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***Inosine 5'-Monophosphate Dehydrogenase 1* Haplotypes and Association with Mycophenolate Mofetil Gastrointestinal Intolerance in Pediatric Heart Transplant Patients**

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Abstract

Objective—Mycophenolate mofetil (MMF), the most commonly used adjuvant immunosuppressant in pediatric heart transplantation, has frequent gastrointestinal (GI) adverse events. Single nucleotide polymorphisms (SNPs) in *inosine 5'-monophosphate dehydrogenase 1* (*IMPDH1*) may contribute to MMF GI intolerance. Phased haplotypes may have more utility than individual SNPs in candidate gene association studies for complex traits. This study defined common *IMPDH1* haplotypes and investigated whether these haplotypes influence MMF GI intolerance in 59 pediatric heart recipients.

Methods—Genotypes were assessed by Taqman analysis of *IMPDH1* rs2288553, rs2288549, rs2278293, rs2278294, and rs2228075 and haplotypes were inferred using Arlequin 3.01 software. GI intolerance was defined as diarrhea, vomiting, nausea or abdominal pain requiring MMF dose holding for >48 hours or MMF discontinuation.

Results—GI intolerance occurred in 21 patients (35.6%). Ten *IMPDH1* haplotypes were identified in this population. In univariable analyses, one haplotype was strongly associated with MMF GI intolerance with 59.1% of carriers of this haplotype experiencing MMF GI intolerance compared to 21.6% of non-carriers ($p=0.005$).

Conclusion—In this study, we identify a common *IMPDH1* haplotype associated with MMF GI intolerance in a population of pediatric heart transplant patients. This haplotype of interest did not demonstrate stronger association with MMF GI intolerance than an individual *IMPDH1* SNP.

Keywords

inosine 5'-monophosphate dehydrogenase I; haplotype; mycophenolate mofetil; pediatric heart transplantation; adverse event

Introduction

Mycophenolate mofetil (MMF; CellCept by Hoffmann-La Roche, Basel, Switzerland) is an adjuvant immunosuppressant commonly used in pediatric heart transplant recipients and is frequently associated with gastrointestinal (GI) adverse events that may require dose reduction, temporary dose holding, or drug discontinuation. Several studies have demonstrated that MMF dose reduction and discontinuation are associated with an increased risk of graft loss and rejection in transplant recipients (1–3). Though some studies have found higher MMF doses related to GI adverse events, no association between the plasma level of MMF's active metabolite, mycophenolic acid (MPA), and GI intolerance has been demonstrated (4,5). Concomitant tacrolimus therapy, female sex, and type 1 diabetes have been identified as potential risk factors of MMF GI intolerance (6). Recipient genetic variation may also contribute to the variation in the development of MMF adverse events, including GI intolerance (7).

Few studies have investigated the role of genetic polymorphisms as risk factors of MMF GI intolerance (8,9). Our recent study investigated associations between the occurrence of MMF related adverse events and several common genetic polymorphisms in genes involved in MPA absorption, excretion and pharmacologic site of action. We identified significant associations between various MMF related adverse events and selected genetic polymorphisms, including an association of MMF GI intolerance and the variant alleles of *IMPDH1* rs2278294 and rs2228075 (10). *IMPDH1* encodes inosine 5'-monophosphate dehydrogenase 1; MPA is a selective inhibitor of this target enzyme. As haplotype association studies may provide more efficient strategies for identifying genetic variants that increase susceptibility to complex conditions (11), we expanded our investigation in this study to examine the association between *IMPDH1* haplotypes and MMF GI intolerance in a population of pediatric cardiac transplant recipients.

Methods

Patient and MMF GI Adverse Event Data

As described previously (10), the study population consisted of pediatric heart transplant recipients (<21 years of age at transplantation) followed at Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center between 1995 and 2007. The protocol for the investigation of DNA polymorphisms in pediatric heart transplant patients was approved by the Institutional Review Board of the University of Pittsburgh. The patients had received mycophenolate mofetil immunosuppression at any point in their post transplantation therapy, and had provided consent and a peripheral venous blood sample for DNA studies. Patients were maintained on primary calcineurin inhibitor therapy with tacrolimus or cyclosporine. Standard MMF dosing (1200mg/m²/day) was adjusted according to patient weight, body surface area, and tolerance of adverse effects as directed by institutional protocols.

