to three ml of peripheral blood samples were obtained from patients in EDTA vacutainers. Ficoll-Paque density gradient centrifugation was used to isolate PBMCs which were stored at -80 °C until RNA extraction. QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) was used for genomic DNA.

Genotyping of NR3C1 gene (rs10482634, rs6877893) polymorphisms by kompetitive allele specific polymerase chain reaction (KASP) assay

Competitive allele-specific PCR was used for genotyping. This PCR is based on two unlabelled allele-specific forward primers and one common reverse primer which contains a universal FRET cassette labelled with FAM and HEX dye.

Expression analysis by real-time PCR

GRα and GRβ expression was checked by Real-time PCR (RT-PCR) using SYBR Master Mix. Primer sequences will be available on request. PCR was performed at 95 °C based on an initial denaturation for 10 min, 10-second denaturation at 95 °C, 10-s annealing, and elongation at 72 °C for 20 s (×45 cycles). For normalization of the mRNA expression, GAPDH was used. At 72–95 °C, melting curves were made to confirm the specificity, at the end of PCR cycling. Furthermore, standard curves were produced by cDNA amplification with dilutions. To quantify the relative expression of target mRNAs, the comparative, well-established threshold cycle (CT) method $2 - \Delta\Delta CT$ was applied.

Statistical analysis

Statistical Package for Social Sciences for Windows (SPSS version 29, Chicago, Illinois, USA) was used for statistical analysis.Hardy Weinberg equilibrium was calculated for each SNP studied. Fisher's exact test was used for the comparison of SNP frequencies between both the groups and ANOVA was applied for subgroup comparison. Student's t-test has been used as the test of significance for quantitative data and the chi-square test has been used as the test of significance for qualitative data. Descriptive statistics are presented as mean and median for measures of central tendency and standard deviation as measures of dispersion. Skewed data is represented in the form of median and Interquartile ranges.

Results

Distributions of sociodemographic and clinicopathological characteristics (Table 1)

In our study, 70% subjects were males. About two-third of the patients experienced symptom onset between 2 and 5 years of age. The mean weight and height of the study population were 21.07 kg (SD = 8.97) and 107.89 cm (SD = 17.28) respectively. The SSNS group also included frequently relapsing nephrotic syndrome (FRNS), and steroid-dependent nephrotic syndrome (SDNS). The SRNS group had a nearly equal distribution of initial resistance (46%) and late resistance (54%) to steroids. Edema (49/50 in SSNS and all cases in the SRNS group) and oliguria (21/50 cases in the SSNS and 27/50 in the SRNS group) were the most common presenting symptoms. A significant proportion of subjects in the SRNS group (21/50) presented with hypertension compared to only 10 out of 50 in the SSNS group (p value = 0.017), possibly due to the prolonged duration of steroid therapy in the former (Table 1).

Workup for nephrotic syndrome

Urine analysis revealed nephrotic range proteinuria in majority of the subjects. Urine Protein-to-Creatinine ratio (UpUc) > 2 was present in approximately 82% (39/50 in SSNS and 43/50 in SRNS groups) of the population. Those who had already received treatment prior to presentation to our center had lesser degrees of proteinuria. Microscopic hematuria was present in 6% of the subjects. None had macroscopic hematuria.

Eighty percent of the children developed hypoalbuminemia (S. albumin < 2.5 g/dL) (Table 1). Those who did not exhibit hypoalbuminemia were children who had already received steroids before presentation to our

