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Spectrum and Frequency of Mutations in IMPDH1 Associated with Autosomal Dominant Retinitis Pigmentosa and Leber Congenital Amaurosis

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Abstract

Purpose—The purpose of this study was to determine the frequency and spectrum of inosine monophosphate dehydrogenase type I (IMPDH1) mutations associated with autosomal dominant retinitis pigmentosa (RP), to determine whether mutations in IMPDH1 cause other forms of inherited retinal degeneration, and to analyze IMPDH1 mutations for alterations in enzyme activity and nucleic acid binding.

Methods—The coding sequence and flanking intron/exon junctions of IMPDH1 were analyzed in 203 patients with autosomal dominant RP (adRP), 55 patients with autosomal recessive RP (arRP), 7 patients with isolated RP, 17 patients with macular degeneration (MD), and 24 patients with Leber congenital amaurosis (LCA). DNA samples were tested for mutations by sequencing only or by a combination of single-stranded conformational analysis and by sequencing. Production of fluorescent reduced nicotinamide adenine dinucleotide (NADH) was used to measure enzymatic activity of mutant IMPDH1 proteins. The affinity and the specificity of mutant IMPDH1 proteins for single-stranded nucleic acids were determined by filter-binding assays.

Results—Five different IMPDH1 variants, Thr116Met, Asp226Asn, Val268Ile, Gly324Asp, and His 372Pro, were identified in eight autosomal dominant RP families. Two additional IMPDH1 variants, Arg105Trp and Asn198Lys, were found in two patients with isolated LCA. None of the

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novel IMPDH1 mutants identified in this study altered the enzymatic activity of the corresponding proteins. In contrast, the affinity and/or the specificity of single-stranded nucleic acid binding were altered for each IMPDH1 mutant except the Gly324Asp variant.

Conclusions—Mutations in IMPDH1 account for approximately 2% of families with adRP, and de novo IMPDH1 mutations are also rare causes of isolated LCA. This analysis of the novel IMPDH1 mutants substantiates previous reports that IMPDH1 mutations do not alter enzyme activity and demonstrates that these mutants alter the recently identified single-stranded nucleic acid binding property of IMPDH. Studies are needed to further characterize the functional significance of IMPDH1 nucleic acid binding and its potential relationship to retinal degeneration.

Retinitis pigmentosa (RP) is a progressive form of retinal degeneration that affects approximately 1.5 million individuals worldwide.¹ Genes and mutations causing RP are exceptionally heterogeneous. To date, 14 autosomal dominant, 15 autosomal recessive, and 5 X-linked forms of RP have been identified, in addition to many other syndromic, systemic, and complex forms (RetNet, <http://www.sph.uth.tmc.edu/Ret-Net>). Further, many distinct pathogenic mutations have been identified in each RP gene, and different mutations in the same gene may cause distinctly different forms of retinal disease. Determining the types of mutations and the range of phenotypes associated with each RP gene is one of the first steps to understanding the pathophysiologic mechanisms that lead to photoreceptor death, a crucial step in the development of treatments.

One RP gene that has not been examined in a wide range of patients with inherited retinal disease but is likely to contribute to distinct phenotypes, is inosine monophosphate dehydrogenase type I (IMPDH1). Mutations in IMPDH1 cause the RP10 form of autosomal dominant RP (adRP).^{2,3} IMPDH1 is located on chromosome 7q32.1 and encodes the enzyme IMPDH1. IMPDH proteins form homotetramers and catalyze the ratelimiting step of de novo guanine synthesis by oxidizing IMP to xanthosine-5'-monophosphate (XMP) with reduction of nicotinamide adenine dinucleotide (NAD). IMPDH genes are found in virtually every organism, and the gene and amino acid sequences are highly conserved across species. Humans have two IMPDH genes, IMPDH1 and IMPDH2, both expressed in a wide range of tissues.³⁻⁶

Two IMPDH1 mutations, Arg224Pro and Asp226Asn, were identified by linkage mapping and positional cloning in three adRP families.^{2,3} A reasonable prediction is that these missense mutations cause photoreceptor degeneration because of reduced enzyme activity and subsequent reduction in guanine nucleotide concentration. However, contrary to expectations, two studies demonstrate that these mutations do not affect enzyme activity or tetramer formation.^{7,8} Thus, an important unanswered question is the nature of the functional consequences of IMPDH1 mutations that lead to retinal degeneration.

