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Review Paper

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Tacrolimus-induced nephrotoxicity and genetic variability: A review

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Summary

Background: Calcineurin inhibition (CNI) is the mainstay of immunosuppressant therapy for most solid organ transplant patients. High tacrolimus levels are related with acute nephrotoxicity, but the relationship with chronic toxicity is less clear. Variation in disposition of tacrolimus is associated with genetic variation in *CYP3A5*. Hence, could genetic variation in *CYP3A5* or other genes involved in tacrolimus disposition and effect be associated with a risk for tacrolimus-induced nephrotoxicity?

To perform a review of the literature and to identify if genetic variation in *CYP3A5* or other genes involved in tacrolimus disposition or effect may be associated with tacrolimus-induced nephrotoxicity and/or renal dysfunction in solid organ transplant recipients.

Material/Methods: Pubmed/Medline, Embase and Google were searched from their inception till November 8th 2010 with the search terms 'tacrolimus', 'genetics', and 'nephrotoxicity' or 'renal dysfunction'. References of relevant articles were screened as well.

Results: We identified 13 relevant papers. In kidney recipients, associations between donor *ABCB1*, recipient *CCR5* genotype and tacrolimus-induced nephrotoxicity were found. *CYP3A5* genotype studies in kidney recipients yielded contradictory results. In liver recipients, a possible association between recipient *ACE, CYP3A5, ABCB1* and *CYP2C8* genetic polymorphisms and tacrolimus-induced nephrotoxicity was suggested. In heart recipients, *TGF-β* genetic polymorphisms were associated with tacrolimus-induced nephrotoxicity. The quality of the studies varied considerably.

Conclusions: Limited evidence suggests that variation in genes involved in pharmacokinetics (*ABCB1* and *CYP3A5*) and pharmacodynamics (*TGF-β, CYP2C8, ACE, CCR5*) of tacrolimus may impact a transplant recipients' risk to develop tacrolimus-induced nephrotoxicity across different transplant organ groups.

Key words: **tacrolimus • nephrotoxicity • genetics • *CYP3A5* • *ABCB1* • *ACE* • *TGF-β***

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Background

Calcineurin inhibition (CNI) is the mainstay of immunosuppressant therapy for most solid organ transplant patients. Having been introduced in 1995 [1,2], the macrolide calcineurin inhibitor drug tacrolimus was included in many treatment protocols from 1997 onwards [1]. Tacrolimus originates from a fungus (*Streptomyces tsukubaensis*) and it is an effective immunosuppressant agent. But its use brings with it serious adverse effects such as renal dysfunction, neurotoxicity, glucose intolerance, liver function abnormalities and hypertension [3–6].

Tacrolimus has a narrow therapeutic window and as a serum concentration-response relationship exists between tacrolimus and acute nephrotoxicity, therapeutic drug monitoring is recommended to minimize tacrolimus-related acute renal dysfunction [7]. Tacrolimus-related acute and chronic nephrotoxicity is a well-recognized adverse effect [7,8] and a serious concern, often leading to permanent renal damage or even kidney loss. In the 2003 landmark study by Ojo et al. [8], the five year cumulative incidence of chronic renal failure for a cohort of 70,000 adult non-renal transplant patients was 16.5% and end-stage renal disease (ESRD) developed in 28.9% of these patients. In that cohort, chronic renal failure was associated with an increased risk of death (relative risk: 4.55; 95% CI: 4.38–4.74). However, most of the patients in this study received cyclosporine. Meanwhile, tacrolimus has become the primary calcineurin inhibitor for immunosuppressant treatment in solid organ transplant recipients [9]. Both cyclosporine and tacrolimus are associated with chronic nephrotoxicity, but the incidence and underlying mechanisms seem to differ [10–12].

Furthermore, the adverse event profiles of the two drugs are different [11–15]. Both cyclosporine and tacrolimus are mainly metabolized by CYP3A4, but there is a bigger role for CYP3A5 in the metabolism of tacrolimus [16]. Although the risk of chronic renal failure in tacrolimus treated transplant patients is increased, not all patients develop renal failure. To identify patients at risk, more in depth knowledge of genetic and non-genetic risk factors is needed.

