MAJOR ARTICLE

Influence of *IFNL3/4* Polymorphisms on the Incidence of Cytomegalovirus Infection After Solid-Organ Transplantation

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Background. Polymorphisms in *IFNL3* and *IFNL4*, the genes encoding interferon λ3 and interferon λ4, respectively, have been associated with reduced hepatitis C virus clearance. We explored the role of such polymorphisms on the incidence of cytomegalovirus (CMV) infection in solid-organ transplant recipients.

Methods. White patients participating in the Swiss Transplant Cohort Study in 2008–2011 were included. A novel functional TT/-G polymorphism (rs368234815) in the CpG region upstream of *IFNL3* was investigated.

Results. A total of 840 solid-organ transplant recipients at risk for CMV infection were included, among whom 373 (44%) received antiviral prophylaxis. The 12-month cumulative incidence of CMV replication and disease were 0.44 and 0.08 cases, respectively. Patient homozygous for the minor rs368234815 allele (−G/−G) tended to have a higher cumulative incidence of CMV replication (subdistribution hazard ratio [SHR], 1.30 [95% confidence interval {CI}, .97–1.74]; $P = .07$), compared with other patients (TT/TT or TT/−G). The association was significant among patients followed by a preemptive approach (SHR, 1.46 [95% CI, 1.01–2.12]; $P = .047$), especially in patients receiving an organ from a seropositive donor (SHR, 1.92 [95% CI, 1.30–2.85]; $P = .001$), but not among those who received antiviral prophylaxis (SHR, 1.13 [95% CI, .70–1.83]; $P = .6$). These associations remained significant in multivariate competing risk regression models.

Conclusions. Polymorphisms in the *IFNL3/4* region influence susceptibility to CMV replication in solid-organ transplant recipients, particularly in patients not receiving antiviral prophylaxis.

Keywords. IL28B; innate immunity; antiviral immunity; cohort study; viral infection.

Cytomegalovirus (CMV) infection remains one of the most common infectious complications after solid-organ transplantation [1]. Several risk factors for the development of CMV infection have been described, with the donor (D) and recipient (R) serostatus at the time of transplantation being the main determinant for predicting the risk for subsequent CMV infection [2]. Additional risk factors include the type and dose of immunosuppressive drug used and previous occurrence of acute rejection [3]. Despite advances in the prevention of CMV replication after transplantation, a significant number of recipients may still develop CMV disease, even in the absence of the risk factors mentioned above [4].

While it is widely accepted that the adaptive immune response is essential in the control of CMV replication,

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particularly the specific $CD8^+$ T-cell response against CMV [5], the importance of innate immunity for CMV control has not completely been determined [6]. After transplantation, when cellular immunity is impaired because of the effect of immunosuppressive drugs, innate immunity may play a more prominent role in controlling viral replication. For example, some studies have identified that polymorphisms of genes involved in innate immunity, such as Toll-like receptor 2 (TLR-2) [7], TLR-4, and mannose binding lectin [8, 9], were associated with an increasing incidence of CMV infection or disease after transplantation.

Type I interferon (IFN) has been long considered to be critical for immune responses to viral infections. However, type III IFN, also called IFN-λ (IFNL), has recently been described to share many biological functions with type I IFN and also to have an important role in response to viral infections [10, 11]. In particular, genome-wide association studies revealed that polymorphisms in the *IFNL3/4* region exert a dramatic influence on the ability to clear hepatitis C virus (HCV), either spontaneously [12] or in response to antiviral therapy [12–15]. A novel TT/-G substitution (rs368234815) was recently identified as possibly the most robust or clinically relevant marker for predicting HCV clearance [16, 17].

The potential influence of IFNL polymorphisms on controlling viral infections other than that due to HCV has not been well characterized. In 2 recent studies involving solid-organ transplant recipients and hematopoietic stem-cell transplant recipients, the presence of a minor allele of IFNL3 polymorphisms was associated with reduced CMV replication after transplantation [18, 19]. This was in contrast with a study involving human immunodeficiency virus (HIV)-infected individuals at high risk fro CMV infection, in which patients harboring the minor allele of rs368234815 had a higher incidence of CMV retinitis [20]. We therefore explored the role of this novel *IFNL3/4* rs368234815 polymorphism on the incidence of CMV infection and disease in a unique nationwide prospective cohort of solid-organ transplant recipients, the Swiss Transplant Cohort Study (STCS).