Recent research shows that IMPDH binds single-stranded nucleic acids, suggesting another possible mechanism by which IMPDH1 mutations could cause retinal disease.⁹ In vitro and in vivo assays demonstrate that several IMPDH species, including human types I and II, bind random pools of single-stranded nucleotides via a protein subdomain composed of two CBS domains, named for homologous domains in cystathionine β -synthase (CBS). IMPDH can be found in both the cytoplasm and the nucleus of cultured cells; it binds both RNA and single-stranded DNA but does not bind to cognate RNA.⁹ Additional studies show that the Arg224Pro and Asp226Asn adRP mutations affect the affinity and the specificity of IMPDH1 nucleic acid binding and suggest that testing this functional property will assist in determining pathogenicity of novel IMPDH1 variants.⁸

In this study, we surveyed a large population of patients with adRP to determine the range and the frequency of IMPDH1 mutations. The clinical heterogeneity of mutations in genes

associated with retinal degeneration has been demonstrated many times.¹⁰⁻¹³ Therefore, we also analyzed patients with a variety of other inherited retinal degenerations, specifically autosomal recessive RP (arRP), macular degeneration (MD), and Leber congenital amaurosis (LCA), to investigate the possibility that mutations in IMPDH1 cause alternate phenotypes. The enzymatic activity and nucleic acid binding properties of each novel IMPDH1 protein variant identified in these patients were also determined and used to infer pathogenicity.

Materials and Methods

Subjects

This study was performed in accordance with the Declaration of Helsinki and informed consent was obtained in all cases. Most subjects examined in this study were diagnosed at one of the following sites: (i) the Anderson Vision Research Center, Retina Foundation of the Southwest, Dallas, Texas; (ii) the Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, California; (iii) Kellogg Eye Center, University of Michigan, Ann Arbor, Michigan; (iv) Cullen Eye Institute, Baylor College of Medicine, Houston, Texas; or (v) Hermann Eye Center, University of Texas Health Science Center, Houston, Texas. A few patients were also ascertained by ophthalmologists and genetic counselors from other institutions. The research at each academic institution was approved by the respective human subjects' review board. DNA was extracted from peripheral blood or buccal swabs by previously reported methods.^{2,12} All of the patients tested in this study had been previously tested for mutations in rhodopsin, peripherin/RDS, and RP1. Patients found to have mutations in those genes were excluded from this study.

Single-Stranded Conformational Analysis

Genomic DNA was amplified with the primers listed in Table 1, Amplitaq Gold polymerase (Applied Biosystems, Foster City, CA), and standard cycling parameters. PCR products were radiolabeled by incorporation of 1 μ Ci of P³²dCTP during amplification. Select PCR products were digested with restriction enzymes before gel analysis (see Table 1). PCR products were denatured and separated on $\times 0.6$ MDE gels (Cambrex BioScience, Walkersville, MD) at room temperature and at 4°C. Gels were dried and subjected to autoradiography after electrophoresis. Gels were analyzed by inspection.

Sequencing

Sequence analysis was also conducted by using the primers listed in Table 1. The amplimers for exons 3 and 4, exons 5 and 6, and exons 12 and 13 were combined for sequencing due to the small intron size. PCR products were treated with shrimp alkaline phosphatase and Exonuclease I (ExoSapIt; USB, Cleveland, OH) and then were sequenced bidirectionally by using BigDye v1.1 (Applied Biosystems) and amplification primers. For exon 7, the following nested sequencing primers were used: 5'-CATCTTCACCCTCCTAACATC-3' and 5'-AACCTCCACTCTGCTGAACCAC-3'. Sequence reactions were purified with sephadex columns (Princeton Separations, Adelphia, NJ) and were run on either an ABI 310 or ABI 3100 Avant Genetic Analyzer (Applied Biosystems). Sequences were compared with consensus IMPDH1 (GenBank accession NT_007933) by using SeqScape (Applied Biosystems).

