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ORIGINAL ARTICLE

Familial steroid-sensitive nephrotic syndrome in Southern Israel: clinical and genetic observations

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Abstract Reports on genetically informative steroid-responsive (sensitive) idiopathic nephrotic syndrome (SSNS) families are lacking. We studied an extended SSNS Bedouin (B) family with a high rate of consanguinity. The clinical presentation and steroid response of its 11 affected individuals were similar to those of sporadic SSNS (spontaneous remission towards puberty and minimal change disease by kidney biopsy). Genome-wide linkage analysis, using a 382 microsatellite-markers mapping set and additional markers adjacent to 80 candidate genes of the index family, did not support linkage to any chromosomal locus. Retrospective analysis of all additional children with SSNS treated by our institution in the past 20 years (n=96, 50% of them of Jewish origin) revealed another five non-related B families with 2–3 first-degree cousins affected with SSNS in each. The overall familial SSNS rate among the B population (excluding the index family) was 28%, compared with 4% among Jews (Js) (OR 1.8–64, P<0.005). There were more Bs with simple SSNS than there were Js (71% and 40%, respectively; OR 3.58, 95% CI 1.41–9.23, P<0.01). In summary, SSNS in this index family was not linked to any of the presently known chromosomal loci nor predicted to be caused by mutation in any one of a list of genes associated with nephrotic syndrome (NS). The presence of other B families affected by SSNS supports the role for susceptibility genes enrichment, exposing highly consanguineous populations to an increased incidence of SSNS.

Keywords Chromosome mapping · Consanguinity · Nephrosis · Lipoid · Arabs · Jews

Abbreviations

B	bedouin
FSGS	focal segmental glomerulosclerosis
GBM	glomerular basement membrane
J	jew
LOD	logarithmic odds
MCNS	minimal change nephrotic syndrome
NS	nephrotic syndrome
SSNS	steroid sensitive idiopathic nephrotic syndrome

Introduction

Minimal change nephrotic syndrome (MCNS) is a sporadic disease with a weak familial tendency [1]. Genetic studies in the past years have identified a family of novel genes mutated in several genetic types of corticosteroid-resistant nephrotic syndrome (NS), including: nephrin (OMIM # 602716) [2], that causes the Finnish type of congenital NS, podocin (OMIM # 604766) [3], causing the familial form of

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focal segmental glomerulosclerosis (FSGS), laminin-beta-2 [4], transient receptor potential cation channel 6 (TRPC-6) [4] and others. These genes encode crucial components of the glomerular podocytes slit membrane. These diseases cause a very early onset of nephrosis (usually before the age of 1 year) and do not respond to corticosteroid or any other immunosuppressive therapy. In contrast, there are very few reported cases of familial steroid-responsive (-sensitive) NS (SSNS) [5]. Isolated inbred populations have been successfully utilized in mapping Mendelian genes and are likely to be particularly useful for the identification of predisposing genes for common complex diseases. We have recently identified a SSNS family of such a population, whose members have been treated at our center for the past 25 years. Since the inheritance pattern of SSNS in this family appeared to be substantially governed by the contribution of a major gene, either via a recessive inheritance model of full penetrance or via a dominant inheritance model with partial penetrance, we chose to use parametric linkage analysis for testing linkage to several seemingly promising candidate genes. In addition, we have summarized our clinical experience with SSNS in two populations living in our area: the consanguineous-prone Arab–Bedouins (Bs), who also carry a high birth rate, and Jews (Js), with lower birth- and consanguinity rates.

Methods

Patients

The Soroka University Medical Center Committee for Human Experimentation approved the study protocol, in adherence to the *Declaration of Helsinki*. Informed consent was obtained from all patients and parents. The Soroka University Medical Center is the only tertiary care center that serves the Negev region in Southern Israel, a semi-arid area inhabited by about 600,000 people (300,000 of them children<18 years of age). It is composed of two major sub-populations: Js (75%), who usually live in urban and rural settlements and Bs (25%), who have been experiencing a process of transition from a semi-nomad to an urban lifestyle in the past 20 years. There is a general difference in life style between these two communities, including high (~65%) consanguineous marriage and high birth rates and overall lower economic conditions among Bs [6]. All inhabitants of this area have easy access to medical services by virtue of a National Health Insurance program for the past 15 years. All patients were treated and followed in our hospital. The younger ones are currently being followed by the Pediatric Nephrology Service. Thus, we expect to have encountered all new cases of familial and non-familial SSNS occurring in this area.