Table 1 Summary of baseline variables

Variable	*SSNS (N = 50)* *N (%)*	*SRNS (N = 50)* *N (%)*	*p*- value
Female, n (%)	13 (26)	17 (34)	0.383
Male, n (%)	37(74)	33(66)	
Weight (kg), Mean (SD)	21.9(9.8)	20.19(8.05)	0.768
Height (cm), Mean (SD)	110.8(17.9)	104.9(16.2)	0.816
Blood Pressure (BP) (mmHg)			
Systolic blood pressure (SBP), Mean (SD)	106(12)	108(16)	0.331
Diastolic blood pressure (DBP), Mean (SD)	69(12)	69(13)	0.996
Age at onset			
< 1	2(4)	3(6)	0.761
1–5 years	32(64)	33(66)	
5–10 years	14(28)	14(28)	
> 10 years	2(4)	0(0)	
Age at enrolment, Median (IQR)	4.25 (3-7.25)	4.0 (2.37-6)	0.08
Initial symptoms			
Edema	49(98)	50(100)	1.000
Hypertension	10(20)	21(42)	**0.017**
Decreased urine output	21(42)	27(54)	0.230
Spontaneous bacterial peritonitis	4(8)	7(14)	0.338
Cumulative dose(mg/kg), median (IQR)	239(148,370.2)	398(183.5,488)	0.111
Steroids side effects			
Weight gain	13(26)	13(26)	1.000
Short stature	3(6)	10(20)	**0.037**
Cataract	4(8)	7(14)	0.338
Glaucoma	1(2)	2(4)	1.000
Hypertension	23(46)	43(86)	**0.000043**
Hyperglycaemia	1(2)	1(2)	1.000
Cushingoid facies	26(52)	38(76)	**0.012**
Acanthosis	3(6)	9(18)	0.065
Immunosuppression	0	2(4)	0.495
Gastritis	5(10)	10(20)	0.161
Hirsutism	6(12)	19(38)	**0.003**
NS related complications			
Peritonitis	6(12)	14(28)	**0.046**
Cellulitis	5(10)	14(28)	**0.022**
Pneumonia	2(4)	3(6)	1.000
Cerebral venous sinus thrombosis (CSVT)	0	4(8)	0.117
Deep vein thrombosis (DVT)	0	1(2)	1.000
Pulmonary thromboembolism (PRES)	0	1(2)	1.000
Acute kidney injury (AKI)	2(4)	3(6)	1.000
Lab investigations			
Nephrotic range proteinuria	39(78)	43(86)	0.297
Serum albumin			
> 3.5	4(8)	0	0.105
< 2.5	36(72)	43(86)	
2.5–3.5	9(18)	7(14)	
Microscopic hematuria	3(6)	3(6)	1.000
Hypercholesterolemia	30(60)	41(82)	**0.032**
Elevated Urea	9(18)	11(22)	0.617
Elevated Creatinine	4(8)	10(20)	0.084
Anaemia (Hb < 10)	14(28)	17 (34)	0.516

**Bold values show significant p-values*

centre. Patients on long-term steroid therapy were monitored for metabolic complications on a 6-monthly basis. Approximately 71% developed hypercholesterolemia (S. Cholesterol > 200 mg/dL). The incidence was significantly higher in the SRNS groups ($p = 0.032$) (Table 1). A quarter showed elevated HbA1c levels (> 5.9%). As the cumulative steroid dose could not be determined for all patients, the correlation with steroid dose could not be ascertained.

At presentation, around 20% of the patients had deranged kidney function. In most cases, the etiology was prerenal, attributed to intravascular volume depletion secondary to edema which improved as the edema subsided. Etiological workup showed ANA positivity in only one subject in the SRNS group. Viral markers were negative in all cases.

Histopathological findings from kidney biopsy

A Kidney biopsy was performed to evaluate the underlying histopathological differences between SSNS and SRNS (Fig. 1b). The timing of biopsy was usually following 6 weeks of steroid therapy in the SRNS-IR group, while it was variable in the SRNS-LR and SSNS groups, as it was based on the different timings at which the subjects developed steroid resistance and dependence respectively. The biopsy which was done following CNI (calcineurin inhibitor) therapy was however not considered in this study.

In the SRNS group, FSGS (40%) and MCD (30%) were the most common findings, while others included mesangioproliferative glomerulonephritis, IgM nephropathy and, membranous nephropathy. However, secondary SRNS should also be considered, where they are likely to have started with MCD, which eventually progressed to FSGS due to lack of steroid responsiveness, resulting in sclerosis mediated by TGF beta. However, this could not be confirmed as we had only a single biopsy, which was taken 6 weeks or later after steroid therapy. In contrast, the SSNS group displayed minimal change disease, podocytopathy, and an unusual case of collapsing glomerulopathy. These findings underscore the complex nature of nephrotic syndrome and the necessity for tailored therapeutic approaches based on precise histopathological diagnosis.