The genetically polymorphic cytochrome P450 3A5 (CYP3A5) is a phase I metabolizing enzyme involved in the metabolism of tacrolimus [17]. Carrying the *CYP3A5*3* allele results in a premature stop codon and the absence of the CYP3A5 protein (non-expressors) [18]. Genetic variation in the drug-metabolizing enzyme cytochrome P450 3A5 (CYP3A5) is associated with interindividual differences in tacrolimus clearance in kidney, lung and heart transplant patients, not only in adults, but also in children [19,20]. CYP3A5 expressors need higher doses of tacrolimus to reach the same therapeutic levels. And as high tacrolimus levels are associated with acute renal failure, genetic variation in *CYP3A5* may also impact the risk to develop chronic renal failure.

The ABCB1 transporter actively transports substrates out of the cell [21,22]. As tacrolimus is a substrate for ABCB1, variation in ABCB1 expression rate is thought to influence the plasma and/or intracellular concentration of tacrolimus [23].

The effect of tacrolimus on the kidney appears to be multi-factorial. Renal failure may occur due to an imbalance in the secretion and metabolism of nitric oxide, prostaglandines (both vasodilatory), thromboxane and endothelin (both vasoconstrictive) together with increased activity of the sympathetic nervous system. Consequently, variation in genes affecting these processes, such as *ACE*, *TGF-β* and *CYP2C8* may also impact on the risk to develop renal failure while using tacrolimus. Transforming growth factor-β (TGF-β) seems to play a role in the development of fibrosis in the kidney, which is a typical histological feature of tacrolimus-induced nephrotoxicity [24,25] CYP2C8 is thought to counter the vasoconstrictive effect of tacrolimus, through a reduction of epoxyeicosatrienoic acids (EETs) formation by CYP2C8 [26,27].

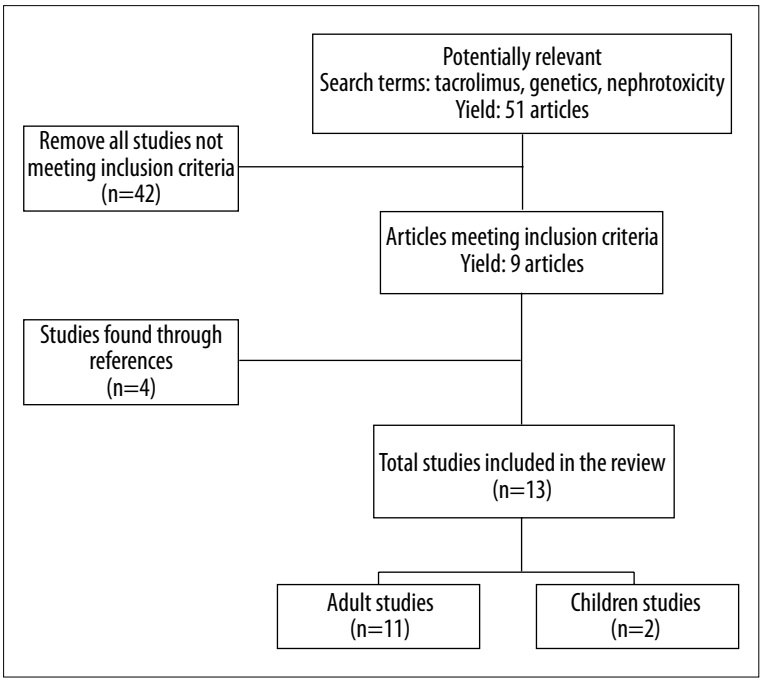

Figure 1. Flow diagram of selection process.

The objective of the present study was to systematically review the current evidence for genetic associations with tacrolimus-induced nephrotoxicity and/or renal dysfunction in adult and pediatric renal and non-renal organ transplant recipients.

Material and Methods

Literature search

The Pubmed/Medline, Embase and Google databases were searched from their inceptions till November 8, 2010. The search terms were: 'tacrolimus', 'genetics', 'nephrotoxicity' and 'renal dysfunction'. Additionally, reference lists of articles were screened.