In addition to the index family, we have summarized clinical data on all children (age <16 years at onset) affected by SSNS and treated at our institution in the past 20 years. Patient identification was based on the Pediatric Nephrology Clinic database and was double-checked with the Hospital's Admissions Department database. Patients with steroid-resistant or secondary NS (such as systemic lupus erythematosus or membranoproliferative glomerulonephritis) were excluded. Data were collected from medical records and transferred to standardized forms, which included age at onset, family history of NS (including an affected first- or second-degree relative), the type of SSNS (steroid-sensitive, frequent-relapsing or steroid-dependent, as defined below), the use of steroid-sparing therapy, complications and accompanying diseases.

Patients had received standard prednisone therapy for their NS, beginning with a 4–6 week full dose (60 mg/m^2 per day) followed by a 4–6 week alternate day reduced dose (40 mg/m^2 per day) course. Each patient was defined as having simple steroid-sensitive (i.e., non-relapsing and not steroid-dependent), frequent-relapsing or steroid-dependent NS, according to standardized criteria about their worst disease period during follow up [7]. Only patients with a minimal follow-up period of 1 year since discontinuation of steroid therapy were included in the final definition regarding their type of steroid response/dependence.

Linkage analysis

DNA was prepared by standard methods from 10–20 ml samples of peripheral blood. A 382 microsatellite-markers mapping set with an average spacing of 10 cM (the ABI PRISM Linkage Mapping Set-MD10 version 2, Applied Biosystems) was used for the whole genome study. Polymerase chain reaction (PCR) amplification of individual markers (fluorescent-dye-labeled forward primer and unlabeled reverse primer) was performed in PTC 225 DNA Engine (MJ Research, Watertown, MA, USA) with 25 ng of genomic DNA, using AmpliTaq Gold DNA polymerase (ABI, Foster City, CA, USA) in a total volume of 10 μl. After amplification, labeled PCR products were pooled, according to the panels' lists, and 2 μl was sampled and diluted in 9 μl of loading buffer (formamide). PCR product electrophoresis and detection were performed with the 3700 automated DNA analyzer (Applied Biosystems, Foster City, CA). Sizing and genotyping were performed with GENES CAN and GENOTYPER software (Applied Biosystems). Alternatively, additional markers, not included in the set, were PCR amplified in the presence of a trace (0.05 μCi) of ^{32}P-dCTP, and the products were separated on sequencing gels and visualized by exposure to a phosphor-imager.

Candidate genes were examined for linkage, using markers at a distance not exceeding 5 cM, and included several categories: genes described as being associated with

human hereditary proteinuria syndromes (nephrin, podocin, etc., $n=7$) [Table 2 (a)] or chromosomal loci linked to NS or other glomerulopathies, including the previously described locus to SSNS in chromosome 2 [Table 2 (b)]; podocyte-related structural proteins (B7-1, fyn, synapyopodin, etc.) or structural proteins of glomerular basement membrane (collagen type IV chains, heparanase) (Table 3); immunity-related genes, associated either with SSNS severity or tendency (Table 4). We also included in the list those genes associated with secondary glomerular fibrosis and deterioration of renal function (Table 5).

Statistical analysis

Annual average NS incidence rate (per 100,000 population at risk) was calculated on the basis of region- and religion-specific national reports [8]. For the comparison of non-parametric clinical data, the chi-square test was used. The data were analyzed with the SLINK* program [9], which allows one to simulate data conditional on the phenotype and the recombination fraction(s) between markers. Logarithmic odds (LOD) scores were computed with Superlink v 1.4 at the PedTool server http://bioinfo.cs.technion.ac.il/superlink/). The calculation was done assuming recessive inheritance with 99% penetrance and incidence of 0.01 or 0.001 for the disease allele in the population.