UTAD391 STR and SNP Analysis

The following short tandem repeat (STR) loci were amplified from patients' DNA by using fluorescent PCR primers, Amplitaq Gold polymerase (Applied Biosystems), and standard multiplex reaction conditions: D1S498, D3S1292, D3S3606, D6S282, D6S1549, D7S484, D7S2252, D7S504, D7S530, D8S532, D11S4191, D11S987, D17S784, D14S972, D17S831, D17S957, D17S944, and D19S902. PCR products were diluted in water, pooled in formamide,

and run on an ABI 3100 Avant Genetic Analyzer (Applied Biosystems). Genotype data were analyzed using GeneMapper version 3.7 (Applied Biosystems).

Single nucleotide polymorphisms (SNP) located in and near the IMPDH1 gene were selected from National Center for Biotechnology Information (NCBI)'s dbSNP (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp>). DNA was genotyped for the following SNPs by using automated fluorescent sequencing as described above: rs53125, rs3828942, rs2290225, rs359652, rs3763398, rs2288550, rs2278294, rs2278293, rs2288553, and rs2288555.

Construction of IMPDH1 Mutants and Protein Isolation

Expression constructs corresponding to IMPDH1 protein variants were created with the previously described pKK223-3 wild-type IMPDH1 vector and site-directed mutagenesis (QuikChange; Stratagene, La Jolla, CA).⁹ The following primers and their complements were used to create the Arg105Trp, Thr116Met, Asn198Lys, Gly324Asp, and His372Pro mutants, respectively: 5'-ccaggccaacgaggtgtggaaggtcaagaag-3', 5'-gaacagggttcattcatggaccctgtgtgc-3', 5'-cggtgaaagaggcaaaggagatcctgcagcgtagc-3', 5'-cgggctgcgcgtggacatgggctcggctcc-3', and 5'-cgggctgcgcgtggacatgggctcggctcc-3'.

Mutant IMPDH1 cDNAs were sequenced by using BigDye v.1.1 (Applied Biosystems) and a 3100 Avant sequencer to verify that no other mutations had been introduced. Wild-type and mutant IMPDH1 protein constructs were induced in *Escherichia coli* with IPTG. Proteins were purified as previously described.^{8,9}

Enzyme Activity

Enzyme activity was determined in standard assay conditions (50 mM Tris-HCl [pH 8.0], 1 mM DTT, 100 mM KCl) and varying amounts of IMP and NAD⁺.¹⁴ NADH production was monitored at $\lambda_{\text{ex}} = 340$ nm and $\lambda_{\text{em}} = 460$ nm with a multiwell plate reader (Cytofour II multiwell plate reader; PerSeptive Biosystems, Framingham, MA) at 37°C. A standard curve of NADH solution in assay buffer was used to calibrate NADH production.

Filter Binding

Single-stranded DNA (ssDNA) binding assays were also done as described previously.⁹ The sequence of ssDNA was 5'-gggaatggatccacatctacgaattc-N30-ttactgcagactgacgaagctt-3', where N30 denotes a random 30 base sequence. 5' P³²-labeled ssDNA (2 nM) and varying concentrations of protein were mixed for 20 minutes at 25°C in an assay buffer of 10 mM Tris-HCl [pH 8.0], 50 mM KCl, and 1 mM DTT. Protein-bound nucleic acids were separated from free nucleic acid by filtration on a vacuum manifold (Schleicher and Schuell, Keene, NH). Protein complexes were captured on a nitrocellulose membrane, and the remaining free nucleic acids were captured on an underlying Hybond membrane (Amersham Biosciences, Piscataway, NJ). Membranes were washed with 100 μ L assay buffer, and the radioactivity bound to each was quantified by a PhosphoImager (Amersham Biosciences). The fraction of nucleic acid bound to nitrocellulose was fit to the following equation by using software (SigmaPlot; Systat Software, Inc., Point Richmond, CA): $f = (R[\text{IMPDH}]/K_d + [\text{IMPDH}]) + B$, where f is the fraction of nucleic acid bound, R is the maximum specific bound fraction, K_d is the dissociation constant, and B is the fraction bound to the membrane in the absence of protein.

