Transcriptome changes in renal allograft protocol biopsies at 3 months precede the onset of interstitial fibrosis/tubular atrophy (IF/TA) at 6 months

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Abstract

Background. Interstitial fibrosis and tubular atrophy (IF/TA) in renal transplants are the major morphological correlates of progressive graft deterioration. Early diagnosis of IF/TA is a pre-requisite for a timely therapeutic intervention in patients at risk. To evaluate events occurring before the overt onset of IF/TA, gene expression profiling of 3-month protocol biopsies from patients with IF/TA was performed in a patient group (n = 8) who developed mild IF/TA [chronic allograft nephropathy (CAN) grade I, by the Banff scoring system] in the subsequent 6-month protocol biopsy (‘progressors’), and in 12 patients without IF/TA at 6 months (‘non-progressors’).

Methods. RNA was extracted, labelled and hybridized to human specific genome wide DNA microarrays. Normalized data were subjected to gene-centric and pathway-centric statistical methods.

Results. Compared to the non-progressors, the 3-month biopsies of the progressor group showed overexpression of several genes that are important in the T- and B-cell activation and immune response. Genes involved in profibrotic processes were identified in the biopsies of the progressors that preceded the observed IF/TA at 6 months. Furthermore, several genes with transporter and metabolic functions were underrepresented in the progressors in the 3-month biopsies.

Conclusion. Gene expression profiling of early protocol biopsies identified changes in the transcriptome of grafts, which may be important for the development of IF/TA. Such early detection of transcriptome changes can facilitate the identification of patients at risk shifting the intervention time point well before the histological diagnosis of irreversible IF/TA.

Keywords: gene expression profiling; IF/TA; protocol biopsy

Introduction

Renal transplantation remains the major life-saving treatment for patients with end-stage renal diseases. Despite improved 1-year graft survival and decreased acute rejection rates with current immunosuppressive protocols, progressive deterioration and failure of grafts in the long term cause a substantial portion of patients to return to dialysis each year [1]. The incidence of chronic renal allograft damage in the first year after transplantation has remained unchanged over recent years, largely because the interplay of the known risk factors for graft deterioration, such as high donor age, long cold ischaemia time, rejection episodes and drug effects, is not yet fully understood [2]. Due to the complex nature of the pathophysiology of chronic allograft deterioration, the biological classification of chronic renal damage is still unspecific and under discussion [3]. Termed as ‘chronic allograft nephropathy’ (CAN) in the Banff classification system [4], cumulative injury due to pathogenic insults and allograft response—either immune or non-immune mediated—results in a histological uniform phenotype of fibrotic tissue damage, which is dominated by tubular atrophy and interstitial fibrosis (IF/TA) [5,6].

Histopathological evaluation of biopsies is an indispensable method for the detection of allograft injury, although it tends to suffer from the subjectivity of the approach [7]. Inspection of molecular changes, for example by using DNA microarrays, a technology that explores the expression levels of thousands of mRNAs under normal and disease conditions, may add important aspects to the complexity and a fine-tuning of classification and diagnosis [8] and may help to identify critical molecules and pathways of graft injury.

We hypothesized that changes on the molecular level may actually precede the onset of histological phenotypic changes of CAN (i.e. IF/TA). In a proof-of-principle study, we employed human whole genome microarray analysis of 3-month protocol biopsies from patients with IF/TA in the
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6-month biopsy (‘progressors’) and compared these expression profiles with those of patients who did not have IF/TA in the 6-month protocol biopsy.

**Material and methods**

**Patients**

All patients in this study received a renal transplant between 2001 and 2004 at the transplant centre of the Medical School Hannover, Hannover, Germany. Protocol biopsies of the renal allografts were available through the protocol biopsy programme of the transplant centre that is part of the routine medical care following transplantation and had been approved by the local ethical committee. Informed consent was obtained from each patient. The participation in the programme was completely voluntarily.

The use of archive material for research had been permitted by the local ethical committee on the understanding of anonymity. All patients were Caucasians. Isometrical vacuolization of tubular epithelial cells suggestive of CNI toxicity was detected in 25% of patients in each group at the 3-month biopsy (four patients) and in 50% of the progressors compared to 17% of the non-progressors in the 6-month biopsies (four patients). CyA trough levels in these patients were not different from the levels in patients without CNI toxicity (not shown). One patient of each group had delayed graft function; one patient of the progressor group had a borderline rejection episode between T0 and Week 6 [MMF (1 g/day) was added to maintenance immunosuppression, consisting of CyA and steroids until the time of the borderline episode]. The two patient groups had similar allograft function (creatinine clearance), during the first 6 months post-TX, without statistically significant time-dependent changes (data not shown). Individual demographic data of the patients in this study and the corresponding donors are shown in Figure 1A–C.