Results

The index extended B family (Fig. 1) showed an inheritance pattern that seemed to be substantially governed by the contribution of a major gene. There were 11 affected family members. The mode of inheritance was analyzed by the software Superlink, and two most likely models were found: recessive and dominant with partial penetrance. The recessive model with full penetrance was supported by all patients in generation V and two of the patients in generation VI that were born to five pairs of healthy parents, at least four of the pairs with reported consanguinity. In generation VI, two nuclear families had affected children, with one affected parent. The healthy parent could have been a carrier, since, in one pair, there was documented consanguinity, and, in the other, it was suspected from the mother's descent from the same tribe. The second mode of inheritance was dominant inheritance, derived from the appearance of affected patients in generation VI born to one affected parent in two cases, with 50–60% penetrance for a very wide range of prevalence (0.1–10%). The age at onset and the clinical characteristics in patients who belonged to this extended family were indistinguishable from those of sporadic childhood MCNS (see Table 1), including a good response to corticosteroids (albeit with the variant of "frequent relapsing/steroid dependency" in some). In addition, long-term remission was achieved in seven of the 11 patients when they entered puberty or early adulthood. The remaining four children were still prepubertal. No child progressed to chronic renal insufficiency. No salient associated immunological abnormalities could be found. Renal biopsy was performed in one patient (# V-NS1 in Fig. 1), owing to numerous relapses of nephrosis, and revealed a histopathological picture indistinguishable from that of MCNS. The patient succumbed to overwhelming sepsis at age 14 years, with normal kidney function.

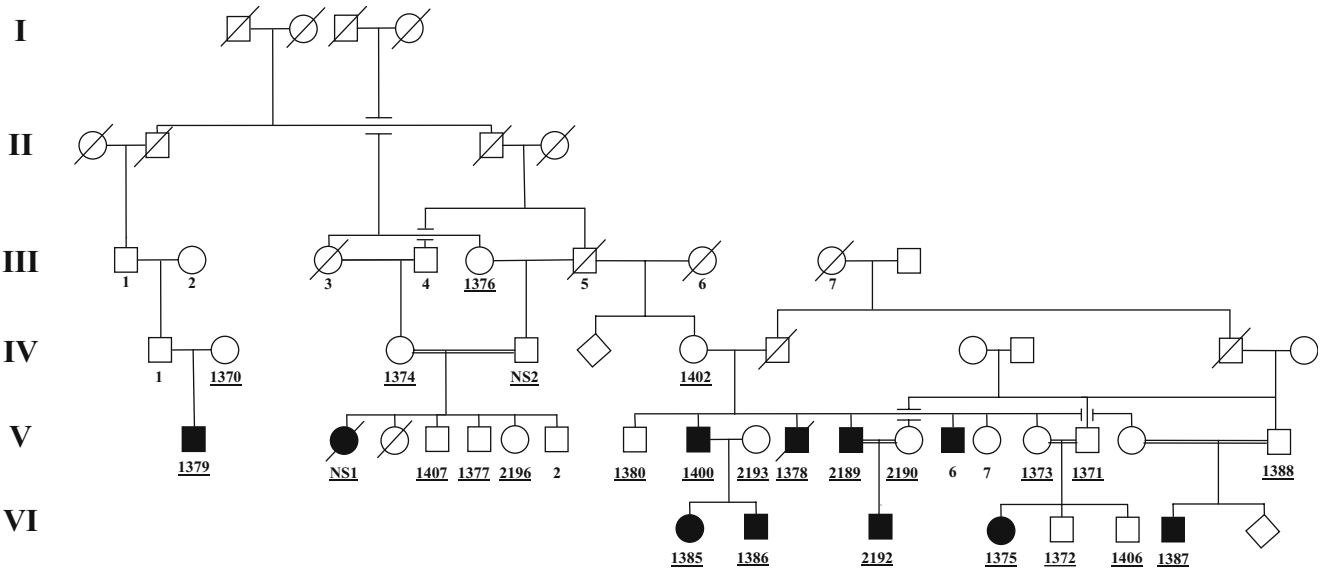

Fig. 1 Index family pedigree. Health status of family members in this pedigree was assessed by direct interrogation in members of generations III to VI. Patient V-1378 died in an accident while in prolonged remission. Patient V-NS1 died of a severe infection, with normal kidney function. The individuals that were tested are underlined

Table 1 Patients' characteristics (*SS* simple steroid-sensitive, *FR* frequent-relapsing, *SD* steroid-dependent, *NA* not available, *NS* not significant)

