Genotype Informed Bayesian Dosing of Tacrolimus in Paediatric Solid Organ Transplant Individuals

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Abstract

Tacrolimus, a calcineurin inhibitor, is an effective immunosuppressant for solid organ transplants (SOT). However, its narrow therapeutic index and high variability in pharmacokinetics can lead to inefficacy, toxicities, and suboptimal outcomes. Genotyping for CYP3A5 gene prior to SOT can identify individuals at risk of high or low tacrolimus levels and guide first-dose dosing. Genotype-guided Bayesian dosing uses population pharmacokinetic data and individual patient characteristics to accurately predict the tacrolimus dose required to achieve a target concentration. This can help achieve target tacrolimus concentrations sooner and maintain them within range, reducing risk of organ rejection or tacrolimus toxicity. This review aims to assess the benefits of genotype-guided Bayesian dosing for tacrolimus and its ability to accurately predict tacrolimus dosing, leading to increased maintenance of therapeutic drug exposure in these individuals. This systematic review identified three studies that incorporated genotyping and Bayesian informed methods to predict tacrolimus dosing in the paediatric population post SOT. The studies included 369 kidney, 231 heart, 246 liver and 16 lung transplant individuals. The review found that combination of clinical, demographic, and genetic data has a significant influence on tacrolimus clearance. Combining these parameters allowed the prediction of first dose tacrolimus post SOT and ongoing therapeutic tacrolimus dosing to optimally maintain target tacrolimus levels. In conclusion, personalised tacrolimus dosing models in paediatric SOT can be developed using clinical, demographic, and genetic data to predict first dose and ongoing adjustments to meet therapeutic tacrolimus targets and reduce the risk of under- and over- exposure.

INTRODUCTION

1.1 Tacrolimus

Tacrolimus is a calcineurin inhibitor classified as a 23-membered macrolide antibiotic produced by the bacterium Streptomyces tsukubaensis(1). It is a potent immunosuppressant used to prevent organ rejection after transplantation. Tacrolimus’ mechanism of action involves binding to the intracellular immunophilin FK506-binding protein 12 (FKBP12) forming a complex that blocks the action of calcineurin, a key enzyme in T-cell activation. By blocking calcineurin, tacrolimus suppresses the immune system and prevents organ rejection post-transplantation(2).

Tacrolimus has a narrow therapeutic index, and can vary highly due to an individual’s pharmacokinetics (PK)(4) leading to incomplete effectiveness, toxicities and suboptimal outcomes(5). To enhance efficacy and reduce adverse effects, therapeutic drug monitoring (TDM) is conducted regularly post solid organ transplant (SOT).
1.2 TDM and the impact of CYP3A5 genotype

The most accurate approach to measuring tacrolimus exposure is area under the serum concentration versus time curve for 0-12 hours (AUC$_{12}$). Although accurate, this is impractical as it requires multiple blood samples over the dosing interval period with patient burden and financial constraints. Therefore, a simpler strategy is adopted for measuring tacrolimus levels in clinical practice. At a single time point called the trough concentration ($C_0$), a blood sample is taken from which dose adjustments are made. TDM cannot predict how a patient will respond to tacrolimus in the immediate post-transplant period, a critical timepoint for preventing graft rejection. With advances in pharmacogenomics, individual genotyping can help predict the most appropriate first dose of tacrolimus, minimising the potential incidence of organ rejection.

Cytochrome P450 (CYP) 3A4 and 3A5 are the main liver and intestinal enzymes that metabolise tacrolimus. Studies have shown a link between an individual’s CYP3A5 genotype and tacrolimus clearance (6). Individuals who are phenotypic expressers (extensive $1/*1$ and intermediate $1/*3$) of the CYP3A5 gene require a lower tacrolimus dose-adjusted $C_0$ and decreased chance of achieving target tacrolimus concentrations and as such require a higher first dose of tacrolimus when compared to those who are CYP3A5 phenotypic non-expressers (poor metabolisers, $*3/*3$) (6). Over 40% of PK variability is predictable based on an individual’s CYP3A5 genotype along with other measurable covariates (7, 8).

