J Am Soc Nephrol 14: 1897–1900, 2003

Identification of the First Gene Locus (*SSNS1*) for Steroid-Sensitive Nephrotic Syndrome on Chromosome 2p

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Abstract. Disease mechanisms of steroid-sensitive nephrotic syndrome (SSNS) remain unknown. Whereas gene identification has furthered the understanding of pathomechanisms in steroid-resistant nephrotic syndrome (SRNS), not even a gene locus is known for SSNS. Total genome linkage analysis was performed in a consanguineous SSNS kindred to identify a gene locus for SSNS. Homozygosity mapping identified a locus for SSNS on chromosome 2p12-p13.2 between markers D2S292 and D2S289 (multipoint LOD score $Z_{max} = 3.01$ at D2S145). The first gene locus for SSNS, as a first step to detect the responsible gene, was thus identified. There was clear evidence for genetic locus heterogeneity upon examination of ten additional families with SSNS.

Nephrotic syndrome (NS), defined as the association of proteinuria and hypoalbuminemia with edema and hyperlipidemia, constitutes one of the most common diagnoses made in nephrology (1). The related pathogenesis has been elusive, however, despite decades of studies into its renal histology and protein biochemistry (2). Whereas gene identification by positional cloning has recently opened inroads into unraveling the pathogenesis of steroid-resistant nephrotic syndrome (SRNS) (2), not even a gene locus is known for the more frequent variant of steroid-sensitive nephrotic syndrome (SSNS). In SRNS, the majority of patients develop renal pathology of focal segmental glomerulosclerosis (FSGS), whereas renal biopsy in SSNS reveals minimal change nephrotic syndrome (MCNS) in 80% and FSGS in 20% (3). We recently ascertained worldwide and characterized clinically 11 kindred with familial occurrence of SSNS (3). This confirmed the existence of autosomal-recessive forms of SSNS and established the basis for a positional cloning approach to its pathogenesis. Linkage to the *NPHS2* locus for SRNS was excluded (3). To identify a gene locus for SSNS, we here conducted a total genome search for linkage in a consanguineous pedigree sufficiently large for linkage.

Received June 23, 2002. Accepted March 17, 2003.

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1046-6673/1407-1897
Journal of the American Society of Nephrology

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DOI: 10.1097/01.ASN.0000070070.03811.02

Materials and Methods

In a consanguineous SSNS kindred (INS6) originating from Germany, the parents of three affected children were second-degree cousins (Figure 1). All three children exhibited characteristic symptoms of SSNS as published previously (3). There was childhood onset proteinuria with acute edema at 5.5, 4.2, and 8.1 yr of age in individuals II:1, II:2, and II:3, respectively (Table 1). Renal function and BP were normal initially. Renal biopsy revealed MCNS in siblings II:1 and II:2. To test for genetic locus heterogeneity, ten additional multiplex families with SSNS, consistent with autosomal recessive inheritance, were examined by haplotype analysis. Clinical inclusion criteria were (*1*) proteinuria exceeding 40 mg/m^2 per h and acute edema; (*2*) initial response to steroid therapy; (*3*) normal renal function and normal BP (3). Genomic DNA was isolated using standard methods after receiving informed consent (3). A total genome search for linkage was performed in family INS6 using 371 microsatellite markers covering all autosomes (Center National de Genotypage, Evry, France; marker list available on request). Physical distances between markers were based on the UCSC Humane Genome Browser (http://genome.ucsc.edu). For further fine mapping on chromosome 2p12-p13.2, seven additional markers with an average distance of 1.24 Mb were used. Order and physical distances (in parentheses) between these markers from p-telomeric to centromeric are as follows: *D2S292* (1.2 Mb), *D2S291* (1.4 Mb), *D2S2111* (0.1 Mb), *D2S145* (0.2 Mb), *D2S2109* (1.9 Mb), *D2S286* (1.3 Mb), *D2S2116* (1.9 Mb), *D2S1777* (3.7 Mb), *D2S289* (0.7 Mb), *D2S1396*. Haplotype data from the total genome search was evaluated in tabular form, searching haplotypes for potential homozygosity by descent (4). LOD score calculations were performed by the LINKAGE program package (5), with the help of the LINKRUN computer program (TF Wienker unpublished). Semi-automated genotyping for further fine mapping at the *SSNS1* locus was performed with a MegaBACE-1000 analysis system. Data were analyzed by Genetic Profiler Software, version 1.1. To conduct multipoint linkage analysis, we used the program GENEHUNTER (6), based on a recessive model with full penetrance for affected individuals. Allele frequencies of tested markers were calculated