Characteristics	Jews	Bedouins Index family		*P* value[a]
Number	48	48	11	
Male (%)	37 (78)	38 (77)	8 (73)	NS
Family history (%)	2 (4.2)	13 (28)	NA	<0.005
Age at onset (years)	4.8±3.4	4.9±2.5	4.4±2.4	NS
NS Type				
SS (%)	19 (40)	34 (71)	4 (36)	<0.005
FR (%)	16 (33)	10 (20)	1 (9)	0.08
SD (%)	12 (25)	4 (8)	4 (27)	0.13
NA (%)	1 (2)	0	2 (18)	NS
Steroid-sparing therapy (%)	17 (35)	10 (21)	1 (10)	0.05
Major complications (%)	8 (18)	8 (17)	4 (36)	NS

[a] Values refer to comparison between Bs and Js

Retrospective NS cohort analysis

The retrospective analysis identified another 96 children diagnosed with SSNS (in addition to the index family) in the past 20 years (Table 1). Of the total 107 children with NS, 59 (55%) were Bs. The annual average NS incidence rate per 100,000 population at risk was 2.44 (95% CI 0.24–7.22) and 4.78 (0.62–8.77) among Js and Bs, respectively (*P*=NS). Table 1 provides separate clinical data for the Js and Bs (including, separately, the index family and the remaining population). The mean age at onset (4.8±3.4 years in Js and 4.9±2.5 years in Bs) and the rate of affected boys (77% in Js and 79% in Bs) were similar. However, 28% of the B children had a family history of NS, with 4.3% among the Js (OR 9.06, 95% CI 1.8–63, $P<0.005$). There were two parent/child-affected pairs in the Js subgroup, whereas, in the B children, all related affected children were cousins in families with high consanguinity (Fig. 2). Associated illnesses at presentation were similar and uncommon and included: bronchial asthma (Bs, three; Js, four), atopic dermatitis and IgA deficiency (in one B child each). Seven children in each group suffered different complications associated with SSNS and corticosteroid therapy, including: spontaneous peritonitis (Bs, three; Js, two), bacteremia ($n=3$), dysentery ($n=2$), bacterial meningitis ($n=1$), arterial thromboembolism ($n=1$) and acute renal failure ($n=1$). None of the patients in the three different study groups developed a chronic impairment in kidney function. Simple (i.e., non-relapsing and not steroid dependent) SSNS was seen in 34 (71%) of the B children and 19 (40 %) of the Js (OR 3.58, 95% CI 1.41–9.23, $P<0.005$). A similar number of children (ten out of 46 Js and ten out of 46 Bs, of those followed for more than 1 year since the discontinuation of steroids) experienced a single attack of NS. Twenty-one percent of the B and 37% of the J children were provided with steroid-sparing therapy (*P*=0.05). Kidney biopsies were performed in four Bs and eight Js and revealed either MCNS (Bs, one; Js, five) or minimal mesangial proliferation with IgM deposits (Bs, three; Js, three).

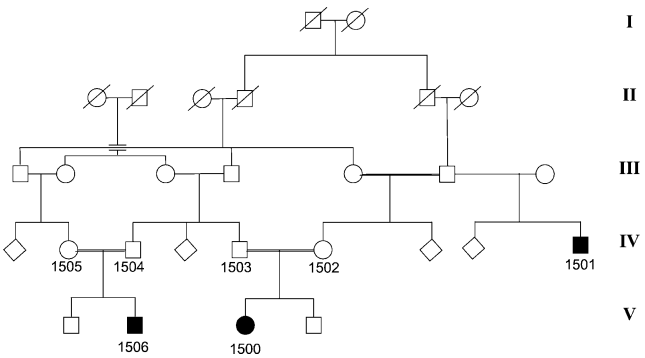

Fig. 2 Representative additional extended family of B origin with three affected SSNS children

Genetic analysis

DNA was extracted from eight affected and 17 non-affected individuals in the index family. Linkage to genes previously implicated in steroid-resistant nephrotic syndromes was tested by markers within the interval (Tables 2, 3, 4 and 5). For each gene, a two-point analysis was performed, using Superlink [7], under two modes of inheritance: a recessive mode with 99% penetrance and a dominant mode with 50% penetrance. For both models, a prevalence of 0.01 of the mutant gene was assumed, and a uniform frequency of marker alleles was used. Tables 2, 3, 4 and 5 show the examined genes and nearby markers. All LOD results under a Mendelian recessive model with 99% penetrance were negative, thus excluding linkage for a recessive pattern of inheritance. A dominant Mendelian model, with 50% penetrance, best fitting the pattern of inheritance observed in the family, was also tested for two-point analysis. The results did not support linkage between the chosen candidate genes and the disease under this mode of inheritance either. In addition, no other suggestive linkage was identified for any of the other genome-wide 382 polymorphic markers contained in the analysis.