Despite the evidence that CYP3A5 genotyping can help with accurately dosing tacrolimus however, to date, routine genotyping is not standard of care for SOT. Standard of care involves weight-based tacrolimus dosing with target concentrations ranges. This “trial and error” approach, as opposed to personalised approach, can substantially delay time to therapeutic target levels in certain individuals (9).

The potential advantages of genotype-informed Bayesian dosing include reducing time to reach and time within target tacrolimus concentrations and thus the opportunity to reduce the risk of rejection (10-12), donor-specific antibody formation (13, 14), post-transplant malignancy and graft loss (15). Guidelines for CYP3A5 genotype and tacrolimus dosing are published by the Clinical Pharmacogenetics Implementation Consortium (CPIC) (6) and Dutch Pharmacogenetics Working Group (DPWG) (16). The recommendations include starting doses 1.5 to 2 times higher than standard tacrolimus dosing (max: 0.3mg/kg/day) in individuals who are intermediate or extensive expressers (6).

1.3 Bayesian dosing

An alternative to measuring single timepoints of tacrolimus concentration alone is to enrich the ability of the measure(s) to predict an individual’s apparent drug clearance using maximum a posteriori Bayesian estimation (MAPBE). MAPBE is a pharmaco-statistical technique that uses information about a drug’s pharmacokinetic behaviour and variability (incorporated in a population PK model, the Bayesian prior), as well as an individual’s characteristics and measured drug concentrations, to accurately predict the dose required to achieve a target concentration (17). Bayesian prediction takes into account previous tacrolimus levels and predicts dose adjustments accordingly. Bayesian dosing is superior to clinician based TDM as proven in randomised controlled trials in renal transplantation (18, 19). This remains to be proven in paediatric practice.

1.4 Adult population models

Adult population models have been created with successful internal and external validation to allow the prediction of tacrolimus PK (20, 21). In adult renal transplant recipients, demographics (age, body surface area, lean bodyweight), clinical (albumin, serum creatinine, haematocrit) and genetics (CYP3A4 and CYP3A5 genotype) are covariates that significantly affect tacrolimus PK in the first 3 months post-transplant (20). Optimal individualised models for first tacrolimus have been created i.e. Andrews et al developed a model incorporating CYP3A5 genotype, age and BSA. This group found that their model allowed more individuals to be within the target range compared to standard body-weight based dose methods (33% vs 26%) (20). Similarly, Llboberas et al (19) model showed a significantly higher proportion of individuals (54.8%) achieved tacrolimus therapeutic target quicker using their model compared with the control group (20%).
A gap in the literature in paediatrics exists, whereby whilst the impact of CYP3A5 genotype has been studied in heart, liver, kidney transplantation, the combination of a MAP Bayesian in conjunction with genotyping is lacking. This systematic review (SR) aims to describe the current literature in genotype-informed Bayesian dosing for tacrolimus in paediatric.

METHODS

This SR was conducted according to the guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement (PRISMA)(22) and registered with the International Prospective Register of Systematic Reviews (PROSPERO)(2023 CRD42023401179)(23).

2.1 Eligibility Criteria

Studies had to include paediatric or adolescent individuals aged less than 18 years old who had received a SOT with tacrolimus included in their post-transplant immunosuppressant. In addition, studies had to investigate genotype-based dosing and Bayesian models to predict tacrolimus dosing. SOTs included liver, heart, kidney or lung. Intestinal transplants were excluded due to complications in using genotype for prescribing. Articles were included if full text and published in English no earlier than 2012. Only original studies were included from randomised, non-randomised and cohort studies and we excluded other reviews, protocols, editorials, conference abstracts, case studies, non-peer reviewed and ongoing studies.

2.2 Search Methods

Advanced literature searches were conducted in March 2023 in Medline (Ovid), Embase (Ovid) and Cochrane CENTRAL (Wiley). Date of coverage was restricted to 2012 onwards and searches were limited to English. In Medline, the search strategy consisted of a combination of exploded subject headings (MESH) and various keywords to identify the literature. Subject headings applied in Medline included: “organ transplantation”, “tacrolimus”, “pharmacogenomic testing”, “genotype”, “bayes theorem”, “pediatrics”. The subjects were combined in their associated cluster groups with keywords where all word variations were searched. These included “tacro”, “pgx”, “cyp3a”, “bayes dosing”. The adjacency operator was used in some instances that linked words in proximity to one another. The “AND” was applied to all separate concepts to yield relevant results. The search in Embase followed a similar strategy to Medline with variations according to its subject thesaurus (Emtree). In Cochrane CENTRAL, keyword combinations were used.

