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Phenotypic variation among seven members of one family with deficiency of hypoxanthine–guanine phosphoribosyltransferase

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ABSTRACT

We describe a family of seven boys affected by Lesch–Nyhan disease with various phenotypes. Further investigations revealed a mutation c.203T>C in the gene encoding HGprt of all members, with substitution of leucine to proline at residue 68 (p.Leu68Pro). Thus patients from this family display a wide variety of symptoms although sharing the same mutation. Mutant HGprt enzyme was prepared by site-directed mutagenesis and the kinetics of the enzyme revealed that the catalytic activity of the mutant was reduced, in association with marked reductions in the affinity towards phosphoribosylpyrophosphate (PRPP). Its K_m for PRPP was increased 215-fold with hypoxanthine as substrate and 40-fold with guanine as substrate with associated reduced catalytic potential. Molecular modeling confirmed that the most prominent defect was the dramatically reduced affinity towards PRPP. Our studies suggest that the p.Leu68Pro mutation has a strong impact on PRPP binding and on stability of the active conformation. This suggests that factors other than HGprt activity *per se* may influence the phenotype of Lesch–Nyhan patients.

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1. Introduction

Lesch–Nyhan disease (LND) is caused by inherited deficiency of the purine recycling enzyme, hypoxanthine–guanine phosphoribosyltransferase (HGprt), which is encoded by the *HPRT1* gene on the long arm of the X-chromosome. Mutations in the *HPRT1* gene are quite varied, with more than 615 so far described [1–3]. These different mutations are associated with varied effects on the biochemical properties of the HGprt enzyme, as well as variations in the clinical phenotype [3–5].

The classical severe form of the disease (LND) includes overproduction of uric acid and its sequelae (nephrolithiasis, gout and tophi), motor and intellectual disability, and self-injurious behavior. Some of these clinical features are attenuated or absent in the milder variant forms of the disease (LNV). Classic LND is thought to occur with mutations that result in null enzyme function, while the milder variants are thought to arise from mutations that permit some degree of residual enzyme function.

Although the *HPRT1* gene and HGprt enzyme have been studied extensively, there are few reports that describe varying phenotypes among multiple members of the same family carrying the same mutation. Hladnik and colleagues [6] described 5 members of a single family with variable phenotypes associated with a splice site mutation, IVS6+2T>C. Here the mechanism for phenotypic variation is likely to be variation in the fidelity of the splicing mechanisms from patient to patient. Sarafoglou et al. [7] described variable clinical phenotypes among three members of one family with the c.500G>T mutation leading to the substitution of arginine to methionine at residue 167 (p.Arg167Met) of HGprt. Here, many of the clinical differences could be attributed to comparing adults more than 50 years of age to toddlers at 2 years of age when the full clinical syndrome has not yet evolved. Sampat [8] also described phenotypic variation among 10 individuals from 8 different families with the c.143G>A mutation, resulting in p.Arg48His. The phenotypic variation for this mutant was associated with an unstable protein, likely leading to variable loss of activity among different patients. Here we describe one family with 7 members carrying the c.203T>C mutation leading to the substitution of leucine to proline at residue 68 (p.Leu68Pro). Some of the family members had clinical phenotypes consistent with the classic LND phenotype including motor disability, intellectual impairment, and self-injury. Others had a milder LNV phenotype

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consistent with less severe motor disability, lack of self-injury, or apparently normal cognition. Interestingly the leucine at position 68 is located next to the stretch of amino acids important for the interaction between the subunits of HGprt (from positions 70 to 101) [3]. Molecular modeling and biochemical studies of the mutant HGprt suggested a novel mechanism to explain the phenotypic variation in this family.

2. Material and methods

2.1. HPRT gene mutation

Genomic DNA was isolated from whole blood and the *HPRT1* mutation evaluated as previously described [9]. In brief, eight PCR products encompassing all nine exons including intron/exon boundaries were amplified and sequenced.

2.2. Molecular modeling

Human HGprt without (PDB code: 1Z7G, [10]) and with (PDB code: 1BZY, [11]) a transition-state analog were downloaded from the Protein Data Bank. Molecular modeling was done with Discovery Studio 3.0 from Accelrys [12]. The impact of the p.Leu68Pro mutation on the stability of the human structures was estimated using the protein stability protocol within Discovery Studio 3.0. With this protocol, a positive value for $\Delta\Delta G_{Mut}$ indicates a destabilizing mutation and a negative value indicates a stabilizing mutation.