We reviewed the titles and abstracts of the retrieved papers for eligibility. The inclusion criteria were: human studies, original genetic research, tacrolimus. For renal transplant patients, we only included papers that used biopsy-proven nephrotoxicity defined as such by the authors of the papers. For the non-renal transplant patients we only included papers that used renal function, as defined by the authors.

Quality assessment

Study quality, in terms of internal and external validity and power, was assessed with the Downs-Black scale (Figure 1) [28]. To our knowledge, quality assessment scales for genomic association studies are lacking. We looked for other scales that could be used alternatively. The Downs Black scale has been identified as a valuable tool to assess methodological quality of non-randomized studies [29]. Two reviewers (VG, PM) applied the Downs-Black scale without blinding of authorship. Discrepancies were resolved by discussion and if necessary by a third reviewer (SW). The scale rated items with '1' if the item was reported, '0' if the item was not reported or 'U' if the reviewers were unable to make a determination. An exception is item number 5 (the distribution of the principal confounders): scored with '2' if all the confounders were listed, '1' if it was only partially reported, '0' if not at all. Another exception is item number 27 (the power of the study): scored from 0–5. The maximum total score is 32.

The reviewers used decision rules. For one, in the Downs-Black scale the term "intervention" was replaced with 'tacrolimus regimen' where applicable. As the scale can also be used for intervention studies, three items (14, 23 and 24) pertaining to blinding and randomization were scored as 'U' and did not contribute to the total scores. The directions for use of the scale were otherwise followed.

Study power was calculated using the data provided in the articles, if sufficient. We compared proportions yes/no renal dysfunction/nephrotoxicity for carriers with and without genetic polymorphism, using the power calculator for proportions from Sigmaplot (version 11.0, Systat software, 2008). We contacted authors of four papers to

obtain more information needed to complete the power calculation. All studies were evaluated for a type-1 error rate with α=set to 0.05. Study power is scored as follows: A=<80% power → 0, B=80% power → 1, C=85% power → 2, D=90% power → 3, E=95% power → 4 and F=99% power → 5.

RESULTS

Our initial search yielded 51 articles, of which 9 met the inclusion criteria. The reference lists yielded 4 more papers, resulting in a total of 13 papers (Figure 1). Table 1 shows detailed characteristics of the study populations, outcome measures and outcomes of all studies.

Adult patients were studied in ten papers, pediatric patients in three papers.

Adults

Twenty-four polymorphisms in nine different genes *(CYP3A4, CYP3A5, ABCB1, CYP2C8, ACE, TGF-β, CYP2J2, AGT1, AT1)* were investigated in adults.

CYP3A

Seven articles studied the association between *CYP3A* polymorphisms and renal toxicity of tacrolimus [30–36].

Kidney transplant

Kuypers et al. [31] reported a higher incidence of biopsy-proven CNI-nephrotoxicity in renal transplant recipients (n=95) carrying the *CYP3A4*1/CYP3A5*1* or *CYP3A4*1B/CYP3A5*1* expressor genotype compared to non-carriers of the alleles (37.5% *vs.* 11.2%, P=0.03 and 42.8% *vs.* 11.2%, P=0.02). With a larger cohort (n=304), three years later, the same group confirmed previous results that carrying the *CYP3A5*1* genotype increases the risk for biopsy-proven tacrolimus-induced nephrotoxicty (HR: 2.38 (1.15–4.92), P=0.01) at 3 months post-transplant [36]. In this second study, *de novo* arteriolar hyalinization was used as of the histological definition of calcineurin-inhibitor toxicity (CNIT). In contrast to the two previous studies, a third study in 67 kidney recipients from a Chinese population showed a higher incidence of nephrotoxicity in the *CYP3A5*3/*3* genotype group, using protocol biopsies at one month post-transplant [32]. CNIT was graded based on histological changes from 0 to 3, with 3 being most severe. In patients with the *CYP3A5*3/*3* genotype, interstitial fibrosis (1.04±0.51 *vs.* 0.53±0.61, p<0.01) and vacuolization (0.89±0.63 *vs.* 0.40±0.63, p<0.05) were more severe than in patients with the *CYP3A5*1/*3* genotype. Similarly, 136 renal transplant recipients with the *CYP3A5*3/3* genotype presented with a non-significant trend towards a higher incidence of developing biopsy proven nephrotoxicity compared to *CYP3A5*1/*3* and *CYP3A5*1/*1* genotypes (33% *vs.* 9% and 10%, P=0.1) [33].