METHODS

Study Population

The STCS is a multicenter nationwide cohort study including solid-organ transplantation performed in Switzerland from May 2008 onward [21]. The STCS comprises 6 transplantation centers in Switzerland. Kidney transplantation is performed in all centers, liver and heart transplantation in 3 centers, and lung and pancreas transplantation in 2 centers. Data on demographic parameters, transplant type, comorbidities, immunosuppressive treatment, antimicrobial drugs, rejection, and infectious and noninfectious events are collected at enrollment, 6 months after enrollment, and every 12 months after enrollment on standardized data forms by local physicians and data managers. Specific data on CMV infection available in the STCS database include the date of the first episode of CMV replication and of each episode of CMV disease, the use of antiviral drugs, and the type of CMV event, which is classified as asymptomatic replication, viral syndrome, and probable or proven end-organ disease.

For the present study, we included all white patients transplanted from May 2008 to March 2011 with at least one post transplant follow-up, a positive donor and/or recipient CMV serostatus, DNA available for genotyping and written informed consent for participation in the STCS. Patients who died within 24 hours of transplantation were excluded. The STCS protocol has been approved by the Ethics Committees of all participating centers.

Antiviral and Immunosuppressive Regimens

The antiviral preventive strategy per protocol varied among centers and type of transplant [2]. Most CMV-seronegative patients who received transplants from seropositive donors (hereafter, "D+/R− patients") received valganciclovir prophylaxis for 3–6 months, except in 2 transplantation programs, in which D+/R− patients were followed preemptively. CMV-seropositive patients who received transplants from CMV-seropositive or CMV-seronegative donors (hereafter, "R+ patients") were managed either by preemptive therapy or antiviral prophylaxis, according to the transplantation program, except for lung transplant recipients, all of whom received antiviral prophylaxis. In patients receiving antiviral prophylaxis, monitoring of CMV replication by polymerase chain reaction (PCR) was done after discontinuation of prophylaxis in 5 of 6 transplantation centers every 2–4 weeks for an additional 3-month period, irrespectively of the CMV serostatus. The protocol of the preemptive approach was decided by each center, but basically consisted of screening for CMV DNAemia by PCR every 1–2 weeks during the first month after transplantation and then every 2 weeks until 3–6 months after transplantation. Five centers used PCR in plasma, and 1 center used PCR in whole blood. All but one center used a homemade PCR; the remaining center used a commercial Abbott Real-Time CMV Assay. Antiviral therapy for asymptomatic patients with CMV replication was generally started at a cutoff of 2–3 $\log_{10}$ copies/mL of plasma or 3–4 $\log_{10}$ copies/mL of whole blood, but this cutoff varied according to the CMV serostatus, the time after transplantation, and whether the patient had received lymphocyte-depleting antibodies. Only results of the first PCR positive CMV DNAemia (and whether DNAemia was treated) were recorded in the STCS database. Immunosuppressive regimens also varied among centers and type of organ transplant.

Genotyping of the Gene Encoding Interleukin 28B (IL-28B)

The rs368234815 polymorphism was genotyped by Competitive Allele-Specific PCR (KASP) system (LGC Genomics, United Kingdom), using the ABI 7500 Fast real-time thermocycler,

according to manufacturer's protocols (available at: http://www.lgcgenomics.com/kaspchallenge). The KASP primers were designed by Kraken assay design and workflow management software (LGC Genomics). Automated allele calling was performed using SDS software (Applied Biosystems).

Clinical Definitions

Antiviral prophylaxis was defined as the initiation of ganciclovir or valganciclovir during the first 2 weeks after transplantation. Patients without such prophylactic treatment who were at risk for CMV disease (ie, D+/R− patients and R+ patients) were considered as being managed by the preemptive approach [2]. The definition of CMV infection followed international guidelines [22], in which active CMV infection was defined as laboratory confirmation of CMV replication irrespective of the presence symptoms, and CMV disease was defined as CMV replication with corresponding signs and symptoms.

Statistical Analysis

The main end point of the study was the incidence of CMV replication (thus including asymptomatic CMV infection and CMV disease). The cumulative incidence of CMV replication by genetic variables was calculated by using the stcompet program implemented in Stata (StataCorp, College Station, Texas). The risk of CMV replication and/or disease for each genetic and demographic variable was assessed by using a semiparametric regression model [23], also implemented in Stata (stcrreg). Death was considered a competing event. A stepwise multivariate regression model ($P < .1$) was used to determine the independent risk factors from the predicted variables. We analyzed the incidence of CMV replication in all solid-organ transplant recipients and in kidney transplant recipients.

