IFNL4 rs368234815 polymorphism does not predict risk of BK virus associated nephropathy after living-donor kidney transplant: A case-control study

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Abstract
Background: BK polyoma virus (BKPyV) associated nephropathy (BKPyVAN) is a major cause of kidney graft loss in renal transplant patients. Interferons (IFNs) are an important innate immune response against viral infections and genetic polymorphisms of the IFN-pathways can affect susceptibility and mortality during viral infection. Here, we investigated whether the dinucleotide polymorphism rs368234815 (ΔG/TT) in the IFNL4 gene contributed to BKPyV reactivation or BKPyVAN after living-donor kidney transplantation.

Methods: This retrospective case-control study determines the prevalence of IFNL4 variants in a Caucasian population of living-donor kidney transplant recipients and donors and explores its association with BKPyV infection and BKPyVAN development. We included 28 recipients with BKPyV reactivation, 10 of which developed BKPyVAN and 30 BKPyV negative controls. Targeted sequencing of the IFNL4 gene from both recipients and their respective donors was performed.

Results: We found IFNL4 rs368234815 ΔG allele frequencies of 41.7% in BKPyV negative and 39.3% in BKPyV positive recipients (P = .85), and 41.7% and 40.4% (P > .99) in their respective donors. IFNL4 rs368234815 ΔG allele frequencies in BKPyVAN developing recipients and their respective donors were 50% and 43.7% (P = .60 and P > .99).

Conclusions: Our results indicate that the IFNL4 rs368234815 ΔG allele is not associated with BKPyV reactivation, nor the manifestation of BKPyVAN.
INTRODUCTION

BK polyoma virus (BKPyV), a non-enveloped double-stranded DNA virus of the polyomavirus family that was first described in 1971,1 has emerged as one of the most challenging pathogens for kidney transplant recipients (KTR). BKPyV is usually acquired during childhood with more than 90% of adults being seropositive for BKPyV by the age of 23 years.2 Following infection, a state of non-replicative asymptomatic infection termed “latency” is established in epithelial cells of the kidney and the urinary tract of most immune-competent hosts. Yet, urinary shedding of BKPyV without viremia can be detected in 7% of healthy blood donors.3 In contrast, the immunosuppression necessary after kidney transplant enables the virus to reactivate even further, thus leading to BKPyV replication and viremia in up to 60% of all KTR.4,5 BKPyV reactivation within the allograft can result in BKPyV associated nephropathy (BKPyVAN), which commonly occurs during the first year after transplantation in up to 10% of KTR. BKPyVAN can lead to a progressive decline in graft function, potentially resulting in allograft loss in up to 50% of those affected.6 Clinical management focuses on active surveillance for reactivation and a modification of immunosuppression in case of replication. The latter poses a substantial risk of acute rejection.

In healthy individuals viral control is achieved through the combined actions of the innate and adaptive immune system. Interferons (IFNs) are part of the early innate immune response against viral infections as they can induce a large group of more than 300 interferon-stimulated genes (ISG), which have potent anti-viral functions.7 IFNs are divided into three subfamilies according to their distinct receptor utilization, namely, type I (e.g., interferons alpha and beta [IFN-α/β]), type II (interferon gamma [IFN-γ]), and type III (interferon lambda [IFN-λ]). The type II interferon IFN-γ is the signature cytokine of type 1 immune responses and regulates innate and adaptive lymphocytes in their cytotoc effectors functions against intracellular pathogens and transformed cells. Both type I and type III IFNs are potent antiviral cytokines, which strongly act on non-hematopoietic cells and play a key role in innate immunity against viral infection.8 Type III interferons were the most recently discovered interferons and humans possess four IFNL genes (IFNL1, IFNL2, IFNL3, IFNL4 encoding for IFN-λ1, IFN-λ2, IFN-λ3 and IFN-λ4, respectively). The heterodimeric IFN-λ receptor is preferentially expressed on epithelial cells, which explains the relevant and more specific anti-microbial role of type III interferons in mucosal organs.9,10 Although, type III interferons can be induced in hematopoietic and non-hematopoietic cells by viral infections or stimulation of Toll-like receptors (TLR),11–13 the most potent producers of IFNLs seem to be myeloid and plasmacytoid dendritic cells (DCs).14 Of note, the IFN-λ response in the kidney is restricted to epithelial cells of the renal tubules and the urinary epithelium, which, conspicuously, also provide the niche for BKPyV.9