Finally, Naesens et al. [35] did not find an association between donor and recipient *CYP3A5* genotype and histological signs of nephrotoxicity in 252 renal transplant recipients.

Liver transplant

In 60 liver transplant recipients, *CYP3A5* non-expressors had a higher risk to develop dysfunction [HR 3.16 ($CI_{95\%}$: 1.01-6.16, p<0.05)] [30].

Heart transplant

In contrast, Klauke et al. [34] found no association between the *CYP3A5* genotype and an increase in serum creatinine (SCr ⩾1.8 mg/dl) in 53 heart transplant patients.

ABCB1

Kidney transplant

In 252 renal transplant recipients, a significant association was found between *ABCB1* genotype and histological signs of nephrotoxicity, as defined by interstitial fibrosis/tubular atrophy [35]. When both donor and recipient are homozygous for the *T* variant of *ABCB1 3435* there was an odds ratio of 3.9 ($CI_{95\%}$: 2.0–7.6, P<0.001) for higher Interstitial Fibrosis/Tubular Atrophy (IF/TA) grades compared to no homozygosity for the *C3435T* polymorphism.

Liver transplant

In liver transplant patients, Hebert et al. [37] showed that 50% of patients with the *ABCB1 11/22* haplotype *(2677G,3435C/2677T,3435T)* experienced renal dysfunction (serum creatinine ⩾1.6 mg/dl) compared to 31% of patients with the *ABCB1 11/11* haplotype *(2677G,3435C/2677G,3435C)* and 11.2% of patients with the *ABCB1 22/22* haplotype *(2677T,3435T/2677T,3435T)*.

Three other studies did not identify a relation with *ABCB1* genotype and nephrotoxicity [31,34,36].

Table 1. Included studies in the review.