RESULTS

Study Population

The characteristics of 840 patients meeting the inclusion criteria are shown in Table 1. Overall, 373 patients (44%) received antiviral prophylaxis, either with valganciclovir upfront or initially with intravenous ganciclovir. Most patients received induction therapy with either basiliximab (60%) or antithymocyte globulins (18%) and a maintenance immunosuppressive regimen including a calcineurin inhibitor, an antimetabolite, and corticosteroids. The 12-month cumulative incidence of CMV infection and disease was 0.44 and 0.08, respectively. The median time from transplantation to CMV replication was longer in patients who received prophylaxis than in patients followed by the preemptive approach (median, 167 days vs 40 days; $P < .0001$).

The baseline characteristics among kidney transplant recipients (n = 526) were similar to those of the whole study population (n = 840).

Impact of the rs368234815 Polymorphism on CMV Replication According to Antiviral Strategy

Overall, 102 solid-organ transplant recipients (12%) were homozygous for the minor allele of rs368234815 (−G/−G carriers). The cumulative incidence of CMV replication tended to be higher among −G/−G carriers (0.52), compared with other patients (TT/TT or TT/−G carriers, 0.43; subdistribution hazard ratio [SHR], 1.30 [95% confidence interval {CI}, .97–1.74]; $P = .07$; Figure 1 and Table 2). The association was significant when considering patients followed by the preemptive approach (SHR, 1.46 [95% CI, 1.01–2.12]; $P = .047$) but not when considering those who received antiviral prophylaxis (SHR, 1.13 [95% CI, .72–1.78]; $P = .6$). The former association was still significant in a multivariate model (SHR, 1.57 [95% CI, 1.10–2.23]; $P = .01$; Table 3), after adjustment for other risk factors associated with CMV replication in solid-organ transplant recipients not receiving prophylaxis, including recipient age, donor/recipient CMV serostatus, transplanted organ type, as well as induction and maintenance immunosuppressive regimen. In patients with asymptomatic CMV replication, the presence of the minor allele of *rs368234815* had no influence on the rate of patients who required antiviral therapy ($P = .5$).

Results were similar when kidney transplant recipients were analyzed separately. Among kidney recipients followed by a preemptive approach, −G/−G carriers had a higher cumulative incidence of CMV replication, compared with TT/TT and TT/−G carriers (SHR, 1.76 [95% CI, 1.10–2.84]; $P = .02$; Figure 1). This association was still observed in the multivariate analysis, in which −G/−G carriage was still associated with CMV replication (SHR, 1.78 [95% CI, 1.18–2.69]; $P = .006$; data not shown). However, no association between the rs368234815 polymorphism and CMV replication was detected among kidney transplant recipients receiving antiviral prophylaxis (SHR, 1.11 [95% CI, .65 −1.89]; $P = .7$; data not shown).

There was no association between the rs368234815 polymorphism and CMV disease in solid-organ transplant recipients (−G/−G vs TT/TT and TT/−G; $P = .4$) and in kidney transplant recipients ($P = 1.0$; Supplementary Figure 1).

Impact of the rs368234815 Polymorphism on CMV Replication, According to CMV Serostatus

We also analyzed the role of the rs368234815 separately, according to CMV serostatus. Among D+ solid-organ transplant and kidney transplant recipients who were followed by a preemptive approach, −G/−G carriers had a higher cumulative incidence of CMV replication, compared with the other patients (all transplant recipients: SHR, 1.92 [95% CI, 1.30–2.85; $P = .001$]; kidney transplant recipients: SHR, 2.28 [95% CI, 1.40–3.71; $P = .0009$]; Figure 2). These associations remained significant in the multivariate Cox regression models, after adjustment for relevant covariates (transplant recipients: SHR, 2.06 [95% CI, 1.40–3.01; $P < .0001$]; for kidney transplant

Table 1. Baseline Characteristics of Cytomegalovirus (CMV)–Seronegative Patients Who Received Transplants From Seropositive Donors (D+R−) and CMV-Seropositive Patients Who Received Transplants From CMV-Seropositive (D+R+) or CMV-Seronegative (D−R+) Donors

