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ORIGINAL ARTICLE

Clinical and genetic heterogeneity in familial steroid-sensitive nephrotic syndrome

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Abstract

Background Familial steroid-sensitive nephrotic syndrome (SSNS) is a rare condition. The disease pathophysiology remains elusive. However, bi-allelic mutations in the *EMP2* gene were identified, and specific variations in *HLA-DQA1* were linked to a high risk of developing the disease.

Methods Clinical data were analyzed in 59 SSNS families. *EMP2* gene was sequenced in families with a potential autosomal recessive (AR) inheritance. Exome sequencing was performed in a subset of 13 families with potential AR inheritance. Two variations in *HLA-DQA1* were genotyped in the whole cohort.

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Results Transmission was compatible with an AR ($n = 33$) or autosomal dominant (AD, $n = 26$) inheritance, assuming that familial SSNS is a monogenic trait. Clinical features did not differ between AR and AD groups. All patients, including primary ($n = 7$) and secondary steroid resistant nephrotic syndrone (SRNS), ($n = 13$) were sensitive to additional immunosuppressive therapy. Both *HLA-DQA1* variations were found to be highly linked to the disease (OR = 4.34 and OR = 4.89; $p < 0.001$). Exome sequencing did not reveal any pathogenic mutation, neither did *EMP2* sequencing.
Conclusions Taken together, these results highlight the clinical and genetic heterogeneity in familial SSNS. Clinical findings sustain an immune origin in all patients, whatever the initial steroid-sensitivity. The absence of a variant shared by two families and the *HLA-DQA1* variation enrichments suggest a complex mode of inheritance.

Keywords Familial nephrotic syndrome · Genetics · *EMP2* · Immunity · Steroid sensitivity · Steroid resistance · Podocyte · *HLA-DQA1*

Abbreviations

AR	Autosomal recessive
AD	Autosomal dominant
EMP2	Epithelial member protein 2
FR	Frequent relapsers
FSGS	Focal and segmental glomerulosclerosis
OR	Odds ratio
SRNS	Steroid-resistant nephrotic syndrome
SSNS	Steroid-sensitive nephrotic syndrome
WES	Whole exome sequencing

Introduction

Idiopathic nephrotic syndrome (NS, i.e., minimal change disease [MCD] or primary focal segmental glomerulosclerosis [FSGS]) is the most prevalent cause of NS in both children and young adults. Idiopathic NS can occur through two main mechanisms. Either a defective expression of podocyte-specific proteins resulting from genetic abnormalities (almost all steroid-resistant NS [SRNS]) [1]; or more frequently, an elusive abnormal response of the immune system to exogenous stimuli (mostly steroid-sensitive NS [SSNS]) [2]. Indeed, the hypothesis of a circulating factor that could derive from immune cells is now broadly accepted, but not proven [3]. Evidence supporting this includes the triggering of relapses by allergy or infections, the unique observation of remission of proteinuria after kidney transplantation from a nephrotic patient to a non-nephrotic recipient, the transient materno-fetal transmission of NS [4], and the immediate recurrence of NS after kidney transplantation in 30–50% of SRNS/FSGS patients [5] that may respond to plasma exchange or immunoadsorption [6, 7]. Furthermore, the

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injection of supernatants of cultured lymphocytes of patients with NS has been shown to induce nephrotic proteinuria in immunocompetent rodents [8, 9]. To date, many candidates have emerged, but none has been definitely incriminated as a cause of the immune-mediated nephrotic syndrome [10].

Affected patients with SRNS have been the subjects of intense genetic research in the past decades. The identification of approximately 40 mutated genes encoding proteins expressed in the podocyte has provided crucial insights into the pathophysiology of the glomerular filtration barrier [11]. Conversely, reported SSNS pedigrees are scarce [12–14]. Several studies investigated SRNS gene mutations (mainly in *NPHS1*, *NPHS2* and *WT1*) in SSNS cohorts, but no causative mutation was identified [12, 15–18]. *EMP2* mutations have been identified in AR SSNS by a combination of linkage analysis and exome sequencing [19]. This gene encodes the epithelial membrane protein 2, a tetraspan protein residing in lipid rafts known to regulate the trafficking of proteins such as integrins and GPI-anchored proteins [20], an unexpected finding in the context of a potential circulating factor as a cause of SSNS.

The aim of the present study was twofold. First, we wanted to assess the clinical spectrum of familial SSNS thanks to a large worldwide cohort developed in the laboratory since the 1990s. Then, we wanted to assess the underlying genetic contribution in this disease through different analyses.

1. We determined the prevalence of *EMP2* mutations in our cohort of SSNS families compatible with an autosomal recessive (AR) mode of inheritance.
2. We sought to identify novel gene(s) involved in SSNS by linkage analysis combined to whole exome sequencing (WES) in a subset of families with AR inheritance.
3. We genotyped two variations in *HLA-DQA1* gene recently linked to a high risk of developing sporadic SSNS [21].

Materials and methods

Patients

Over the past three decades, we have established a worldwide cohort of patients with hereditary NS. Among them, 59 families of SSNS were identified with ≥2 individuals with NS. In each family, at least 1 patient presented SSNS at diagnosis. Clinical information and DNA samples were available for 131 and 102 patients respectively. Families with at least two affected members in only one generation were considered to be compatible with an autosomal recessive (AR) mode of inheritance, although an X-linked transmission is also possible in several families. Conversely, when the pathology involved two subjects in two successive generations, the mode of inheritance was considered to be autosomal dominant (AD).

Steroid sensitivity was defined as the absence or trace of proteinuria on the dipstick on 3 consecutive days [22, 23] after steroid therapy (4 weeks of prednisone at a dose of 60 mg/m^2/d ± three 1 g/1.73 m^2 methylprednisolone pulses) according to the French Society of Pediatric Nephrology protocol [24]. Secondary steroid resistance was defined as the failure to achieve complete remission after 8 weeks of corticosteroid therapy in patients with a previous steroid sensitivity.