Polymermorphisms in IFNL4, including rs12979860 (C/T) and rs368234815 (ΔG/TT) (Figure 1A), were first described in association with spontaneous or treatment-induced clearance of hepatitis C virus (HCV).15 Rs368234815 (ΔG/TT) is a dinucleotide polymorphism (DNP) located in exon 1 of IFNL4, formed by the tightly linked single nucleotide polymorphisms (SNP) rs11322783 (ΔA/T) and rs74597329 (T/G). The IFNL4 rs368234815 ΔG allele forms an open reading frame in IFNL4, and carriers of IFNL4 rs368234815 TT therefore do not express functional IFN-λ416 (Figure 1B). Intriguingly, patients who harbor a functional IFNL4 gene (IFNL4 rs368234815 ΔG) show reduced clearance of HCV leading to progression of liver inflammation and fibrosis.17,18 Mechanistically, current data indicates that hepatic expression of IFN-λ4 (i.e., rs368234815 ΔG) impairs responsiveness towards further type I interferon stimulation,17,19 which prevents efficient viral clearance. In addition, IFNL4 rs368234815 ΔG was identified in high linkage disequilibrium (LD) with the IFNL4 rs12979860 T SNP (Figure 1B), which is particularly the case in individuals of European (r² = .98) and Asian ancestry (r² = .99). In line with that, IFNL4 rs12979860 T is also associated with an impaired HCV clearance.15 Finally, studies in the hematopoietic stem cell and solid organ transplant setting have reported an association of CMV replication with the IFNL4 rs368234815 ΔG as well as the rs12979860 T allele.20–22

Currently, there are no reports that interrogate the association between BKPyV replication and IFNL4 polymorphisms in KTR and their donors. With this study, we aim (i) to determine the prevalence of the different SNP variants of the IFNL4 gene, and (ii) to evaluate the association of genetic IFNL4 variants, with the incidence of BKPyV reactivation and BKPyVAN in a Caucasian population of KTR. Importantly, we analyzed the IFNL4 SNPs in both, recipients and donors, as IFN-λs can potentially be secreted by donor and recipient derived cells in the allograft. Considering the highly relevant findings for HCV clearance, we hypothesized that polymorphisms in the IFNL4 region, especially the presence of the rs368234815 ΔG allele, which leads to the production of the IFN-λ4 protein, might increase the risk of BKPyV replication and BKPyVAN. To this end, genotyping of SNPs might identify patients at risk allowing individualized treatment strategies to prevent BKPyV reactivation and progression to BKPyVAN.

PATIENTS AND METHODS

2.1 Study population, BKPyV and BKPyVAN screening

Between 2010 and 2017, 267 living-donor kidney transplants were performed at the University of Freiburg, Germany. Demographic, clinical and immunological data were recorded. The primary inclusion criterion was living-donor kidney transplantation in adults (≥18
years. Deceased and patients treated with an mTOR-inhibitor were excluded, since use of the latter may be protective against BKPyV replication. 23 240 patients were eligible for inclusion. Of these, 56 patients developed BKPyV replication (> 500 copies/ml). Informed consent and blood samples could be obtained in 28 cases. Together with their corresponding donors they were sub-grouped according to recipients' BKPyV status. 18 recipients did not develop BKPyV viremia (group 1), 10 recipients presented with biopsy-proven (immunohistochemically SV-40 positive) BKPyVAN (group 2). The follow-up period of recipients that developed BKPyV viremia was 66 months (IQR 47–77 months) after transplantation (Table 1). Altogether, 58 recipients and 56 corresponding donors could be included in this retrospective case-control-study (Figure 2). The rs12879860 (C/T) SNP lies in intron 1, the rs368234815 (ΔG/TT) DNP lies in exon 1. (B) Common haplotypes of functional variants of the IFNL4 gene and their role in HCV infection. Note that rs12879860 T and rs368234815 ΔG are in strong LD, that is, inherited together, especially in subjects of Asian and Caucasian ancestry.