Results

Genetic Analyses

DNA from 203 adRP, 55 arRP, 7 isolated RP, 17 MD, 20 isolated LCA, and 4 multiplex LCA patients was tested for mutations in the 14 coding exons and flanking intron-exon junctions of

IMPDH1. No probable disease-causing mutations were identified in any of the arRP, isolated RP, or MD patients tested.

As expected, several IMPDH1 variants were identified in the adRP samples, including the previously described Asp226Asn mutation that was found in the UTAD030, UTAD177, and UTAD557 families (Table 2 and Fig. 1).^{2,13} Analysis of DNA from nine additional members of the UTAD557 family demonstrated that the mutation segregated with disease. Samples were not available from additional members of the UTAD030 and UTAD177 families.

Five novel, potentially disease-causing IMPDH1 variants were also identified in the adRP cohort (Table 2). One of these mutations, Val268Ile, was reported previously in our original report characterizing mutations in IMPDH1 as the cause of RP10. We report it again here for accurate frequency calculations but did not include it in other analyses.^{2,8}

A Thr116Met mutation was identified in the UTAD083 proband and in her affected son. This mutation alters a residue in the first CBS domain of the flanking region. A His372Pro was identified in the UTAD026 proband. This mutation alters a highly conserved residue in the core region of the protein (Fig. 1). Unfortunately, no additional family members were available for testing from the UTAD026 family.

A Gly324Asp variant was identified in the adRP proband from the UTAD067 and UTAD904 families. This variant alters a residue adjacent to the active site of the enzyme (Fig. 1). DNAs from three additional UTAD904 family members were tested and found to also contain the Gly324Asp mutation. One of these family members was diagnosed with RP and another with fundus flavimaculatus. The third family member was reported to be unaffected, although definitive clinical testing, such as fundus examination and electroretinogram (ERG), had never been done. No additional UTAD067 family members were available at this time.

Two novel heterozygous IMPDH1 variants were identified in the isolated LCA patients (Table 2). An Arg105Trp was found in UTAD463 and an Asn198Lys was found in UTAD391 (Fig. 1). No mutations in AIPL1, CRB1, CRX, GUCY2D, LRAT, MERTK, or RPE65 were identified in either of these patients when tested by Asper Ophthalmics (Tartu, Estonia) with their LCA microarray chip. Additional family members were not available from UTAD463. DNA was obtained from the unaffected sister and parents of the UTAD391 proband. None of these individuals had the Asn198Lys change, indicating that Asn198Lys is a new mutation or the result of germ-line mosaicism. Parentage of the affected child was confirmed by SNP and STR markers (data not shown). PCR and subcloning was used to determine which of the informative SNP rs2288550 genotypes carried the mutant allele. This analysis suggests that the mutation occurred on the allele inherited from the patient's mother (Fig. 1).

A His296Arg change was found in one of the arRP probands. This variant was heterozygous in this patient, and no other protein-altering variants could be identified. Subsequent testing in our diagnostic laboratory identified this variant in an adRP patient who had a rhodopsin mutation, further substantiating reports that this variant is nonpathogenic.¹⁵

DNA samples from 116 unrelated individuals from the Centre d'Etude du Polymorphisme Humain were sequenced to check for the presence of the novel IMPDH1 variants and to determine the background variation found in IMPDH1. None of the variants described above were found in these samples. Only one rare amino-acid substitution, Ala285Thr, was found in this population, further demonstrating the high conservation of IMPDH1 (Table 3) and increasing the likelihood that most of the observed amino acid changes in retinal degeneration patients are pathogenic.

Enzyme Activity

Wild-type and mutant IMPDH1 were expressed in *E. coli* strain H712 (which lacks endogenous IMPDH) and purified to >95% homogeneity. The enzyme activity of each IMPDH1 protein was determined by monitoring NADH production. Each mutant IMPDH1 protein tested showed a specific activity similar to wild type (Table 4). This is consistent with reports of other IMPDH1 mutations associated with retinal degeneration.^{7,8}

Nucleic Acid Binding

Previous studies have demonstrated that IMPDH1 binds singlestranded nucleic acids with nanomolar affinity.⁹ Several adRP-associated IMPDH1 mutations have been shown to alter this property, thereby suggesting a common functional abnormality that can aid in classifying variants as pathogenic or benign.⁸ Each novel IMPDH1 mutant identified in our cohort of patients was tested to determine single-stranded nucleic acid binding affinity and specificity.