**Biopsy sampling/histology**

Two biopsy cores were obtained with a 16-gauge automated biopsy needle by ultrasound guidance. Both cores were immediately checked for the presence of at least seven glomeruli with a stereo microscope. One of the cores was snap-frozen for scientific investigation, without further histologic evaluation. The second core was fixed in 4% formalin and processed for semi-quantitative light microscopic evaluation. Assessment of IF/TA was done according to the updated Banff classification [4]. All biopsies were stained for C4d using a commercially available polyclonal rabbit-anti-human antiserum on paraffin sections.

Inclusion criteria for the retrospective selection of the patients and their tissue samples for this study were complete availability of the clinical data and sufficient tissue according to the Banff requirements of biopsy adequacy [4], as well as absence of IF/TA until the 6-month protocol biopsy, and the absence of AR episodes (including C4d positive rejection) during the entire study period. Patient and sample selection was otherwise performed at random.

**RNA extraction and microarray analysis**

Frozen biopsy samples were homogenized in an RLT-lysis buffer (Qiagen, Hilden, Germany), using a motor-driven homogenizer. Total
RNA was extracted using RNeasy affinity resin columns with an on-column DNase digestion according to the manufacturer’s instructions (Qiagen). Integrity and concentration of the RNA samples were determined using the Agilent RNA 6000 Nano LabChip kit (Agilent Technologies, Palo Alto, CA, USA). Total RNA (50 ng) was amplified and labelled using the Affymetrix Two-Cycle cDNA Synthesis kit (Affymetrix, Inc., Santa Clara, CA, USA). The cRNA fragments were hybridized on GeneChip Human Genome U133 Plus2 arrays. Image files (.dat files) were processed using the Microarray Analysis Suite 5 (MAS5) software (Affymetrix, Inc.). The average intensity of each chip was scaled to a target intensity of 150.

Data processing and Statistical analysis

MAS5-normalized signal intensities below 25 were set to 25. To account for experimental microarray-wide variations in intensity, all measurements on each array were normalized by dividing them by the 50th percentile of that array. Only probe sets that passed all following thresholds were used in the analysis: raw expression of >100 in either of 8 samples (progressor group) or 9 of 12 samples (non-progressor group, controls), trimmed mean expression level >100 in either of the two patient groups, the coefficient of variance <30% in either patient group and the minimum ratio of the trimmed means at 1.5. Comparisons of these data were performed using the t-test, with P < 0.1 considered as statistically significant. The significance is indicated by a P-value, and false discovery rate was calculated as qDR [9].

Gene-set enrichment analysis (GSEA) was adapted from Mootha [10] and modified [11]. Quantitative real-time PCR analysis was performed on the same total RNA samples by TaqMan Low Density Arrays (TLDA) run on a 7900HT PCR system (Applied Biosystems, Foster City, CA, USA) after reverse transcription of RNA samples with the High Capacity cDNA Archive kit (Applied Biosystems). The analysis by RQ Manager in the SDS 2.3 software package (Applied Biosystems) provided relative gene expression levels after normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and calibration to the non-progressor samples.

Immunohistochemistry

Paraffin-embedded tissue sections (2 µm) were stained with periodic acid–Schiff (PAS) and trichrome stain and subjected to histologic analysis as described previously according to the updated Banff criteria [12]. The paraffin sections (2 µm) were stained on a LabVision™ automated stainer using the following antibodies: mouse-anti-human CD45 (clone UCHL-1, Dako, Hamburg, Germany), and anti-human Collagen III (clone MU167-UC, BioGenex, CA, USA). The sections were heated (CD45) and enzyme (Collagen III) pretreated incubated with primary antibodies, followed by respective biotinylated secondary antibody incubation. Staining was developed by an avidin-biotin-based polymer detection system (Zytovision, Germany) with peroxidase and DAB visualization. For CD45, the number of stained cells in three high-power fields was counted. For Collagen III, the percentage of stained cortical interstitium was semi-quantitatively assessed by one of the authors (M.M.)

