A novel variant L263F in human inosine 5′-monophosphate dehydrogenase 2 is associated with diminished enzyme activity

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Background and objective **Inosine 5′-monophosphate dehydrogenase 2 is required for purine synthesis in activated lymphocytes. Variants in the *IMPDH2* gene may account for the large inter-individual variability in baseline enzyme activity, immunosuppressive efficacy and side effects in transplant recipients receiving mycophenolic acid. Therefore, the objective of this study was to identify and functionally characterize *IMPDH2* variants.**

Methods **DNA samples from 152 solid organ transplant patients were screened at exons and exon/intron junctions of the *IMPDH2* genes by PCR amplification followed by bidirectional direct DNA sequencing. Genetic variant was constructed by site-directed mutagenesis and transformed to an inosine 5′-monophosphate dehydrogenase-deficient strain of *Escherichia coli* h712. Proteins were purified to homogeneity and the enzymatic activity was measured by reduced nicotinamide adenine dinucleotide production.**

Results **Nine genetic variants were identified in the *IMPDH2* gene, with frequencies of the rarer alleles ranging from 0.5 to 10.2%. A novel nonsynonymous variant L263F was identified, and the kinetic assay demonstrated that the inosine 5′-monophosphate dehydrogenase activity of L263F variant was decreased to 10% of the wild-type. The K_i for mycophenolic acid inhibition of the L263F variant was comparable with the wild-type, and the variant K_m for inosine 5′-monophosphate and nicotinamide adenine dinucleotide did not change significantly.**

Conclusions ***IMPDH2*** **has low genetic diversity, but the nonsynonymous variant L263F has a significant impact on inosine 5′-monophosphate dehydrogenase activity. This novel functional variant may be one of the factors contributing to the inter-individual difference of baseline inosine 5′-monophosphate dehydrogenase activity as well as drug efficacy and adverse events in transplant patients.** ***Pharmacogenetics and Genomics*** **17:283–290 © 2007 Lippincott Williams & Wilkins.**

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Introduction

Inosine 5′-monophosphate dehydrogenase (IMPDH) (EC 1.1.1.205) is the target of mycophenolic acid (MPA), the active metabolite of the prodrug mycophenolate mofetil (MMF), which is widely used clinically to prevent allograft rejection in solid organ transplantation [1]. MPA is also used as an immunomodulator in diseases such as AIDS, lupus nephritis, myasthenia gravis and immune thrombocytopenic purpura [2–6]. Unlike other cell types, which can use the salvage pathway for purine synthesis, lymphocytes are exclusively dependent upon the de-novo pathway catalyzed by IMPDH for the generation of guanosine nucleotide [7,8]. Drugs active against purine synthesis also have broad applications in oncology, and new agents such as nelarabine [9] continue to advance treatment of T-cell-related diseases.

MPA can uncompetitively, selectively and reversibly inhibit this pathway and therefore inhibit cell proliferation. IMPDH has two isoforms, namely IMPDH type 1 and type 2, and the genes that encode these proteins are located on two different chromosomes [10]. IMPDH1 is constitutively expressed in all tissues, whereas IMPDH2 is required for purine synthesis in activated lymphocytes [11,12]. Although MPA is demonstrated to inhibit the activities of both IMPDH1 and IMPDH2 isoforms *in vitro*, IMPDH2 is 4.8 times more sensitive to MPA inhibition than IMPDH1 [13].

Owing to the difficulty in maintaining the balance between rejection and over-immunosuppression, pharmacokinetic and pharmacodynamic monitoring of MMF has been recommended to optimize drug dosage and

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administration schedule. Previous studies, however, have not provided a scientific basis for the accurate prediction of MPA's efficacy or side effects [14]. Moreover, large inter-patient and intra-patient variability has been observed in both MPA concentrations and IMPDH activity, and no correlation was found between the two parameters [15]. In some patients with rejection, no inhibition of IMPDH activity was observed despite seemingly adequate MPA concentrations [16]. Considering the significant inter-patient and intra-patient variability of the effects of a given concentration of MPA, the therapeutic range for the desired pharmacologic effect without adverse effects in most patients is difficult to discern [17].