As it was not possible to classify all patients as steroid-dependent or frequent relapsers according to the ISKDC definitions [22] owing to a lack of chronological details in this retrospective study, we used the following classification:

1. Patients who presented >2 relapses or needed additional immunosuppressive drugs for remission were classified as frequent relapsers (FRs)
2. Patients who presented ≤2 relapses and only received steroids were classified as nonfrequent relapsers (NFRs)

EMP2 and *HLA-DQA1* Sanger sequencing

The four coding exons and the intronic flanking sequences of the *EMP2* gene were Sanger sequenced in the index patient of the 33 families with a compatible AR mode of inheritance. Furthermore, the second exon of the *HLA DQA1* was sequenced to analyze the rs1129740 and rs1071630 frequencies in all patients, and in 33 European controls suffering from sporadic SSNS. Sequence chromatograms were analyzed using Sequencher software (Gene Codes, Ann Arbor, MI, USA).

Linkage analysis

Genome-wide linkage analysis was performed using the Human Mapping 250 k *Nsp*I array (Affymetrix). Multipoint Lod Scores were calculated across the whole genome using MERLIN software, assuming autosomal recessive inheritance with complete penetrance.

Whole exome sequencing and mutation calling

Whole exome sequencing was performed using the Agilent SureSelect All Exon 51 Mb V5 capture-kit on a HiSeq2500 (Illumina) sequencer (paired-end reads: 2 × 75 bases). Obtained sequences were aligned to the human genome (National Center for Biotechnology build 37/hg19) using the Lifescope suite from Life Technologies. Substitution and variant calls were made using the Genome Analysis Toolkit pipeline. Variants were then annotated using the Paris Descartes University Bioinformatics platform software system. We assumed the causal variant:

1. Segregates with disease status

2. Is novel or has a minor allele frequency <1/1,000 in dbSNP [25], 1,000 Genome Project [26], and ExAC [27]
3. Was not found in >10/1,226 projects from our in-house database

The pathogenicity of missense variants was evaluated using PolyPhen2, SIFT, and Mutation Taster. When DNA was available, segregation analysis of selected variations was performed by Sanger sequencing.

SRNS gene screening

Steroid-resistant nephrotic syndrome gene screening was performed using a targeted gene panel designed for specific sequencing of the coding exons and ≥10 bp flanking intronic sequences of 34 known FSGS genes (Multiplicom Kit-FSGS MASTR, Supplementary Table 1 contains RefSeq [NCBI] accession numbers) [28]. Sequence alignments and data analysis were performed as described above.

Statistical analyses

Graphpad Prism software was used for statistical analyses. Differences between categorical data were evaluated using Fisher's exact test, and means were analyzed using Student's t test. Correlation coefficient was calculated using Pearson's method. Risk factors were expressed as odds ratios. Data were examined at the 95% confidence level, and statistical significance was set at $p < 0.05$. When necessary, p values were adjusted using the Bonferroni approach as the most stringent test for controlling false-positive results.

Results

Clinical features in familial SSNS

Mode of inheritance

Fifty-nine families with ≥2 individuals with NS, and ≥1 with SSNS at diagnosis were identified. Clinical information was available for 131 patients, 77 of whom were male (58.8%). All patients presented nephrotic syndrome with clinical edema discovered at a median age of 7.85 years (1–58; Table 1).

The cohort was split into two groups based on the most likely potential mode of inheritance: AR (71 patients from 33 unrelated families) or AD (60 patients from 26 unrelated families). However, in some AR cases, an autosomal dominance with incomplete penetrance could not be ruled out. Clinical data are shown in Table 1, and Supplementary Fig. 1a, b.

Autosomal recessive pedigrees mostly consisted of 2–3 siblings (Supplementary Fig. 1a). Notably, the cohort comprised only one large kindred of 2 siblings and 3 cousins involving three generations and several loops of consanguinity. They all presented a similar disease course with steroid-sensitivity and complete remission without secondary steroid-resistance (SS20, Supplementary Fig. 1a). Two sibships consisted of triplets, including one set of monozygotic triplets among whom only two brothers were affected, with a similar age of onset (7.1 and 7.3 years respectively), but a very different disease course (SS6, Supplementary Fig. 1a). Four other twin pairs consisted of two monozygotic and two dizygotic affected siblings.

Most families with an apparent AD inheritance were composed of a parent and a child, whatever the sex (15 of the 26 families). No large pedigree was noted in this group (Supplementary Fig. 1b).

Most patients developed NS during childhood (89.7%). Mean age at onset was 6.52 ± 1.06 years and 10.21 ± 1.94 years in the AR and AD groups respectively ($p = 0.08$). Intra- and inter-familial variability regarding the age at diagnosis was low in each group (Fig. 1). The mean difference in the age at onset between relatives was 5.4 years. However, in one family from the AR group and six from the AD group, affected relatives had a difference in age at onset of more than a decade (Fig. 1).

Disease course and response to therapy

Histological data did not differ among patients from each group and are reported in Table 1. Minimal change disease was the most commonly represented pathological condition in the cohort (81.8%).

All patients received initial steroid therapy, and the steroid-sparing agents mostly used were calcineurin inhibitors, whatever the group (Table 1). Patients and families from each group were sorted regarding their relapsing status. The number of FR and NFR patients did not significantly differ between groups, but a total of 25 families (42.4%) comprised both FR- and NFR-affected relatives (Table 1, Fig. 2).

