Influence of interleukin-18 polymorphisms on kidney transplantation outcomes: a meta-analysis

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Abstract

Objective: Kidney transplantation (KT) procedures are confronted with adverse outcomes that include allograft failure. Allograft survival are in large part attributed to genetics, which render the recipient susceptible or protected from allograft rejection. The genetics of KT outcomes point to single nucleotide polymorphisms (SNPs) where studies have reported the role of cytokines in allograft survival, one of which is *interleukin-18* (*IL-18*). Reported associations of *IL-18* with KT outcomes have been inconsistent. This prompted a meta-analysis to obtain more precise estimates.

Methods: From four included articles, we posed two hypotheses about *IL-18* SNPs: (1) they are either high in patients (hp) /controls (hc) based on genotype distribution (GD) and (2) they either increase or decrease the risks of allograft rejection. To this end, we compared the *IL-18* genotypes to estimate odds ratios [ORs] and 95% confidence intervals using standard genetic models (homozygous, recessive, dominant and codominant). Subgrouping was ethnicity-based. Heterogeneous (random-effects) associations were subjected to outlier treatment which split the outcomes as pre- (PRO) and post- (PSO) outlier. Stability and robustness of the outcomes were analyzed by Bonferroni-correction and sensitivity treatment, respectively.

Results: Our results revealed two core outcomes based on significance ($P^a < 0.05$): (1) genotype frequency was hp than hc (OR 1.34, $P^a = 0.0007$) in the codominant model (PSO) based on stability and robustness and (2) protection from allograft rejection (OR 0.74, $P^a = 0.04$) in the dominant model (PRO) based on homogeneity. Subgroup analysis showed that Caucasian and Asian outcomes validated the GD and allograft outcomes, respectively.

Conclusions: The *IL-18* SNPs showed associations (hp) with KT up to 1.3-fold and protected KT recipients from allograft rejection (26%). Subgroup outcomes delineated the Asian and Caucasian effects. Enabled by outlier treatment, these findings were supported by non-heterogeneity. More studies should confirm or counter our findings.

Background

The end-stage of renal failure resulting from kidney disease [1] points to kidney transplantation (KT) as the optimal therapeutic choice [1, 2]. The transplanted material (allograft) in the recipient is successful only if it is not rejected [3]. Unrejected allografts are expected to perform the functions as normal kidneys. Normal post-KT graft outcomes depend on immunology where variation in immune responses of the recipient is genetically influenced [4]. This variation may help individualize immunosuppressive regimens by identifying alleles that could increase risk or confer protection for immune-mediated complications [5]. Cytokine proteins modulate and mediate the immune response [6] and the gene that encode these proteins influence transcription, yielding differences in cytokine production [7]. Of the cytokine-related factors, interleukin-18 (IL-18) has been identified as a post-KT biomarker [8]. Single nucleotide polymorphisms (SNPs) have been reported to be associated with KT outcomes [9, 10]. Studies on the role of IL-18 SNPs and KT outcome have helped us to better understand the immunology of renal disease, providing greater insight into the biology of transplantation. However, the primary studies that have examined the role of IL-18 SNPs with KT have varied in their degree of concurrence. Performing a meta-analysis serves to address this variation and may yield clearer estimates of the role of IL-18 SNPs in renal allograft survival post-KT. In this meta-analysis, we examine the genotype distribution behavior between patients and healthy controls in order to establish an association between the polymorphisms and KT outcomes. Operating on the hypothesis that IL-18 SNPs reduce or increase the risk for allograft rejection, we compare rejector (RJ) with non-rejector (NRJ) patients which might provide useful information for transplant genetics.

Methods

Selection of studies

Three databases (PubMed, Google Scholar and Science Direct) were searched for genetic association articles as of September 24, 2019. Search terms included: "*interleukin*", "*IL-18* ", "*cytokine* ", "*polymorphisms*", "*allograft*" and "*renal transplantation*". Where duplicate articles were encountered, the later dated one was selected. Inclusion criteria were (1) studies that associated *IL-18* SNPs with KT outcomes; (2) *IL-18* genotype frequencies that compare KT patients and healthy controls, NR and NRJ. (3) genotype frequency data that allowed calculation of the odds ratios (ORs) and 95% confidence intervals (CIs). Exclusion criteria were studies that (1) did not examine renal allografts or KT outcomes; (2) were reviews; (3) were not about the *IL-18* SNPs and (4) had unusable genotype or allele frequencies.

