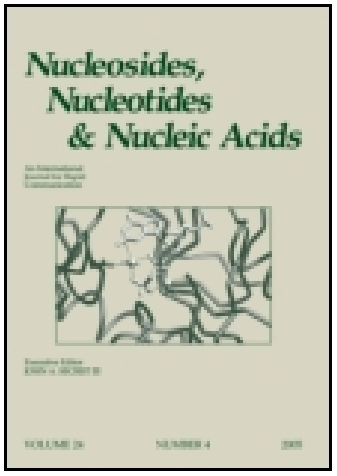

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HPRT Deficiency in Spain: What Have We Learned in the Past 30 Years (1984-2013)?

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HPRT DEFICIENCY IN SPAIN: WHAT HAVE WE LEARNED IN THE PAST 30 YEARS (1984–2013)?

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□ *Since 1984, we have diagnosed at the La Paz University Hospital, Madrid, Spain, 41 patients with hypoxanthine phosphoribosyltransferase (HPRT) activity deficiency. These patients belonged to 34 families. We have also performed molecular and enzymatic diagnosis in three patients from India, one from Belgium, and three from Colombia. About 1/3 of these patients were followed up at La Paz University Hospital at least every year. This fact has allowed us to examine the complete spectrum of HPRT deficiency as well as to perform a more accurate diagnosis and treatment. In the present review, we also summarized our studies on the basis of physiopathology of the neurological manifestation of Lesch Nyhan disease (LND).*

Keywords DNA/RNA analysis; gout; hyperuricemia; Lesch Nyhan; purinergic receptors; purinergic signaling

CLINICAL PHENOTYPES

Hypoxanthine phosphoribosyltransferase (HPRT) deficiency causes uric acid overproduction-related symptoms in all patients, however, other manifestations of the complete Lesch Nyhan disease (LND) as neurological and behavioral symptoms and hematological disturbances may be absent in partial forms.[1,2] HPRT deficiency phenotypes can be classified in classical LND and partial HPRT deficiency or LND variants (LNDV). Other authors propose four grades depending on severity (Grades 1 to 4).

The most frequent phenotype was the complete enzyme deficiency or classical LND (Grade 4) phenotype.[1,3] It was recognized in 25 patients

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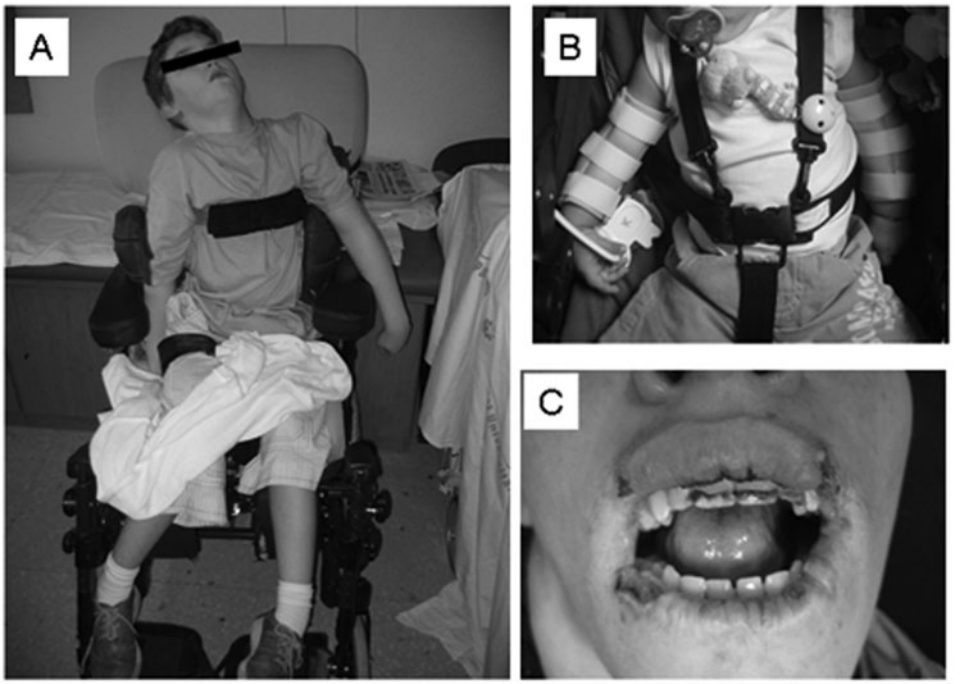

FIGURE 1 Complete Lesch Nyhan disease phenotype. (A) The patient presents severe generalized dystonia, with coreoathetosis and ballismus, which confined him to a wheelchair and made him completely dependent for daily activities. (B) Arm restrictions to prevent blending the elbows and bite the fingers. (C) Important self-mutilating lesions.