By definition, at least one affected relative had typical SSNS. However, in 19 families, one patient was either primary ($n = 7$–7 families) or secondary ($n = 13$–12 families) steroid-resistant with a similar proportion in the two groups (primary 5.6 vs 5.0%, and secondary 11.3 and 8.3%, $p = 1$ and 0.77 respectively). Patients with primary steroid-resistance (4 in the AR group and 3 in the AD group, Table 2) achieved remission with intensive immunosuppressive therapy. Thirteen (10%) patients from 12 families developed secondary steroid-resistance. Remission was obtained in 6 out of 13 using intensive immunosuppressive therapy, and 6 others progressed to end-stage kidney disease (ESKD; Table 2). Among them, 4 did not experience any recurrence after transplantation. The other two recurred and achieved remission after intensified

Table 1 Epidemiological and therapeutic characteristics of autosomal recessive (*AR*) (*AD*) nephrotic syndrome (*NS*) groups

		AR group		Total AR	Total AD	*p* Total AR vs total AD
		Exome	No exome			
Families (patients)		13 (30)	20 (41)	33 (71)	26 (60)	
Consanguineous families (known/suspected)		4/3	4/1	8/4	2	
Mean age at onset (years) ± SEM		4.57 ± 0.65	8.08 ± 1.81	6.52 ± 1.06	10.21 ± 1.94	*0.08*
Sex ratio (male/female)		4	1.2	1.85	1.04	
Therapy (patients)						
Steroids		30	41	71	60	
Steroids pulses		3 (10%)	3 (7.3%)	6 (8.5%)	3 (5.0%)	*0.51*
Alkylating agents		9 (30%)	10 (24.4%)	19 (26.8%)	9 (15.0%)	*0.13*
Calcineurin inhibitor		6 (20%)	21 (50.2%)	27 (38%)	23 (40.0%)	*0.86*
Mycophenolate mofetil		5 (16.7%)	4 (9.8%)	9 (12.7%)	7 (11.7%)	*1.00*
Plasma exchanges		0	2 (4.9%)	2 (2.8%)	0	–
Ergamisol/levamisole		4 (13.3%)	0	4 (5.6%)	2 (3.3%)	*0.45*
Rituximab		2 (6.7%)	1 (2.4%)	3 (4.2%)	4 (6.7%)	*0.70*
Frequent relapsers		18 (60%)	22 (53.7%)	40 (56.3%)	34 (56.7%)	*0.90*
Nonfrequent relapsers		12 (40%)	19 (46.3%)	31 (43.7%)	26 (43.3%)	
Steroid resistance	At onset	0	4 (9.8%)	4 (5.6%)	3 (5.0%)	*0.90*
	Secondary	2 (6.7%)	7 (14.6%)	8 (11.3%)	5 (8.3%)	*0.77*
Pathology		9	21	30	25	
MCD		8 (88.9%)	16 (76.2%)	24 (80%)	21 (84%)	*0.74*
FSGS		1 (11.1%)	5 (23.8%)	6 (20%)	4 (16.0%)	
ESKD	Initial steroid-sensitivity	0	4	4	2	*0.69*
	Initial steroid-resistance	0	0	0	0	–
Renal transplantation		0	4	4	2	
Recurrence after transplantation		0	1	1	1	
Associated auto-immune diseases		1	0	1	2	*0.90*
Geographical origin (families)						
Europe				14	19	
Maghreb				13	5	
Iran/Oman/India/Turkey				5	0	
USA				1	0	
Southeast Asia				0	2	

No difference was found between AR and AD groups regarding the disease course, treatments, and kidney disease

Statistical significance was set at $p < 0.05$

SEM Standard error of mean, *ESKD* end-stage kidney disease, *MCD* minimal change disease, *FSGS* focal and segmental glomerulosclerosis

immunosuppression (plasma exchanges+/−IV cyclosporine+/−rituximab). The last one was lost to follow-up.

Associated features

Few associated autoimmune diseases were identified: one type 1 diabetes with the *HLA-DQB1* risk allele, one monoclonal gammopathy, and one leukocytoclastic vasculitis. Besides, in a nonconsanguineous family, two children and their father presented with hypophosphatemic nephrolithiasis/osteoporosis-1 (NPHLOP1 - # OMIM-612286) without any mutation in the *SLC34A1* and *SLC9A3R1* genes. One patient had progressive bilateral neurological deafness associated with right renal hypoplasia without renal insufficiency.

Overall, clinical features are consistent with an immune-mediated NS in all cases. We observed wide intra- and inter-familial variability regarding the disease course, apart from the age at onset, between related cases.