Linkage disequilibrium and data extraction

The included articles examined two *IL-18* SNPs, -137G/C (rs187238) and -607A/C (rs1946518), each presented with genotype data (Table 1 and S1 Table). Observed phenotypic associations have been attributed to the proximity of two SNPs [11, 12]. NCI LDLINK

(https://ldlink.nci.nih.gov/) results shows that the two SNPS are in linkage equilibrium (LD) based on both European (CEU) and Han (CHB) genotypes [13]. LD is defined as the correlation between alleles located near each other [14] which is measured in terms of D' with a value of 1 indicating complete LD [15]. Therefore, *IL-18* SNPs with D' values of 1.00 in this study were reported to be in LD (S1 Table) and combined in the analysis (S2/S3 Table). Given these conditions, rs187238 and rs1946518 SNPs in *IL-18* were combined. This combination allowed analysis by GD and allograft as well as subgrouping by ethnicity (Tables 2 and 3).

Two investigators (TE and NP) independently extracted data and arrived at a consensus. The following information was obtained from each publication: first author's name, year of the study, country of origin, ethnicity, age of the subjects, *IL-18* SNPs (rs number) and the Clark-Baudouin (CB) score (Table 1). Sample sizes as well as genotype data between the RJ and NRJ were also extracted along with calculated outcome of the minor allele frequency (maf) (S2 and S3 Tables).

Power calculations and HWE assessment

Using the G*Power program [16], we evaluated statistical power as its adequacy bolsters the level of associative evidence. Assuming an OR of 1.5 at a genotypic risk level of α = 0.05 (two-sided), power was considered adequate at ≥ 80%. The Hardy-Weinberg Equilibrium (HWE) was assessed using the application in https://ihg.gsf.de/cgi-bin/hw/hwa1.pl.

Methodological quality of the studies

We used the CB scale to evaluate methodological quality of the included studies [17]. The CB criteria include P-values, statistical power, correction for multiplicity, comparative sample sizes between cases and controls, genotyping methods and the HWE. In this scale, low, moderate and high have scores of < 5, 5-6 and ³ 7, respectively.

Meta-analysis

We estimated odds ratios (ORs) and 95 % confidence intervals (CIs) of association using two overall approaches: (i) genotype distribution (GD) between cases and healthy controls and (ii) allograft wherein RJ were compared with NRJ. Calculated pooled ORs for GD were either higher in patients (hp) or higher in controls (hc); in allograft, they were either increased (in) or decreased (de), indicating risk for rejection. Standard genetic modeling was used, wherein we compared the following, (i) variant (*var*) genotype compared with the wild-type (*wt*) genotype (homozygous: Ho). To address importance of the heterozygous genotype, we evaluated recessive (Rc: *wt-wt* versus *wt-var* + *var-var*), dominant (Do: *wt-wt* + *wt-var* versus *var-var*) and codominant (Co: *wt* versus *var*) effects. Heterogeneity between studies was estimated with the c^2-based Q test [18], with threshold of significance set at $P^b < 0.10$. Heterogeneity was also quantified with the I^2 statistic which measures variability between studies [19]. I^2 values of > 50% indicate more variability than those £ 50% with 0% indicating zero heterogeneity. Evidence of functional similarities in population features of the studies warranted using the fixed-effects model [20], otherwise the random-effects model [21] was used. Sources of heterogeneity were detected with the Galbraith plot [22] followed by re-analysis (outlier treatment). Of note, outlier treatment dichotomized the comparisons into pre-outlier (PRO) and post-outlier (PSO). Sensitivity analysis, which involves omitting one study at a time and recalculating the pooled OR, was used to test for robustness of the summary effects. The low number of studies precluded assessment of publication bias. Data were analyzed using Review Manager 5.3 (Cochrane Collaboration, Oxford, England), SIGMASTAT 2.03 (Systat Software, San Jose, CA, USA) and SPSS 20.0 (IBM Co., Armonk, NY, USA).

Results

Search outcomes and study features

Figure 1 outlines the study selection process in a PRISMA-sanctioned flowchart (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). Initial search resulted in 39 citations, followed by a series of omissions that eventually yielded four articles for inclusion [23-26].

Characteristics of the included studies

Three [24-26] of the four included articles examined the two *IL-18* polymorphisms (rs187238 and rs1946518). S2 and S3 Tables show seven studies each for GD and allograft analyses. Under these two analyses, the following studies comprised the two ethnicities: (1) GD: Asian and Caucasian: three and four studies from two [23, 24] and two articles [25, 26], respectively; (2) allograft: Asian and Caucasian, five from two articles and two studies[23, 24] from one article [25] respectively (Tables 2 and 3). Table 1 shows that the methodological quality of the component studies was moderate based on mean and SD (6.37 ± 1.24) of the normally distributed CB scores (SW test: P = 0.33). This meta-analysis followed the PRISMA guidelines (S5 Table).

