

## Podocin Mutation in Steroid Resistant Nephrotic Syndrome

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Proteinuric kidney diseases are as major problem in pediatric nephrology. Despite extensive research the pathogenesis of proteinuria has been poorly understand. Recently functional or structural defects in the slit diaphragm components lead to protein leakage through the podocyte slit pore. The glomerular capillary wall is composed of three layers; a fenestrated endothelium, glomerular basement membrane (GBM) and the epithelial cell (podocyte) layer with distal foot processes. The foot processes are connected about the GBM by slit diaphragm which bridges the filtration pores between the adjacent podocyte foot processes (1).

The component of the glomerular filtration barrier has recently been identified through genetic approaches. Neph-1 (2), CD2AP (3),  $\alpha$  actinin 4 (4), and podocin (5) are critical to the functioning of a highly specialized glomerular cell type the podocyte and hence maybe involved in pathogenesis of nephrotic syndrome (NS) or heavy proteinuria. Kestilä et al. isolated a novel gene (NPHS1) responsible for congenital nephrotic syndrome of Finnish type (CNS). Soon after identification of the neph-1 gene, Boue et al. firstly found mutation of another gene in NPHS2 locus. Neph-1 and podocin are not the only components in the podocyte slit pore. The slit diaphragm also contains other proteins, such as p-catherin, and FAT, whose functions are unresolved. Neph-1 and Neph-1 interact with the intracellular adaptor molecules podocin, CD2 AP and ZO-1, which connect the slit diaphragm to the actin cytoskeleton of foot processes. The adapter molecules also enhance the signaling function of neph-1 and neph-1 (9).

Podocin is an integral membrane protein and is encoded by NPHS2 which is mapped to 1q25-31 and is exclusively expressed in glomerular podocytes. The genetic work has revealed that mutations in the podocin gene can be responsible for autosomal recessive familial steroid resistant nephrotic syndrome (SRNS) with minor glomerular abnormalities or focal segmental glomerulosclerosis (FSGS) which is characterized by early childhood onset and rapid progression to chronic renal insufficiency (5). This gene mutation is also responsible for an adolescent/adult onset form of autosomal recessive familial FSGS with heavy proteinuria it has been demonstrated that sporadic SRNS and heavy proteinuria are also due to NPHS2 gene mutations (6). Several groups from European countries recently showed that in 10%-30% of patients with sporadic SRNS the SRNS was caused by NPHS2 gene mutations. Frishberg et al. reported that among children of Israel-Arabic descent, a specific NPHS2 mutation (R 138X) was responsible for cases of SRNS (7,10). Tsukaguchi et al. (8) studied NPHS2

mutations in adult FSGS patients. Many cases were compound heterozygotes carrying a R229Q variant in one allele. Also we analysed NPHS2 gene in Turkish children with steroid resistant nephrotic syndrome. This study is the first systemic investigation of NPHS2 gene mutations in familial and sporadic SRNS in Turkish children. Sixtyfive (33 girls and 32 boys) diagnosed as steroid resistant nephrotic syndrome according to ISKDC criteria were included the study. The mean NS diagnosis age was 46±42 months (range 4-187 months) and median follow-up time was 4.2 years. The characteristic features included family occurrence, onset of proteinuria, response to therapies, renal histology, permanent renal functional failure, progression to end-stage renal failure (ESRD) and recurrence after transplantation was noted. Table 1. Twelve patients from 10 families had positive history for NS in their relatives and 53 patients was sporadic SRNS. Autosomal recessive inheritance pattern was defined in the all families. We could find podocin mutation in 34% of sporadic and 33% of familial SRNS. Also we found new podocin mutations (901-903GCT, P118L, 460/7insT). These new mutations are waiting confirmational studies. Three of 5 patients who reached ESRD were transplanted in our group. There was no any nephrotic syndrome recurrence in 3 patients.

In conclusion we could find;

- Podocin mutations are frequently seen in both familial and sporadic Turkish SRNS patients.
- Podocin mutation carrying patients represents broad clinical and histological spectrum.
- Clinical and histological findings are not significantly different between podocin mutation carriers and other SRNS patients. But reaching to ESRD is faster in podocin mutation carrying patients.
- Distribution of ACE I/D, AGT M235T and AT1 A1166c genotype related to renal fibrosis genotypes are same in patients carried podocin mutation and other SRNS in our study group.
- Determining of podocin mutation may be helpful to prevent unnecessary treatment modalities that can be hazardous.
- Podocin mutation carriers are good candidate for renal transplantation. But recurrence of proteinuria or nephrotic syndrome must be in mind.
- Some other factors effecting to renal injury and fibrosis may play role in disease progression in podocin mutation carrying patients.