The considerable variability of baseline IMPDH activity and MPA response may logically be under the control of genetic variation within the *IMPDH* genes or in gene expression. Although several mutations in the IMPDH-binding site that confer variable degrees of resistance to MPA were found in parasite *Tritrichomonas foetus* and varying drug-sensitive forms of this enzyme were detected in *Saccharomyces cerevisiae*, few studies have been reported on *IMPDH* gene variants in humans [18–20]. Five different variants of the *IMPDH1* gene have been identified in eight autosomal-dominant retinitis pigmentosa families, but their functional significance remains to be defined [21–23]. One *IMPDH2* SNP was recently identified in an abstract as having an association with biopsy-proven rejection in renal transplant patients [24].

Analysis of genetic variants could provide explanation for the variability of IMPDH activity and MMF response in transplant patients. Owing to its important function in lymphocytes, *IMPDH2* was the focus of our initial work to establish genotype–phenotype relationships for genetics variants. The objective of this study was to identify novel *IMPDH2* variants, to establish the relationship of this variant to in-vitro activity, and to look for possible clinical correlates for altered IMPDH activity.

Methods

Patient population

A total of 30 DNA samples from liver transplant patients at the University of Southern California, and 30 lung transplant patient as well as 92 heart transplant patient DNA samples from the University of Pittsburgh were included in this study. The protocol was approved by the institutional review boards of the University of Southern California and the University of Pittsburgh, and all patients or guardians provided informed written consent to undergo genotyping. Anticoagulated venous blood was obtained from each patient, and DNA was extracted from whole blood using a commercially available DNA extraction procedure (Qiagen, Valencia, California, USA).

Table 1 Sequences of primers used for amplification and sequencing of *IMPDH2* and for site-directed mutagenesis

Name	Primer sequence (5′ to 3′)
Primers used for long-range PCR amplification	
IMPDH2-for	5′- CCA CAG GGG AAC GAT GTT CTT TTC T-3′
IMPDH2-rev	5′- CAG GAG GAA CTT TTT GGA CCT GGA A-3′
Primers used for DNA direct sequencing	
Seq1-for	5′- CCA TGT GTT CCT CCA TCT CAA-3′
Seq1-rev	5′- CCC ACC TGT CTG TTG AAA GAT-3′
Seq2-for	5′- AGT CGA TGA CTG GCC CTT CT-3′
Seq2-rev	5′- TGA AAC TGG GGT CTC TGT GG-3′
Seq3-for	5′- TAA GGG ATG CTT TCC CAC ACT-3′
Seq3-rev	5′- CAG AAG CCC CTT GTC TTC AAC-3′
Seq4-for	5′- TGT GCC TGA TGG AAT TCT TG-3′
Seq4-rev	5′- CAA GCC CAA TCT GGT GAG TT-3′
Primers used for L263F site-directed mutagenesis	
L263F-for	5′-TAG GCT GGA CTT GTT CGC CCA GGC TGG TG-3′
L263F-rev	5′-CAC CAG CCT GGG CGA ACA AGT CCA GCC TA-3′

For, forward; IMPDH2, inosine 5′-monophosphate dehydrogenase 2; Rev, reverse; Seq, sequence.

Genotyping

IMPDH2 was PCR-amplified and followed by bidirectional direct DNA sequencing [25]. PCR was carried out in a total volume of 50 µl using 50 ng of genomic DNA, 5 pmol of each forward and reverse primer (Sigma, St Louis, Missouri, USA), 0.2 mmol/l deoxyribonucleotide triphosphate (Promega, Madison Wisconsin, USA), 1 × PCR buffer and 1.5 units of thermostable Taq DNA polymerase and Tgo DNA polymerase with proofreading activity and high fidelity (Expand 20 kb^{PLUS} PCR system, Roche Applied Science, Penzberg, Germany). The primers used are described in Table 1. The PCR process included initial denaturation at 92°C for 2 min and 10 cycles of denaturation at 92°C for 10 s, annealing at 57°C for 30 s and synthesis at 68°C for 7 min followed by additional 25 cycles of denaturation at 92°C for 10 s, annealing at 57°C for 30 s and synthesis at 68°C for 7 min with the time extended by 10 s for each successive cycle. The final elongation was carried out for 5 min at 72°C. Amplified PCR products were purified by using Qiagen PCR purification kit (Qiagen), and sequenced by conventional means using the BigDye Teminator Cycle Sequencing Ready Reaction Kit on ABI 377 XL Sequencer (Applied Biosystems, Foster City, California, USA). SNPs were identified by transferring the chromatograms to sequence assembly software Sequencher 4.1.4 (Gene Codes, Ann Arbor, Michigan, USA). Each base call was compared with the consensus sequence, and the SNPs were confirmed by visual inspection of the chromatograms.