HLA-DQA1 sequencing in familial SSNS

Two common variations in *HLA-DQA1* gene (rs1129740 and rs1071630) were recently reported to be risk factors for developing sporadic SSNS [21]. All patients in our cohort were
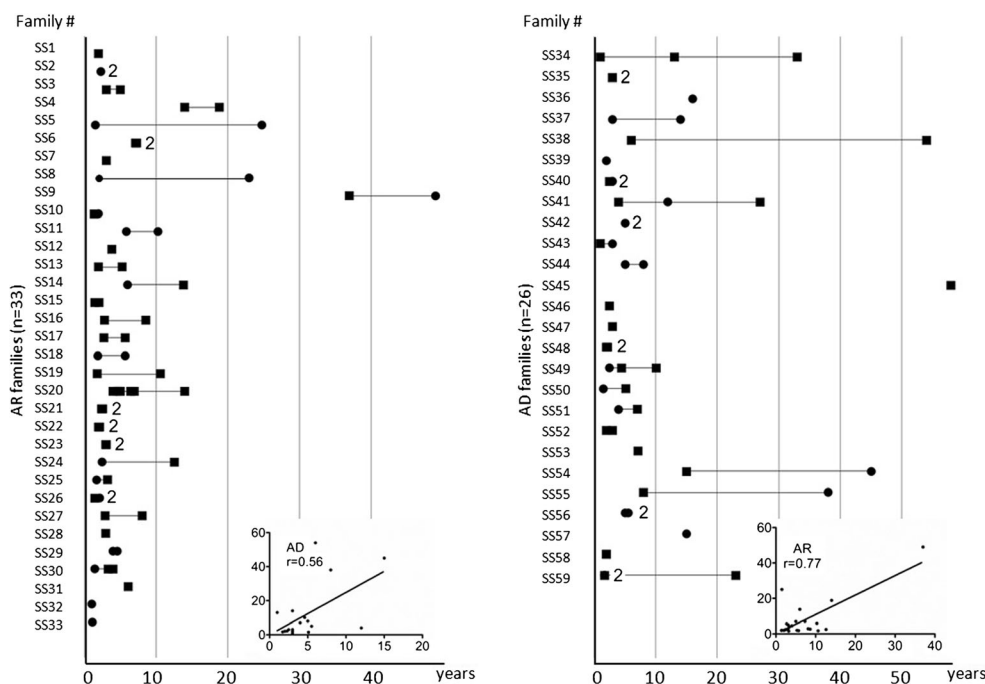
Fig. 1 Intra- and interfamilial variability for age of onset of steroid-sensitive nephrotic syndrome (SSNS). Age at onset of nephrotic syndrome (NS) between the affected relatives in both the **a** autosomal recessive (AR) and **b** autosomal dominant (AD) families shows a correlation. Mean age at onset is 6.52 ± 1.06 years and 10.21 ± 1.94 years in the AR and AD group respectively. Pearson's correlation coefficient was calculated in all families with available age at onset in at least two affected cases. Each *horizontal line* represents a family. Family numbers are given along the vertical axis. Males are shown as *squares*, females as *circles*. The number "2" is noted when two square(s)/circle(s) are indistinguishable. Raw data are reported in Supplementary Table 3

genotyped for *HLA-DQA1* variations, including a total of 49 patients with European ancestry. This population was compared to 23 sporadic SSNS control cases and 503 healthy European controls from the 1000genomes database. Results are summarized in Table 3. Both variations were found to be enriched in the familial SSNS cohort. A strong effect was observed compared with healthy controls: OR = 5.54 [3, 29] and OR = 6.57 [3.88–11.12] for rs1129740 and rs1071630 respectively, and $p < 0.001$ for both after Bonferroni correction. Patients with an atypical disease course (i.e., primary or secondary SRNS) were at the same risk as others: OR = 1 (0.36–2.81). No

difference was found between familial and sporadic SSNS for the two variations (OR = 0.56 [0.19–1.52]). Notably, patients who exhibited a homozygous state for both variations ($n = 73$) had a lower age at diagnosis than heterozygous patients ($n = 24$; $age^{HO} = 6.13 \pm 0.93$ and $age^{HE} = 11.54 \pm 3.82$, $p = 0.04$).

EMP2 sequencing in familial SSNS

EMP2 gene mutations have been previously reported in three families with AR SSNS or SRNS [19]. The four coding exons and their intronic-flanking sequences were Sanger-sequenced
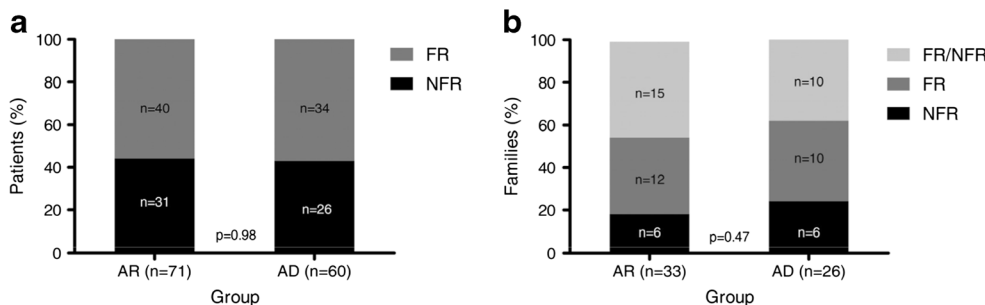

Fig. 2 Disease course according to the number of relapses. **a** Proportion of patients individually classified as frequent relapsers (FRs) or nonfrequent relapsers (NFRs) sorted by mode of inheritance. The groups were similar with regard to the course of the disease. **b** Proportion of families who presented FRs, NFRs, or both FR–NFR patients. Most families had both FRs and NFRs

Table 2 Outcome of patients with primary (*n* = 7) or secondary steroid-resistance (*n* = 13)

	Autosomal recessive	Autosomal dominant
Primary steroid-resistance	4	3
Evolution to ESKD	0	0
Therapy used to induce remission on native kidneys		
Calcineurin inhibitors	2	2
Rituximab	0	1
Deceased under therapy	1	0
Lost to follow-up	1	0
Secondary steroid-resistance	8	5
Evolution to ESKD	4	2
Transplantation	4	2
Recurrence after transplantation	1	1
Therapy used to induce remission		
On native kidneys		
Cyclophosphamide	1	0
PE	1	0
Rituximab	2	0
Calcineurin inhibitors	0	1
MMF	0	1
Loss to follow-up	0	1
After post-transplantation recurrence		
PE + IV calcineurin inhibitors + rituximab	1	0
PE only	0	1

Among patients with primary steroid-resistance, none evolved to ESKD, whereas among the 13 patients who developed a secondary steroid-resistance, 6 progressed to ESKD and were finally transplanted; 2 recurred after transplantation: No statistical difference was found between the two groups

ESKD end-stage kidney disease, *PE* plasma exchanges, *MMF* mycophenolate mofetil, *IV* Intra-venous

in the 33 index patients of each AR family. No disease-causing mutation could be detected. These results suggest that *EMP2* mutations might not be frequently involved in familial AR SSNS.