Author	Organ	Total n	N on TAC	Age (years)	Renal outcome measurement	DNA origin	Genes of interest	Outcomes	Total score
van de Wetering et al. [38]	Heart	402	21	49 (15–64)	ESRF defined as need to start renal replacement	Recipient	*TGF-β*	*TGF-β* Pro^{10} carriers: RR 2.9 for CNIT *TGF-β* Pro^{25} carriers: RR 2.6 for CNIT *Data only analyzed pooled with cyclosporine*	22
Klauke et al. [34]	Heart	53	21	50.2 ±14.6	Serum creatinine ≥1.8 mg/dl at 3 ≥occasions median 20 months post-transplant	Recipient	*CYP3A5, ABCB1, TGF-β*	No relationship found between any of the genotypes and the renal outcome. *Data only analyzed pooled with cyclosporine*	16
Fukudo et al. [30]	Liver	60	60	55 (29–70)	Initial serum creatinine increase of > 0.5 mg/dl above pre-transplant baseline at 3,6,9,12 months post-transplant	Recipient Donor	*CYP3A5*	Recipient *CYP3A5* expressors *vs.* non-expressors CNIT 17% *vs.* 46%. Donor *CYP3A5* no association was found with CNIT.	20
Smith et al. [39]	Liver	163	41	48±10	Serum creatinine ≥1.6 mg/dl at 3 yrs post-transplant	Recipient	*CYP2C8, CYP2J2*	*CYP2C8*3* OR 16.67 for CNIT. No relationship was found for the *CYP2J2* genotype and CNIT.	21
Gallon et al. [40]	Liver	143	100	55±14	Serum creatinine ≥1.5 mg/dl at most recent follow-up (median 60 months post-transplant)	Recipient	*ACE, AGT1, AT1*	*ACE D/D* carriers: 57% nephrotoxicity *vs.* 20% non nephrotoxicity (RR 4.3). No relationship was found for the *AGT1*, *AT1* genotype and CNIT. *Data only analyzed pooled with cyclosporine*	25
Hebert et al. [37]	Liver	120	31	48±12	Serum creatinine ≥1.6 mg/dl 3 years after transplantation or subjects who required hemodialysis or a kidney transplantation before 3 years post-transplantation	Recipient	*ABCB1*	*ABCB1* 2677 GT, *3435* CT 50%; *2677* GG, 3435 CC 31%; 2677 TT, 3435 TT 19% incidence of CNIT. *Data only analyzed pooled with cyclosporine*	19
Hawwa et al. [42]	Liver	51	51	2 (0.6–16)	30% reduction in GFR (Counahan-Barrat formula taken at 3,6,12 months) compared with pre-transplant baseline	Recipient	*ABCB1*	CNIT at 6 months post-transplantation (1236T allele: frequency =63.3% in nephrotoxic patients *vs.* 37.5% in controls, P=0.019; 2677T allele: frequency =63.3% *vs.* 35.9%, P=0.012; 3435T allele: frequency =60% *vs.* 39.1%, P=0.057). T-T-T haplotype 52.9% incidence vs 29.4% in controls, P = 0.029. None of *ABCB1* alleles or haplotypes were associated with renal toxicity 1 year post-transplantation	17
Kuypers et al. [31]	Kidney	95	95	51.3 ±14.1	Biopsies taken when clinically indicated. Biopsy proven using Banff 2001 any time up to 5 years post-transplant	Recipient	*CYP3A5, ABCB1*	Both *CYP3A4*1/CYP3A5*1* and *CYP3A4*1B/CYP3A5*1* were associated with a higher incidence of CNIT compared to non-carriers (37.5% vs 11.2%, P=0.03 and 42.8% *vs.* 11.2%, P=0.02 or combined *CYP3A4*1/CYP3A5* 1* and *CYP3A4*1B/CYP3A5*1*: 40% *vs.* 11.2%, P=0.005. No significance association found for the *ABCB1* genotype and CNIT	19

Table 1 continued. Included studies in the review.

Author	Organ	Total n	N on TAC	Age (years)	Renal outcome measurement	DNA origin	Genes of interest	Outcomes	Total score
Chen et al. [32]	Kidney	67	67	39.28 ±12.46	Protocol biopsy at 1 month, Banff 1997 criteria. Serum creatinine at 1 wk, 1,3,6,12 months post-transplant	Recipient	*CYP3A5*	*CYP3A5*3/*3* higher scores on all the histopathological changes for nephrotoxicity. No difference in serum creatinine between genotypes	11
Quteineh et al. [33]	Kidney	136	136	45±11	Biopsy was obtained when possible and biopsy-proven nephrotoxicity defined according to Banff 2005 criteria	Recipient	*CYP3A5*, *ABCB1*	*CYP3A5*3/*3* trend for higher risk compared to patients with *CYP3A5*1/*3* and *CYP3A5*1/*1* (33% *vs.* 9% and 10%, P=0.1). No significant association was found for the *ABCB1* genotype and CNIT	19
Kuypers et al. [36]	Kidney	304	304	52.9 ±14.1	Biopsy-proven nephrotoxicity based on the presence of de novo afferent arteriolar hyaline thickening at 3 months and every 12 months post-transplant.	Recipient	*CYP3A4*, *CYP3A5*, *ABCB1*	Carrying the *CYP3A5*1* genotype results in a HR of 2.38 (1.15–4.92, P=0.01) No relationship was found for the *ABCB1* and *CYP3A4* genotypes and CNIT	24
Naesens et al. [35]	Kidney	252	252	54.5 ±13.9	Histological signs of nephrotoxicty (IF/TA and arteriolar hyalinosis) at 3 years post-transplant	Recipient Donor	*ABCB1*, *CYP3A4*, *CYP3A5*	A significant association was found for carriers of the *ABCB1 3435TT* genotype for recipients (OR: 1.8 (1.11–2.93), P=0.0175) and donors (OR: 1.76 (1.07–2.89), P=0.0263). The OR for a higher IF/TA grade is 3.9 (2.0–7.6, P<0.001) if both donor and recipient are homozygous for *ABCB1 3435TT* genotype vs no homozygosity for *C3435T* polymorphisms. OR is 3.7 (1.8–7.7, P<0.001), if both donor and recipient are homozygous for *T* variant of *3435 vs.* mixed combinations. No relationship was found between *ABCB1 3435* genotypes and arteriolar hyalinosis or *ABCB1 2677* genotypes and any signs of nephrotoxicity. No relationship was found for the *CYP3A4* and *CYP3A5* genotypes and CNIT	23
Grenda et al. [41]	Kidney	207	61	11±5	Renal biopsy, observed consistent deterioration of renal function, accompanied with hyperuricaemia and/or tubuluar acidosis.	Recipient	*ABCB1*, *CYP3A5*, *IL10*, *IL6*, *CCR5*, *TNF-alpha*, *IL1B*, *IL1RN*, *MCP-1*, *VEGF*, *TGF-β*	100% of the Tac patients with nephrotoxicity carried the *CCR5Δ32* polymorphism vs 21% controls. No relationship was found for the other genotypes and CNIT, *Data only analyzed pooled with cyclosporine*	11

