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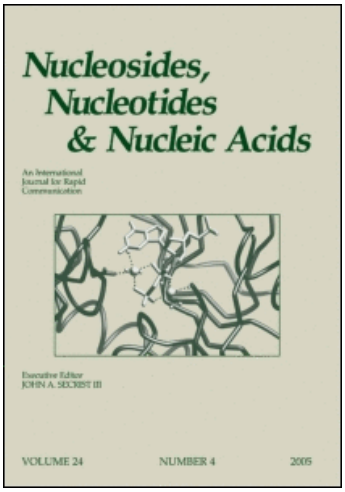

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Unusual Presentation of Kelley-Seegmiller Syndrome

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UNUSUAL PRESENTATION OF KELLEY-SEEGMILLER SYNDROME

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□ *Female carriers of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency have somatic cell mosaicism of HPRT activity and are healthy. We report a 50-year-old woman without gout or nephrolithiasis. She was never on allopurinol. Normal serum uric acid concentrations, increased plasma hypoxanthine, and xanthine were found. HPRT activity in erythrocytes was surprisingly low: at 8.6 nmol* h^{-1} *mg* $^{-1}$ *haemoglobin. Mutation analysis revealed a heterozygous HPRT gene mutation, c.215A > G (p.Tyr72Cys). Assessment of X-inactivation ratio has shown that > 75% of the active X-chromosome bears the mutant allele and could explain these unusual, previously undescribed findings.*

Keywords Hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency; Lesch-Nyhan disease variants; hyperuricemia; gout

INTRODUCTION

Hypoxanthine-guanine phosphoribosyltransferase (HPRT,E.C.2.4.2.8) deficiency is one of the most frequent inborn errors of purine metabolism. This defect is characterized by an increase in uric acid synthesis. The overproduction of uric acid results in hyperuricemia and hyperuricosuria. These biochemical abnormalities may cause nephrolithiasis, gout, and in the severe form acute renal failure. In addition, the most severe manifestation is severe neurologic disability and self-injurious behavior. This genetic defect causes three overlapping clinical syndromes, depending on the amount of residual enzyme activity. Less than 1.5% of residual enzyme activity, leads to neurologic impairment and impulsive, self-injurious behaviors along with

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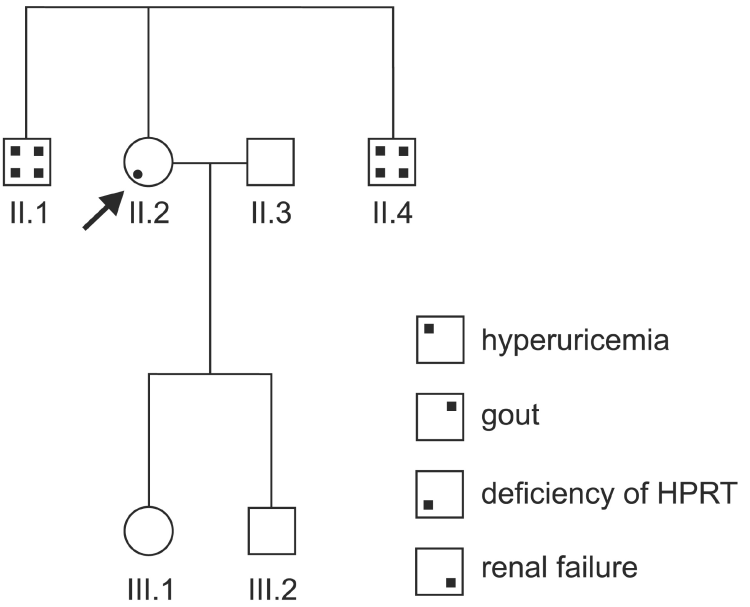

FIGURE 1 Family pedigree.