Complications of nephrotic syndrome

Complications related to the disease were also studied. The most common complication was edema (70.3%) requiring diuretics and/or albumin infusions in some cases (Table 1). This was followed by infections in about 53.1% of the subjects, followed by thrombotic complications (7.8%) and AKI (6%) secondary to third space volume loss (Fig. 1a). Thrombotic complications were seen in 5 subjects of which 4 had CSVT and 1 had DVT.

Side effects of steroid therapy

As expected, patients in the SRNS group received a higher median cumulative dose of steroids compared to the SSNS group ($p = 0.11$). Anthropometry was recorded at 3 monthly intervals. Short stature was defined as height for age < -2SD. None of the subjects had short stature to begin with, however eventually with prolonged steroid therapy, some of them developed short stature. A significantly higher proportion of children in the SRNS group compared to the SSNS group had short stature (10/50 children vs. 3/50) ($p = 0.037$). Similarly, a higher proportion had hypertension in the SRNS group compared to the SSNS group (43/50 vs. 23/50) ($p = 0.001$) Table 2. These findings could be attributed to the higher cumulative doses of steroid therapy in the SRNS group (398 mg/kg) as compared to the SSNS group (239.5 mg/kg).

All children on long-term steroid therapy underwent ophthalmological screening at 6 monthly intervals. 11% of the subjects (4/50 from the SSNS and 7/50 from the SRNS groups) developed cataract after a mean duration of 2.3 years after starting steroid therapy. Only 3% (1/50 in SSNS and 2/50 in SRNS groups) had glaucoma, which developed after a mean duration of 1.8 years of starting steroid therapy. These complications were higher in the SRNS group, though not statistically significant.

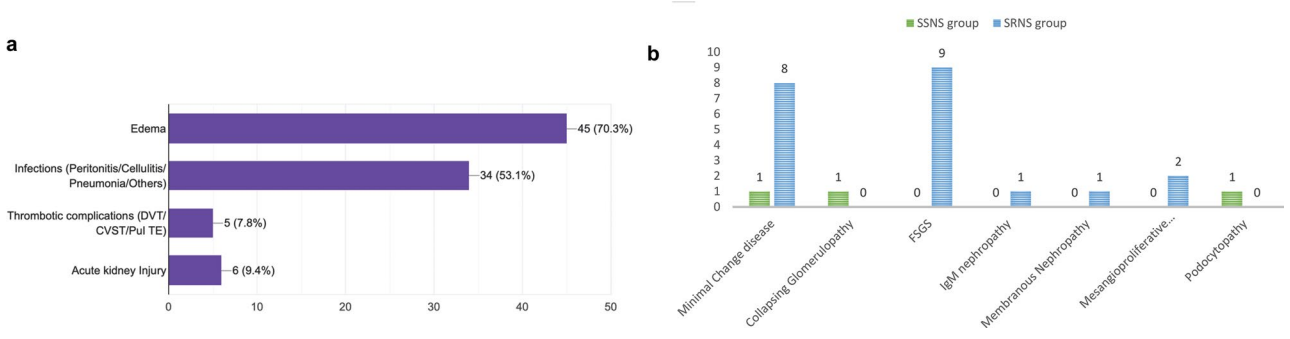

Fig. 1 (**a**) Complications associated with NS. (**b**) Histopathological Differences between SSNS and SRNS

