Lesch-Nyhan Disease (LND) is a rare X-linked recessive metabolic and neurological syndrome due to the deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT). Besides its well known “housekeeping” function this purine salvage enzyme has revealed an unexpected role in neurodevelopment, unveiled by the peculiar neurological symptoms flanking hyperuricemia in LND: dystonia, choreoathetosis, compulsive self-injurious behaviour. Several lines of research have tried to find the molecular basis for the neurological phenotype after the disease was first described in 1964. Dopaminergic deficit was then found to underlie the neurologic symptoms but the aetiology for such alteration seemed inexplicable. A number of detailed studies in the last 50 years addressed the genetic, metabolic, cognitive, behavioral and anatomical features of this disease. Initial investigations sought for accumulation of toxic metabolites or depletion of essential molecules to disclose potential connections between purine recycling and neuronal dysfunction. In the last two decades sophisticated biotechnological methods were used for a deeper insight in the genetic and molecular aspects, unveiling a network of combined gene dysregulations in neuronal development and differentiation producing neurotransmission defects. These studies, conducted with several different approaches, allowed consistent steps forward, demonstrating transcriptional aberrations affecting different metabolic pathways in HPRT deficiency, yet leaving many questions still unsolved.

Lesch-Nyhan disease • neurological syndrome • hyperuricemia • therapies

Introduction

A “bizarre” metabolic and neurological syndrome, characterized by marked hyperuricemia and hyperuricuria, was identified as an X-linked recessive disease and named “Lesch-Nyhan Disease” (LND) after the two physicians who first described it [1,2]. Later, the biochemical cause was identified as a single enzyme deficiency: hypoxanthine-guanine phosphoribosyltransferase (HPRT; EC 2.4.2.8) [3]. HPRT catalyzes the salvage of the purine bases hypoxanthine and guanine converting them into their respective monophosphate nucleosides (IMP and GMP) by a PRPP-dependent phosphoribosyl transfer reaction (Fig. 1). It is a cytoplasmic enzyme ubiquitously expressed in human tissues, displaying different specific activity in different tissues and during development, with highest activities in testis and brain [4-6]. The human enzyme is encoded by a single structural gene (HPRT1) located at Xq26-27. The gene has been sequenced and more than 400 different mutations in the coding region have been described causing different degrees of deficiency [7]. HPRT aminoacid sequence has been determined and various alterations of the physical and kinetic properties of the enzyme have been reported in patients bearing different mutations [8,9], leading to complete or partial deficiency. In few cases deficiency of HPRT activity in intact cultured fibroblasts was reported not to be related to any mutation in the HPRT coding sequence but to markedly decreased HPRT expression of mRNA [10-12]. In these cases deficiency was attributed to a defect in gene regulation of unknown cause.

Megaloblastic anaemia unresponsive to folate therapy is also common in LND patients [13]. The basic diagnostic criterion for LND and its variants is HPRT activity assay in lysates or intact erythrocytes or fibroblasts; diagnostic suspect can be raised by grossly increased uric acid amount in plasma and urine, accompanied by increased hypoxanthine and xanthine [13].

Genetic aspects of LND

The prevalence of LND is approximately estimated to be 1/380,000 live births. It appears to occur in all populations
with equal frequency and is the less rare disease among those described for purine metabolism. Since HPRT deficiency is inherited as a recessive X-linked trait, males are generally affected and women may be asymptomatic carriers. Nevertheless at least five females with complete HPRT deficiency and full LND [14-17] and one with partial deficiency [18] have been reported. In female LND a variety of molecular mechanisms causing the deficiency have been described, affecting the second allele or X-inactivation ratio [16].