Meta-analysis outcomes

Table 2 delineates the overall pooled ORs by direction of effect, where GDs were hp (OR > 1.00) and decreased risk in the allograft analysis (OR < 1.00). The results show five statistically significant ORs ($P^a < 0.05$), three (all PSO) and two outcomes in GD and allograft, respectively. Of the five significant overall pooled ORs, only one survived the Bonferroni correction. This Bonferroni-surviving pooled OR became our core finding in GD, the PSO-derived Co model outcome indicating hp (OR 1.34, 95% CI 1.13-1.58, $P^a = 0.0007$). This finding was validated in Caucasian subgroup (OR 1.32, 95% CI 1.04 to 1.66, $P^a = 0.02$). Based on homogeneity ($I^2 = 0\%$) and initial fixed-effects features, the other core outcome was found in the Do model of the allograft analysis (OR 0.74, 95% CI 0.55-0.98, $P^a = 0.04$). This finding was validated in the Asian subgroup (OR 0.70, 95% CI 0.50-0.98, $P^a = 0.04$). The mechanism of outlier treatment for *IL-18* in the Co model of allograft analysis is visualized in Figs 2-4. Fig 2 shows the PRO forest plot with a non-significant ($P^a = 0.48$) and heterogeneous ($P^b = 0.02$, $I^2 = 60\%$) pooled effect indicating reduced risk (OR 0.89, 95% CI 0.63-1.25). The Galbraith plot identified the two studies [24, 25] as the sources of heterogeneity (outliers), located above the +2 confidence limit (Fig 3). In Fig 4, the PSO outcome (outliers omitted) shows reduced heterogeneity ($P^b = 0.16$, $I^2 = 39\%$); reduced risk effect (OR 0.73, 95% CI 0.56-0.93) and gained significance ($P^a = 0.01$). This operation is numerically summarized in Table 2. Table 3 shows the outcomes from sensitivity analysis where two of the nine (22%) comparisons were robust, one of which was the core finding (GD overall PSO).

Discussion

Summary of associations

The main findings of this study point to a dichotomized pattern of significant effects where all GD ORs favored patients (hp) over that of controls (up to 1.6-fold). In contrast, all outcomes in the allograft analysis indicated reduced risks of rejection (as much as 30%). Subgroup validation of these effects indicate consistency. Bonferroni correction and sensitivity analysis indicate stability and robustness, respectively, of the core outcomes.

In this meta-analysis, subgroup and outlier treatments have unraveled significant and non-heterogeneous and homogeneous associations that were not present in the component single-study outcomes. Conflicting outcomes between primary studies may be attributed to their lack of power and small sample sizes. Underpowered outcomes appear to be common in candidate gene studies [27] and are prone to the risk of Type 1 error.

IL-18 SNPs in allografts

The crucial role of *IL-18* in kidney physiology lies in its involvement in the filtration, integrity and permeability of the glomerular basement membrane [28]. IL-18 expression in renal epithelium might be important in triggering specific immune response manifested as acute graft rejection [29]. Post-KT hypoxia stimulates IL-18 production from the immune cells (neutrophils and macrophages) which permeate the allograft [30]. Moreover, rising levels of IL-18 enable the endothelial cells to ease entry of leukocytes into the allograft which sets the stage for rejection [24]. A study demonstrated an upregulation of IL-18 production in patients with acute rejection of kidney allograft [29]. Moreover, another study found significantly higher levels of IL-18 in culture biopsies from patients with acute rejection in comparison to stable KT patients [31]. Urinary IL-18 has been found to be an early, noninvasive, and accurate predictor for dialysis within the first week of KT [32, 33].

Strengths and limitations

Interpreting our findings should consider its limitations and strengths. Limitations include: (i) All the component studies were underpowered and (ii) most significant outcomes were non-robust. On the other hand, the strengths comprise of the following: (i) the combined sample sizes translated to adequate statistical power (80%); (ii) none of the articles had control frequencies that deviated from HWE. Confining the analysis to studies in HWE did not materially alter the pooled ORs; in fact, HWE-analysis validated the overall pooled effects. Given this outcome, the risk of genotyping errors appears to be a minor issue which minimizes methodological weakness in our study. Outlier treatment was key to generating significance and reducing heterogeneity. This demonstrates the utility of this meta-analysis tool in elevating the level of evidence for associations.

Conclusions

We have shown that associations of the *IL-18* SNPs with KT outcomes were genetic model dependent, with significance confined to the Do/Co models in the allograft analysis. Consistency of the GD outcomes due to outlier treatment and subgroup analysis pointed to the Do/Co models. The evidence presented here may render *IL-18* useful as prognostic markers in allograft failure post-KT. In spite of the evidence for associations, the complexity of allograft rejection involves interactions between genetic and non-genetic factors allowing for the possibility of environmental involvement. Interactions of the IL-18 gene with other genes and environmental factors have been reported

in post-KT allograft rejection. One article [24] examined another gene polymorphism (vascular endothelial growth factor (*VEGF*)). All articles acknowledged gene-environment interaction. Addressing gene-gene and gene-environment interactions may help address the pathophysiological significance of *IL-18* in allograft failure post-KT. All articles mentioned haplotype analysis with one presenting haplotype data [24]. Focus on *IL-18* haplotypes have been suggested for future association studies [10]. Future studies that focus on aspects other than those covered in this study would facilitate better understanding of *IL-18* associations with KT outcomes.

