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Consequences of Impaired Purine Recycling on the Proteome in a Cellular Model of Lesch-Nyhan Disease

Eric B. Dammer^{#1}, Martin Göttle^{#2}, Duc M. Duong¹, John Hanfelt³, Nicholas T. Seyfried¹, and H. A. Jinnah^{2,4}

¹ Department of Biochemistry, Emory University, Atlanta, GA

² Department of Neurology, Emory University, Atlanta, GA

³ Department of Biostatistics and Bioinformatics, Emory University, Atlanta, GA

⁴ Department of Human Genetics & Pediatrics, Emory University, Atlanta, GA

[#] These authors contributed equally to this work.

Abstract

The importance of specific pathways of purine metabolism for normal brain function is highlighted by several inherited disorders, such as Lesch-Nyhan disease (LND). In this disorder, deficiency of the purine recycling enzyme, hypoxanthine-guanine phosphoribosyltransferase (HGprt), causes severe neurological and behavioral abnormalities. Despite many years of research, the mechanisms linking the defect in purine recycling to the neurobehavioral abnormalities remain unclear. In the current studies, an unbiased approach to the identification of potential mechanisms was undertaken by examining changes in protein expression in a model of HGprt deficiency based on the dopaminergic rat PC6-3 line, before and after differentiation with nerve growth factor (NGF). Protein expression profiles of 5 mutant sublines carrying different mutations affecting HGprt enzyme activity were compared to the HGprt-competent parent line using the method of stable isotopic labeling by amino acids in cell culture (SILAC) followed by denaturing gel electrophoresis with liquid chromatography and tandem mass spectrometry (LC-MS/MS) of tryptic digests, and subsequent identification of affected biochemical pathways using the Database for Annotation, Visualization and Integrated Discovery (DAVID) functional annotation chart analysis. The results demonstrate that HGprt deficiency causes broad changes in protein expression that depend on whether the cells are differentiated or not. Several of the pathways identified reflect predictable consequences of defective purine recycling. Other pathways were not anticipated, disclosing previously unknown connections with purine metabolism and novel insights into the pathogenesis of LND.

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Corresponding author H. A. Jinnah, M.D., Ph.D. 6300 Woodruff Memorial Research Building Department of Neurology Emory University Atlanta GA, 30322 Phone: 404-727-9107 Fax: 404-727-8576 hjinnah@emory.edu.

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Keywords

Lesch-Nyhan disease; dopamine neurons; proteome; hypoxanthine-guanine phosphoribosyltransferase

Introduction

The purines are a class of organic molecules that include adenine-based derivatives (e.g. ATP, ADP, AMP, cAMP, NAD, adenosine), guanine-based derivatives (e.g. GTP, GDP, GMP, cGMP, guanosine), and related metabolites (hypoxanthine, xanthine, and uric acid). Various purines play critical roles in many cellular functions such as serving as cofactors for enzymatic reactions and building blocks for DNA and RNA synthesis. Purines also are involved in many aspects of neuronal development and function. Multiple purines have trophic influences on neurons and glia [1-4]. Neurite extension and synaptic remodeling is regulated by a group of GTP-dependent GTPases [5, 6]. There also are intercellular signaling pathways involving adenine or guanine metabolites, and G-proteins that require cAMP or cGMP.

The importance of purines for normal brain function is highlighted by several rare inherited disorders of purine metabolism [7-10]. For example, defects in purine recycling mediated by the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGprt) cause the severe neurobehavioral problems of Lesch-Nyhan disease (LND). However, the mechanisms by which HGprt deficiency cause the clinical problems are not well understood [11]. Clinical studies have suggested that dysfunction of specific dopaminergic neurons projecting to the basal ganglia underlie many of the neurological and behavioral abnormalities seen in patients with LND [12, 13], and autopsy studies have revealed significant reductions in basal ganglia dopamine and related indices of the dopamine neuron phenotype [14-16]. Prominent abnormalities of dopamine neurons have been documented for an HGprt knockout mouse [17-20], and HGprt⁻ neuron-like cells in culture [21-30]. These findings imply that HGprt deficiency causes a cell-intrinsic defect that can be modeled in vitro, in the absence of complex inter-cellular relationships in the developing nervous system. Although clinical and experimental studies both have pointed towards prominent involvement of dopamine neurons, the mechanism by which HGprt deficiency influences these neurons remains enigmatic, because there are no known biochemical connections linking purine metabolism with dopamine pathways [12].