Plasmids and site-directed mutagenesis

The plasmid pHIA5 carrying wild-type human *IMPDH2* was kindly provided by Dr Liz Hedstrom (Department of Biochemistry, Brandeis University) [26]. Nonsynonymous variant was made with QuickChange site-directed mutagenesis kit (Strategene, La Jolla, California, USA) using pHIA5 construct as a template. Two complementary primers were designed for the L263F variant as described in Table 1. PCR cycle conditions were as follows: 95°C for 1 min followed by 18 cycles of 95°C for

50 s, 60°C for 50 s and 68°C for 6 min. The final elongation was carried out for 7 min at 68°C. After digesting the parental DNA template with *Dpn*I at 37°C for 1 h, the PCR product was transformed into DH10B bacteria, and plasmids containing the variant *IMPDH2* inserts were isolated. The variant *IMPDH2* cDNA was fully sequenced to verify that only the desired mutation had been introduced.

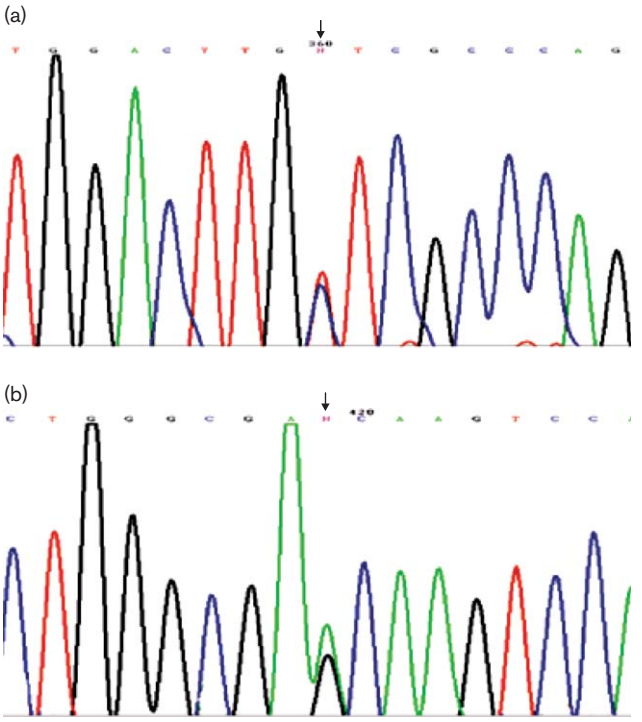
Expression and purification of the inosine 5′-monophosphate dehydrogenase 2 wild-type and L263F variant

The plasmids containing the *IMPDH* gene were transformed and expressed in H712 cells, an IMPDH-deficient strain of *Escherichia coli*. H712 cells carrying pHIA5 were grown overnight in 3 l of Luria–Bertani medium containing 1 mmol/l isopropyl β-D-1- thiogalactopyranaside (IPTG) and 100 μg/ml ampicillin. The cells were harvested by centrifugation and resuspended in Buffer A with 100 mmol/l Tris (pH 7.5), 1 mmol/l dithiothreitol and 10% glycerol. The cells were frozen, thawed and then disrupted by sonication with 2 × 20 pulses at power setting 5 (Misonix 3000 with microtip probe Misoniz, Inc., Farmingdale, New York, USA). Cell debris was removed by centrifugation at 18 000*g* for 30 min. The crude lysate was applied to a Cibacron Blue Sepharose column (Sigma, St. Louis, Missouri, USA) pre-equilibrated in Buffer A. IMPDH was eluted in a linear gradient of 0–1 mol/l KCl. The IMPDH containing fractions were pooled, diluted in Buffer A and applied to an inosine 5′-monophosphate (IMP) affinity column prepared by coupling IMP to epoxy-activated Sepharose as described [27]. IMPDH was eluted in 80 ml of Buffer A containing 2 mmol/l IMP. The fractions were collected and judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and proteins were pooled and concentrated using Amicon Centriprep filters (Millipore, Billerica, Massachusetts, USA). IMP was removed by dialysis against 4 l of buffer A overnight at 4°C. Protein purity was evaluated by Bio-Safe Coomassie Blue staining (Bio-Rad, Hercules, California, USA). Protein concentration was determined by microBCA protein assay kit (Pierce, Rockford, Illinois, USA) [26].