Declarations

-Ethics approval and consent to participate Not applicable

-Consent for publication Not applicable

-Availability of data and material In Supporting information

-Competing interests The authors declare that they have no competing interests

-Funding This study was unfunded

-Authors' contributions

Conceptualization: TE, PT

Formal analysis: PT, NP

Investigation: TE, PT, NP

Methodology: PT, NP, RM, AB

Resources: PT, AT

Supervision: AT

Validation: TE, PT, NP, RM, AB, AT

Writing – original draft: TE, PT, NP

Writing – review & editing: TE, PT, NP, RM, AB, AT

All authors have read and approved the manuscript

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Supporting information

S1 Table LD matrix DOCX

S2 Table Quantitative features GD DOCX

S3 Table Quantitative features Allograft DOCX

S4 Table HWE analysis DOCX

S5 Table PRISMA checklist DOCX

Abbreviations

é robust; all other significant outcomes were non-robust

û significant outcomes that did not survive the Bonferroni correction

P significant outcome that survived the Bonferroni correction

A adenine

AM analysis model

C cytosine

CB Clark-Baudouin

CC homozygous genotype

CEU genomic datasets representing individuals of European ancestry

CHB genomic datasets representing individuals of Han Chinese ancestry

CI confidence interval

Co codominant genetic model

de decreased risk

Do dominant genetic model

du duplicate

EH eliminated heterogeneity

Fe fixed-effects

G guanine

GD genotype distribution

GS gained significance

hc higher in controls

het heterogeneity

Ho homozygous genetic model

hp higher in patients

HWE Hardy-Weinberg Equilibrium

I^2 measure of variability

IL-18 interleukin-18 gene

IL-18 interleukin-18 protein

in increased risk

KT kidney transplantation

LD linkage disequilibrium

Log OR logarithm of standardized odds ratio

m controls matched with cases

maf minor allele frequency

n number of studies

NRJ non-rejector

OR odds ratio

P^a P-value for association

P^b P-value for heterogeneity

PRO pre-outlier

PSO post outlier

[R] Reference

Rc recessive genetic model

Re random-effects

RH reduced heterogeneity

RJ rejector

RNS retained non-significance

SD standard deviation

SE standard error

Sig significant

SNP single nucleotide polymorphism

var variant

wt wild-type homozygotes

wt-var heterozygote

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Tables

Table 1 Characteristics of the included studies in *IL-18*

First author	[R]	Year	Country	Ethnicity	Age (years) mean ± SD	RJ / NRJ	*IL-18* SNPs	CB
Kim	[23]	2008	Korea	Asian	33.9 ± 9.4 / 36.1 ± 11.1		rs187238	10
Mittal	[24]	2011	India	Asian	33.2 ± 12.6 / 38.2 ± 11.1		rs187238, rs1946518	6
Kolesar	[25]	2007	Czechlovakia	Caucasian	49.6 (patients)		rs187238, rs1946518	7
do Nascimento	[26]	2014	Brazil	Caucasian	33.1 ± 12.4 / 40.5 ± 13.0		rs187238, rs1946518	7

[R] Reference; *IL*-18: interleukin-18; SD: standard deviation; RJ: rejector; NRJ: non-rejector; SNP: single nucleotide polymorphism; CB: Clark-Baudouin

Table 2 Summary outcomes for *IL-18*

	Test of association					Test of heterogeneity			Test of association					Test of heterogeneity			Effect of outlier treatment	
	n	OR	95% CI	P[a]		P[b]	I^2 (%)	AM	n	OR	95% CI	P[a]		P[b]	I^2 (%)	AM	Sig	Het
	PRO								PSO									
GD					**Status**								**Status**					
Ho	7	1.39	0.87-2.22	0.17	hp	0.07	49	Re	5	**1.63**	**1.16-2.30**	**0.005** û	hp	0.13	44	Fe	GS	RH
Rc	7	1.33	0.99-1.79	0.06	hp	0.12	41	Fe	---	---	---	---	---	---	---	---	---	---
Do	7	1.24	0.93-1.65	0.14	hp	0.03	57	Re	6	**1.39**	**1.13-1.70**	**0.002**éû	hp	0.23	28	Fe	GS	RH
Co	7	1.17	0.96-1.44	0.12	hp	0.04	54	Re	5	**1.34**	**1.13-1.58**	**0.0007**éP	hp	0.22	31	Fe	GS	RH
Allograft					**Risk**								**Risk**					
Ho	7	0.75	0.47-1.19	0.22	de	0.27	20	Fe	---	---	---	---	---	---	---	---	---	---
Rc	7	0.84	0.55-1.29	0.43	de	0.14	38	Fe	---	---	---	---	---	---	---	---	---	---
Do	7	**0.74**	**0.55-0.98**	**0.04** û	de	0.46	0	Fe	---	---	---	---	---	---	---	---	---	---
Co	7	0.89	0.63-1.25	0.48	de	0.02	60	Re	5	**0.73**	**0.56-0.93**	**0.01** û	de	0.16	39	Fe	GS	RH