FIGURE 1 IFNL4 locus, investigated SNP (A), linkage disequilibrium and functional relevance in HCV infection (B). (A) Schematic depiction of the IFNL locus on chromosome 19 and the IFNL4 SNP investigated in this study. The rs12879860 (C/T) SNP lies on the chromosome 19 locus, investigated SNP (A), linkage disequilibrium and functional relevance in HCV infection (B). (A) Schematic depiction of the IFNL locus on chromosome 19 and the IFNL4 SNP investigated in this study. The rs12879860 (C/T) SNP lies in intron 1, the rs368234815 (ΔG/TT) DNP lies in exon 1. (B) Common haplotypes of functional variants of the IFNL4 gene and their role in HCV infection. Note that rs12879860 T and rs368234815 ΔG are in strong LD, that is, inherited together, especially in subjects of Asian and Caucasian ancestry.

2.2 | PCR and Sanger sequencing

Historical blood samples were used for genotyping. Genomic DNA was extracted using a QIAamp Blood DNA Midi Kit (QIAGEN, Venlo, Netherlands). DNA was quantified using NanoDrop Microvolume Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). DNA was PCR-amplified with Taq DNA Polymerase (VWR Life science, Radnor, PA, USA) using primer pairs for IFNL4 (FW 5'-CCCTCTTTGGCTTCCCTGAC-3' and REV 5'-CCAGCAGCTCCAGGATCG-3') and 20-100 ng of DNA in a total volume of 20 μl. The amplification mixture consisted of water, 10 mM Tris-HCl, pH 8.5, 50 mM KCl, .75 mM MgCl2, .0625 mM of deoxynucleoside triphosphates, 10 μM of each primer and 1 U of Taq DNA polymerase (QIAGEN). All reactions were performed with the following cycling parameters: initial denaturation cycle (95°C for 5 min) followed by 35 amplification cycles (95°C for 15 s; 64°C for 30 s; 72°C for 50 s), followed by a final extension step (72°C for 10 min). PCR-amplified fragments were sequenced by Sanger sequencing using GATC Eurofins Genomics Germany GmbH services, Ebersberg.
### Table 1
Baseline characteristics of transplant recipients and donors

|                              | All patients \(n = 58\) | BKPyV negative \(n = 30\) | BKPyV positive \(n = 28\) | \(P\) |
|------------------------------|--------------------------|--------------------------|--------------------------|------|
| **Recipients**               |                          |                          |                          |      |
| age, years\(^a\)             | 50 (42–57)               | 50 (40–56)               | 52 (41–61)               | .465\(^c\) |
| female                       | 22 (38)                  | 14 (47)                  | 8 (29)                   | .184\(^d\) |
| time after transplant, months\(^a\) | 76 (58–94)              | 88 (70–102)              | 66 (47–77)               | .002\(^e\) |
| autoimmune renal disease     | 29 (50)                  | 12 (40)                  | 17 (61)                  | .189\(^d\) |
| HLA I mismatches, mean\(^b\) | 2.24 (1.06)              | 2.10 (1.19)              | 2.39 (0.92)              | .299\(^e\) |
| HLA II mismatches, mean\(^b\) | 1.17 (0.68)             | 1.27 (0.64)              | 1.07 (0.72)              | .270\(^e\) |
| ABO incompatible              | 18 (31)                  | 11 (37)                  | 7 (25)                   | .402\(^d\) |
| high dose steroid bolif\(^f\) | 17 (29)                  | 6 (20)                   | 11 (39)                  | .151\(^d\) |
| ATG application               | 4 (7)                    | 2 (7)                    | 2 (7)                    | >.999\(^d\) |
| **BKPyV**                    |                          |                          |                          |      |
| time till viremia, months\(^a\) |                      | 3.50 (3.0–5.8)          |                          |      |
| change of immunosuppression   |                          | 25 (89)                  |                          |      |
| BKPyVAN                       |                          | 10 (36)                  |                          |      |
| graft loss due to BKPyVAN     |                          | 4 (14)                   |                          |      |
| **CMV**                      |                          |                          |                          |      |
| viremia                       | 6 (10)                   | 4 (13)                   | 2 (7)                    | .671\(^d\) |
| high risk status (D*/R-*)      | 8 (14)                   | 4 (13)                   | 4 (14)                   | >.999\(^d\) |
| **Matched donors**           |                          |                          |                          |      |
| median age, years\(^a\)      | 53 (47–59)               | 53 (47–59)               | 52 (46–60)               | .719\(^a\) |
| female                       | 38 (66)                  | 21 (70)                  | 17 (61)                  | .582\(^d\) |

Data generally reported as counts (% of recipients per column) unless indicated otherwise.

