The genetics of late-onset steroid-resistant nephrotic syndrome and nephronophthisis

Ph.D. thesis

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Introduction

The most common causes of end-stage renal disease (ESRD) in chidhood are monogenic diseases and congenital anomalies of the kidney and urinary tract. The detailed percentage distribution of the causes is missing in the literature. According to previous studies steroid-resistant nephrotis syndrome (SRNS) and nephronophthisis accounts for 6-7% and 6-10% of ESRD cases, respectively.

Based on its etiology, two forms of SRNS can be distinguished, an immune and a genetic form. As the treatment of these two forms is different, their differentiation is clinically important. In the genetic form the immunosuppressive treatment is unnecessary and the recurrence of nephrotic syndrome in a kidney graft is unlikely. The differentiation is primarily based on the identification of the causative mutation. *NPHS2* is the most frequently mutated gene, it may cause congenital, infantile, childhood and late-onset SRNS in function of the mutation. The late-onset form develops in patients who are compound heterozygous for a mutation and the p.R229Q polymorphism. The significance of this latter is evidenced by its high allele frequency in Europe (MAF=3%). The pathogenicity of homozygous p.R229Q is questionable.

The major gene of nephronophthisis is *NPHP1*. Nephronophthisis is often diagnosed at the stage of end-stage renal

disease as the symptoms of nephronophthisis (polyuria-polydypsia, anemia and failure to thrive) are typically subtle. The genetic diagnosis in nephronophthisis therefore plays an important role in the diagnosis and makes unnecessary the kidney biopsy.

Only genetic investigations make the clinical diagnosis of SRNS and nephronophthisis certain. However, genetic investigations in SRNS and nephronophthisis were not available in Hungary, and in some cases, it might be difficult to ascertain the causal role of an identified genetic variant.

Objectives

1. To determine the clinical etiology of ESRD in the cohort of children treated at the Ist Department of Pediatrics, Semmelweis University.
2. To establish an online registry for children with SRNS and cystic kidney disease in Hungary.
3. To introduce the mutational screening of *NPHS1*, *NPHS2*, *WT1* and *PAX2* genes among children with SRNS in Hungary.
4. To determine the role of *NPHS2* p.V290M mutation in late-onset SRNS.
5. To determine the pathogenicity of the *NPHS2* p.R229Q polymorphism.
6. To introduce the mutational screening of *NPHP1* in nephronophthisis.
7. To determine which clinical symptoms are specific for nephronophthisis based on the clinical data of patients with different genetically proven cystic kidney diseases.

Methods

Patients

To determine the etiology of ESRD, the clinical data of 88 families treated between 2002 and 2012 in the first Department of Pediatrics, Semmelweis University was used.

Sixty-three patients with SRNS or nephrotic range proteinuria (>40mg/m^2/h) were enrolled in the SRNS cohort.

Ninety-five children from 85 family with cystic kidney or chronic kidney failure without hematuria, nephrotic range proteinuria or urinary tract malformation were involved in the nephronophthisis study. Among them the specific symptoms of nephronophthisis were investigated in children with genetically proven nephronophthisis (n=19), autosomal recessive polycystic kidney disease (n=14), renal hypoplasia (n=9), autosomal dominant tubulointerstitial kidney disease (n=2) and autosomal dominant polycystic kidney disease with positive family history (n=26). The prevalence of anemia, polyuria, failure to thrive, hypertension and ametropia were compared between with and without nephronophthisis.

Laboratory methods

In patients with SRNS or nephrotic range proteinuria the 8 exons of *NPHS2* (n=43), the 29 exons of *NPHS1* (n=4), exon 14, 22 and 24 of *NPHS1* (n=13), exon 8 and 9 of *WT1* (n=20) and the 12 exons and the intronic junctions of *PAX2* (n=6) were sequenced. In late-onset SRNS only the *NPHS2* p.R229Q polymorphism and the p.V290M mutation (n=6) were screened.

The *NPHS2* p.V290M mutation was examined in late-onset SRNS in two large cohorts: (1) in 83 patients originating mainly from Europe and North Africa (the French cohort) and (2) in 95 patients originating mainly from Italy, Turkey, Germany and Poland (the PodoNet cohort). The mutational screening of the PodoNet consortium were performed in several foreign laboratories.

Family members of patients carrying p.V290M mutations were genotyped using markers D1S3760, D1S215, D1S3759 and D1S2751 neighboring the *NPHS2* gene locus.

