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Clinical and Genetic Evaluation of Familial Steroid-Responsive Nephrotic Syndrome in Childhood

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Abstract. Steroid-responsive idiopathic nephrotic syndrome (SSINS) is the most common form of nephrotic syndrome in childhood. This article reports a cohort of familial SSINS with disease onset in childhood. The clinical course in terms of age at onset, symptoms during the initial phase, renal morphology, and outcome was evaluated. Furthermore, linkage to *NPHS2*, the gene for autosomal-recessive steroid-resistant INS on chromosome 1, was examined. Two families with haplotypes consistent with linkage to *NPHS2* were evaluated for mutations in the *NPHS2* gene. Familial SSINS (32 patients from 15 families, minimal change NS in 12 of 12 biopsies) was found to be a clinically homogeneous entity. Interfamilial and intrafamilial variability with respect to the age at disease onset was low, indicating a strong genetic influence on disease onset. By linkage studies and mutational analysis, familial SSINS was found to be genetically distinct from *NPHS2*. This is the first report of a large cohort of familial SSINS. Exclusion of linkage to *NPHS2* makes likely the existence of a distinct gene locus for SSINS.

The most common cause of nephrotic syndrome in children is idiopathic nephrotic syndrome (INS), with the pathohistologic equivalents of minimal change NS (MCNS) or focal segmental glomerulosclerosis (FSGS). Most children with sporadic INS respond to steroid treatment; the disease outcome is favorable without progression to end-stage renal disease (ESRD). Numerous case reports (1–3) as well as larger population studies of familial occurrence of INS (4–8) have been published since 1970; however, reports on larger cohorts of familial SSINS are lacking.

Several genes involved in familial steroid-resistant INS (SRINS) are known. *NPHS2*, located on chromosome 1q25, has been mapped in a subgroup of autosomal-recessive SRINS with disease onset in early childhood and rapid progression to ESRD (8). In most of the patients, renal biopsies showed FSGS; recurrence of the disease in the renal allograft was not observed. The *NPHS2* gene has recently been identified (9). It is predicted to be an integral membrane protein (podocin), which is exclusively expressed in glomerular podocytes. Podocin is supposed to interact with other proteins involved in nephrotic syndromes, such as nephrin and α-actinin-4, and thereby play a major role in the regulation of the actin cytoskeleton. α-actinin-4, an isoform of α-actinin located on chromosome 19q13, causes an autosomal-dominant variant of FSGS (FSGS1), with later onset in adulthood (10,11). A second autosomal-dominant FSGS gene (FSGS2) maps on chromosome 11q21-q22 and has not yet been identified (12).

The aim of our study was to characterize a further subgroup of autosomal-recessive INS. Familial steroid-responsive INS (SSINS) seems to be clinically homogeneous and genetically distinct from other variants of nephrosis and therefore appropriate for genetic analysis by using positional cloning approaches.

Materials and Methods

Patients

Families with at least two affected members presenting with SSINS (including frequent relapsing and steroid-dependent INS) were included in the study (for standard definitions of response and recurrence as well as frequent relapsing and steroid-dependent INS, see references 13 and 14). The following features were evaluated by using a standard questionnaire: age of onset, clinical symptoms (edema, BP), urine status (proteinuria, hematuria), renal function (GFR according to Schwartz *et al.* (15)), response to steroid therapy including alternative drug treatment (alkylating agents, cyclosporine), and report of the renal biopsy, if performed. Renal biopsy specimens—when available—were reassessed by one reference pathologist. Intrafamilial variability regarding the age of onset was evaluated by calculating the Spearman rank correlation coefficient.

Linkage Studies

Genomic DNA was extracted from leukocytes according to standard laboratory protocol. PCR was performed as described previously (8). Five polymorphic microsatellite markers, D1S416, D1S480, D1S215, D1S2883, and LAMC2 (16,17), spanning the region of interest were analyzed. Genetic linkage analysis was carried out using

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LINKAGE package, version 5.3 (18), assuming an autosomal-recessive mode of inheritance. The disease frequency was set to 0.001;, penetrance was assumed to be complete.

The mutation analysis in the *NPHS2* gene was performed in patients INS3II:1 and INS30II:1. Exons 1 to 8 where amplified by using flanking intronic primer pairs as described previously (9). Amplified DNA segments were subjected to direct sequencing of both strands by using an automated ABI 373 A sequencer (Applied Biosystems, Norwalk, CT). Before sequencing, the amplified DNA segments were cleaned with the QIAquick system (Quiagen, Hilden, Germany) and precipitation (ethanol) protocol described previously (19).