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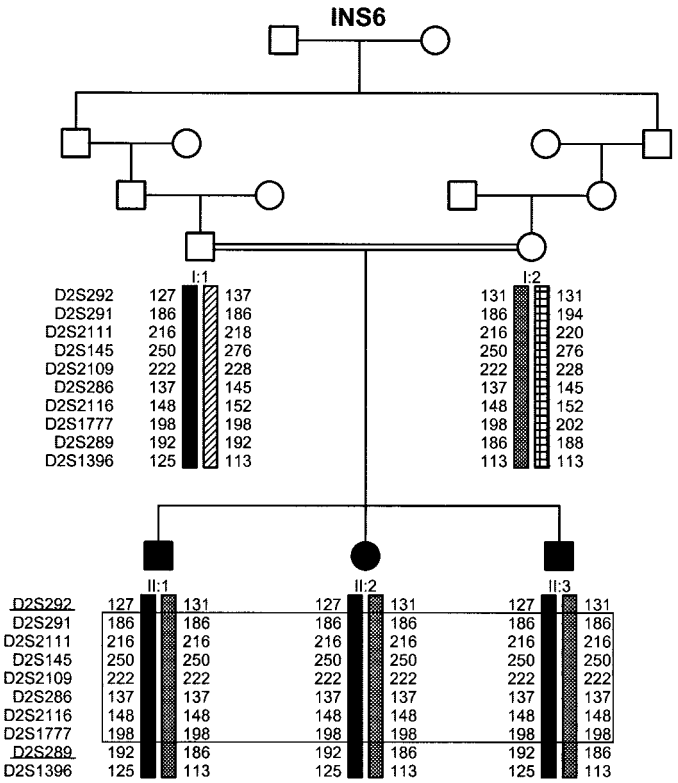

Figure 1. Pedigree and haplotype analysis in consanguineous family INS6. The parents of three children affected with steroid-sensitive nephrotic syndrome (SSNS) are second-degree cousins. Haplotypes are given for ten consecutive microsatellite markers in the critical *SSNS1* region on chromosome 2p12-p13.2 from top to bottom in telomere to centromere orientation. Marker names are shown with individual II:1. Alleles for consecutive markers (*i.e.*, haplotypes) are given as columns of Arabic numerals, representing the length of the alleles in bp. Paternal haplotypes are drawn to the left, maternal ones to the right. In addition, differently shaded bars symbolize haplotypes for clarity. Marker alleles continuously homozygous in all three patients are encased in a frame. Markers flanking the *SSNS1* locus by lack of homozygosity are underlined. Circles, females; squares, males; black symbols, affected individuals; white symbols, unaffected individuals.

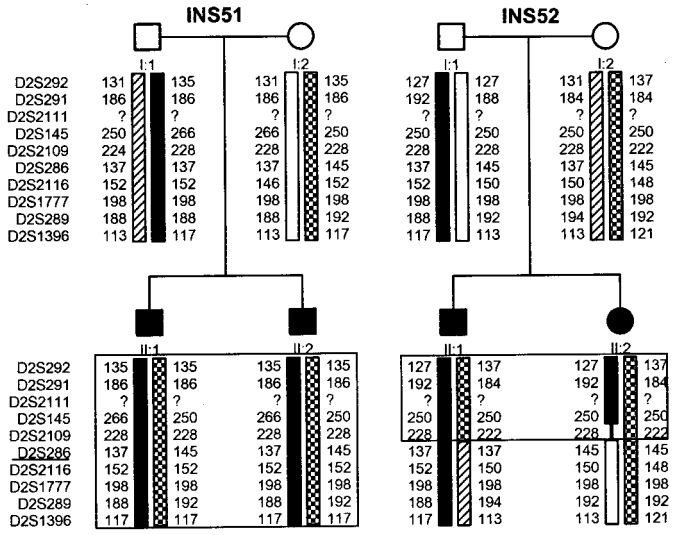

Figure 2. Pedigree and haplotype analysis in families INS51 and INS52. Haplotypes are given for ten consecutive microsatellite markers in the critical *SSNS1* region on chromosome 2p12-p13.2 from top to bottom in telomere to centromere orientation. Marker names are shown with individual II:1 of family INS51. Alleles for consecutive markers (*i.e.*, haplotypes) are given as columns of Arabic numerals, representing the length of the alleles in bp. Paternal haplotypes are drawn to the left, maternal ones to the right. In addition, differently shaded bars symbolize haplotypes for clarity. Markers compatible with linkage are encased in a frame. Circles, females; squares, males; black symbols, affected individuals; white symbols, unaffected individuals.

according to Center d'Etude du Polymorphisme Humain (CEPH) Database (http://www.cephb.fr/cgi-bin/wdb/ceph/systeme/form). Disease frequency was estimated at 10^{-4} (3). CYRRILIC version 2.1.3 (Cherwell Scientific, Oxford) was used for haplotype display.