Wild-type IMPDH1 binds random pools of ssDNA oligonucleotides with a $K_d = 6$ nM (Fig. 2 and Table 4; IMPDH concentrations refer to tetramers). The Arg105Trp, Thr116Met, and Asn198Lys mutations show 8-fold, 17-fold, and 12-fold reductions in binding affinity, respectively. The His372Pro mutant also shows a decrease in binding affinity, although only 2-fold, much less than the other mutants. The Gly324Asp did not affect the affinity of IMPDH1 for ssDNA.

In our current studies, wild-type IMPDH1 binds approximately 7% of the random pool of nucleic acids, which is consistent with previous findings.⁸ Just as with other reported adRP mutants, most of the IMPDH1 mutants tested in this study affect ssDNA binding specificity.⁹ This is true for the Arg105Trp, Thr116Met, and Asn198Lys mutants, which bind 2.9-, 2.3-, and 3.6- more of the random oligonucleotide pool, respectively. The binding specificity decreased most with the His372Pro mutant that bound 4-fold more of the random pool than wild-type IMPDH1. As with binding affinity, the Gly324Asp mutant was indistinguishable from wild-type and did not show loss of binding specificity.

Assessing Pathogenicity

Determining pathogenicity of novel protein variants is a frequent problem due to small family size or a lack of DNA samples from additional family members. This study used a combination of segregation analysis, absence in unaffected controls, cross-species comparisons, and alteration of the nucleic acid binding property to assess pathogenicity of IMPDH1 variants. Grading of a variant as benign, probably pathogenic, or pathogenic was based on the number of criteria that each mutant fulfilled and is summarized in Table 5.

There is little doubt as to the pathogenicity of the Asp226Asn mutation. This mutation has been identified in many families, including two of the three very large families originally used to identify and refine the RP10 locus (Wada Y, *IOVS* 2005;45:ARVO E-Abstract 2456).^{2,15} Furthermore, this mutation has never been seen in unaffected controls, is 100% conserved in other IMPDH proteins, and alters the ability of the protein to bind single-stranded nucleic acids.^{2,8}

Only one additional family member was available to test segregation of the Thr116Met mutation, and no additional family members were available from the His372Pro family. As with previously reported IMPDH1 mutations, neither of these mutants affected IMPDH1 enzyme activity, but they do decrease the affinity and the specificity of single-stranded nucleic acid binding. This, in conjunction with the high sequence conservation of each residue (Fig. 3) and the lack of these variants in unaffected controls (Table 3), leads us to conclude that the

Thr116Met and His372Pro mutations are likely to be pathogenic, as is the previously described and tested Val268Ile.^{2,8}

The glycine at residue 324 is also 100% conserved in other IMPDH proteins, but segregation analysis of the Gly324Asp mutation shows a probably unaffected individual with the variant, which raises doubts as to pathogenicity. Functional analysis of the Gly324Asp mutation shows no effect on enzyme activity or nucleic acid binding, suggesting that this is a rare, benign variant and not a likely cause of retinal degeneration.

Pedigree and functional analysis demonstrates that the Asn198Lys mutation in the CBS domain is pathogenic and the result of a new mutational event. This residue is conserved in the mammalian IMPDH type I and type II proteins and in *Drosophila* and *C. elegans* IMPDH (Fig. 3). Like Asn198Lys, the Arg105Trp mutation is located at the junction of the CBS subdomain and alters the nucleic acid binding properties of IMPDH1. This residue is found in a large number of IMPDH proteins but is not as conserved as the Asn198 residue. This leads us to conclude that the Arg105Trp mutation is likely to be pathogenic. Furthermore, the isolated nature of the UTAD463 patient suggests the possibility that this could be the result of a new mutation.

