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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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Keywords

Lesch-Nyhan disease; variants; PRPP; phenotype-genotype; HGprt; molecular modeling

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 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 HGprts without (PDB code: 1Z7G, [10]) and with (PDB code: 1BZY, [11]) a transition-state analogue 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 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 *E. 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 concentration 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 $1770 \text{ M}^{-1} \text{ cm}^{-1}$. The production of GMP from guanine was measured 257 nm with an extinction coefficient of $5146 \text{ M}^{-1} \text{ cm}^{-1}$. The Michaelis-Menton 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 includes 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.

AD (III-6) was 11 years old at evaluation with a history very similar to that of his older brother (AH) with abnormal motor signs beginning before 2 years of age (prior report [4]), difficulties in school that required special education classes, but no sign of self-injury or other difficult behaviors. His examination was also very similar to that of his brother, with moderate generalized dystonia and corticospinal signs limited to the legs.

FN (IV-3) was 9 years old at evaluation with signs of delayed motor development as early as 9 months of age, when he was unable to sit on his own (prior report [4]). Crawling skills also were delayed, and he never gained the ability to walk independently. His speaking and hand skills were similarly impaired. He spends much of his time in a wheelchair, but has sufficient hand skills to perform transfers and basic hygiene. His cognitive skills were impaired, and he required special education classes. He never displayed signs of self-injury or other difficult behaviors. Instead, his caretakers noted inappropriately friendly or affectionate behavior, even with strangers.

Case KY (II-8) was 45 years old with a history of hypotonia and delayed development in the first year of life. He was able to walk by 3 years of age, but required assistance because of a stiff and labored quality. Speech was dysarthric from an early age, and hand skills also were impaired. His motor disability did not progress after 6 years of age, but he increasingly used a wheelchair for convenience with advancing age. He completed college, although records of performance and formal neuropsychological testing were not available. He never displayed self-injury or other difficult behaviors. His examination revealed generalized dystonia. Speech was hesitant and stuttering, and it was produced in short phrases with a fixed smile. Hand and arm movements showed severe stiffening with use, although he was able to grasp objects and perform simple tasks. Arm tone was normal at rest, increased with action, and had a rate-dependent spastic catch. Walking was quite labored with mild forward bending at the pelvis, with very slow and stiff leg movements, and occasional scissoring. Muscle stretch reflexes were brisk in the arms and the legs.

Case KY reported a brother with severe LND, case MY (II-7), who unfortunately could not participate in further clinical investigations. However the neurological symptoms were obtained from a questionnaire revealing a phenotype more severe than his brother KY.

In addition, case FN reported two cousins, cases SD (III-20) and DD (III-21), both showing a severe phenotype of LND associated with no HGprt residual activity in erythrocytes.

3.2. Structural analysis of the p.Leu68Pro mutation

The kinetic mechanism for human HGprt is proposed to be sequential with binding of PRPP followed by binding of the purine base [14–16]. A covalent reaction between the two substrates occurs in a third step. The two newly formed products are released stepwise, starting with pyrophosphate and followed by the newly formed nucleotide.

Large conformational changes occur at each step of the reaction [10; 17]. In particular the motion of the flexible loop (residues 100–128) opens and closes the active site of the enzyme (Fig 2a and 2b). The closed conformation of the enzyme induces a distortion of the docked purine base that decreases the activation energy of the reaction [15]. In this context, the reduced functional activity of the p.Leu68Pro mutant could be explained by a misfolding of the protein leading to reduced enzymatic activity, a less stable protein decreasing

endogenous level of active enzyme, or an enzyme with modified kinetic properties towards its endogenous substrates.

Calculations based on molecular modeling suggested that the p.Leu68Pro mutation may be destabilizing ($\Delta G_{\text{Mut}} = 10.95 \text{ kcal.mol}^{-1}$ using the chain A of the apo structure 1Z7G, 11.27 with chain B). Additionally, Keough *et al* [10] have shown that the first step of the enzymatic reaction involves the recruitment of PRPP as co-factor which occurs concomitantly with an isomerisation of the L68-K69 peptide bond from a *trans* to a *cis* conformation (Fig. 2c and 2d). This isomerisation leads to the removal of K69 from the catalytic site by a 180° rotation of the side chain allowing the next H-bond to stabilize the loop in a closed conformation. Experimental evidence shows that the p.Lys69Ala mutation has a low impact on the catalytic activity of the enzyme because the chain is not involved directly in the stabilization of the PRPP substrate [16]. However, the p.Leu68Pro mutation could have a major impact on the *cis/trans* isomerisation process and/or the thermodynamic stability of the closed state, since two key stabilizing interactions are lost, the hydrogen bond between both backbones of L68 and I100 and the hydrophobic interaction between both side chains of L68 and F99 (Fig. 2e and 2f).

