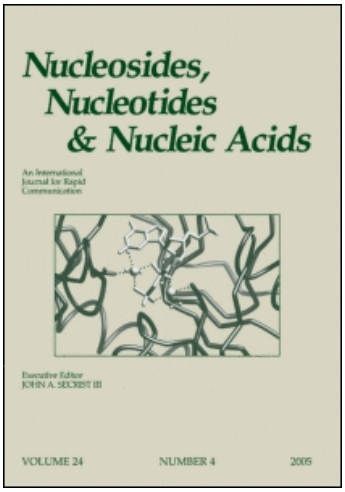

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The Spectrum of Mutations Causing HPRT Deficiency: An Update

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ABSTRACT

Mutations in the gene encoding hypoxanthine-guanine phosphoribosyltransferase (HPRT) cause Lesch–Nyhan disease, which is characterized by hyperuricemia, severe motor disability, and self-injurious behavior. Mutations in the same gene also cause less severe clinical phenotypes with only some portions of the full syndrome. A large database of 271 mutations associated with both full and partial clinical phenotypes was recently compiled. Since the original database was assembled, 31 additional mutations have been identified, bringing the new total to 302. The results demonstrate a very heterogeneous collection of mutations for both LND and its partial syndromes. The differences between LND and the partial phenotypes cannot be explained by differences in the locations of mutations, but the partial phenotypes are more likely to have mutations predicted to allow some residual enzyme function. The reasons for some apparent exceptions to this proposal are addressed.

Key Words: Genotype-phenotype correlation; Diagnostic testing; Kelley-Seegmiller syndrome.

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INTRODUCTION

Mutations in the gene encoding hypoxanthine-guanine phosphoribosyltransferase (HPRT) cause a spectrum of clinical phenotypes in humans. The most severe phenotype, Lesch–Nyhan disease (LND), is characterized by hyperuricemia, severely disabling motor disability, cognitive impairment, and self-injurious behavior. The least

Table 1. New mutations.

Name	Phenotype	Mutation	Result	Ref.
Family 5	LND	A50G	T17C	New
NA	LND	T194C	L65P	[2]
NA	LND	G209A	G70E	[2]
Jerusalem	HRH	T410A	I137T	[3]
NA	HRND	T440C	L147P	[2]
Potenza	HRH	C439T	L147F	[4]
Family 32	LND	G419A	G140D	New
NA	HRND	A475G	K159E	[2]
Family 39	LND	T541C	F181K	New
Patient 3	LND	G569A	G190E	[5]
NA	HRH	C575T	A192V	[2]
NA	HRND	A584G	Y195C	[2]
NA	LND	IVS1 + 1G > T	Splice defect, 49 bp added to mRNA	[2]
CS	HRH	IVS1 + 47G > T	Splice defect, 49 bp added to mRNA	[6]
NA	LND	IVS1-1G > C	Splice defect excludes exon 2	[2]
NA	LND	IVS8-1G > A	Splice defect excludes bp 610–626	[2]
NA	LND	IVS3-1G > T	Splice defect excludes exon 4	[2]
Patient 4	LND	IVS4-1G > A	Splice defect excluded exon 5	[5]
Family 28	LND	IVS4-2A > C	Splice defect excludes exon 5	New
NA	LND	IVS5-2A > G	Splice defect, inserts IVS5-1G into mRNA	[2]
Patient A	LND	IVS6 + 1G > C	Splice defect excludes exon 6	[7]
NA	HRND	IVS7 + 2T > C	Splice defect excludes exon 7 with early chain termination	[2]
Patient B	LND	IVS7-9T > G	Splice defect excludes exon 8	[7]
NA	LND	IVS7-9T > A	Splice defect excludes exon 8	[2]
Patient C	LND	E1 deleted	One exon deleted	[7]
Patient D	LND	E1 deleted	One exon deleted	[7]
Family 56	LND	292–298delGATTTTA	7 bp deletion	New
NA	LND	317–318delGT	Splice defect excludes exons 2–3	[2]
Family 72	LND	E4–E9 deleted	6 exon deletion	New
NA	LND	IVS8 + 1–2delGT	Splice defect excludes exon 8	[2]
NA	Female HRH	T158C with non-random X inactivation	T158C plus inactive X-chromosome	[8]