Results

To obtain insight into early gene expression changes preceding IF/TA, we conducted a genome-wide transcriptome analysis in human 3-month renal allograft protocol biopsies from 20 transplant patients. Based on the diagnosis IF/TA in the subsequent 6-month protocol biopsy, patients were classified into two groups: progressors (Figure 1A, patients A–H) were diagnosed with IF/TA at month 6; non-progressors (Figure 1A, patients I–T) were without IF/TA. Banff grades are listed in Figure 1A.

Gene expression analysis of biopsies

High-density oligonucleotide microarray analysis resulted in a total of 997 probe sets that passed the raw data filtering and statistical analysis (Figure 2A). A low-stringent feature-selection method (P-value < 0.1, fold change ≥1.5) was chosen to capture relevant biological information on biological themes, pathways and networks while accepting the risk a few individual false positives.

The most significantly overrepresented functional categories in the progressor group according to gene ontology (GO) were defense response (30% of upregulated genes), response to wounding (10%), response to stress (18%) and cell communication (45%) (Figure 2B). Genes that were underrepresented in the progressor group compared to the non-progressor group belonged mostly to gene ontologies referring to tissue and cell function (Figure 2C).

We grouped the probe sets into functional categories, which reflect some of the pathophysiological processes observed during allograft injury, such as induction of immune response and tissue remodelling.

HLA expression, antigen presentation and leukocyte activation that are linked by the complement system were found to be activated in the allografts of the progressors including various complement cascade members, interferon gamma receptor, and HLA-DQ and HLA-DR genes (supplementary Table 1A). Many genes coding for the T cell receptor pathway were overexpressed in the progressor group, for instance T cell receptor alpha and beta loci, CD3d, CD3z, linker for activation of T cells (LAT) and lymphocyte-specific protein tyrosine kinase (LCK). Interestingly, while most of the above-mentioned genes showed an expression increase of ~2-fold, B cell-expressed immunoglobulin IgA, IgG and IgM genes were up to 17-fold overexpressed in allografts of the progressors.

Increased complement activity and immune insults activate endothelial cells, which respond by secretion of leukotactic and lymphotactic chemokines and cytokines [13]. In the allografts of progressors, genes involved in the homing process of leukocytes were overexpressed (supplementary Table 1A). This included the initial phase of leukocyte rolling (e.g. selectin L, selectin P ligand, vascular cell adhesion molecule 1 and CD44), and the subsequent phase of integrin-mediated firm adhesion through induction of chemokine receptors (e.g. integrin alpha 4 and beta 2, and chemokine receptors CCR2, CCR7, CXCR4). The successive disintegration of intercellular junctional structures and concomitant increase of intercellular permeability are indicated e.g. by the overrepresentation of stabilin 1, a protein associated with endothelial inflammation [14], and by the underrepresentation of molecules such as claudins that under normal conditions prevent the transmigration of leukocytes into the tissue. In addition to these local factors, a gene expression signature was found that indicates graft infiltration with macrophages by exhibiting overexpression of macrophage expressed gene 1, osteonectin, fatty acid binding protein 5 and cathepsin S (supplementary Table 1A). Moreover, genes representing other leukocyte subtypes, e.g. neutrophils, were differentially overrepresented in progressor biopsies, implying a net infiltration and/or activation of these cell types in the graft.

Inflammation and vascular changes through tissue remodelling are connected processes [15]. In our dataset, genes coding for proteins involved in cell-to-cell adhesion and epithelial integrity and function were generally
underrepresented in the progressors, such as claudins, KSP-cadherin, shroom 2 and aquaporin 2 (supplementary Table 1B). Genes encoding proteins believed to modulate the injury-induced transdifferentiation of tubulo-epithelial cells (TECs) into fibroblasts were differentially higher expressed in the progressor group, some of them belonging to the BMP signalling pathways, others to the Wnt signalling pathway (bone morphogenetic protein inducible kinase, BMP6, sclerostin and dickkopf 3, Wnt inducible signalling protein 1, respectively). Many fibroblast-associated genes were expressed higher in the progressors, such as periostin, lumican, plasminogen activator inhibitor, urokinase, heat shock protein HSP47, fibronectin and its receptor ITGA4. In addition, genes that are involved in smooth muscle differentiation and proliferation were found dysregulated (mostly overexpressed) in the progressor group: adipocyte enhancer binding protein 1 (AEBP1), cathepsin S, versican, smooth muscle associated protein 2, thrombospondin 1 and 2 and transgelin (SM22). These changes in gene expression suggest increased fibroblast numbers and activation of gene expression, leading to increased ECM deposition. Accordingly, a large number of genes representing ECM deposition and remodelling were found overexpressed, including collagens (e.g. COL III), versican and its receptor CD44, decorin, biglycan and lumican, similar to findings in experimental glomerulonephritis[16].