\(^a\) Data reported as median (IQR).
\(^b\) Data reported as mean values (SD).
\(^c\) \(P\)-values were calculated using Mann-Whitney test.
\(^d\) \(P\)-values were calculated using Fisher’s exact test.
\(^e\) \(P\)-values were calculated using Student’s t test.
\(^f\) Administration in case of rejection.

Germany. The sequencing chromatogram was analyzed applying ApE software (ApE, A plasmid Editor – V 2.0.61).\(^26\)

### 2.3 Statistical analysis

#### 2.3.1 Baseline characteristics and DNP/SNP distributions

Patients were stratified into three subgroups: biopsy proven BKPyVAN (group 2), BKPyV viremia without BKPyVAN (group 1), and BKPyV negative control group (group 0). If not otherwise indicated, data were expressed as counts (%), median (IQR) or mean (SD).

Baseline characteristics of recipients and donors were compared according to BKPyV status (group 0 vs. group 1+2) using the nonparametric Mann-Whitney test (median age), Fisher’s exact test (frequency distributions) or the unpaired student’s t test (HLA-mismatches). Due to a log-normal distribution, viral loads within the BKPyV positive subgroups (group 1 vs. 2) were compared after logarithmic transformation using the unpaired student’s t test and presented as means with corresponding 95% confidence intervals.

Analysis of allelic variants as risk factors of BKPyV disease was performed individually for recipients and donors. In the first step, all patients with BKPyV viremia (group 1+2) were analyzed together and compared to BKPyV negative patients (group 0). In a second step, only BKPyVAN patients (group 2) were compared to BKPyV negative patients (group 0). Analysis was performed in three different models, an allele based, a genotype based, and a \(\Delta G\)-dominant model. Allele frequencies were obtained by counting of individual alleles. Distribution of allelic variants within the different subgroups was evaluated by Fisher’s exact test (comparison of two groups) or the chi-square test (comparison of three groups).

All statistical analyses were performed using GraphPad Prism V8.4.0 software (GraphPad Software, San Diego, CA, USA). A two-sided \(\alpha\) of less than .05 was considered statistically significant.
2.3.2 Linkage disequilibrium

Linkage disequilibrium (LD) depicts the non-random association of genetic marker alleles. Pairwise LD between the genetic IFNL4 rs368234815 (ΔG/TT) DNP and IFNL4 rs12979860 (C/T) SNP polymorphisms was estimated using the LD-function of the Population Genetics R package version 1.3.8.1.2 in R statistical computing environment.27,28 Since Sanger sequencing supplies unphased genotypes, the LD-function uses maximum likelihood estimation to compute the haplotype frequencies prior to estimating the LD. To compare and verify the obtained LD of our study cohort with a reference population, LD between rs11322783 (Δ/T) SNP (one position of the rs368234815 DNP) and rs12979860 (C/T) SNP was estimated using the LDpair module of the LDlinkR suite and data obtained from a European population included in the 1000 Genomes Project (Phase 3; Version 5).29,30 SNP nomenclature is based on The Single Nucleotide Polymorphism Database (dbSNP) build 151. The standardized estimators of LD, D prime ($D'$) and $r^2$ and corresponding goodness-of-fit statistics ($X^2$ and $P$-value) are reported. A two-sided $α$ of less than .05 was considered statistically significant.
3 RESULTS

3.1 BK disease manifestation

From 2010 to 2017, 267 living-donor kidney transplantation were performed at the University hospital of Freiburg, Germany. 240 were eligible for screening: 56 (23.3%) patients developed BKPyV viremia, of these 28 cases could be included by obtaining informed consent and material. This group consisted of 18 recipients who showed viral replication without organ disease (group 1) and ten recipients who developed biopsy-proven BKPyVAN (group 2). 30 matched, persistently BKPyV viremia negative recipients, were selected as controls (group 0). In total, we analyzed 58 KTR and their corresponding 56 donors (two donor samples could not be provided) (Figure 2).

