A Gene Locus for Steroid-Resistant Nephrotic Syndrome with Deafness Maps to Chromosome 14q24.2

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Abstract. Steroid-resistant nephrotic syndrome (SRNS) leads to end-stage renal disease (ESRD) in childhood or young adulthood. Positional cloning for genes causing SRNS has opened the first insights into the understanding of its pathogenesis. This study reports a genome-wide search for linkage in a consanguineous Palestinian kindred with SRNS and deafness and detection of a region of homozygosity on chromosome 14q24.2. Multipoint analysis of 12 markers used for further fine mapping resulted in a LOD score $Z_{\text{max}}$ of 4.12 ($\theta = 0$) for marker $D14S1025$ and a two-point LOD score of $Z_{\text{max}} = 3.46$ ($\theta = 0$) for marker $D14S77$. Lack of homozygosity defined $D14S1065$ and $D14S273$ as flanking markers to a 10.7 cM interval. The identification of the responsible gene will provide new insights into the molecular basis of nephrotic syndrome and sensorineural deafness.

Nephrotic syndrome (NS) is defined as the association of proteinuria, hypoalbuminemia, edema, and hyperlipidemia. Autosomal recessive steroid-resistant nephrotic syndrome (SRNS) has been described as childhood onset of proteinuria, rapid progression to ESRD, resistance to steroid therapy, and absence of recurrence after renal transplantation (1). It remains the most intractable cause for ESRD in the first two decades of life. Whereas renal biopsy mostly shows minimal change nephrotic syndrome (MCNS) in steroid-sensitive nephrotic syndrome (SSNS), renal biopsy in SRNS reveals different morphologic changes, such as MCNS, mesangial sclerosis, or focal segmental glomerulosclerosis (FSGS) (2). Treatment ranges from early bilateral nephrectomy and transplantation in the congenital nephrotic syndrome of the Finnish type (3), and mutations in $NPHS2$, encoding podocin (OMIM 604766), cause SRNS type 1 (4). Mutations in $ACTN4$, encoding $\alpha$-actinin 4 (OMIM 604638) have been identified as an autosomal dominant cause of SRNS (5). An additional locus has been mapped to chromosome 11q21-q22 for an autosomal dominant form of nephrotic syndrome (OMIM 603965) (6). Gene identification underlined the importance of genetic factors in the pathogenesis of nephrotic syndrome. Through identification of these three genes as causative for SRNS, their gene products, nephrin, podocin, and $\alpha$-actinin 4, were identified as important for the function of the glomerular podocyte (7). Specifically, these gene products were shown to represent integral components of the glomerular slit membrane localized between podocyte foot processes, which represents the primary glomerular filter of the kidney (7). We here performed a total genome search for linkage, applying the paradigm of homozygosity mapping to a Palestinian kindred with the association of SRNS and sensorineural deafness (SND), and identified a new gene locus (SRN2) for SRNS with SND within a 10.7 cM interval on chromosome 14q24.2.

Materials and Methods

Blood samples and clinical data of the family with SRNS and SND were obtained after informed consent was obtained from patients and their parents and siblings. Ethnic origin of the family was Palestinian. Affected individuals all showed symptoms of nephrotic syndrome (gross proteinuria, hypoalbuminemia, edema, and hyperlipidemia). Diagnosis was established by a pediatric nephrologist. Onset of disease ranged from 0.3 to 6.4 yr (Table 1). The histopathologic diag-
nosis was FSGS. All affected individuals entered ESRD within the range of 1.25 to 9.3 yr (Table 1). In three patients, kidney transplantation was performed and no recurrence of FSGS was described. Congenital SND was diagnosed in all four patients. Patient IV: 2 died at the age of 15 yr from acute heart failure when restarting dialysis after chronic rejection of the kidney transplant. Patient V: 1 died at the age of 1.7 yr from peritonitis and cerebral candida infection while on peritoneal dialysis (Table 1). Genomic DNA was isolated, by standard methods (8), either directly from blood samples or after Epstein-Barr virus transformation of peripheral blood lymphocytes (9). For the methods (8), either directly from blood samples or after Epstein-Barr virus transformation of peripheral blood lymphocytes (9). For the genome-wide search for linkage, DNA was available in two affected individuals, four unaffected siblings, and both parents who were first cousins and for two parents and their children, one affected, who were more distantly related to the first pedigree (Figure 1). All affected individuals were offspring of consanguineous marriages; therefore, an affecteds-only strategy was defined as the genetic map positions intersecting the LOD interval –1 support interval” was defined as the genetic map positions intersecting the LOD curve at Zmax –1 (13). For haplotyping and computation of multipoint LOD scores, the program SIMWALK (14) was used, allele frequencies were calculated according to the database of the Gene Mapping Center and Department of Molecular Genetics, Max-Delbrueck Center for Molecular Medicine, Berlin-Buch, Germany (unpublished data). The comprehensive data collected during hundreds of total genome searches representing a wide range of different ethnicities are included in the database, which represents an appropriate population to estimate allele frequencies. In addition, the LOD Score of 4.1 obtained in these data is robust enough for a wide range of allele frequencies (15).