IL-18: *interleukin-18* gene; GD: genotype distribution; Ho: homozygous; Rc: recessive; Do: dominant; Co:co dominant; n: number of studies; OR: odds ratio; CI: confidence interval; P[a]: P-value for association; in: increased risk; de: decreased risk; hp: higher in patients; P[b]: P-value for heterogeneity; I^2: measure of variability; AM: analysis model; Re: random-effects; Fe: fixed-effects; PRO: pre-outlier; PSO: post-outlier; GS: gained significance; RH: reduced heterogeneity; values in bold indicate significant associations; é robust; all other significant outcomes were non-robust (Table 3); û significant outcomes that did not survive the Bonferroni correction; significant outcome that did P

Table 3 Subgroup outcomes for *IL-18*

	Test of association					Test of heterogeneity			Test of association					Test of heterogeneity			Effect of outlier treatment	
	n	OR	95% CI	P^a		P^b	I^2 (%)	AM	n	OR	95% CI	P^a		P^b	I^2 (%)	AM	Sig	Het
	PRO								PSO									
GD																		
Asian					Status								Status					
Ho	3	1.46	0.62-3.46	0.38	hp	0.05	68	Re	2	1.12	0.66-1.88	0.68	hp	0.25	23	Fe	RNS	RH
Rc	3	1.47	0.71-3.04	0.29	hp	0.09	58	Re	2	1.25	0.76-2.04	0.38	hp	0.16	49	Fe	RNS	RH
Do	3	1.12	0.71-1.77	0.62	hp	0.03	71	Re	2	0.90	0.67-1.22	0.51	hc	0.16	50	Fe	RNS	RH
Co	3	1.17	0.86-1.60	0.32	hp	0.07	63	Re	2	0.99	0.78-1.26	0.94	hc	0.69	0	Fe	RNS	EH
Caucasian																		
Ho	4	1.24	0.77-2.00	0.37	hp	0.16	42	Fe	---	---	---	---	---	---	---	---	---	---
Rc	4	1.09	0.70-1.67	0.71	hp	0.25	26	Fe	---	---	---	---	---	---	---	---	---	---
Do	4	**1.37**	**1.04-1.80**	**0.03** û	hp	0.11	50	Fe	---	---	---	---	---	---	---	---	---	---
Co	4	1.17	0.86-1.61	0.32	hp	0.06	60	Re	3	**1.32**	**1.04-1.66**	**0.02** û	hp	0.16	40	Fe	GS	RH
Allograft																		
Asian					Risk								Risk					
Ho	5	0.74	0.44-1.24	0.25	de	0.11	47	Re	---	---	---	---	---	---	---	---	---	---
Rc	5	0.87	0.38-2.00	0.75	de	0.05	58	Re	4	0.62	0.35-1.10	0.10	de	0.12	49	Fe	RNS	RH
Do	5	**0.70**	**0.50-0.98**	**0.04** û	de	0.25	25	Fe	---	---	---	---	---	---	---	---	---	---
Co	5	0.78	0.52-1.16	0.22	de	0.05	58	Re	4	**0.70**	**0.53-0.92**	**0.01** û	de	0.10	52	Fe	GS	RH
Caucasian																		
Ho	2	0.79	0.30-2.07	0.63	de	0.89	0	Fe	---	---	---	---	---	---	---	---	---	---
Rc	2	0.88	0.35-2.20	0.78	de	0.82	0	Fe	---	---	---	---	---	---	---	---	---	---
Do	2	0.85	0.50-1.47	0.57	de	0.73	0	Fe	---	---	---	---	---	---	---	---	---	---
Co	2	1.20	0.82-1.75	0.35	in	0.10	63	Fe	---	---	---	---	---	---	---	---	---	---

IL-18: *interleukin-18* gene; GD: genotype distribution; Ho: homozygous; Rc: recessive; Do: dominant; Co:co dominant; n: number of studies; OR: odds ratio; CI: confidence interval; P^a: P-value for association; in: increased risk; de: decreased risk; P^b: P-value for heterogeneity; I^2: measure of variability; AM: analysis model; Re: random-effects; Fe: fixed-effects; PRO: pre-outlier; PSO: post-outlier; GS: gained significance; RNS: retained non-significance; RH: reduced heterogeneity; EH: eliminated heterogeneity; values in bold indicate significant associations; hp: higher in patients; hc: higher in controls; all significant ORs were non-robust (Table 3); û significant outcomes that did not survive the Bonferroni correction