Table 3 *HLA DQA1* genotyping (rs1129740 and rs1071630) in familial SSNS compared with sporadic SSNS and the public database (1000genome)

Origin	Variation in *HLA-DQA1*	Allele	MAF		OR (95% CI)	Allele	MAF		OR (95% CI)
			Familial SSNS	1000Genome Healthy			Familial SSNS	Sporadic SSNS	
Worldwide	rs1129740	G	32 (0.157)	2,388 (0.477)	OR = 4.89 (3.35–7.18)	G	32 (0.157)	9 (0.122)	OR = 0.74
		A	172 (0.843)	2,620 (0.523)	*p* < 0.001	A	172 (0.843)	65 (0.878)	(0.33–1.63)
									p = 0.46
	rs1071630	T	32 (0.157)	2,238 (0.447)	OR = 4.34 (2.96–6.36	T	32 (0.157)	9 (0.122)	OR = 0.74
		C	172 (0.843)	2,770 (0.553)	*p* < 0.001	C	172 (0.843)	65 (0.878)	(0.33–1.63)
									p = 0.46
Europe	rs1129740	G	18 (0.184)	448 (0.445)	OR = 5.54 (3.27–9.38)	G	18 (0.184)	5 (0.109)	OR = 0.56
		A	80 (0.817)	558 (0.555)	*p* < 0.001	A	80 (0.817)	41 (0.891)	(0.19–1.62)
									p = 0.26
	rs1071630	T	18 (0.184)	406 (0.404)	OR = 6.57 (3.88–1.12)	T	18 (0.184)	5 (0.109)	OR = 0.56
		C	80 (0.817)	600 (0.596)	*p* < 0.001	C	80 (0.817)	41 (0.891)	(0.19–1.62)
									p = 0.26

MAF minor allele frequency, *OR* odds ratio, *CI* confidence interval, *SSNS* steroid-sensitive nephrotic syndrome (http://www.internationalgenome.org)

Exome sequencing in familial SSNS

We searched for new genes involved in hereditary SSNS, focusing on AR forms. In four different families with a known (SS26 and SS20) or suspected (SS27 and SS23) consanguinity, linkage analysis failed to demonstrate any common region of homozygosity. In addition, we failed to find any common region of homozygosity in the multiplex family (SS20) either. In the latter, analysis of the pedigree suggested an AR mode of inheritance, although an X-linked transmission could not be ruled out. No region with a significant LOD score was identified.

Whole exome sequencing was performed in a subset of 13 AR families in which DNA was available in affected siblings and their parents for segregation studies (Supplementary Fig. 1). According to the filtering strategy, and after segregation studies, 8 variants (in 7 genes) were identified in a total of 4 families (1 homozygous, 5 hemizygous, 2 compound heterozygous). No mutated gene was common to two families (Supplementary Table 2). None of the candidate genes identified are known to have specific protein expression in lymphocytes or podocytes according to ProteinAtlas® (Supplementary Table 2). Furthermore, despite the assumption of a circulating factor, none of the gene-associated protein presented a signal peptide for secretion [30]. These results were submitted to GeneMatcher [31], but no match was identified.

SRNS genes screening

Five out of six patients who reached ESKD were screened for the 34 genes known to be involved in SRNS (Supplementary Table 1). The last one had no more DNA available, but presented a steroid-sensitivity (SS12, Supplementary Fig. 1a). Three other patients with loss to follow-up ($n = 1$) or uncommon disease course ($n = 2$) were also screened. Among them, one presented primary steroid-resistance with a notion of complete remission with chronic kidney disease without other information. The other presented a steroid-sensitive NS with a sensorineural deafness. No damaging mutation was found in any of the eight patients.

Discussion

In the past few years, genetic analyses of familial SRNS led to the discovery of many causative mutated genes. Compared with inherited SRNS, familial SSNS is a very uncommon condition, and the rare published studies failed to decipher the pathophysiology of this disease. We report herein 131 affected cases from 59 unrelated families, which to our knowledge represents the largest familial SSNS cohort published to date. Excluding the present report, 139 familial SSNS cases (>

53 families) have already been described in a total of 16 published cohorts (Table 4).

All patients in our cohort were sensitive to any immunosuppressive therapy, which strongly supports immune-mediated mechanisms underlying the pathophysiology of this disease [2]. Indeed, rare observations of primary SRNS finally responded to other immunosuppressive drugs.

Our cohort characteristics are consistent with most series of sporadic idiopathic SSNS [24, 44]. Indeed, we observed a male predominance (59.5%), a median age at onset of 7.85 years (1–58), 35.6% of steroid-dependent and 14.2% of steroid-resistant patients, and 78.3% of MCD. We report here about 56% of FR, regardless of AR or AD group, whereas Harambat et al. reported 60% of FR and steroid-dependent patients in a French cohort of sporadic NS in 2013 [45]. In 1974, Bader et al. already showed a similar clinical course in 16 patients with familial SSNS (9 families) and 54 sporadic cases [35].

Conflicting data exist regarding the intra-familial variability of the disease course in familial SSNS. In 2001, Fuchshuber et al. reported 32 cases (15 families) with familial childhood-onset SSNS [12]. Disease course was favorable for all. Conversely, the clinical course could be very heterogeneous among affected relatives, including monozygotic twins in Bader's series [35]. Our series confirms this intra-familial variability, as 42.4% of families shared both FR and NFR in their affected relatives. It is noteworthy that among the monozygotic triplets, one was unaffected, one was steroid-sensitive, and the third exhibited secondary steroid-resistance. This strongly argues against a monogenic cause of familial SSNS, but rather suggests the role of external environmental factors.

However, we and others have reported the low intra-familial variability regarding the age at onset. The Pearson correlation coefficient was 0.77 and 0.56 in the AR and AD groups respectively. Similarly, in Fuchshuber's series, the Spearman rank coefficient correlation test was 0.60 and the difference in the age at onset between siblings did not exceed 4 years, except in 2 families. This suggests a genetic influence on the age of familial SSNS onset. Indeed, a common environmental cause would result in the synchronous onset of the disease within relatives, but not at the same age. Overall, if environment seems to play a major role in disease course, age at onset could be linked to a genetic predisposition.