Variable	SOT Recipients (n = 840)[a]	Kidney Transplant Recipients (n = 526)
Age, y		
Recipient	54 (18)	54 (20)
Donor[b]	53 (22)	53 (20)
Recipient sex		
Male	534 (64)	336 (64)
Female	306 (36)	191 (36)
White ethnicity	840 (100)	526 (100)
Transplanted organ		
Kidney	493 (59)	493 (94)
Liver	163 (19)	. . .
Lung	78 (9)	. . .
Heart	56 (7)	. . .
Pancreas/islets/small bowel[c]	17 (2)	. . .
Combined[d]	33 (4)	33 (6)
Donor type		
Deceased	629 (75)	325 (62)
Living related and unrelated	211 (25)	201 (38)
HLA full mismatch[e]	294 (59)	209 (52)
CMV serostatus		
D+/R−	218 (26)	134 (26)
D−/R+	263 (31)	159 (30)
D+/R+	359 (43)	235 (44)
Induction therapy[f]		
Basiliximab	488 (60)	308 (62)
Antithymocyte globulin	143 (18)	88 (18)
None	179 (22)	103 (21)
Maintenance regimen[g]		
Tacrolimus	495 (65)	379 (77)
Cyclosporine	205 (27)	100 (20)
MMF/MPA	616 (81)	429 (87)
Azathioprine	22 (3)	16 (3)
mTOR inhibitors	52 (7)	11 (2)
Corticosteroids	517 (68)	366 (74)
CMV management approach		
Prophylaxis	373 (44)	248 (47)
Preemptive	467 (56)	278 (53)
Acute rejection episode (12-mo cumulative incidence)	259 (31)	134 (25)
CMV infection, cases, no. (12-mo cumulative incidence)	0.44	0.47
CMV disease, cases, no. (12-months cumulative incidence)	0.08	0.08
IFNL3/4 −G/−G	102 (12)	61 (12)

Data are median value (interquartile range) or number (%) of patients, unless otherwise indicated.

Abbreviations: CMV, Cytomegalovirus; *IFNL3/4*, interferon λ3 and interferon λ4; IQR, interquartile range; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; SOT, solid-organ transplant.

[a] Among 1119 white individuals enrolled in the Swiss Transplant Cohort Study genetic analysis, 279 were excluded because the CMV serostatus of the donor and recipient was negative (n = 253), results of CMV serologic analysis were missing or incomplete (n = 13), death occurred within 24 hours of transplantation (n = 7), or the *IFNL3/4* genotype was missing (n = 6).

[b] Donor age was missing for 163 solid organ transplant recipients and 0 kidney recipients.

[c] Including 12 islet, 4 pancreas, and 1 small bowel transplants.

[d] Including 19 kidney-pancreas, 6 kidney-liver, 4 kidney-kidney, 2 kidney-islets, 1 kidney-lung, and 1 kidney-kidney-pancreas transplants.

[e] Data were missing for 341 SOT recipients and 124 kidney transplant recipients.

[f] Data were missing for 30 SOT recipients and 27 kidney transplant recipients.

[g] Treatment at 12 months was assessable for 763 SOT recipients and 494 kidney transplant recipients.

