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ORIGINAL ARTICLE

Eye involvement in children with primary focal segmental glomerulosclerosis

Fatih Ozaltin · Saskia Heeringa · Ceren Erdogan Poyraz · Yelda Bilginer · Sibel Kadayifcilar · Nesrin Besbas · Rezan Topaloglu · Seza Ozen · Friedhelm Hildebrandt · Aysin Bakkaloglu

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Abstract Distinct eye abnormalities have been described in children with nephrotic syndrome, particularly in diffuse mesangial sclerosis (i.e. Pierson syndrome). The aim of the study was to investigate whether there were any associated ocular anomalies in children with steroid-resistant nephrotic syndrome (SRNS), all of whom had revealed primary focal segmental glomerulosclerosis in biopsy. Thirty-three SRNS patients (16 male, 17 female) with a median age of 10.5 years (range 3–25 years) were enrolled in the study. Twenty steroid-sensitive nephrotic syndrome (SSNS) patients (ten male, ten female) with a median age of 8 years (range 3–15 years) served as controls. All SRNS patients were examined by mutational analysis for mutations in the *NPHS2*, *WT1,* and *LAMB2* genes. Nine out of 33 SRNS patients (27.2%) showed various eye abnormalities. However, no abnormal ocular findings were detected in any of the SSNS patients. Abnormal eye findings detected in SRNS patients were anisometropic amblyopia (n=4), Mittendorf's dots (n=4), myopic astigmatism (n=3) and exotropia (n=1). Macular pigment changes (n=1), posterior subcapsular opacities (n=1) and cataract (n=1) were considered as steroid-induced side effects. In four patients, more than one eye abnormality was found. Mutational analysis for the *NPHS2, WT1* and *LAMB2* genes revealed disease-causing mutations in 24.2% of patients. Homozygous *NPHS2* mutations were detected in five patients (15.1%), all of whom had parental consanguinity. In three patients (9%) from non-consanguineous parents, heterozygous de novo *WT1* mutations were detected as disease-causing mutations. No *LAMB2* mutation was detected in any patient. While four out of five (80%) patients with homozygous *NPHS2* mutations showed at least one abnormal ocular finding (i.e. Mittendorf's dot or anisometric amblyopia), none of the patients with a *WT1* mutation had ocular involvement. In conclusion, ocular involvement may accompany SRNS caused by primary focal segmental glomerulosclerosis (FSGS). Ophthalmologic evaluation at the time of diagnosis might be beneficial to characterize further the spectrum of this possible association.

Keywords Childhood · Focal segmental glomerulosclerosis · Eye · Steroid-resistant nephrotic syndrome

F. Ozaltin (✉) · Y. Bilginer · N. Besbas · R. Topaloglu · S. Ozen · A. Bakkaloglu
Unit of Nephrology, Department of Pediatrics, Hacettepe University Faculty of Medicine, Sihhiye, 06100 Ankara, Turkey
e-mail: fozaltin@hacettepe.edu.tr

S. Heeringa · F. Hildebrandt
Department of Pediatrics, University of Michigan, Ann Arbor, MI, USA

C. E. Poyraz · S. Kadayifcilar
Department of Ophthalmology, Hacettepe University Faculty of Medicine, Ankara, Turkey

Introduction

Nephrotic syndrome (NS), characterized by proteinuria, hypoalbuminemia, edema, and hyperlipidemia, is one of the most frequent glomerular diseases encountered in children [1, 2]. For decades, NS has been separated into two broad categories on the basis of the response to standard steroid therapy, i.e. steroid-sensitive NS (SSNS) and steroid-resistant NS (SRNS) [1, 2]. NS is a clinical heterogeneous condition characterized by histologic variants [3, 4] and different genetic backgrounds [5–10]. In SRNS, approximately 75% of patients exhibit renal histologic features of

focal segmental glomerulosclerosis (FSGS) and 20% demonstrate minimal-change NS (MCNS). Conversely, in SSNS, renal histologic features indicate MCNS in 80% of cases and FSGS in 20% [11]. In recent years, advances in the molecular genetics of familial NS have led to the discovery of specialized molecules located on podocytes that are mutated in SRNS. Several genes have been identified by positional cloning as causing SRNS in humans. These include the recessive genes, *NPHS1* (nephrin) [5], *NPHS2* (podocin) [6], *LAMB2* (laminin-β2) [8], *PLCE1* (phospholipase C epsilon 1) [10] as well as the dominant genes *WT1* (Wilms tumor suppressor gene 1) [12, 13], *ACTN4* (actinin alpha-4) [7] and *TRPC6* (canonical transient receptor potential 6 ion channel) [9]. However, in most cases of isolated SRNS and in the majority of patients with syndromic forms associated with neurological, ocular, skeletal, and other abnormalities, the genetic basis remains unclear [14].