Table 1 depicts baseline characteristics, HLA-matching, immunosuppression, and CMV risk constellation of transplant recipients and donors in respect to recipients BKPyV status. Before diagnosis of BKPyV viremia all patients received a maintenance triple immunosuppression (tacrolimus, mycophenolate mofetil, and low dose steroids). 25 patients with high BKPyV viremia (> 3.7 log10 copies/ml) required a reduction of immunosuppression, four of whom eventually lost their graft due to BKPyVAN. The median age of donors was 53 years, 66% were women. The median age at transplantation of recipients was 50 years, 38% were women. Validating our matching strategy, gender and age distribution of recipients and donors was not correlated with BKPyV status after transplantation (Figure 3A and B). Furthermore, there were no significant differences concerning HLA mismatches, induction treatment with antithymocyte globulin or application of intravenous steroids for acute cellular rejections between BKPyV positive and negative recipients. Neither CMV reactivation nor CMV serostatus showed a significant association with BKPyV replication (Table 1).

In accordance with previous reports, patients who developed BKPyVAN showed significantly higher levels of BKPyV viremia (5.94 log10 copies/ml; 95% CI 5.30; 6.58) than those who did not develop BKPyVAN (4.30 log10 copies/ml; 95% CI 3.98; 4.62) (Figure 3C).31

3.2 Linkage disequilibrium

Due to their close chromosomal proximity and potential evolutionary advantage many of the polymorphisms of the IFNL4 locus exist in high LD, which makes it difficult to single out the protective functions of individual SNPs. For example, it has been shown in patients with HCV infection that the favorable IFNL4 rs12979860 C allele is in strong LD with the favorable IFNL4 rs368234815 TT allele, which results in a frameshift mutation and disrupts IFN-λ4 expression. Hence, we analyzed the distribution of the IFNL4 rs368234815 (ΔG/TT) DNP and IFNL4 rs12979860 (C/T) SNP genotypes of our cohort (recipients and donors; n = 114) and calculated the LD of these polymorphisms. In line with previous reports, we were able to demonstrate a strong LD (D' = .98, r2 = .95) in our cohort between the rs368234815 ΔG
TABLE 2  Linkage disequilibrium between rs368234815 (ΔG/TT) DNP and rs12979860 (C/T) SNP

(A). IFNL4 genotypes in study population

| IFNL4 rs12979860 SNP | IFNL4 rs368234815 DNP |
|-----------------------|------------------------|
| C/C                   | ΔG/ΔG                  |
| 0 (0)                 | 0 (0)                  |
| C/T                   | ΔG/TT                  |
| 1 (1)                 | 60 (53)                |
| T/T                   | TT/TT                  |
| 35 (30)               | 2 (2)                  |

(B). Pairwise LD estimation

| LD statistics         |
|-----------------------|
| n = 114               |
| D' = .981             |
| r² = .946             |
| χ² = 215.692          |
| P < .0001             |

Data reported as genotype counts (%) in total study population (recipients and donors). Pairwise LD estimation calculated using Population Genetics R package.

TABLE 3  Linkage disequilibrium between rs11322783 (Δ/T) SNP and rs12979860 (C/T) SNP in a European reference population published in the 1000 Genomes Project

(A). IFNL4 haplotypes in European population

| IFNL4 rs12979860 SNP |
|----------------------|
| IFNL4                |
| Δ                    |
| C                    |
| 692 (69)             |
| T                    |
| 310 (31)             |

(B). Pairwise LD estimation

| LD statistics         |
|-----------------------|
| n = 1006              |
| D' = .995             |
| r² = .982             |
| χ² = 987.413          |
| P < .0001             |

Data reported as haplotype counts (%) in European reference population (1000 Genomes Project). Pairwise LD estimation calculated using LDlinkR R package. Note that rs11322783 (Δ/T) SNP refers to the first position of rs368234815 (ΔG/TT) DNP.

and rs12979860 T, as well as the rs368234815 TT and rs12979860 C haplotypes, respectively (Table 2).

Our results are also representative of a wider European population. Comparable results are obtained, estimating LD of these polymorphisms from sequencing data of the 1000 Genome Project (Table 3).30

3.3  Association between IFNL4 rs368234815 (ΔG/TT) DNP and BKPyV disease

Next we compared the prevalence of the rs368234815 (ΔG/TT) DNP genotypes in recipients and donors depending on recipients’ BKPyV disease (BKPyV negative: group 0 vs. BKPyV positive: groups 1+2). The allele and genotype frequencies among recipients and donors are summarized in Tables 4 and 5. The IFNL4 rs368234815 ΔG allele frequency in recipients and donors was 40.5% and 41.1%, respectively.