Prior studies addressing the relationship between HGprt deficiency and dopamine systems have taken a largely hypothesis-driven approach, exploring proposed connections between purine metabolism and the function of dopamine neurons such as uric acid toxicity [31], oxidative stress [25, 32], reduced tetrahydropterin synthesis [33], abnormal purinergic neurotransmission [34-37], G-protein dysfunction [38, 39] micro RNAs [40, 41], or dysregulation of neurodevelopmental processes [26, 27, 29, 41-43]. These studies have provided a wealth of conflicting data, so the most relevant pathways for pathogenesis remain to be established. In the current studies, we applied a proteomic approach unencumbered by specific hypotheses to disclose novel potential connections between purine recycling and

neuronal dysfunction in a recently developed model of HGprt-deficient dopamine-like neurons. The results revealed that HGprt deficiency has an unexpectedly broad influence on many biochemical pathways. Several of these pathways could be predicted from the known consequences of HGprt deficiency, while others were not anticipated.

Experimental Design & Methods

Cells and culture

The current studies focused on the PC6-3 cell line, a subline of the rat PC12 pheochromocytoma line [44]. This cell line was chosen because it expresses pathways involved in dopamine synthesis and metabolism, and it exhibits rapid and high efficiency neuronal differentiation after exposure to nerve growth factor (NGF), permitting evaluation of both proliferating and post-mitotic neuron-like cells. It is well known that subclones of established cell lines exhibit idiosyncratic properties that differ from the parental line because of genetic drift during cell culture [25, 45-48]. Such idiosyncratic variation was apparent in prior studies of the PC6-3 line used in the current studies [28], raising concerns for studying only a single HGprt⁻ subline. To guard against this problem of idiosyncratic clonal heterogeneity, 5 independent HGprt⁻ PC6-3 subclones were compared against the PC6-3 parent line, a strategy used in prior studies of HGprt⁻ mouse MN9D [25] and human SK-N-BE(2) M17 [48] sublines. The analytical strategy focused on delineating abnormalities that were abnormal across the HGprt⁻ sublines as a group, rather than idiosyncratic abnormalities among individual mutants.

The 5 HGprt⁻ mutant sublines were established and characterized earlier [28]. Each has a different mutation in the *HPRT1* gene, but all have <1% residual HGprt enzyme activity. All cells routinely were grown at 37 °C in an atmosphere of 5% CO₂ and 95% air in RPMI 1640 medium supplemented with 5% fetal bovine serum (Thermo Fisher Scientific, Logan UT), 10% horse serum (Invitrogen, Carlsbad CA), 1 U/mL penicillin, 2 mM L-glutamine, and 100 µg/mL streptomycin. When cells were to be harvested for studies, they were grown on flasks coated with rat tail collagen type I (BD Biosciences, Bedford MA) to promote adherence as previously described [28]. Because the consequences of HGprt deficiency may be influenced by neuronal differentiation, all cell lines were evaluated before and 4 days after exposure to 50 ng/mL of 2.5S NGF from the mouse submaxillary gland.

Protein measurements

The SILAC method was used for proteomic analyses [49]. The culture medium for labeling consisted of RPMI-1640 without arginine or lysine (89984, Thermo Fisher Scientific, Rockford IL) and dialyzed animal sera as described above. Parent cells were grown for at least 10 doublings in “heavy” medium containing 1.15 mM labeled (U-13C6, U-15N4) arginine (CNLM-539-H, Cambridge Isotope Laboratories, Andover MA) and 0.274 mM labeled (U-13C6, U-15N2) lysine (CNLM-291-H, Cambridge Isotope Laboratories). Mutant cells were grown for at least 10 doublings in “light” medium containing 1.15 mM unlabeled L-arginine (ULM-8347, Cambridge Isotope Laboratories) and 0.274 mM unlabeled lysine (ULM-8766, Cambridge Isotope Laboratories). To compensate for slight differences in growth rates among the lines, the density of initial seeding of the cultures was varied so that

all cultures had a similar level of confluency at harvest. Subsequently, the supernatant was removed and cells were detached by trypsinization and washed once in RPMI-1640 without arginine, lysine or animal sera. Cells then were disrupted by suspending in 600 μ L of 8M urea plus 50 mM ammonium bicarbonate. Homogenates were sonicated for 30 sec (Branson Sonifier 450, Danbury, CT) and stored at -80°C . Total protein concentrations were estimated with the Pierce BCA kit (Thermo Fisher Scientific, Rockford, IL).

Mass spectrometry (MS) was performed as previously described [50]. Briefly, samples were homogenized and solubilized in 8M urea supplemented with Halt protease and phosphatase inhibitor cocktail (Thermo Scientific, Rockford, IL). Equal protein weights of light total protein from each of the 5 HGprt⁻ cell lines were mixed separately with heavy SILAC control internal standard protein, and run separately in a 10% SDS-PAGE gel. Five bands were excised separately for each of the 5 cell lines and in-gel digested with trypsin. Peptides were extracted in 50% acetonitrile and 5% formic acid and lyophilized (Thermo Scientific Savant ISS110 SpeedVac concentrator). Lyophilized peptides were resuspended in a loading buffer consisting of 1% acetonitrile, 0.1% formic acid, and 0.03% trifluoroacetic acid.

