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Interleukin-1 alpha genotype and outcome of unrelated donor haematopoietic stem cell transplantation for chronic myeloid leukaemia

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Summary

Interleukin-1 alpha (IL-1α) is a pro-inflammatory cytokine that is implicated in the initiation/maintenance of graft-versus-host disease (GVHD) and the immune response to infection. A cytosine (C) to thymine (T) transition at position −889 is believed to influence gene transcription. A previous single institution study showed that the presence of at least one *IL1A* T allele in the donor was associated with improved survival after unrelated donor haematopoietic stem cell transplant and lower transplant-related mortality if the donor and recipient each possessed the *IL1A* T allele. The present study sought to confirm these results in a larger homogeneous population. Thus the study population included 426 patients older than 18 years with chronic myeloid leukaemia (CML), transplanted in first chronic phase and receiving a total body irradiation and cyclophosphamide preparative regimen. Donor recipient pairs were categorised into four groups according to the presence or absence of an *IL1A* T allele in the donor and recipient. There were no significant differences in patient, donor and transplant characteristics between the groups. We did not observe an association with IL-1α genotype in donor and/or recipient and transplant-outcome. These data suggest that the outcome of unrelated donor transplant for CML is not influenced by IL-1α genotype.

Keywords

interleukin-1; polymorphisms; chronic myeloid leukaemia; unrelated donor; haematopoietic stem cell transplantation

Allogeneic haematopoietic stem cell transplantation (HSCT) offers a potential cure for a variety of malignant and non-malignant conditions. However, regimen-related toxicity,

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graft-versus-host disease (GVHD), and opportunistic infections (Kernan *et al*, 1993) still remain the major cause of morbidity and mortality after unrelated donor (URD) allogeneic HSCT. In addition to identifying patients and transplant-related prognostic factors, identifying favourable factors in the donor may aid in donor selection and may improve outcomes after HSCT.

Non-human leucocyte antigen (HLA) genetic polymorphism has been shown to influence risk of GVHD and HSCT mortality. Middleton *et al* (1998) showed that the homozygous d3 genotype of the tumour necrosis factor (TNF)-α microsatellite, and the presence of *IL10* alleles with greater numbers of dinucleotide repeats were preferentially associated with grade III/IV GVHD in sibling transplant recipients. In studies of 993 recipients of matched sibling donor transplant, Lin *et al* (2003, 2005 showed that interleukin 10 (IL-10) genotype of the recipient and IL-10 receptor genotype of the donor modified the risk of GVHD. Associations between IL-6 and interferon-gamma genotype and susceptibility to GVHD have also been shown in sibling donor HSCT recipients (Cavet *et al*, 2001; Socie *et al*, 2001). Data addressing this issue in the unrelated donor HSCT setting are scarce, however Keen *et al* (2004) have reported association of TNF-α and IL-10 genotypes with toxicity after unrelated donor transplant. Taken together, these data suggest that non-HLA genetic variation influences risk of GVHD and HSCT mortality, and that it might be possible to predict HSCT outcome from a profile of donor or recipient risk factors, including cytokine polymorphisms.

Interleukin-1 is a pro-inflammatory cytokine implicated in the initiation and maintenance of GVHD and the immune response to infection (Ferrara & Deeg, 1991). The IL-1 gene family includes three members [*IL1A* (IL-1α), *IL1B* (IL-1β) and *IL1N* alias *IL1RA* (IL-1 receptor agonist; IL-1RA)] that mediate immune and inflammatory responses through two specific cell surface receptors. IL-1α and IL-1β are agonists and IL-1RA is a competitive receptor antagonist. A cytosine (C) to thymine (T) transition at position −889 in the *IL1A* promoter is believed to influence gene transcription. The T/T genotype creates the consensus site for a novel transcription factor (Skn-1) and is associated with a significant increase in promoter activity compared with the C/C genotype.

Three previous studies have examined IL-1 polymorphism and outcome of sibling donor bone marrow transplantation. Cullup *et al* (2001) studied *IL1B* and *IL1N* polymorphism and showed modest evidence for an association between donor IL-1RA genotype and incidence of acute GVHD. In a larger study, Lin *et al* (2000) reported little association between donor or recipient IL-1β and IL-1RA genotypes and frequency of GVHD. Cullup *et al* (2003) have also reported the association of *IL1A* −889 polymorphism in the donor genotype with the occurrence of chronic GVHD. Neither of these studies investigated *IL1A* polymorphism or examined URD HSCT recipients.