TGF-β

Heart transplant

In 402 heart transplant recipients, of whom 21 were treated with tacrolimus, carrying the Pro allele at codon 10 or codon 25 of the *TGF-β* gene was associated with a relative risk of 2.9 ($CI_{95\%}$: 1.5–5.8, P=0.002) and 2.6 ($CI_{95\%}$: 1.4–4.8, P=0.002), respectively, to develop end-stage renal dysfunction [38]. In contrast, in 106 heart transplants (n=42 on tacrolimus), no relation

was found between this genotype and renal dysfunction [34]. For both cohorts, it was not possible to determine the association for the tacrolimus patients separately, as this was not reported in the papers.

CYP2C8 and *CYP2J2*

Liver transplant

In 41 liver transplant recipients, carrying the *CYP2C8*3* polymorphism was associated with a higher risk [OR 16.67 ($CI_{95\%}$: 2.8–99.6)] to develop renal dysfunction [39]. In the same cohort, no association was found with *CYP2J2* genotype and renal dysfunction.

ACE, AGT1 and *AT1*

Liver transplant

In 143 liver transplant recipients, (100 on tacrolimus), the *ACE D/D* genotype was significantly associated with a higher risk of renal dysfunction [RR 4.3 ($CI_{95\%}$: 1.9–9.7, P=0.0001)] [40].

In the same cohort, no correlation was found between *AGT1* or *AT1* genetic variation and renal dysfunction.

Children

We identified only two studies in children. Grenda et al., studied twenty-four polymorphisms in ten different genes *(CYP3A5, ABCB1, TGF-β, IL10, IL6, CCR5, TNF-alpha, IL2B, IL1RN, MCP-1, VEGF)* in 207 (61 on tacrolimus) pediatric kidney transplant recipients [41]. The rationale for studying these genotypes was not given by the authors of the paper. Except from *CCR5*, no relation between any of these genotypes and nephrotoxicity, defined by clinically indicated biopsy or deterioration of renal function, was found. We identified one other study in pediatric liver transplant recipients [42].

ABCB1

Kidney transplant

In contrast to adults, in 207 (61 on tacrolimus) pediatric kidney transplant recipients no association between *ABCB1* genotype and nephrotoxicity, as defined by biopsy or reduced renal function was found [41].

Liver transplant

In 51 paediatric liver recipients, *T-T-T* haplotype (*C1236T, G2677T, C3435T*) was associated with an increased risk of a reduction in creatinine clearance at 6 months post-transplant [42]. At 6 months post-transplant, 52.9% of the patients with renal dysfunction were carriers of the *T-T-T* haplotype compared to 29.4% in the patients without renal dysfunction (P=0.029).

CCR5

Kidney transplant

In the Grenda study, all transplant recipients with nephrotoxicity (n=18) carried *CCR5* wild-type compared to 79% of the no-nephrotoxicity group (n=28, P=0.041) [41].