At the molecular level, Gee et al. identified *EMP2* mutations in three patients with SSNS from two families, and in one patient with SRNS [19]. The encoding protein EMP2 is expressed in podocyte [19] and non-podocyte glomerular cells [46]. However, the underlying defect in hereditary SSNS would be expected to lie in a gene involved in the immune system. Moreover, the identification of *EMP2* mutations in both SSNS and SRNS patients is an unexpected finding. We did not find any *EMP2* mutation in 33 affected individuals (from 33 AR families), suggesting that *EMP2* mutations might

Table 4 Reported SSNS cohorts since 1970

Reference	Year	Country	Males/Females	Familial cases with SSNS
Roy and Pitcock [32]	1971	Cases from the USA	0/2	Identical twins
Moncrieff et al. [33]	1973	Cases from two hospitals in England	NA	14 cases in 7 families
White [34]	1973	Cases from 24 hospitals in Europe	NA	12 cases
Bader et al. [35]	1974	Cases from a hospital in the USA	12/4	16 cases in 9 families
McEnery and Welch [36]	1989	Cases from a hospital in the USA	NA	8 cases in 4 families
Awadalla et al. [37]	1989	Cases from Kuwait	3/0	3 cases in a family
Mallmann [38]	1998	Cases from a hospital in Pakistan	2/0	2 cases in a family; post-axial hexadactyly
Fuchshuber et al. [12]	2001	Cases from seven Caucasian countries	25/7	32 cases in 15 families
Kari et al. [29]	2001	Cases from Bengali	5/6	11 cases in 3 families
Ruf et al. [14]	2003	Cases from Germany	2/1	3 cases in a family
Landau et al. [13]	2007	Cases from two Bedouin families	10/4	14 cases in 2 families
Roberts and Gleadle [39]	2008	Cases from Australia	1/1	2 cases in a family
Motoyama et al. [40]	2009	Cases from Japan	1/3	4 cases in 2 families
Xia et al. [41]	2013	Cases from China	7/2	9 cases in 3 families
Tusgaard Petersen et al. [42]	2012	Cases from Denmark	2/0	2 cases in a family
Chehade et al. [43]	2013	Cases from Portugal and France	5/0	5 cases in 2 families
Total			75/30 (71.4% males)	139 familial cases

One hundred and thirty-nine familial cases have been reported. The sex ratio was 2.5. The largest cohort was published in 2001 and presented 32 cases in 15 families

NA not available, *SSNS* steroid-sensitive nephrotic syndrome

not be a frequent cause of familial SSNS. Further studies are needed to decipher its link with the immune disorders observed in SSNS during relapses. Besides, data suggest that in addition to their effect on T-cell function, glucocorticoids might also have an impact on the cytoskeleton, and might be potentially beneficial in non-circulating factor NS [47].

Although most hereditary SRNS have a classical monogenic heritability, our results suggest a more complex pattern of inheritance of familial SSNS. Indeed, although SSNS represents more than 90% of patients with NS (both SSNS and SRNS) [48], familial SSNS is very scarce and represents less than 10% of families with NS in our cohort of 570 pedigrees with either familial SSNS or familial SRNS. Furthermore, in the present cohort, only two brothers of a monozygotic male triplet developed SSNS, at the same age, the third one being unaffected (family SS6). Last, we failed to identify any *EMP2* mutation nor any common variant in coding genes. Altogether these data demonstrate that a monogenic inheritance is very unlikely in familial forms of SSNS, although private

mutations in coding sequences or non-coding RNAs cannot be excluded.

On the other hand, several studies reported the HLA loci to be risk factors for SSNS supporting the assumption of a polygenic inheritance [49]. We described herein a strong correlation between SSNS and the two variations in the *HLA-DQA1* gene (rs1129740 and rs1071630), and higher frequencies of these alleles in SSNS patients than that observed in healthy controls. Our findings replicated results reported by Gbadegesin et al. in 2015 [21]. Owing to the difficulty in finding ancestry-identical controls, and because of the lack of familial cases, *HLA-DQA1* variations could not be compared in populations other than European. However, comparisons between our cohort and data from the whole 1000genome database showed similar results (Table 3).

Advances in the pathophysiology underlying nephrotic syndrome emphasize the importance of distinguishing patients with an immune-mediated disease from those related to a podocyte–gene defect. As we report here, steroid-sensitivity is not an exclusive criterion for supporting immune

mediation of the disease, as, in this study, all primary and secondary steroid-resistant patients had a disease course consistent with an immune-mediated NS, and were sensitive to at least one immunosuppressive drug. Monogenicity seems to be a very rare cause of immune-mediated NS, at least in familial forms, supporting the hypothesis of a complex disease. In this sense, new molecular findings in immune-mediated NS, such as *HLA-DQA1* variations, would support disease heterogeneity and help clinicians with regard to patients' classifications. However, these results must be confirmed in larger cohorts. Indeed, only large-scale genetic analysis could help to decipher the pathophysiology of the disease by discovering other genetic susceptibility to developing immune-mediated NS.