2.3 Article Appraisal

Results generated were uploaded to SR software, Covidence©, a web-based collaboration software platform that streamlines the production of systematic and other literature reviews(24) . Two reviewers independently reviewed abstracts and full text for inclusion and exclusion criteria in a two-stage screening process. Any conflicts were resolved through collaborative consensus.

Reference lists of included studies as well as any relevant review articles were cross-checked to ensure that all studies had been obtained. Each study was assessed for risk of bias by both reviewers using the Newcastle-Ottawa Scale (NOS), a tool for evaluating the quality of non-randomised studies, such as cohort and case-control studies(25).

2.4 Data Extraction and Analysis

The data was extracted independently by two reviewers using Covidence focusing on pred-defined variables. These extracted variables included general information, study characteristics (country, study design, aim), patient demographics, covariates tested, study outcome, type of pharmacogenomic testing, and any data for dosing algorithms.
The primary study outcomes of interest were discovering genotype-informed Bayesian dosing of tacrolimus and the outcomes included tacrolimus doses, levels, significant covariates, and clinical outcomes. Data was analysed for common themes including significant covariates.

RESULTS

3.1 Study Selection

Following a systematic search strategy, 999 articles were identified of which 897 underwent title and abstract screening. Using the Covidence© software, only 16 were identified as duplicates with a further 89 duplicates identified during title and abstract screening. Full text review was then carried out on 110 articles. Following a two-reviewer screening, three studies fulfilled eligibility criteria (Table 1).

3.2 Study Characteristics

The SR of the three (26-28) studies included 369 kidney, 231 heart, 246 liver and 16 lung transplant individuals enrolled across one retrospective study and two prospective studies. The intervention was genotype-informed Bayesian dosing of tacrolimus and the outcomes included tacrolimus doses, levels, significant covariates, and clinical outcomes. (Table 2) describes the characteristics included in each study.

3.3 Quality assessment of the included studies

NOS was used by the reviewers to independently assess the risk of bias for the three studies. The NOS consists of categories including selection, comparability and outcome or exposure for quality assessment.

3.4 Overview of included studies

The three articles differed in their findings and methods, but all used twice-daily dosing regimen of the immediate-release tacrolimus preparation, and developed or used a predictive model that incorporated genotyping. The first two articles were written by the same author. The first article developed a model, and the second article applied the model prospectively.

3.4.1 Retrospective study by Andrews et al (2018)

This retrospective study was conducted in the first 6 weeks post-transplant creating a PPK model validated for 2–18-year-olds. A total of 722 blood samples were collected from 46 post renal transplant individuals during the 6 weeks post renal transplant. All individuals were treated with the TWIST protocol (basiliximab, tacrolimus, mycophenolic acid, and a 5-day course of glucocorticoids) and were started on a first dose tacrolimus dose of 0.3mg/kg/day in 2 divided doses. Genotyping for CYP3A5 and CYP3A4 was recorded. Covariates used in model development were selected based on its theoretical relation with tacrolimus PK and included demographic, clinical, and genetic factors as listed in (Table 4). Any covariates that significantly improved the model (p<0.05) were added to the full model. The covariates; CYP3A5 genotype, donor (deceased or alive), hematocrit, bodyweight, and estimated glomerular filtration rate (eGFR) together accounted for 41% of the apparent variation in tacrolimus clearance. The final algorithm for predicting the starting dose of tacrolimus post renal transplant included CYP3A5, bodyweight and donor status. The tacrolimus dosing guidelines in this study ranged from 0.27 – 1.33mg/kg/day.

A strength of this paper was the extensive evaluation of the final model which included visual predictive checks (VPCs), bootstrap analysis and a normalised prediction distribution errors (NPDE) together with an external validation(29). Another advantage of this study was the uniformity of immunosuppressive therapy streamlining the PPK model development.