Table 2 Side effects of steroid therapy

Side effects	SSNS group	SRNS group	Total	Chi-square value	*p*-value
	No. of cases (%)	No. of cases (%)	N		
Weight gain	13(26%)	13(26%)	26	0.000	1.000
Short stature	3(6%)	10(20%)	13	4.332	**0.037**
Cataract	4(8%)	7(14%)	11	0.919	0.338
Glaucoma	1(2%)	2(4%)	3	0.344	0.558
Hypertension	23(46%)	43(86%)	66	17.825	**0.001**
Hyperglycaemia	1(2%)	1(2%)	2	0.000	1.000
Cushingoid facies	26(52%)	38(76%)	64	6.250	**0.012**
Acanthosis	3(6%)	9(18%)	12	3.409	0.065
Immunosuppression	0(0%)	2(4%)	2	2.041	0.153
Gastritis	5(10%)	10(20%)	15	1.961	0.262
Hirsutism	6(12%)	19(38%)	25	9.013	**0.005**

**Bold values show significant p-values*

Table 3 Association of rs10482634 and rs6877893 with steroid resistance

SNPs	Genotype	SSNS group *N* (%)	SRNS group *N* (%)	Chi-square value	OR 95% CI	*p*-value
rs10482634	AA (wild)	29 (58.0%)	21 (42.0%)	6.613		**0.037***
	AG (hetero)	19 (38.0%)	19 (38.0%)		1.381(0.59–3.22)	
	GG (mutant)	2 (4.0%)	10 (20.0%)		6.905(1.36–34.8)	
rs6877893	AA (wild)	19 (38.0%)	15 (30.0%)	2.066	-	0.356
	GA (hetero)	22 (44.0%)	20 (40.0%)		1.15(0.46–2.8)	
	GG (mutant)	9 (18.0%)	15 (30.0%)		2.1(0.72–6.14)	

**Bold values show significant p-values*

The incidence of other side effects such as cushingoid facies($p = 0.012$), hirsutism ($p = 0.003$), and hypertension ($p = 0.000043$) were significantly higher in the SRNS group compared to the SSNS group. This could be explained by the higher cumulative dose of steroids in the SRNS group.

Need for additional therapies and complications

For patients who experienced multiple relapses, adjuvant therapies were introduced to maintain remission. About 16% of the patients in SSNS and 6% in the SRNS group were started on Levamisole. Similarly, 18% of SSNS and 22% of the SRNS groups were started on MMF. It was found that 50% of the patients on MMF developed GI complications or infections. A small number of patients in the SSNS group and 98% in the SRNS group were started on Calcineurin inhibitors. Among them, about 12% developed kidney injury, 8% developed infections and, 2% developed hair pigmentation as complications of therapy.

Correlation of genetic variants with SRNS

The association of NR3C1 gene polymorphisms rs10482634, and rs6877893 with steroid resistance was analysed as shown in Table 3.

Association of rs10482634 and rs6877893 with steroid resistance

In the case of rs10482634 SNP, AA genotype frequency was higher (58%) in the SSNS group, while AG frequency was similar in both the groups and GG was higher (20%) in the SRNS group as compared to the SSNS group (4%). This difference was statistically significant between the two study groups (p value-0.037, OR: 6.90). Hence this polymorphism showed a significant association with steroid resistance and can be used as a predictor of pediatric SRNS (Table 3). An odds ratio of 6.905, 95% CI (1.36–34.8) indicates that individuals with the GG genotype have 6.905 times higher odds of having SRNS compared to those with the AA genotype, and this is statistically significant (p-value- 0.037) (Table 3). To minimize the influence of ethnic variability on the inheritance of these SNPs, subjects were recruited exclusively from the northwestern region of the subcontinent.

It has been observed that in the case of rs6877893 SNP, the frequency of wild-type genotype (AA) was higher (38%) in the SSNS group as compared to the SRNS group (30%), while the mutant genotype GG was higher (30%) in SRNS group as compared to SSNS group (18%). However, no significant correlation could be derived between the two groups for this SNP (p-value- 0.356) Table 3.