**Table 1. The findings of transplanted podocin mutation carrying patients. Renal transplantation was performed in 4 of 13 ESRD patients.**

Case No	Mutation	Tx age (mo)	Donor	Proteinuria after TX	Follow-up (month)	GFR (ml/sc/1.73 m <sup>2</sup> )
1	R138X Hom/419delG Het	54	Cadaveric	Yes (22 mg/m <sup>2</sup> /hr)	4	66
7	S96S Hom	111	Cadaveric		12	45
13	460/7insT Hom	120	LRD (mother)		40	50

**References**

1. Tryggveson K: Unraveling the mechanisms of glomerular ultrafiltration. Nephrin; a key component of the slit diaphragm. *J. Am Soc. Nephrol.* 10:2440-2445, 1999.
2. Kestila M, Lenkkeri U, Mannikko M, Lamberdin J, McCready P, Puteola U et al: Positionally cloned gene for a novel glomerular protein nephrin is mutated in congenital nephrotic syndrome. *Mol Cell* 1:575-582, 1998.
3. Shik NY, Li J, Karpitskii V, Nguyen A, Dustin ML, Kanagawa O et al: Congenital nephritic syndrome in mice locking CD2 associated protein. *Science* 286: 312-315, 1999.
4. Kaplan SM, Kim SH, North KN, Rennke H, Correia LA, Tang HQ et al: Mutations in ACTN4, encoding alpha-actinin-4 cause familial focal segmental glomerulosclerosis. *Net Genet.* 24: 251-256, 2000.
5. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fushshuber A et al : NPHS2, encoding the glomerular protein podocin is mutated in autosomal recessive steroid-resistant nephritic syndrome. *Net. Genet.* 24: 349-354, 2000.
6. Tsukaguchi H, Yager H, Dawborn J, Jost L, Cohlmi J, Abreu PF et al: A locus for adolescent and adult onset familial focal segmental glomerulosclerosis on chromosome 1q 25-31 *J. Am. Soc. Nephrol.* 11: 1674-1680, 2000.
7. Frishberg Y, Rinet C, Megged O, Shapira E, Feinstein S, Raas-Rothschild A: Mutation in NPHS2 encoding podocin are a prevalent cause of steroid resistant nephrotic syndrome among Israeli-Arab children. *J. Am Soc. Nephrol.* 13: 400-405, 2002.
8. Tsukaguchi H, Sudhekar A, Le TC, Nguyen T, Yao J, Schwimmer JA et al: NPHS2 mutation in late-onset focal segmental glomerulosclerosis; R229Q is a common disease-associated allele. *J. Clin Invest.* 110: 1659-1666, 2002.
9. Salanko H: Pathogenesis proteinuria: Lessons learned from nephrin and podocin. *Pediatr Nephrol.* 18: 487-491, 2003.
10. Meruyoma K, Lijima K, Ikeda M, Kitamura A, Tsukaguchi H, Yoshiya K: NPHS2 mutation in sporadic steroid resistant nephrotic syndrome in Japanese children. *Pediatr. Nephrol.* 18: 412-416, 2003.

# Which One is More Effective: Ethylene Vinyl Alcohol (EVAL) or Polysulphone Dialyzer?

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## Introduction

The principle of hemodialysis (HD) was first described over a century ago while the first human HD treatment was performed in 1923 with collodion tubes (1). Since that time a variety of different hemodialyzer configurations and membranes have been used. In 1967, Lipps et al. helped develop the hollow fiber artificial kidney (HFAK). Membranes made from synthetic polymers, in general, are considered as being biocompatible membranes and tend to be treated as a homogenous group. Over time, there has been a progressive increase in the use of synthetic and modified cellulosic dialyzers, and a corresponding decrease in the utilization rate of unmodified cellulosic dialyzers (2). However, all of these membranes have multiple and different characteristics. In the present prospective study, we investigated the effects of polysulfone (PS) and ethylene vinyl alcohol (EVAL) membranes on some of the serum biochemical parameters (albumin, total protein, calcium, phosphorus, uric acid, cholesterol, trygliceride etc.), mean arterial pressure (MAP) in before and after the HD session, urea reduction ratio (URR), complete blood count, recombinant human erythropoietin (rhEPO) dose, and iron sucrose dose.