Results
By using haplotype analysis data of the genome-wide search, the known loci for SRNS on chromosomes 1q25–31 (NPHS2), 11q21–q22 (6), and 19q13 (NPHS1 and ACTN4) were excluded. Of all 380 markers employed in the genome-

Table 1. Clinical data of affected individuals in Palestinian kindred with SRNS and SND

<table>
<thead>
<tr>
<th>Individual</th>
<th>Gender</th>
<th>Age at Onset (yr)</th>
<th>PU</th>
<th>Renal Biopsy</th>
<th>Age at ESRF (yr)</th>
<th>Age at Renal Transplant (yr)</th>
<th>SND</th>
<th>Exitus Letalis (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV:2</td>
<td>F</td>
<td>1.8</td>
<td>+</td>
<td>FSGS</td>
<td>4.0</td>
<td>4.4</td>
<td>+</td>
<td>15.0</td>
</tr>
<tr>
<td>IV:3</td>
<td>F</td>
<td>6.4</td>
<td>+</td>
<td>FSGS</td>
<td>9.3</td>
<td>10.1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>IV:6</td>
<td>M</td>
<td>0.3</td>
<td>+</td>
<td>ND</td>
<td>1.7</td>
<td>2.2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>V:1</td>
<td>F</td>
<td>1.2</td>
<td>+</td>
<td>FSGS</td>
<td>1.2</td>
<td>–</td>
<td>+</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* ESRF, end-stage renal failure; FSGS, focal segmental glomerulosclerosis; ND, not done; PU, proteinuria; SND, sensorineural deafness.

Table 2. Two-point LOD scores at various recombination fractions for 12 microsatellite markers versus SRN2 on chromosome 14q24.2

<table>
<thead>
<tr>
<th>Marker</th>
<th>0</th>
<th>0.001</th>
<th>0.01</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>Zmax (at θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D14S1069</td>
<td>−0.115</td>
<td>−0.038</td>
<td>0.327</td>
<td>0.710</td>
<td>0.760</td>
<td>0.597</td>
<td>0.360</td>
<td>0.148</td>
<td>0.760 (0.1)</td>
</tr>
<tr>
<td>D14S1065</td>
<td>−0.125</td>
<td>−0.048</td>
<td>0.315</td>
<td>0.683</td>
<td>0.712</td>
<td>0.521</td>
<td>0.276</td>
<td>0.085</td>
<td>0.712 (0.1)</td>
</tr>
<tr>
<td>D14S1029</td>
<td>1.528</td>
<td>1.524</td>
<td>1.486</td>
<td>1.321</td>
<td>1.118</td>
<td>0.736</td>
<td>0.411</td>
<td>0.166</td>
<td>1.528 (0.0)</td>
</tr>
<tr>
<td>D14S588</td>
<td>2.946</td>
<td>2.939</td>
<td>2.882</td>
<td>2.621</td>
<td>2.289</td>
<td>1.609</td>
<td>0.951</td>
<td>0.397</td>
<td>2.946 (0.0)</td>
</tr>
<tr>
<td>D14S258</td>
<td>2.901</td>
<td>2.895</td>
<td>2.837</td>
<td>2.577</td>
<td>2.246</td>
<td>1.571</td>
<td>0.923</td>
<td>0.383</td>
<td>2.901 (0.0)</td>
</tr>
<tr>
<td>D14S1002</td>
<td>2.879</td>
<td>2.874</td>
<td>2.822</td>
<td>2.587</td>
<td>2.284</td>
<td>1.651</td>
<td>1.009</td>
<td>0.436</td>
<td>2.879 (0.0)</td>
</tr>
<tr>
<td>D14S77</td>
<td>3.460</td>
<td>3.453</td>
<td>3.392</td>
<td>3.116</td>
<td>2.758</td>
<td>2.002</td>
<td>1.221</td>
<td>0.514</td>
<td>3.460 (0.0)</td>
</tr>
<tr>
<td>D14S71</td>
<td>2.997</td>
<td>2.990</td>
<td>2.932</td>
<td>2.667</td>
<td>2.327</td>
<td>1.628</td>
<td>0.948</td>
<td>0.382</td>
<td>2.997 (0.0)</td>
</tr>
<tr>
<td>D14S1025</td>
<td>3.246</td>
<td>3.240</td>
<td>3.180</td>
<td>2.910</td>
<td>2.563</td>
<td>1.839</td>
<td>1.115</td>
<td>0.481</td>
<td>3.246 (0.0)</td>
</tr>
<tr>
<td>D14S1047</td>
<td>2.741</td>
<td>2.735</td>
<td>2.683</td>
<td>2.448</td>
<td>2.145</td>
<td>1.517</td>
<td>0.893</td>
<td>0.364</td>
<td>2.741 (0.0)</td>
</tr>
<tr>
<td>D14S273</td>
<td>−0.243</td>
<td>−0.132</td>
<td>0.315</td>
<td>0.719</td>
<td>0.761</td>
<td>0.587</td>
<td>0.347</td>
<td>0.139</td>
<td>0.761 (0.1)</td>
</tr>
<tr>
<td>D14S270</td>
<td>−0.015</td>
<td>0.119</td>
<td>0.611</td>
<td>1.012</td>
<td>1.028</td>
<td>0.788</td>
<td>0.475</td>
<td>0.199</td>
<td>1.028 (0.1)</td>
</tr>
</tbody>
</table>
wide search, only marker D14S588 showed homozygosity for
the same allele in all affected individuals. No homozygosity
was found in the unaffected family members. The maximum
two-point LOD score at D14S588 was 2.94 (θ = 0). Further
fine mapping with additional ten markers confirmed this region
as contiguously homozygous by descent extending from
marker D14S1029 to D14S1047. Homozygosity was present
in all affected individuals and was absent in unaffected individ-
uals. Lack of homozygosity in individual V:1 defined marker
D14S1065 as the centromeric flanking marker, and marker
D14S273 as flanking on the q-telomeric side (Figure 1), thus
restricting the critical genetic region for SRNS2 to a 10.7 cM
interval on chromosome 14q24.2. Multipoint analysis of the 12
markers resulted in a $Z_{\text{max}}$ of 4.12 for marker D14S1025 at
relative position 71.6 (Figure 2). The 95% CI at $Z_{\text{max}} - 1$ (13)
extends over a 10.1 cM interval flanked by marker D14S1029
and D14S1047. Marker D14S77 showed the highest two-point
LOD score with $Z_{\text{max}} = 3.46$ (θ = 0) (Table 1).

