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Biochemical and molecular study of mentally retarded patient with partial deficiency of hypoxanthine-guanine phosphoribosyltransferase

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Abstract

Nucleotide metabolism was studied in erythrocytes of a mentally retarded child and family members. Partial hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency was found in the propositus and an asymptomatic maternal uncle. Studies in crude lysates demonstrated decreased apparent V_{max} and slightly decreased apparent K_m for hypoxanthine in both HPRT-deficient subjects. Genomic DNA analysis revealed a single nucleotide change with leucine-147 to phenylalanine substitution in both subjects; mother and grandmother were heterozygous carriers of the same defect. This new variant has been termed $HPRT_{Potenza}$. Increased erythrocyte concentration of NAD and rate of synthesis by intact erythrocytes were found in the patient; increased activities of nicotinic acid phosphoribosyltransferase (NAPRT) and NAD synthetase (NADs) were demonstrated in erythrocyte lysates, with normal apparent K_m for their substrates and increased V_{max}. These alterations were not found in any member of the family, including the HPRT-deficient uncle. These findings show multiple derangement of nucleotide metabolism associated with partial HPRT deficiency. The enzyme alteration was presumably not the cause of neurological impairment since no neurological symptoms were found in the HPRT-deficient uncle, whereas they were present in the propositus' elder brother who had normal HPRT activity.

Keywords: Hypoxanthine-guanine phosphoribosyltransferase deficiency; Human HPRT mutation; Lesch–Nyhan syndrome; Purine nucleotide; Pyridine nucleotide; NAD

1. Introduction

Hypoxanthine-guanine phosphoribosyltransferase (HPRT) (EC 2.4.2.8) catalyses salvage of the purine bases hypoxanthine and guanine to their respective monophosphate nucleosides (IMP and GMP) by a 5′ -phosphoribosyl-1-pyrophosphate (PPRibP)-dependent reaction. It is a cytoplasmic enzyme ubiquitous in humans, displaying different specific activities during development and in different tissues (highest activities in testis and brain tissue) [1–3]. The genetically determined deficiency of HPRT is associated with a number of clinical phenotypes mainly depending on the degree of deficiency. Marked uric acid overproduction resulting in hyperuricemia, nephrolithiasis and gout presenting at an early age is a common feature. Neurological abnormalities may be absent (previously called Kelley–Seegmiller syndrome, KSS) [4], or mild in cases with intermediate severity (some degree of mental retardation, spasticity, dystonia), while the most severely affected cases display a disabling neurological syndrome, characterized by choreoathetosis, spasticity and compulsive self-injurious behavior [5,6]. The latter most severe form is usually associated with virtually complete deficiency and is known as Lesch–Nyhan syndrome (LNS) [7]. The connection between the neurological syndrome described in LNS patients and HPRT deficiency is still unclear.

Human HPRT is encoded by a single structural gene at Xq26–27 [8], consisting of nine exons and eight introns. The entire HPRT gene has been sequenced [5], and several

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different alterations in the coding region have been described as responsible for HPRT deficiency [5,9]. The amino acid sequence of HPRT has been determined [10], the physical and kinetic properties have been studied, and different alterations of the enzyme in patients with various degrees of deficiency have been reported [1,11–17]. Additional biochemical tissue-specific alterations have been reported in patients with different degrees of HPRT deficiency. They have mostly been detected in erythrocytes, and include increased PPRibP concentrations (also found in lymphoblasts and mainly fibroblasts) [18], increased UDP-sugars (UDPs) and appreciable levels of 5-amino-4-imidazole-carboxamide riboside 5′-triphosphate (ZTP) [19], increased activities of adenine phosphoribosyltransferase (APRT) and IMP dehydrogenase [20], grossly increased NAD concentrations [21] and GTP depletion. Involvement of NAD and its precursors in different neurological syndromes has been reported also in connection with the neurotransmitter serotonine [22,23] and has stimulated research in this rather neglected field. The increased NAD concentrations in erythrocytes of patients with HPRT deficiency have not yet been explained. Altered intracellular stability of the coenzyme can be ruled out [24], and underutilization has not been found. We reported altered NAD synthesis in intact erythrocytes of a patient with partial HPRT deficiency and of an LNS patient [25] and in fibroblasts of an LNS patient [26].