However, the main limitation of this study is that it was retrospective and relied heavily on data available in the medical records. Due to its retrospective nature, cohort control was limited resulting in fewer CYP3A5
expressors in the validation group. Furthermore, tacrolimus analysis methodology changed from immunoassay to liquid chromatography–tandem mass spectrometry (LC-MS/MS) partway through study. This was accounted for in the error model. The study concluded that the tacrolimus weight-normalized starting dose should be higher in (i) CYP3A5 expressors (CYP3A5*1), (ii) individuals with lower bodyweight and, (iii) individuals receiving a cadaveric kidney donation.

3.4.2 Prospective study by Andrews et al (2020)

The authors from the previous study used the developed PPK model to predict the individual first dose of tacrolimus for each individual in this single arm, prospective study. The study was powered to include 28 individuals however, an interim analysis revealed inaccuracy in the predicted exposure leading to early study termination.

All individuals were treated with the TWIST protocol and had pre-emptive CYP3A4 and CYP3A5 genotyping. The primary endpoint was the proportion of individuals with target tacrolimus C0 range at Day 3. Secondary endpoints included i) proportion of individuals with target tacrolimus ranges at Day 7 and 10 and, ii) proportion of individuals with sub- and supra- therapeutic tacrolimus levels at Day 3.

At interim analysis (n=16 children), at Day 3 post-transplant, five individuals (31%) had a tacrolimus C0 within the target range (10–15 ng/mL), 31% had a supratherapeutic C0, and 38% a subtherapeutic C0. Ideally, this primary endpoint should have been 55%. 20% of the individuals had a tacrolimus C0 within the target range at day 7, compared with five individuals (36%) at day 10.

A strength of this study was using a previous model that had been extensively evaluated, including VPCs, bootstrap analysis, NPDEs and external validation. However, the model had been validated on a small sample size. In the original study Andrews 2018, two out of the 46 individuals were CYP3A5 expressors and a recipient of a cadaveric kidney. This was insufficiently powered to determine prediction in this model. This resulted in the algorithm predicting significantly higher doses (i.e., 0.80 mg/kg/day) in individuals who were CYP3A5 expressors and received a cadaveric kidney (n=3). This led a new dosing algorithm creation (Table 5). The authors in the previous study claimed that one the key covariates they found was donor type (deceased or living donor), in saying this, there is no theoretical basis for inclusion of this covariate, and as they report no prior identification this could have contributed to the inaccuracy of the original algorithm.

This study showed that even though a dosing algorithm may be validated in a study, it does not guarantee that it will be effective in clinical practice, emphasizing the importance of testing algorithms in prospective studies prior to implementation. This study led to an improved dosing algorithm in which tacrolimus weight-normalized first doses were higher in individuals with a lower bodyweight and in CYP3A5 expressors (CYP3A5*1). Although not yet externally validated, this second algorithm shows promise and warrants prospective testing.

3.4.3 Prospective observational study by Min et al (2022)

This study was a prospective observational study that included enrolment criteria for paediatric patients with any SOT with a follow-up period of > 12-months. The study was a genome-wide association study (GWAS) to identify genetic variants associated with tacrolimus levels in paediatric transplant recipients. The GWAS showed that 25 single nucleotide polymorphisms (SNPs) were correlated with tacrolimus C0. Eight of these SNPs were significant at a genome-wide level. A machine learning approach was used to build a predictive model. A significant interaction between age and organ type with rs776476*1 was identified.

Tacrolimus levels were observed to be higher in liver, lung and heart in CYP3A5 non expressors (p<0.01), but greater in heart transplant compared to other organs (p<0.01). 14 SNPs linked with tacrolimus C0 in heart, 11 in kidney and 6 in liver (p<0.05). Lung transplantation was not analysed due to low numbers in both the validation and discovery group.

There was no association between donor genotype for the 14 SNPs with tacrolimus C0 in either liver or non-liver transplant recipients, however the study was underpowered to prove or disprove this association.
The age of the individual also played an important role in predicting tacrolimus levels, with younger children having higher levels and therefore at increased risk of toxicity. Although CYP3A5 expression is independent on age, the same cannot be said for CYP3A4. This enzyme demonstrates low activity in fetuses and gradually increases to reach adult levels in children between 1 and 5 years old. Interestingly, CYP3A4 shows higher activity in adults compared to CYP3A7, which is more active during infancy.

