Cytokine 58 (2012) 321–326

Contents lists available at SciVerse ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/issn/10434666

Short Communication

Genetic polymorphisms located in *TGFB1*, *AGTR1*, and *VEGFA* genes are associated to chronic renal allograft dysfunction

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ARTICLE INFO

Article history:
Received 11 August 2011
Received in revised form 9 January 2012
Accepted 24 February 2012
Available online 19 March 2012

Keywords:
Kidney transplant
Allograft rejection
SNPs
Grow factors
Biomarkers

ABSTRACT

Background: Persistent inflammation and fibrosis have been related to active progression of renal deterioration and reduced survival of kidney transplant. The aim of this study was to determine the impact of single-nucleotide polymorphisms (SNPs) located in regions related to inflammatory and immune processes on the development of chronic renal allograft dysfunction (CRAD).

Methods: A retrospective study was carried out on 276 patients who received kidney transplant (KT). SNPs were genotyped via the SNPlex platform. Statistical analysis was performed with SNPstat and regression logistic analyses were adjusted by age and gender of recipients and donors, cold ischemia time and the number of human leukocyte antigen (HLA) mismatches.

Results: From 276 patients with KT, 118 were non-CRAD and 158 were CRAD. Three SNPs showed significant associations with CRAD development: rs1800471 in *transforming growth factor beta 1* (*TGFB1*), rs5186 in *angiotensin II receptor type 1* (*AGTR1*), and rs699947 in *vascular endothelial growth factor A* (*VEGFA*). GC genotype of rs1800471 was associated with increased odds of CRAD compared to GG genotype (OR = 2.65 (95% confidence interval (CI) = 1.09; 6.47), $p = 0.025$), as well as AC and AA genotype of rs699947 assuming a dominant model (OR = 1.80 (95% CI = 1.02; 3.20), $p = 0.044$). Besides, AC and CC genotypes of rs5186 were associated with reduced odds of CRAD assuming a dominant model (OR = 0.56 (95% CI = 0.33; 0.96), $p = 0.033$).

Conclusion: Our findings suggest that three genes related to immunity and inflammation (rs1800471, rs5186 and rs699947) are associated to susceptibility or protection to CRAD, and might have diagnostic utility in predicting the likelihood of developing CRAD.

1. Introduction

Chronic kidney disease and end-stage renal disease (ESRD) are global public health problems with important social and economic impact, due to their high prevalence and substantial effect on morbidity and mortality [1]. Renal replacement therapy for ESRD includes hemodialysis, peritoneal dialysis, and kidney transplant (KT), where KT is the best therapeutic alternative for most causes of chronic renal failure [1,2]. Currently, strategies to maintain transplant function and to improve long-term graft survival are important goals in renal transplantation in order to prevent chronic renal allograft dysfunction (CRAD) [3], which is defined as functional and morphologic deterioration of a renal allograft at least 3–6 months after transplantation.

There are several factors that influence graft survival such as the age of recipient and donor, degree of human leukocyte antigen (HLA) matching, cold ischemia time and delayed renal graft function, type of immunosuppression, incidence of acute rejection and time on dialysis before transplantation among others [4]. Persistent inflammation and fibrosis have also been related to active progression of renal deterioration and reduced KT survival [5], therefore both factors provide important diagnostic and therapeutic information for patient management [6]. Besides, KT outcomes may also be influenced by the production of immune mediators, which display genetic inter-individual differences. For example, single-nucleotide polymorphisms (SNPs) located on cytokines may have a crucial impact on graft survival by affecting

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http://dx.doi.org/10.1016/j.cyto.2012.02.017

gene expression [7]. Indeed, it has been proved that changes in pro-inflammatory cytokines levels lead to increase interstitial fibrosis and tubular atrophy and therefore, the risk of allograft rejection [8].

To date, the influence of many inflammatory markers on the KT outcome is not well understood [7,9]. For this reason, in the present study we have try to determine the impact of SNPs, located in regions related to inflammatory and immune processes, on CRAD development in patients with KT.

2. Patients and methods

2.1. Study design and patients

A retrospective observational study was carried out among patients with ESRD who received KT in the "Hospital Clínico Universitario" from Valladolid (Spain), between December 1995 and October 2008. The total number of kidney transplants performed in this period was 440. Only those patients who gave their written consent were included in the study, being approved by the Institutional Ethics Committee. Demographic and clinical data were obtained from medical records.

Patients who were younger than 18 years, those that had unavailable DNA sample and/or that unsigned informed consent, were ruled out. In total, 276 patients were included and divided into two groups of study according to the kidney graft outcomes: (a) Non-CRAD: patients with stable transplant or absence of CRAD and (b) CRAD: patients that developed CRAD. We have considered as CRAD any decrease $\geqslant$30% of the inverse of creatinine (1/Cr) after the third month of transplantation, using as baseline the highest value of 1/Cr in the first three months after transplantation [10]. In our hospital, biopsy is not performed to monitor the evolution of renal transplantation per protocol.