Many transporters and cell polarity genes were differentially dysregulated in the progressor group, suggesting early functional changes in tubulointerstitial structures before structural alterations like IF/TA become manifest in the 6-month biopsies (supplementary Table 2). Specifically, the expression of transporters for urea (SLC14A1), glucose (SLC2A12), amino acids (e.g. SLC1A4), water and ions (e.g. aquaporin 2, Barttin, calcium-sensing receptor, potassium channel tetramerization domain containing 1) and other metabolites were lower in the progressors than in the non-progressors.

**Gene-set enrichment analysis**

To take the analysis a step further and analyse the data on the pathway level, we applied GSEA. GSEA is a pathway-centric approach, based on the assumption that gene expression changes not only affect singular entities (i.e. genes)
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but rather affect pathways and signalling networks as a whole [9,10]. The approach compares the unfiltered expression changes of predefined regulatory or otherwise related functional groups of genes between two biological sample cohorts. Comparison of progressors and non-progressors confirmed the importance of immune response-related pathways and networks that were overrepresented in the progressor group (Figure 3A). A network comprising genes related to extracellular matrix (ECM) deposition, tissue remodelling, smooth muscle and fibrosis was overexpressed in progressors that involved collagens, decorin, smooth muscle actin, vinculin and von Willebrand factor (Figure 3B). An aspect, which GSEA added to the results of the conventional statistical analysis, was the underrepresentation of genes involved in mitochondrial function, such as members of the NADH dehydrogenase subcomplex, ATPase’s and cytochromes (Figure 3C). While only some of these genes were significantly changed and passed the statistical thresholds, GSEA was able to identify this entire network of oxidative phosphorylation as underrepresented.

**TaqMan quantitative real-time PCR analysis**

To independently confirm changes in the median gene expression level of some genes of the previous analyses, TaqMan quantitative real-time PCR was performed on selected genes. We selected genes with functional involvement of the immune response, tissue remodelling and tissue function, and with a fold change range that would be representative of the fold changes seen in the entire dataset. RNA samples from 5 progressors and 12 non-progressors were available. The relative gene expression changes as obtained in the TLDA analysis were compared to the DNA microarray data of the same samples. As seen in Figure 4, all trends in relative gene expression could be confirmed, most with a statistical significance of $P < 0.1$.

**Immunohistochemistry**

Gene expression analysis from 3 months after transplantation suggested an increased lymphocyte number or lymphocyte activation, and a stronger involvement of tissue remodelling, especially ECM, in allografts with latent IF/TA compared to quiescent allografts. To further evaluate whether our findings on gene expression level correlate with protein level, we performed a proof-of-principle immunohistochemistry analysis on available paraffin-embedded sections of some of the corresponding biopsies that were used for the gene expression study. We stained for CD45, a general marker of lymphoid cells (but not of erythrocytes), and for collagen III, which serves a marker...
Fig. 4. Confirmation of gene expression changes by PCR. Semi-quantitative real-time PCR analysis of the relative expression differences between the progressor and the non-progressor group of patients was applied to 20 genes using a TaqMan Low Density Array (TLDA, ABI Inc). Genes with functional involvement in the immune response, tissue remodelling and tissue function ontologies in a range of fold changes representative of the fold changes seen in the entire dataset were chosen. The deduced relative fold changes were then compared to the relative expression changes that were measured in a DNA microarray analysis with the same RNA samples. Only gene expression data from samples with sufficient amount of RNA for TaqMan analysis were used in the comparison (5 progressors, 12 non-progressors). (A) Gene annotation and numerical representation of the relative fold changes for TLDA and microarrays, including the probe set IDs and TLDA assay IDs. ∗P-value < 0.1, ∗∗P-value < 0.05; (B) Graphical representation of the relative fold changes in (A) ∗P-value < 0.1, ∗∗P-value < 0.05.

for extracellular remodelling and matrix deposition. Both mRNA species are differentially overrepresented in progressors at month 3. As shown in Figure 5, CD45- and COL III-protein expression were found overrepresented in individual sections of progressors at month 3 compared to non-progressors, corresponding to gene expression data.