Hyperuricemia in LND

HPRT deficiency, either partial or complete, causes marked overproduction of uric acid with consequent nephrolithiasis, renal failure and juvenile gout. Several mechanisms contribute to uric acid overproduction resulting in hyperuricemia: the ready conversion of uric acid into xanthine and hypoxanthine by means of guanase and xanthine oxidase (Fig. 1), and the consistent increase of de novo purine synthesis. The latter is likely due to raised availability of PRPP and to decreased amounts of IMP, AMP and GMP, feedback inhibitors of phosphoribosylpyrophosphate-amidotransferase (the first and rate-limiting enzyme of de novo synthesis, Fig.1) [19]. Elevated adenine phosphoribosyltransferase (APRT; E.C. 2.4.2.7) activity is a common finding and may also contribute to purine overproduction [20].

Hyperuricemia, often leading to renal failure, is a serious problem in LND and its variants. Usual treatment is based on inhibitors of xanthine oxido-reductase (XOR: XDH, E.C. 1.17.1.4; XO, E.C. 1.17.3.22) such as allopurinol and febuxostat, effectively lowering UA, but accumulating hypoxanthine and xanthine. The latter may form stones with frequent renal failure [21,22]; moreover, the increased hypoxanthine and xanthine concentrations occurring in LND cerebrospinal fluid have been related to the neurological manifestation (reviewed below). Despite the use of allopurinol to control hyperuricemia, some patients still succumb to the consequences of persistent nephrolithiasis, such as renal failure or urosepsis. Rasburicase, a recombinant urate oxidase converting uric acid into allantoin, is also sporadically used for rapid prevention of renal failure. Alternative treatments avoiding hypoxanthine accumulation have been recently proposed, based on recombinant enzyme therapy restoring the uricolytic pathway, lost in humans [23], or on upstream PNP inhibition to slower purine breakdown [24].

Neurological aspects in LND

LND patients present severe neurological and motor disability, and most of them are confined to wheelchair. The neurological picture, closely resembling athetoid cerebral palsy, encompasses a spectrum of extrapyramidal signs including dystonia, choreoathetosis, dysarthria, dysphagia, opisthotonos, and occasionally ballismus. Some patients also develop pyramidal signs, such as spasticity and hyperreflexia. The most striking aspect of the disease concerns behavioral problems: a peculiar severe sort of compulsive self-injurious behavior is common, with self mutilation (lip, tongue or finger biting), and other occasional different means of self-harm. Self-injury is not the result of a lack of sensation (the patients feel pain and are relieved when protected from themselves) but can be ascribed to an obsessive-compulsive behavior often revealing new and unexpected forms [25] and still under study. Without restrictions, most patients can develop important auto-mutilating lesions [26]. Difficult behaviours such as impulsivity, striking or spitting at others, or use of socially unacceptable language is also rather frequent. Despite their periodic aggressive behavior, LND patients are frequently happy and engaging children when they are restrained. Affected individuals have often been described as cognitively impaired, but such feature is difficult to assess and is often misdiagnosed due to the behavioral disturbances, motor deficits, and attention problems [27]. Individuals with LND usually have a normal prenatal and perinatal course and psychomotor delay may become evident within 3 to 6 months. Self mutilation can appear as soon as teeth are present or later [28,29]. Few patients live beyond 40 years, death occurring due to different causes, including pneumonia and sudden, unexpected death with respiratory origin [30].

Depending on the amount of residual HPRT activity the neurological syndrome can be full-spectrum (LND, virtually no residual activity), or less severe, with mild or no neurological impairment [28]. The term “Lesch-Nyhan variants” has been introduced to describe a continuous spectrum of neurological involvement present in HPRT-deficient patients, with some degree of cognitive impairment, spasticity, dystonia, but without the complete syndrome. It is general opinion that genotype-phenotype correlations are based on HPRT residual activity, the severity of neurological symptoms being inversely proportional to enzyme activity [31-33]. Site-directed mutagenesis and in vitro expression of mutant HPRT (44 mutations associated with a wide spectrum of clinical phenotypes) was used to confirm correlation between disease severity and residual catalytic activity of the enzyme [34]. The deficiency of HPRT activity on guanine was described to correlate more strictly with clinical aspects of LND phenotype than that on hypoxanthine, suggesting different direct roles for guanine and hypoxanthine in the pathogenesis [35]. In some cases members of affected families bearing identical mutations have been described to present surprisingly different range of phenotype [36,37].
The connection between the neurological syndrome described in LND patients and HPRT deficiency appeared inexplicable: the role of HPRT in purine metabolism was well known and no connection with neurotransmitters was evident. A number of detailed studies in the last decades addressed the genetic, metabolic [25,28,38], cognitive [26,39], behavioral [26,27,40] and anatomical [6,29,41] features of the disease. Initial investigations sought for the possible accumulation of toxic metabolites or depletion of essential molecules to disclose potential connections between purine recycling and neuronal dysfunction. Further on sophisticated methods were used to have a deeper insight in the genetic and molecular aspects, unveiling a network of combined dysregulations in cell development and differentiation producing neurotransmission defects (Fig. 2).