Table 2 Genes associated with (*a*) hereditary proteinuria syndromes and (*b*) chromosomal loci linked to NS or other glomerulopathies

Parameter	Gene	Proximal marker	Distal marker	Distance to proximal marker (cM)	Distance to distal marker (cM)
2a	Actinin alpha 4	D19S220	D19S420	0.85	4.2
2a	CD2-associated protein	D6S452	D6S257	1.07	5.72
2a	Laminin beta 2	D3S3647	D3S1289	2.3	2.9
2a	Nephrin (*NPHS1*)	D19S245	D19S220	4	2
2a	Podocin (*NPHS2*)	D1S218	D1S238	3.4	8
2a	TRPC6	D11S898		0	
2a	Wilms' tumor 1 (*WT1*)	D11S1977	D11S935	3.9	6.1
2a	LMX1B	D9S1682	D9S290	5	3
2b	SSNS1	D2S211	D2S286	0	0
2b	MPGN type III	D1S1614	D1S1660	0	0
2b	Familial FSGS	D11S901	D11S898	0	0

Discussion

The pathogenesis of MCNS is ill defined, but the lack of significant histopathological changes, together with a good response to corticosteroid therapy and a tendency for bacterial infections, has raised the hypothesis of the existence of an immune derangement, possibly of the cellular system, as a reason for this disease [10–12]. The

Table 3 Genes encoding podocyte- or glomerular basement membrance (GBM) related structural proteins

Gene	Proximal marker	Distal marker	Distance to proximal marker (cM)	Distance to distal marker (cM)
Actin alpha 2	D10S1686	D10S185	5	4
Actin beta	D7S517	D7S641	8.3	1
Actin gamma 1	D17S784	D17S928	1	5
Actin gamma 2	D2S2368	D2S286	10	1
B7-1 (CD80)	D3S1303	D3S3023	0.3	0.5
Beta 3 integrin	D17S1299	D17S1868	3	2
Collagen type IV alpha 1 and 2	D13S1265	D13S285	4.5	5.5
Collagen type IV alpha 3 and 4	D2S126	D2S396	9.1	2.6
COX2	D1S218	D1S238	10	2
Endosulfatase hSulf2 (D4)	D20S119	D20S178	2	8
Erythropoietin receptor	D19S884	D19S221	6	2
FAT-1	D4S1535	D4S426	11	2
FAT-2	D5S436	D5S410	8	2
Fyn	D6S278	D6S287	2	5
Heparanase	D10S185	D10S192	5	2
Myelin protein zero	D1S484	D1S2878	0.5	7.5
Nck adaptor protein 1	D3S1292	D3S1764	5	2
Nck adaptor protein 2	D2S436	D2S160	1.2	4
Neuropilin1	D10S208	D10S196	2	7
Neuropilin2	D2S117	D2S325	7	2
Plexin A1	D3S1267	D3S1292	3	4
Plexin A2	D1S249	D1S425	3	5
Plexin A3	Chr X			
Plexin B1	D3S3647	D3S1289	2.2	2.8
Plexin D1	D3S1267	D3S1292	7	1
Semaphorin 3A	D7S820	D7S644	0	1.17
Semaphorin 3B	D3S3647	D3S1289	3.5	1.5
Semaphorin 3C	D7S2204	D7S2212	5	1
Semaphorin 3D	D7S820	D7S644	0.5	0.5
Semaphorin 3E	D7S2212	D7S820	0	1.4
Semaphorin 3F	D3S3647	D3S1289	3	2
Synaptopodin	D5S436	D5S410	7	3

Table 4 Genes encoding immunity-related genes associated with SSNS severity (*a*) or SSNS tendency (*b*)