Quality assessment

Overall, the quality of the studies was moderate: the mean score on the Downs-Black scale [28] was 17.8±4.1 (range 11–25). Separate scores are given in the Table 1.

Appropriateness of the statistical tests used, the compliance of the intervention and whether patients were recruited from the same population were the best-reported items. These aspects were reported in all 13 studies. The worst reported items were on determining if the outcomes were blinded to genotype of the patients (only 3 papers) and the population pool subjects were recruited from (5 papers) and whether losses to follow-up were taken into account (4 papers).

Statistical power

Four papers [32,33,39,42] lacked sufficient data to recalculate the power. We were able to contact the authors of two of these papers [[39,42], however only one [42] provided additional data. For those without sufficient data, the item was scored with a 'U' as in 'unable to determine'.

Only three studies [36,38,40] had sufficient power, rated with 4 or 5 points. All studies considered a $p<0.05$ as statistically significant, and none made adjustment for multiple testing of genetic variants.

Discussion

This literature review shows that specific polymorphisms in six different genes have been

implicated to be associated with the variation in the incidence of renal dysfunction in adults or children who have received tacrolimus for immunosuppression after solid organ transplant. The identified genes are *CYP3A5, ABCB1, CYP2C8, ACE, TGF-β* and *CCR5*.

Five studies suggest a role for the *CYP3A5* gene (four kidney [31–33,36] one liver [30]), whereas three studies (two kidney [35,41], one heart [34]) could not confirm such an association. For two papers, this lack of an association may be due to the small number of patients studied (n=61 and n=21, respectively).

In two studies researching kidney recipients, patients who are *CYP3A5* expressors had a higher risk of nephrotoxicity while the opposite was found in two other studies. The discrepancy between these studies may be explained by the outcome measure used. In the largest study to date, calcineurin inhibitor induced nephrotoxicity was defined as *de novo* arteriolar hyalinosis at 3 months after transplant and yearly thereafter. Hence, patients who received a donor kidney with arteriolar hyalinosis at transplant, would not be scored as CNIT, while this may have been the case in the other studies where any arteriolar hyalinosis was scored or other histological signs, according to Banff 2001, such as interstitial fibrosis/tubular atrophy. The disparate results may further be explained by the timing of biopsies, e.g. early (at month) versus late (over 3months after transplant). When taking these differences and inherent limitations of the different studies into account, the strongest evidence points towards a positive association between *CYP3A5* expressors and a higher risk for calcineurin induced nephrotoxicity in adult renal transplant patients. For liver transplant patients only one paper (n=60) was identified, with the opposite finding for recipient *CYP3A5*. This may be explained by the effect of genetic differences in renal CYP3A5 activity, versus the genetic differences in hepatic *CYP3A5* in the renal transplant patients.

There is also a discrepancy with regard to *ABCB1*. Only 3 (one in kidney [35] and two in liver [37,42]) of the 8 papers on the association of *ABCB1* polymorphisms and tacrolimus-induced nephrotoxicity found a positive association. Again, similar reasons as for the *CYP3A5* associations for these discrepancies apply: small patient populations, mixed tacrolimus/cyclosporine cohorts, follow-up time, different organs and different *ABCB1* haplotypes. Taking these differences/limitations into account, no conclusion can be drawn regarding the association between *ABCB1* genotype and renal dysfunction in adult heart or liver transplant recipients. For pediatric liver transplant patients, an association for early (less than 1 year after transplant) renal dysfunction and *ABCB1* genotype in patients on tacrolimus may be present. In adult and renal transplant patients, no association between recipient *ABCB1* genotype and CNIT seems to be evident, although this is dependent on the definition. If IF/TA are also considered due to tacrolimus, such an association may exist.

The discrepancies in the findings regarding *CYP3A5* and *ABCB1* could also be related to one another. A possible interaction between the CYP3A and ABCB1 expression has been proposed in the past, showing higher hepatic and intestinal CYP3A4 expression in *ABCB1 2677TT* carriers than homozygotes for *ABCB1 2677G* [43]. A gender effect on this interaction has been suggested as well, with women carrying the *ABCB1 2677TT-3435TT* haplotype showing significantly higher CYP3A4 mRNA expression levels in the native intestine than *ABCB1 2677GG-3435CC* carriers. This was not found in men [44].