Table 4 Sensitivity analysis for *IL-18* associations with KT outcomes

Comparison		Outlier status	Genetic model	P^a	Sensitivity outcome
GD	Overall	PSO	Ho	0.005	[24]*
GD	Overall	PSO	Do	0.002	Robust
GD	Overall	PSO	Co	0.0007	Robust
Allograft	Overall	PRO	Do	0.04	[23, 24]*
Allograft	Overall	PSO	Co	0.01	[23, 24]*
GD	Caucasian	PRO	Do	0.03	[26]
GD	Caucasian	PSO	Co	0.02	[26]
Allograft	Asian	PRO	Do	0.04	[23, 24]*
Allograft	Asian	PSO	Co	0.01	[23, 24]*

IL-18: *interleukin-18* gene; GD: genotype distribution; KT; kidney transplantation; PRO:

pre-outlier; PSO: post-outlier; Ho: homozygous; Do: dominant; Co: codominant; P^a: P-value

for association; Reference under sensitivity outcome indicates non-robustness; [24]* Mittal rs1946518; [23, 24]* Kim rs187238, Mittal rs187238; [26] do Nascimento rs187238 and rs1946518

Figures

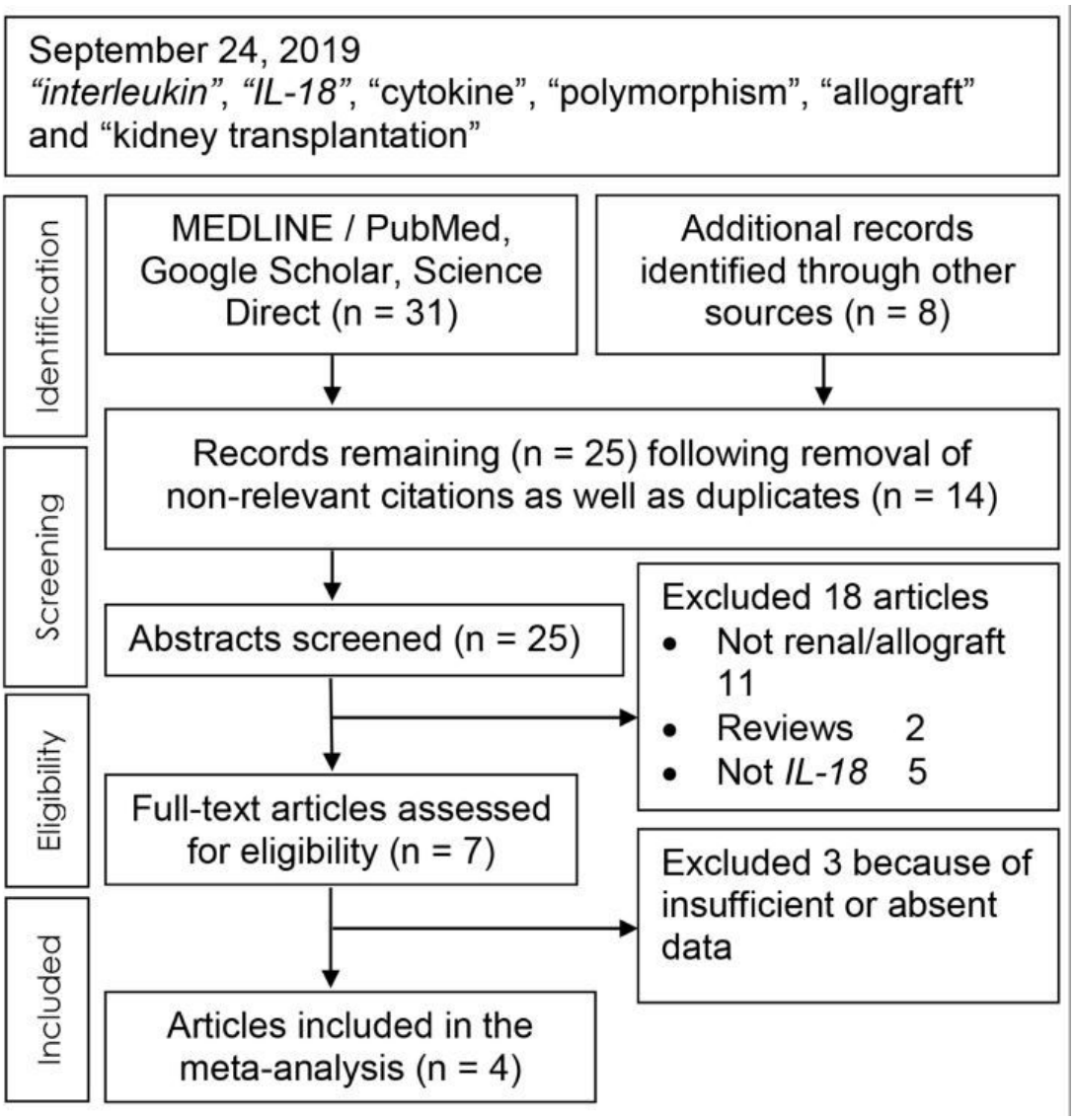
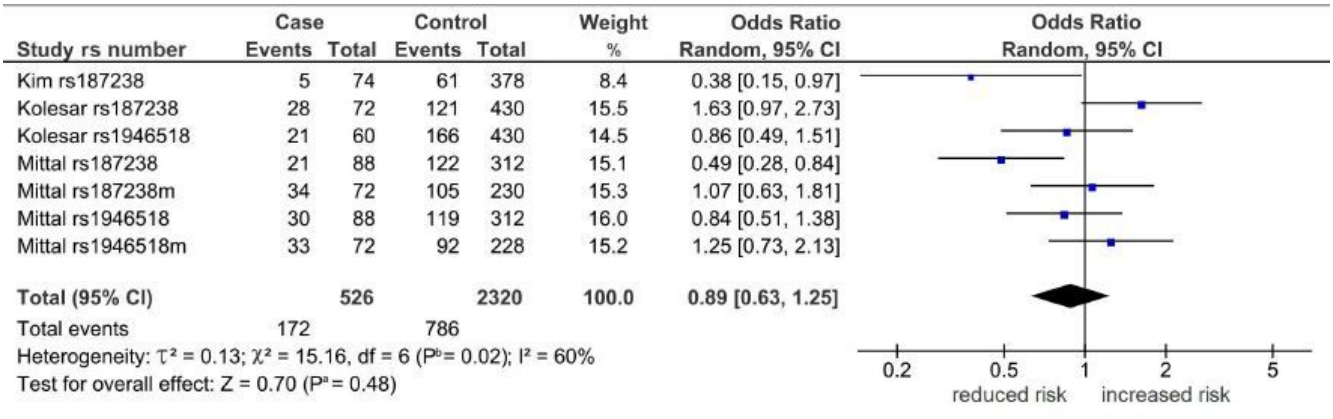