Compliance with ethical standards

References

1. Machuca E, Benoit G, Antignac C (2009) Genetics of nephrotic syndrome: connecting molecular genetics to podocyte physiology. Hum Mol Genet 18(R2):R185–R194
2. Ding WY, Koziell A, McCarthy HJ, Bierzynska A, Bhagavatula MK, Dudley JA, Inward CD, Coward RJ, Tizard J, Reid C, Antignac C, Boyer O, Saleem MA (2014) Initial steroid sensitivity in children with steroid-resistant nephrotic syndrome predicts post-transplant recurrence. J Am Soc Nephrol 25(6):1342–1348
3. Shalhoub RJ (1974) Pathogenesis of lipoid nephrosis: a disorder of T-cell function. Lancet 2(7880):556–560
4. Kemper MJ, Wolf G, Muller-Wiefel DE (2001) Transmission of glomerular permeability factor from a mother to her child. N Engl J Med 344(5):386–387
5. Hoyer JR, Vernier RL, Najarian JS, Raij L, Simmons RL, Michael AF (1972) Recurrence of idiopathic nephrotic syndrome after renal transplantation. Lancet 2(7773):343–348
6. Dantal J, Bigot E, Bogers W, Testa A, Kriaa F, Jacques Y, Hurault de Ligny B, Niaudet P, Charpentier B, Soulillou JP (1994) Effect of plasma protein adsorption on protein excretion in kidney-transplant recipients with recurrent nephrotic syndrome. N Engl J Med 330(1):7–14
7. Savin VJ, Sharma R, Sharma M, McCarthy ET, Swan SK, Ellis E, Lovell H, Warady B, Gunwar S, Chonko AM, Artero M, Vincenti F (1996) Circulating factor associated with increased glomerular permeability to albumin in recurrent focal segmental glomerulosclerosis. N Engl J Med 334(14):878–883
8. Boulton Jones JM, Tulloch I, Dore B, McLay A (1983) Changes in the glomerular capillary wall induced by lymphocyte products and serum of nephrotic patients. Clin Nephrol 20(2):72–77
9. Koyama A, Fujisaki M, Kobayashi M, Igarashi M, Narita M (1991) A glomerular permeability factor produced by human T cell hybridomas. Kidney Int 40(3):453–460
10. Davin JC (2015) The glomerular permeability factors in idiopathic nephrotic syndrome. Pediatr Nephrol 31(2):207–215
11. Lovric S, Ashraf S, Tan W, Hildebrandt F (2016) Genetic testing in steroid-resistant nephrotic syndrome: when and how? Nephrol Dial Transplant 31(11):1802–1813
12. Fuchshuber A, Gribouval O, Ronner V, Kroiss S, Karle S, Brandis M, Hildebrandt F (2001) Clinical and genetic evaluation of familial steroid-responsive nephrotic syndrome in childhood. J Am Soc Nephrol 12(2):374–378
13. Landau D, Oved T, Geiger D, Abizov L, Shalev H, Parvari R (2007) Familial steroid-sensitive nephrotic syndrome in southern Israel: clinical and genetic observations. Pediatr Nephrol 22(5):661–669
14. Ruf RG, Fuchshuber A, Karle SM, Lemainque A, Huck K, Wienker T, Otto E, Hildebrandt F (2003) Identification of the first gene locus (SSNS1) for steroid-sensitive nephrotic syndrome on chromosome 2p. J Am Soc Nephrol 14(7):1897–1900
15. Caridi G, Gigante M, Ravani P, Trivelli A, Barbano G, Scolari F, Dagnino M, Murer L, Murtas C, Edefonti A, Allegri L, Amore A, Coppo R, Emma F, De Palo T, Penza R, Gesualdo L, Ghiggeri GM (2009) Clinical features and long-term outcome of nephrotic syndrome associated with heterozygous NPHS1 and NPHS2 mutations. Clin J Am Soc Nephrol 4(6): 1065–1072
16. Dhandapani MC, Venkatesan V, Rengaswamy NB, Gowrishankar K, Ekambaram S, Sengutavan P, Perumal V (2016) Report of novel genetic variation in NPHS2 gene associated with idiopathic nephrotic syndrome in south Indian children. Clin Exp Nephrol 21(1):127–133
17. Mao J, Zhang Y, Du L, Dai Y, Gu W, Liu A, Shang S, Liang L (2007) NPHS1 and NPHS2 gene mutations in Chinese children with sporadic nephrotic syndrome. Pediatr Res 61(1): 117–122
18. Ruf RG, Schultheiss M, Lichtenberger A, Karle SM, Zalewski I, Mucha B, Everding AS, Neuhaus T, Patzer L, Plank C, Haas JP, Ozaltin F, Imm A, Fuchshuber A, Bakkaloglu A, Hildebrandt F, Group APNS (2004) Prevalence of WT1 mutations in a large cohort of patients with steroid-resistant and steroid-sensitive nephrotic syndrome. Kidney Int 66(2):564–570
19. Gee HY, Ashraf S, Wan X, Vega-Warner V, Esteve-Rudd J, Lovric S, Fang H, Hurd TW, Sadowski CE, Allen SJ, Otto EA, Korkmaz E, Washburn J, Levy S, Williams DS, Bakkaloglu SA, Zolotnitskaya A, Ozaltin F, Zhou W, Hildebrandt F (2014) Mutations in EMP2 cause childhood-onset nephrotic syndrome. Am J Hum Genet 94(6):884–890

20. Forbes A, Wadehra M, Mareninov S, Morales S, Shimazaki K, Gordon LK, Braun J (2007) The tetraspan protein EMP2 regulates expression of caveolin-1. J Biol Chem 282(36):26542–26551

21. Gbadegesin RA, Adeyemo A, Webb NJ, Greenbaum LA, Abeyagunawardena A, Thalgahagoda S, Kale A, Gipson D, Srivastava T, Lin JJ, Chand D, Hunley TE, Brophy PD, Bagga A, Sinha A, Rheault MN, Ghali J, Nicholls K, Abraham E, Janjua HS, Omoloja A, Barletta GM, Cai Y, Milford DD, O'Brien C, Awan A, Belostotsky V, Smoyer WE, Homstad A, Hall G, Wu G, Nagaraj S, Wigfall D, Foreman J, Winn MP, Mid-West Pediatric Nephrology C (2015) HLA-DQA1 and PLCG2 are candidate risk loci for childhood-onset steroid-sensitive nephrotic syndrome. J Am Soc Nephrol 26(7):1701–1710