Table 4 Association of steroid toxicity with SNP rs10482634

rs10482634				Chi-square value	*p*-value
	AA (wild)	AG (hetero)	GG (mutant)		
Weight gain	15	6	5	**8.397**	**0.015**
Short stature	8	1	4	**8.397**	**0.015**
Cataract	7	3	1	0.921	0.631
Glaucoma	1	1	1	1.363	0.506
Hypertension	34	24	8	0.228	0.892
Hyperglycaemia	1	0	1	3.231	0.199
Cushingoid facies	36	18	10	**7.898**	**0.019**
Acanthosis	7	4	1	0.42	0.81
Immunosuppression	1	1	0	0.322	0.851
Gastritis	9	5	1	0.872	0.646
Hirsutism	13	8	4	0.787	0.675

**Bold values show significant p-values*

Association of rs10482634 and rs6877893 with adverse effects of steroids

As shown in Table 4, it was found that the AA genotype of SNP rs10482634 was significantly associated with short stature, with a p-value of 0.015. It was also found that the genotype GA was significantly associated with cushingoid facies as a complication of steroid therapy. While in the case of rs6877893, no significant association was seen with steroid toxicity (data not shown).

Expression of the glucocorticoid receptors (GR) α and B

GR-α and GR-β expression levels were also studied in both the study groups. GR-α was significantly expressed in the SSNS group with a fold change value of 38.7 ($p = 0.049$). However, GR-β expression did not show significant difference between the two groups (Fold change = 1.054, $p = 0.253$) (Fig. 2). However, the extreme diversity in the dose and treatment regimens, made it impossible to standardize or account for these variables in the gene expression assay. Some developed serious complications with steroid therapy such as cataract, glaucoma, and short stature with lower cumulative doses, while some tolerated higher cumulative doses without any side effects, which suggests that the difference in expression of GR-α and GR-β could determine their response to GC therapy.

Discussion

Steroids remain the mainstay of therapy in pediatric NS, the initial response to which dictates the prognosis of the disease. Large inter-individual variability in steroid response leads to issues like inadequate therapeutic action in some and significant side effects in others. This has been attributed to various genetic polymorphisms in GC receptors, drug-metabolizing enzymes, and components of inflammatory pathways. As of now, there is minimal data on genetic markers to predict the response to steroid therapy. The primary goal of this study was to ascertain a genetic marker that could aid in the early identification of steroid-resistant cases, thereby avoiding prolonged steroid therapy and related complications.

The two study groups did not differ concerning sociodemographic factors like age, gender distribution, geography, etc. A significant proportion of patients in our study suffered from various steroid-related side effects like hypertension (66%), cushingoid features (64%), cataracts (11%), and glaucoma (3%). The occurrence of these side effects correlated with higher cumulative steroid doses in the SRNS group as compared to the SSNS group. Nearly half of the patients developed NS-related complications which included infections (cellulitis and SBP), thrombosis, severe hypertension, and kidney injury. These were associated with a protracted course of the disease as evidenced by higher occurrence in the SRNS, FRNS/SDNS subjects. 13% of the subjects suffered from short stature. Several studies have shown that children with nephrotic syndrome may have altered serum levels of IGFs and IGFBPs which offers a reasonable explanation for the association of this disease with short stature [12, 13].

FSGS was the most common histopathological finding in SRNS. Widiasta et al. found that in patients with