Results

Total genome haplotype analysis in family INS6 revealed within the entire human genome only one region on chromosome 2p, where all three affected children were homozygous for two consecutive markers (*D2S286* and *D2S2116*). To test

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

Family	Individual	Gender	Predominant symptoms at presentation				Age at Renal Biopsy (yr)	Symptoms at Last Examination	
			Age at Onset (yr)	PU (g/24 h)	HU	Creatinine (mg/dl)		Creatinine (mg/dl)	PU (mg/m^2 per h)
INS 6	II:1	M	5.5	3.3	Neg	0.50	MCNS (7.5)	0.68	13
	II:2	F	4.2	10.0	ND	0.45	MCNS (5)	0.64	120
	II:3	M	8.1	3.6	Neg	0.56	ND	0.4	Neg
INS 51	II:1	M	5.3	3.8	Neg	0.34	ND	0.34	Neg
	II:2	M	3.4	5.4	Neg	0.43	ND	0.43	Neg
INS 52	II:1	M	1.7	11.8	Neg	0.30	ND	0.30	Neg
	II:2	F	1.9	14.5	Neg	0.40	ND	0.40	Neg

[a] INS, idiopathic nephrotic syndrome; PU, proteinuria; HU, hematuria; ND, not determined. All patients presented with edema during the acute phase. BP was normal in all patients at onset of disease, as well as at last examination.

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Table 2. Two point LOD scores generated in the SSNS kindred at various recombination fractions for markers at the *SSNS1* locus

Marker	Two-Point LOD Scores at Recombination Fraction θ =								
	0	0.05	0.1	0.15	0.2	0.25	0.3	0.4	Z_{max} (θ)
D2S292	−1.63	−0.01	0.16	0.2	0.2	0.17	0.12	0.03	0.2 (0.2)
D2S291	1.49	1.3	1.11	0.93	0.74	0.56	0.4	0.12	1.49 (0)
D2S2111	1.79	1.56	1.34	1.11	0.89	0.68	0.48	0.15	1.79 (0)
D2S145	2.98	2.72	2.44	2.16	1.87	1.57	1.26	0.64	2.98 (0)
D2S2109	1.93	1.69	1.45	1.21	0.97	0.74	0.52	0.16	1.93 (0)
D2S286	2.14	1.89	1.62	1.36	1.09	0.82	0.56	0.13	2.14 (0)
D2S2116	1.71	1.49	1.27	1.05	0.84	0.63	0.44	0.13	1.71 (0)
D2S1777	1.61	1.42	1.23	1.04	0.85	0.67	0.5	0.2	1.61 (0)
D2S289	−1.63	−0.01	0.16	0.2	0.2	0.17	0.12	0.03	0.2 (0.2)
D2S1396	−1.65	−0.05	0.12	0.17	0.17	0.14	0.1	0.02	0.17 (0.2)

the hypothesis of potential homozygosity by descent (4), fine mapping by haplotype analysis was performed, using the ten consecutive polymorphic microsatellite markers on chromosome 2p12-p13.2 listed in Figure 1. Of these, seven consecutive markers were homozygous in all three affected children (Figure 1), thus corroborating the hypothesis of homozygosity by descent. LOD score analysis was performed to test these data for significance for linkage. Two-point linkage analysis using the ten markers produced a maximum LOD score of Z_{max} = 2.98 at a recombination fraction of theta = 0 for marker *D2S145* (Table 2). Multipoint linkage analysis yielded a maximum LOD score of Z_{max} = 3.01 for marker *D2S145* (Figure 3), which is sufficient for proof of linkage. We thus identified the first gene locus (*SSNS1*) for SSNS. Through lack of homozygosity by descent, markers *D2S292* and *D2S289* restrict the *SSNS1* locus to a genetic interval of 16 cM (http://research-.marshfieldclinic.org/genetics) (Figure 1), equivalent to a physical distance of 10.5 Mb according to the UCSC Human Genome Browser (http://genome.ucsc.edu) (Figure 3). In ten additional multiplex families (3), two were compatible with linkage to *SSNS1* (Figure 2), thus revealing evidence for genetic locus heterogeneity.

