Phosphoribosylpyrophosphate synthetase superactivity

Author: Doctor Michael A. Becker
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1University of Chicago Medical Center, MC0930, 5841 South Maryland Avenue, Chicago, Illinois 60637 USA.
mbecker@medicine.bsd.uchicago.edu

Abstract
Phosphoribosylpyrophosphate (PRPP) synthetase superactivity is an X chromosome-linked disorder of purine metabolism in which excessive Phosphoribosyl Pyrophosphate Synthetase (PRS) activity results in accelerated purine nucleotide and uric acid synthesis. PRS superactivity appears to be rare, with less than 30 affected kindreds described since 1972. Hyperuricemia and hyperuricosuria are demonstrable in all affected individuals who are thus predisposed to gout with uric acid overproduction and uric acid urolithiasis. In affected men presenting in late adolescence or early adulthood, gouty arthritis and urinary tract stones are usually the sole manifestations of PRS superactivity. Other families show a more severe phenotype in which affected male children display uric acid crystalluria, hyperuricemia, and hyperuricosuria in conjunction with neurodevelopmental impairment, and heterozygous carrier women may show milder metabolic and neurological abnormalities. Genetic heterogeneity underlies the different phenotypic expressions of inherited PRS superactivity. The severe clinical phenotype is usually associated with point mutation in the translated region of the PRPS1 gene that results in defective allosteric control of PRS1 isoform activity. In contrast, the later-onset, less severe disorder is usually associated with selective acceleration of PRPS1 transcription, resulting in increased concentration of normal PRS1 isoform. Treatment of uric acid overproduction with allopurinol successfully reverses or prevents the consequences of hyperuricemia and hyperuricosuria. Success in managing associated neurodevelopmental impairments, however, awaits pathogenetic understanding of these manifestations.

Keywords
PRPP; phosphoribosylpyrophosphate synthetase; X-linked inheritance; PRPS genes; point mutation; transcriptional dysregulation; hyperuricemia; gout; sensorineural deafness; neurodevelopmental impairment.

Definition
Phosphoribosylpyrophosphate (PRPP) synthetase (PRS; EC 2.7.6.1) superactivity (also called Phosphoribosyl Pyrophosphate Synthetase – PRS—overactivity) is an X chromosome-linked inborn error of purine metabolism. The disorder is characterized biochemically by accelerated rates of PRPP, purine nucleotide, and uric acid synthesis in conjunction with excessive PRS activity and clinically by gout with uric acid overproduction and,
in some affected families, neurodevelopmental impairment, especially sensorineural hearing deficit (1). In both the X-linked mode of inheritance and the clinical manifestations of uric acid overproduction, PRS superactivity closely resembles deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT; EC 2.4.2.8)(2).

Although the two disorders also share neurologic and developmental aberrations in affected male children, the cardinal features of severe HPRT deficiency (Lesch-Nyhan syndrome) have not been encountered in PRS superactivity. Distinction between these enzyme defects requires either specific enzymatic or functional assays for activities of the respective enzymes or, where appropriate, demonstration of altered genotype.

Prevalence
PRS superactivity appears to be a rare disorder, with less than 30 affected kindreds described since 1972. Among patients with gout, the prevalence of PRS superactivity is less than 1 to 2% and, even among the 10 to 15% of patients with primary gout, PRS superactivity is demonstrable in less than 10% of individuals tested to date. In contrast, severe HPRT deficiency appears to be substantially more common. The striking clinical features of Lesch-Nyhan syndrome, however, may have served to maximize identification of severe HPRT deficiency. In fact, no screening of children with sensorineural deafness for PRS superactivity (or even for uric acid overproduction) has been reported, perhaps because of the complexity of screening for this disorder (discussed below).

PRPP and PRS
PRPP is a substrate in the synthesis of virtually all nucleotides and is also an important regulator of the de novo pathways of purine and pyrimidine nucleotide synthesis (3). Formation of PRPP is catalyzed in mammalian cells by a family of PRS isoforms in the reaction:

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\text{Mg-ATP} + \text{Ribose-5-phosphate} \rightarrow \text{PRPP} + \text{AMP}
\]

The reaction requires magnesium ions (Mg2+) and inorganic phosphate (Pi). Intracellular synthesis of PRPP is regulated in a complex manner that includes - but is not limited to- allosteric control of PRS enzymatic activity (by purine nucleotide inhibitors and Pi) and PRS isoform concentrations (3,4).

Multiple isoforms of PRS were first identified by Tatibana and colleagues (5) who cloned and sequenced 3 distinct PRS cDNAs (numbered 1, 2, and 3) encoding highly homologous polypeptides of identical length. Each human PRS cDNA is encoded by a separate PRPS gene: human PRPS1 and PRPS2 map to different regions of the X chromosome (Xq22-q24 and Xp22.2-p22.3, respectively) (6) and are widely expressed; PRPS3 maps to human chromosome 7 and appears to be transcribed only in the testes. X-linked human PRS1 and PRS2 cDNAs show 80% nucleotide sequence identity throughout their 954 bp translated regions but show no homology in the corresponding 5' and 3' untranslated regions. PRS1 and PRS2 cDNAs hybridize with transcripts of 2.3 and 2.7 kb, respectively (6). Human organs, tissues, and cell lines contain both PRS1 and PRS2 transcripts (7) and isoforms (3), but the relative abundances of these gene products vary with the cell source. Studies of tissue-specific expression of PRPS1 and PRPS2 genes indicate that PRPS1 may be a constitutively expressed gene while PRPS2 expression may be responsive to mitogenic stimulation and/or transformation. Both X-linked PRPS genes exceed 20 kb and contain 7 exons with virtually identical exon-intron borders. 5'-Promoter regions of the genes are, however, structurally distinct (8).