SSNS category	Gene	Proximal marker	Distal marker	Distance to proximal marker (cM)	Distance to distal marker (cM)
4a	Arachidonate 5 lipoxygenase activating protein	D13S217	D13S171	5	5
4a	Dystroglycan	D3S3647	D3S1289	2.5	2.7
4a	Fcgr3	D1S484	D1S2878	1	7
4a	GRO alpha (CXCL1)	D14S292	D14S1007	1	2.76
4a	Nitric oxide synthase	D12S79	D12S86	6	1
4a	PDGF-C	D4S1548	D4S413	7.5	0.5
4b	Complement component 4a	D6S273	D6S1610	0.14	7.5
4b	IL2 receptor alpha	D10S591	D10S189	5	1
4b	Interleukin 4, 13	D5S1984	D5S2115	1.3	2
4b	Interleukin 4 receptor	D16S3068	D16S3136	2	9
4b	IL10	D1S249	D1S425	2	6
4b	IL8	D4S392	D4S2964	4	5
4b	Leukotriene A4 hydrolase (Arachidonate 5 lipoxygenase)	D12S351	D12S346	6	5
4b	Macrophage migration inhibitory factor	D22S1685	D22S1174	0.5	0.5
4b	Vascular endothelial growth factor	D6S1549	D6S1632	4.6	4.4
4b	HLA-DQA1	D6S273	D6S1610	0.64	7
4b	HLA-DQA2	D6S273	D6S1610	0.72	6.92
4b	HLA-DQB1	D6S273	D6S1610	0.71	6.93
4b	HLA-DQB2	D6S273	D6S1610	0.73	6.91
4b	HLA-DQB3	D6S273	D6S1610	0.76	6.88
4b	HLA-f	D6S273	D6S1610	0.65	6.9

existence of a circulating substance (most probably produced by a component of the immune system) that may interfere with glomerular permeability has been proposed as an explanation for this disease {and also to some cases of FSGS [13]}. This clinical finding has been recently reiterated in an animal model [14]. It has been proposed that MCNS reflects a disorder of T lymphocytes as well as other immune cells [15]. These T cells, which are presumably sensitive to corticosteroids and other immunosuppressive agents, such as cyclosporine, may release a cytokine that induces rearrangement of the unique 3D structure of the glomerular epithelial cells [16]. In accordance with this immune pathogenesis for MCNS and FSGS, most studies that tried to identify mutations in podocytes or other glomerular structural proteins in these diseases (mainly in the steroid-responsive forms) were

Table 5 Genes associated with renal function deterioration/fibrosis (all species)

Gene	Proximal marker	Distal marker	Distance to proximal marker (cM)	Distance to distal marker (cM)
Aldose reductase (D3)	D7S640	D7S684	2	5
Angiotensin-converting enzyme (ACE)	D17S944	D17S2193	1.5	4.9
Alpha 1 proteinase inhibitor	D14S280	D14S65	5	7
Bcl-2	D181357	D18S814	4	1
Complement C6, C7	D5S426	D5S418	6	0
Endothelin 1	D6S287	D6S262	4	7
Endothelin 2	D1S255	D1S2797	5	5
Endothelin 3	D20S171	D20S173	5	3
Fibrinogen Aa	D4S1548	D4S1585	4	2
Metalloproteinase 9	D20S119	D20S178	1	4
Paraoxonase 1, 2, 3	D7S657	D7S515	1	7
Smurf	D7S657	D7S515	2	6
Transforming growth factor beta 1	D19S220	D19S420	3	2
TNFRSF11A	D18S1357	D18S814	3	1
Trans-thyretin (TTR) (prealbumin)	D18S36		0	

unsuccessful. For example, two current reports failed to identify mutations in the *WT1* and other podocyte-related genes for SSNS in a large number of patients [17] and in four candidate genes for congenital NS patients [18]. In agreement with this theory and results we did not find linkage between SSNS and any of the candidate genes associated with steroid-resistant nephrotic syndrome, including: structural podocyte-derived proteins such as *WT1*, nephrin, podocin, actinin alpha 4 [19], vascular endothelial growth factor (VEGF) [20], laminin beta 2 [21] and CD2AP [22]. No linkage was found to the proteins in the human leukocyte antigen (HLA) system that have been found to be related to the severity of SSNS [23] or the tumor necrosis factor (TNF) receptor [24], or to other genes related to the cellular immune system, such as B7-1 (CD80) [13]. Genes associated with an increased tendency for renal amyloidosis, such as fibrinogen A alpha [25] and transthyretin (or prealbumin) [26], or for glomerulonephritis, such as C4a [27] were also tested and found to be negative.

