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High Risk of FSGS Recurrence in Kidney Allograft Recipients Independent of Heterozygous *NPHS2* Mutation*

Duże ryzyko nawrotu ogniskowego segmentalnego stwardnienia kłębuszków nerkowych u biorców przeszczepu nerki niezależnie od obecności heterozygotycznej mutacji genu podocyny

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Abstract

Background. A high recurrence rate of focal segmental glomerulosclerosis (FSGS) is one of the most frequent events after kidney transplantation, with a risk of graft loss in more than 50% of the affected patients, but patients with a homozygous podocin mutation (*NPHS2*) are at low risk of FSGS recurrence because of proper podocine structure in the allograft. The mechanism of recurrence is still obscure, and a multifactorial origin has been proposed.

Objectives. The purpose of the study was to identify recipients at risk of recurrence of FSGS or FSGS *de novo* in terms of podocin gene mutations (*NPHS2*).

Material and Methods. Twelve patients (4 females, 8 males, mean age at transplantation: 35.5 ± 10.4 years) were analyzed. Of these, 5 of the 9 recipients (55%) with pre-transplant FSGS had recurrence of proteinuria and 3 developed *de novo* FSGS. Delayed graft function was observed in 3 patients with proteinuria and primary non-function with immediate recurrence of heavy proteinuria and graftectomy in one patient. Acute rejection occurred in 5 of the 8 patients with recurrent FSGS and in only one of the 4 without proteinuria. After 1 year the mean serum creatinine concentration (1.6 mg/dl) in six patients with significant proteinuria (6.2 ± 1.9 g/d) was higher than in the 4 patients with no proteinuria (0.97 mg/dl). During the observation period, 4 patients lost graft function in an average of 51 months and one patient died.

Results. Mutational analysis of *NPHS2* (5'UTR, coding sequences and flanking region) was performed in 10 patients and revealed two heterozygous mutations in exon 5 (R229Q) with recurrence of FSGS during the first month after transplantation in the first and no recurrence in the other recipient.

Conclusions. These observations confirm the high risk of FSGS recurrence after kidney transplantation (55.5%), which was not affected by the presence of a heterozygous *NPHS2* mutation but is connected with worse graft function after one year (**Adv Clin Exp Med 2008, 17, 2, 207–212**).

Key words: FSGS, podocin, *NPHS2* mutation, recurrence after kidney transplantation.

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Streszczenie

Wprowadzenie. U chorych z ogniskowym segmentalnym stwardnieniem kłębuszków nerkowych (FSGS – *focal segmental glomerulosclerosis*) w nerkach własnych często wystepuje nawrót choroby po przeszczepie nerki, z utratą czynności przeszczepu u około połowy z nich. Pacjenci z homozygotyczną mutacją genu podocyny (*NPHS2*) mają jednak małe ryzyko nawrotu ze względu na przeszczepienie narządu z prawidłową strukturą podocyny.
Cel pracy. Chociaż mechanizm nawrotu FSGS jest niejasny, to uważa się, że ma podłoże wieloczynnikowe. Celem pracy była identyfikacja pacjentów z nawrotem FSGS lub FSGS *de novo* pod kątem mutacji genu dla podocyny (NPHS2).
Materiał i metody. Przeprowadzono analizę danych 12 pacjentów (4 kobiet i 8 mężczyzn) w wieku 35,5 ± 10,4 lat podczas przeszczepiania nerki. Nawrót FSGS po przeszczepie nerki obserwowano u 5 z 9 chorych (55%), u których przyczyną niewydolności nerek własnych było FSGS oraz u 3 pacjentów z obrazem FSGS *de novo*. U 3 pacjentów obserwowano opóźnione podjęcie czynności przez przeszczepioną nerkę z obecnością białkomoczu, a u jednego pacjenta natychmiastowe wystąpienie ciężkiego białkomoczu było przyczyną usunięcia przeszczepu. Ostre odrzucanie przeszczepu wystąpiło u 5 z 8 pacjentów z nawrotem FSGS i tylko u 1 z 4 pacjentów bez białkomoczu. Po roku obserwacji u 6 pacjentów ze znaczącym białkomoczem (6,2 ± 1,9 g/dobę) średnie stężenie kreatyniny w surowicy wynosiło 1,6 mg/dl, podczas gdy u 4 pacjentów bez białkomoczu wynosiło 0,97 mg/dl. Podczas obserwacji stwierdzono, że u 4 pacjentów przeszczepiona nerka utraciła czynność średnio po 51 miesiącach, a jeden pacjent zmarł.
Wyniki. Analiza mutacji genu *NPHS2* (region 5'UTR, sekwencje kodujące oraz sąsiadujące z nimi obszary intronowe) była wykonana u 10 chorych. U żadnego chorgo nie wykazano homozygotycznej mutacji w obrębie genu podocyny. W dwóch przypadkach wykazano natomiast heterozygotyczną mutację w 5 egzonie (R229G), z wczesnym nawrotem FSGS (w pierwszym miesiącu po przeszczepie u jednej osoby i brakiem nawrotu u drugiej).
Wnioski. Opisane obserwacje potwierdziły duże ryzyko nawrotu FSGS po przeszczepie nerki (55,5%), niezależnie od obecności heterozygotycznej mutacji genu dla podocyny (*NPHS2*). Nawrót FSGS wiązał się z gorszą czynnością nerki po roku (**Adv Clin Exp Med 2008, 17, 2, 207–212**).