The prevalence of BKPyV disease showed no association with IFNL4 rs368234815 ΔG allele frequency, neither with recipients (P = .85) nor donors (P = .99). Likewise, analyses stratified according to the rs368234815 genotypes (ΔG/ΔG, ΔG/TT, and TT/TT) showed no significant differences between the BKPyV negative and the BKPyV positive group (P = .77 in recipients and P = .8 in donors). To further evaluate a possible ΔG dominant effect, we also applied a ΔG-dominant model, in which the IFNL4 rs368234815 ΔG/ΔG and ΔG/TT genotypes are grouped together and compared to the IFNL4 rs368234815 TT/TT genotype. Again, no significant differences between the groups could be detected. The proportion of recipients (Table 4) with the IFNL4 rs368234815 ΔG/ΔG or ΔG/TT genotypes were nearly identical (P = .99) in the BKPyV negative (group 0; 20/30 = 66.7%) and the BKPyV positive group (group 1+2; 19/28 = 67.9%). Similar results were seen for the respective donor polymorphisms (Table 5). Here, the proportion of donors (Table 5) with the IFNL4 rs368234815 ΔG/ΔG or ΔG/TT genotypes in the BKPyV negative (group 0; 20/30 = 66.7%) and the BKPyV positive group (group 1+2; 18/26 = 69.2%) also showed no significant differences (P = .99).

3.4  Association between IFNL4 rs368234815 (ΔG/TT) DNP and BKPyVAN

Finally, to exclude potentially confounding results from recipients with BKPyV replication not developing BKPyVAN (group 1), we restricted the analysis to recipients with a biopsy proven BKPyVAN (group 2) and compared them to recipients without any reactivation of the virus (group 0). No significant results could be identified, neither in recipients (Table 4) nor donors (Table 5). Thus, the data provide no evidence that the IFNL4 rs368234815 (ΔG/TT) DNP, on its own, affects susceptibility to BKPyV replication or nephropathy.

4  DISCUSSION

In this case-control-study of 58 kidney transplant recipient-donor pairs, no associations were observed between IFNL4 rs368234815 (ΔG/TT) DNP and the occurrence of both, BKPyV replication and BKPyVAN. Hence, it does not appear that the IFN-λ4 protein, which is
TABLE 4 Allele and genotype frequencies of IFNL4 rs368234815 (ΔG/TT) DNP in recipients

| Recipients          | All recipients | BKPyV negative | BKPyV positive |
|---------------------|----------------|----------------|----------------|
|                     | n = 58         | Group 0 n = 30 | Group 1+2 n = 28 | Group 2 n = 10 | P1 (OR1) | P2 (OR2) |
| Allele model        |                |                |                |                |          |
| ΔG                  | 47 (40.5)      | 25 (41.7)      | 22 (39.3)      | 10 (50.0)      | .85 (1.10) |
| TT                  | 69 (59.5)      | 35 (58.3)      | 34 (60.7)      | 10 (50.0)      | .61 (.71) |
| Genotype model      |                |                |                |                |          |
| ΔG/ΔG               | 8 (13.8)       | 5 (16.7)       | 3 (10.7)       | 2 (20.0)       | .77       |
| ΔG/TT               | 31 (53.4)      | 15 (50.0)      | 16 (57.1)      | 6 (60.0)       | .73       |
| TT/TT               | 19 (32.8)      | 10 (33.3)      | 9 (32.1)       | 2 (20.0)       |           |
| ΔG dominant model   |                |                |                |                |          |
| ΔG/ΔG + ΔG/TT       | 39 (67.2)      | 20 (66.7)      | 19 (67.9)      | 8 (80.0)       | >.99 (.95) |
| TT/TT               | 19 (32.8)      | 10 (33.3)      | 9 (32.1)       | 2 (20.0)       | .69 (.50) |

Data reported as counts (% of all recipients).
\(^a\) Totals of allele model n = 116 / n = 60 / n = 56 / n = 20.
\(^b\) P-values were calculated using Fisher’s exact test: p1(OR1): group 0 vs group 1+2; p2(OR2): group 0 vs group 2.
\(^c\) P-values were calculated using Chi-square test: p1(OR1): group 0 vs group 1+2; p2(OR2): group 0 vs group 2.

generated in subjects carrying the IFNL4 rs368234815 ΔG allele, influences susceptibility to BKPyV.