The biochemical aspects of LND were extensively explored in patient cells (erythrocytes, lymphoblasts, fibroblasts), in patient autopsied brain specimens, in different HPRT-deficient cultured cell lines and in animal models. The results are often inconsistent, suggesting significant differences depending upon cell types and tissue source [42]. Several metabolic abnormalities are known to accompany HPRT deficiency, including the above mentioned grossly increased de novo purine synthesis. Peculiar features have been reported in erythrocytes, such as GTP depletion, increased UDP-glucose and PRPP concentration [43] (also found in lymphoblasts and fibroblasts) and appreciable levels of ZTP (5-amino-4-imidazole carboxamide ribotide triphosphate), a phosphorylated intermediate of de novo synthesis normally undetectable [44,45]. Some enzyme activities have also been reported to be abnormally increased in HPRT-deficient erythrocytes, namely APRT, IMP dehydrogenase (E.C. 1.1.1.205) [20] and cN-II (E.C. 3.1.3.5) [46]. Grossly increased NAD concentration was reported [47,43] with normal or lower NADH/NAD+ ratio in LND erythrocytes [48]. Increased utilization of exogenous NAD precursor nicotinic acid by intact erythrocytes [49,50], with increased or normal erythrocyte activities of the enzymes committed to its synthesis were reported in LND patients [51, 52], and low NAD glycohydrolase activity [53] (Fig. 1). Decreased activity of PARP (Poly-ADPribose polymerase) was found in LND lymphoblasts [54], possibly accounting for the high levels of NAD and also suggesting defective DNA repair mechanisms. By contrast decreased NAD, ATP and GTP concentrations and increased NAD production from nicotinic acid were measured in LND fibroblasts [38]. NAD concentration and related enzyme activities were found to be significantly increased in liver, but not in brain or blood, of HPRT-deficient erythrocytes, namely APRT, IMP dehydrogenase and cN-II (E.C. 3.1.3.5).

Reduced levels of the dopamine (DA) metabolite homovanillic acid, together with increased hypoxanthine and xanthine concentrations were found in LND patient’s cerebrospinal fluid [57]. PET neurochemical and neuroimaging in vivo studies performed in LND patients demonstrated significant abnormalities of DA neuron function in the basal ganglia: decreased dopaminergic neuron terminals in the striatum and increased amount of serotonin and 5-hydroxyindololacate [56]. Decreased levels of the dopamine (DA) metabolite homovanillic acid, together with increased hypoxanthine and xanthine concentrations were found in LND patient’s cerebrospinal fluid [57]. PET neurochemical and neuroimaging in vivo studies performed in LND patients demonstrated significant abnormalities of DA neuron function in the basal ganglia: decreased dopaminergic neuron terminals in the striatum and increased amount of serotonin and 5-hydroxyindololacate [56]. 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Hypoxanthine excess is a prominent biochemical feature described in the central nervous system of LND patients, and its role has been extensively investigated. Hypoxanthine has been reported to alter adenosine transport [68] and to decrease sensitivity at the post-synaptic DA receptors [69]. Studies conducted in LND peripheral blood lymphocytes exposed to hypoxanthine revealed increased expression of DRD5 dopamine receptor, variably aberrant expression of ADORA2A adenosine receptor and decreased expression and protein level of 5-HTR1A serotonin receptor. This would support the hypothesis that the pathogenesis of neurological manifestations of LND patients may be related to an imbalance of neurotransmitters, rather than to the isolated disturbance of one of them. In fact, adenosine, DA and serotonin receptors, belonging to the G-protein-coupled superfamily, seem to be integrated through intermembrane receptor–receptor interactions [70,71]. Hypoxanthine excess has also been reported to alter Na+/K+ ATPase activity [72] in isolated cells, thus suggesting its implication in the pathogenesis of the neurological dysfunction. Implication of hypoxanthine in the morphogenesis impairment and proliferation enhancement in cultured HPRT deficient neuroblastoma cells, a neuronal model of LND, has also been proposed [73]. Intrastriatal hypoxanthine administration to 60-day-old rats altered neuroenergetic parameters, resulting in ATP depletion and mitochondrial dysfunction and cell death by apoptosis, suggesting that these processes may be associated, at least in part, with neurological symptoms found in LND patients [74].