The screening of the *NPHS2* p.R229Q polymorphism was performed in 212 control subjects and in 7 relatives of the 3 patients with p.V290M mutation, who were heterozygous for the p.V290M mutation.

In patients with nephronophthisis, the *NPHP1* homozygous deletion was first screened by the amplification of exons 7, 19, introns 2, 18 and a control region (n=26). Second, a heterozygous deletion was investigated by QMPSF and MLPA (n=16). Once a

heterozygous deletion was identified, the 20 coding exons and the intronic junctions were directly sequenced (n=7).

A part of the mutational screening was performed in INSERM U983, Hôpital Necker-Enfants Malades, at the University of Michigan, Ann Arbor, MI and at the University of Debrecen, Department of Laboratory Medicine, Laboratory of Molecular Genetics.

Statistical analysis

All statistical analyses were performed using Statistica package. A value of $p<0.05$ was considered to be significant. Normality of data distribution was analyzed by the Shapiro–Wilk's test. Parameters with abnormal distribution were evaluated using Mann–Whitney test. Fisher's exact test was used in the analysis of contingency tables and haplotype frequencies.

Results

The determination of the clinical etiology of ESRD in children treated at the first Department of Pediatrics, Semmelweis University.

Among the investigated 88 families ESRD was caused by SRNS (30%), nephronophthisis (10%), renal hypoplasia (14%), congenital anomalies of the kidney and urinary tract (8%), Alport syndrome (7%), autosomal recessive polcystic kidney disease (3%), glomerulonephritis (7%), other (17%) and unknown disease (3%). In this cohort half of the families suffered from monogenic disease. We found SRNS and nephronophthisis to be responsible for ESRD in 40% of the cases.

The creation of an online registry for children with SRNS and cystic kidney disease in Hungary.

Clinical data of 81 patients with SRNS and that of 141 with cystic kidney disease (26 with nephronophthisis) were uploaded.

The introduction of the mutational screening of NPHS1, NPHS2, WT1 and PAX2 gene among children with SRNS in Hungary.

32% of the investigated 57 families were found to carry a causative mutation in the *NPHS1, NPHS2, WT1* or *PAX2* genes in

2%, 14% 9% and 7% of the families, respectively. The *NPHS2* p.E264* mutation was novel.

The investigation of the role of NPHS2 p.V290M mutation in late-onset SRNS.

The two patients carrying the homozygous or compound heterozygous *NPHS2* p.V290M mutation followed a markedly milder clinical course compared to the classical *NPHS2*-related phenotype. Hypalbuminemia and edema has never developed in the 18-year-old boy. The second patient developed nephrotic syndrome at the age of 27 years. Currently, at the age of 21 and 31 years, their kidney function is normal. As the allele frequency of p.V290M mutation was important in the Hungarian cohort, we examined its allele frequency in late-onset SRNS in two large European cohorts. While we did not fint it in any of the 83 patients in the French cohort, two of the 95 patients in the PodoNet cohort (a German and a Turkish person) were found to carry the p.V290M mutation. To investigate whether a founder effect could explain the observed geographical differences, the 12 parental alleles of the three patients carrying the p.V290M were genotyped. While the eight p.290V alleles were all different from each other, three of the four p.290M allele shared a common haplotype. Both this finding and the isolated geographic distribution shows that the p.V290M mutation is a founder mutation.

The determination of the patogenicity of homozygous NPHS2 p.R229Q polymorphism.

We found a patient with nephrotic-range proteinuria to carry the *NPHS2* p.R229Q polymorphism in the homozygous state. He was incidentally diagnosed with proteinuria at the age of 7 months. Renal biopsy later showed focal segmental glomerulosclerosis (FSGS). His proteinuria persisted but his serum albumin level never dropped below 30 g/l, nor did he develop edema. He reached ESRD at the age of 33 years. The patient has nystagmus associated with a poor visual acuity on the left side. Both the father and the healthy brother carried the p.R229Q variant in the homozygous state, with no proteinuria at the age of 59 and 40 years, respectively. Haplotype analysis revealed that the paternal allel of the index patient was different from that of his brother's. Therefore, the role of a cryptic mutation in the *NPHS2* gene could not be excluded. An extended ophthalmological examination was performed, "Morning glory" disk anomaly was detected, which is specific for *PAX2* mutations. The mutational screening revealed a de novo, heterozygous frameshift *PAX2* mutation (c.76dupG, p.V26Gfs*28), which could explain both the renal insufficiency and the ocular involvement of the index patient. Although *PAX2* mutations are rarely associated with FSGS.