Importantly, the predictive model developed by Min et al. is a statistical model that is used to predict tacrolimus dosing based on clinical and genetic data, as opposed to Andrews et al (31, 32) which used clinical and genetic information in developing the popPK model.

This study adjusted the final algorithm for genetic ancestry differences between ethnic groups by including covariates in the initial discovery analysis.

One of the limitations of this study was the heterogeneity of standard immunosuppressant regime dosing post transplantation. Immunosuppression varied by SOT type, treating center, and starting dose of mycophenolate, tacrolimus, and glucocorticoids. There was also statistically significantly more Caucasians in this study and more kidney transplants in validation than intervention (29% vs 47%, p<0.001). This predictive model focused on first dose tacrolimus dosing as opposed to maintenance dosing.

One of the benefits of using GWAS pharmacogenetic discovery was that it allowed the identification of additional predictive SNPs on chromosome 7 (on CYP3A4, CYP3A5, CYP3A7, and CYP3A7-CYP3AP1 genes) and not just the common rs776746 (CYP3A5). This study developed an integrated predictive model that incorporated organ-specific differences, demonstrating the value of incorporating age, organ type, and genotype into tacrolimus dosing algorithms. The model demonstrated a 30% accuracy in predicting tacrolimus levels, significantly outperforming clinical or genetic models alone. The finding of this study suggests that it is possible to personalize tacrolimus dosing for paediatric SOT individuals based on genetic makeup, potentially leading to improved individual outcomes.

3.5 Commonalities amongst studies for genotype and significant covariates discovered.

All three studies had several covariates investigated that have proven importance for tacrolimus dose algorithms (Table 4 and 5). CYP3A5 genotype plays a vital role in the first dose prediction of tacrolimus dose. Body weight and organ type (living vs. deceased) are also pivotal covariates that are needed for Bayesian dosing.

**DISCUSSION**

4.1 Main Findings

This review is the first to summarise the use of Bayesian dosing of tacrolimus combined with genotype in paediatric SOT. CYP3A5 genotyping before tacrolimus initiation is needed to determine if Bayesian dosing improves clinical outcomes in paediatric SOT with successful predictive tacrolimus models in adult SOT.

Bayesian forecasting algorithms of tacrolimus have predominately been conducted in adult kidney transplant. A recent RCT by Liboberas et al(19) found CYP3A5 genetic variability linked to individual differences in tacrolimus metabolism and exposure, can affect an individual’s drug responses. This supports the idea that CYP3A5 genetic variability is a key factor in determining tacrolimus metabolism and exposure, which can affect drug response, as has been described in the literature.

With respect to paediatric SOT our review shows that there is a paucity of literature to date. Whilst Andrews et al.(26) developed a predictive model in paediatric renal transplant. When tested clinically(27) the results were sub-optimal, likely related to small validation cohort sizes and most likely due to model misspecification on the use of donor type as covariate. This highlights the importance of power calculations in mechanistic modelling, together with the need to think about broad heterogenous groups within validation cohorts. Whilst larger cohort sizes are needed to develop predictive algorithms across all SOT, there is a need for collaboration given the paucity of patients globally.
The spectrum of individual CYP diplotypes and the effect on tacrolimus levels remains an ongoing area of research in the international literature(30). CYP3A4*22 diplotype may warrant inclusion in predictive algorithms but requires further study. The genome-wide association study comprising 1,345 European Americans (a sub-cohort from the Deterioration of Kidney Allograft Function (DeKAF) genomics study) carrying CYP3A5 loss-of-function alleles (*3,*6, or *7) found an additional association of the CYP3A4*22 with tacrolimus C0 levels(1). The recent study by Lloberas et al.(19) found that in adults those with CYP3A4*22 allele required 20% less tacrolimus than CYP3A4*1 to reach therapeutic levels, independent of CYP3A5 genotype. Andrews et al (2018)(26) did not include any patients with CYP3A4*22 and thus could not verify the association in children. In August 2023, via direct correspondence, CPIC discussed the potential evolving evidence of using both CYP3A4 and CYP3A5 diplotypes to guide dosing recommendations and this is the subject of ongoing guideline development(30).