A previous single institution report of 90 recipients of unrelated donor HCT showed improved survival at 1 year if at least one *IL1A* T allele was present in the donor. Lower TRM was seen if both donor and recipient possessed the *IL1A* T allele (MacMillan *et al*, 2003). While these data showed a striking reduction in TRM associated with IL-1 genotype, numbers were small and these studies were performed in a heterogeneous patient population. In this study, we sought to replicate these findings in a larger and more uniform population.

Methods

Inclusion criteria

The study population included 426 recipients of unrelated donor HSCT, aged 18–60 years, transplanted in first chronic phase of chronic myeloid leukaemia (CML), between 1990 and

2002 in the US, using donors facilitated by the National Marrow Donor Program (NMDP). Patients had to have received a total body irradiation-containing conditioning regimen. Excluded were recipients of peripheral blood stem cells, disease status other than first chronic phase CML, and recipients of second transplant.

IL-1 genotyping of donor and recipient

Donor and recipient DNA were obtained from the NMDP repository and normalised to 10 ng/μl. The Institutional Review Board of the NMDP approved release of donor and recipient samples from the repository for genotyping of *IL1A* polymorphism and subsequent correlation with transplant-outcome. Donor-recipient pairs were genotyped for the *IL1A* polymorphism using a high throughput polymerase chain reaction (PCR) assay (5′nuclease allelic discrimination assay-TaqMan; Applied Biosystems, Foster City, CA, USA). We use gene-specific PCR primers (forward primer – 5′-ACT AGG CTG GCC ACA GGA ATT AT-3′; reverse primer – 5′-CCA GAA GCC AGT GGC TAA GTT T-3′) and fluorogenic probes [VIC (wild type)-CTT CAA TGG TGT TGC; FAM (variant)-CCT TCA ATG ATG TTG] for allelic discrimination. PCR cycling reactions were performed in 96-well microtitre plates in a GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT, USA). For each 25 μl reaction, a 10 ng DNA template was added to the reaction mixture containing wild-type VIC and variant FAM probe, PCR Mastermix (Applied Biosystems) along with forward and reverse primer (final concentration of each primer 7·5 μmol/l). Thermocycling was performed with initial 50°C incubation for 2 min followed by a 10-min incubation at 95°C. A two-step cycling reaction was performed for 40 cycles with denaturation at 95°C for 15 s, and annealing and extension at 60°C for 1 min. Results were analysed by the automated TaqMan allelic discrimination assay using sequence detection system software 2.1 (ABI TaqMan 7700; Applied Biosystems). Genotyping results were duplicated in 10% of samples; concordance between repeats was 100%. Further-more, 10% of the samples were also genotyped using direct sequencing; concordance with TaqMan genotyping was 100%.

Donor and recipient pairs were grouped into four mutually exclusive categories for analyses of genotypes (Hurme & Santtila, 1998). Similar to MacMillan *et al* (2003), the four groups were based on the presence or absence of a T-allele in donor and/or in recipient (only recipient has T-allele, only donor has T-allele, both donor and recipient have T-allele and neither donor or recipient have a T-allele).

Definition of clinical endpoints

Diagnosis of acute and chronic GVHD was based on local institutional criteria with overall grade of acute GVHD assigned retrospectively by the NMDP based on stage of involvement reported for each individual organ (Przepiorka *et al*, 1995). Transplant-related mortality was defined as death during a continuous remission. Relapse was defined as haematological leukaemia recurrence.

Statistical analysis

Baseline variables were compared between the four donor-recipient IL-1 genotype groups as described earlier using the chi-square statistic for categorical variables and the Kruskal–Wallis test for continuous variables. The probability of overall survival was calculated using the Kaplan–Meier estimator (Klein & Moeschberger, 2003). For overall survival, death from any cause was considered an event, and patients surviving at last follow-up were censored. Probabilities of acute and chronic GVHD, transplant-related mortality and relapse were calculated using the cumulative incidence function estimator (Klein & Moeschberger, 2003). For GVHD, death without the event was the competing risk; for transplant-related mortality, relapse was the competing event; and, for relapse, transplant-related mortality was the competing event.