Here we present data on purines, pyridines and pyrimidines in the erythrocytes of a mentally retarded child and several family members. Partial deficiency of HPRT was found in the propositus and an asymptomatic maternal uncle. At molecular level, a mutation was found in exon 6. We also detected biochemical alterations of purines and pyridines and a significant increase in the activities of two enzymes, nicotinic acid phosphoribosyltransferase (NAPRT) (EC 2.4.2.11) and NAD synthetase (NADs) (EC 6.3.5.1) catalysing NAD synthesis from nicotinic acid (NA), previously reported in LNS patients [27] (Fig. 1). These findings support the hypothesis that the biochemical basis for increased NAD concentrations in erythrocytes of all HPRT-deficient patients examined so far is increased synthesis. The clinical manifestations are peculiar since neither the propositus nor the uncle with the same HPRT alteration had classical KSS. At our knowledge the propositus' uncle is the second case with apparently no symptoms related to enzyme deficiency [28].

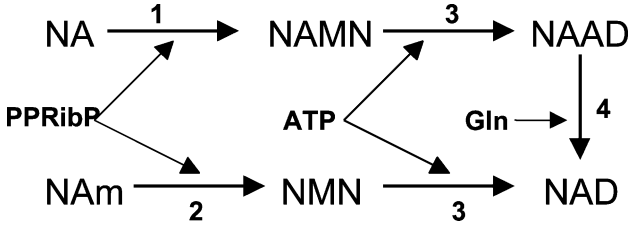

Fig. 1. Synthesis of NAD in human erythrocytes. NA: nicotinic acid; NAm: nicotinamide; NAMN: NA mononucleotide; NMN: NAm mononucleotide; NAAD: NA adenine dinucleotide; NAD: NAm adenine dinucleotide; PPRibP: 5-phosphoribosyl-1-pyrophosphate; gln: glutamine. (1) NA phosphoribosyltransferase (EC 2.4.2.11); (2) NAm phosphoribosyltransferase (EC 2.4.2.12); (3) NAMN adenylyltransferase (EC 2.7.7.18) and NMN adenylyltransferase (EC 2.7.7.1); (4) NAD synthetase (EC 6.3.5.1).

2. Materials and methods

2.1. Patients and relatives

The propositus, L.C., male, Italian, was the second child of apparently healthy parents. At age 9 months he developed epileptic seizures and hypotonia. At age 11 months, he showed severe neurodevelopmental retardation, was unable to walk and spoke only a few words. His purine metabolism was first investigated during a screening of 150 children with neurological impairment and partial HPRT deficiency was found. Analyses were repeated on blood samples drawn at the age of 14, 21, 36 months and 5 years. At 5 years he could hardly sit, with frequent falls on his right hand side, and could only stand and walk few steps with support. Speech was virtually absent, and frequent stereotypes (rolling) and attempts to bite himself appeared. Seizures were frequent, with short attacks of impaired consciousness. The following family members of L.C. were examined: the elder brother (G.C.), age 8 years when first investigated, who had severe neurodevelopmental retardation, drug-resistant seizures and autistic features but could sit and walk properly; father, mother, grandmother and mother's sisters (A, C and R) and brother (G.M.). The latter, maternal uncle of L.C., age 34 years, had no clinical symptoms, not even related to any kidney impairment, but turned out to have partial HPRT deficiency.

Control children without metabolic dysfunction, 1–14 years of age were also examined (14 females and 12 males). Control adults were 32 healthy volunteers 18–64 years of age (17 females and 15 males). All subjects, including the propositus' parents and relatives, gave their informed consent to the study. Blood samples were obtained as part of the treatment programme or provided by voluntary donors and thus ethical committee approval was not required.