4.2 Limitations of the review

Few studies met the criteria for this review. The small number of studies reflects the paucity of literature and the need for further research. Having only searched for studies in English text may have excluded other relevant studies. All three studies were single centered based in Netherlands and Canada, with a significant population of Caucasians. Therefore, results may not be generalisable globally.

Our search was limited to studies published in the last 10 years, and all studies had a minimum age requirement of 2 years. This means that the predictive algorithms are not relevant for those < 2 years, whom arguably have the greatest ontogeny and variance in achieving tacrolimus levels. Furthermore, studies outside of these dates, may have developed predictive algorithms relevant to those < 2 years old. As the field of predictive algorithms is rapidly evolving, it is important to consider studies from a variety of SOT timepoints, and global immunosuppression approaches to gain a complete picture of the impact of genotype informed tacrolimus dosing and significant covariates.

Not everyone expresses the CYP3A5 enzyme, but CYP3A5 expressors metabolize tacrolimus at faster rates. Unsurprisingly, individuals with CYP3A5 expression typically need 1.5 to 2.5 times the usual dose of tacrolimus to achieve the desired level of exposure(6). CYP3A5 is expressed in 10-15% of Caucasians, 40-50% in Africans, and 50-70% in the Asian populations(31). Ethnicity can play a role in the CYP3A5 allele variation as can been seen in various studies. In the Mexican population this gene is expressed in 50% of individuals(32).

Andrews et al(26) cohort had limited CYP3A5 expressors (likely a reflection of cohort ethnicity) hence the dosing algorithm was inaccurate.

4.3 Future directions

One important gap in the literature is the lack of studies on Bayesian prediction of tacrolimus dosing in paediatric SOT individuals that consider pharmacogenomic factors. We recognize that one model may not be applicable to all SOT types, and there is a lack of validation of predictive models in liver, lung, and heart transplant individuals. While an individual’s genotype is a factor in predicting tacrolimus dosage, other factors, both clinical and genetic, play a role.

Overall, genotype-informed Bayesian dosing is a promising approach to predicting tacrolimus dosage in paediatric SOT individuals. It can help to improve the accuracy of dosing and reduce the need for dose adjustments. However, further implementation of such models is required to validate this approach in larger-scale clinical trials.

CONCLUSIONS

Although prospective studies are lacking, literature to date has demonstrated that the combination of clinical, demographic, and genetic data, can be used to develop a personalised model to estimate tacrolimus dosing.
CYP3A5 genotype-informed initial dose and ongoing Bayesian revised dosing using a combination of clinical and genetic prediction models are needed to better understand and guide individualised tacrolimus dosing in the paediatric SOT population and prove the benefits on time to therapeutic dose, time in range and avoidance of SOT related complications and rejection.

REFERENCES


24. Veritas Health Innovation M, Australia. Covidence systematic review software. Veritas Health Innovation, Melbourne, Australia. p. Covidence is a web-based collaboration software platform that streamlines the production of systematic and other literature reviews.


<table>
<thead>
<tr>
<th>Inclusion</th>
<th>Exclusion</th>
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<tbody>
<tr>
<td>Population</td>
<td>Adults (&gt;18yo)</td>
</tr>
<tr>
<td>Intervention/ Condition of interest</td>
<td>Not SOT. Tacrolimus dose is IV.</td>
</tr>
<tr>
<td>Outcome</td>
<td>Tacrolimus levels. Tacrolimus dose. Significant Covariates.</td>
</tr>
<tr>
<td>Other</td>
<td>Published in last 10 years. English Language. Full text.</td>
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Table 1: Eligibility criteria for systematic review. Abbreviations: IV: intravenous; SOT: solid organ transplant.
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country</th>
<th>Study design</th>
<th>Sample size</th>
<th>Organ Transplant</th>
<th>Purpose of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrews et al, 2020</td>
<td>Netherlands (2 sites)</td>
<td>single-arm, prospective</td>
<td>16 (at interim analysis)</td>
<td>Kidney</td>
<td>To use a PPK model developed by the same author to prospectively predict tacrolimus dosing in paediatric renal transplant recipients.</td>
</tr>
<tr>
<td>Min et al, 2022</td>
<td>Canada (7 sites)</td>
<td>prospective observational</td>
<td>Discovery group (n=445) Validation group (n=322)</td>
<td>Kidney Heart Liver Lung</td>
<td>To identify genetic variants that are associated with tacrolimus levels and to develop a predictive model for tacrolimus levels in paediatric SOT recipients.</td>
</tr>
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</table>

Table 2: Characteristics included in each study. Abbreviations: PPK: population pharmacokinetic; SOT: solid organ transplant.