(61%). These patients presented with severe generalized dystonia plus choreoathetosis and ballismus, which confined them to a wheelchair and made them completely dependent for daily activities. They also showed the characteristic behavioral disturbances with self-injurious behavior (Figure 1). Cognitive impairment and megaloblastic anemia are present in very different degrees.[3–5]

Partial HPRT deficiency or LNDV patients may present a variety of neurological manifestations but they do not show self-injurious behavior[3,5–7] (Figure 2). They are classified as HPRT-related neurological disease (HRND) if they present clinically apparent neurological manifestations, or as HPRT-related hyperuricemia (HRH) if the patient does not present neurological manifestations (Grade 1, 14.7%).

Some of HRND patients present a severe generalized dystonia similar to that of LND patients and, although they do not show overt self-injurious behavior, they are dependent for mostly daily activities (Grade 3, Figure 2A) (14.6%). However, in other HRND patients, dystonia is mild to moderate (9.7%), being the patient able to walk (Figure 2B). This allows them to carry on independent lives (Grade 2). Patients with HRND also may present macrocytic anemia and, in some cases, slight mental retardation.

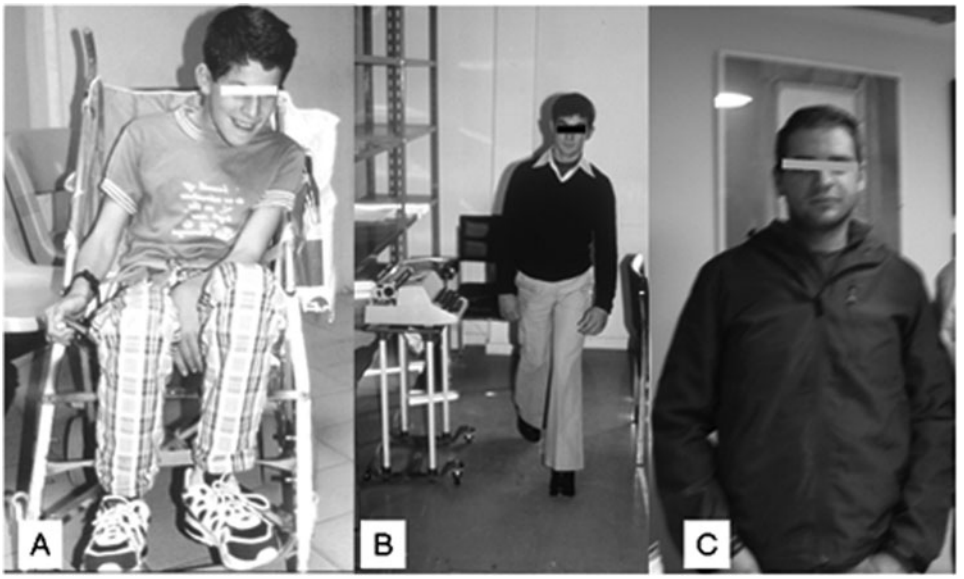

FIGURE 2 Lesch Nyhan disease variants or partial HPRT deficiency. (A) Some of variants present severe HPRT related neurological disease (HRND) with severe generalized dystonia similar to that of LND patients and, although do not show overt self-injurious behavior, are dependent for most daily activities (grade 3). (B) Dystonia may be mild to moderate, and in some patients allow walking and being independent (grade 2). (C) Grade 1 patients with HPRT related hyperuricemia (HRH) may only show manifestations of uric acid overproduction, with no neurological symptoms.