Various types of ocular defects involving the lens, retina and cornea have been described in syndromes that are associated with renal glomerular defects [15–19]. Moreover, in certain glomerular or tubular diseases, some particular ocular findings have been reported that are specific for that disease or are being encountered more frequently than those seen in the general population. Since certain genes are involved in the early development of both eye and renal glomerulus, it is rational to detect ocular involvement in patients with genetic kidney diseases.

In the present study, we aimed to evaluate whether there is an association with a certain eye phenotype in children with biopsy proven primary FSGS presenting with steroid-resistant nephrotic syndrome (SRNS) along with mutational analysis by direct sequencing of *NPHS2*, *WT1* and *LAMB2*.

Patients and methods

Patients

This study was approved by the local Ethics Committees of Hacettepe University Faculty of Medicine and of the University of Michigan. Informed consent/assent of the parents and/or the patients was obtained. Inclusion criteria were (1) biopsy proven primary FSGS presented with steroid-resistant nephrotic syndrome, which was diagnosed according to published criteria [20], (2) patients/parents who gave written informed consent/assent for the study. Criteria for exclusion from the study were (1) secondary and syndromic FSGS, (2) presence of any systemic illness other than FSGS, (3) any clinically severe co-morbid condition, including marked hypertension.

Overall, 33 patients (16 male, 17 female) with biopsy proven primary FSGS were enrolled in the study. The median age of the patients was 10.5 years (range 3–25 years), and the median age at disease onset was 6 years (range 1–13 years). Consanguinity between parents was noted in 15 patients (45%) (Table 1). Detailed demographic and clinical data were ascertained from the hospital records of each patient (Table 1). Twenty patients (ten male, ten female), with a median age of 8 years (range 3–15 years) with steroid-sensitive nephrotic syndrome (SSNS) served as controls. The median age at onset for the control group was 5 years (range 2–11 years).

Detailed ophthalmologic examination was performed by two experienced ophthalmologists (C.E.P. and S.K.).

Ophthalmological examination

All patients underwent ophthalmological examination consisting of visual acuity determination with the most advanced chart (Teller, Lea or Snellen visual acuity chart). According to the patient's age, refractive error was determined by cycloplegic retinoscopy, approximately 45 min following instillation of cyclopentolate 1% drops twice (10 min apart) into each eye. Evaluation of strabismus (cover–uncover tests and eye movements), slit lamp biomicroscopy of the anterior segment before and after cycloplegia, and fundus examination after dilatation were also performed.

Mutational analysis

All SRNS patients were screened by mutational analysis for *NPHS2*, *WT1* and *LAMB2*. Genomic DNA was isolated from blood samples with the Puregene DNA purification kit (Gentra, Minneapolis, MN, USA) in accordance with the manufacturer's guidelines. Mutation analysis was performed by exon-flanking direct sequencing of all eight exons of *NPHS2*, exons 8 and 9 of *WT1* and all 32 exons of *LAMB2*. Exon primers are available from the authors on request. Polymerase chain reaction (PCR) products were sequenced with an ABI automated sequencer (Applied Biosystems, CA, USA). For sequence analysis the software SEQUENCHER (Gene Codes, Ann Arbor, MI, USA) was used. For all detected mutations and other sequence variants, sequencing of both strands was performed. The absence of previously unpublished mutations was shown in 160 control chromosomes from healthy individuals of matched ethnic origin.

Statistical analysis

The results were analyzed with SPSS, version 11.0 (SPSS Inc., Chicago, IL, USA) and were expressed as median (minimum–maximum) for data not showing normal distribution and as mean ± SD for data showing normal

Table 1 Demographic characteristics of 33 patients with primary FSGS (*A* acute presentation, *Cre* creatinine, *F* female, *M* male, *N* no, *Y* yes, *Pr* protein, *R* during regular examination)