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overproduction of uric acid. This most severe form is known as Lesch-Nyhan disease (LND), previously Lesch-Nyhan syndrome. Residual activity, between 1.5 and 8%, is responsible for milder neurologic disabilities, such as minor clumsiness or even extrapyramidal and pyramidal motor dysfunction. Patients with at least 8% of residual activity suffered only from the clinical consequences of uric acid overproduction, such as nephrolithiasis and gout. The last two clinical forms, were previously named Kelly-Seegmiller syndrome or partial HPRT deficiency. HPRT deficiency has an X-linked mode of inheritance. The *HPRT* gene has been mapped to Xq26-q27 and more than 200 mutations responsible for the disease have been described. Female carriers have somatic cell mosaicism of HPRT activity and are healthy, with enzyme activity in erythrocytes within normal limits.[1] Few female patients with clinical symptoms of Lesch-Nyhan disease have been described.[1,2]

FAMILY STUDIES

Proband (II.2. in Figure 1), a 50-year old asymptomatic woman (sister of II.1 and II.4) did not experienced gout, nephrolithiasis, or hyperuricemia. She was never on allopurinol or any other therapy affecting serum urate levels. No evidence of any neurologic involvement had been observed. She came to our attention because of an investigation of other family members; partial HPRT deficiency had been previously diagnosed in her brother.

Case II.1 (Brother of the Proband)

This 48-year old man with 7-year history of chronic tophaceous gout was frequently hospitalized because of recurrent acute attacks of arthritis in his first metatarsophalangeals, heels, knees, elbows, carpal and distal interphalangeal joints. Laboratory tests revealed severe hyperuricaemia (more then 800 μmol/l). He had experienced one episode of renal colic, caused by radiolucent nephrolithiasis 25 years ago. Advanced chronic renal insufficiency appeared in 1998 and he was admitted to the Department of Internal Medicine. As the etiology of the chronic renal insufficiency was uknown, renal biopsy was indicated. The pathologist described advanced AA amyloidosis and gouty nephropathy, as described previously.[3] There was no evidence of any neurologic impairment. Due to severe hyperuricemia, the patient was referred to our department for detailed purine metabolic investigations, where partial HPRT deficiency was confirmed.

Case II.4 (Brother of the Proband)

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This 56-year old man with a 10-year history of recurrent renal colic caused by radiolucent nephrolithiasis, proteinuria, chronic polyarticular arthritis, and polyneuropathy, was hospitalized because of clinical symptoms of nephrotic syndrome. Large tophi were present on the right elbow. The examination of a tophus aspirate confirmed the presence of urate crystals. Hyperuricaemia (612 μmol/l), increased ESR, CRP, and nephrotic range proteinuria were the most alarming of the laboratory results. His renal function was normal. X-rays depicted erosive bone changes in the interphalangeal and metatarsophalangeal joints, wrists, and knees. Chronic tophaceous gout was diagnosed. No neurologic disability was observed. Because of unexplained proteinuria, a renal biopsy was performed, which confirmed chronic urate nephropathy and AA amyloidosis, as described previously.[3] Subsequently, the patient was referred to our department. A detailed purine metabolic invetigation revealed partial HPRT deficiency.

Case III.2 (Son of the Proband)

He was an asymptomatic, 27-year-old man. He had not experienced either gout, nephrolithiasis, nor hyperuricemia. He was never on allopurinol, and has no neurologic involvement.

METHODS

Uric acid in serum was measured by a specific enzymatic method. Creatinine in the plasma was measured by the Jaffé reaction, adapted to the autoanalyser. Endogenous plasma purines and purine enzymes activities in lysed

erythrocytes were measured as described.[4] Genomic DNA was isolated from peripheral leukocytes. Mutation analysis of *HPRT* gene was performed using cDNA of the Case II.1 (the first affected family member who came to medical attention). One RT/PCR product, containing entire coding sequence and parts of 5′ UTR and 3′ UTR of the *HPRT* gene, was subjected to direct sequencing. The mutation identified was verified by sequencing analysis using genomic DNA as a template. Other family members were genotyped for the mutation using genomic DNA. For X-inactivation studies, genomic DNA from peripheral blood leukocytes was modified with sodium bisulfite to allow active and inactive X-chromosomes to be distinguished (HUMARA assay). Briefly, highly polymorphic promoter region of the androgen receptor (AR) gene has been amplified using methylation-specific PCR with fluorescently-labeled primer. Amplified PCR products have been analyzed as described previously.[5]

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RESULTS

The family pedigree is shown on Figure 1. Table 1 shows the results of the detailed purine metabolic investigations of the family members. Normal serum urate levels (due to allopurinol therapy), elevation of plasma hypoxanthine and xanthine, and low HPRT activity in erythrocytes are evident in case II.1. These tests together with identified mutation c.215A > C (p.Tyr72Cys) in the third exon of the HPRT gene confirmed a diagnosis of partial HPRT deficiency. The same findings including mutation analysis were observed in case II.4 (brother of the proband). Severe hyperuricemia in this patient was evident. Our proband (II.2) had repeatedly normal serum urate levels, increased hypoxanthine and xanthine levels, and low HPRT activity in erythrocytes. Activities of phosphoribsylpyrophosphate synthetase (PRibPP synthetase) in all family members were within normal limits.