Discussion

Our study results provide novel insight into early processes of IF/TA at a time point where conventional histological evaluation of the allograft is without pathological findings.

We found a panel of significant changes in the transcriptome that preceded the histological phenotype of IF/TA by 3 months. Especially, transcriptional levels of genes involved in complement activation, leukocyte homing, T- and B-cell infiltration and activation, beginning profibrotic events, functional changes in specific metabolic and transport properties of the nephron, or in epithelial-to-mesenchymal transition (EMT), the process of transdifferentiation of epithelial cells to cells with fibroblast phenotype [17,18], were significantly different in early protocol biopsies of patients with subsequent development of irreversible IF/TA. This further corroborates the assumption that inflammation that is quantitatively below the diagnostic threshold of acute rejection is involved in early stages of progressive renal allograft damage [19].
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Fig. 5. Immunohistochemistry. Representative CD45 (A) and Collagen III (B) immunostainings of individual paraffin sections. Increased number of CD45 positive cells and COLIII-positive areas correspond to increased levels of CD45 and COLIII mRNA levels in progressors at week 12.

Early tissue injury in renal allografts may be related to many factors, such as donor graft condition (age, gender, pre-existing disease), cold and warm ischaemia time and drug toxicity. Tubular injury itself may then trigger immune and repair responses, thereby further contributing to the damage [20]. At the crossroads of these pro-inflammatory events is the complement system, which is enhanced under conditions of tissue injury [13]. We identified many overexpressed genes in the progressors with functional association to activated immune system. Antibodies that are reactive to MHC are most likely harmful to the endothelium. Through GSEA analysis, it became clear that mitochondrial function is negatively affected as well. Mitochondrial dysfunction seems to be involved in the aetiolo-phy pathophysiology of a broad spectrum of renal diseases [21].

Damaged endothelial cells react by upregulating the expression of adhesion molecules like selectin E and P, and secretion of leukocyte attracting factors such as chemokine ligands CCL2 and CCL5 and PECAM1, which mediate leukocyte and lymphocyte homing to the damaged sites [22]. Increase in their expression indicates that increased leukocyte homing and transendothelial migration processes may be activated already before development of fibrosing changes in the allograft.

Tissue remodelling is a common sequel of the pathogenesis of many chronic illnesses. Intimal thickening of arterial vessels, proliferation of smooth-muscle cells (SMC) and the synthesis and deposition of matrix proteins lead to the formation of neointima and interstitial fibrosis. Many genes characteristic of smooth muscle or fibroblast expression were overexpressed in the progressor group, implying that during early phases of IF/TA development myofibroblast numbers are increased or have activated transcriptional activity. Transforming growth factor beta-induced (TGFBI) and AEBP1 may be of particular importance. TGFBI regulates the adhesion and migration of vascular SMCs during the pathogenesis of atherosclerosis [23], and AEBP1 promotes transdifferentiation of preadipocytes into smooth muscle-like cells [24]. Osteonectin, which stimulates TGF-beta expression and is expressed in neointima during chronic allograft rejection [25], and smooth muscle associated protein 2, which is upregulated following intraluminal balloon injury of the rat carotid artery and accompanied the proliferation and migration of medial vascular smooth muscle cells into the intima [26], were overrepresented in the allografts of the progressors. Versican, which was overexpressed in the progressors as well, creates pericellular matrices that are required for pro-fibrotic, arterial SMC migration and proliferation [27]. In turn, the expression of versican is regulated by beta-catenin [28], a mediator of EMT [28], and it binds to gene products of other genes that were upregulated in the progressor group, such as fibulin-1, fibrillin-1, L-selectin, P-selectin ligand 1 and CD44 [29]. CD44-expression is increased during inflammatory renal diseases [30], and contributes to fibrogenesis, at least in part through enhancement of hepatocyte growth factor (HGF) and TGF-beta 1 signalling [31]. HGF was found upregulated in allografts of the progressors, as were RANTES (CCL5) and PAI-1 (plasminogen activator inhibitor 1), which are altered in experimental and human chronic renal allograft rejection [32,33].