2.2. Materials

Reagents of analytical grade were purchased from Sigma (St. Louis, MO, USA). Chemicals for HPLC separation were of the highest available quality.

2.3. Biochemical analysis

Non-radiochemical HPLC-linked methods previously described [27,29–32] were used to measure purine and pyridine nucleotide content in erythrocyte extracts, nucleosides and bases in plasma extracts and urine, as well as enzyme activities in erythrocyte lysates. The activities of the

following enzymes were detected by previously described methods: HPRT, APRT [27], PPRibP synthetase (PRPS) [30], adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP) [21], NAPRT [29], nicotinic acid mononucleotide (NAMN) and nicotinamide mononucleotide (NMN) adenylyltransferase (NAMN/NMN-AT), NADs [31], orotate phosphoribosyltransferase (OPRT) and OMP decarboxylase (ODC) [32]. Urine samples were prepared according to Simmonds et al. [33]; 24-h urine specimens collected after 3 days of low-purine diet, preserved with toluene, were warmed at 56 °C for 30 min with frequent shaking to dissolve precipitated compounds, and diluted 1:31 with HPLC eluant A for HPLC processing (see below).

Conversion of ^{14}C-labelled precursors hypoxanthine (Hyp), adenine (Ade), NA and nicotinamide (NAm) into nucleotides was investigated after incubation of intact cells in isotonic PPRibP-producing medium, as previously described [27]. Incubation time with Hyp and Ade was 1 h, and that with NA and NAm was 6 h.

Two HPLC apparatus were used, consisting of a Beckman System Gold Module 126, with a mod. 167 dual channel scanning detector module (System 1), or a Beckman 420 controller, equipped with a mod. 163 variable wavelength detector (System 2) in-line with a mod. 171 radioisotope detector with a packed solid scintillation cell of 125 or 300 μl capacity (Beckman, San Ramon, CA, USA). Supelcosil LC-18 columns (3 μm particle size, 75 × 4.6 mm) or Beckman ODS Ultrasphere columns (3 μm particle size, 70 × 4.6 mm) or Phenomenex Luna C18 (3 μm particle size, 75 × 4.6 mm) equipped with guard columns (Supelguard, 5 μm, 2 cm, or Phenomenex Security guard 4 mm L × 3 mm ID) were used.

The RP-HPLC elution procedures for assays conducted on erythrocytes and plasma have been described [27]. A gradient of 0.01 M potassium phosphate buffer pH 5.5 (eluant A) and methanol (eluant B), as described for plasma analysis, was used for the analysis of urine samples. Peak identities were confirmed by retention time, coelution with internal standards and 280/260 nm absorbance ratios; concentration/area linear plots were developed for quantification. A mixture of all standard solutions was injected daily to check the reliability of separation and any modification of RTs due to the chromatographic system. Radioactive compounds were separated by Sys 2; the UV trace at 260 nm and the radioactivity trace were monitored. Radioactive peak area was converted to count per minute and then nanomoles according to the specific activity of the precursor used.

2.4. DNA analysis

Genomic DNA was purified from peripheral blood lymphocytes according to Miller [34]. DNA fragments of each exon, amplified by symmetric followed by asymmetric PCR [35], were purified with glasspowder (Geneclean, La Jolla) and directly sequenced (Sequenase, USB, Amersham). SSCP analysis was performed as described previously [36].

3. Results

3.1. Purine and pyridine enzymes

The activities of the enzymes of purine (PRPS, HPRT, APRT) and pyridine (NAPRT, NMN/NAMN-AT, and NADs) metabolism of the propositus and relatives are reported in Table 1. HPRT activity in erythrocyte lysate of L.C. was 17% of the normal value and APRT activity was increased more than twofold. L.C.'s uncle, G.M., showed an even lower HPRT activity (10%) with APRT activity in the normal range. HPRT/APRT activity ratio was very low in both the propositus and G.M. (0.45 and 0.38, respectively) compared with controls (5.8 ± 1.6). PRPS activity was within the normal range in all subjects except G.M., in whom it was low. NAPRT and NADs activities were higher than normal in the propositus, but not in G.M.; NMN/NAMN-AT activities were within the normal range in all subject. The activities of the purine enzymes ADA and PNP,

