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Original Article

Genetic predisposition of donors affects the allograft outcome in kidney transplantation: Single-nucleotide polymorphism of *aquaporin-11*

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Abstract

Background: Aquaporin-11 (AQP11) is a novel member of the aquaporin family. Disruption of the murine *Aqp11* gene causes severe proximal tubular injury and renal failure. The rs2276415 (G > A) single-nucleotide polymorphism in the human *AQP11* gene results in glycine to serine substitution in a functionally important domain. In this study, the role of the genetic predispositions of *AQP11* rs2276415 (G > A) on renal allograft outcomes was evaluated.

Methods: A total of 198 pairs of donors and recipients were enrolled in this study. Long-term graft survival was traced and clinical parameters that could have influenced graft outcome were collected through the electronic medical record system.

Results: The genotype distribution and allele frequency of rs2276415 polymorphism were not different between donors and recipients. Despite similar allele frequencies between donors and recipients, the minor allele rs2276415 (GA + AA) of *AQP11* from the donors, but not from the recipients, had a harmful effect on the graft survival compared with the wild-type donor (GG; $P = 0.029$). This association was significant after adjusting for several risk factors including age, sex, human leukocyte antigen mismatch, donor type, hypertension, and diabetes mellitus ($P = 0.032$).

Conclusion: A donor-derived, not recipient-derived, genetic *AQP11* polymorphism has different effects on graft outcome. Thus, the genetic influence from donors should be carefully considered for proper management of allografts after kidney transplantation.

Introduction

Aquaporins (AQPs) are a family of membrane water channel proteins found throughout the animal and plant kingdoms [1]. Currently, 13 aquaporin family members have been identified in mammals. Aquaporin-11 (AQP11) is one of the newly described members, and its function is still not clearly understood.

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Northern blot analyses showed that AQP11 expression was highest in the testis, and moderate in the kidney, liver, and brain. In the kidney, AQP11 is most abundantly expressed in the proximal tubule. A previously conducted study showed that AQP11-null mice died due to advanced renal failure with polycystic kidneys [2]. Vacuoles originated prior to cysts from the endoplasmic reticulum (ER), and AQP11 was considered to be important for ER homeostasis. These results imply that AQP11 is important in kidney development and function.

An unpublished study reported that rs2276415 (G > A) single-nucleotide polymorphism (SNP) in the human *AQP11* gene, which results in Gly102Ser substitution in a functionally important domain, is associated with increased risk of acute kidney injury and chronic kidney disease [3]. These results indicate that AQP11 insufficiency predisposed the kidney to renal dysfunction.

Various immunological and nonimmunological determinants may affect the outcome of a renal allograft in kidney transplantation. The genetic interactions between donors and recipients are also an important issue. In this study, it is assumed that the graft outcome is dependent not only on the recipient's response but also on the responses of the graft as an active participant. Therefore, it was hypothesized that genetic variation in the AQP11 proteins of both donors and recipients might affect long-term graft survival in kidney transplantation. In this study, the role of genetic predisposition of *AQP11* rs2276415 (G > A) on renal allograft outcomes is analyzed.

Methods

Study population

A total of 198 pairs of Korean recipients and donors who were followed up for at least 1 year were recruited for this study. They had received kidney transplants at Seoul National University Hospital, Seoul, Korea between 1987 and 2008. Whole-blood samples from the recipients and their donors were collected as follows: 96 samples from donors and recipients; 25 samples from recipients only, and 77 samples from donors only.

The research protocol used for this study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (IRB No. C-1205-061-410). All clinical investigations were conducted according to the guideline in the Declaration of Helsinki.

Medical record of recipients based on the electronic medical record system was reviewed. Clinical parameters that could have influenced graft outcome were collected, which included recipient's sex and age at transplantation, history of hypertension, diabetes mellitus, and donor type. Estimated glomerular filtration rate of donor was calculated by the Modification of Diet in Renal Disease equation [4]. Delayed graft function was defined as need for dialysis in the 1st week after transplantation. Cytomegalovirus (CMV) and BK virus infections were evaluated with CMV antigenemia and plasma BK virus polymerase chain reaction, respectively.

The primary outcome of this study was graft loss defined as graft dysfunction that necessitated renal replacement therapy after transplantation.

Genotyping

DNA was extracted from whole blood, and genotyping for *AQP11* rs2276415 (C_12041092_1) was carried out by the TaqMan SNP genotyping assays (7900HT fast real-time PCR system; Applied Biosystems, Foster city, CA, USA).

A different fluorescence label (6-carboxyfluorescein for wild type and 4,7,2′-trichloro-7′-phenyl-6-carboxyfluorescein for mutant) was used to label the 5′ segment of allelic probes. The TaqMan minor groove binder probe sequence was as follows: *AQP11* rs2276415 5′-GGTGGGCACGTCCAGCAACCCGTGC[A/G]GCG TGATGATGCAGATGATGCTGGG-3′. Reaction mixtures consisted of a 1.0 μL 10 × AmpliTaq buffer, 1.0 μL deoxynucleotide triphosphates (2.5mM each), 0.2 μL forward primer (20 pmol/μL), 0.2 μL reverse primer (20 pmol/μL), 1.0 μL genomic DNA (50 ng/μL), and 0.15 μL iMax II Taq polymerase. Polymerase chain reactions were carried out under the following conditions: 5 minutes at 94°C (1 cycle); 30 seconds at 94°C; 30 seconds at 56°C (35 cycles); 50 seconds at 72°C; and 7 minutes at 72°C (1 cycle).

Tissue immunohistochemical staining and analysis

To evaluate the AQP11 expression in kidney tissue according to the SNP, 15 kidney biopsy samples that showed the least pathology among recipients were selected. For immunohistochemical study, paraffin-embedded graft blocks of recipients were cut into 4 μm slices. For deparaffinization and hydration, xylene and ethanol were used. Endogenous streptavidin activity was blocked by 0.3% hydrogen peroxide (H_2O_2). To examine the expression of human AQP11, deparaffinized sections were stained with rabbit anti-AQP11 antibody (Novus Biologicals, Littleton, CO, USA). Antigen retrieval was carried out by heating paraffin-embedded sections in 10% citrate buffer in a microwave oven three times (each of 5 minutes duration). streptavidin and 3,3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louise, MO, USA) were used for immunohistochemical detection. For each kidney sample, three fields were viewed at 200 × magnification under a light microscope. Sections were then counterstained with Mayer's hematoxylin and examined by light microscopy. All morphometric parameters were determined using a microscope coupled to a computerized morphometry system (Qwin3; Leica, Rijswijk, The Netherlands).

Statistical analysis

SPSS for Windows package 12.0 K (SPSS Inc., Chicago, IL, USA) was used for all analyses and calculations. Student *t* test was used for continuous variables, and the results were presented as mean ± standard deviation. The Chi-square test was used for categorical variables. Graft survival was analyzed using the Kaplan–Meier method, and comparison among groups was performed by the log-rank test. Values of $P < 0.05$ were considered statistically significant.

Results

Frequency of genetic variants of AQP11

The genotype frequencies of the renal transplant recipients and donors did not show significant deviation from the Hardy–Weinberg equilibrium ($P > 0.05$). The genotype and allele