<table>
<thead>
<tr>
<th>Study, year</th>
<th>Selection*</th>
<th>Comparability+</th>
<th>Outcome*</th>
<th>Overall</th>
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<tbody>
<tr>
<td>Andrews et al, 2018</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>7 / 9</td>
</tr>
<tr>
<td>Andrews et al, 2020</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>7 / 9</td>
</tr>
<tr>
<td>Min et al, 2022</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>8 / 9</td>
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Table 3: Quality assessment using Newcastle-Ottawa Scale.

Abbreviations: *Maximum rating 4, †Maximum rating 2, #Maximum rating 3.
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<tr>
<th>Author, Year</th>
<th>Study Period</th>
<th>Assay</th>
<th>Sample times post-dose</th>
<th>Method of analysis</th>
<th>Covariates investigated</th>
<th>PK Model and Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrews et al. (2018) (26)</td>
<td>24 hours before transplantation until 6 weeks post-transplantation</td>
<td>LC-MS/MS and immunoassay methods</td>
<td>Samples: 722 tacrolimus levels At least 1 PK profile after 2 weeks transplant: tacrolimus bloods at : pre-dose, and at 10, 30, 90, 120, and 240 min post-dose (4h tacrolimus concentration vs time profile data)</td>
<td>Weight, height, time post-transplant, sex, age, ethnicity, hematocrit, creatinine, AST, albumin, C-reactive protein, total protein, CYP3A4 CYP3A5, concomitant medications, glucocorticoid dose, primary diagnosis, previous transplantation, renal replacement therapy prior to transplantation, donor status, HLA-mismatched.</td>
<td>PK analysis: two-compartment model with allometric scaling for bodyweight. NONMEM. Validation of PK model: bootstrap, VPCs, and an NPDE were performed, and the model externally validated.</td>
<td></td>
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<tr>
<td>Author, Year</td>
<td>Study Period</td>
<td>Assay</td>
<td>Sample times post-dose</td>
<td>Method of analysis</td>
<td>Covariates investigated</td>
<td>PK Model and Evaluation</td>
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<tr>
<td>Andrews et al. (2020) (27)</td>
<td>Followed for 10 days post organ transplant</td>
<td>LC-MS/MS</td>
<td>Samples: tacrolimus levels on Day 3, 7 and 10</td>
<td></td>
<td>Weight, height, time post-transplant, sex, age, ethnicity, haematocrit, creatinine, est GFR, AST, albumin, C-reactive protein, CYP3A4, CYP3A5, concomitant medication, steroid dose, primary diagnosis, number of transplantations, renal replacement therapy prior to transplantation, donor status, HLA- mismatch.</td>
<td>PK analysis: two-compartment model with inter-individual variability, allometric scaling. Validation of PK model: VPCs, and an NPDE were performed, Final model internally validated.</td>
</tr>
<tr>
<td>Min et al, 2022 (28)</td>
<td>12months +/- 3 months</td>
<td>LC-MS/MS</td>
<td>Samples: 3649 tacrolimus levels. Taken at 6 time-points: 36-48 hours post-dose, at 7 days, 14 days, 30 days, 3 months and 12 months post-transplant</td>
<td></td>
<td>Weight, height, sex, age, Race/ethnicity, Organ type, concomitant medications (e.g., corticosteroids, CYP3A4 inhibitors), est GFR, albumin, C-reactive protein, total protein, genotype for 25 SNPs associated with tacrolimus levels</td>
<td>No PK modelling. <strong>Multivariate analysis</strong>: linear regression, logistic regression, and random forest. Validation of predictive model: holdout set, a validation cohort, cross-validation, and bootstrapping.</td>
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</table>
## TABLE 4: Data collected, investigation and analysis included in each study. Abbreviations: LC-MS/MS: liquid chromatography–tandem mass spectrometry; PK: Pharmacokinetic; Non-linear mixed-effects modelling (NONMEM); AST: Aspartate transferase; CYP: Cytochrome P450; est GFR: estimated Glomerular filtration rate, HLA: human leukocyte antigens; SNPs: single nucleotide polymorphisms; VPC: visual predictive checks; NDPE: normalised prediction distribution errors.