We also included 288 normal subjects (control-group) from the same hospital. These subjects were routinely discharged from the general medicine service with an age and gender distribution similar to the case-group patients. In order to ensure homogeneity, all patients were Caucasian.

Regarding immunosuppression protocol, an anti-interleukin-2 receptor monoclonal antibody (basiliximab) and steroids were used as induction treatment. For maintenance immunosuppression, cyclosporine and steroids were used until 2002; tacrolimus and steroids after 2002. In addition to this, mycophenolate mofetil was also applied when transplant came from an elderly donor, it was a second transplant and/or patients developed acute tubular necrosis.

2.2. SNPs selection

Published SNPs at Pubmed database (http://www.ncbi.nlm.nih.gov/pubmed/) located in genes involved in inflammatory and immune pathways, as well as those related to kidney transplantation toleration or associated processes, were selected. SNPs located at regulatory regions were prioritized. Furthermore, several databases were reviewed: (a) SNPs: HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) and dbSNP databases (http://www.ncbi.nlm.nih.gov/SNP/); and (b) Pathways: GeneOntology (http://www.geneontology.org/) and KEGG (http://www.genome.jp/kegg/pathway.html) for pathways analysis.

Moreover, for each significant SNP, the biological implications were analyzed "in silico" via web-tools: SMART (http://smart.embl-heidelberg.de/) for identifying the protein domains, PolyPhen (http://genetics.bwh.harvard.edu/pph/) for predicting possible impact of an amino acid substitution on the structure and function of a protein, PATROCLES (http://www.patrocles.org/) for identifying putative microRNA binding sites, ESEfinder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home) to identify exonic splicing enhancers binding sites and TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) for searching transcription factor binding sites.

2.3. DNA samples and genotyping

DNA was extracted from whole blood by the Chemagic Magnetic Separador Module1, CHEMAGEN® which uses a magnetic particles system to obtain DNA. The quantity of recovered DNA was quantified by using PicoGreen® dsDNA Quantitation Reagent (Molecular Probes Inc., Eugene, OR, USA).

All SNPs were genotyped at the Spanish National Genotyping Centre (CeGen: http://www.cegen.org/) by the SNPlex genotyping system 48-plex (Applied Biosystems, Foster City, CA, USA) following the manufacturer's recommended protocol (http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_042019.pdf). As quality control, two Centre d'Etude du Polymorphisme Humain (CEPH) samples (NA10860 and NA10861) from the HapMap database were included in all genotyping assays [11].

2.4. Statistical analysis

All SNPs from the control group were analyzed for Hardy–Weinberg equilibrium (HWE) by using the Pearson Chi-square (χ^2) statistic. Only SNPs that fulfilled HWE were included in the association analysis.

In order to perform the association analysis, a logistic regression model was carried out by using SNPstat software (http://bioinfo.iconcologia.net/SNPStats_web) [12]. We have considered the most influential clinical factors as adjusted variables (age and gender of recipients and donors, cold ischemia time and the number of HLA mismatches), in order to correct the association results. Five inheritance models were tested (co-dominant, dominant, recessive, over-dominant and additive). For each SNP, likelihood ratio test (LRT), and Akaike's information criteria (AIC) were used to select the inheritance model that best fits the data. The equation that defines the logistic model is: $\log(p/1-p) = \alpha + \beta G + \gamma Z$; p being the probability, G the categorical variable with the polymorphisms codified, Z the variables to adjust the model (α, β and γ must be estimated). Odds ratios (OR) and 95% confidence intervals (CI) were calculated to test the relative risk for association.

Besides, multiple testing corrections were performed by SFDR (Stratified False Discovery Rate) software version 1.6. (http://www.utstat.utoronto.ca/sun/Software/SFDR/index.html). Significance was fixed at p value <0.05.

3. Results

3.1. Patients

Table 1 shows the clinical characteristics of all patients included in our study: 118 were in the Non-CRAD group, and 158 in the CRAD. Chronic glomerulonephritis was the most frequent cause of ESRD in both groups. The most frequent causes of death of donors were cerebrovascular accident followed by traumatic brain injury. Finally, CRAD groups had higher cold ischemia time than non-CRAD group ($p < 0.05$).

The mean follow up of patients after transplantation was 5.32 ± 3.02 years. The percentage of positive panel-reactive antibodies (PRA) was 10% in no-CRAD versus 13% in CRAD group. Induction treatment was used in 86% no-CRAD patients versus 71.2% CRAD ($p > 0.05$).