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Sex and Haplotype Associations with Adverse Effects of Calcineurin Inhibitors Post-Renal Transplant

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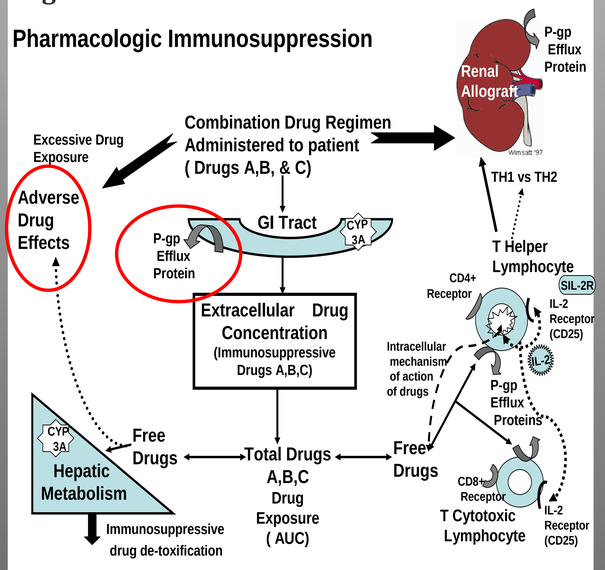
ABSTRACT

BACKGROUND: P-glycoprotein (P-gp), an ABC transport protein contributes to the interpatient pharmacokinetic and pharmacodynamic variability of calcineurin inhibitors(CNI), tacrolimus(TAC) and cyclosporine (CYA). *ABCB1* encodes P-gp and the single nucleotide polymorphisms (SNP) 1236C>T, 2677G>T/A, 3435C>T may alter protein expression or function. Our objective was to examine the association of *ABCB1* haplotypes, sex and race with chronic CNI adverse effects (AE) in renal transplant recipients (RTR).
METHODS: A meta-analysis of 3 prospective observational studies was completed in 149 stable RTR [GFR= 51 ±17 ml/min/1.73m2] using identical inclusion and exclusion criteria in 62 African Americans (AA) and 81 Caucasians (C) treated with CYA (troughs: 50-150 ng/ml) and mycophenolate mofetil or TAC (troughs: 5-10 ng/ml) and mycophenolate sodium. Each RTR had AE assessed using standardized objective scales by study physicians. A Cumulative AE ratio was determined using 14 AE. Separate gastrointestinal (GI), central nervous system (CNS), and aesthetic AE ratios were also assessed. DNA from peripheral blood mononuclear cells was collected to characterize *ABCB1* SNPs completed on 11/15/12. Haplotype computation and association with AE was completed by THESIAS program on 12/3/12.
RESULTS: All genotypes were in Hardy-Weinberg equilibria. AA had a greater frequency of the C-G-C haplotype (SNPs: 1236-2677-3435) compared to C (71.6% vs. 44.2%; p<0.001). A gender difference was noted for Cumulative (p<0.001); GI (p=0.046); aesthetic (p=0.0002) and CNS (p=0.051) AE ratios with greater AE ratios in females. The Aesthetic AE ratio was associated with haplotype T-T-C (p=0.008). Haplotype C-T-T was associated with increased GI AE ratio (p=0.02) though the effect was not significant when sex was included as a covariate (p=0.13). Race had no associations with AE.
CONCLUSION: RTR receiving CNI based immunosuppression within the therapeutic range exhibited interpatient variability in AE with associations to sex and *ABCB1* haplotypes.

INTRODUCTION

Figure 1:



METHODS

Study Design
- Meta-analysis of three observational pharmacokinetic-pharmacodynamic studies

Objectives
- Determine influence of sex and *ABCB1* haplotypes on cumulative incidence of adverse effects (AEs) associated with calcineurin inhibitor based maintenance immunosuppression post-renal transplant

Adverse Effects
- Quantitated using objective, standardized, nephrologist administered assessment tool

Genomics
- Genomic DNA isolated from 600mcl of peripheral blood mononuclear cells (Wizard® Genomic DNA Purification)
- 10ng of genomic DNA used to characterize SNPs in *ABCB1* (1236, 2677, 3435)
  - Taqman™ allelic discrimination assay
  - CFX96 Real-Time PCR detection system
- Genotype results for 141 patients utilized for haplotype analysis
- Haplotype frequencies computed by THESIAS®

Statistics
- General linear modeling employed for Cumulative, GI, and Aesthetic AE Ratios
- Logistic regression model employed for individual AEs and CNS AE Ratio
  - Type of CNI, sex, race, and race X sex interaction as base model grouping variables
- Association of haplotypes with AEs using maximum likelihood estimates with 95% confidence intervals and Chi-Square test for comparison
- Statistical analysis performed with SAS® version 9.3 and THESIAS® version 3.1