Phenotypic Description of Patients with adRP

Asp226Asn—The proband from the UTAD030 family was seen in the clinic at age 32 years, after an initial diagnosis of RP. At that time, she had been an insulin-dependent diabetic for 4 years, had 20/20 corrected vision OU, and complained of night blindness. Biomicroscopy was normal, except for mild posterior subcapsular and paracentral cortical haze. The anterior vitreous showed syneresis with +1 pigmented cells. Fundus examination demonstrated granular pigmentary changes, pigment clumping, and vascular attenuation. The macula was intact in each eye. Goldmann visual fields showed concentric constriction to ~20° in all meridians (Fig. 4). One year later, her visual fields had constricted to 15° and were essentially the same 2 years later.

The proband from the UTAD177 family was initially diagnosed with RP at 10 years of age. When examined at 41 years of age, he had diffuse RPE atrophy, bone spicules at the equator and central RPE hypertrophy in the macula. ERGs were nonrecordable, and visual fields were 3° OD and 2° OS with the IV-4e isopters.

Clinical details are unavailable for most of the UTAD557 family. Affected members of this family were diagnosed with RP between 9 and 54 years of age. One affected male who was diagnosed at age 14 years was blind by age 39 years; while another was diagnosed at age 23 years and still had significant vision at age 65 years, although optic disc pallor, vascular attenuation and RPE abnormalities were present on examination.

Thr116Met—The proband from UTAD083 began experiencing night blindness in her early 40's and was first seen in the clinic at age 50 years. At this time, her visual acuity was 20/20 OU. Goldman visual fields were severely and symmetrically contracted OU with the I-4-e isopter subtending 8° and her IV-4-e isopter approximately 20° (Fig. 4). Fifteen years later the visual acuity was 20/40 OU. The visual fields were decreased to 2° with the I-4-e isopter, and 12° with the IV-4-e isopter.

His372Pro—The proband began experiencing night blindness around age 42 years. When examined at age 67 years, she had severe loss of vision, with the visual acuity of OD 20/80 and OS hand motions at 3 feet. Retinal examination showed RPE dropout like choroidal sclerosis. Optic disc pallor and bull's-eye maculopathy were present (Fig. 4). Geographic atrophy was seen in the fluorescein angiography.

Phenotypic Description of Patients with LCA

Arg105Trp—The affected child in the UTAD463 family was first seen at 8 months of age when he was diagnosed with LCA and developmental delay with severe hypotonia. He had roving nystagmus with no fixation to light. Macular reflex was present in both eyes with the retina showing diffuse RPE mottling. No pigmentary deposits were present.

Asn198Lys—The affected female in the UTAD391 family was seen after referral at age 33 months. The parents had noted the child could not see things in her peripheral vision, and she could not find her food in dimly lighted conditions. Refractive error was OD +3.50+1.50 × 85, and OS +3.50+1.50 × 95. By Allen cards, her vision was 20/40 (Fig.4).

Discussion

Several IMPDH1 mutations were identified in our patient cohort. The Asp226Asn mutation has been reported by several groups and is the most common IMPDH1 mutation seen in our adRP patient cohort and by other laboratories collectively (Wada Y, *IOVS* 2005;45:ARVO E-Abstract 2456).¹⁵ Our data suggest that the Asp226Asn mutation alone causes 1% of adRP. Haplotype evidence suggests that this mutation has arisen independently multiple times and, hence, is a hot spot for adRP mutations (Bowne S. *IOVS* 2003;44:ARVO E-Abstract 2307).

Realizing that members of our adRP cohort were previously excluded from having mutations in rhodopsin, peripherin/RDS and RP1 (which collectively cause 39% of adRP [Sullivan LS. *IOVS* 2005;46:ARVO E-Abstract 2293]), we calculate that mutations in IMPDH1 cause approximately 2% of adRP. This estimate is consistent with other reports of American and Japanese adRP populations (Wada Y, *IOVS* 2005;45:ARVO E-Abstract 2456),¹⁵ although analysis of an Italian adRP cohort failed to find any IMPDH1 mutations (Ciccodicola C. *IOVS* 2005;46:ARVO E-Abstract 1817).