The origin of myofibroblasts during development of tubulointerstitial fibrosis is not fully understood. Yet, EMT has recently been proposed to be one of the sources of myofibroblast cells in the kidney [17,18]. During EMT, injured epithelial cells lose their polarity and their transporter function, reorganize their cytoskeleton into stress fibres, disrupt the tubular basement membrane and migrate into the interstitium where they synthesize increasing amounts of ECM [34]. There is an increasing body of evidence that endoplasmatic reticulum stress protein HSP47 (SERPINH1) is involved in the initiation of EMT processes. Increased expression of HSP47, as observed in the progressor group, may be important in pro-fibrotic processes including up-regulation of collagen expression, and has been found during complement-mediated glomerular epithelial cell injury, and in deteriorating and atrophic tubules, along with other markers of EMT like vimentin and alpha-SMA [35,36]. Vimentin and alpha-SMA were upregulated in the progressor group, but did not meet the analysis thresholds (data not shown). The importance of EMT is further stressed by a recent study showing that protein levels of the EMT marker vimentin are elevated in tubular cells of kidney allografts at 3 months post-TX, which was associated with the development of fibrosis at month 12 [37].

In a previous study, we reported on a predictive set of 10 probe sets as marker for (CAN, Banff 2003) in biopsies 6 months before the diagnosis of CAN [38]. In the present proof-of-principle study, which was aimed at gaining insight into mechanistic processes leading to IF/TA,
HoxB7 and opioid binding protein/cell adhesion molecule-like (OPCML), a tumour suppressor protein, were found again. HoxB7 has recently been related to EMT, as HoxB7 overexpression in both MCF10A and Madin-Darby canine kidney (MDCK) epithelial cells leads to loss of epithelial proteins, claudin 1 and claudin 7, mislocalization of claudin 4 and E-cadherin, and the expression of mesenchymal proteins, vimentin and alpha-smooth muscle actin [39]. TNF-α13B (TALL-1) and APRIL appear to stimulate B and T cell proliferation in vitro [40]. A reason for the observed diversity of the results may be the different biopsy time points (6 months and 12 months in our previous study; and 3 months and 6 months in the present study), taking into account that disease progression is not static, but rather a dynamic process.

In summary, using two specifically selected patient groups with sequential protocol biopsies from renal allografts, we were able to show that significant changes in the transcriptome precede the histological phenotype of tubular atrophy and interstitial fibrosis, the visible hallmark of chronic allograft deterioration. We identified gene signatures that support previous findings that immune- and non-immune-related events contribute to the initiation of tubular atrophy and interstitial fibrosis. Most importantly, our data indicate, to our knowledge for the first time, that these processes occur already before they are detectable with conventional histopathological examination of renal allografts. Thus, microarray technology applied to protocol biopsies provides insight into the pathophysiology of IF/TA and might allow for a more timely therapeutic intervention.

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Conflict of interest statement. None declared.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

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A population-based assessment of the familial component of acute kidney allograft rejection

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Abstract

Background. The genetic determinants of acute kidney transplant rejection (AR) are not well studied, and familial aggregation has never been demonstrated. The goal of this retrospective case-control study was to exploit the unique nature of the Utah Population Database (UPDB) to evaluate if AR or rejection-free survival aggregates in families.

Methods. We identified 891 recipients with genealogy data in the UPDB with at least one year of follow-up, of which 145 (16.1%) had AR and 77 recipients had biopsy-proven rejection graded ≥1A. We compared the genealogical index of familiality (GIF) in cases and controls (i.e. recipients with random assignment of rejection status).

Results. We did not find evidence for familial clustering of AR in the entire patient population or in the subgroup with early rejection (n = 52). When the subgroup of recipients with rejection grade ≥1A (n = 77) was analysed separately, we observed increased familial clustering (GIF = 3.02) compared to controls (GIF = 1.96), although the p-value did not reach the level of statistical significance (p = 0.17). Furthermore, we observed an increase in familial clustering in recipients who had a rejection-free course (GIF = 2.45) as compared to controls (GIF = 2.08, p = 0.04). When all recipients were compared to non-transplant controls, they demonstrated a much greater degree of familiality (GIF = 2.03 versus GIF 0.63, p < 0.001).

Conclusions. There is a familial component to rejection-free transplant course and trend to familial aggregation in recipients with AR grade 1A or higher. If a genetic association study is performed, there are families in Utah identified in the current study that can be targeted to increase the power of the test.

Keywords: acute rejection; familial; kidney transplant; outcome; genealogy

Introduction

Renal transplant is the preferred modality of treatment for end-stage renal disease (ESRD), as it is associated with lower morbidity and greater recipient survival [1], improved quality of life [2] and lower medical expenses [3] compared to patients remaining on the transplant waiting list. However, since organ transplantation requires surgical...