HRH patients present only with the manifestations of uric acid overproduction (Figure 2C). They did not show anemia or macrocytosis and they are intellectually normal, although an exercise-induce dystonia, slight attention deficit, or certain obsessive behavior has been noted in some of them when carefully examined. Although Grades 1 to 3 are designated as partial HPRT deficiency, only HRH patients exhibit a residual HPRT activity in hemolysates, while it is extremely unusual in Grades 2 to 4 patients. Some residual HPRT activity has been described in fibroblasts from patients with Grades 2 to 3 (HRND).[3]

DIAGNOSIS

In this series, psychomotor delay at different ages prompted the diagnosis of LND but, in the last 5 years, HPRT deficiency was established before age 2 years in most Spanish patients. We encourage determining serum and particularly urinary uric acid to assess psychomotor delay in all infants. A spot urine sample with a uric acid to creatinine ratio elevated should prompt determination of HPRT activity in a hemolysate (Figure 3). Although Grades 1 to 3 are designated as partial HPRT deficiency, only HRH patients exhibit a residual HPRT activity in hemolysates, while it is extremely unusual in

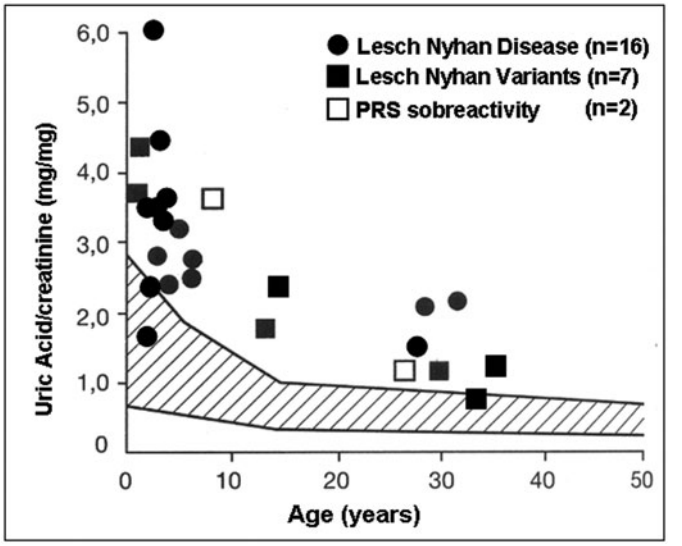

FIGURE 3 Urinary uric acid creatinine ratio in the diagnosis of HPRT deficiency. Shadow area represents normal values of urinary uric acid/creatinine ratio relating to age.

Grades 2 to 4 patients. Some residual HPRT activity has been described in fibroblasts from patients with Grades 2 to 3 (HRND).[3]

In the past 30 years, we have focused on the questions elicited by this clinical experience in close contact with the patients. This is what we call "translational research." One of the first questions we faced when we diagnosed our initial HPRT-deficient patient was the carrier status of female relatives. Carrier diagnosis is an important issue for most HPRT-deficient families. As human HPRT is encoded by a single structural gene on the long arm of the X chromosome and HPRT deficiency is inherited as a recessive X-linked trait, males are affected and women may be carriers. Female carriers are usually asymptomatic and carrier status cannot be accurately assessed by biochemical and enzymatic methods, since HPRT activity is most often normal in the hemolysate of female carriers due to selection against HPRT-deficient erythrocyte precursors.[8] Enzymatic diagnosis of the carrier state can be performed in hair follicles or cultured fibroblasts, although such diagnosis is not infallible. Proliferation assay of peripheral blood T-lymphocytes in the presence of 6-thioguanine can select HPRT-deficient cells from carrier females based on their 6-thioguanine resistances. This method is diagnostic in most cases. However, faster and more accurate carrier diagnosis can be performed by molecular methods.

As HPRT1 gene is a housekeeping gene expressed in peripheral blood and in most HPRT-deficient patients HPRT mRNA is expressed, molecular diagnosis can be accomplished by RNA extraction, RNA transcription

into DNA by a viral inverse transcriptase, and sequencing of the entire coding HPRT region.[9] In other cases, genomic DNA sequencing of the nine HPRT1 exons, with its intronic flanking sequences, may be necessary. In some cases, the HPRT coding region is normal and the patients may present a decreased HPRT mRNA expression of unknown origin. In these patients, quantification of HPRT mRNA by real-time polymerase chain reaction (PCR) may be employed for molecular diagnosis.[10,11] This fact highlights the importance of enzyme diagnosis in patients with HPRT deficiency, since a normal HPRT coding region does not rule out HPRT deficiency.