This is the first report of IMPDH1 variants associated with LCA. Given the severity of the disease in adRP patients with certain IMPDH1 mutations, it is reasonable to predict that other IMPDH1 mutations could cause an earlier onset, more severe form of retinal degeneration.^{16,17} Studies have shown that, despite common misconceptions, not all isolated retinal degeneration is recessive. Isolated incidence rates do not equate with the recessive carrier frequency, supporting the notion that de novo dominant mutations may be an infrequent cause of disease.¹⁸ The identification of two pathogenic mutations in our isolated LCA cohort suggests an incidence of de novo, pathogenic IMPDH1 mutations of approximately 10%. This small cohort comprises all the isolated LCA patients in our diagnostic laboratory. Testing of a larger patient cohort will provide a better idea of the types and frequencies of IMPDH1 mutations associated with LCA.

None of the retinal disease-associated mutations tested to date affects the enzyme activity of IMPDH, but most alter the recently identified single-stranded nucleic acid binding property. Subsequent studies are needed to further elucidate the nucleic acid binding property of IMPDH1 and its relevance to photoreceptor biology and retinal disease.

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Figure 1.

IMPDH1 variants found in retinal degeneration cohort. **(A)** Pedigrees of families with IMPDH1 variants. *Closed symbols* represent affected individuals, *open symbols* represent unaffected individuals, *arrows* indicate proband. Genotypes for each tested family member are listed *below*: +, wild-type allele; -, mutant allele. **(B)** Localization of variants in the human IMPDH1 monomer crystal structure. The catalytic domain is in *gray* and the subdomain is in *blue* (some of which is disordered). Variants found in adRP patients are in *yellow*, and LCA-associated variants are in *purple*. **(C)** UTAD391 pedigree with SNP and STR genotypes used to determine parental origin of allele with de novo Asn198Lys mutation. Based on rs2288550, the mutant allele (shaded in *gray*) must have come from the patient's mother.

Figure 2. Nucleic acid binding affinity of IMPDH1 variants in a filter binding assay. Each graph is representative of three experiments. IMPDH1 concentration is shown as tetramers.

Figure 3.

Alignment of IMPDH proteins from multiple species. Proteins were aligned by using ClustalW and were formatted with ESPrpt 2.0. Sequence numbering is based on the human IMPDH1 sequence. Completely conserved residues are shown as *white letters on black background*; highly conserved residues are *boxed*. CBS domains are shown with *gray bars*, and IMPDH1 variants identified in this study are shown with *arrows*.

Figure 4.

Selected fundus photographs from patients with IMPDH1 mutations. **(A)** Asp226Asn; 32-year-old female with moderately advanced paucipigmentary RP. Each eye manifested vitreous syneresis, cells and condensations, vascular attenuation, and pigment epithelial atrophy concentrically from the periphery. The macula in each eye was spared, consistent with residual, formal visual fields of approximately 20° in all meridians. *Left photograph* shows optic disc pallor and spared macula; *right eccentric view* shows vascular attenuation, RPE thinning, and pigment clumping. **(B)** His372Pro; 67-year-old female with advanced RP. The disease is symmetric between eyes. The *left photograph* shows the left macula with severe loss of the macula, sclerotic-appearing loss of the adjacent retina, vessel attenuation, and pallor of the

optic nerve-head. *Right photograph* shows typical pigmentary retinal degeneration in the equatorial retina. **(C)** Thr116Met; 54-year-old female demonstrating a relatively normal posterior pole with mild choroidal sclerotic changes (*left photo*) while the equatorial regions has heavy pigmentary bone spicule-like deposits (*right photo*). **(D)** Asn198Lys; photographs, right eye (on *left*) and left eye (on *right*) of 33-month-old female with severe visual loss in periphery and 20/40 vision OU. There is a generalized depigmentation of the fundus, vascular attenuation, pallor of the optic nerve, and a diffuse hypopigmented ring around the discs.