Smith et al. [39] reported a higher risk of developing renal dysfunction in adult liver recipients carrying the *CYP2C8*3* polymorphism. The patient group was a mixed one, but the patients receiving tacrolimus were also analyzed separately. The odds ratio for these patients was much higher at 16.67 ($CI_{95\%}$: 2.8–99.6). In spite of the very wide confidence interval, the risk is considerate since the lower limit is almost 3. The results of this study strongly suggest a role for *CYP2C8* in the risk of tacrolimus-related renal dysfunction in adult liver recipients.

The *ACE* study [40] was assigned the highest overall quality score of 25 and the highest power (99%). The liver transplant recipients group was mixed (tacrolimus or cyclosporine), but tacrolimus treatment was predominant (100 of 142 patients). The results suggest an association between *ACE* and renal dysfunction in adult liver recipients who receive tacrolimus.

The results for *TGF-β* in adult heart transplant patients are interesting with an association between this genotype (*Pro10* and *Pro25* carriers) and an increased risk for end-stage renal failure. Only a small minority received tacrolimus (21 of 402), but considering the proposed mechanism

of action in the kidney of both cyclosporine and tacrolimus, it is reasonable to assume that results may be similar if only patients on tacrolimus are studied.

For the other genes not mentioned in detail that were studied by different groups, no correlation between genotype and renal dysfunction and/or nephrotoxicity in solid organ transplant recipients was reported. Considering the limitations of the different studies discussed above, it can, however not be ruled out that these genes do not play a role in the risk to develop renal dysfunction after tacrolimus exposure.

However, the candidate-gene approach used in all of the included papers has a limitation in its design, as it only targets the genes thought to be important to the researcher are included in the design of the study. A Genome-Wide Association Study (GWAS) or a proteomic study might give a better understanding of the mechanisms involved in the pathophysiology of transplant-related diseases.

The first limitation of this review resides in the multifactorial background of renal dysfunction after transplantation. Besides the use of calcineurin-inhibitors, such as tacrolimus, other possible causes (i.e. diabetes, atherosclerosis, HCV-associated glomerulonephritis) might be the reason for the renal dysfunction experienced after transplantation [45]. Furthermore, renal biopsies are not regularly done creating difficulties tracking the progress of the renal dysfunction. Yet, even if they were, controversy exists on the specific histological signs for calcineurin-inhibitor induced nephrotoxicity [10,46]. Secondly, the study design and reporting of the some of the studies that we identified are suboptimal. Reporting can be improved by using a recently published guideline on the reporting in genetic association studies (STREGA) [47]. As we have discovered in our review, reporting of the methods in particular is sub standard. The laboratory methods, the genotyping methods as well as the error rates and call rates should be reported. The laboratory where genotyping was done should be identified as well. Additionally, it should be specified whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches, and whether or how the Hardy-Weinberg equilibrium was considered. Although the papers in this review lacked much of this information and often results were not adjusted for multiple testing, they did report on other aspects mentioned in the guideline, especially in the results section. For example, the proportions of successfully genotyped patients were reported in 9 of the 13 papers.

Conclusions

Despite the fact that the factors discussed may limit overall conclusions on pharmacogenomic variation in tacrolimus-induced nephrotoxicity, we believe that interesting candidate genes have been identified. The pharmacokinetic genes (e.g. *CYP3A5, ABCB1*) and some pharmacodynamic genes (e.g. *CYP2C8, TGF-β*) deserve further investigation – also in children. Critically, it will be important to include all the target genes in a single predictive analysis, and not isolated ones. An important finding of the present analysis is that most studies had little statistical power to detect any genotype-phenotype associations. Although we realize that obtaining a large enough sample size is difficult regarding (pediatric) transplant recipients, we feel that future studies should try and increase the number of subjects studied, by genetic meta-analysis and/or consortia formation.

Conflict of interests

The authors declared no conflict of interest.

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