Table 1
Purine and pyridine enzyme activities in crude lysates of patient and relatives

Subjects	HPRT	APRT	PRPS	NAPRT	NMN-AT	NAMN-AT	NADs
L.C.	23	51	38.2	2.50	0.37	0.29	0.89
Mother	121	20	38.8	1.49	0.25	0.27	–
Father	143	18	43.1	1.42	0.23	0.24	0.24
Brother	118	19	36.4	1.02	0.38	0.26	0.37
Uncle G.M.	11	29	14.1	1.19	0.20	0.22	0.27
Grandmother	77	22	31.3	1.09	0.20	0.19	0.20
Aunt A.	114	24	44.5	1.01	0.20	0.14	0.59
Aunt C.	121	22	43.2	1.25	0.24	0.13	0.45
Aunt R.	98	23	40.1	0.92	0.17	0.14	0.20
Controls							
Adults	113 ± 11	21 ± 4	37 ± 9	1.01 ± 0.27	0.32 ± 0.08	0.29 ± 0.08	0.39 ± 0.11
Children	127 ± 30	21 ± 3	41 ± 10	0.87 ± 0.37	0.437 ± 0.122	0.35 ± 0.12	0.45 ± 0.2

Activities in nmol/h/mg Hb (mean ± standard deviation).

Table 2
Nucleotide concentration in erythrocytes of patients and relatives

Subjects	AMP	ADP	ATP	GDP	GTP	UDPs	ADPR	NAD	NADP
L.C.	8	111	1530	14	68	132	4.6	125	39
Uncle (G.M.)	11	120	995	7.5	68	55	12.5	66	41
Mother	11	135	1067	5	64	79	3.6	55	36
Father	24	111	1300	10	80	99	2.3	60	41
Brother	6	126	1526	10	78	82	5.4	72	46
Controls									
Adults	16 ± 14	147 ± 57	1099 ± 264	12 ± 7	71 ± 29	67 ± 24	7.2 ± 3.9	42 ± 12	36 ± 9
Children	10 ± 4	151 ± 20	1270 ± 98	13 ± 9	85 ± 23	69 ± 4	9 ± 6.1	56 ± 22	41 ± 16

Concentrations in nmol/ml packed cells (mean ± standard deviation).

assayed in the propositus L.C. and brother G.C., were within the control range (control values for ADA: 78 ± 18 nmol/h/mg Hb; L.C.:106, G.C.:138; control values for PNP: 4402 ± 965 nmol/h/mg Hb; L.C.: 5254, G.C.: 4871). The activities of the pyrimidine enzymes OPRT and ODC were also within control range in the propositus (OPRT 0.12, ODC 0.18; children control range 0.33 ± 0.18 and 0.31 ± 0.14 nmol/h/mg Hb, respectively) and uncle G.M. (OPRT: 0.31, ODC 0.28; adult control values 0.19 ± 0.03 and 0.18 ± 0.03 nmol/h/mg Hb, respectively).

3.2. Metabolites in erythrocyte and plasma extracts and in urine

Erythrocyte concentrations of nucleotides are reported in Table 2. NAD levels were twice the normal and UDPs concentrations were above normal in the propositus, but not in G.M.; all the other nucleotides were within the age-matched control range. The finding of the above alterations in the propositus' erythrocytes led us to suspect either HPRT or PNP deficiency [21], but the latter was ruled out. ADP-ribose (ADPR), produced by NAD breakdown, was also in the normal range, though at the upper limit in G.M.