Słowa kluczowe: FSGS, podocyna, mutacja genu *NPHS2*, nawrót po przeszczepie nerki.

Focal segmental glomerulosclerosis (FSGS) is a glomerular disease with a high recurrence rate after kidney transplantation. Proteinuria appears in approximately 30% of patients within a few days after kidney transplantation with a relatively good response to plasmapheresis (PF), but with graft loss in more than 50% of the affected patients [1–3]. The risk of recurrence differs in children and adults (50% vs. 11%, respectively) [4]. Some patients with no FSGS in native kidneys develop late-onset *de novo* FSGS (over 6 months) after transplantation. Although recurrence of FSGS negatively impacts kidney allograft survival, it has also been noted that some individuals with proteinuria have adequate kidney function for a number of years. The mechanisms of FSGS in native kidneys as well as FSGS recurrence in kidney allografts have not been fully elucidated. It is currently believed that primary FSGS is caused by alterations in glomerular epithelial cells (podocytes) induced by a circulating vascular permeability factor (VPF) produced by T lymphocytes or by an intrinsic podocyte cellular defect [5, 6].

The discovery of a mutation of podocin, exclusively expressed by podocytes, showed new pathogenic mechanisms of the disease [7, 8]. Mutations of the *NPHS2* gene, which encodes podocin, cause a steroid-resistant nephrotic syndrome (SRNS) in children with autosomal recessive inheritance and nonfamilial sporadic FSGS in adults indistinguishable from idiopathic FSGS on clinical grounds, but in which proteinuria is determined by homozygous mutations of podocin [7, 9–12]. Typically, *NPHS2* mutations require two defective alleles for the clinical manifestation of the disease [10, 13]. Among patients with a homozygous *NPHS2* mutation, a molecular defect of podocin is considered to be the disease-causing mechanism. Since this mechanism should vanish after kidney transplantation, patients bearing homozygous mutations are at low risk of FSGS recurrence, with only the possibility of other proteinuria-inducing factors (i.e. VPF or autoantibodies against unmutated podocin) [14]. In patients with only a single *NPHS2* mutation, FSGS does not seem to be related to *NPHS2*, assuming that a second mutation has not been omitted. However, heterozygous *NPHS2* mutations, sequence variants, and polymorphisms may play a role in atypical cases of SRNS with a later onset, mild clinical course, and recurrence after kidney transplantation [8, 14]. The recurrence of FSGS in patients carrying homozygous or heterozygous mutations support the general idea of a multifactorial origin of the primary disease. The clinical course of kidney allografts with recurrence or *de novo* FSGS varies and it is controversial which factors are of importance in determining the outcome. The aim of the study was to identify kidney allograft recipients at risk of FSGS recurrence in terms of *NPHS2* mutations.

Material and Methods

The clinical data of 12 patients (4 females, 8 males, mean age at transplantation: 35.5 ± 10.4 years) with pre-transplant history of FSGS (9 patients) or with no FSGS in the native kidneys but *de novo* FSGS after transplantation (3 patients) were retrospectively analyzed and mutational analyses of their *NPHS2* gene were compared. Three patients underwent a second transplantation with loss of the first graft due to recurrent FSGS in two and surgical complications in the third. The initial immunosuppressive therapy consisted of prednisone, calcineurin inhibitors (cyclosporin A/tacrolimus: 9/3 patients), azathioprine or mycophenolate mophetil (5 and 7 patients, respectively), and anti-IL2 receptor antibodies in 3 patients. In recurrent FSGS, the treatment was based mainly on cyclosporin A and plasmapheresis in particular patients. Acute rejection episodes were treated with intravenous methylprednisolone pulses. The diagnosis of FSGS in 8 patients was confirmed by kidney allograft biopsy. The remaining patients without rejection or proteinuria had no protocol biopsies.