Enzyme kinetic assays

IMPDH2 activity was assayed after protein purification as previously described [26]. IMP, NAD^+ and NADH were purchased from Sigma. For the kinetic analysis, assays were performed at 37°C in 200 μl buffer of 100 mmol/l Tris HCl, 10 mmol/l KCl, 3 mmol/l ethylenediaminetetraacetic acid, 2 mmol/l dithiothreitol, pH 8.0. The reaction was started by the addition of enzyme (15–30 nmol/l), and the rates were obtained by monitoring NADH formation at 340 nm for 30 min in a temperature-controlled 96-well microtiter

Fig. 1



Representative chromatogram of the L263F heterozygote for inosine 5′-monophosphate dehydrogenase gene showing the (a) sense sequence and (b) antisense sequence of a transplant patient. The arrows indicate the variant is C/T in the sense sequence and A/G in the antisense sequence (A, adenine; G, guanine; C, cytosine; T, thymine).

Table 2 **Genetic variants of *IMPDH2* in transplant patients detected by bidirectional DNA sequencing**

Variants[a]	Position	Flanking sequence	AA change[b]	Frequency (%)	Reported[c]
IVS1-162 C>T	Intron 1	CCGCCCCCGC[C/T]GCAGCGAGGC		1.1	N/A
IVS1+91T>G	Intron 1	GCCTTGGGCG[T/G]GGGCGTGGGG		1.7	N/A
IVS4+197C>T	Intron 4	ACCCATGTCT[C/T]TAAGATGTGT		1.2	N/A
IVS5-62G>A	Intron 4	CCATGCTTCA[G/A]ATCAAGAGCC		0.5	N/A
417 C>T	Exon 5	GGCATGGTTT[C/T]TGCGGTATCC	Phe139Phe	1.2	N/A
787C>T	Exon 7	GCTGGACTTG[C/T]TCGCCCAGGC	**Leu 263Phe**	1.0	N/A
IVS7+10T>C	Intron 7	GGTGAGCTGC[T/C]ACACAGGTGG		10.2	rs11706052
IVS8-7G>A	Intron 7	CTTACTTCTT[G/A]TCCTAGGACT		0.7	N/A
IVS12-23C>T	Intron 11	TGACCTTGCC[C/T]GTGTCTCTGC		1.8	N/A

[a]The position is relative to ATG start site with A as nucleotide +1 and based on mRNA sequence from GeneBank accession number NM_000884. Designation of genetic variations: IVS1-162 C>T denotes the C to T substitution at nucleotide −162 of intron 1; 417 C>T implies that a C is changed to T at nucleotide 417.
[b]Amino acid change with its position shown in middle. Nonsynonymous change is shown in bold.
[c]N/A, not available.

plate reader (Bio-Rad). The extinction coefficient of 6.2 mmol/l/cm at 340 nm was used for NADH. Michaelis constants for IMP and NAD^+ were calculated from substrate titration data at saturating concentrations of the other substrate using the Michaelis–Menten equation (equation 1). The inhibition of IMPDH2 by MPA was assayed at varying concentrations of NAD and MPA in the presence of saturating IMP (400 µmol/l). The inhibition constants of MPA were determined by fitting the data to the equation for standard uncompetitive inhibition (equation 2) using the program Prism version 3.02 (GraphPad, San Diego, California, USA).

$$v = k_{cat}[E][S]/(K_m + [S])$$

$$v = V_m[S]/(K_m + [S](1 + [I]/K_i))$$

where v is the initial velocity, k_{cat} is the turnover number, E is IMPDH enzyme concentration, K_m is the Michaelis constant, S is the substrate concentration, v_m is the maximum velocity, K_i is the inhibition constant and I is the inhibitor concentration.

