New Mutation Affecting Hypoxanthine Phosphoribosyltransferase Responsible for Severe Tophaceous Gout

To the Editor:

Hypoxanthine phosphoribosyltransferase (HPRT) deficiency, which is due to mutations of the *HPRT1* gene, is a rare cause of inherited hyperuricemia and gouty arthritis[1]. Different *HPRT1* mutations induce various levels of residual HPRT enzymatic activity, resulting in clinical symptoms of various severity[2]. Complete HPRT deficiency leads to the classic clinical phenotype of Lesch-Nyhan disease (LND), characterized by uric acid overproduction and its sequelae (nephrolithiasis, gout, and tophi), motor dysfunction, and behavioral problems including recurrent self-injury[3]. However, there also are attenuated clinical variants in which some of these clinical features are either absent or clinically insignificant. Collectively, patients with attenuated phenotypes are designated Lesch-Nyhan variants (LNV)[4]. The mildest form of LNV includes only overproduction of uric acid and its associated problems. These patients do not have clinically overt neurological or behavioral abnormalities, and most often are described as having HPRT-related hyperuricemia (HRH). Between the 2 extreme phenotypes of LND and HRH is a spectrum of phenotypes with varying degrees of neurological abnormalities, designated HPRT-related neurological dysfunction (HRND). Patients with HRND have an overproduction of uric acid along with some neurological difficulties, but they do not exhibit the self-injurious behaviors seen in classic LND. Patients with LND or LNV have both increased monosodium urate monohydrate (MSU) crystal formation that leads to gouty arthritis and arthropathy, tophi, and/or