Subsequent mutation analysis revealed that the proband is heterozygous for the family-specific mutation c.215A > C (p.Tyr72Cys). No other mutation within the *HPRT* gene was observed.The son of the proband (III.2) has no elevation of uric acid in the serum, nor elevation of purine metabolites in the plasma, and has normal activity of HPRT. The mutation c.215A > C was not detected. Figure 2 demonstrates the results of X-inactivation studies in the proband and her son. Fluorescent-labelled PCR product of the HUMARA gene are shown. Different lengths of alleles correspond to paternal and maternal X chromosomes.

We were not able to obtain DNA samples from the parents of the heterozygous female studied. It is obvious, however, that the X chromosome that has been inherited by the healthy son (presumably the chromosome without mutation) has been preferentially inactivated. Hence, the X chromosome with a mutation is preferentially active. The results showed a

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TABLE 1 Results of purine metabolic investigations in blood, including enzyme activities in erythrocytes and mutation analysis

Patient	Age (years)	Urate (μmol/l)	Creatinine (μmol/l)	Hypoxanthine (μmol/l)	Xanthine (μmol/l)	HPRT (nmol/h/mg Hb)	PRibPP synthetase (nmol/h/mg Hb)	Presence of c.215A > G (p.Tyr72Cys) in *HPRT* gene
II.1	48	268*	71	55.3	19.3	1.0	58	+ (hemizygote)
II.2	50	314	55	19.8	7.5	8.6	127	+/− (heterozygote)
II.4	56	797	124	–	–	3.9	105	+ (hemizygote)
III.2	27	345	77	2.1	2.9	105	168	−(normal)
ref. ranges		female 120–340 male 120–416	28–100	<3.5	<3.1	50–500	20–170	

*–on allopurinol therapy, Hb–haemoglobin.