3.3. Kinetics of p.Leu68Pro mutant *HGprt* enzyme

To evaluate predictions from the molecular models, the p.Leu68Pro *HGprt* mutant was reconstructed and its biochemical kinetics tested *in vitro*. As shown Table 2, the catalytic activity of the mutant was reduced, in association with marked reductions in the affinity towards PRPP. Its K_m for PRPP was increased 215-fold with hypoxanthine as substrate and 40-fold with guanine as substrate. In contrast, affinity constants were increased only 5-fold for hypoxanthine and 4-fold for guanine. Enzyme stability could not be assessed because of the very low starting activity. These results show the most prominent defect to be reduced affinity towards PRPP, with associated reduced catalytic potential.

Several other LND and LNV case have been linked to a reduced affinity of *HGprt* towards PRPP. The different clinical features of these other cases are summarized in Table 3.

4. Discussion

Here we describe clinical variation among seven members of one family carrying a c. 203T>C mutation in the *HPRT1* gene, predicting the replacement of leucine by proline at codon 68. To our knowledge, this is the largest family ever reported. Two of the family members had the severe classic form of LND, including severe motor disability, some cognitive disability, and recurrent self-injury. This clinical picture is consistent with a prior report of an unrelated patient with the classic phenotype of LND who had the p.Leu68Pro substitution [3]. However, 5 other members of this family had attenuated phenotypes without self-injury. They all had motor disability, although some were wheelchair-bound with severe disability while others had milder disability and could walk. Although formal neuropsychological testing was not available, most had cognitive disability as judged by the need for special education. However, one completed college and was considered by family members to have normal intelligence. Molecular modeling and biochemical kinetic experiments on the mutant p.Leu68Pro *HGprt* revealed a mechanism that may explain the clinical variations observed.

Understanding genotype-phenotype correlations is a fundamental goal in human genetics because these correlations help to understand basic mechanisms of pathogenesis and may provide prognostic value. Prior studies have suggested that mutations causing null enzyme activity most often are associated with classical phenotype of LND, while mutations allowing residual enzyme activity typically cause milder clinical phenotypes. This concept is

supported by prior studies where HGprt enzyme activity has been measured in living cell preparations that mimic the natural state *in vivo* [18–20]. Less than 1.5% of residual activity typically causes LND. Approximately 2 to 8% residual activity is associated with less severe phenotypes with varying motor or cognitive disability but not self-injury. More than 8% activity typically causes an even milder phenotype with overproduction of uric acid, but the neurobehavioral manifestations are minimal or absent. Studies employing live cell assays therefore have provided good evidence that *HPRT1* mutations cause disease primarily via an influence on HGprt enzyme function, with relatively minor influences from other factors. Although there are rare examples where the severity of the clinical phenotype does not correlate with residual enzyme function, these exceptions almost always occur when HGprt is measured via assays that do not replicate natural conditions that occur *in vivo* [21–24].

If residual HGprt enzyme activity is the critical factor that determines the clinical phenotype, then identical mutations should cause the same clinical phenotype, particularly when they occur in different members of a family. Observations of phenotypic variation occurring among individual members of the same family are important because they may provide evidence that factors other than HGprt activity *per se* may influence the phenotype. These factors may act independently from HGprt, but some may act by influencing residual HGprt activity.

Prior studies have revealed at least two factors that may alter residual HGprt activity and thereby cause variable clinical outcomes. For example, phenotypic variations may occur with mutations that affect splice site junctions in the *HPRT1* gene [6, 25–26, 27]. For these mutants, the major mRNA species transcribed is either absent or incorrect in size, and it encodes a mutant HGprt enzyme with no function. However, a small proportion of transcripts may occur due to variation in the accuracy of the splicing machinery. Since splicing mechanisms are inherited independently from *HPRT1*, the amount of normal transcripts encoding functional HGprt may vary among individuals carrying the same splicing mutation. This mechanism may explain why splice site mutations are associated with variable clinical outcomes.

Other studies have shown that different clinical outcomes also may occur with *HPRT1* mutations that encode an unstable HGprt protein [8]. For unstable enzymes, residual activity depends on repair of damaged protein or synthesis of new protein. Since mechanisms for repair and synthesis are inherited independently from *HPRT1*, different individuals carrying the same mutation that influences enzyme stability may exhibit variable residual HGprt activity. With unstable enzymes, variable residual activity also may depend on environmental conditions, such the occurrence of recurrent or severe fever that may exaggerate instability and thereby cause lower HGprt enzyme activity.