Plasma and urine concentrations of some metabolites are reported in Table 3. Uric acid (UA) concentrations in plasma were above the normal range in G.M., while hypoxanthine (Hyp) concentrations were normal; the reverse situation was found in the propositus, who showed raised levels of hypoxanthine and normal levels of uric acid; no alteration was detected in the propositus' brother. In the propositus, urinary hypoxanthine and xanthine (Xan) levels were also above control values; uric acid was also elevated on the basis of creatinine (crea) excretion (creatinine was 37 mg/dl in the propositus and 32 mg/dl in the brother; control values were 81 ± 45 mg/dl). Plasma concentrations of tryptophan and Nam, possible NAD precursors, were in the control range.

3.3. Intact cell studies

The rate of incorporation of radiolabeled hypoxanthine, adenine, NA and NAm into nucleotides by intact erythrocytes is reported in Table 4. The uptake of hypoxanthine was less than half the control in the propositus, and that of adenine was double. The ratio of hypoxanthine uptake versus adenine uptake was lower than controls in the propositus (0.3, adult controls ranging 1.5 ± 0.5; and age-matched controls 0.99 ± 0.28). Incubation with NA and NAm yielded mono- and di-nucleotides. NAD was obtained from both precursors; NAMN and NA adenine dinucleotide (NAAD) from NA; NMN from NAm. The uptake of NA and NAm was slightly above that of age-matched controls

Table 3
Metabolites in plasma and urine of patients and relatives

Subjects	Plasma					Urine			
	UA	Hyp	Xan	Trp	NAm	UA	UA/crea	Hyp	Xan
L.C.	212	54	1.7	51	3.4	61.1	1.66	4.7	22.2
Uncle (G.M.)	356	5.3	3.3	36.8	1.5				
Mother	181	2.3	–	35.9	1.0				
Father	245	2.5	1.3	34.8	1.0				
Brother	143	9.3	6.1	50.1	1.0	24.2	0.75	0.2	1.5
Controls									
Adults	263 ± 57	3.8 ± 2.9	1.7 ± 0.8	40.7 ± 7	3.2 ± 2.1	48 ± 24	0.41 ± 0.2	1.12 ± 0.4	2.8 ± 1.7
Children	209 ± 50	4.2 ± 2.5	1.9 ± 0.9	39.5 ± 11	2.0 ± 1.8	69 ± 32	0.98 ± 0.39	1.13 ± 0.7	3.7 ± 2

Concentrations in plasma in nmol/ml; in urine mg/dl (mean ± standard deviation).
Trp: tryptophan; Nam: nicotinamide.

Table 4
Production of purine nucleotides from radiolabeled Hyp and Ade (1 h incubation), and of pyridine nucleotides from NA and NAm (6-h incubation) in intact erythrocytes

Precursor	Hyp	Ade	NA			NAm	
Products	IMP	AMP + ADP + ATP	NAMN	NAAD	NAD	NMN	NAD
Subjects							
L.C.	76	235	410	11	105	8.9	11.2
Brother	215	169	370	14	98	11.0	5.1
Controls							
Adults	173 ± 56	106 ± 27	346 ± 37	15.1 ± 8.4	55.9 ± 27.0	6.1 ± 2.3	7.2 ± 2.3
Children	160 ± 39	169 ± 37	296 ± 53	16.3 ± 8.6	56.7 ± 17.8	6.5 ± 4.4	5.04 ± 3.3

Production rates in nmol/ml erythrocytes (mean ± standard deviation).

in the propositus (NAMN and NAD productions were elevated).

3.4. HPRT studies

3.4.1. Apparent kinetic characteristics

The apparent V_{max} and K_m for both substrates of HPRT (hypoxanthine and PPRibP) were determined in crude hemolysates of the propositus and his uncle, parents and brother. Markedly decreased apparent V_{max} (14 and 11 nmol/h/mg Hb in L.C. and G.M., respectively, compared to 76.3 ± 3.2 nmol/h/mg Hb in controls) and slightly decreased apparent K_m for hypoxanthine (0.5 and 1.8 μmol/l in L.C. and G.M., respectively, compared to 5.2 ± 0.2 μmol/l in controls) were found. To our knowledge, this type of kinetic alteration had not been reported previously. The K_m for PPRibP was within the control range (43.9 and 37.9 μmol/l in L.C. and G.M., respectively, compared to 37.5 ± 8.5 μmol/l in controls).