Results

Families with autosomal-recessive SSINS occurring in infancy and childhood were examined. In total, 15 families with 32 affected individuals and 17 healthy siblings were assessed. They originated from Germany, Switzerland, Italy, and Czech Republic. Pedigrees are shown in Figure 1. The occurrence of the disorder in siblings—but not in previous generations—and the incidence of inbreeding in three families (INS6, 15, and 24) made an autosomal-recessive mode of inheritance very likely. The two affected siblings of kindred INS15 presented with the association of SSINS and postaxial hexadactyly. All patients initially presented with proteinuria, which exceeded 40 mg/m^2 per h, and acute edema (Table 1). Renal function and BP were always normal; microhematuria was observed in five patients (families INS1, 15, 30, and 45; Table 1). No or less than four relapses were reported in 20 of 32 patients; more than four relapses occurred in 12 patients, 3 of whom have been designated as frequent relapsers (INS15II:2, 23II:3, 30II:1) and five as steroid dependent (INS15II:3, 20II:2, 24II:1, 39II:1, 48II:4). In addition to corticosteroids, drug treatment in frequent relapsing and steroid dependent NS included cyclophosphamide (15 patients), cyclosporin A (4 patients), and levamisole (2 patients). Renal biopsies were performed in 12 children (9 of 15 families; Table 1) and showed MCNS in all cases.

The age of onset varied between 7 mo and 14 yr with a median age of 3.4 yr (quartiles 2 to 5 yr; Table 1). Except for 3 of 32 children (families INS6, 20, and 39), the disease occurred within the first 7 yr of life. Furthermore, with the exception of two families (INS20 and 39), the difference in the age of onset between siblings did not exceed 4 yr (Figure 2). To evaluate the hypothesis of low intrafamilial variability regarding the age of onset, we computed the Spearman rank correlation coefficient for two ranks, defined as the first and the second affected child, respectively. The correlation was $r_s = 0.60$, which corresponds to a $t_{(13;97,5)}$ value of 2.72. This result is highly significant on the 5% level, indicating strong intrafamilial concordance, consistent with a strong genetic influence on disease onset.

The disease outcome was favorable in all children with normal renal function and normal BP at the last examination. All patients remained steroid responsive. With the exception of three children with relapse of nephrotic syndrome at the time of the last examination and in whom proteinuria was observed,

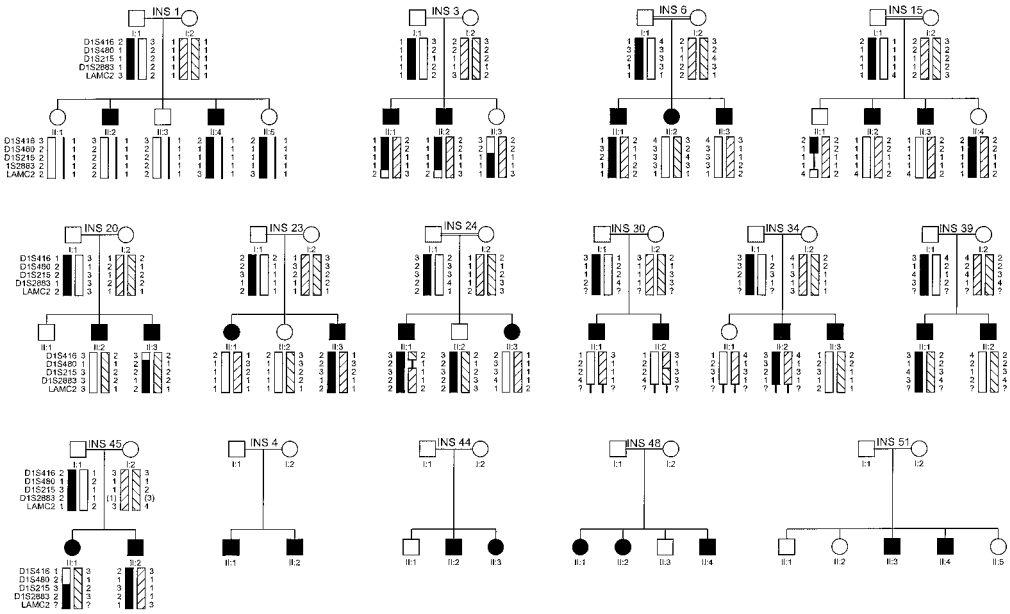

Figure 1. Pedigrees of 15 families with familial steroid-responsive idiopathic nephrotic syndrome (SSINS). In 11 families, DNA was available for haplotype analysis at the *NPHS2* locus. Haplotypes were generated by using five consecutive microsatellite markers that span the critical genetic *NPHS2* region on chromosome 1q25. Haplotypes were generated by minimizing recombinants and are shown as differently shaded bars. Affected individuals are indicated by black symbols. Males are shown as squares, females as circles. Consanguinity is indicated by double bars connecting parents. Paternal haplotypes are drawn to the left, maternal haplotypes to the right. Marker order from top to bottom: cen- D1S416, D1S480, D1S215, D1S2883, and LAMC2-tel (markers that flank the *NPHS2* region are underlined). Note that in SSINS families INS3 and 30, haplotype data were compatible with linkage to the *NPHS2* locus on the basis of cosegregation of the respective haplotypes.