Figure 1

Summary flowchart of literature search



Study rs number	Case Events	Case Total	Control Events	Control Total	Weight %	Odds Ratio Random, 95% CI
Kim rs187238	5	74	61	378	8.4	0.38 [0.15, 0.97]
Kolesar rs187238	28	72	121	430	15.5	1.63 [0.97, 2.73]
Kolesar rs1946518	21	60	166	430	14.5	0.86 [0.49, 1.51]
Mittal rs187238	21	88	122	312	15.1	0.49 [0.28, 0.84]
Mittal rs187238m	34	72	105	230	15.3	1.07 [0.63, 1.81]
Mittal rs1946518	30	88	119	312	16.0	0.84 [0.51, 1.38]
Mittal rs1946518m	33	72	92	228	15.2	1.25 [0.73, 2.13]
Total (95% CI)		**526**		**2320**	**100.0**	**0.89 [0.63, 1.25]**
Total events	172		786			

Heterogeneity: $\tau^2 = 0.13$; $\chi^2 = 15.16$, df = 6 ($P^b = 0.02$); $I^2 = 60\%$
Test for overall effect: Z = 0.70 ($P^a = 0.48$)

Figure 2

Forest plot outcome in the allograft analysis of the Co model. Diamond denotes the pooled odds ratio (OR) indicating reduced risk (0.89). Squares indicate the OR in each study. Horizontal lines on either side of each square represent the 95% confidence intervals (CI). The Z test for overall effect was non-significant (Pa = 0.48). The χ2-test shows the presence of heterogeneity (Pb = 0.02, I2 = 60%); I2: a measure of variability expressed in %; m: controls matched with cases