3.5. Enzyme stability

Recovery of HPRT activity after storage of erythrocytes at −80° and after heating lysates to 60 °C was measured in order to check enzyme stability. No difference was found among patients L.C. and G.M. and controls in either case (no decay of enzyme activity after storage for up to 3 years nor after heating for up to 8 min; data not shown).

3.6. Molecular studies

The complete nucleotide sequence of the coding region of the HPRT gene of patient L.C. showed substitution of C538 to T in exon 6. This mutation, confirmed by sequencing of the complementary strand and SSCP, changes leucine-147 into phenylalanine. This is a new variant, not reported previously, and has been termed $HPRT_{Potenza}$. The same mutation was confirmed in uncle G.M. No alterations were found in any other exon. The mother and grandmother were heterozygous for the mutation, whereas the father, brother and mother's sisters were homozygous for the normal sequence. Fig. 2 shows sequence analysis of PCR-amplified DNA of exon 6 of the HPRT gene in closest relatives. To our knowledge this is the first Italian patient with partial deficiency in whom mutation of the HPRT gene has been investigated.

3.7. NAPRT and NADS studies

The increased activity of NAPRT and NADs in crude lysate of the propositus may be due to an increased number of active molecules, or the presence of altered molecules, or

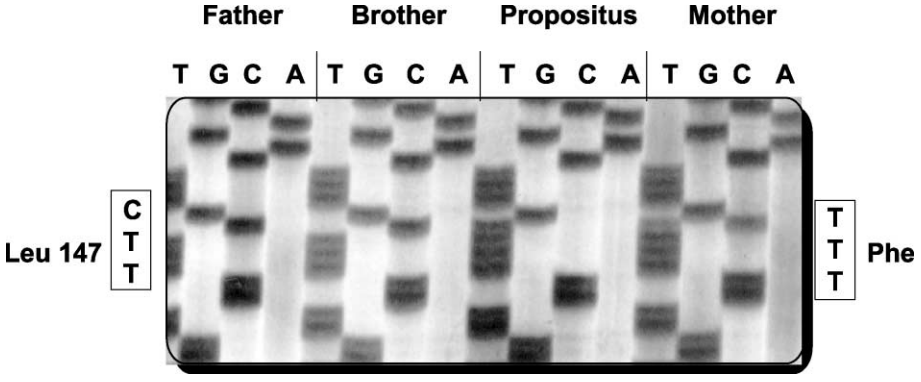

Fig. 2. SSCP analysis of PCR-amplified DNA of exon 6 of the HPRT gene in closest relatives.

even the presence of endogenous metabolites activating both enzymes. The latter hypothesis was ruled out by comparing the activity measured in lysates from L.C. with that measured in mixtures of equal volumes of lysate from the patient and control lysate. No significant increase in activity with respect to the expected mean value was found for either enzyme (NAPRT—expected mean for L.C. plus control 1.28 nmol/h/mg Hb, measured activity 1.37 nmol/h/mg Hb; NADs—expected mean for L.C. plus control 0.62 nmol/h/mg Hb, measured activity 0.67 nmol/h/mg Hb). Storage of washed erythrocytes for up to 1 year at $-80\,^{\circ}C$ did not show any difference in enzyme stability (no significant decay in patients or controls; data not shown).