Patient no.	Age (years)	Gender	Age at onset of nephrotic syndrome (years)	Parental consanguinity	Clinical presentation	Initial serum albumin (g/dl)[a]	Urinary Pr/Cre ratio	Hematuria
1	11	F	1	Y	R	2.40	42	Y
2	16	M	10	Y	A	1.40	24	N
3	10	M	9	Y	A	3.40	3.40	Y
4	13	M	5	N	A	2.20	28	N
5	15	F	5	N	A	3.10	2	N
6	8	M	3	Y	A	2.30	5.87	N
7	10	F	2	N	A	1.80	24	N
8	3	F	2	Y	R	2.90	1	N
9	14	F	11	N	R	3.20	10	N
10	9	F	7	Y	A	1.50	15	N
11	9	F	6	N	A	1.40	22	N
12	13	M	7	N	A	1.30	36	N
13	14	F	13	N	A	3.50	1.60	N
14	10	M	9	N	A	2.40	4.20	Y
15	9	M	6	Y	A	3.50	8	N
16[b]	15	F	5	Y	R	2.10	21	Y
17[b]	5	M	3	Y	R	2.20	8.40	Y
18	9	M	2	N	A	1.30	73	N
19	12	M	8	Y	A	1.40	24	N
20	11	F	9	Y	A	1.50	22	N
21	10	M	7	N	A	3.30	21	N
22	14	M	11	N	A	2.60	16	N
23	11	M	3	Y	R	1.50	46	Y
24	9	F	4	N	A	1.2	32	Y
25	8	F	7	N	R	2.91	8.20	N
26	12	M	10	N	A	3	9	N
27	11	M	6	Y	A	1.6	16.20	Y
28	16	F	3	N	A	3.7	16	N
29	9	F	1	N	A	1.30	2.25	N
30	6	M	3	Y	A	1.60	12.10	Y
31	15	F	4	N	R	2.10	8.20	N
32	25	F	12	N	A	1.80	7.40	N
33	4	F	3	Y	A	1.80	56	Y

[a] Milligrams per milligram
[b] Patients 16 and 17 are siblings

distribution. The Mann–Whitney U test was used to compare study groups. A P value less than 0.05 was considered significant.

Results

Clinical characteristics

Twenty-five patients (75.7%) with SRNS presented acutely with nephrotic range proteinuria; eight patients were identified by non-nephrotic proteinuria during regular examination (Table 1). Mean initial serum albumin and urinary protein/creatinine ratio (milligrams per milligram) were 2.2±0.8 g/dl and 18.5±16.6, respectively, in the SRNS group, and 1.9±0.69 g/dl and 4.8±5.9, respectively, in the SSNS group ($P>0.05$). Ten patients (30.3%) also had hematuria (Table 1). Hypertension was noted in two patients (patients 2 and 21). Renal biopsy revealed FSGS in all SRNS patients.

Initial treatment for all patients consisted of oral prednisone administration (60 mg/m^2 per day, in divided doses for 4 weeks, followed by 40 mg/m^2 every other day in a single dose for 4 weeks) according to the protocols of the International Study of Kidney Disease in Children (ISKDC) and the Arbeitsgemeinschaft für Pädiatrische Nephrologie [21, 22]. When this treatment failed to induce remission, then cyclophosphamide (2 mg/kg per day, 12 weeks) and/or pulse doses of methyl prednisolone (20–30 mg/kg, three consecutive days, once a month for

6 months) and/or cyclosporine A (2–3 mg/kg per day) were added to low-dose alternate-day oral steroid therapy. Overall, while 13 patients showed primary resistance to initial steroid treatment, partial response was noted in 20 patients (Table 2). Median follow-up time was 54 months (range 4–180 months) for the SRNS patients and 33 months (range 12–120 months) for the SSNS patients. The duration of steroid treatment and time of ophthalmologic examination for each patient are given in Table 2.

Eye findings

Nine out of 33 patients (27.2%) had various abnormal eye findings, which were not related to drug therapy. These were anisometropic amblyopia (n=4), Mittendorf's dots (n=4), myopic astigmatism (n=3) and exotropia (n=1). Macular pigmentary changes in patient 15, cataract in patient 16 and posterior subcapsular opacities in patient 21 were thought to be secondary to steroid treatment, and, therefore, these findings were not included in the calculations. Four patients had two distinct abnormal eye findings at the same time (Table 3).