*NA = not available.

severe phenotype is characterized by hyperuricemia without any neurological or behavioral abnormality, designated HPRT-related hyperuricemia (HRH). In between these two extreme phenotypes are cases with hyperuricemia and vary degrees of neurobehavioral abnormality but without self-injury, designated HPRT-related neurological dysfunction (HRND). To better understand genotype-phenotype relationships, a large database of all known mutations associated with defined clinical phenotypes was compiled.

MATERIALS AND METHODS

Mutations were determined as previously described or collected from published literature.[1] The clinical phenotypes were classified into three groups described above.

RESULTS

Thirty-one new mutations associated with HPRT deficiency in humans have been identified (Table 1). Twenty-two of these were associated with the LND phenotype, including 6 missense mutations, 10 base substitutions leading to splicing errors, 4 deletions of coding sequence, and 2 deletions leading to splicing errors. Nine of the mutations were associated with a partial clinical phenotype (HRND or HRH), including 6 misssense mutations and 2 base substitutions leading to splicing errors. One new female case with HRH was determined to have a missense mutation with non-random X-inactivation. In total, 302 mutations in the HPRT gene have been associated with clinical disease of varying severity (Table 2).

Table 2. All mutations (from reference 1 plus Table 1).

Mutation	LND (n = 223)	LNV (n = 71)	NA (n = 8)	Total (n = 302)
Single base substitution				
Missense	69	54	4	127
Nonsense	23	1	1	25
Splice error	36	7	0	43
Deletion				
Coding sequences	61	2	3	66
Splice error	5	0	0	5
Insertion				
Coding sequences	18	1	0	19
Splice error	1	0	0	1
Others				
Duplication	3	3	0	6
Substitutions	2	0	0	2
Females	5	1	0	6
Double	0	2	0	2

DISCUSSION

In contrast to some genetic diseases in which one or a small number of mutations account for the majority of patients, LND and the related partial syndromes are caused by multiple different mutations affecting nearly all parts of the gene. The clinical differences between patients with LND and those with partial phenotypes cannot be explained by differences in the locations of mutations, since the locations of these mutations overlap considerably between the groups.[1] However, patients with partial phenotypes are more likely to have mutations predicted to allow some residual enzyme function. More specifically, patients with partial syndromes are only rarely found to have early stop mutations, deletions, insertions, or more complex rearrangements.

The concept that the full phenotype of LND occurs when enzyme activity is completely or near-completely eliminated while the partial syndromes occur when significant residual enzyme function persists is supported by the majority of studies in which HPRT enzyme activity has been empirically measured. In general, the severity of disease appears inversely correlated with the amount of residual enzyme function.[9–11] However, several examples have been reported in which the severity of the phenotype does not appear to correlate with measured residual HPRT enzyme activity. For example, there are several reports describing cases with the most severe phenotype in combination with large amounts of residual HPRT enzyme activity.[12,13] Conversely, there are also reports describing cases with a very mild clinical phenotype and no apparent residual HPRT enzyme activity.[14,15] These reports of significant discrepancies between the severity of the clinical phenotype and predictions from genetic studies or empirically determined biochemical measures, albeit infrequent, could be construed as evidence against a strict relationship between disease severity and residual enzyme function.

There are several potential explanations for these discrepancies. All of these explanations relate to the fact that most current biochemical measures of enzyme activity do not precisely replicate the conditions found in relevant tissues in vivo. First, mutations causing enzymes to be structurally unstable outside of the normal cellular environment will be associated with inaccurate results with some enzyme assays. In these cases, assays based on cell lysates will underestimate the enzyme's activity in vivo. Several discrepancies between assays based on cell lysates and assays that more closely mimic the in vivo state have already been described.[10,16,17]

