INFLUENCE OF APOPTOSIS AND INFLAMMATION GENE POLYMORPHISMS ON TRANSPLANTED KIDNEY FUNCTION

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OBJECTIVES

The progressive deterioration of kidney allograft function which leads in most cases to transplant failure remains a central clinical challenge for improving long-term graft survival rate. In the decade, polymorphisms in genes involved in inflammation and apoptosis have been suggested as potential genetic markers for graft dysfunction, representing one conceivable explanation for interindividual differences in kidney transplant outcomes.
This work was aimed at identifying genetic risk profiles for transplanted kidney function by studying the impact of interleukin 6 (IL-6), transforming growth factor beta 1 (TGFB1) and Fas gene polymorphisms.

METHODS

The study recruited a total of 376 kidney transplant recipients transplanted at our center from January 2005 to June 2011. The follow-up period was 2.6 ± 1.4 years. A case-control study was carried out to assess potential associations between polymorphisms in inflammation- and apoptosis-related genes and the risk for chronic impairment of kidney graft function: the control group included 256 renal transplant recipients with stable graft function (SGF group), whereas the group of the cases was composed by 120 patients with worsening graft function (WGF group) within the follow-up period, based on the observation of a constant and irreversible increase in serum creatinine at least 30% above baseline in the absence of recurrent primary nephropathy or other ascertained causes. After genomic DNA isolation from white blood cells, all the patients were genotyped for IL-6/G–174C, TGFB1/L10P, TGFB1/R25P, Fas/G-670A polymorphisms using PCR-RFLP (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism) and direct sequencing.

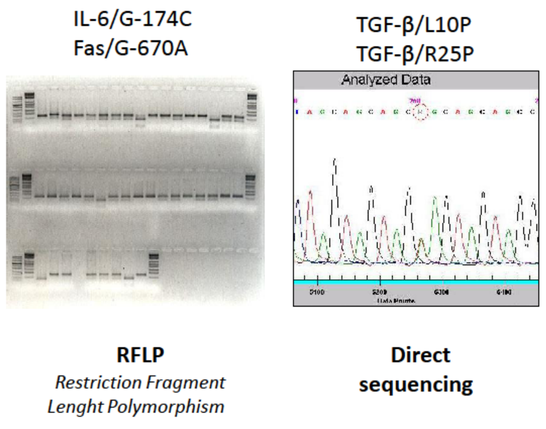
Table 1. Demographic, clinical and transplant-related parameters in SGF group and WGF group.

Parameter	SGF group (n= 256)	WGF group (n= 120)	P
Sex	M: 120; F: 136	M: 56; F: 64	ns
Recipient age, years	43.3 ± 13.2 (range: 19-70)	41.9 ± 14.3 (range: 20-72)	ns
Donor age , years	42.8 ± 17.2 (range: 15-74)	42.8 ± 15.8 (range: 17-77)	ns
Dialysis vintage, months	26.6 ± 24.4 (range: 1.6-117.8)	24.9 ± 20.8 (range: 2.5-76.4)	ns
Cold ischemia time, hours	16.9 ± 6.2 (range: 1-31)	17.5 ± 8.2 (range: 2-32)	ns
HLA DR mismatches	0.68 ± 0.59	0.66 ± 0.58	ns
HLA A, B, DR mismatches	3.12 ± 1.15	3.20 ± 1.13	ns
Number of acute rejections	24/256	41/120	<.001
Serum creatinine at discharge, mg/dL	1.52 ± 0.48	1.65 ± 0.58	.023
Serum creatinine at 1 year, mg/dL	1.55 ± 0.64	1.71 ± 0.41	.013
Serum creatinine at 2 years, mg/dL	1.48 ± 0.53	1.82 ± 0.57	<.001
Serum creatinine at 3 years, mg/dL	1.49 ± 0.47	1.97 ± 0.56	<.001
Serum creatinine at 4 years, mg/dL	1.59 ± 0.81	2.21 ± 0.63	<.001
Immunosuppressive therapy			
– Steroids	158/256	38/120	ns
– Cyclosporin A	131/256	31/120	ns
– Azathioprine	33/256	8/120	ns
– Mycophenolate mofetil	46/256	11/120	ns
– Tacrolimus	123/256	30/120	ns
– Sirolimus/Everolimus	26/256	6/120	ns
Primary disease			
– Glomerulonephritis	71/256	36/120	ns
– Polycistic kidney disease	55/256	29/120	ns
– IgA nephropathy	20/256	13/120	ns
– Interstitial nephritis	27/256	12/120	ns
– Vascular nephropathy	31/256	10/120	ns
– Hereditary nephropathy	16/256	9/120	ns
– Not diagnosed	39/256	14/120	ns

Table 2. Contingency table for genotype combination of IL-6 and Fas variants.

Genotype combination	SGF group (n=256)	WGF group (n=120)
IL-6/GG Fas/GG	37 (14.8%)	0 (0%)
IL-6/GG Fas/AA	26 (10.2%)	13 (10.8%)
IL-6/CC Fas/AA	9 (3.5%)	15 (12.5%)
Other (heterozygous)	184 (71.5%)	92 (76.7%)

Figure 1. Genotype analysis on genomic DNA from 376 kidney transplant recipients.



RFLP
Restriction Fragment Lenght Polymorphism

Direct sequencing

RESULTS

Considering the single IL-6, TGFB1 and Fas polymorphisms, we found similar allelic and genotype frequencies between the two groups of patients (SGF *vs* WGF group).
To test the hypothesis of mutual effects of polymorphisms, multiple logistic regression was performed incorporating data for all the possible dual genotypic associations. The association of IL-6 high producer and Fas low producer genotype resulted in a protective effect against graft dysfunction (OR = 0.79; 95% C.I. = 0.72-0.86).

CONCLUSIONS

These data confirmed the significant prognostic value of gene polymorphisms involved in inflammatory response and programmed cell death on long term kidney allograft survival and indicated the carriage of IL-6 high producer/Fas low producer genotype as a positive prognostic factor on kidney transplant outcome.
This kind of approach may represent a helpful tool for the stratification of kidney recipients according to their individual susceptibility to graft dysfunction and for the application of genotyping in the tailoring of immunosuppressive interventions.

References

1. Kreis HA, Ponticelli C. Causes of late renal allograft loss: chronic allograft dysfunction, death, and other factors. Transplantation. 2001 Jun 15;71(11 Suppl):SS5-9.
2. Pawlik A, Domanski L, Rozanski J, et al. The association between cytokine gene polymorphisms and kidney allograft survival. Ann Transplant. 2008;13(2):54-8.
3. La Manna G, Cappuccilli ML, Cianciolo G, et al. Cardiovascular disease in kidney transplant recipients: the prognostic value of inflammatory cytokine genotypes. Transplantation. 2010 Apr 27;89(8):1001-8.
4. Carstens J, Markussen N, Madsen M. The granule exocytosis and Fas/FasLigand pathways at the time of transplantation and during borderline and acute rejection of human renal allografts. Transplant Proc. 2005 Oct;37(8):3294-7.
5. Huang QR, Morris D, Manolios N. Identification and characterization of polymorphisms in the promoter region of the human Apo-1/Fas (CD95) gene. Mol Immunol. 1997 Jun;34(8-9):577-82.
6. La Manna G, Cappuccilli ML, Capelli I, et al. The impact of apoptosis and inflammation gene polymorphisms on transplanted kidney function. Ann Transplant. 20013, in press.