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Patient</th>
<th>Dose prediction Final Algorithm</th>
<th>Significant covariates discovered</th>
<th>Results</th>
<th>Findings and conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Andrews et al. (2018) (26)</strong></td>
<td>Model group (n=46) Age: 9.1 (2.4-17.9) Validation group (n=23) Age: 8.2 (1.6-17.1)</td>
<td>Dose = 54.9 x [weight/ 70]^{0.75} X [1.8 if CYP3A5*1/<em>3 OR CYP3A5</em>1/*1] X [0.74 if living donor] X AUC</td>
<td>CYP3A5 genotype, bodyweight, and donor type.</td>
<td>Dosing algorithm that includes genotype was created which is to be tested prospectively.</td>
<td>The tacrolimus weight-normalized starting dose should be higher in individuals who are CYP3A5 expressors (CYP3A5*1), with a lower bodyweight and in individuals receiving cadaveric kidney</td>
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<td><strong>Andrews et al. (2020) (27)</strong></td>
<td>16 (at interim analysis) Age: 15.0 (4.6–16.8) (Living donor 11, Deceased donor 5)</td>
<td>Dose = 34.5 x [weight/ 70]^{0.56} X [1 if CYP3A5*3/*3] OR [1.46 if CYP3A5 *1/*3 or CYP3A5 *1/*1 ] X AUC.</td>
<td>CYP3A5 genotype and bodyweight.</td>
<td>Interim results: On Day 3, 31% had a tacrolimus C₀ within the target range (10–15 ng/mL), 31% had a supra-therapeutic C₀, and 38% had subtherapeutic C₀. 20% had a tacrolimus C₀ within the target range on day 7, compared with 36% on day 10.</td>
<td>Validation on paper does not necessarily means effective in clinical practice. Dosing algorithms should first be tested in prospective studies. The tacrolimus weight-normalized starting dose should be higher in individuals who are CYP3A5 expressors (CYP3A5*1), with a lower bodyweight.</td>
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<tr>
<td>Author, Year</td>
<td>Patient</td>
<td>Dose prediction Final Algorithm</td>
<td>Significant covariates discovered</td>
<td>Results</td>
<td>Findings and conclusions</td>
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<td>Min et al, 2022 (28)</td>
<td>Discovery group (n=445) Age: 4.5 (1.0–11.7) Validation group (n=322) Age: 6.1 (2.0–13.1) Kidney n = 133 Heart n = 151 Liver n = 161 Lung n = 10 Discovery Kidney n = 151 Heart n = 80 Liver n = 85 Lung n = 6 (validation)</td>
<td>Organ type, age at transplant, and genotype; SNPs rs776746 (CYP3A5), rs12333983 (CYP3A4), and rs12957142</td>
<td>Genome-wide association study (GWAS) Identified 25 SNPs of which 8 SNPs significantly linked with tacrolimus levels. Organ specific: Tacrolimus levels higher in liver, lung and heart in CYP3A5 non expressors (p&lt;0.01), but greater in heart compared to other organs (p&lt;0.01). 14 SNP linked with tacrolimus level in heart, 11 in kidney and 6 in liver (p&lt;0.05) lung-too small. NO association of donor genotype for the 14 SNPs with tacrolimus level in both liver and non-liver individuals.</td>
<td>GWAS approach to pharmacogenetic discovery that included all paediatric solid organ transplant recipients. This allowed the identification of additional SNPs on chromosome 7 (on CYP3A4, CYP3A5, CYP3A7, and CYP3AP1 genes) beyond rs776746 (CYP3A5) and also allowed the development of an integrated prediction model that included organ-specific difference.</td>
<td></td>
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</table>

**TABLE 5:** Significant Covariates and results found in each study. Abbreviations: CYP: Cytochrome P450; AUC: area under the curve; SNPs: Single nucleotide polymorphisms, GWAS: Genome-wide association study; ng/mL: nanograms per millilitre.

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