## Patients and Methods

The study included 18 patients (male, 11; female, 7; age [years] =  $64.0 \pm 13.1$ , the duration of hemodialysis [months] =  $43.0 \pm 44.9$ ; frequency and time of hemodialysis : 3 times a week, 4 hours in 18 pts) on the hemodialysis program. In the first 6-month period, only EVAL membranes (KF201, Kawasumi, Japan) were used to treat the patients. In the second 6-month period, we used PS (Hemoflow F6S, Fresenius, Germany) membranes. The data were obtained through 12 months. In an attempt to deliver the same dose of dialysis to all patients, we used dialysers having similar hollow fibre configurations, clearance characteristics, and surface area. Blood flow rate was maintained between 300-350 ml/min. Dialysis water was obtained from reverse osmosis. Bicarbonate-based dialysate was used in all cases, and dialysate flow rate was 500ml/min.

In the laboratory assessments, the levels of albumin, uric acid, total cholesterol, trygliceride, calcium, phosphorus, complete blood count, serum iron, and the total iron-binding capacity (TIBC) were measured with conventional autoanalyzer in the blood samples taken prior to hemodialysis following a period of one night-fasting. Pre- and

post-dialysis the level of urea of the same HD session were also measured to calculate the urea reduction ratio (URR) as the indicator of hemodialysis efficacy. Arterial blood pressure (ABP) was measured in the pre- and post-dialysis periods (Sphygmomanometer, Erka, Germany). All the patients were treated with rhEPO and parenteral iron sucrose. Statistical Analysis

The Pearson correlation test was used to assess the relationship between the values obtained from all patients. Mann-Whitney U test was used to compare the mean values obtained from the EVAL and PS dialyzers periods. The values were given as mean  $\pm$  standard deviation (SD), and  $p < 0.05$  was considered statistically significant. All statistical processes were performed on Windows using SPSS 11 software.

## Results

The clinical characteristics of the patients are shown in Table 1.

**Table 1 Clinical characterization of the patients**

	Patients (n: 18)
Gender (male/female)	11/7
Age (years)	$64.0 \pm 13.1$
Duration of dialysis (months)	$43.0 \pm 44.9$

The clinical and biochemical characteristics of the patients in pre- and post-EVAL and PS periods are shown in Table 2.

Compared to pre-EVAL period, the mean URR was lower in post-EVAL period,  $60.7 \pm 14.8$  and  $52.7 \pm 8.9$ , respectively ( $p < 0.05$ ). The level of calcium was higher in post-EVAL period than pre-EVAL period,  $9.9 \pm 0.7$  and  $9.1 \pm 0.7$ , respectively ( $p < 0.05$ ). Compared to pre-EVAL period, post-HD MAP value in the post-EVAL period was significantly higher,  $97.4 \pm 10.7$  and  $87.9 \pm 12.4$ , respectively ( $p < 0.05$ ).

Compared to pre-PS period, the mean URR was higher in post-PS period,  $65.7 \pm 8.3$  and  $52.7 \pm 8.9$ , respectively ( $p < 0.001$ ). The level of calcium was lower in post-PS period than pre-PS period,  $9.2 \pm 0.7$  and  $9.9 \pm 0.7$ , respectively ( $p < 0.05$ ).

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**Table 2 The clinical and biochemical characteristics of the patients in pre- and post-EVAL and PS periods**

Parameters	Pre-EVAL period	Post-EVAL and Pre-PS period	Post-PS period
Albumin (mg/dl)	3.5±0.4	3.5±0.3	3.5±0.2
Calcium (mg/dl)	9.1±0.7	9.9±0.7 <sup>a,b</sup>	9.2±0.7
Phosphorus (mg/dl)	5.6±1.7	4.9±1.3	5.6±1.6
Total cholesterol (mg/dl)	176.7±43	176.1±35.3	166.2±32.6
Trygliceride (mg/dl)	127.3±61.5	130.1±40.2	133.2±50
Uric acid (mg/dl)	9.2±1.1	8.3±1.5	8.2±1.6
Leucocyte (cell/mm <sup>3</sup> )	7.3±1.8	7±2 <sup>b</sup>	8.8±2.6
Hemoglobin (g/dl)	11.7±1.6	12±1.8	12.1±1.4
Hematocrit (%)	35.5±4.9	36.3±5.8	35.5±4.3
Platelet (cell/mm <sup>3</sup> )	206.8±60.7	213.7±65.4	183.8±76.8
URR (%)	60.7±14.8	52.7±8.9 <sup>a,c</sup>	65.7±8.3
Transferin Saturation Index (TSI) (%)	41.8±18.1	38±18.5	52.5±28.4
rhEPO dose (U/month)	16000±16392	18944.4±17739.7 <sup>b</sup>	8000±14712.9
Iron sucrose dose (mg/month)	238.8±383.6	438.8±318.3	327.7±239.6
Pre-HD mean arterial presurre (MAP) (mmHg)	98.7±10.6	101.3±11	98.1±6
Post-HD mean arterial presurre (MAP) (mmHg)	87.9±12.4	97.4±10.7 <sup>a,b</sup>	86±7.8