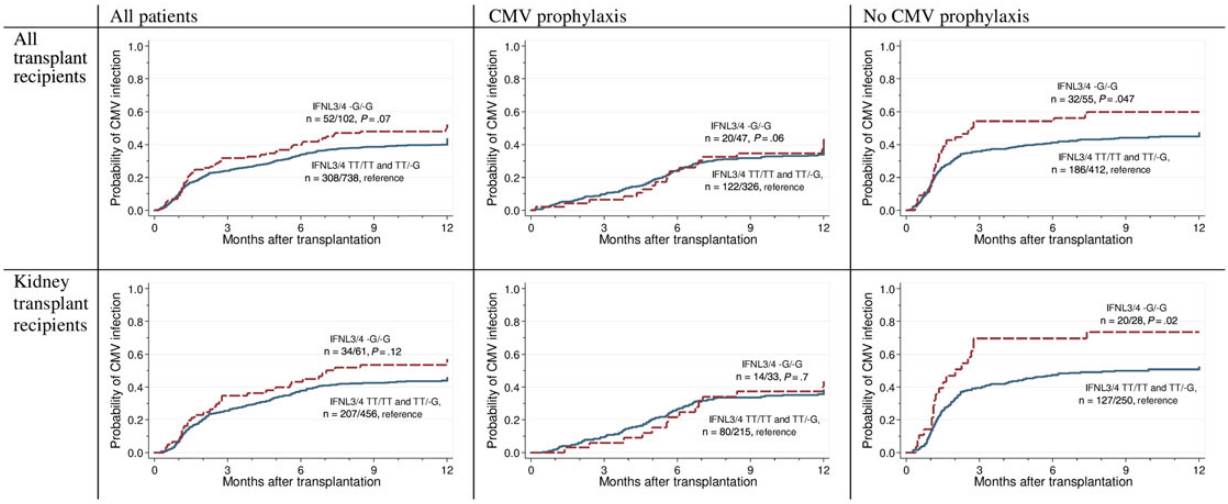

Figure 1. Cumulative incidence of cytomegalovirus (CMV) replication, according to prophylaxis and transplanted organ, in patients homozygous for the minor allele of rs368234815 (—G/—G carriers), compared with TT/TT or TT/—G carriers. A semiparametric regression model published by Fine and Gray [23] was used to evaluate the relative hazards associated with demographic factors or genetic variants and the end points. Proportions denote the number of patients with CMV infection/total number of patients in the group. Abbreviation: *IFNL3/4*, interferon λ3 and interferon λ4.

recipients: SHR, 2.24 [95% CI, 1.40–3.58; $P = .001$], respectively; Supplementary Table 1). Again, no significant associations were observed among patients receiving antiviral prophylaxis (transplant recipients: $P = .053$; kidney transplant recipients: $P = .2$; Figure 2).

DISCUSSION

In this nationwide cohort study of solid-organ transplant recipients, we assessed the potential influence of polymorphisms in the *IFNL3/4* region on the incidence of CMV replication. We found that the TT/-G rs368234815 substitution, which was recently identified as the best predictor of HCV clearance [16, 17], was an independent risk factor for developing CMV replication, specifically in the group of patients not receiving antiviral prophylaxis. This was also found for kidney transplant recipients in particular, a more homogeneous group of solid-organ transplant recipients in terms of immunosuppression and antiviral strategies. We did not observe any difference in the development of CMV disease between patients with and those without rs368234815, probably because of the overall low incidence of CMV disease in our cohort.

While extensive literature exists on the role of *IFNL3/4* polymorphisms in influencing spontaneous or treatment-induced clearance of HCV [12, 13, 15], there are few data assessing a potential association between such polymorphisms and CMV replication, particularly in the transplant recipients. In a recent study of 151 hematopoietic stem-cell transplant recipients, donor carriage of the minor TT genotype of *IFNL3* rs12979860 (a SNP in strong linkage disequilibrium with rs368234815) was associated with a shorter duration of CMV replication in the recipient [18]. However, no differences in the overall incidence of CMV infection were observed according to this SNP. In a study of 38 D+R− solid-organ transplant recipients, the minor G allele of rs8099917 (which is also in linkage disequilibrium with rs368234815) was associated with a lower risk of CMV replication after discontinuation of antiviral prophylaxis [19]. Finally, in concordance with our data, we found that the same rs368234815 polymorphism was significantly associated with the occurrence of CMV retinitis in a cohort of 1217 of HIV-infected individuals at risk (HR, 2.54 [95% CI, 1.20–5.40]; $P = .02$) [20]. Discrepancies between studies may be explained by several factors, including the use of different study populations and/or groups at risk and the differences in sample size.

The exact mechanisms by which rs368234815 may influence susceptibility to CMV replication among solid-organ transplant recipients receiving no antiviral prophylaxis are not well established. However, increasing evidence suggests that IFNLs contribute to antiviral responses against viruses other than HCV. The antiviral activity of IFNLs was highlighted in a series of cell culture models, including herpesvirus infection [24–27]. In an intestinal cell model of CMV infection, IFNL1 and IFNL3 were shown to activate STAT1, thereby inducing the production of antiviral proteins and inhibiting the expression of CMV [28]. The administration of recombinant IFNLs inhibited replication in mice models of herpes simplex virus infections [26], thereby confirming the role of these molecules in vivo. However, blockade of the IL-28 R1 subunit of the IL-28B receptor was recently reported to decrease CMV replication in foreskin fibroblast [19]. The rs368234815 polymorphism was associated with reduced HCV clearance in peripheral blood