The current studies provide evidence for a third mechanism whereby variable clinical outcomes may be associated with the same *HPRT1* mutation. The c.203T>C mutation does not affect the splicing of mRNA transcripts. Instead, the major defect appears to be marked reduction of its affinity for the co-substrate PRPP. As a result, residual enzyme activity will depend heavily on the amount of PRPP made by cells *in vivo*. Because the synthesis of PRPP and any regulatory mechanisms that maintain intracellular PRPP levels are inherited independently from *HPRT1*, residual enzyme activity may vary among different individuals carrying the same mutation. More specifically, the residual activity of HGprt will be higher among cells that maintain higher steady-state PRPP levels. A loss of PRPP binding is not limited to the p.Leu68Pro mutant, since there are multiple isolated case reports of other mutants with a similar defect. The clinical phenotypes of associated cases ranged from the mildest phenotype of hyperuricemia alone to the severest phenotype of LND [9, 22, 28–33, 35, 36].

Structurally, the leucine at codon 68 is conserved from bacteria to humans and is critically important for interactions with PRPP at the active site of HGprt, together with phenylalanine at codon 99 [34]. The p.Leu68Pro substitution would interfere with PRPP binding and reduce flexibility in the loop. Since PRPP has a stabilizing influence on HGprt, this mutation also could affect enzyme stability [33, 35]. Interestingly, the first key step of the mechanism, which consists of the recruitment of PRPP, induces the isomerisation of the L68-K69 peptide bond from *trans* to *cis* (Fig 2c and 2d). Experimental evidence shows that the K69A mutation has a low impact on the catalytic activity of the enzyme because the side chain is not involved directly in the stabilization of PRPP. No data have been reported concerning mutation of L-68. Our studies suggest that the p.Leu68Pro mutation has a strong impact on PRPP binding, and perhaps also on the *cis/trans* isomerisation and stability of the active conformation.

In conclusion, in most cases, patients' phenotype largely correlates with the residual HGprt enzyme activity, caused by *HPRT1* mutations. We show, here, that the substitution of leucine at position 68 by proline in seven members of a family leads to variable phenotypes. Kinetics of the mutant HGprt enzyme and molecular modeling revealed that the catalytic activity was reduced, associated with a marked reduction in the affinity towards PRPP. Therefore the potential activity of HGprt may rely on the concentration of intracellular PRPP, which may vary from patient to patient. Our study unravels a new mechanism whereby regulation of HGprt activity by its co-substrate directly influences the phenotypes of Lesch-Nyhan patients.

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HIGHLIGHTS

- We describe a family of seven boys with different phenotypes sharing the same mutation c.203T>C of the *HPRT1* gene.
- Kinetic studies of the mutant enzyme and molecular modeling suggest that factors others than the nature of genetic mutation *per se* may influence the phenotype in this family.
- the enzymatic activity may contribute to the phenotypic variability.
- These results provide a mechanistic explanation that help to understand genotype-phenotype correlations in Lesch-Nyhan disease and its attenuated variants.

Fig. 1. Pedigree of the family

Fig. 2. Molecular modeling of the mutation p.Leu68Pro

The panels of the first column (a, c and e) illustrate 3 representations of the open form of human HGprt (PDB code : 1Z7G). The panels of the second column (b, d and f) depict 3 representations of the same enzyme in its closed conformation (PDB code: 1BZY). Carbons of the side chain of L68 (or P68 for the mutant) are in yellow. The enzyme is depicted as a solid ribbon and for clarity and only key residues are shown. Panels a and b represent a full view of one monomer, highlighting the large motion of the flexible loop. The active site is indicated in green. The rectangle in black dashed line corresponds to the closer view of the 4 other panels.

Panels c and d represent a closer view on the loop containing residue L68. In the open form (c), the L68-K69 peptide bond is in a *trans* conformation with K69 going deeper inside the catalytic site while in the closed form (d), this peptide bond is in a *cis* conformation and K69 side chain is removed from the active site and an additional hydrogen bond is formed. Panels e and f represent the same view as panels c and d respectively, but after a p.Leu68Pro mutation. This mutation induces a lost of positive interactions (hydrophobic contact with F99 and hydrogen bond with I100), but also highlights that the conformation of the proline in the closed form is constraint and not favourable, thereby contributing to the destabilization of this conformation.

Table 1

Clinical phenotype associated with c.203T>C (p.Leu68Pro)

Clinical features of the seven affected family members.