<sup>a</sup> Significant differences between pre- and post-EVAL periods are  $p < 0.05$

<sup>b</sup> Significant differences between pre- and post-PS periods are  $p < 0.05$

<sup>c</sup> Significant differences between pre- and post-PS periods are  $p < 0.001$

Compared to pre-PS period, post-HD MAP value in the post-PS period was significantly lower,  $86 \pm 7.8$  and  $97.4 \pm 10.7$ , respectively ( $p < 0.05$ ). The mean rhEPO dose (U/month) was lower in post-PS period than that of pre-PS period,  $8000 \pm 14712$  U/month vs  $18944.4 \pm 17739$  U/month, respectively ( $p < 0.05$ ).

### Discussion

Much work has been aimed at highlighting the different results achieved with cellulose-based or synthetic membranes. The conclusions reached by the various studies are far from unanimous and are often markedly discordant. Many parameters taken into account for comparative evaluation: firstly survival, then various forms of morbidity, nutritional status, metabolic alterations, hospitalization time, etc. In most of studies have reported that there are no major or even significant differences between different forms of synthetic membranes (3).

An obvious difference between synthetic and cellulosic membranes is chemical composition. Synthetic membranes are manufactured polymers that are classified as "thermo-plastics". In fact, for most of the synthetic membranes, the hemodialysis market represents only a small fraction of their entire industrial utilization (2).

Many of the synthetic polymers used in the manufacturing of the synthetic membranes are hydrophobic (4). PS membrane is hydrophobic, but EVAL has both a hydrophilic and a hydrophobic component in its molecule. Therefore EVAL membrane has been recognized as having excellent biocompatibility (5).

In this study, we measured the dose of delivered dialysis, but we did not determine the course and outcome of renal failure. Most of the clinical studies had investigated the effects of the dialysis membranes on mortality in patients with acute renal failure (6). The investigators compared synthetic membranes (approximately 50 % of them) with cuprophane membranes and found no significant difference between these membranes in patient outcome (6).

In literature, we could not find a study group who compared the effects of the dialysis membranes which belong to the same "thermoplastic" family. In conclusion, we investigated that the patients dialysed with PS membranes needed much less rhEPO than the patients dialysed with EVAL membranes. We also found that, compared to EVAL era, a more adequate dialysis dose could be reached by using PS dialyzer. But the present study is a kind of mini-survey including an insufficient number of patients. Therefore, further investigations are needed to clarify this issue.

### References

1. Clark WR. Hemodialyzer membranes and configurations: A historical perspectives. *Seminars in Dialysis* 2000; 13(5): 309-311.
2. Clark WR, Gao D. Properties of membranes used for hemodialysis therapy. *Seminars in Dialysis* 2002; 15(1): 191-195.
3. Stefoni S, Scolari MP, Cianciolo G et al. Membranes, Technologies and long-term results in chronic haemodialysis. *Nephrol Dial Transplant* 2000; 15[Suppl 2]: 12-15.
4. Ghol H, Buck R, Strathmann H. Basic features of polyamide membranes. *Contrib Nephrol* 1992; 96: 1-25.
5. Nakaji S, Yamamoto T. Membranes for therapeutic apheresis. *Ther Apher* 2002; 6(4): 267-270.
6. Gastaldello K, Melot C, Kahn RJ, Vanherweghem JL, Vincent JL, Tielemans C. Comparison of cellulose diacetate and polysulfone membranes in the outcome of acute renal failure. A prospective randomized study. *Nephrol Dial Transplant* 2000; 15: 224-230.