Table 2. Univariate Analysis of Risk Factors for the Development of Cytomegalovirus (CMV) Replication in CMV-Seronegative Patients Who Received Transplants From Seropositive Donors (D+R−), CMV-Seropositive Patients Who Received Transplants From CMV-Seropositive (D+R+) or CMV-Seronegative (D−R+) Donors, Overall and for Patients Not Receiving Antiviral Prophylaxis

	All Patients (n = 840)		Patients Not Receiving Antiviral Prophylaxis (n = 467)	
Variable	SHR (95% CI)[a]	*P* Value	SHR (95% CI)[a]	*P* Value
Recipient age, per 10-y increase	1.12 (1.04–1.21)	.003	1.11 (1.01–1.22)	.02
Donor age, y	1.03 (.96–1.11)	.4	1.05 (.96–1.14)	.3
Recipient male sex	0.97 (.79–1.20)	.8	0.99 (.75–1.31)	1.0
Transplanted organ				
Kidney	Reference		Reference	
Liver	0.79 (.58–1.07)	.13	0.58 (.41–.82)	.002
Other/combined	0.83 (.64–1.07)	.15	0.85 (.58–1.24)	.4
HLA full mismatch	1.17 (.90–1.52)	.2	1.15 (.82–1.62)	.4
Rejection episode	1.05 (1.01–1.09)	.007	1.04 (.98–1.11)	.18
CMV serostatus				
D+/R−	Reference		Reference	
D−/R+	0.81 (.61–1.08)	.15	0.85 (.54–1.32)	.5
D+/R+	1.33 (1.04–1.69)	.02	1.53 (1.03–2.28)	.04
Induction therapy				
None	Reference		Reference	
Basiliximab	1.03 (.78–1.35)	.9	1.15 (.83–1.58)	.4
Antithymocyte globulin	1.19 (.86–1.65)	.3	1.99 (1.29–3.08)	.002
Maintenance regimen				
Tacrolimus	0.97 (.94–1.01)	.13	0.94 (.87–1.00)	.06
Cyclosporine	1.05 (1.01–1.08)	.01	1.09 (1.01–1.17)	.02
MMF/MPA	1.08 (1.02–1.14)	.009	1.10 (.99–1.22)	.08
mTOR inhibitors	0.99 (.90–1.09)	.8	0.97 (.83–1.14)	.7
Corticosteroids	1.12 (1.04–1.21)	.003	1.33 (1.01–1.76)	.04
Exposure to antiviral drug[b]	0.90 (.81–0.99)	.02	0.96 (.78–1.18)	.7
IFNL3/4 -G/-G[c]	1.30 (.97–1.74)	.07	1.46 (1.01–2.12)	.047

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; *IFNL3/4*, interferon λ3 and interferon λ4; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin.

[a] The subdistribution hazard ratio (SHR) was calculated using the semiparametric regression model published by Fine and Gray [23].

[b] Risk of CMV infection during concurrent exposure to valganciclovir or ganciclovir.

[c] Genetic association with *rs368234815* is for recessive mode of inheritance (patients homozygous for the rare alleles [−G/−G] are compared to the other patients [TT/TT and TT/−G]).

mononuclear cells (PBMCs) stimulated with poly(I:C) [17]. It was also associated with the expression of a novel IFNL analogue, IFNL4, that exerts antiviral activities similar to those of other type I and type III IFNs in vitro [16, 29]. Therefore, increased CMV events among solid-organ transplant recipient carrying rs368234815 may be due in part to insufficient IFNL3 expression or to the expression of IFNL4 itself. Yet the reason why the expression of IFNL4 would be associated with increased rather than decreased viral replication in vivo remains to be elucidated [30]. In a mouse model of lymphocytic choriomeningitis virus (LCMV), differences in the IFN-stimulated gene background were observed in acute versus latent infection, determining the control of LCMV replication [31]. Further investigations are needed to understand the exact role of IFNL3 and IFNL4 in antiviral immune responses.