Table 1. Clinical data of the patients with familial steroid-responsive INS[a]

Family No.	Individual	Gender	Predominant Symptoms at Presentation				Age (yr) at Renal Biopsy	Symptoms at Last Examination	
			Age at Onset (yr)	PU (mg/m^2/h)	HU	Creatinine (mg/dl)/GFR (ml/min/1,73 m^2)		Creatinine (mg/dl)/GFR (ml/min/1,73 m^2)	PU (mg/m^2/h)
INS 1	II:2	m	0,7	125	1	0,3/155	n.d.	0,77/94	neg.
	II:4	m	3,4	166	neg.	0,5/110	MCNS (5)	0,63/104	neg.
3	II:2	m	6,0	410	neg.	0,67/92	n.d.	0,68/121	neg.
	II:3	m	3,7	280	neg.	0,4/130	n.d.	0,56/162	neg.
4	II:1	m	2,0	60	neg.	0,67/88	MCNS (14)	0,83/119	neg.
	II:2	m	2,5	80	neg.	0,54/91	n.d.	0,76/110	neg.
6	II:1	m	5,5	250	neg.	0,5/130	MCNS (7,5)	0,68/133	13
	II:2	f	4,2	700	n.d.	0,45/120	MCNS (5)	0,64/135	120
	II:3	m	8,1	180	neg.	0,56/120	n.d.	0,44/173	neg.
15	II:2	m	2,5	500	1	0,3/196	MCNS (3,6)	0,77/112	neg.
	II:3	m	3,8	330	neg.	0,3/170	MCNS (4)	1,08/86	neg.
20	II:2	m	10,0	80	neg.	0,49/175	n.d.	0,63/154	25
	II:3	m	4,5	110	neg.	0,37/145	n.d.	0,38/188	neg.
23	II:1	f	3,0	60	neg.	0,37/167	n.d.	normal	neg.
	II:3	m	5,5	70	neg.	0,59/85	n.d.	normal	neg.
24	II:1	m	2,1	130	neg.	0,4/157	MCNS (3)	0,6/128	neg.
	II:3	f	4,0	220	neg.	0,3/179	n.d.	0,5/161	neg.
30	II:1	m	5,0	210	neg.	0,45/134	MCNS (7,5)	0,8/95	neg.
	II:2	m	1,8	200	1	0,3/179	n.d.	0,5/125	neg.
34	II:2	m	3,1	160	neg.	0,6/110	n.d.	normal	neg.
	II:3	m	3,5	240	neg.	0,6/193	n.d.	normal	neg.
39	II:1	m	14,0	110	neg.	0,5/158	n.d.	0,74/134	neg.
	II:2	m	5,0	160	neg.	0,4/135	MCNS (5,5)	0,87/125	neg.
44	II:1	m	1,5	80	neg.	0,32/144	MCNS (5,5)	normal	neg.
	II:3	f	2,0	120	neg.	0,38/130	MCNS (2,5)	normal	neg.
45	II:1	f	1,9	160	1	0.39/120	MCNS (3,5)	0,9/99	neg.
	II:2	m	1,5	480	1	0,42/108	n.d.	0,76/96	neg.
48	II:1	f	1,9	>200	neg.	0,6/86	n.d.	0,68/135	neg.
	II:2	f	2,0	>100	neg.	0,38/123	n.d.	0,73/115	neg.
	II:4	m	2,8	>200	neg.	0,4/130	n.d.	0,39/175	neg.
51	II:3	m	5,3	200	neg.	0,34/186	n.d.	0,38/165	neg.
	II:4	m	3,4	350	neg.	0,43/121	n.d.	0,45/122	neg.

[a] INS, idiopathic nephrotic syndrome; PU, proteinuria: nephrotic-range proteinuria is defined as >40 mg/m^2 per h; HU, hematuria; 1, microhematuria; n.d., not determined; normal, normal renal function, exact values not available. All patients presented with edema during the acute phase. BP was normal in all patients at disease onset, as well as at last examination.

the last urine examination was normal with respect to proteinuria and hematuria.