Multivariate analysis was performed using the Cox proportional hazards regression model (Cox, 1972). All multivariate models were constructed using stepwise forward selection, with a *P*-value ≤ 0·05 considered significant. All variables met the proportional hazards assumption. The variable for donor-recipient IL-1 genotype was retained in all steps of model building. Other variables considered were recipient and donor age, sex of the recipient and donor, cytomegalovirus (CMV) serostatus of the recipient and donor, time from diagnosis to transplantation, recipient performance score at transplantation, GVHD prophylaxis, year of transplant and donor- recipient HLA compatibility. Disease status and conditioning regimen were not tested as all patients were in first chronic phase and received a total body irradiation based regimen. All reported *P*-values are two-sided. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results

Patient and transplant characteristics

Patient, donor and transplant characteristics are shown in Table I. The median age at transplantation was 39 years (range 18–59 years) and 57% of patients were male. The median age of donors was 38 years (range 18–57 years). Sixty-four per cent of patients received grafts from donors matched at HLA-A, -B and -DRB1 (allele-level typing), 22% were mismatched at ≥1-loci (allele-level typing) but matched by antigen-level typing and 14% were mismatched at 1-locus by antigen-level typing. There was no difference in the genotype frequency between recipients and donors (c/c: 48% vs. 46%, c/t: 44% vs. 45%, t/t: 8% vs. 9%) and were consistent with those predicted under the conditions of the Hardy–Weinberg equilibrium. The median time from diagnosis to transplantation was 13 months. All patients received total body irradiation (myeloablative dose) and cyclophosphamide for conditioning. The median follow-up of surviving patients was over 7 years.

Graft-versus-host disease

Rates of grade 2–4 and grade 3–4 acute GVHD did not differ by donor-recipient IL-1 genotype (Table II). Similarly, rates of chronic GVHD did not differ by donor-recipient IL-1 genotype (Table II). The probabilities of grade 2–4 acute GVHD at day-100 were 62%, 60%, 56% and 55% when T-allele was present in only the recipient, present in only the donor, present in both the donor and recipient or absent in both donor and recipient, respectively. Corresponding probabilities of chronic GVHD at 5-years were 52%, 51%, 54% and 54%.

Transplant-related mortality

Transplant-related mortality rates did not differ by donor-recipient IL-1 genotype (Table II, Fig 1). In all groups, transplant-related mortality was higher in recipients of mismatched transplants [≥1-allele mismatch: relative risk (RR) 1.99, $P < 0{\cdot}001$; 1-antigen mismatch: RR 2·05, $P < 0{\cdot}001$] compared with matched transplants.

Relapse

Relapse rates did not differ by donor-recipient IL-1 genotype (Table II). The probabilities of relapse at 5-years were 7%, 5%, 5% and 4% when T-allele was present in only the recipient, present in only the donor, present in both the donor and recipient or absent in both donor and recipient respectively. As expected, relapse rates were higher in recipients of T-cell depleted bone marrow grafts regardless of donor-recipient IL-1 genotype (RR 4·10, $P < 0{\cdot}001$). Of the 23 patients with haematological relapse, three received donor leucocyte infu- sion (DLI) and three underwent a second transplant. Two patients who received DLI and one of the patients receiving a second transplant had received T-cell depleted bone marrow grafts.

Overall mortality

Overall mortality rates did not differ by donor-recipient IL-1 genotype (Table II, Fig 2). Mortality rates were higher after mismatched transplants (≥1-allele mismatch: RR 1·92, $P < 0·001$; 1-antigen mismatch: RR 1·94, $P < 0·001$) and in recipients of T-cell depleted grafts (RR 1·43, $P = 0·017$) regardless of donor-recipient IL-1 genotype. Frequent causes of death included infection (23%), GVHD (19%) and interstitial pneumonitis (17%). Others include: recurrent leukaemia (4%), graft failure (5%), haemorrhage (8%), organ failure (11%) and other causes (13%). Causes of death did not differ by donor-recipient IL-1 genotype.

Discussion

A number of studies have investigated the role of cytokine gene polymorphisms in susceptibility to post-HSCT complications, such as GVHD and transplant-related mortality. For example, possible roles for polymorphisms in TNFα, IL-10 and members of the IL-1 family in outcome of sibling donor HSCT have been addressed (Middleton *et al*, 1998; Cavet *et al*, 1999; Lin *et al*, 2000; Cullup *et al*, 2001, [2003],).