An important finding of our study is that the influence of *IFNL3/4* polymorphisms on CMV replication disappeared when considering patients receiving CMV prophylaxis. A plausible hypothesis is that, in patients managed by the preemptive approach, CMV replication develops earlier and more frequently after transplantation than in patients receiving antiviral prophylaxis. As cell-mediated immunity is impaired after transplantation, the role of innate immunity might be more evident when viral replication occurs early and more often after transplant receipt. This concords with previous studies evaluating the role of natural killer immunoglobulin-like receptor polymorphisms, in which the influence of such polymorphisms in determining CMV replication was significant also during the first 3 months after transplantation but decreased over time [32]. We also found that donor CMV serostatus was more

Table 3. Multivariate Analysis of Risk Factors for the Development of (CMV) Replication in CMV-Seronegative Patients Who Received Transplants From Seropositive Donors (D+R−), CMV-Seropositive Patients Who Received Transplants From CMV-Seropositive (D+R+) or CMV-Seronegative (D−R+) Donors, Overall and for Patients Not Receiving Antiviral Prophylaxis

	All Patients (n = 840)		Patients Not Receiving Antiviral Prophylaxis (n = 455)[a]	
Factor	SHR (95% CI)[b]	*P* Value	SHR (95% CI)[b]	*P* Value
Recipient age, per 10-y increase	1.14 (1.06–1.23)	.001	1.12 (1.02–1.24)	.02
Transplanted organ				
Kidney	. . .		Reference	
Liver	. . .		0.66 (.45–.98)	.04
Other/combined	. . .		0.66 (.42–1.04)	.07
CMV serostatus				
D+/R−	Reference		Reference	
D−/R+	0.75 (.56–1.01)	.06	0.91 (.55–1.49)	.7
D+/R+	1.29 (.01–1.70)	.04	1.60 (1.02–2.60)	.04
Induction therapy				
None	. . .		Reference	
Basiliximab	. . .		1.14 (.82–1.58)	.5
Antithymocyte globulin	. . .		2.08 (1.21–3.57)	.008
Acute rejection	1.05 (1.02–1.09)	.005	. . .	
Maintenance therapy				
Corticosteroids	1.13 (1.04–1.22)	.003	1.31 (1.01–1.70)	.04
Tacrolimus			0.91 (.84–.99)	.02
MMF/MPA	1.07 (1.01–1.13)	.02	. . .	
Cyclosporine	1.08 (1.04–1.12)	<.0001	. . .	
Exposure to antiviral drug[c]	0.85 (.77–.93)	.001	. . .	
IFNL3/4 -G/-G[d]	1.32 (.99–1.75)	.06	1.57 (1.10–2.23)	.01

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; *IFNL3/4*, interferon λ3 and interferon λ4; IQR, interquartile range; MMF, mycophenolate mofetil; MPA, mycophenolic acid.

[a] The number of patients in the multivariate analyses (n = 455) is slightly lower than the number of patients included in the univariate analysis (n = 467) because of missing data on induction therapy for 12 patients.

[b] The subdistribution hazard ratio (SHR) was calculated using the semiparametric regression model published by Fine and Gray [23]. Covariates with a *P* value of < .1 were kept in the multivariate analyses.

[c] Risk of CMV infection during concurrent exposure to valganciclovir or ganciclovir.

[d] Genetic association with rs368234815 is for recessive mode of inheritance (patients homozygous for the rare alleles [−G/−G] are compared to the other patients [TT/TT and TT/−G]).

important than recipient CMV serostatus in determining the influence of the IFNL variants. Actually, a wide majority of patients harboring the rs368234815 polymorphism who received an organ from a seropositive donor developed CMV replication independently, whether they were R− or R+. These data suggest that new CMV strains transmitted by the donor [33] may be more difficult to control in recipients harboring the rs368234815 polymorphism.

Our study have some limitations. First, because data from only the first episode of asymptomatic CMV replication were recorded in the STCS database, we were not able to investigate, in the present study, whether the *IFNL3/4* polymorphisms had an impact on the overall duration of CMV infection and, particularly, on the response to antiviral therapy. Second, patients who received antiviral therapy were not monitored for CMV replication by use of the same schedule as that for patients managed by the preemptive approach just after transplantation; this difference could partially account for the different impact of the *IFNL3/4* polymorphisms on CMV replication observed according to the preventive strategy used. Third, the low number of CMV disease events prevented us from drawing any conclusion about a potentially higher risk of progression from asymptomatic viral replication to overt CMV disease in patients with −G/−G carriage. Fourth, the association between CMV replication and the IFNL3 polymorphism was observed for the recessive mode of inheritance, while the association observed among HCV-infected patients is usually dominant. This difference may be due to some threshold effect of the amount of *IFNL3/4* in response to specific pathogens. The recessive model was chosen in a post hoc analysis, which could also be a limitation of our study. Finally, the prevention strategies and immunosuppressive regimens were somewhat variable