Western blot

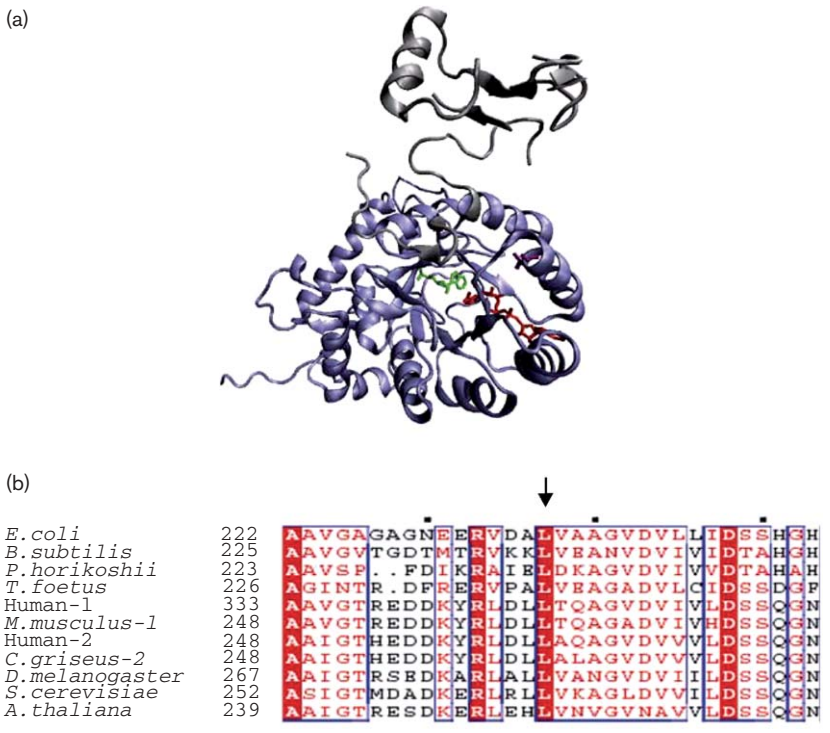
Wild-type or L263F variant of IMPDH2 proteins were incubated with or without 400 µmol/l of IMP and NAD^+ in a final volume of 200 µl at 37°C for 8 h. MPA was also added in a final concentration of 0 or 10 µmol/l. Samples were size fractionated on a 7.5% polyacrylamide gel containing 0.1% SDS (w/v) and transferred onto Immobilon polyvinylidene difluoride membranes (Millipore, Billerica, Massachusetts, USA) using a Transblotter (Bio-Rad). The membrane containing proteins was incubated with the anti-hIMPDH antibody (Antibody solutions, Mountain View, California, USA) at 1 : 200 dilutions (5 ug/ml) in Tris-buffered saline, supplemented with 0.1% Tween 20 and 5% milk. The proteins were visualized using the ECL kit (Amersham Biosciences, Piscataway, New Jersey, USA), and the band signal density was analyzed by Discovery Series Quantity One 1-D Analysis software (Bio-Rad).