Mutational Analysis

Blood samples for mutational analysis were obtained from the patients after informed consent. The study was approved by the Ethics Committee of Silesian Piasts University of Medicine in Wrocław. Mutational analysis of *NPHS2* (5'UTR, coding sequences and flanking region) was performed in 10 patients. Genomic DNA was extracted from peripheral blood samples with a QiaAmp Blood Kit (Qiagen) according to standard procedure. Molecular analysis of podocin was performed by DNA sequencing. All eight exons of the *NPHS2* gene were amplified by PCR using flanking intronic primers, subjected to automatic sequence analysis by a dye-terminator reaction, and compared with gene bank sequences for *NPHS2* (NC_000001.9 reg. 177786299--177811691). Primer sequences and PCR conditions are available on request.

Results

The analyzed data revealed that delayed graft function (DGF), lasting a mean of 18 days, was observed in only 3 patients with proteinuria. One recipient of a second kidney allograft with primary graft non-function and immediate onset of heavy NS required graftectomy after 2.5 months. Acute rejection was observed in 6 of the 12 patients (50%) in the first or second month posttransplant, predominantly in patients with recurrent FSGS (5 patients), and was successfully treated with methylprednisolone (mean dose: 2.3 g per patient). Nephrotic--range proteinuria developed in 8 of the 12 patients after kidney transplantation, with a mean observation period of 32 ± 26 months. Five of the 9 patients (55%) with pre-transplant FSGS experienced recurrence and 3 of the remaining patients developed *de novo* FSGS. Proteinuria relapsed in 5 patients at a mean of 42 days (range: 1–180 days), with onset within the first 2 weeks posttransplant in 4 patients and in the 6th month in 1 patient, while *de novo* FSGS had later onset: after the 6th month in two and in the 36th month in one patient. Normal serum creatinine concentration (< 1.4 mg/dl = 128 μmol/l) was achieved in all patients but one at a mean of 21 days (range: 2–90 days). A follow-up analysis was conducted in 10 recipients after 1 year. Six of them had significant proteinuria (6.2 ± 1.9 g/d) with a mean serum creatinine concentration of 1.6 mg/dl (range: 1.1–2.0 mg/dl) and 4 patients with no proteinuria had lower creatinine concentration (0.97 mg/dl, range: 0.7–1.4 mg/dl). During the observation period, 4 patients lost graft function after an average of 51 months (range: 22–82 months) and one patient died due to cerebral stroke.

Mutational analysis of *NPHS2* was performed in 10 patients and revealed solely heterozygous mutations in exon 5 (R229Q) in two of them (Fig. 1). Early recurrence of FSGS (during the first two weeks posttransplant) and a relatively mild course with ultimate loss of the allograft after 7 years was observed in the first patient, while the second has no proteinuria after 6 years despite a second kidney transplant (the first was lost because of surgical complications).

Discussion

The discovery of mutations of podocin, exclusively expressed by podocytes, in sporadic FSGS has shed new light on its pathogenic mechanisms. Typically, the clinical manifestation of the disease requires two functional mutations (homozygosity or compound heterozygosity) of the *NPHS2* gene. The association of single mutations of podocin with less severe course than in homozygosity raises the possibility that the encoded peptide may retain some functional aspects and determine a mild phenotype [8]. On the other hand, it is speculated that proteinuria may be associated with missing the second NPHS2 mutation due to the methodological approach (e.g. mutation in noncoding or regulatory regions). Heterozygous mutations of podocin in a subset of proteinuric patients with

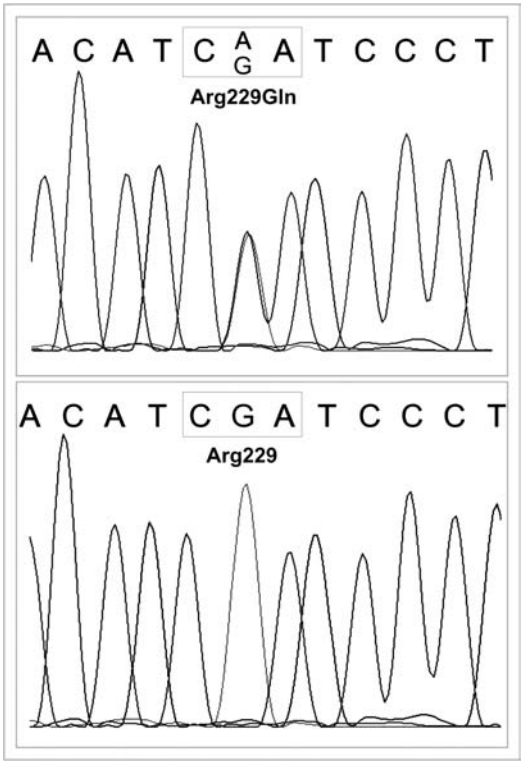

Fig 1. Mutational analysis of *NPHS2* (5'UTR, coding sequences and flanking region) with heterozygous R229Q (Arg229Gln) mutations in exon 5 (upper panel) compared with the normal gene sequence (lower panel)