2.3. HGprt enzyme activity

HGprt enzyme activity was assessed as recently described [8–13]. In brief, a cDNA construct encoding the normal enzyme was altered by PCR to add a polyhistidine tag at the amino terminus for purification. The cDNA was then subcloned into the pET24d(+) vector (Novagen, New Canaan, CT). The mutant enzyme was created by site-directed mutagenesis using the PCR-based QuickChange kit from Stratagene (La Jolla, CA). Constructs encoding the normal or mutant HGprt were expressed in *Escherichia coli*, and the enzyme was purified to near homogeneity by affinity chromatography. Concentrated protein was frozen in liquid nitrogen and stored at −80 °C until used. Protein purity was determined by Coomassie blue staining after SDS-PAGE. Protein quantification was conducted using the Bradford method. The kinetics of the enzyme towards its substrates were examined using a spectrophotometric assay with varying concentrations of hypoxanthine, guanine, or PRPP. Kinetic values for the purine bases were determined by measuring initial velocities with the concentration of PRPP fixed at 1 mM and varying concentrations of hypoxanthine or guanine from 2 to 200 μM. Kinetic values for PRPP were determined with the concentration of either hypoxanthine or guanine fixed at 200 μM and varying concentrations of PRPP from 5 μM to 1000 μM. The assay involved monitoring the rate of production of IMP or GMP in 96-well UV-compatible microplates with a SpectraMax M5e spectrophotometer (Molecular Devices, Sunnyvale, CA). The production of IMP from hypoxanthine was measured at 245 nm with an extinction coefficient of 1770 M^{-1} cm^{-1}. The production of GMP from guanine was measured at 257 nm with an extinction coefficient of 5146 M^{-1} cm^{-1}. The Michaelis–Menten K_m and k_{cat} were calculated with SigmaPlot (Systat Software Inc., San Jose, CA) by non-linear regression of initial velocities at each substrate concentration.

3. Results

3.1. Clinical features associated with p.Leu68Pro

The family included 7 affected members spanning 3 generations (Fig. 1). Their clinical features are summarized in Table 1. Four of the seven were available for direct clinical evaluation by a neurologist. Clinical histories for the remaining 3 were obtained from clinical data extracted from a questionnaire filled out by neurologists or primary consultants.

AH (III-4) was a 27 year-old man who displayed signs of motor delay as early as 2 years of age, when persistent toe walking was noted (prior reports [3,4]). His gait abnormality became progressively more apparent in the next few years, with a slow and stiff appearance. However, worsening of the gait did not progress beyond 4 years of age. His motor skills were not limited to gait abnormalities. He had difficulties with speech and hand skills too. He also had some difficulty in school, requiring special education. He never displayed any tendency towards self-injury or other difficult behaviors. At 16 years of age, he developed a kidney stone. His examination revealed generalized dystonia. Speech was dysarthric with slowing and overflow contraction of multiple facial muscles. There were subtle multidirectional movements of the head/neck, with slight tonic hyperextension of the back that worsened with walking. Movements of the hands and arms also were slowed and stiff, with overflow posturing when performing fine manual tasks. The gait also was slow, stiff and labored. He tended to walk with a high step, excessive adduction of the thighs, and intorsion of the feet. Muscle stretch reflexes were normal in the arms but brisk in the legs, with clonus at both ankles.

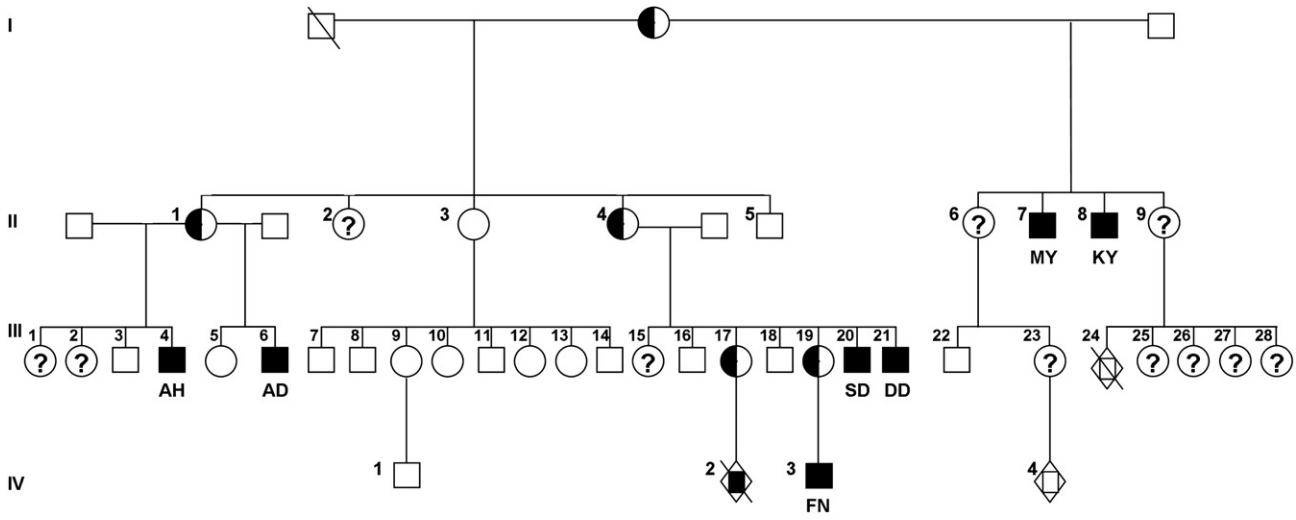

Fig. 1. Pedigree of the family.

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