Comparison between the B and J children affected by SSNS in our area revealed an overall typical manifestation of childhood SSNS: a mean age at onset of 5 years and male predominance, with no significant differences between Js and Bs. The disease manifestations among the B children seemed to be milder than in Js: there was a higher proportion of simple SSNS attacks as well as a lower proportion of children who needed steroid-sparing therapy or experienced a phase of steroid dependency of frequent relapses during their disease. In spite of the overall similarity in SSNS characteristics, there was a marked increase in familial cases of the disease among Bs. In addition, the index family was not different in its SSNS clinical manifestations from the background B or J populations studied. The only salient clinical characteristic for this index family (and the B patients in general) was the high rate of family history. Therefore, this familial tendency for SSNS in the index family should be seen in the context of a similar higher tendency for the disease among the B population. Similar observations about the apparent higher rate of an immunity-based disease among the B population in our area have been made for celiac disease [28, 29]. Interestingly, no clear single genetic locus has yet been identified for celiac disease either (a disease with known familial tendency), in spite of numerous efforts [30]. This observation supports the hypothesis of a higher tendency for regular–sporadic NS in this closed community.

Gene mapping for complex traits is a major challenge, since factors such as genetic heterogeneity, small effects of disease alleles on risk and confounding effects caused by gene–environment and gene–gene interactions hamper it. Numerous susceptibility genes are likely to be involved in common diseases such as SSNS, and most of those genes may be common in populations, suggesting interactions of different allelic variants in this disease's etiology. Selection of homogeneous study populations with reduced genetic variability and etiological heterogeneity is therefore important to increase the possibility of identification of susceptibility genes. Isolated populations are likely to be particular useful for the identification of predisposing genes for common complex diseases [31]. This is because a small number of founding individuals and a high rate of consanguineous and endogamous marriages, typical of small communities, increase genetic homogeneity and highlight susceptibility genes. This is the typical situation in the Israeli Bedouins [32], where there is still a very high rate of consanguineous marriages and small communities. Furthermore, since individuals are exposed to a common environment and a relatively uniform lifestyle, non-genetic variability is also minimized, and the noise caused by other etiological determinants is therefore reduced.

We assumed that, in the index family, there was a founding father effect, even if not fully evident from the pedigree analysis, due to the very high rate of consanguinity prevalent in our area among the B population [33, 34]. In small founder populations, like the one we studied, the linkage disequilibrium may extend over relatively large intervals [35] and, therefore, may be detectable in the proximity of a disease gene. Therefore, a genome-wide linkage is warranted to detect such a locus, even after the exclusion of the candidate genes or the previously SSNS1 locus. The latter showed linkage in only three families out of 11 tested [36]. The extended family investigated in this report was suitable for the detection of a major gene that confers a relatively high disease risk in the population. However, it lacks the power to detect "weak-effect" disease genes, which are likely to be missed when a small sample of individuals is tested through genome-wide screening. A genome-wide linkage analysis, with a denser marker set, in these and similar other families may be of help in the future.

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Appendix

Tables 2, 3, 4 and 5: markers were used for linkage analysis to the candidate genes. The genetic distance between the gene and the marker was calculated on the basis of the genetic map [37] and the physical distance to the marker in the Human UCSC genome browser http://genome.ucsc.edu/cgi-bin/hgGateway), assuming 1 cM equals 0.5 Mb. Candidate genes were chosen on the basis of literature search.

A detailed list of the references can be provided by D.L. upon request. See suggested review [38] for podocyte physiology and specific proteins potentially involved in NS. The genes are depicted by categories. Table 2: (a) Hereditary proteinuria syndromes and (b) chromosomal loci linked to NS or other glomerulopathies; Table 3: podocyte-related and GBM structural proteins; Table 4: (a) immunity-related genes, associated with SSNS severity and (b) immunity-related genes, associated with SSNS tendency; Table 5: genes associated with deterioration/fibrosis of renal function (all species).

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