Deficit of other purine compounds due to HPRT defect is controversial, and altered nucleotide concentrations have been postulated as a possible cause of changes in G-protein-mediated signal transduction [75]. This hypothesis was supported by the finding of changes in the expression and function of adenylate cyclase C isoform as a result of HPRT deficiency in B103 neuroblastoma cells [76]. Another line of research hypothesized that GTP depletion in HPRT deficiency may affect tetrahydrobiopterin (BH₄) synthesis through GTP cyclohydrolase, but BH₄ limitation was not demonstrated to be responsible for the dopamine loss in patients or animal models [77].

Investigations on the pathogenesis of neurological and behavioral alterations in LND: role of hypoxanthine

Transcriptional aberrations in a number of genes were described in the HPRT knockout mice, suggesting a role of genes other than HPRT in the HPRT-deficiency phenotype. In this hypothesis HPRT deficiency would induce secondary transcriptional aberrations in other genes, that could play an important role in the development of some aspects of the HPRT-deficiency phenotype, especially the neurological deficits [78]. Such hypothesis was confirmed by several studies conducted in the last decade in HPRT-deficient cell models [79,80] and in HPRT-deficient human neural stem cells [81]. Aberrant expression of several vital transcription factors involved in DA-neuron development and in pan-neuronal differentiation has been demonstrated in cultured HPRT-deficient human teratocarcinoma NT cells (NT2). Such studies provided direct experimental evidence for aberrant neurogenesis in HPRT deficiency and suggested impaired upregulation of tyrosine hydroxylase (TH, the rate-limiting enzyme in DA production) and of aromatic L-amino-acid decarboxylase, the final step in DA synthesis [79]. Investigations conducted by microarray and quantitative PCR in 10 different HPRT-deficient mouse cell sublines also demonstrated that HPRT deficiency influences early developmental processes controlling the dopaminergic phenotype by increasing transcription factors which play a key role in the specification and survival of DA neurons [80]. Altered expression of several transcription factors and DA markers was found in human neural stem cells (hNSCs) isolated from human LND fetal brain, providing direct experimental evidence for aberrant neurogenesis [82]. Studies in SH-SY5Y neuroblastoma cells made HPRT-deficient by shRNA revealed broad pleiotropic neuro-regulatory defects, demonstrating dysregulated Wnt signaling and presenilin-1 expression together with impaired expression of dopaminergic transcription factors [83]. Other authors found that hypoxanthine excess influences the Wnt/β-catenin pathway by both increasing WNT11 and WNT4 expression and reinforcing the WNT4 and EN1 expression induced by retinoic acid in NT2/D1 cells, thus deregulating early neuronal differentiation [84]. Aberrant over-expression of miR181a was found in HPRT-deficient human dopaminergic SH-SY5Y neuroblastoma cells, which significantly reduced endogenous expression of genes known to be required for neural development, including EN1, EN2, LMX1a and BRN2, and suggested that miRNAs may play a role in the pathogenesis of LND [85]. Dysregulated microRNAs from the miR-17 family cluster and guanine-based cellular functions were found in differentiating HPRT-deficient human neuron-like cell lines by microRNA array and gene ontology analysis. In the same study, dysregulated expression of exchange protein activated by cAMP (EPAC) in the cortex, the midbrain and the striatum of HPRT ko mice and in HPRT-deficient human neuron-like cell lines and fibroblast cells from LND patients were found, and also a marked impairment in the activation of small GTPases. Collectively these aberrations were hypothesised to contribute to the complex LND neurological phenotype [86]. Another
line of research demonstrated that HPRT-deficient neuronal cell lines have reduced CREB (cAMP response element-binding protein) expression and lower intracellular cyclic AMP (cAMP), which correlates with attenuated CREB-dependent transcriptional activity and reduced phosphorylation of protein kinase A (PKA) substrates such as synapsin (p-syn I). Increased expression of phosphodiesterase 10A (PDE10A) was also found and the overall conclusion was that HPRT-deficiency alters cAMP/PKA signaling pathway [87].