Data, including demographic data, and serial height, weight, predose MPA plasma levels, MMF dose, concomitant medications, and all instances of temporary MMF dose holding or permanent MMF discontinuation, were collected from a transplant specific database and paper and electronic records from the time of transplant to last follow up. Gastrointestinal intolerance was defined as abdominal pain, nausea, vomiting or diarrhea, not due to other specific causes, requiring MMF dose-holding >48 hours or MMF discontinuation. The decision to hold or discontinue MMF was made by the primary transplantation physician based on patients' current symptoms, past history and clinical laboratory results. If symptoms failed to rapidly improve after discontinuation or temporary hold of MMF, other causes of GI symptoms were sought. Physicians were blinded to the genotype and haplotype

status of the patients. Data collection ceased at re-transplantation, patient death or the end of the study period.

Haplotype Analysis

DNA was extracted from whole blood by a standard phenol/chloroform procedure. Genotypes were assessed for *IMPDH1* intron 1 rs2288553, intron 4 rs2288549, intron 7 rs2278293, intron 7 rs2278294, and exon 15 rs2220875 by validated TaqMan allele discrimination assays, as described previously (Applied Biosystems, USA) (10). Data analysis was processed by SDS 2.2.2 software (Applied Biosystems, USA). Sequenced controls were used to validate the assays. Haplotypes were inferred using Arlequin 3.01 software. This program tested linkage disequilibrium (LD) between paired polymorphisms using an extension of the Fisher's exact probability test through contingency tables. Arlequin estimated the gametic phase of multi-locus genotypes with the (maximum-likelihood) EM algorithm and assigned a specific pair of haplotypes to each tested sample (12).

Statistical Analysis

Haplotypes present in $\geq 15\%$ of the population were investigated for association with MMF GI intolerance. Wilcoxon non-parametric rank sum tests and Fisher's exact tests were used to compare the distribution of categorical and continuous patient characteristics, respectively, between patients who experienced MMF GI intolerance and patients free from MMF GI intolerance and between haplotype carriers and non-carriers. The percent of patients who experienced MMF GI intolerance were compared between haplotype carriers and non-carriers using Fisher's exact tests. All statistical analyses were performed using Stata IC version 11 (College Station, Texas).

Results

The patient population has been described previously (10). Briefly, 59 (33 males, 26 females) pediatric heart transplant recipients were included in this study. Most patients were White, non-Hispanic (86.4%); 3.4% were White of Hispanic ethnicity, and 10.2% were Black, non-Hispanic. The majority of patients received tacrolimus-based maintenance immunosuppression (89.8%) and 6 patients received a cyclosporine-based regimen. The median age at transplantation was 8.2 years (interquartile range 1.9, 13.5 years). The median length of time from transplantation to initiation of MMF therapy was 1.3 months (interquartile range 0.5, 8.5 months) with 27 patients (48.5%) starting MMF within a month post-transplantation. The average length of exposure to MMF therapy was 3.4 ± 2.7 years (range: 1 week to 10.2 years) with 43 (72.9%) patients having at least one year of cumulative exposure to MMF.

Symptoms of MMF GI intolerance lead to MMF dose holding >48 hours or MMF discontinuation in 21 patients (35.6%). Nine patients were discontinued from MMF therapy for symptoms of GI intolerance, and 13 patients temporarily held MMF dose for nausea, vomiting, diarrhea or abdominal pain. Only 1 patient who had temporarily held MMF dose for GI intolerance was eventually discontinued from MMF for GI symptoms during the study period. The median time from starting MMF therapy to the development of GI intolerance leading to dose holding or drug discontinuation was 9.9 months (interquartile range: 1.6, 21.7 months). The distribution of the following patient characteristics did not differ between patients who experienced GI intolerance and those who were free from GI intolerance ($p>0.10$): race, gender, age at transplantation, time from transplantation to start of MMF therapy, average daily MMF dose per kilogram body weight, and average predose MPA plasma level (Table 1). The percent of patients who received tacrolimus therapy was

95% (20 out of 21 patients) among patients with GI intolerance and 87% (33 out of 38 patients) of patients without GI intolerance; this difference was also not significant ($p>0.10$).