Every mutation in each Spanish HPRT-deficient family has been different.[3,12] Around the world, more than 500 disease-associated mutations (deletions, insertions, duplications, and point mutations) have been found disperse within the gene.[13] Molecular diagnosis is also useful for prognostic purposes. Missense point mutations are the main cause of partial deficiency of the enzyme and Lesch Nyhan variant (LNV) phenotypes, whereas mutations that modify the size of the predicted protein are usually related to LND.[13,14]

When the family propositus mutation is known, carrier diagnosis can be achieved by sequencing the mutated HPRT1 gene region from the female genomic DNA.[8,15] When the propositus mutation is not available, amplification of the nine HPRT1 exons, with its intronic flanking sequences, may be necessary. If a deletion has been found in the propositus, gene dosage may be accomplished by quantitative PCR or multiplex ligation-dependent probe amplification (MLPA). Molecular diagnosis has allowed carrier diagnosis in 99 females from 33 HPRT-deficient families. Remarkably, only four HPRT-deficient patient mothers resulted in a noncarrier diagnosis. Thus, in this series of HPRT-deficient patients, 88% of the mutations were inherited in an X-linked manner. These results highlight the importance of carrier diagnosis.

TREATMENT

In our experience, uric acid overproduction is effectively controlled with allopurinol. However, the major side effect is xanthine lithiasis. This may be prevented by titrating allopurinol doses to maintain high-normal serum uric acid levels (close to the serum saturating level of 7.0 mg/dL).[16] In acute cases, three LND patients were treated with rasburicase during a short period of time with renal function recovery and improvement of severe acute lithiasis.[17] We have great expectation with other therapies now under investigation that could obviate the xanthine lithiasis as PEG uricase or purine nucleoside phosphorylase (PNP) inhibitors.

Spasticity and dystonia can somehow be alleviated with benzodiazepines and gamma-amino butyric acid inhibitors, such as baclofen. Dopamine replacement therapy in Lesch Nyhan patients is associated with intolerable

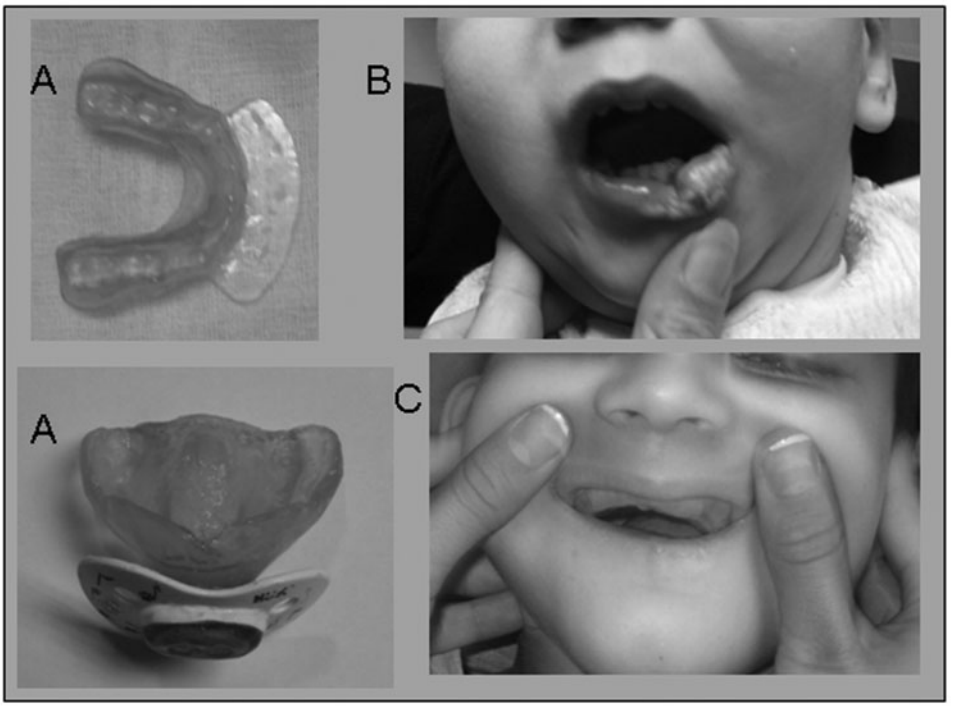

FIGURE 4 Lesch Nyhan disease (LND) self-injurious behavior. (A) Different protective oral devices designed to avoid self-biting lesions. (B) Severe lip lesions in an LND patient. (C) Teeth removal in a patient in whom different oral devices, in addition to behavioral education and botulinum toxin, was not useful to avoid lip damage.

side effects.[18] Today, physical rehabilitation is the key for the management of neurological manifestations.