The objective of the current study was to examine the association between the presence of the *IL1A* T allele and outcomes after URD transplantation. Previously MacMillan *et al* (2003) reported lower transplant-related mortality and improved overall survival after URD transplantation when an *IL1A* T allele was present in both the recipient and the donor. We sought to confirm these findings in a larger and relatively homogenous cohort by limiting the study population to patients with CML in first chronic phase who received a uniform conditioning regimen, and by focussing on the *IL1A* −889 polymorphism. Our findings differed from that reported by MacMillan *et al* (2003). We did not observe a significant association between the presence of an *IL1A* T allele in the recipient and/or donor and transplant-related mortality, GVHD or overall survival after URD transplant for CML in first chronic phase after adjusting for significant prognostic factors. We speculate that the differing results of these two studies reflected the relatively small (97 cases) and heterogeneous population in the first study. A limitation of this analysis is the heterogeneity of HLA matching. Although the number of cases with HLA mismatch did not differ by IL-1 categories, it remains possible that undetected HLA disparity outside of HLA-A, -B and -DRB1 could have confounded the ability to measure independent effects of *IL1A* polymorphism. These findings illustrate the need for replication of association studies in an independent dataset before clinical application.

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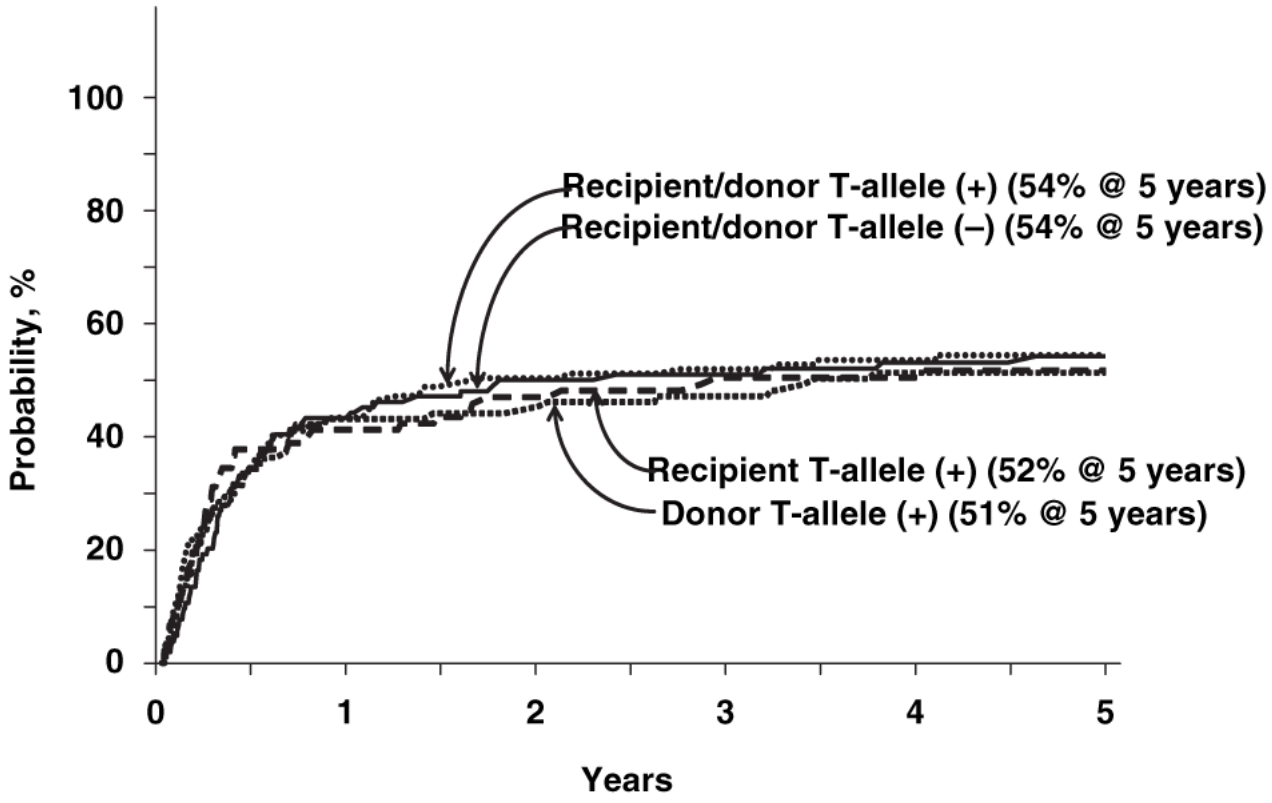

Fig 1.
Probability of transplant-related mortality by donor and recipient IL-1 genotype.