Ten different haplotypes were identified in this population. Five of these haplotypes were present in $\geq 15\%$ of the population (Table 2). No significant differences were detected between haplotype carriers and non-carriers with respect to gender, race, age at transplantation, primary immunosuppression, time to start of MMF following transplantation, length of exposure to MMF, average daily MMF dose per kilogram body weight, and average predose MPA plasma level for any of the five specified haplotypes ($p>0.05$).

We found haplotype B to be significantly associated with MMF GI intolerance leading to dose holding >48 hours or drug discontinuation. Among carriers of haplotype B, 13 out of 22 (59.1%) patients experienced MMF GI intolerance; in comparison, only 8 out of 37 (21.6%) non-carriers of haplotype B experienced MMF GI intolerance ($p=0.005$) (Figure 1). Among non-carriers of haplotype A, 48.4% experienced MMF GI intolerance whereas 21.4% of carriers of haplotype A experienced GI intolerance ($p=0.055$). None of the other common haplotypes were significantly associated with MMF GI intolerance.

Table 3 compares the test properties of haplotype B with the two individual SNPs that we found to be significantly associated with MMF GI intolerance in this population in our previous study (10). In this population, the rs2278294 A allele had the highest sensitivity for MMF GI intolerance (71.4%). Haplotype B and the rs2228075 A allele each had a specificity of 76.3% for MMF GI intolerance. An A allele at rs2228075 had the best positive (60.9%) and negative predictive values (80.6%) for MMF GI intolerance.

Discussion

MMF GI intolerance was common in our study population with 36% of patients holding MMF dose or discontinuing MMF due to GI intolerance. This prevalence of MMF GI intolerance is comparable to rates of GI intolerance previously reported in pediatric transplant patients (13,14). No difference in the average daily MMF dose or MPA plasma levels between patients experiencing GI complications and those free from GI symptoms was observed. However, one of the five common *IMPDH1* haplotypes in our population was significantly associated with GI intolerance with a positive predictive value of 59.1% and a sensitivity of 61.9% for MMF GI intolerance. Included in this haplotype were the variant alleles of the two SNPs, *IMPDH1* rs2278294 and rs2228075, that we found to be associated with MMF GI intolerance in our initial study (10). In particular, we previously found the A allele of *IMPDH1* rs2228075 to be associated with a positive predictive value of 60.9% and a sensitivity of 66.7% for MMF GI intolerance (10). Thus, in this small, single-center population, we found the results of our haplotype association study to be comparable to the results of our single SNP analysis.

Haplotype analysis in candidate gene association studies can provide several potential advantages over the single SNP approach. Haplotypes may more accurately represent the functional properties of the gene product. The biologic function of a protein is determined by how it is folded and its stability, which is delineated by its amino acid sequence, which, in turn, is dictated by the final RNA transcript that was transcribed and processed in a complex manner from the linear DNA sequence defined by the haplotype. Intronic SNPs, such as the majority of those investigated in the present study, may participate in the processing of the RNA transcript. As several polymorphisms in the *cis* position within one gene can interact, the influence of each polymorphism on the phenotype may vary depending on the presence/absence of, and its relationship with, other polymorphisms along

the haplotype. Additionally, multiple SNPs may “tag” an untyped variant more effectively than a single typed variant (15). As our results demonstrate no increase in utility of haplotype analysis compared to genotyping of a single SNP in measuring association with MMF GI intolerance, this pilot study suggests that genotyping of rs2228075 may be adequate to identify patients at high risk of MMF GI intolerance.

The pathogenicity of MPA GI intolerance is not well-defined. MPA is present in relatively high concentrations in the stomach, intestines and cecum (16). Studies in animals have demonstrated MPA-induced inhibition of proliferation of the basal epithelial cells of the intestine (16), and there is one case-report of MMF-related villous atrophy with associated diarrhea (17). Neerman and Boothe hypothesize that MPA inhibition of IMPDH in enterocytes of the rapidly dividing GI tract or epithelial cells of the gastric mucosa may lead to cytotoxicity even in the presence of the purine salvage pathway (18). Thus, an IMPDH variant resistant to MPA inhibition could be expected to exhibit less GI cytotoxicity and resultant GI intolerance to MMF. Previous work with *IMPDH1* and kidney transplant rejection is consistent with this proposed mechanism (19). The rs2278294 genotype associated with biopsy proven kidney rejection and probable resistance to MPA inhibition in that study (19) was found to be associated with increased tolerance to the GI effects of MMF in our study (10).