Self-injurious behavior must be managed with a combination of physical restraints, behavioral, and pharmacological medications. The atypical antipsychotic drug risperidone reduces self-injurious behavior in some patients but restrains remain the main weapon for self-injurious management. Concerning self-biting, prior to teeth removal which may be an option in certain patients, botulinum toxin may be very helpful, with or without protective oral devices[19] (Figure 4). We believe that different oral devices should be tried first in addition to behavioral education.

PHYSIOPATHOLOGY

We do not have an effective treatment to eliminate or ameliorate the neurological and behavioral symptoms. One of the reasons for this fact is that we do not really know why HPRT deficiency causes these manifestations. Neurological manifestations suggest a dysfunction in the basal ganglia but we

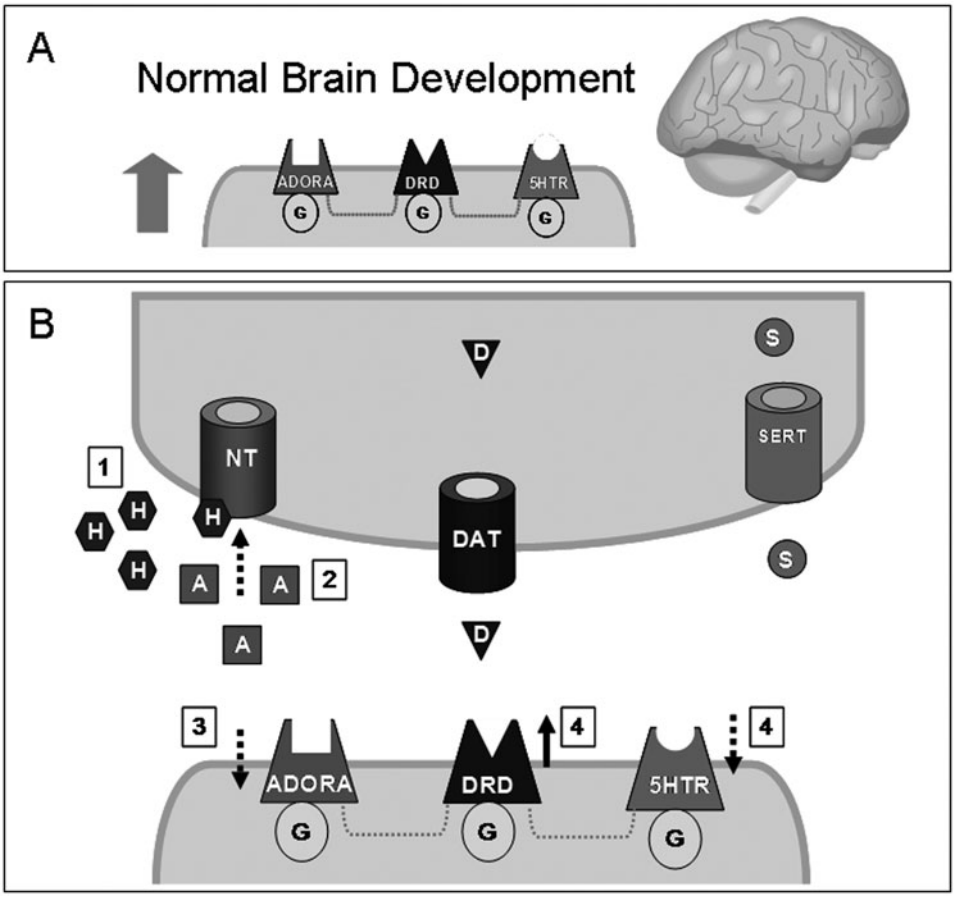

FIGURE 5 Physiopathological hypothesis of brain dysfunction in HPRT deficiency. (A) Adenosine, dopamine, and serotonin receptors are markers of neurogenic differentiation and their receptors seem to be a marker of neuronal fate. (B) HPRT deficiency related brain dysfunction may arise during brain development. Event sequence on the light of the authors' research: (1) hypoxanthine excess due to HPRT deficiency may compete with adenosine transport; (2) the diminished adenosine transport causes an increase in adenosine availability in the synaptic space; (3) increased adenosine may determine a diminished expression of adenosine receptors; (4) a diminished expression of adenosine receptors may disrupt the neurotransmitter receptors' balance modifying dopamine and serotonin receptor expression. ADORA: Adenosine receptor; DRD: Dopamine receptor; 5HTR: Serotonin or 5-hydroxy-tryptamine receptor; G: G-protein; NT: Nucleoside transporter; DAT: Dopamine transporter; SERT: Serotonin transporter; H: Hypoxanthine; A: Adenosine; S: Serotonin.

only have a small number of the intriguingly puzzle pieces that may contain the solution.