Table 2 Clinical characteristics of the patients (*PR* partial response to steroid, *SR* primary steroid resistance, *Y* yes, *N* no)

Patient no.	Duration of steroid therapy (months)	Response to steroid	Follow-up time and the time of ophthalmologic examination (months)	Steroid treatment at the time of eye examination
1	62	SR	72	Y
2	36	PR	72	N
3	8	PR	9	N
4	60	PR	96	Y
5	24	PR	84	N
6	12	SR	66	N
7	72	PR	96	Y
8	8	PR	12	N
9	36	PR	36	Y
10	24	PR	24	Y
11	24	PR	36	N
12	36	PR	72	N
13	8	PR	16	N
14	14	SR	28	Y
15	16	SR	32	Y
16	60	SR	116	Y
17	12	SR	39	N
18	64	PR	84	N
19	48	PR	48	Y
20	14	PR	26	N
21	84	PR	108	N
22	12	PR	12	Y
23	48	SR	96	Y
24	24	PR	60	N
25	11	PR	11	Y
26	24	PR	24	Y
27	54	SR	60	Y
28	30	PR	156	N
29	54	SR	96	N
30	26	SR	26	Y
31	96	SR	132	Y
32	72	SR	180	N
33	12	SR	12	Y

Mutational analysis

Mutational analysis for the *NPHS2*, *WT1* and *LAMB2* genes revealed disease-causing mutations in eight out of 33 patients with SRNS (24.2%). Homozygous *NPHS2* mutations were detected in five patients from different families (15.1%), all of whom had parental consanguinity (patients 6, 16, 17, 23, 33; see Table 3). One patient (patient 5) was carrying the Arg229Gln variant heterozygously, which did not explain the disease phenotype. In three patients (9%) from non-consanguineous parents, heterozygous de novo *WT1* mutations were detected as disease-causing mutations (patients 12, 25, 31; Table 3). No *LAMB2* mutation was detected in any patient. Four out of five (80%) patients with homozygous *NPHS2* mutations showed at least one abnormal ocular finding (i.e. Mittendorf's dot or anisometric amblyopia). Interestingly, Mittendorf's dot was present in two siblings (patients 16 and 17) who were carrying the same homozygous *NPHS2* mutation. In six patients without mutations in *NPHS2* or *WT1,* seven different eye abnormalities were found: anisometropic amblyopia (patients 11, 14), myopic astigmatism (patients 13, 20), macular pigmentary changes (patient 15), posterior subcapsular opacities (patient 21) and Mittendorf's dot (patient 20) (Table 3). However, the ocular changes (i.e. macular pigmentary changes and posterior subcapsular opacities) in patients 15 and 21 were considered to be steroid-induced side effects. No abnormal ocular findings were found in three patients with a disease-causing mutation in *WT1*. In the control group (SSNS patients), no ocular involvement was detected.

Discussion

In this study we examined a single-center cohort of 33 SRNS children with biopsy-proven primary FSGS for mutations in *NPHS2*, *WT1* and *LAMB2* for the association with ophthalmologic symptoms. While ocular involvement was found to be present in nine out of 33 (27.2%) children with SRNS, in the SSNS control group no ocular changes were found. Four out of five patients (80%) with disease-causing *NPHS2* mutations showed at least one ocular abnormality. No eye involvement was found in patients with a *WT1* mutation.

Table 3 Abnormal eye findings, and mutational analysis performed for *WT1* and *NPHS2*

Patient no.	Eye findings	*WT1*	*NPHS2* mutation (amino acid exchange)
1	None		
2	None		
3	None		
4	None		
5	Mittendorf's dot		Ex 5 c.686G->A=Arg229Gln (het)
6	Anisometropic amblyopia, exotropia		Ex 7 c.803T->G =Val268Gly (homo)
7	None		
8	None		
9	None		
10	None		
11	Anisometropic amblyopia		
12	None	Ex 9 IVS9+5 G/A (het)	
13	Myopic astigmatism		
14	Anisometropic amblyopia		
15	Macular pigmentary changes		
16	Mittendorf's dot, cataract		Ex 4 c. 460-467 insT = Val165X (homo)
17	Mittendorf's dot, myopic astigmatism		Ex 4 c. 460-467 insT = Val165X (homo)
18	None		
19	None		
20	Mittendorf's dot, myopic astigmatism		
21	Posterior subcapsular opacities		
22	None		
23	Anisometropic amblyopia		Ex2 c.353C->T = Pro118Leu (homo)
24	None		
25	None	Ex 9 c.1228+4C/T (het)	
26	None		
27	None		
28	None		
29	None		
30	None		
31	None	Ex 9 c.1228+4C/T (het)	
32	None		
33	None		Ex 2 c.353C->T = Pro118Leu (homo)

Mutational analysis was performed for the *NPHS2*, *WT1*, and *LAMB2* genes in each patient. *Ex* exon, *homo* homozygous, *het* heterozygous