4. Discussion

Metabolic and molecular studies were conducted in a child (L.C.) with severe neurodevelopmental retardation and his relatives. The child was found to have partial HPRT deficiency without hyperuricemia, possibly due to the early age of the child [1], and with mild hyperuricuria. His elder brother, also mentally retarded with different features including autistic behavior, had no deficiency in HPRT activity. Partial deficiency was found in the propositus' maternal uncle (G.M.) who showed no neurological or gout symptoms. Metabolic studies involving purine, pyrimidine and pyridine metabolism, devised to compare results with findings in complete HPRT deficiency [21,27], were performed in the erythrocytes. Molecular studies aimed at understanding the predictable consequences of mutation on HPRT activity were performed on genomic DNA. SSCP analysis of PCR-amplified DNA of the two partially HPRT-deficient subjects (L.C., G.M.) confirmed a point mutation C538T in both and the full nucleotide sequence of genomic DNA was determined [37]. This mutation has not been reported previously and the new mutant form of human HPRT was named $HPRT_{POTENZA}$. The propositus' mother and grandmother were found to be heterozygous carriers. To our knowledge, L.C. is the first Italian patient in whom a mutation of the HPRT gene causing partial deficiency has been investigated. Moreover, the propositus' uncle G.M. is probably the second case of completely asymptomatic partial HPRT deficiency [28].

Almost 300 different mutations occurring throughout the HPRT gene have been identified so far and correlation between mutations and phenotype investigated [5,6]. Studies lead to the conclusion that identification of mutations provides a poor tool to predict the phenotypic manifestation. Mutations leaving some residual enzyme activity are typically associated with less severe clinical manifestations. Deletions and duplications within the gene have mostly been demonstrated to cause complete enzyme deficiency and LNS; point mutations have been identified in most patients with either partial or complete deficiency. Conserved amino acid substitutions are likely to alter HPRT protein conformation less severely than nonconserved substitutions. In the mutation described here, the resulting protein differs from normal HPRT by a single amino acid (nonpolar leucine vs. nonpolar phenylalanine), yet the enzyme activity is as little as 17% of normal. The amino acid substitution found in $HPRT_{Potenza}$ is close to the hypothesized binding-domain of hypoxanthine, and is included in a homology area common to other phosphoribosyltransferases [38], which suggests that also Leu147 might be involved in the binding of hypoxanthine. These data are consistent with the slightly decreased apparent K_m for hypoxanthine found in crude hemolysates of the two affected subjects also showing decreased V_{max}. Different kinetic alterations have been described in mutant enzymes such as increased K_m for PPRibP [15,39,40] and for hypoxanthine [41] To our knowledge, the type of kinetic alteration found in $HPRT_{Potenza}$ had not been reported previously.

The appreciable conversion of hypoxanthine to IMP in L.C.'s intact erythrocytes suggests that the deficiency in the activity of crude lysates may be related to molecular instability, though the activity did not show significant decay either after storage or heating. The patient might therefore have sufficient activity in his cells, as demonstrated by the ratio of hypoxanthine uptake to adenine uptake, which was one-third the normal value, whereas the ratio of HPRT activity to APRT activity in lysates was one-tenth the normal value.

The erythrocytes of the propositus (but not his uncle) had the typical biochemical features reported in LNS cells [21,27]: increased APRT activity, increased NAD concentrations and related activities of NAPRT and NADs. We recently reported increased erythrocyte 5′-nucleotidase activity in the partially deficient child L.C., as well as in a group of LNS patients [42]. NAD concentrations in erythrocytes of L.C. were twice the controls, possibly due to increased synthesis through raised activities of NAPRT and NADs, as suggested in LNS patients [27]. The alteration did not seem to be caused by increased stability of the two enzymes [43] or by the presence of intracellular activators, and is probably related to molecular modifications or increased synthesis of the two enzymes. OPRT and ODC activity, often reported to be dramatically elevated in LNS patients and possibly related to allopurinol treatment [44], were within the normal range in L.C. and G.M., neither of whom was on allopurinol therapy. The lack of any biochemical alteration, except for low HPRT activity in G.M.'s erythrocytes, is puzzling and suggests that partial deficiency may have different features and vary from one patient to another. The neurological manifestations in the child were likely not related to partial HPRT deficiency. In fact they were absent in the partially HPRT-deficient uncle. Moreover, a severe neurological impairment, though with different clinical features, was also present in the elder brother who had normal HPRT activity. The role of the biochemical

alterations identified in patient L.C. on the neurological manifestations is thus unknown.

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