Case ID	Age	Consequences of increased uric acid	Motor impairments	Cognition	Self-Injury
AH ^{*,#}	27	Kidney stones	Moderate: -motor delay, -difficulties with speech and hand skills, -generalized dystonia	Impaired	No
AD ^{*,#}	11	Kidney stones	Moderate: -abnormal motor signs, -moderate generalized dystonia	Impaired	No
FN ^{*,#}	9	Kidney stones	Severe: -delayed motor development, -speech and hand skills impaired, -use of wheelchair	Impaired	No
KY [*]	45	Kidney stones	Moderate: -delayed development, -difficulties with speech and hand skills, -generalized dystonia	Normal	No
MY	42	Kidney stones	Severe: -use of wheelchair	Impaired	No
SD	31	Kidney stones	Severe: -choreic movements, -hypotonia, -generalized dystonia	Impaired	Yes
DD	10	Kidney stones	Severe: -hypotonia, -dystonia	Impaired	Yes

Asterisks denote patients who were directly evaluated, while those without asterisks indicate patients where clinical information was extracted from a questionnaire completed by neurologists or primary consultants. Cognition was assessed indirectly since formal neuropsychological testing was not available.

[#] previously reported [3].

Table 2
Apparent HGprt enzyme kinetics

Kinetic properties of the normal human HGprt and p.Leu68Pro mutant HGprt enzyme were determined separately for hypoxanthine and guanine. Parameters for hypoxanthine or guanine were determined with varying concentrations of either purine with PRPP fixed at 1 mM, or with varying PRPP concentrations with the purine based fixed at 200 μ M.

	Normal HGprt	p.Leu68Pro Mutant
Hprt activity		
k_{cat} in s^{-1} (hypoxanthine)	9.02 ± 0.95	0.14 ± 0.03
K_{m} in μM (hypoxanthine)	8.23 ± 1.43	108.06 ± 10.51
k_{cat} in s^{-1} (PRPP)	11.14 ± 1.89	0.58 ± 0.07
K_{m} in μM (PRPP)	26.81 ± 5.29	6076.55 ± 745.64
Gprt activity		
k_{cat} in s^{-1} (guanine)	14.69 ± 1.21	0.04 ± 0.00
K_{m} in μM (guanine)	5.08 ± 0.79	38.68 ± 10.84
k_{cat} in s^{-1} (PRPP)	15.17 ± 0.63	0.11 ± 0.02
K_{m} in μM (PRPP)	55.67 ± 3.20	2375.88 ± 152.20

Table 3
Clinical phenotype associated with other HGGprt mutants affecting its interaction with PRPP from literature survey

Reference	Consequences of increased uric acid	Motor impairments and Cognition	HGGprt enzymatic activity	Km (PRPP)	Self-injury	Associated mutation
[28]	Gout Renal failure	Mild neurological deficit	Normal in erythrocyte lysate	Increased 10 times in erythrocyte lysate	No	ND
[22]	Renal failure	Severe neurological problems: -dysphagia, -dysarthria, -choreo-athetoid movements and opisthotonos	Reduced in erythrocyte and fibroblast lysates	Increased 7 times in erythrocyte and fibroblast lysates	Yes	ND
[29]	ND	Severe neurological problems: -choreo-athetoid and dystonic movements, -dysarthria	Reduced in erythrocyte and fibroblast lysates	Increased 100 times in fibroblast lysate	Yes	ND
[30]	Gouty attack, renal failure, multiple trophies	Mild neurological problems: -increased tone of the lower limbs with symmetrical brisk reflexes, -slow learner	Reduced in erythrocyte lysate	Increased 20 times in erythrocyte lysate	No	ND
[31]	ND	Mild neurological problems: -developmental delay, -difficulties in speech and walking, -poor coordination of movement	Reduced in erythrocyte lysate	Increased	No	ND
[32]	Gouty arthritis, renal failure, renal calculi	No neurological problems: -no evidence of abnormality in mental and intellectual development, nor in physical development	Reduced in erythrocytes	Increased 2 times	No	c. 68G>T (p.Cys23Phe)
[33,35]	Renal failure	Mild neurological problems: -delay in acquisition and language skills	Reduced in fibroblast lysate [35] and intact fibroblasts [33]	Increased 20 times in fibroblast lysate	No	c.410T>C (p.Ile137Thr)
[36]	Gout, renal failure	No neurological problems	Reduced in erythrocytes	ND	No	c.410T>C (p.Ile137Thr)
[9]	Gout, Renal calculi	Mild neurological problems: -mild developmental delay, -mild dysarthria	Reduced in erythrocyte lysate	ND	No	c.596T>G (p.Phe199Cys)
[37]	ND	ND	No activity in live lymphoblast	Increased 200 times	yes	c.580G>A (p.Asp194Asn)
[38]	ND	ND	Reduced activity in erythrocyte lysate	Increased 44 times	no	c.582C>G (p.Asp194Glu)
[39]	ND	ND	ND	Increased >450 times	ND	c.602A>G (p.Asp201Gly)

ND: Not Determined