Table 1

SSCP Amplimers and Conditions Used to Screen Retinal Degeneration Cohort

Exon	Primer Sequences 5'-3'	Amplimer Size	Restriction Enzyme
1	GGCGCGGCGTCAGCAGTAGCAG TCGCCGTGCCACGTCCGTCTGCTC	367	<i>AluI</i>
2	ACCCAGTAGACCTTTTCGCT ATGCCCTGCCCTGAGCAAG	323	<i>NlaIV</i>
3	TGGGTGATAAACTCTTTAGCTGG GGAAGTGTGGTCAGAGCCG	255	
4	CCGGCTCTGACCACACTT GCCTCTGAGGTGGGGACT	163	
5	GCTTCTTCCAGCCTGTTTCCT ACACCCAGCCCTGCTTCCC	281	
6	CCTTCTCTCTCACCTGCCAAC AACAACGGGACTGTGGAC	202	
7	AGTGAATCTCTGGAGTGGTC CCTGGGTCCTCATAAACCTC	378	
8 & 9	TTCATCCACTCAGGCTCTCC CTGGGAACAAAAGGCGAGG	560	<i>Cac81</i>
10 & 11	ACACTCATCTGGTGGTATTTG CCATCTGGGGAAGTCGGTG	643	<i>Eco01091</i>
12	AAGAGGTGGGGTGGGGT CAAGGTGGAGAAGAGCG	267	<i>BamHI</i>
13	GCTTCCTTCTCTCGCTCTTC ACCTCGCCAACCCACTGC	202	
14	GGGAAAGGGTTCTGGGAAG TGTGCCCCAAAAGTGGACAC	153	

Table 2
 IMPDH1 Variants Seen in Patients with Retinal Degeneration

Family	Diagnosis of Proband	Nucleotide	Protein
UTAD030 UTAD177 UTAD557	adRP	676 G>A	Asp226Asn
UTAD026 UTAD083 UTAD067	adRP adRP adRP	1115 A>C 349C>T 971 G>A	His372Pro Thr116Met Gly324Asp
UTAD904 UTAD389 UTAD463	adRP LCA LCA	802 G>A 313 C>T 594 T>G	Val268Ile Arg105Trp Asn198Lys
UTAD391 RFS246	LCA arRP	887 A>G	His296Arg

Table 3

IMPDH1 Variants Seen in CEPH Controls

Protein	Nucleotide	Frequency
Cys215Cys	C>T 645	0.004
Leu244Leu	G>C 789	0.226
Ala285Thr	G>A 53	0.004
Ala440Ala	G>A 320	0.241
Ile471Ile	C>T 413	0.004

Table 4
Enzymatic Activity and Nucleic Acid Binding of Novel IMPDH1 Variants Identified in This Study

Variant	Specific Enzyme Activity (μ moles NADH/ min-mg)	Nucleic Acid Binding	
		K_d (nM)	Maximum % Bound
Wild-type	0.6 ± 0.2	6 ± 1	7 ± 0.4
Arg105Trp	0.9 ± 0.4	> 50	> 20
Asn198Lys	0.9 ± 0.3	> 73	> 25
Thr116Met	1 ± 0.3	100 ± 20	16 ± 2
Gly324Asp	1 ± 0.3	6 ± 1	8 ± 0.3
His372Pro	1 ± 0.5	12 ± 1	30 ± 1

Table 5

Assessing Pathogenicity of IMPDH1 Variants

IMPDH1 Protein Variant	Tests to Determine Pathogenicity				Pathogenicity		
	Segregation Analysis Agreement	Presence in Unaffected Controls	Residue Conservation	Alteration of Functional Property	Benign	Probably Pathogenic	Pathogenic
Arg105Trp	NA	No	++	+		X	
Thr116Met	+	No	++	+		X	
Asn198Lys	+	No	+++	+			X
Asp226Asn	+	No	+++	+ ⁸			X
Val268Ile	NA	No	++	+ ⁸		X	
His296Arg	NA	Yes	+	NT	X		
Gly324Asp	Uninformative	No	+++	-	X		
His372Pro	NA	No	+++	+		X	

Pathogenicity of variants was determined by grading each variant in four categories: segregation analysis, presence in unaffected controls, residue conservation in IMPDH proteins and alteration of the nucleic acid binding property. Residue conservation was based on alignments: +, conserved only in mammalian IMPDH1; ++, conserved in mammalian IMPDH1 and IMPDH2; +++, conserved in mammalian IMPDH1 and IMPDH2 and some lower organisms; +++++, conserved in all IMPDH proteins. Any variant not passing the segregation analysis or found in unaffected controls was designated benign. NA, not available for testing. NT, not tested.