Results

Genetic variants in inosine 5′-monophosphate dehydrogenase 2

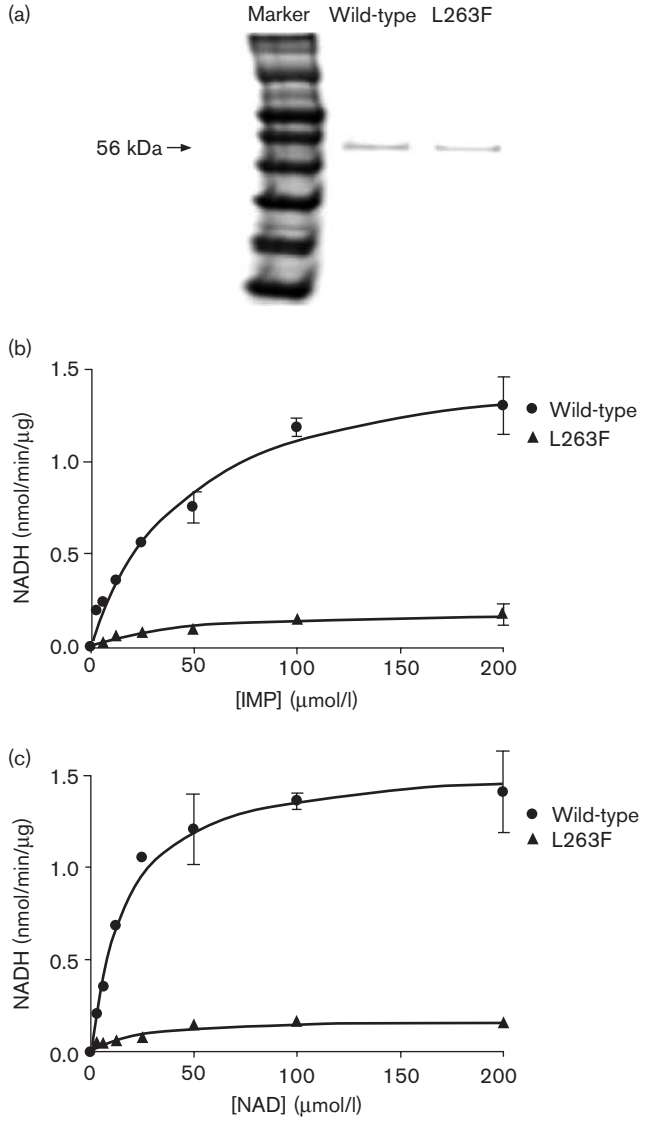
To examine *IMPDH2* genetic variation, we directly sequenced the 300 bp upstream of the proximal promoter region, 14 exons and the intron–exon boundary regions from 152 genomic DNA samples from these transplant patients. Nine genetic variants including eight novel SNPs were identified and confirmed by bidirectional

Fig. 2



Structure and sequence alignment of inosine 5′-monophosphate dehydrogenase (IMPDH) proteins. (a) Location of the L263F on a monomer of human IMPDH2 crystal structure (protein data bank code: 1NFB). The catalytic domain is shown in ice blue and the subdomain in silver. 6-Cl-IMP is shown in green and nicotinamide adenine dinucleotide in red. Residue Leu263 is shown in purple. (b) Sequence alignment of IMPDH proteins from different organisms using ClustalW and ESPript 2.0. The amino acid at position 263 is highly conserved, indicating its potential functional importance.

Fig. 3



Functional analysis of inosine 5′-monophosphate dehydrogenase 2 (IMPDH2) protein for wild-type and L263F variant. (a) Sodium dodecylsulphate (SDS)–PAGE gel electrophoresis of purified wild-type IMPDH2 protein and L263 variant. Proteins were separated by 12% Tris–HCl polyacrylamide gel and stained by Coomassie blue. Plots depict best fit of a Michaelis–Menten model for wild-type (circle) and L263F variant (triangle). Each point represents the mean of replicate samples from two independent experiments. Error bars represent the SEM. Proteins purified from H712 *Escherichia coli* were assayed for IMPDH activity at 400 μmol/l NAD^+, while varying inosine 5′-monophosphate (IMP) (b) or at 400 μmol/l IMP while varying NAD^+ (c).

sequencing (Table 2). Two variants are located in exons and seven are in the intronic regions. One novel variant 787C > T was identified in exon 7 of *IMPDH2*, leading to an amino acid change from leucine to phenylalanine at amino acid residue 263 (Fig. 1). This residue is located within the α-helix of the α/β barrel core domain of IMPDH2 protein which contains the entire machinery for enzyme catalytic activity (Fig. 2a).