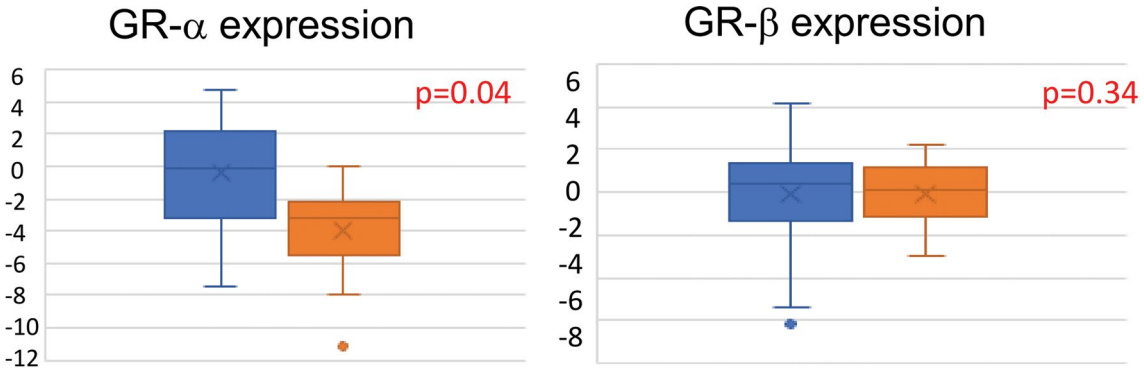

Fig. 2 Expression of GR-α and GR-β in SSNS (blue) and SRNS (orange) groups

LR-SRNS, the lack of steroid response is associated with increased extracellular matrix (ECM) protein production and dysregulation of cell-matrix interaction mediated by TGF- β. This suggests that these children could have MCD, which eventually evolved into FSGS, due to TGF-mediated sclerosis in response to the ongoing inflammation and proteinuria [14].

The correlation between *NR3C1* polymorphisms, gene expression, and steroid responsiveness in pediatric NS patients emphasizes the need for personalized treatment approaches. Genetic screening for *NR3C1* variants in North Indian children with nephrotic syndrome could help identify those at risk of developing steroid resistance, allowing for early intervention with alternative therapies, such as calcineurin inhibitors, rituximab, or other immunosuppressive agents. In an in vitro model, Sher et al. demonstrated the influence of *NR3C1* gene polymorphisms on GC responsiveness [15]. Similarly, Russcher et al. showed that glucocorticoid-regulated gene expression was directly impacted by two *NR3C1* SNPs (ER22/23EK and N363S) [16]. Yang et al. found that TG haplotype of rs6877893 and rs4912905 was linked to a lower risk of infantile spasms ($p = 0.038$), and that SNP rs6877893 was linked to adrenocorticotropic hormone responsiveness [17]. Prednisolone resistance was linked to the GA haplotype of rs10482634 and rs6877893 by Nazma Parvin et al. Similarly, we have found a positive association of GG genotype of rs10482634 polymorphism with steroid resistance. Additionally, several investigations, including those conducted on the Finnish [18], Dutch [19], Chinese [20], and Korean populations [21], found no correlation between various NR3C1 SNPs and steroid resistance. Similar to this, we have found no significant association of SNP rs6877893 with steroid resistance.

In our study, we found that GR-α showed relatively higher expression in the SSNS group, indicating its association with positive steroid response. Gene expression analysis showed significant expression of GR-α in the SSNS group with a fold change value of 38.7 ($p = 0.049$). Several studies have already confirmed the association of GR subtype expression with steroid responsiveness. GR-α expression has been linked to increased steroid responsiveness [22], while GR-β expression has been demonstrated to have a dominant-negative effect on the function of the glucocorticoid receptor [23, 24]. Liu et al. showed increased expression of GR- β in SR patients [25].

GR polymorphisms have also been linked to the incidence of drug-related side effects like obesity and metabolic syndrome [26]. In this study, a significant association has been ascertained between the AA genotype of SNP rs10482634 with the prevalence of short stature ($p = 0.015$) and GA genotype with cushingoid habitus($p = 0.019$) as a side effect of steroid therapy.

The relationship between NR3C1 polymorphisms and steroid resistance in pediatric NS patients in North India underscores the importance of genetic research in understanding treatment variability. The main clinical implication of this study lies in the early identification of SRNS patients, using a novel genetic biomarker. This would in turn help in reducing the cumulative dose of steroids in these patients and thereby the drug-related complications. Some individuals develop serious complications with steroid therapy, such as cataracts, glaucoma, cushingoid habitus, and short stature, even at lower cumulative doses, while others tolerate higher doses without adverse effects. This variability suggests differences in the expression of glucocorticoid receptor (GR) isoforms, particularly GRα and GRβ, which may influence their response to glucocorticoid therapy. This would mean that analysing the isoform distribution and sensitivity can help in individualizing therapy, where those having higher levels of GRα isoform might respond to smaller doses of steroid, challenging the conventional arbitrary standard dose of 2 mg/kg. This in addition can also help in avoiding the side effects associated with the drug therapy. This insight opens new avenues for therapeutic exploration and highlights the potential for precision medicine to optimize glucocorticoid therapy based on individual receptor profiles, improving outcomes for pediatric patients with nephrotic syndrome.