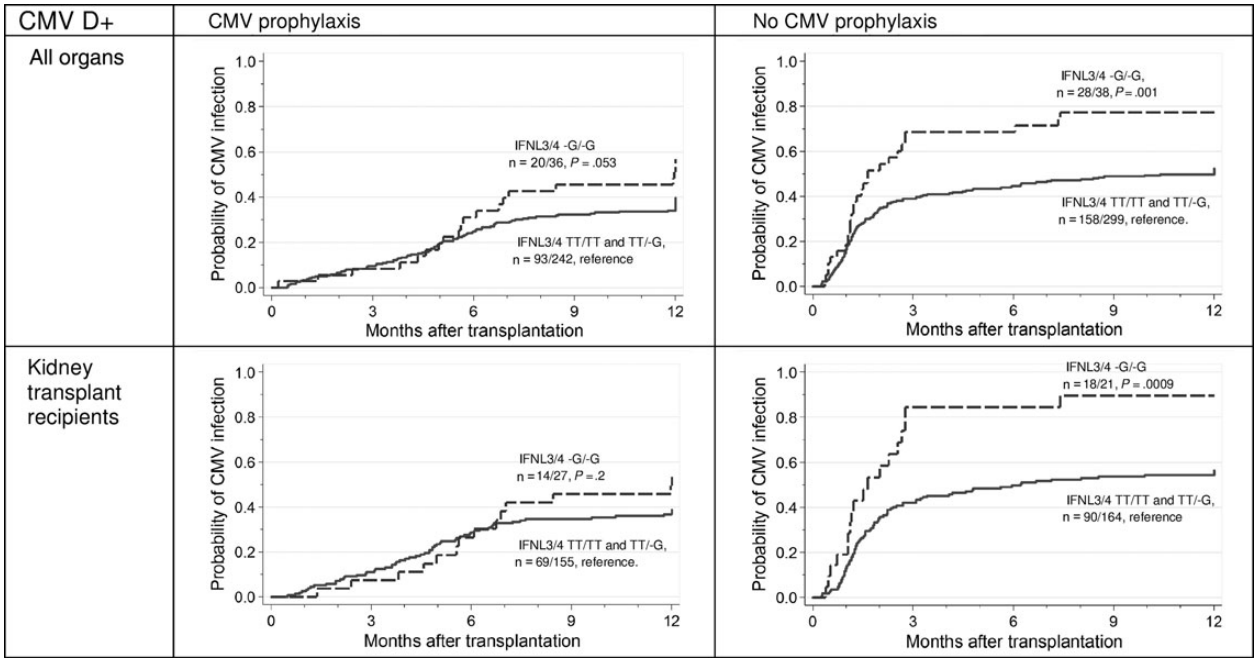

Figure 2. Cumulative incidence of cytomegalovirus (CMV) replication, according to antiviral preventive strategy, in patients who received solid-organ transplants and kidney transplants from CMV-seropositive donors (D+) and were homozygous for the minor allele of rs368234815 (−G/−G carriers), compared with TT/TT or TT/-G carriers. A semiparametric regression model published by Fine and Gray [23] was used to evaluate the relative hazards associated with the demographic factors or genetic variants and the end points. Proportions denote the number of patients with CMV infection/total number of patients in the group. Abbreviation: *IFNL3/4*, interferon λ3 and interferon λ4.

among transplantation programs, so it is possible that some remaining biases specifically related to the transplantation center were not corrected by the multivariate analysis. Nevertheless, because of the large number of patients included, the strict and homogeneous definitions used for CMV infection, and the use of a novel polymorphism with predicted functional activity, the data indicate novel evidence of a relationship between IFNL polymorphisms and CMV infection.

In conclusion, in this large cohort of solid-organ transplant recipients, we found that CMV infection in patients not receiving antiviral prophylaxis was influenced by IFNL genetic variants. This effect was stronger in recipients who received an allograft from a CMV-seropositive donor. These results indicate that the INFL rs368234815 polymorphism might be considered a novel risk factor for developing CMV-associated complications after organ transplantation. Validation of this association in further clinical studies has the potential to improve risk stratification for CMV reactivation and eventually influence future prevention strategies and guidelines, particularly in patients followed up by the preemptive approach.

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Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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