Discussion

We here describe the first gene locus for SSNS, thus demonstrating that SSNS can arise on the basis of a monogenic defect. Although the recombination in individual II:1 and II:2 of family INS52 refined the region further to a 3.6 Mb interval, this cannot be taken as a secure flanking marker, because this family is a non-consanguineous kindred with two affecteds and

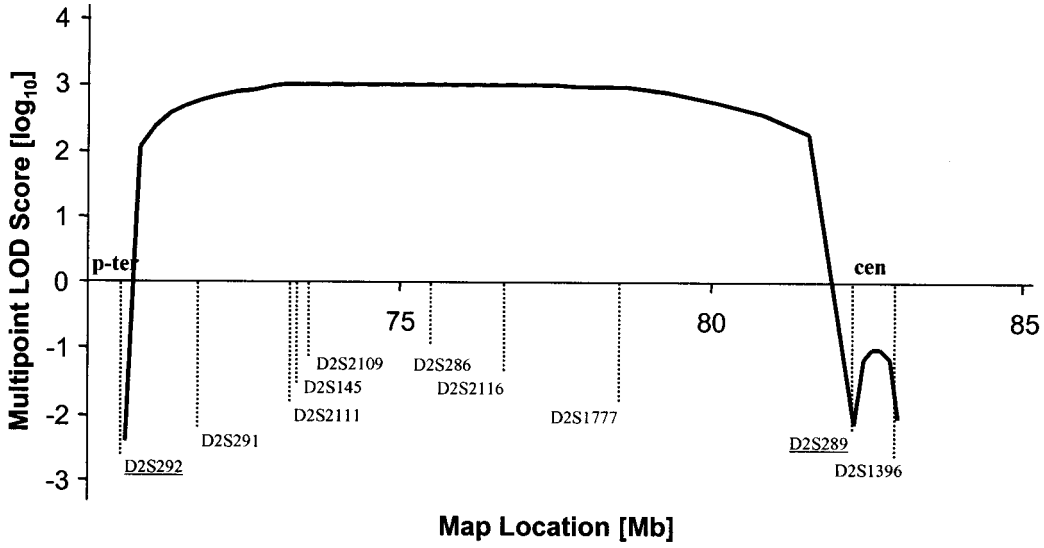

Figure 3. Multipoint linkage analysis for the *SSNS1* gene locus in a consanguineous kindred with SSNS. The ordinate symbolizes multipoint LOD scores. The abscissa gives the marker map on chromosome 2p in cM of physical distance, positions taken from the UCSC Human Genome browser (http://genome.ucsc.edu). Position of markers is shown below the abscissa. Note that LOD_{max} = 3.01 at marker D2S145 represents proof of linkage for the gene locus for SSNS1. Markers flanking the critical region by lack of homozygosity are underlined.

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linkage to the *SSNS1* locus could be coincidental. Further refinement of this locus through additional recombinants in other families linked to this locus is needed to narrow the candidate region sufficiently for direct candidate gene analysis. Approximately 58 currently known and 23 predicted genes have been annotated to the 13 Mb critical interval (http://www.ensembl.org), among which are the candidate genes *transforming growth factor alpha* (*TGFA*), and *semaphorin 4F* (*SEMA4F*). *TGFA* has been implicated in the pathogenesis of chronic glomerulonephritis, whereas functional defects in the immune system have been described in class IV semaphorin (*Sema4D*)-deficient mice (7), although there were no abnormalities in other tissues apparent at the time of examination. The identification of the first locus for *SSNS1* presented here provides a first step toward the identification of the causative gene, which will further our understanding of the pathomechanisms of SSNS.

Whereas gene identification by positional cloning has recently opened inroads into unraveling the pathogenesis of SRNS, not even a gene locus for the more frequent variant of SSNS has been described. The most prominent concept for the pathogenesis of SSNS has been an immunopathogenic hypothesis. It was based on the observations of abnormal T lymphocyte function, based on the remission induced by measles, the susceptibility to pneumococcal and other bacterial infections, the response to steroids and cyclophosphamide, and the association with Hodgkin disease (8). This hypothesis is supported by the finding of associations of SSNS and SRNS with major histocompatibility class I (MHCI) alleles. Significantly increased allele frequencies have been described for the HLA-B12 (9), HLA-A1, B8 (10), and HLA-B27 (11) alleles. These associations, however, are not due to linkage disequilibrium between an SSNS or SRNS locus and the MHC. In general, the question remains unanswered, whether in genetic SSNS the primary defect rests in a gene (or genes) that play a central role in the function of the immune system, or whether the primary defect is expressed in renal cells, like podocytes, in which case the immune phenomena would be secondary to the pathogenesis.

Acknowledgments

We thank the families studied and colleagues of the Arbeitsgemeinschaft für Paediatrische Nephrologie (APN) for contributing clinical data and blood samples. F.H. is a Heisenberg Scholar of the German Research Foundation (Hi 381/7-2).

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