Genetic Analyses

To evaluate genetic variants of familial autosomal-recessive SSINS, we tested 11 SSINS families (INS1, 3, 6, 15, 20, 23, 24, 30, 34, 39, and 45) in whom genomic DNA was available for linkage to the gene locus *NPHS2* on chromosome 1q25 (8). Haplotype analysis was performed by using five consecutive polymorphic microsatellite markers spanning the critical genetic interval (Figure 1). Only 2 of 11 families (INS3, 30) were compatible with linkage to *NPHS2*. The results of the two-point linkage analysis are shown in Table 2. LOD scores of <-2 excluded linkage to *NPHS2* for all markers tested within a distance of ± 5 to 10 cM to the disease locus and within an interval of 1.5 cM between flanking markers D1S480 and D1S2883. To confirm that the respective *NPHS2* gene is not causative for the disease, we performed mutational analysis of the *NPHS2* gene in one affected individual (INS3II:1 and INS30II:1) out of the two families, INS3 and INS30, whose haplotypes were compatible with linkage to *NPHS2*. No mutation was detected in these patients (data not shown).

Discussion

In the present study, 15 multiplex families with autosomal-recessive SSINS were investigated. Our observations regarding

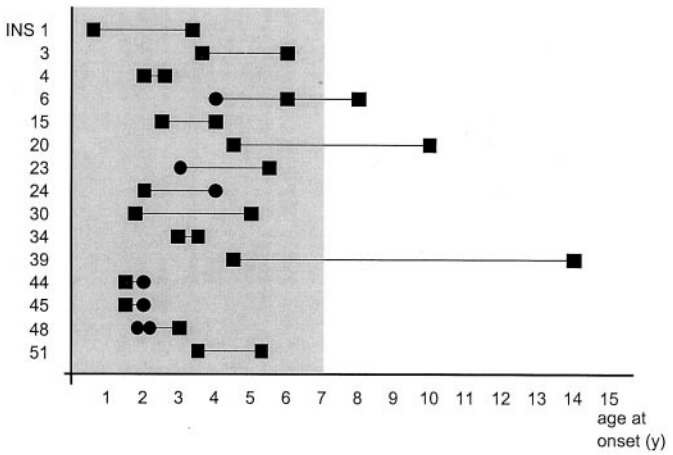

Figure 2. Intra- and interfamilial variability for age of onset of INS. Siblings are connected by horizontal lines. Males are shown as squares, females as circles. Family numbers are given along the vertical axis. The gray shaded area indicates the time period in which the disease occurs in the majority of the cases.

the clinical course of familial SSINS correspond to reports on sporadic SSINS. The presented cohort was found to be homogeneous in terms of renal morphology. The low number of renal biopsies in this cohort (12 of 32 patients) is explained by the fact that in children with SSINS and favorable disease course (only few relapses, no alternative drug treatment), renal biopsies are no longer routinely performed (20). The disease outcome regarding renal function and BP is favorable in all children. This is also true in patients who have been defined as frequent relapsers and steroid dependent. In terms of age at onset of the disease, we observed a low intrafamilial and interfamilial variability, reinforcing the assumption that familial SSINS is a clinically distinct subgroup of autosomal-recessive nephrosis.

Recent genetic findings confirmed the existence of hereditary forms of SRINS (8–10,12). However, corresponding reports in SSINS are lacking. The present study of a large cohort of familial SSINS revealed a disease entity appropriate for genetic analysis. In a first step toward identification of a gene involved in SSINS, we wanted to confirm the hypothesis that familial SSINS is not only clinically but also genetically distinct from SRINS. Linkage to *NPHS2* could be excluded in 9 of 11 SSINS families (82%; Figure 1), and in the remaining two families (INS3 and 30) no mutations in the *NPHS2* gene could be detected. These data corroborate the disparity to SRINS in addition to steroid responsiveness and favor the hypothesis that one or several distinct genes must be involved in hereditary SSINS. The association of familial SSINS with extrarenal symptoms represents an attractive additional tool for genetic investigation. They allow for investigation of candidate chromosomal regions or even direct candidate gene analysis.

We are planning a whole genome-wide linkage analysis in familial SSINS. Identification of a hitherto unknown gene involved in autosomal-recessive nephrosis will help us to provide new mechanisms of disease development in INS.

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Table 2. Two-point LOD scores between *NPHS2* locus and chromosome 1 markers of 11 SSINS families (INS1, 3, 6, 15, 20, 23, 24, 30, 34, 39, 45)[a]

Theta	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D1S416	−∞	−9.26	−3.53	−1.53	−0.23	0.04	0.03
D1S480	−∞	−11.67	−5.65	−3.28	−1.29	−0.46	−0.10
D1S215	−∞	−12.42	−5.80	−3.26	−1.20	−0.40	−0.08
D1S2883	−∞	−9.78	−4.50	−2.48	−0.87	−0.27	−0.05
LAMC2	−∞	−6.81	−2.91	−1.49	−0.44	−0.11	−0.02

[a] Markers that flank the *NPHS2* locus within a 1.5 cM interval are underlined. LOD, logarithm of odds. SSINS, steroid-responsive INS.

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