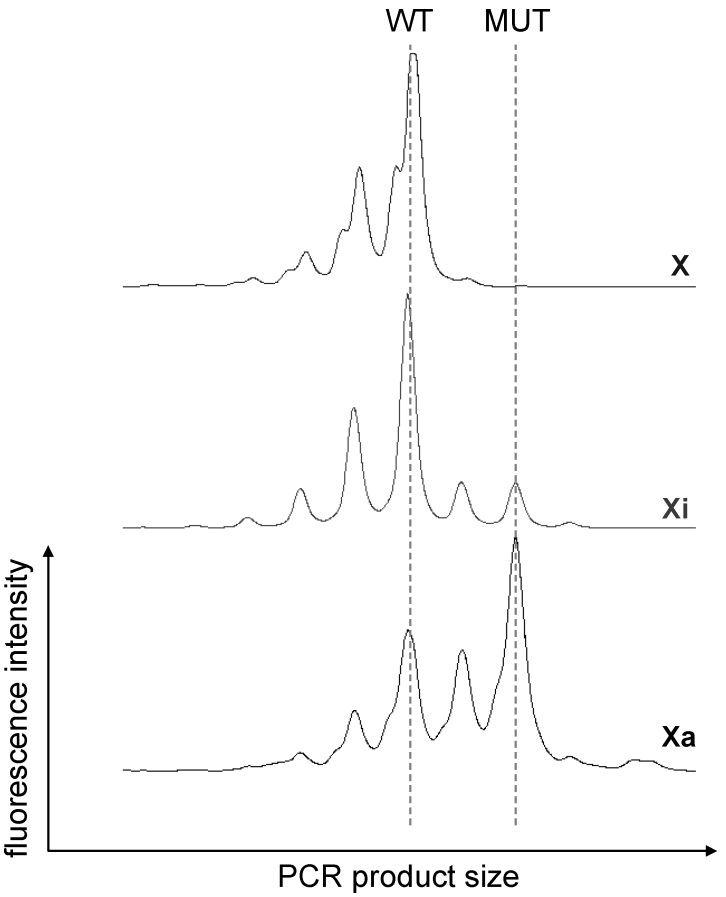

FIGURE 2 Skewing of X inactivation of the proband. As examined by HUMARA assay, the healthy son of the proband (**X**) has inherited shorter, presumably widtype allele (**WT**). In the proband, the wildtype allele seems to be preferentially inactivated (**Xi**) and reciprocally, mutant allele (**MUT**) is more frequent among PCR products of active X-chromosome (**Xa**). Actual percentage of skewing has been obtained as decribed[5] and equalizes for the preferential amplification of shorter PCR products. Multiple minor peaks preceding the main peak are commonly seen, inheritant artefact of the method arising from errors produced by DNA polymerase during amplification of repetitive (CAG)n sequence and do not influence the results.

skewed inactivation ratio (25:75) in favor of the mutant allele, which could explain our findings of very low HPRT activity.

DISCUSSION

The full biochemical and clinical manifestations of Lesch-Nyhan disease in females are quite rare. Previously, only seven female cases with complete HPRT deficiency have been reported.[1] Moreover, only two additional patients with LND variants (partial deficiency, without neurologic symptoms) were described. Inokuchi et al.[6] reported a 29-year-old woman

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with gouty attacks and a history of urinary calculi. We had previously found a 16-year-old girl with gouty arthritis.[7] These unusual findings expands the differential diagnosis of gout in females, and highlights the need to perform detailed purine metabolic investigations in females with unexplained hyperuricemia or gout.

The mechanistic aspects of the primary cause of symptomatic females were highlighted by X-inactivation studies. X chromosome inactivation is widely believed to be random in early female development, resulting in a mosaic distribution of cells. Females are thus expected to have varying X inactivation ratios, defined as the proportions of cells expressing alleles from one or the other X chromosome. These ratios can range from a completely skewed ratio of 0:100, where the same X chromosome is active for all cells, to a 50:50 ratio, where each X chromosome is active in an equal number of cells. Recent data showed that the X chromosome inactivation ratio can be considerabely skewed in a relatively small proportion of females.[8,9] Therefore, in heterozygous females, X chromosome inactivation can fundamentally influence the course of X-linked diseases. In unfortunate cases, when most cells have a transcriptionally active X chromosome with a deleterious mutation, symptoms can be as severe as in hemizygous males. A recent report of a female with LND documented the absence of transcription of the two HPRT alleles.[2]

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In our reported asymptomatic woman, we have found an abnormal elevation of hypoxanthine and xanthine, very low activity of HPRT in erythrocytes, and normal uric acid. Such findings have not previously been described. Our results of the X-inactivation study in this patient show a skewed inactivation ratio (25:75) in favor of mutant allele, which could explain these abnormal findings. Although enzyme deficiency with urate overproduction (presenting as high plasma oxypurines) is evident, the reason for normal serum urate concentrations remains uncertain. One could speculate about possible increased disposal of uric acid in the gut, but this has not been demonstrated in this individual. The mutation found in our proband was previously described in two Korean siblings, where one boy developed acute renal failure.[10] Abnormal findings in our proband raise the question of whether the reported biochemical abnormalities are in agreement with the carrier status of HPRT deficiency.

There have been only a few studies of purine metabolism in HPRT carriers. One comprehensive study[11] showed that an elevated hypoxanthine and/or xanthine excretion rate differentiates most heterozygotes for HPRT deficiency from non-carrier women, and thus could be useful for carrier diagnosis. Our biochemical findings are different from those previously reported[11] in the following two aspects: a) we have found very low HPRT activity in erythrocytes (unlike the reported mean difference of HPRT activity in red cells 21 nmol h^{-1} mg^{-1} haemoglobin); and b) elevated plasma oxypurines (unlike normal levels in heterozygotes carriers). These findings

provide additional information about purine metabolism in HPRT deficient females. More data from females with this disorder are needed.

Another aspect of our family study is evident. The variants of LND (previously partial HPRT deficiency) are still underrecognized conditions. Both of our two reported brothers came to attention for purine metabolic investigation with damaged kidneys and joints and an overproduction of uric acid. Available therapy could have prevented the clinical consequences of hyperuricemia and hyperuricosuria had earlier diagnosis been made. There is a need for purine metabolic investigations in asymptomatic family members of subjects with LND variants, and a greater awareness of this disorder is needed for an efficient diagnosis.

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