The mechanisms by which HPRT deficiency influences the expression of different genes and leads to transcriptional aberrations might be direct or indirect, the latter following the changes in purine metabolism when HPRT is missing. A general problem in the field of LND research concerns the selection of a suitable model system. Indeed, disruption of purine levels is known to have an important influence on neuronal differentiation [81]. Purine pools and their metabolism were examined in rat PC6-3 cells, a PC12 pheochromocytoma subclone. The loss of HPRT-mediated purine recycling is associated with significant loss of dopamine and related metabolites in the HPRT mutant PC6-3 lines, suggesting an important connection between purine and dopamine pathways [88].

HPRT-deficient pluripotent human stem cells induced by shRNA targeted to the HPRT gene showed aberrant purinergic signaling occurring at least partly through aberrant P2Y1-mediated expression and signaling. Such mechanisms may play a role in the neuropathology of HPRT-deficiency LND [89]. A proteomic approach revealed changes in protein expression in HPRT-deficient dopaminergic rat PC6-3 line, before and after differentiation with nerve growth factor, with an unexpectedly broad influence on many biochemical pathways (neurotransmission, protein synthesis and metabolism, mitochondrial function, single methyl donor pathways involving SAM or folate) possibly related to the cell cycle [90]. Transcriptomic studies conducted on LND fibroblasts and on induced pluripotent stem cells (iPS) by microarray based methods together with qPCR also revealed that HPRT deficiency is accompanied by perturbations in specific processes. Twenty-five transcripts were found with significantly altered expression level that are involved in specific processes known to regulate cell cycle and cell-division, metabolic and nucleic acid processes [91]. Global transcriptomic analyses confirmed that several mechanisms are severely affected during neuronal differentiation of HPRT-deficient murine ESD3 embryonic stem cells: beside large number of developmental and cell signaling pathways regulating CNS development, most particularly mechanisms that determine neuronal/glial cell fate decisions during neurogenesis were altered, with a major transcriptional switch away from neuronal almost entirely to a glial gene expression program, though with at least some of the principal molecular properties of dopaminergic neurons [92].

A role for the amyloid precursor protein (APP) has been suggested in the development of LND epigenetic modifications, due to gene-gene interactions (epistasis) between mutated HPRT and APP genes, which could affect the regulation of alternative APP pre-mRNA splicing in favor of APP isoforms responsible for the disease, though no experimental evidence at present proven the direct link between LND and APP [93]. Many combined results deriving from such many different approaches seem to indicate that the housekeeping gene HPRT is a vital neurodevelopmental gene and that it plays a number of important non-“housekeeping” functions in some pathways of mammalian neurogenesis. In summary, various studies have led to understand that the neurological symptoms of LND are related to a dysfunction of the dopaminergic neurotransmitter system in the basal ganglia, and possibly extended beyond this area. The great bulk of recent observations shed a new light on the mysterious relationship between the dopamine deficit and the purine metabolic disorder, though the final step is still lacking.