Second, mutations resulting in a change in the kinetic properties of the enzyme will be associated with inaccurate results with lysate-based assays unless the assays are conducted with exact concentrations of substrates available in vivo. Very frequently, lysate-based assays are conducted with maximal enzyme activity being driven by substrate concentrations above physiologically relevant levels. Examples of such kinetic mutations have also already been reported.[18,19]

Third, some mutations may be ''unstable'' and revert in vivo. Examples of unstable mutations include two duplications, which have been shown to recombine in vivo, eliminating the duplication and restoring a normal gene in a small proportion of cells.[20–22] In the case of unstable mutations, the patient is actually a somatic mosaic with an unknown distribution of cells making no HPRT mixed with cells making a normal amount of HPRT. Depending on the source of the clinical sample, residual HPRT activity could vary from nil to normal.

Fourth, some mutations may be ''leaky'' and allow variable amounts of normal HPRT mRNA transcription in different cells or tissues. Such ''leaky'' mutations include some splice site-mutations, where the major mRNA species are either absent or aberrant, but a small proportion of correctly spliced transcripts allow a very low level of normal HPRT to be translated.[23,24] The problem with accurate enzyme measurements from the ''leaky'' mutations is analogous to that described for somatic mosaicism; it is not easy to determine if the clinical sample being testing has an expression of HPRT that representative of the relevant tissues in vivo.

The discrepancies arising from mutations resulting in structurally unstable enzymes or enzymes with altered kinetic properties can be minimized by employing assays that most closely mimic the in vivo state. The assays that have been developed for this purpose are based on the metabolism of physiologically relevant concentrations of labeled hypoxanthine or guanine by HPRT in live cells. Traditionally, the cells used have included those derived from the blood (erythrocytes and lymphocytes) or fibroblasts, since these are readily obtainable tissue sources from patients.[9,16,25] For both structurally unstable enzymes or enzymes with altered kinetic properties, the live cell assays will provide more accurate results than assays based on cell lysates.

Unfortunately, the live cell assays do not address the discrepancies that may arise from somatic mosaicism. In theory, somatic mosaicism should not present a problem for assays based on live blood cells, since any blood sample will include a large and mixed population of cells derived from bone marrow sites throughout the body. Unfortunately, there is a strong selective growth disadvantage for HPRT-negative bone marrow stem cells. The most dramatic example of this selection comes from female heterozygous carriers, where random X-chromosome inactivation should produce 50% normal and 50% HPRT-deficient erythrocytes and leukocytes. However, empirical determinations have shown heterozygotes to have 1–10% HPRT-negative blood cells, indicating a strong selective growth disadvantage for the HPRT-negative cells.[26–29] As a result of the selective growth disadvantage of HPRT-deficient bone marrow cells, assays based on live blood cells do not provide a representative sample of most other body tissues in vivo. Specifically, the live blood cell assays will overestimate the amount of residual HPRT activity existing in tissues where such selective pressures are less prominent. The problem of somatic mosaicism could be avoided in the fibroblast assays. HPRT-postive skin cells demonstrate only minor growth advantage,[30] and collecting tissue from multiple sampling sites should obviate any significant differences from different sites. Practically, however, this solution is rarely realized in clinical testing, since the live fibroblast assays are usually conducted with cells derived from a single skin biopsy.

The live cell assays also do not guarantee accurate results for cases with ''leaky'' mutations. The problem here is analogous to that described for somatic mosaicism. In short, these biochemical assays do not necessarily provide estimates for residual HPRT enzyme activity in relevant tissues in vivo. Since the most variable elements of the phenotype include the behavioral and neurological abnormalities, the relevant tissue for which residual HPRT estimates are most important is brain. Unfortunately, it is not feasible to devise a live cell assay analogous to the erythrocyte or fibroblast assays for brain cells. These cells are not readily biopsied and do not easily grow in tissue culture.

Until an assay is developed that more closely estimates the residual HPRT enzyme activity in brain, we can expect to find clinical phenotypes that do not appear to match

residual HPRT enzyme activities. Such apparent discrepancies do not necessarily refute the concept that the amount of residual enzyme activity being expressed in the brain plays the dominant role in determining disease severity.

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