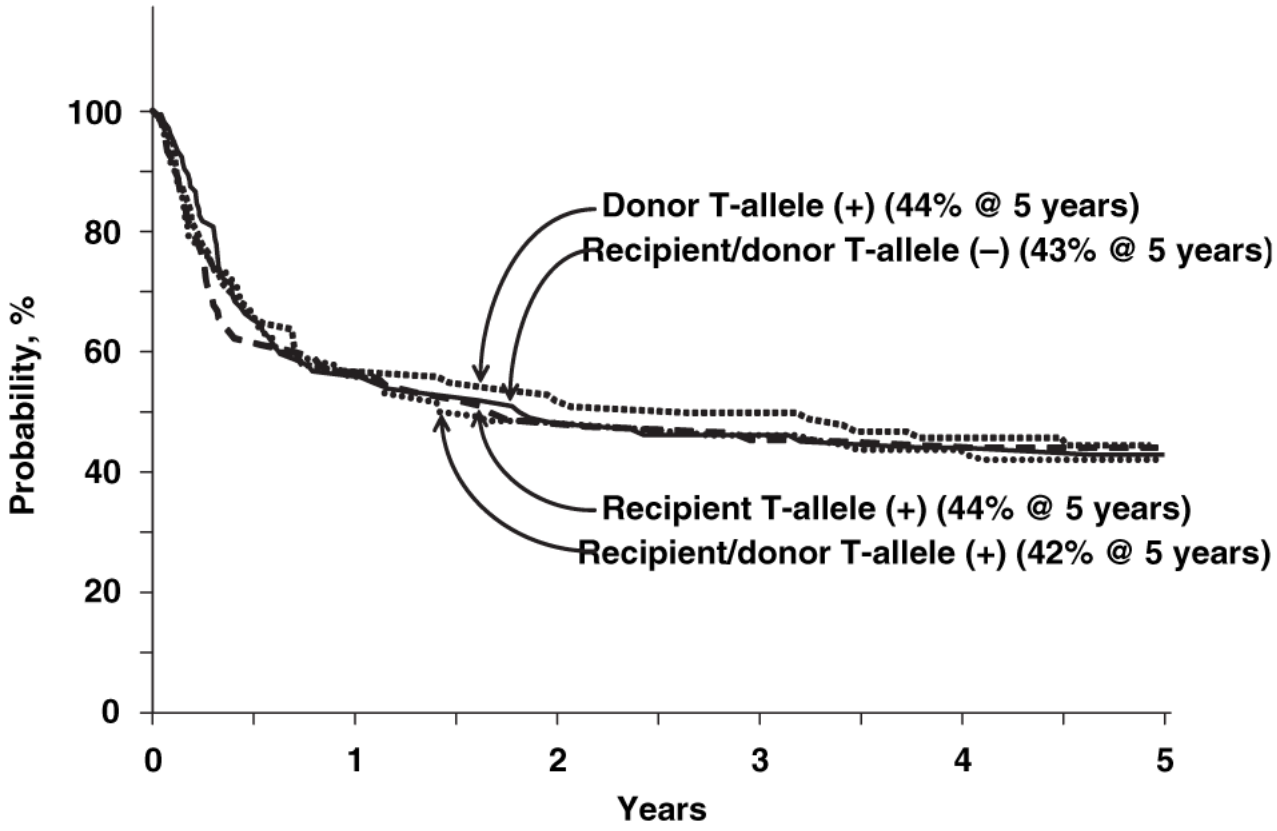

Fig 2.
Probability of overall survival by donor and recipient IL-1 genotype.