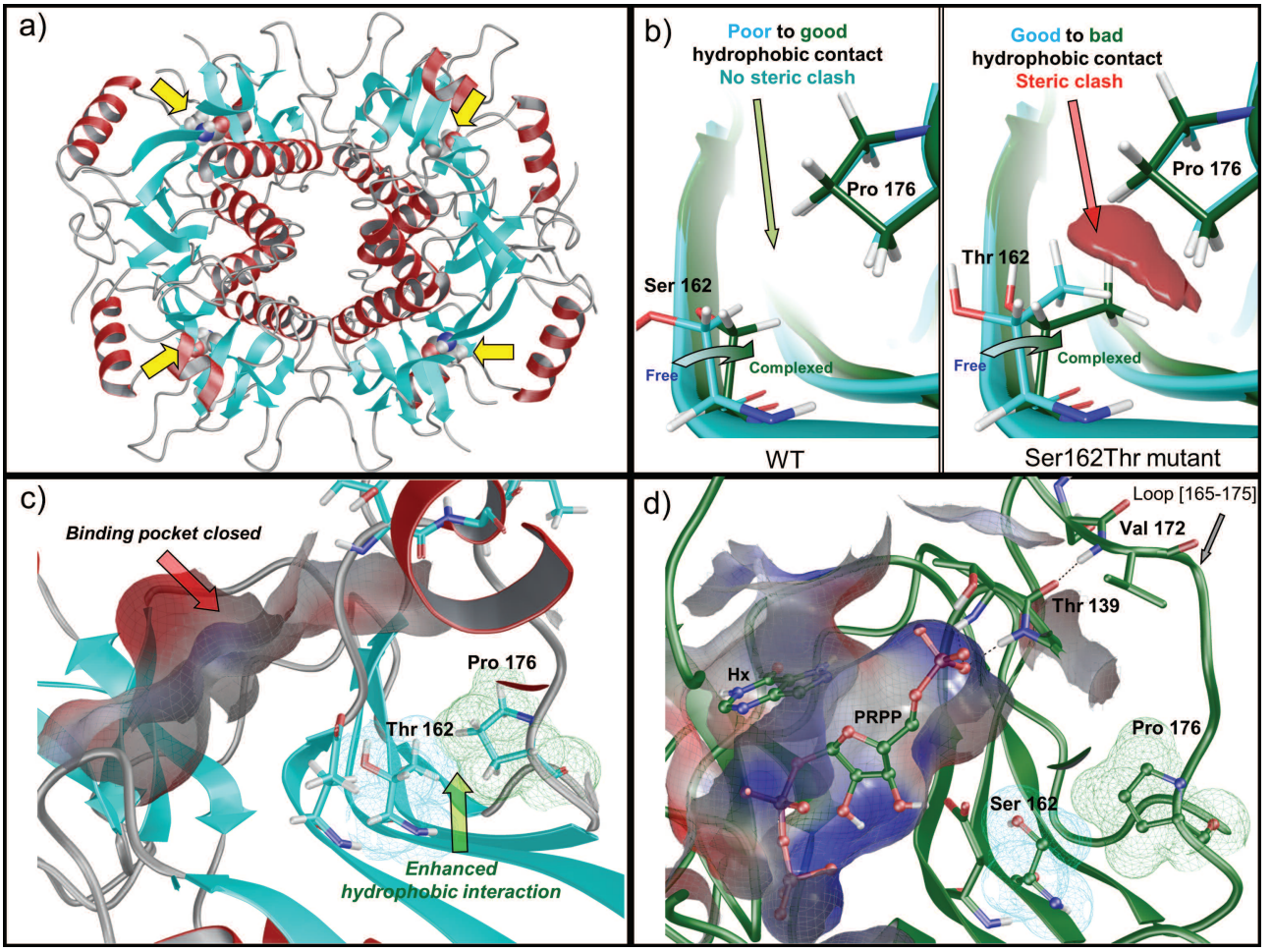

Figure 1. Structural analysis of the hypoxanthine phosphoribosyltransferase (HPRT) Ser162Thr mutation. (a) Ribbon representation of the free human HPRT tetramer [Protein Data Bank (PDB) code 1Z7G]. β-sheets are in light blue and α-helix are in red. The 4 Ser162 are represented in CPK Atomic Models and located by yellow arrows. In panels b, c, and d, carbons and ribbons of the free enzyme (PDB code 1Z7G) are in light blue while they are in dark green for the enzyme containing its substrates (PDB code 1D6N). (b) Representation of the superimposition of the free and complexed form of HPRT for the wild type HPRT (left) and for the Ser162Thr mutant model (right). This representation focuses on the short rotation of Ser162, as a consequence of substrates binding, which stabilizes the complexed form by adding hydrophobic contact with Pro176. This rotation leads to a steric clash in the mutant of the Ser162Thr model, destabilizing the complexed form. (c) Representation of the free human Ser162Thr HPRT mutant. An enlargement of Thr162 is shown for only 1 of the 4 monomers. It highlights the enhanced hydrophobic interaction between Thr162 and Pro176, which stabilizes the free conformation, and as a consequence contributes to stabilization of the close conformation of the enzyme. (d) Representation of human HPRT containing its 2 substrates, phosphoribosyl pyrophosphate (PRPP) and hypoxanthine (Hx). An enlargement of Ser162 for only 1 of the 4 monomers is shown, and highlights the binding mode of PRPP, which is close to Ser162 but not in direct interaction. The short allosteric rearrangement of the protein necessary to stabilize PRPP has modified the relative position of Ser162 toward Pro176, which are in close contact, enhancing hydrophobic interaction.

nephrolithiasis[4,5,6]. The gout resulting from HPRT deficiency is remarkable in its severity and its propensity to form tophi[5,6]. Its juvenile onset in a male is an important diagnostic clue for HPRT deficiency. The discovery of more than 600 mutations in the *HPRT1* gene and their consequences for HPRT activity in patients with LND or LNV has resulted in a better understanding of the diversity of resulting phenotypes[7] (www.lesch-nyhan.org).

A 24-year-old man was seen for a recurrent migratory arthritis that had evolved over the 5 previous years, affecting the knees, wrists, and hands. His childhood psychomotor development was considered normal, and there were no affected family members. No self-injurious behavior was reported. The patient had no history of kidney stones or renal colic. The diagnosis of gout was made following an arthritis flare of the right knee, from which MSU had been isolated. The serum uric acid (SUA) level was 760 μmol/l (normal values 210–430 μmol/l). Serum creatinine level was 102 μmol/l (Modification of Diet in Renal Disease maximum 83.9 ml/min/1.73 m^2). Upon examination, he had synovitis of the first and fifth right proximal interphalangeal (PIP) joints, the third left PIP, and an effusion of the left knee. He also had multiple tophi of the ears and hands. Radiographs showed an arthropathy of the fifth left metacarpophalangeal joint, first PIP joint, and third right metatarsophalangeal joint. The neurological examination revealed generalized hypertonia, as well as hyperactive tendon reflexes, while the Babinski signs were negative. Magnetic resonance imaging of the brain was normal. These neurological signs led us to classify our patient's disorder as an LNV-HRND form.

In erythrocyte lysates, HPRT activity was 30% of control subjects (patient: 0.6; controls: 2.0 to 2.9 nmol/min/mg hemoglobin). The patient's *HPRT1* gene had a missense c.485G > C mutation (exon 6), leading to a Ser162Thr substitution in the HPRT protein. His mother was an asymptomatic carrier.