**Therapy**

The lack of precise understanding of the neurological dysfunction in LNS has precluded development of specific therapies, though several attempts have been made in different directions. Treatment with allopurinol has no effect on the neurologic or behavioural manifestations of the disease [62]. Following the hypothesis that deficiency of IMP and GMP might be the cause for LND, treatment with adenine (readily transported and converted to AMP, which in turn can be converted to IMP by AMP deaminase) was tried without effect [94] as well as treatment with AICA or AICAR expected to raise nucleotide levels [95]. Actually no specific decrease in purine level was found in any model studied. Many pharmaceutical treatments, often useful, but never resolving (e.g.: benzodiazepines, carbamazepines and gamma-aminobutyric acid inhibitors, gabapentin, dopamine replacement) often leading to inconsistent outcomes have been reported and reviewed [29, 13, 96]. Limited studies have been conducted on S-adenosylmethionine (SAM) as a medication for LND with contradictory but promising findings, at least offering an additional therapeutic means to current symptomatic therapy [97-100]. Dopamine neurons likely fail to mature properly, which is consistent with several gene expression studies of HPRT cell models showing disruption of molecular pathways for dopamine neuron development. Thus, the loss of dopamine or TH seems to reflect an aspect of a broader developmental defect in these neurons, and restoration of dopamine alone seems insufficient [101, 102].
Therapeutic efforts have often mainly focused on symptom control. Intrathecal baclofen therapy ameliorated the motor and behavioral symptoms [103].

Good results have been obtained by chronic deep brain stimulation of the globus pallidus which may be a promising method to treat self-mutilating behavior and dystonia associated with LND [104-107].

Attempts to develop gene therapy have also been made [108] but could not be applied in patients.

An enzyme replacement approach by TAT transduction domain and by liposome mediated protein transfer into HPRT deficient leukemia T-cells (CEM/HPRT) was also reported in an in vitro study [109].

### Conclusion

The present review aimed to highlight the great deal of research moved by LND, a rare disease displaying many puzzling aspects. First of all, the connection between the deficiency of HPRT activity and the neurological syndrome: no direct link exists between this enzyme and the dopaminergic transmission which has been demonstrated to be impaired, and several molecular pathways are likely involved as a pathogenic cause. Nevertheless the intimate mechanism is not clear yet. Another unresolved problem is the finding of different phenotypes in patients bearing the same genetic mutation, which pointed at the involvement of epigenetic mechanisms. On the whole many studies led to the conclusion that the consequences of HPRT deficiency are far beyond the metabolic function of this enzyme, and that the pathogenesis of this monogenic but yet very complex neurodevelopmental disease results from combinatorial and multigenic defects.

Resolutive therapy is the main goal of overall research: the understanding of times and modes of neurological lesion occurrence would suggest times and modes of clinical intervention. The common belief is that any therapeutic action should be very early; times for effective intervention (e.g. dopamine restoration) must be ascertained and new therapeutic molecules investigated. In the meantime symptomatic therapies providing some relief from the invalidating disturbances of LND are under clinical trial.

Research is in continuous progress and accurate reviewing is necessary for frequent update and for an overall vision allowing a deeper insight in the disease.

### Conflict of Interest Statement

V. Micheli, M. Bertelli, G. Jacomelli, A. Santucci, G. Bernardini declare that the submitted work was not carried out in the presence of any personal, professional or financial relationship that could potentially be construed as a conflict of interest.

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**FIGURE LEGENDS**

**Figure 1.** Purine salvage/breakdown (grey inset) and diagram of purine and pyridine metabolism.

SAICAR: succinylaminoimidazolcarboxamide ribotide; AICAR: aminoimidazolcarboxamide riboside; PRA: phosphoribosylamine; BH4: tetrahydrobiopterin; FH4: formyltetrahydrofolate; SAM: S-adenosyl methionine; SAH: S-adenosyl homocysteine; NA: Nicotinic acid; Nam: Nicotinamide; NAMN: Nicotinic acid mononucleotide; NMN: nicotinamide mononucleotide.

Figure 2. HPRT deficiency: metabolic alterations which may lead to LND.