We focused our studies more than 10 years ago on the physiopathology of the neurological manifestation of LND. The first puzzle piece we found was that adenosine transport was decreased in HPRT-deficient cells.[20] Adenosine is a purine compound that also functions as a brain neurotransmitter. As a nucleoside, adenosine is transported across the cell membrane by a

group of transport proteins named nucleoside transporters or NT. ENT2 types of NTs are also hypoxanthine transporters. A huge amount of hypoxanthine (over 10 times) is present in the HPRT-deficient cell culture medium, as compared to normal cell medium, due to the base recycling defect, and this competes with adenosine for its transport.[21] Thus, our results suggested that the decrease in adenosine transport was due to the hypoxanthine excess present in the medium, which competes with adenosine and, as a consequence, adenosine may remain in the synaptic cleft for longer period of time. We have found that in presence of hypoxanthine incubation, production of cyclic adenosine monophosphate (AMP) by the A2 adenosine receptor agonist CGS-21680 is significantly increased.[22]

Adenosine and dopamine are neurotransmitters that need to bind to specific G-protein-coupled receptors in the synaptic membrane. Their specific receptors seem to be integrated through receptor–receptor interactions in the membrane. In our studies, we found that adenosine receptors were decreased in most HPRT-deficient cells.[23] This decrease was probably a consequence of a diminished transport.[24] We also found that dopamine receptors were significantly increased in HPRT-deficient cells, although they seem functionally altered.[23] Dopamine deficiency has been found in experimental models of HPRT deficiency and dopamine receptors has been found to be up-regulated in LND brain.

Serotonin is also a neurotransmitter that exerts its effects by binding to specific G-protein-coupled receptors in the synaptic membrane. Their receptors seem to be integrated through receptor–receptor interactions in the membrane and it has been reported that they form heterodimers with other G-protein-coupled receptors that modulate G-protein-mediated intracellular signaling. Serotonin has been related to behavioral disturbances and we have found that serotonin receptors are significantly decreased in HPRT-deficient cells.[25]

In summary (Table 1), from our studies we conclude that three neurotransmitters seem to be implicated in the pathogenesis of severe HPRT

TABLE 1 Summary of the pathophysiological studies on the neurological manifestations of Lesch Nyhan disease

1. Adenosine transport is decreased in HPRT-deficient cells.[20]
2. Hypoxanthine excess due to HPRT deficiency competes with adenosine for its transport.[22]
3. Adenosine may remain in the synaptic cleft for a prolonged period of time.[20,21]
4. Adenosine receptors are decreased in most HPRT-deficient cells. This decrease is probably a consequence of a diminished adenosine transport.[23,24]
5. Dopamine receptors are significantly increased in HPRT-deficient cells.[23,24]
6. Serotonin receptors are significantly decreased in HPRT-deficient cells.[24]
7. Adenosine, dopamine, and serotonin receptors are markers of neurogenic differentiation and their receptors seem to be a marker of neuronal fate.

deficiency (LND): dopamine, adenosine, and serotonin.[25] These neurotransmitters act by binding to specific G-protein-coupled receptors in the synaptic membrane where they are integrated through receptor–receptor interactions. These studies allowed us to hypothesize that HPRT-deficiency-related brain disorder may develop during brain development when hypoxanthine excess due to HPRT deficiency may compete with adenosine transport. This fact may cause an adenosine increase in the synaptic space, which may influence the expression of adenosine receptors. This may in turn disrupt the balance between neurotransmitter receptors modifying dopamine and serotonin receptor expression (Figure 5). We are now studying how HPRT deficiency could modify brain development. Our very preliminary results indicate that adenosine, dopamine, and serotonin receptors are markers of neurogenic differentiation and their receptors seem to be as a marker of neuronal fate.

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