Table 3 Kinetic parameters and inhibition constants of human IMPDH2 wild-type and L263F variant

Enzyme	$k_{cat}(s^{-1})$	IMP K_{m1} (μmol/l)	NAD^+ K_{m2} (μmol/l)	MPA K_i (nmol/l)
Wild-type	1.47 ± 0.08	45.8 ± 8.4	16.3 ± 3.1	11.0 ± 1.3
L263F	0.17 ± 0.02	42.0 ± 16.8	22.7 ± 5.6	18.2 ± 5.7

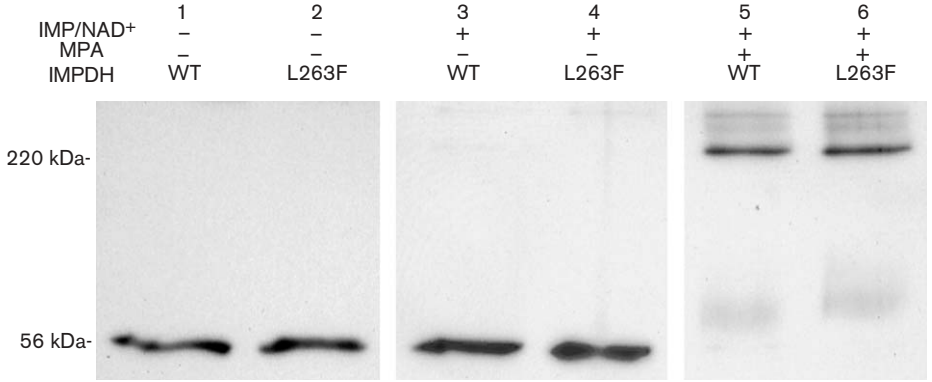
Enzyme activities of inosine 5′-monophosphate dehydrogenase 2 wild-type and L263F variant

An alignment of IMPDH amino acid sequences from different species demonstrates that the leucine residue 263 is highly conserved through a diversity of organisms, indicating its potential functional importance (Fig. 2b). Site-directed mutagenesis was used to generate the variant. Compared with the wild-type, L263F variant expressed less than 50% of the protein level from the IMPDH-deficient *Escherichia coli*. The purified protein had > 95% homogeneity as determined by densitometry of SDS–PAGE gels stained with Coomassie blue (Fig. 3a). Enzyme kinetic analysis demonstrated that the L263F variant had a 10-fold decrease in k_{cat} value relative to the wild-type (Table 3; Fig. 3b and c). The K_m value for IMP and NAD^+ did not change significantly, although there was a slight increase for NAD^+ (Table 3). The K_i for MPA inhibition of L263F variant was also comparable with wild-type (Table 3). Western-blot analysis was performed after incubating purified recombinant human *IMPDH2* with IMP and NAD^+ in the absence or presence of MPA. Our results demonstrated a significant gel mobility shift of the proteins from the 56 kDa to more than 220 kDa upon MPA treatment at 37°C for 8 h (Fig. 4). The shift was evident in both the wild-type protein and the L263F variant.

Discussion

Drug dosing and monitoring for an agent such as MPA is critical for obtaining an antiproliferative response without significant drug toxicity. Drug levels alone do not predict freedom from rejection or long-term graft function in transplant patients [28,29]. The measurement of IMPDH activity in peripheral blood has been proposed as a measure of MPA effect, and high pretransplant IMPDH activity with MMF dose reductions have been associated with the development of acute rejection [30]. Significant inter-individual variability in IMPDH activity can, however, be observed in the isolated lymphocytes of both healthy participants and dialysis patients [15]. IMPDH2 mRNA from peripheral blood has even been explored as a biomarker for MPA activity [31]. In the end, side effects such as leukopenia or failure to exert an antirejection effect by MPA in patients could possibly be explained by the genetic variance in IMPDH genes leading to functional differences. This report is the first study of *IMPDH2* genetic variants in the transplant patients. We found nine genetic variants separately located in the introns and exons with allele frequencies

Fig. 4



Effect of mycophenolic acid (MPA) on inosine 5′-monophosphate dehydrogenase (IMPDH) mobility *in vitro*. Western-blot analysis was performed using anti-hIMPDH antibody at 1 : 200 dilution. Extract of cells expressing wild-type (WT) or L263F variant IMPDH2 protein was incubated in the absence (lanes 1, 2) or presence (lanes 3, 4) of 400 μmol/l of IMP and nicotinamide adenine dinucleotide (NAD^+) in a final of 200 μl at 37°C for 8 h. MPA was also added at a final concentration of 10 μmol/l (lanes 5, 6).

ranging from 0.5 to 10.2%. The nonsynonymous variant 787C > T located in exon 7 leads to amino acid substitution from Leu to Phe at residue 263.