A molecular modeling suite (Maestro, Schrodinger) was used to investigate the effect of the mutation at a molecular level. The cristallographic structure of HPRT, in its apoenzyme form and in complex with its 2 substrates [phosphoribosyl pyrophosphate (PRPP) and hypoxanthine], was downloaded from Protein Data Bank (PDB; codes 1Z7G and 1D6N). Ser162 is not a key catalytic residue and is located on the structural β–sheet that crosses the core of the enzyme (Figure 1a). Mutating this residue cannot directly affect the catalytic reaction nor the protein-protein binding interfaces (the functional tetramer should not be disrupted). We propose that the Ser162Thr mutation affects the allosteric rearrangement that occurs prior to the catalytic reaction by stabilizing the free conformation and then destabilizing the complexed one. The backbone of Ser162 is involved in 2 H-bonds and contributes to the stability of the β–sheet while the side chain is weakly involved in a hydrophobic interaction with Pro176 (Figure 1b). The Ser162Thr mutation does not modify the interaction of the backbone while reinforcing the hydrophobic interaction with Pro176 because of the additional methyl group (Figure 1b and 1c). Prior to the catalytic reaction, a large conformational change occurs to stabilize the complex. In particular, a shift of loop 165-175 leads to the formation of a new hydrogen bond between Val172 and Thr139, and a reinforcement of the hydrophobic interaction between Ser162 and Pro176 (Figure 1b and 1d) stabilizes the complex. The Ser162Thr mutation causes a steric clash with Pro176, which has a negative effect on the allosteric rearrangement that stabilizes loop 165–175 and therefore on the overall stability of the HPRT-PRPP-hypoxanthine complex (Figure 1b). Taken together, these 2 phenomena tend to displace the thermodynamic equilibrium between the free and the complexed form toward the free form, and consequently influence the global kinetics variables of the catalytic reaction.

The failure of allopurinol treatment (400 mg/day) and above-guidelines doses of febuxostat (240 mg/day, successfully used by Schumacher, *et al*[8]) led us to try monthly and then bimonthly administration of rasburicase (15 mg). It was used in association with allopurinol 700 mg/day, colchicine 1 mg/day, and probenecid 1 g per day. Following 12 months of intensive treatment, the patient had no arthritis, a normal SUA level, and regressing tophi as reported by Roche, *et al*[9] (Figure 2).

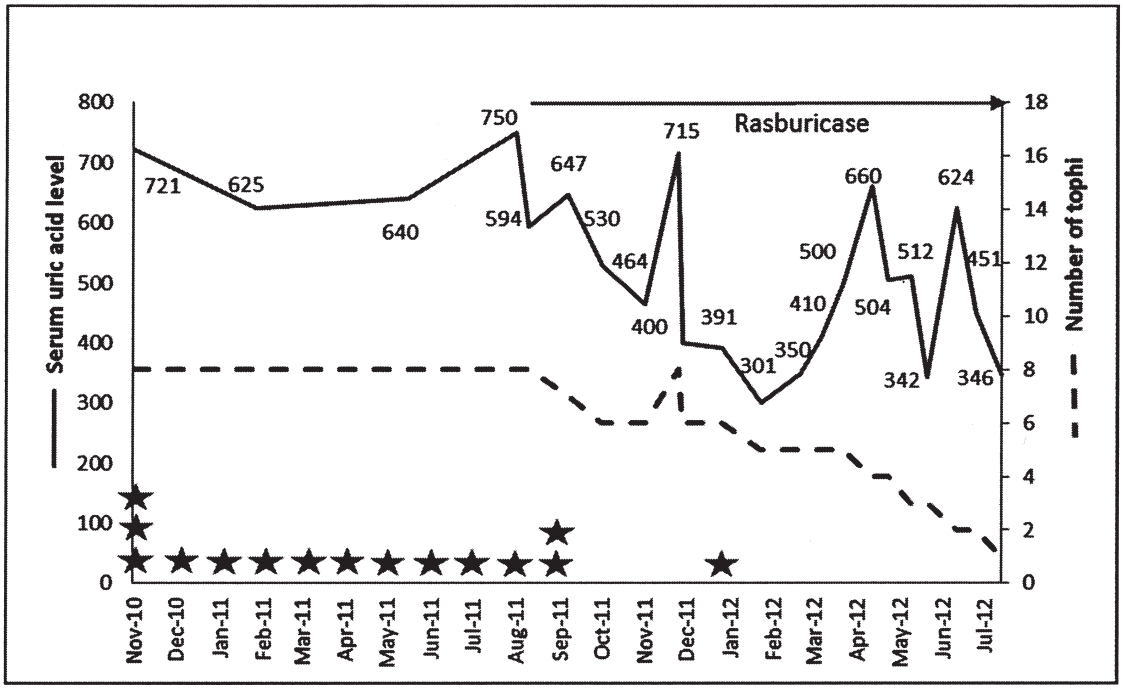

Figure 2. Clinical and biological evolution during treatment. During the first 10 months, single use of allopurinol did not show any efficacy. Daily dose of allopurinol was gradually increased from 200 to 700 mg. A progressive clinical improvement was observed after the introduction of rasburicase. Number of tophi and active synovitis are represented respectively by dashed line and by stars. Serum uric acid level (solid line; μmol/l) returned to normal values. Its variation was possibly related to a lack of compliance to oral medications.

While the unusual occurrence of gout in young men in itself should suggest HPRT deficiency, the diagnosis of LNV is even more likely in the added presence of motor disorders. Biochemical or molecular investigations should be conducted in all such cases. Molecular modeling focusing on how a particular HPRT mutation affects the conformation of HPRT protein and consequently its enzymatic function provides a new tool to understand genotype/phenotypic manifestations in LND and could lead to new therapeutic approaches.

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