22. Kidney Disease Improving Global Outcome (KDIGO), Glomerulonephritis Work Group (2012) Anti-glomerular basement membrane antibody glomerulonephritis. Kidney Int Suppl 2:240–242

23. Gipson DS, Massengill SF, Yao L, Nagaraj S, Smoyer WE, Mahan JD, Wigfall D, Miles P, Powell L, Lin JJ, Trachtman H, Greenbaum LA (2009) Management of childhood onset nephrotic syndrome. Pediatrics 124(2):747–757

24. Dossier C, Sellier-Leclerc AL, Rousseau A, Michel Y, Gautheret-Dejean A, Englender M, Madhi F, Charbit M, Ulinski T, Simon T, Jacqz-Aigrain E, Deschenes G (2014) Prevalence of herpesviruses at onset of idiopathic nephrotic syndrome. Pediatr Nephrol 29(12): 2325–2331

25. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K (2001) dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 29(1):308–311

26. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR (2015) A global reference for human genetic variation. Nature 526(7571):68–74

27. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Exome Aggregation C (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature 536(7616):285–291

28. Boyer O, Lipska-Zietkiewicz B, Gribouval O, Nitschke P, Bole C, Rothier A, Schaefer F, Antignac C (2014) Rationalizing the genetic diagnosis of SRNS/FSGS using next generation sequencing kits. J Am Soc Nephrol 25:172A

29. Kari JA, Sinnott P, Khan H, Trompeter RS, Snodgrass GJ (2001) Familial steroid-responsive nephrotic syndrome and HLA antigens in Bengali children. Pediatr Nephrol 16(4):346–349

30. Petersen TN, Brunak S, von Heijne G, Nielsen H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 8(10):785–786

31. Sobreira N, Schiettecatte F, Valle D, Hamosh A (2015) GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat 36(10):928–930

32. Roy S 3rd, Pitcock JA (1971) Idiopathic nephrosis in identical twins. Am J Dis Child 121(5):428–430

33. Moncrieff MW, White RH, Glasgow EF, Winterborn MH, Cameron JS, Ogg CS (1973) The familial nephrotic syndrome. II. A clinico-pathological study. Clin Nephrol 1(4):220–229

34. White RH (1973) The familial nephrotic syndrome. I. A European survey. Clin Nephrol 1(4):215–219

35. Bader PI, Grove J, Trygstad CW, Nance WE (1974) Familial nephrotic syndrome. Am J Med 56(1):34–43

36. McEnery PT, Welch TR (1989) Major histocompatibility complex antigens in steroid-responsive nephrotic syndrome. Pediatr Nephrol 3(1):33–36

37. Awadalla NB, Teebi AS, Elzouki AY, Shaltout A (1989) Frequent relapser minimal change nephrosis: an unrecognized X-linked disorder? Eur J Pediatr 149(3):205–207

38. Mallmann R (1998) Idiopathic nephrotic syndrome and hexadactyly in two brothers. Pediatr Nephrol 12(5):417–419

39. Roberts IS, Gleadle JM (2008) Familial nephropathy and multiple exostoses with exostosin-1 (EXT1) gene mutation. J Am Soc Nephrol 19(3):450–453

40. Motoyama O, Sugawara H, Hatano M, Fujisawa T, Iitaka K (2009) Steroid-sensitive nephrotic syndrome in two families. Clin Exp Nephrol 13(2):170–173

41. Xia Y, Mao J, Jin X, Wang W, Du L, Liu A (2013) Familial steroid-sensitive idiopathic nephrotic syndrome: seven cases from three families in China. Clinics 68(5):628–631

42. Tusgaard Petersen B, Frydensbjerg Andersen R, Rittig S (2012) Family occurrence of steroid-sensitive idiopathic nephrotic syndrome. Ugeskr Laeger 174(46):2868–2869

43. Chehade H, Cachat F, Girardin E, Rotman S, Correia AJ, Fellmann F, Bonny O (2013) Two new families with hereditary minimal change disease. BMC Nephrol 14:65

44. Dossier C, Lapidus N, Bayer F, Sellier-Leclerc AL, Boyer O, de Pontual L, May A, Nathanson S, Orzechowski C, Simon T, Carrat F, Deschenes G (2016) Epidemiology of idiopathic nephrotic syndrome in children: endemic or epidemic? Pediatr Nephrol 31(12): 2299–2308

45. Harambat J, Godron A, Ernould S, Rigothier C, Llanas B, Leroy S (2013) Prediction of steroid-sparing agent use in childhood idiopathic nephrotic syndrome. Pediatr Nephrol 28(4):631–638

46. Takemoto M, He L, Norlin J, Patrakka J, Xiao Z, Petrova T, Bondjers C, Asp J, Wallgard E, Sun Y, Samuelsson T, Mostad P, Lundin S, Miura N, Sado Y, Alitalo K, Quaggin SE, Tryggvason K, Betsholtz C (2006) Large-scale identification of genes implicated in kidney glomerulus development and function. EMBO J 25(5): 1160–1174

47. Gbadegesin R, Lavin P, Foreman J, Winn M (2011) Pathogenesis and therapy of focal segmental glomerulosclerosis: an update. Pediatr Nephrol 26(7):1001–1015

48. International Study of Kidney Disease in Children.(1981) The primary nephrotic syndrome in children. Identification of patients with minimal change nephrotic syndrome from initial response to prednisone. A report of the International Study of Kidney Disease in Children. J Pediatr 98(4):561–564

49. Karp AM, Gbadegesin RA (2016) Genetics of childhood steroid-sensitive nephrotic syndrome. Pediatr Nephrol. https://doi.org/10.1007/s00467-016-3456-8