Our results demonstrate an association between MMF GI intolerance and an *IMPDH1* haplotype. This study furthers progress toward tailoring immunosuppressive therapy to individual patient needs defined by genetic, clinical and demographic risk factor profiles. Our study is limited by its small, single-center population and retrospective analysis. This small sample size and exploratory study design precluded multivariable analyses adjusting for tacrolimus use, gender and other potential confounding factors. Future studies will attempt to confirm these findings in the larger, multi-center patient cohort of six pediatric transplant centers part of the Specialized Centers for Clinically Oriented Research program investigating the genetic contribution to graft and patient outcomes following pediatric cardiac transplantation (20,21). As an increased number of immunosuppressants become available for use in pediatric heart transplant patients, *IMPDH1* polymorphisms may provide one means of developing a drug selection algorithm.

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Percent of patients experiencing MMF GI intolerance by *IMPDH1* haplotype. Number of patients with GI intolerance and number of patients in category are given as numerator and denominator, respectively, above each column. Asterisk signifies significant difference in percent of patients with GI tolerance ($p < 0.05$).

Table 1

Patient characteristics compared between patients who experienced MMF GI intolerance and patients free from MMF GI intolerance

Characteristic	GI Intolerance N=21	No GI Intolerance N=38	p-value
Male sex, % (N)	47.6 (10)	28.9 (11)	0.42
Black race, % (N)	9.5 (2)	10.5 (4)	1.00
Age at transplant in years, median (IQR)	6.6 (0.8, 11.7)	9.4 (2.8, 14.4)	0.29
<1 year old at transplant, % (N)	28.6 (6)	15.8 (6)	0.32
Cyclosporine primary immunosuppression, % (N)	4.8 (1)	13.2 (5)	0.41
Time from transplant to MMF start in months, median (IQR)	1.2 (0.9, 3.4)	1.45 (0.5, 15.9)	0.98
≤1 month from transplant to MMF start, % (N)	47.6 (10)	44.7 (17)	1.00
Total MMF exposure in years, median (IQR)	1.6 (0.6, 4.9)	3.45 (1.0, 5.4)	0.96
Daily MMF dose in mg per kg body weight, median (IQR)	26.5 (21.0, 34.9)	28.1 (19.9, 33.9)	0.90
Predose MPA plasma level, median (IQR)	1.4 (0.9, 2.0)	1.7 (1.4, 2.47)	0.24

MMF mycophenolate mofetil, GI gastrointestinal, IQR interquartile range, mg milligrams, kg kilograms, MPA mycophenolic acid, BSA body surface area

Table 2

IMPDH1 haplotypes of rs2288553, rs2288549, rs2278293, rs2278294, and rs2220875

<i>IMPDH1</i> Haplotype ID	Haplotype Definition	Haplotype Frequency in Patient Population (%) N=118	Number of Patients with Haplotype (%) N=59
A	T C C C C	30 (25.4)	28 (47.5)
B	T C T T T	26 (22.0)	22 (37.3)
C	T T C C C	22 (18.6)	20 (33.9)
D	T C T C C	21 (17.8)	19 (32.2)
E	A C C C C	9 (7.6)	9 (15.3)
F	T C C T C	4 (3.4)	4 (6.8)
G	T C T T C	3 (2.5)	3 (5.1)
H	A C T T C	1 (0.8)	1 (1.7)
I	T C T C T	1 (0.8)	1 (1.7)
J	T T C C T	1 (0.8)	1 (1.7)

Haplotypes above the dashed line (frequency > 15% in this population) were investigated for association with MMF GI intolerance.

Test properties of *IMDPHI* Haplotype B, rs2228075 and rs2278294 for mycophenolate mofetil gastrointestinal intolerance

Table 3

	Frequency in Study Population, N=59, n (%)	Sensitivity %	Specificity %	Positive Predictive Value %	Negative Predictive Value %
Haplotype B	22 (37.3)	61.9	76.3	59.1	78.4
rs2228075 A allele	23 (39.0)	66.7	76.3	60.9	80.6
rs2278294 A allele	30 (50.8)	71.4	60.5	50.0	79.3