Despite 95% amino acid homology, recombinant PRS1 and PRS2 isoforms differ in several kinetic and physical properties, including kinetic constants for substrates, activators, and inhibitors and isoelectric points (9). The latter difference has allowed the development of an isoelectric focusing (IEF)-immunoblotting method for separation and quantitation of the isoforms in tissue and cell samples (10).

PRS superactivity
PRS superactivity was initially identified by Sperling et al. (11) in brothers with early adult-onset uric acid urolithiasis and gout associated with severe hyperuricemia and hyperuricosuria. In these patients, purine nucleotide overproduction, reflected by markedly increased daily urinary uric acid excretion, was accompanied by an accelerated rate of intracellular PRPP synthesis and a variant form of PRS with defective allosteric regulation of enzyme activity by the purine nucleotide inhibitors, ADP and GDP.

Among kindreds with PRS superactivity, the kinetic mechanisms leading to excessive enzyme activity are heterogeneous (3). These mechanisms include: 1) impaired allosteric regulation of PRS activity by purine nucleotide inhibitors and Pi; 2) "catalytic" overactivity in which excessive enzyme activity results from an overabundance of the normal PRS1 isoform; 3) combined regulatory defects and "catalytic" overactivity;
4) increased affinity for ribose-5-phosphate, which is normally present in cells at concentrations below the apparent dissociation constant of PRS for this substrate. "Catalytic" superactivity is the most common class of kinetic aberration reported to date. Despite the variety of functional abnormalities in the enzyme, purine nucleotide and uric acid overproduction are demonstrable in all affected individuals and result from accelerated PRPP synthesis (12). Increased PRPP availability activates amidophosphoribosyltransferase, the rate-limiting enzyme in the pathway of purine synthesis de novo, resulting in acceleration of purine nucleotide and uric acid synthesis (4). Affected males with defective allosteric regulation of PRS activity generally have higher rates of PRPP production and, ultimately, greater acceleration of purine nucleotide and uric acid synthesis than individuals with isolated overabundance of normal PRS1 (12).

Clinical description
Like HPRT deficiency, PRS superactivity is inherited as an X-linked trait expressed in two clinical phenotypes (3,13). In families with the more severe phenotype, affected hemizygous males show infantile or early childhood symptoms and signs of uric acid overproduction in association with an inconstant array of neurodevelopmental abnormalities, frequently, but not invariably, including sensorineural deafness (13). Gout and deafness may develop in heterozygous female carriers in these families during the reproductive period. In all but one of the kindreds with these clinical features, cultured cells from affected persons show allosteric regulatory or combined defects in the enzyme (14), along with severe derangements in PRPP and purine metabolism. In the remaining family, PRS is catalytically superactive and overproduction of PRPP and purine nucleotides is apparently no more severe than is encountered in the later-onset phenotype. Abnormal purine metabolic and neurologic features in this family appeared in late childhood. Conversely, not all families with mutations disrupting allosteric regulation of PRS show neurologic impairment or even childhood presentation, as exemplified by the family initially described (11,14). As discussed below, the genetic basis of impaired allosteric regulation of PRS has been identified (14); nevertheless, the mechanisms underlying neurologic derangements in PRS superactivity remain unknown.

The late juvenile/early adulthood-onset variety of PRS superactivity has to date been restricted to males who show gout and/or uric acid urolithiasis but no overt neurologic deficits (2). Overabundance of the normal PRS1 isoform (10) is, with the single exception noted above, associated with this clinical pattern. Functional defects in the control of PRPP and purine nucleotide synthesis in cells from such patients are less severe than is the case in cells with allosteric regulation-impaired forms of the enzyme (12).

The genetic heterogeneity suggested by differences in the kinetic abnormalities and phenotypic expressions of inherited PRS superactivity has been confirmed. Point mutations in the translated region of PRPS1 provide the genetic basis for altered allosteric control of PRS activity (14); in contrast, PRS catalytic superactivity reflects altered regulation of the expression of the normal PRS1 isoform (10).

RT-PCR analysis of patient and normal fibroblast and lymphoblast RNA identified single base substitutions in the PRS1 cDNAs derived from each of 6 unrelated male patients with superactive, allosterically altered PRSs (14) and one heterozygous affected female (15). In each instance, the base change in PRS1 cDNA predicted a single amino acid substitution in PRS1, ranging from amino acid residue 51 to 192 of the 317 residue PRS1 polypeptide. The functional significance of these mutations in PRS1 was established by demonstrating that each male patient recombinant PRS1 showed the pattern and magnitude of aberrant allosteric responses to purine nucleotide inhibitors and Pi characteristic of PRS in cells from that patient. Thus, the genetic basis of inherited PRS superactivity associated with altered allosteric regulation is point mutation in the PRS1-coding region of the PRPS1 gene.