The determination of the patogenicity of NPHS2 [p.R229Q;p.V290M] *association.*

As other p.R229Q-mutation association, the [p.R229Q;p.V290M] was considered as pathogenic. In case of its pathogenicity, several cases carrying [p.R229Q;p.V290M] should have been identified, but it was never reported, making its pathogenicity unlikely. We indeed found two second-degree relatives of a patient homozygous for p.V290M mutation who carried [p.R229Q;p.V290M] with no proteinuria at the ages of 37 and 43 years, confirming its non-pathogenic nature.

The introduction of the mutational screening of NPHP1 gene among children with nephronophthisis.

Sixty-two percent of the families with nephronophthisis carried *NPHP1* mutations. Nine of them harboured a homozygous deletion of the whole *NPHP1* gene. Five patients carried a heterozygous deletion associated with either a point mutation (c.489delT, p.Phe163Leufs*19), a short deletion (c.84_87delTTCT, p. Ser29Argfs*4) or a deletion of exons 18-20. Two of these associated mutations were novel.

The characterisation of the clinical symptoms specific for nephronophthisis based on the clinical data of patients with different genetically proven cystic kidney diseases.

Clinical symptoms specific for nephronophthisis were investigated in patients with nephronophthisis (n=19) and with other cystic/tubulointerstitial kidney disorders (n=51). The prevalence of anemia did not differ in the two group (1/16, 6% vs. 4/49, 8%, $p>0,05$). However polyuria/polydypsia, failure to thrive, and ametropia was significantly frequent in the nephronophtisis group (PU/PD: 15/18, 83% vs. 2/37, 5%, $p\leq0,05$, failure to thrive: 5/19, 26% vs. 2/44, 5%, $p\leq0,05$, ametropia: 9/17, 53% vs. 4/20, 20%, $p\leq0,05$). Hypertension was more frequent in the non-nephronophthisis group (1/18, 6% vs. 15/46, 33% $p\leq0,05$). Half of the patients with nephronophthisis developed three of the four symptomes (polyuria-polydypsia, failure to thrive, ametropia, normotension) but nobody in the non-nephronophthisis group. We found that besides the polyuria and failure to thrive, not the generally accepted anemia, but the normotension and ametropia are the main characteristics of nephronophthisis among patients with cystic/tubulointerstitial kidney disorders.

Conclusions

1. SRNS and nephronophthisis are responsible for 40% of end-stage renal disease in our cohort.

2. *NPHP1* was mutated in ⅔ of the patients with nephronophthisis. Based on its frequency its screening should be the first step in patients with nephronophthisis-specific symptoms even in the era of next-generation sequencing.

3. In the Hungarian SRNS cohort pathogen mutation was identified in 32% of the cases.

4. The *NPHS2* p.V290M mutation can cause nephrotic syndrome even in the third decade of life. Therefore, in contrast of previous diagnostic algorithm patients with late-onset (>14 years of age) SRNS or nephrotic range proteinuria should be screened not only for the *NPHS2* p.R229Q polymorphism but also for the p.V290M mutation in Central and Eastern Europe.

5. In contrast to the previous view the *NPHS2* [p.V290M];[p.R229Q] association is not pathogenic, therefore the partners of patients carrying p.V290M mutation should not be screened for p.R229Q polymorphism.

6. The *NPHS2* homozygous p.R229Q is not pathogenic. Patients with SRNS and homozygous p.R229Q should be screened for the causal mutation in a second gene.

7. Besides the polyuria and failure to thrive, not the generally accepted anemia, but the normotension and ametropia are the specific symptoms of nephronophthisis among patients with cystic/tubulointerstitial kidney disorders.

List of publications

The PhD thesis is based on the following publications (in a chronological order):

Kerti A, Csohany R, Szabo A, Arkossy O, Sallay P, Moriniere V, Vega-Warner V, Nyiro G, Lakatos O, Szabo T, Lipska BS, Schaefer F, Antignac C, Reusz G, Tulassay T , Tory K (2013) *NPHS2* p.V290M mutation in late-onset steroid-resistant nephrotic syndrome. PEDIATRIC NEPHROLOGY 28:(5) pp. 751-757.

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chronic kidney disease. PEDIATRIC NEPHROLOGY 28:(6) pp. 875-884.

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