Ryc. 1. Analiza mutacji genu *NPHS2* (region 5'UTR, sekwencje kodujące oraz sąsiadujące z nimi obszary intronowe) obejmująca heterozygotyczną mutację R229Q (Arg229Gln) w 5 egzonie (panel górny) w odniesieniu do prawidłowej sekwencji genu (panel dolny)

higher incidence of R229Q and a milder phenotype was demonstrated by Caridi et al. [8]. The observed R229Q alleles were only heterozygous and R229Q polymorphism is not thought to cause disease when present alone as a heterozygous variant, but in combination with another heterozygous mutation it contributes to autosomal-recessive FSGS [9]. In addition, the R229Q polymorphism was identified in 3.75% of healthy control cohorts, but never in the homozygous state [14]. Tsukagushi et al. found that the R229Q heterozygous variant (with the same frequency as in a normal population) did not appear to cause FSGS, but rather to enhance the susceptibility to FSGS in association with a second mutant *NPHS2* allele [9]. It is believed that patients bearing a homozygous mutation of the *NPHS2* gene are not at risk of recurrence after kidney transplantation [14]. However, delayed recurrence of mild proteinuria after kidney transplantation in some patients with *NPHS2* mutations has been observed by Bertelli et al. [15].

Several pathogenic factors have been postulated in such cases of recurrent FSGS, among these a yet unidentified lymphokine (VPF) derived from T cells [5, 16]. Evidence for a circulating VPF in FSGS originates from clinical observations of immediate recurrence (within a week) of FSGS after kidney transplantation and good response to plasmapheresis. Under normal conditions, VPF activity is neutralized by serum components such as apolipoproteins. Therefore, loss of inhibitors may play a central role leading to a recurrence of FSGS after transplantation [3, 16]. The estimated frequency of FSGS recurrence after kidney transplantation varies from 20% to 40% (average: 30%) and up to 80% after a second kidney transplantation [1–3]. The patients of the present study developed a high, but allowable, recurrence rate (55%) of FSGS after kidney transplantation, especially since two patients with second kidney allografts and loss of the previous grafts due to FSGS were included. Moreover, there were no patients with a homozygous mutation, so the protective mechanism could not be disclosed. In the 5 relapsed patients, proteinuria appeared later than observed by Pardon et al. (42 days vs. 22 days, respectively) [17]. The difference is probably strongly influenced by the small number of patients. Delayed graft function is likely related to proteinuria early after transplantation and occurred only in recipients with relapse. Similar observations were also reported by others [17, 18]. Acute rejection was observed more often among recipients with relapse than in non-proteinuric patients (5 vs. 1 patient, respectively). Similar data were also reported by Kim et al. but not confirmed by Pardon et al. [17, 18]. Because of the low number of patients, these results have to be interpreted with caution. The four patients of the present study with recurrent FSGS lost their graft function after an average of 51 months, which is like the patients with posttransplant glomerulonephritis reported by Ostrowska et al. [19]. Lack of homozygous *NPHS2* mutations affecting both alleles in our small group of kidney transplant patients is consistent with low FSGS recurrence after transplantation, while heterozygous mutations have no protective impact. In one recipient with a heterozygous R229Q mutation, proteinuria developed early after transplantation and probably arose from circulating VPF and not from mutation. The second one is free of proteinuria after 6 years of observation despite the heterozygous mutation.

The authors concluded that the recurrence rate of FSGS after kidney transplantation is high (55%), but it is independent of a heterozygous mutation of the *NPHS2* gene. Patients with recurrent FSGS more often experience delayed graft function and acute rejection and have worse graft function after one year than patients without pro-

teinuria. The genetic assessment of the mutation should be advised to avoid ineffective treatment of nephrotic syndrome and to refer homozygous patients to kidney transplantation, but in the case of heterozygosity the course is unpredictable. Patients should be aware of the mutation, but in kidney transplantation a lack of mutation or heterozygosity should rather be taken into account.

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