Strengths and limitations

In our study, we have found a significant association of SNP rs10482634 with steroid resistance, which can be used for early therapeutic intervention with CNIs instead of long-term steroid therapy. We have also studied the expression of the GC receptor subtypes, which when done in nephrotic syndrome patients, can help us predict the steroid responsiveness and hence can be used to tailor individualized therapeutic protocols. Standard steroid therapy protocols were followed for the first episode and relapse and a wide range of adverse effects associated with steroid therapy were assessed. The need for alternative medications and the adverse effects associated with those were also studied, which gives us an idea about various clinical implications of therapy apart from the therapeutic benefits.

However, a larger study with a bigger sample size would have represented the associations more reliably. We couldn't assess the cumulative steroid dose for all the patients due to a lack of adequate treatment records from outside hospitals, which if present could help us correlate the association of cumulative dose with the adverse effects. We couldn't obtain a statistically significant difference in the receptor expression of GR b subtype between the two groups, because of a large number of outliers and inadequate sample size. Appropriate

responsiveness could not be ascertained in some patients who were on both steroids as well as CNI, as CNIs tend to alter responsiveness to steroids.

Conclusion

Our findings suggest that NR3C1 gene variants may have a significant role in determining steroid responsiveness in pediatric nephrotic syndrome based on the current sample size. However, further studies with larger cohorts are required to validate these results and explore additional gene–environment interactions that may influence steroid sensitivity.

Abbreviations

CNI	Calcineurin inhibitor
IFRNS	Infrequently Relapsing Nephrotic Syndrome
FRNS	Frequently Relapsing Nephrotic Syndrome
FSGS	Focal Segmental Glomerular Sclerosis
GC	Glucocorticoid"
GR	Glucocorticoid Receptor
IgAN	IgA nephropathy
ISKDC	International Study of Kidney Disease in Children
MCD	Minimal Change Disease
MMF	Mycophenolate Mofetil
MN	Membranous Nephropathy
MPGN	Membranoproliferative Glomerulo Nephropathy"
NR3C1	Nuclear receptor superfamily 3, group C, member 1
NS	Nephrotic Syndrome"
PBMC	Peripheral Blood Mononuclear Cell
PLA2R	Phospholipase 2 A Receptor
PRG	Prednisolone Resistant Group
PSG	Prednisolone Sensitive Group
RFLP	Restriction fragment length polymorphism
RT-PCR	Reverse Transcriptase – Polymerase Chain Reaction
SDNS	Steroid Dependent Nephrotic Syndrome
SNP	Single Nucleotide Polymorphism
SRNS	Steroid Resistant Nephrotic Syndrome
SRNS-IR	Steroid Resistant Nephrotic Syndrome- Initial Resistance"
SRNS-LR	Steroid Resistant Nephrotic Syndrome- Late Resistance
SSNS	Steroid Sensitive Nephrotic Syndrome

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Author contributions

Conceptualization: P.S. L.D., K.T.; methodology, S.P., A.K., C.B., P.B.; software, S.P., P.S.; validation, P.S., K.T.; formal analysis, S.P., A.K., P.S.; resources, K.T., L.D., I.P., A.K.; writing—original draft preparation, S.P., P.S.; writing—review and editing, P.S., C.B.; supervision, P.S., K.T.; funding acquisition, P.S. All authors have read and agreed to the published version of the manuscript.

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Data availability

The datasets (SNPs) analysed during the current study are available in the dbSNP repository, [https://www.ncbi.nlm.nih.gov/snp/rs6877893 & https://www.ncbi.nlm.nih.gov/snp/rs10482634].

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Postgraduate Institute of Medical Education and Research (PGIMER) Institutional Ethical Committee (Ref No: IEC-INT/2022/MD-817). Informed, written consent to participate in this study was obtained in accordance with the Declaration of Helsinki from all study participants or by the parents or guardians when participants were under 16 years of age or unable to provide informed consent.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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