The investigation of IFNL4 genetic variants and BKV in the living organ transplant setting, regarding both recipients and donors, is a strength of the study and of potential relevance as the allograft will harbor hematopoietic cells of the donor and the recipient, the latter migrating into the allograft early after transplantation. In this setting, myeloid cells that contribute to allograft recognition might be potent producers of IFN-λ4.\(^{14}\) Thus, it is likely that the recurrence of BKPyV is not only influenced by the recipient’s but also by the donor’s genetic characteristics. While we cannot provide a mechanistic link between IFN-λ4 and BKV, we do shed light on the local phenomena by including donor data.

A strength of this case-control-study is the homogeneity of the cohort in terms of ethnicity, age and gender. Furthermore, we have focused on living-donor organ transplantation, thus avoiding...
potentially bias created by the different transplantation procedure with longer ischemia times, a different inflammatory milieu and higher baseline risk for BKPyV in the post mortem setting. Additionally, the immunological risk profile of our BKPyV positive and BKPyV negative groups is comparable, as there were no significant differences in HLA mismatches, rejection treatment and history of CMV (Table 1).

One of the major limitations is the small sample size, especially in the BKPyVAN group, which limits the statistical power. Nevertheless, our groups are representative, since allele frequency of IFNL4 rs368234815 ΔG in our study (40.5% in recipients and 41% in donors) is similar to that reported in the European population of the 1000 Genome Project (31%). Furthermore, previous studies have reported that LD between IFNL4 rs368234815 ΔG (leading to IFN-λ4 expression), and the IFNL4 rs12979860 T allele is very high for Asians (r² = .99) and Europeans (r² = .98), which means that in these ethnic groups, the ΔG and T alleles are almost always inherited together. In contrast, LD is much weaker in Africans (r² = .83). Our findings with a LD of r² = .95 in this transplant cohort are therefore in accordance with these previous reports and we can deduce that the IFNL4 rs12979860 T allele does not correlate with the occurrence of BK viremia after transplantation.

The retrospective design of our study may also be considered a limitation; however, we were able to obtain accurate data owing to the fact that all the kidney transplanted patients were regularly followed up at our center and have a complete series of medical reports. Unfortunately, due to the unavailability of blood and urine samples from the time of diagnosis we were not able to determine the expression and concentration of IFNL4 and IFN-λ4 protein, which could therefore not be correlated with the respective polymorphisms.

Polymorphisms in IFNL4, including rs368234815 (ΔG/TT), were first described in association with spontaneous or treatment-induced clearance of HCV. Yet, the role of IFNL4 polymorphisms is still controversial for other viruses, including (HBV, HCV, HPV, CMV).

Intriguingly, the IFNL4 locus underwent a strong evolutionary selection against the IFN-λ4 protein-generating rs368234815 ΔG allele after the migration out of Africa 60 000 years ago. As a result 91.8% of Asians, 68.8% of Europeans and only 29.2% Africans express IFNL4 ΔG and T alleles are almost always inherited together. In contrast, LD is much weaker in Africans (r² = .83). Our findings with a LD of r² = .95 in this transplant cohort are therefore in accordance with these previous reports and we can deduce that the IFNL4 rs12979860 T allele does not correlate with the occurrence of BK viremia after transplantation.

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In conclusion, the presented data does not suggest an IFNL4 associated predisposition for BKPyV disease. Thus, there is an ongoing need to identify genetic risk factors, which will enable physicians to determine which patients are at risk of BKPyVAN and allow new preventive measures and patient-tailored immunosuppression.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Ursula Tanriver, Frederic Arnold, and Yakup Tanriver conceived the study and its design, had full access to the patients’ records, and take responsibility for the accuracy and integrity of the presented data. Ursula Tanriver screened the electronic patient records, organized the clinical data, performed DNA isolation and amplification, and curated the sequencing results. Jonas Florian Hummel supported DNA isolation and amplification. Florian Emmerich provided patients’ samples. Marcus Panning provided routine virological data. Frederic Arnold performed the statistical data analysis, LD analysis and generated the figures. Bernd Jänigen, Frederic Arnold, and Yakup Tanriver were involved in the clinical management of the patients. Ursula Tanriver, Frederic Arnold, and Yakup Tanriver drafted the manuscript. All authors critically revised the drafted manuscript and approved of the submission. FA and Yakup Tanriver equally contributed as shared senior authors.

DATA AVAILABILITY STATEMENT

The datasets analyzed in this study and further information on used reagents and techniques, beyond their description in the materials and methods section are available from the corresponding author on reasonable request.

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