Association between SRNS and other organs are increasingly being recognized. In our study, we report the prevalence of ocular findings in patients with SRNS. The question is whether this association is really true or just an incidental finding. Recent evidence suggests that genetic defects causing SRNS might also be a reason of ocular involvement [8]. Ocular involvement, in fact, has been well known in various hereditary diseases associated with certain glomerular disorders characterized by nephrotic syndrome, such as Pierson syndrome, in which mutations in the *LAMB2* gene are causative [8, 23]. There is growing body of evidence that slit diaphragm proteins are also expressed outside of the kidney and might have a functional significance in the development of other organs [5, 24]. For podocin, recent studies have demonstrated its expression in fetal heart [6]. Frishberg et al. [25] have recently confirmed this observation clinically, demonstrating various cardiac anomalies in 89% of patients with homozygous *NPHS2*

mutations at diagnosis of SRNS. In our study, we found ocular involvement in 4/5 (80%) of the patients with homozygous *NPHS2* mutations, which might be attributed to a possible role of *NPHS2* in eye development. Evidence supporting this suggestion might be that: (1) none of the patients with SSNS had ocular involvement, (2) none of the patients with *WT1* mutation had any ocular anomalies, (3) Mittendorf's dot was diagnosed in four out of 33 patients (12%) with FSGS and in 4/9 patients (44.4%) with abnormal eye findings. Moreover, it was present in two siblings (patients 16 and 17) who were carrying the same homozygous *NPHS2* mutation. Although the true prevalence and incidence of Mittendorf's dot in healthy populations is not known, it is believed to be extremely rare [26]. Mittendorf's dot, also called the hyaloid body, represents the remains of the anterior end of the hyaloid artery and is not related to any drugs. It appears as a small axial or nasally paraxial gray-white dot opacity at the posterior apex of the lens. It is, in itself, visually insignificant unless it is large, which is rare, and may then represent a mild form of persistent hyperplastic primary vitreous (PHPV). Occasionally, it may be associated with posterior lenticonus and sometimes with a persistent hyaloid artery. Usually, it is stable, not requiring surgery, but progression has been noted [26]. Another important eye finding was anisometropic amblyopia, which is a common cause of amblyopia that has an estimated prevalence in children ranging from 2% to 5% [27]. However, in our study, we found it in four out of 33 patients (12.1%). Unequal refractive error between two eyes could produce abnormal binocular interaction and/or visual deprivation, when severe [28]. Astigmatism is a common refractive anomaly. Myopic astigmatism is a condition in which the eye is affected with myopia in one meridian only. Its prevalence has been reported to be between 1.4% and 19.8% in different population-based studies [29]. In our patient population, we found it to be 9%, which did not differ from that seen in the general population. We also found macular pigmentary changes in one patient. It can be an isolated finding, maybe associated with many different systemic disorders. However, there is one report on an association with corticosteroid use [30], so we cannot exclude definitely that it might be a steroid-induced side effect. Similarly, posterior subcapsular opacities and cataract formation in two patients were thought to be adverse effects of corticosteroids.

In our study we detected disease-causing mutations in *NPHS2* and *WT1* in eight out of 33 patients with SRNS (five with mutations in *NPHS* and three in *WT1*). The number of detected *NPHS2* mutations (15.1%) is in agreement with that in another Turkish study, in which five different *NPHS2* mutations in four of 30 (13.3%) families was detected [31]. In a large cohort, Ruf et al. [32] reported homozygous or compound heterozygous mutations in *NPHS2* for 29% of familial and sporadic SRNS patients. Caridi et al. [33] observed homozygous or compound heterozygous mutations in *NPHS2* for 12% of SRNS patients. In contrast, Maruyama et al. [34] performed mutational analysis for 36 Japanese children with SRNS without detecting any mutation in *NPHS2*. These discrepancies stem from genetic heterogeneity between different ethnic groups. Thus, every population should establish its own genetic profile for particular diseases. We detected *WT1* mutation as an underlying genetic defect in 3/33 (9%) of all patients, which is in agreement with previous studies [12, 13]. In 75% of all SRNS patients, we did not find mutations in *NPHS2*, *WT1* or *LAMB2*. This further confirms the clinical and genetic heterogeneity of the disease and suggests the existence of more disease-causing gene(s).

In conclusion, ocular involvement may accompany primary FSGS manifested by SRNS independent of treatment. Ophthalmologic evaluation at the time of diagnosis might be beneficial to characterize further the spectrum of this possible association. Further studies in larger cohorts are needed to draw a certain conclusion.

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