In contrast, mutations in the translated region of X-linked PRPS1 or PRPS2 genes do not account for PRS catalytic superactivity. Rather this variety of PRS overactivity involves increased abundance of PRS1 transcripts and isoforms with normal primary sequences (10). Direct measurement of rates of PRPS1 transcription (by nuclear runoff analysis) in patient lymphoblasts and fibroblasts has established that inherited overexpression of the normal PRS1 isoform in PRS catalytic superactivity results from selectively increased rates of PRPS1 gene transcription (16). To date, however, no differences have been found in the sequence of DNA immediately 5' to the transcription initiation site of PRPS1 when promoter region sequences of patient and normal PRPS1s, were compared. This finding raises the possibility of altered regulation of structurally normal PRPS1 promoters in PRS catalytic superactivity. Extended cloning and sequencing of PRPS1 5' and intron DNA and measurements of PRPS1 promoter activities by reporter gene analysis may help resolve this question.
Diagnostic methods
Measurement of PRS activities in dialyzed erythrocyte lysates (17) has usually been the initial approach to identifying PRS superactivity, with subsequent confirmatory kinetic studies being undertaken in extracts of cultured fibroblasts. The diversity of kinetic mechanisms underlying PRS superactivity, however, mandates evaluation of both kinetic and allosteric properties of the enzyme. The consequent lack of a simple diagnostic screening test for PRS superactivity has discouraged wider screening by routine enzyme assay. Alternative approaches to identification of defects in allosteric regulation of PRS have been utilized and include:
1) measurement of purine base incorporation into intact erythrocytes during incubation at 1.0 and 30 mM Pi (18). Ordinarily, purine base incorporation (a PRPP-dependent process) is substantially greater at the higher than the lower Pi concentration. In cells from patients with allosteric regulatory defects in PRS, however, there is little or no difference in rates of base incorporation at the two Pi concentrations.
2) demonstration of deficient PRS activity in hemolysates (14). In some families, male patients with defective allosteric regulation of PRS1 show deficiency of PRS activity in hemolysates as a result of a labile mutant enzyme. In these patients, the residual enzyme activity, (sometimes <3% of normal hemolysate PRS activity) demonstrates abnormal allosteric properties.
3) RT-PCR amplification and sequence analysis of PRS cDNAs carried out on small numbers of cultured fibroblasts or lymphoblasts. This approach to direct PRS sequencing is also applicable to normal peripheral blood leukocytes, although we have not had occasion to study comparable cells from affected individuals. It is important to note, however, these clues to structural defects in PRS in enzyme superactivity are, unfortunately, not applicable to confirmation of the diagnosis of the majority of patients with PRS superactivity in whom the amounts of normal PRS1 transcript and isoform are excessive as a result of accelerated transcription of PRPS1. In such patients, determination of increased PRS1 transcript and/or isoform levels currently provides the only definitive means to confirm PRS "catalytic" superactivity.

Management
Hyperuricemia and hyperuricosuria are the consequences of purine nucleotide and uric acid overproduction in PRS superactivity and underlie the metabolic manifestations of the disorder: gouty arthritis, uric acid urolithiasis, tophus formation, and, potentially, renal impairment arising from uric acid crystal deposition in the renal collecting system or from urate crystal deposition in the renal parenchyma. Prophylaxis, prevention of recurrence, and even reversal of established manifestations of these events can usually be achieved by measures aimed at reducing purine nucleotide and uric acid formation, combined with efforts to prevent or reverse urate and uric acid crystal deposition. These measures include: administration of the xanthine oxidase inhibitor allopurinol; a high daily fluid intake; and urinary alkalization (with potassium citrate), when uric acid urinary tract stone formation or excretion of uric acid gravel in the urine has occurred. In addition to direct inhibition of xanthine oxidase and thus diminished uric acid formation from hypoxanthine and xanthine, allopurinol administration results in deceleration of purine nucleotide synthesis de novo. This process potentiates the uric acid lowering effect of xanthine oxidase inhibition, apparently as a result of HPRT-mediated conversion of allopurinol to allopurinol nucleotide metabolites. Since allopurinol and its major metabolite oxipurinol are of limited solubility, vigorous efforts at maintaining hydration are necessary throughout the course of allopurinol administration. The potential toxicity of allopurinol as well as guidelines for its use in states of renal impairment have been published (19), as have principles of management of acute events accompanying urate crystal-induced arthritis and of acute and chronic uric acid renal diseases (1).

In contrast to success in the management of clinical consequences of hyperuricemia and hyperuricosuria in PRS superactivity, no specific management modalities for treatment of accompanying neurodevelopmental manifestations have been identified, and management is thus symptomatic. It is likely that more specific management recommendations will await the understanding of the basis of neurodevelopmental impairment in this disorder.

References

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