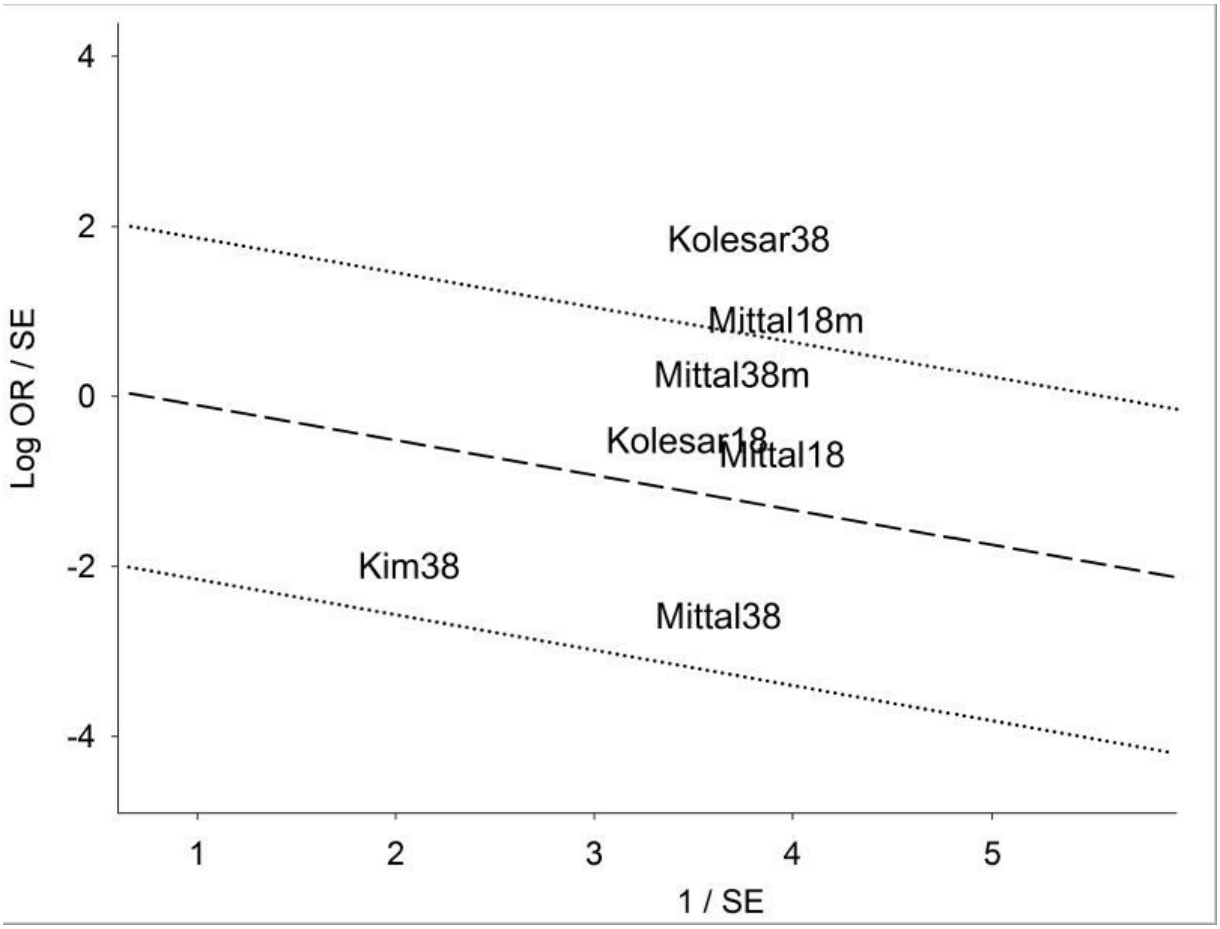


Figure 3

Galbraith plot of the allograft analysis in the Co model. Log OR: logarithm of standardized odds ratio; SE: standard error; The two studies above the +2 confidence limit are the outliers; m: controls matched with cases

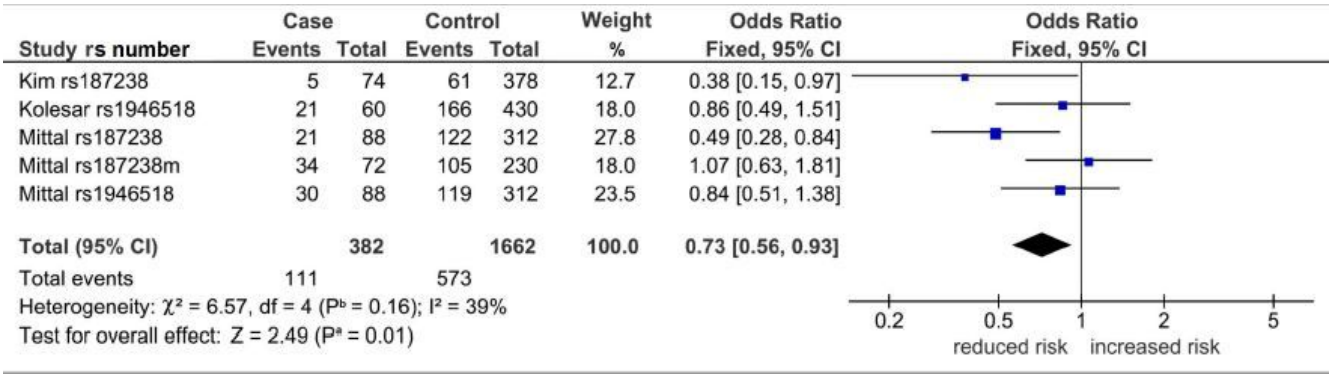


Figure 4

Forest plot outcome of outlier treatment in the allograft analysis of the Co model. Diamond denotes the pooled odds ratio (OR) reduced risk (0.73). Squares indicate the OR in each study. Horizontal lines on either side of each square represent the 95% confidence intervals (CI). The Z test for overall effect shows significance (Pa = 0.01).The χ2-test indicates reduced heterogeneity (Pb = 0.16, I2 = 39%); I2: a measure of variability expressed in %; m: controls matched with cases