A subsequent report has recently appeared in abstract form, and reports on a previously unidentified *IMPDH2* single nucleotide polymorphism (SNP) in renal transplant patients [24]. The *IMPDH2* 3757 C allele, presumably in intron 9, reportedly increased the odds of developing biopsy-proven acute rejection in renal transplant patients by three-fold. Other gene polymorphisms may affect the disposition of MPA and its glucuronide metabolite, and include the multidrug resistance-associated protein 2 transporter [32,33] and the uridine diphosphate glucuronosyl transferases [34].

Two prominent domains have been identified in the X-ray crystal structure of IMPDH [35,36]. Residue 263 is highly conserved in various organisms, and is located in the α-helix of the IMPDH core domain encompassing amino acids 1–108 and 244–514 (Fig. 2a). These segments contain the entire enzymatic machinery necessary for IMPDH catalytic function. Kinetic analysis demonstrated that L263F mutation produced a dramatic drop in IMPDH enzyme activity *in vitro*, indicating that a leucine at residue 263 is important for normal enzyme function (Table 3; Fig. 3b, c). Therefore, lymphocytes carrying this variant may have decreased baseline IMPDH activity *in vivo*. The bulky phenylalanine, unlike leucine, inserts an aromatic ring within the sequence, which could lead to secondary structure changes. Alignment results demonstrated that this amino acid is highly conserved in various organisms (Fig. 2b). No homozygous TT genotype was found in our sample population, possibly because this mutation could be incompatible with life although no studies to date have confirmed this.

The K_i value for MPA inhibition showed that wild-type and L263 variant IMPDH2 had comparable sensitivity to MPA (Table 3). Previous studies indicated that gel mobility shift could provide a surrogate marker for IMPDH inhibition in clinical studies [20,37]. Therefore, we performed Western-blot analysis for human IMPDH2 incubating with IMP and NAD^+ in the absence or presence of MPA (Fig. 4). The gel mobility shift demonstrated that MPA could lead to self-aggregation of both wild-type and L263F variant, possibly through protein conformational changes [37]. These data, together with the MPA inhibition assay, indicate that the L263F variant was inhibited by MPA in a similar manner as the wild-type *IMPDH2*.

To determine whether the *IMPDH2* L263F variant is associated with any clinical relevance in MPA-treated transplant patients, we reviewed the clinical information in 30 liver transplant patients. Two patients were found to be heterozygous CT at the L263F site on *IMPDH2*. Both of these two patients had received living donor liver transplants, and similar immunosuppressive regimens consisting of MMF, tacrolimus and low-dose corticosteroids. At the time of their lowest white blood cell count (WBC) in the first postoperative year, both patients were receiving 0.5 g of MMF daily, or approximately 10 mg/kg/day. Dosing in the other patients ranged from 6 to 44 mg/kg/day of MMF given orally. Neither patient was receiving antiviral therapy or acute antirejection therapy with high dose corticosteroids or antibody therapy at the time of their leukocyte nadir. Patient 1 had mild

leukopenia with a WBC of 3700/ml, 1.7 months after liver transplantation. Patient 2 had moderate leukopenia with a WBC of 2500/ml, 2 and 1.5 months after transplantation. In Patient 2, the MMF was discontinued owing to leukopenia, and rechallenge with MMF after a second transplant also produced leukopenia necessitating drug discontinuation. Further investigation of the IMPDH genotype–phenotype relationship may provide useful information for the clinical management of IMPDH-targeted drugs.

In conclusion, we identified a novel L263F variant in the *IMPDH2* gene. To our knowledge, this is the first reported variant associated with decreased enzyme activity. Future clinical studies are warranted to determine whether this variant and others are associated with MPA efficacy and toxicity, and then to develop and test prospective treatment algorithms that will improve the use of the drug in organ transplant patients.

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