Table 1. Adverse Effect Grouping

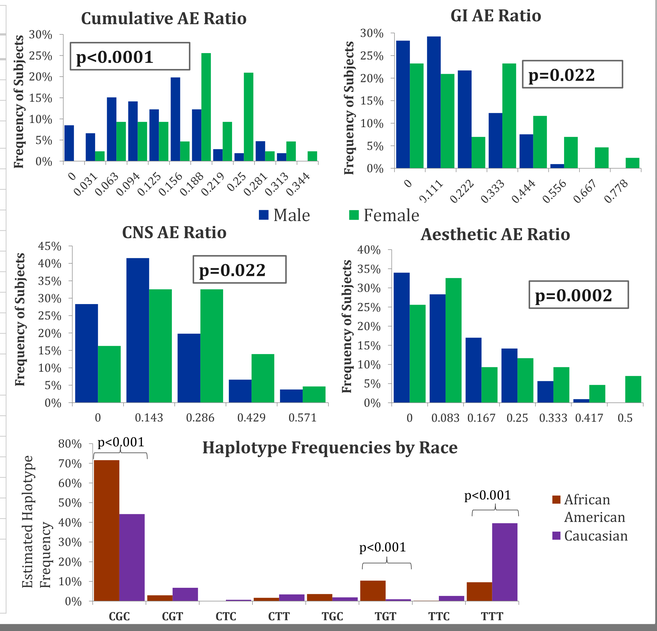
AE Group	AEs Included
Cumulative	Acne, tremor, myopathy, hirsutism, skin changes, insomnia, headache, vomiting, diarrhea, dyspepsia, PPI, H2RA, gingival hyperplasia, post-transplant diabetes
Gastrointestinal (GI)	Vomiting, diarrhea, dyspepsia, PPI, H2RA
Central Nervous System (CNS)	Headache, tremor, insomnia
Aesthetic	Acne, gingival hyperplasia, skin changes, hirsutism

RESULTS

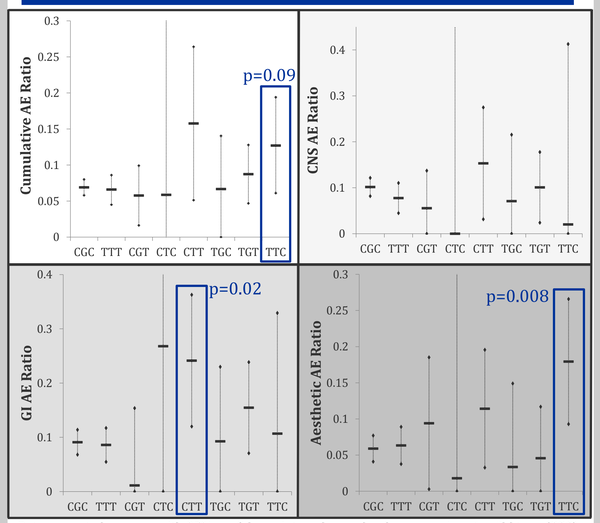
Table 2. Demographic, Laboratory, and Clinical Parameters

	Males (n=106)	Females (n=43)	P value
CYA+ MMF	**68 (64.2%)**	**14 (32.6%)**	**0.0004**
TAC+EC-MPS	**38 (35.8%)**	**29 (67.4%)**	**0.0004**
Age (years)	50.9 (10.5)	51.0 (12.1)	0.937
Time post-transplant (years)	4.28 (3.45)	3.23 (2.63)	0.089
Total weight (kg)	**94.1 (19.1)**	**76.2 (20.0)**	**<0.0001**
BMI (kg/m2)	30.5 (5.79)	28.8 (6.80)	0.063
Systolic BP (mmHg)	139 (22)	139 (24)	0.901
Diastolic BP (mmHg)	80 (12)	81 (9)	0.550
Glucose (mg/dl)	**115 (63)**	**109 (62)**	**0.037**
Total cholesterol (mg/dl)	171 (50)	182 (48)	0.220
Total WBC (cells/mm3)	5.8 (2.0)	5.6 (1.9)	0.486
Hemoglobin (g/dl)	**12.9 (1.4)**	**11.9 (1.2)**	**<0.0001**
Albumin (g/dl)	**4.1 (0.4)**	**3.9 (0.4)**	**0.010**
Serum creatinine (mg/dl)	**1.68 (0.54)**	**1.40 (0.45)**	**0.0008**
Adjusted eGFR (ml/min/1.73m2)	49.0 (16.8)	49.1 (17.3)	0.987
MPA 12hr conc. (mg/l)	**2.71 (1.96)**	**3.64 (2.20)**	**0.008**
MPAG 12 hr con. (mg/l)	84.8 (52.6)	91.8 (56.8)	0.546
Adjusted MPA Dose (mg)	**701 (212)**	**607 (180)**	**0.011**
TAC trough (ng/ml)	7.3 (2.0)	7.1 (1.8)	0.604
CYA trough (ng/ml)	131 (47)	127 (46)	0.675
Prednisone use	40 (37.7%)	14 (32.6%)	0.551
Statin use	46 (43.4%)	18 (41.9%)	0.864

CYA=Cyclosporine; MMF=Mycophenolate mofetil; TAC=tacrolimus; EC-MPS=Enteric coated mycophenolate sodium; BMI=Body mass index; BP=Blood pressure; eGFR=estimated glomerular filtration rate (using 4 factor MDRD equation); MPA=Mycophenolic acid; MPAG=Mycophenolic acid glucuronide



RESULTS CONTINUED



Data presented as mean with 95% confidence intervals; p-value for comparison to wild type (CGC)

Main effects p value with sex as covariate:
- **TTC** haplotype: p=0.30 for Cumulative; ***p=0.02 for Aesthetic***
- **CTT** haplotype: p=0.13 for GI

CONCLUSIONS

- Female sex significantly increases the cumulative incidence of adverse effects associated with calcineurin inhibitor based immunosuppression
- A racial difference in *ABCB1* haplotype distribution exists
- The common *ABCB1* haplotype TTT, often identified as having decreased P-gp function, is not significantly associated with increased adverse effects
- CTT and TTC haplotypes are associated with increased GI and Aesthetic AEs, respectively
  - This association is largely driven by sex as a covariate

FUTURE DIRECTIONS

- Validation of AE assessment tool
- Additional genotyping
  - ABCC2, CYP3A4, CYP3A5, OATP1, UGTs
- Pharmacokinetic analysis

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