Table I

Patient, donor and transplant characteristics by recipient and donor IL-1 genotype

Variable	IL-1 genotype of the donor and recipient			
	T-allele present in the recipient	T-allele present in the donor	T-allele present in recipient and donor	T-allele absent in recipient and donor
Number of patients	90	102	130	104
Male	48 (53)	60 (59)	76 (58)	60 (58)
Performance score, ≥90	77 (86)	87 (85)	117 (90)	90 (87)
Year of transplant				
1990–1994	34 (38)	42 (41)	38 (29)	32 (31)
1995–1999	45 (50)	46 (45)	79 (61)	55 (53)
2000–2002	11 (12)	14 (14)	13 (10)	17 (16)
GVHD prophylaxis				
T-cell depletion	21 (23)	19 (19)	23 (18)	19 (18)
Ciclosporin-based	59 (65)	70 (69)	94 (72)	71 (70)
Tacrolimus-based	10 (11)	13 (13)	13 (10)	14 (13)
D-R CMV serostatus				
D (−)/R (−)	33 (37)	37 (36)	44 (34)	27 (26)
D (+)/R (+)	19 (21)	18 (18)	25 (19)	19 (18)
D (+)/R (−)	14 (16)	16 (16)	17 (13)	24 (23)
D (−)/R (+)	24 (27)	26 (25)	43 (33)	32 (31)
Unknown		5 (5)	1 (1)	2 (2)
D-R HLA disparity				
Allele-matched	63 (70)	60 (59)	82 (63)	68 (65)
Allele-mismatch	15 (17)	26 (25)	30 (23)	22 (21)
Antigen-mismatch	12 (13)	16 (16)	18 (14)	14 (13)
Median follow-up of survivors (months)	90 (12–144)	98 (9–170)	84 (22–168)	91 (31–168)

The numbers in brackets denote percentages.

Patient, donor and transplant characteristics were similar among the four cohorts using the chi square test for categorical values and the Kruskal–Wallis test for continuous variables.

D, donor; R, recipient; M, male; F, female; CMV, cytomegalovirus.

Table II

Results of multivariate analysis of transplant outcome by donor and recipient IL-1 genotype

Outcome	N1/N2	Relative risk (95% confidence interval)	*P*-value
Grade 2–4 acute GVHD			
T-allele absent in recipient and donor	56/94	1·00	0·682*
T-allele present in the recipient	50/79	1·19 (0·81–1·75)	0·369
T-allele present in the donor	50/83	1·01 (0·69–1·49)	0·947
T-allele present in recipient and donor	63/112	0·95 (0·66–1·36)	0·790
Grade 3–4 acute GVHD			
T-allele absent in recipient and donor	28/94	1·00	0·095*
T-allele present in the recipient	33/79	1·52 (0·92–2·53)	0·104
T-allele present in the donor	20/83	0·78 (0·44–1·39)	0·397
T-allele present in recipient and donor	33/112	0·98 (0·59–1·61)	0·923
Chronic GVHD			
T-allele absent in recipient and donor	53/101	1·00	0·739*
T-allele present in the recipient	43/85	1·01 (0·67–1·52)	0·958
T-allele present in the donor	53/94	1·22 (0·83–1·79)	0·313
T-allele present in recipient and donor	64/122	1·10 (0·76–1·59)	0·611
Transplant-related mortality			
T-allele absent in recipient and donor	58/104	1·00	0·504*
T-allele present in the recipient	49/90	1·14 (0·78–1·67)	0·508
T-allele present in the donor	53/102	0·90 (0·62–1·31)	0·592
T-allele present in recipient and donor	75/130	1·16 (0·82–1·64)	0·397
Relapse			
T-allele absent in recipient and donor	5/104	1·00	0·942*
T-allele present in the recipient	6/90	1·41 (0·43–4·64)	0·567
T-allele present in the donor	5/102	1·06 (0·31–3·68)	0·923
T-allele present in recipient and donor	7/130	1·21 (0·38–3·82)	0·742
Overall mortality			
T-allele absent in recipient and donor	61/104	1·00	0·528*

Outcome	N1/N2	Relative risk (95% confidence interval)	*P*-value
T-allele present in the recipient	54/90	1·16 (0·80–1·68)	0·427
T-allele present in the donor	57/102	0·93 (0·65–1·33)	0·691
T-allele present in recipient and donor	81/130	1·16 (0·83–1·62)	0·385

N1, number of events; N2, number evaluable.

* 3-degree freedom test.