**Discussion**

Employing a homozygosity mapping strategy, we thus iden-
tified by total genome search a new gene locus (SRN2) for
a new disease entity of SRNS occurring in association
with SND. According to the UCSC Genome Browser, the interval
between flanking markers D14S1065 and D14S273 spans a
physical distance of approximately 7 Mb (relative marker
positions are 66,310,876 bp and 73,183,615 bp, respectively)
and contains approximately 50 known genes. Among these
genes, the α-actinin 1 (ACTN1) gene is located in the region
suitable for linkage in this family at 66,846,267 to 66,951,188
Mb according to the UCSC Genome Browser. ACTN1 shows
over 70% amino acid homology to ACTN4 (16), which has
been identified as an autosomal dominant cause of SRNS (5).
ACTN1 was found to be expressed in Corti’s organ of the adult

**Figure 1.** Haplotypes on chromosome 14q24.2 of the Palestinian kindred with steroid-resistant nephrotic syndrome (SRNS) and sensorineural deafness (SND). Twelve microsatellites are shown from cen to q-ter (top to bottom). Haplotypes were generated by minimizing recombinants. Circles denote females, squares males; filled symbols indicate affected status. A slash through a symbol marks a deceased individual. Haplotypes are interpreted as differently shaded bars. Paternal haplotypes are drawn to the left, maternal ones to the right. Double lines indicate consanguineous marriages. Regions of contiguous homozygosity are denoted in boxes. The Proband is indicated with an arrow. Note that markers D14S1065 and D14S273 (underlined) are flanking the SRN2 locus.

**Figure 2.** Multipoint LOD scores for the SRN2 locus versus the twelve markers shown in Figure 1 were calculated for the Palestinian SRNS family with SND. Relative positions are given in cM according to the Genethon map (10). The two markers, D14S1065 and D14S273, flanking the SRNS region (see Figure 1) are underlined. q-ter, q-
terminal orientation; cen, centromeric orientation.

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rat and more specifically in the cuticular plate supporting the stereocilia as well as in the stereocilia of the outer and inner hair cells. In addition, it is expressed in the pillar cells and supporting cells (17). No expression of ACTN1 in the kidney has been described so far (5,18). No mutation was found when directly sequencing the 21 exons of ACTN1 for individuals III:10 and IV:6 of the Palestinian pedigree (data not shown).

In the search for the gene responsible for the disease in this family, genes that encode components of the podocyte foot process cytoskeleton and genes that encode proteins interacting with proteins already described to be impaired in NS should be excellent candidate genes. In addition, a role in the function of the inner ear will have to be postulated for candidate genes. Identification of the gene responsible for SRN2 will provide new insights into disease mechanisms of SRNS as well as into the function of the inner ear.

Acknowledgments

We thank all family members and their physicians for the participation in this study.


References