Samples were loaded and eluted over a 130 min reverse-phase gradient on a Waters NanoAcquity UPLC fitted with a 15 cm 100 μ m ID nanoLC column packed with 1.9 μ m ReproSIL Pur C18 beads (Dr. Maisch HPLC GmbH, Ammerbuch-Entringen Germany) and electrospray ionized at 2kV into a hybrid LTQ Orbitrap XL mass spectrometer (Thermo). One survey MS scan was collected on the Orbitrap (300-1600 m/z, 1×10^6 AGC target, 500 ms maximum ion time, at 60000 resolution followed by 10 data-dependent LTQ MS/MS scans (2 m/z isolation width, 35% collision energy, 5,000 AGC target, 150 ms maximum ion time). Raw files were converted and searched on a Sorcerer 2 IDA (Sage-N Research) with Sorcerer Sequest version 4.0.3. The searches were performed with a concatenated target-decoy rat reference database (REFSEQ version 54, containing 29735 target proteins) from the National Center for Biotechnology Information (NCBI). Search parameters included: partial tryptic restriction, parent ion mass tolerance (± 20 ppm), and dynamic modifications of oxidized methionine (+15.9949 Da), SILAC labeled lysine (+8.014199) or arginine (+10.020909). Peptides were first filtered by a mass accuracy of ± 10 ppm after correction of instrumental mass shift and then grouped into trypticity (full or partial) and charge state. This was followed by a dynamic XCorr and Cn filtration algorithm that reduced the protein false discovery rate to less than 1% according to the target-decoy strategy.

Quantitative analyses of the proteome and pathways

Quantitative pair-wise comparisons of parent and mutant cell lines were performed according to previously reported methods [51-53]. Results for proteomics were analyzed by automated extracted ion current (XIC) SILAC ratio comparisons. In brief, the ratio of each peptide pair (light/heavy) was transformed into logarithmic ($\log_2$) values and the detection of outliers determined using Dixon's Q test. If a peptide was identified in multiple MW fractions the intensities for the peptide pair (light and heavy) were summed to give a single cumulative peptide ratio (light/heavy). Any proteins with fewer than 2 unique peptides quantified were eliminated (n=142 proteins) to increase the reliability of the final results. The local false discovery rate was controlled at 5% by using locFDR software [54]. The

Database for Annotation, Visualization and Integrated Discovery (DAVID) functional annotation chart analysis [55] of the gene symbols was then performed to identify overrepresented biochemical pathways and processes or subcellular compartments.

Results

A preliminary LC-MS/MS run of SILAC internal standards derived from total protein from the control PC6-3 cell line, followed by analysis of the population of all proteins identified and quantified for light/heavy peptide ratios revealed approximately 95% efficiency of heavy labeling. After confirming labeling efficiency, light (mutant) and heavy (control) proteins were mixed 1:1, resolved into 5 bands by SDS-PAGE, and tryptic digests analyzed by LC-MS/MS. Despite equal loading of heavy and light proteins, the overall distribution of protein abundance changes in HGprt⁻ cell lines was asymmetric, with a bias toward protein decrease in the mutant lines. The distribution of proteins was bimodal for most of the HGprt⁻ lines, unlike comparative protein distributions from synthetic null comparisons, which display the effects of biological and technical noise from both components of the SILAC mixture, including normal control PC6-3 cells (Figure 1). These results imply an unexpectedly broad abnormality of protein synthesis and metabolism in the HGprt⁻ lines.

In order to identify the most significantly affected proteins, initial normalization of the total quantified protein population relied upon centering the higher expressed subpopulation of proteins of the bimodal peak in the mutants at zero change ($\log_2$ light/heavy ratio of zero). For each experiment, 95% confidence intervals were calculated for variance within the Gaussian fit to the higher expressed subpopulation, and proteins falling outside of this range were considered candidates for significant change in the HGprt⁻ cell lines compared to the parent line. Then, additional steps were applied to enforce a local false discovery rate of <5% across the 5 mutant HGprt⁻ cell lines both before and after NGF-induced differentiation.

Using these criteria, and the additional requirement that reliable quantification was made using 2 or more peptides in at least 3 mutants, a total of 1055 proteins were expressed at significantly abnormal levels in the HGprt⁻ lines in the undifferentiated state. A total of 736 proteins were abnormally expressed in the mutant lines following NGF-mediated differentiation. Many proteins were abnormal in both the undifferentiated and differentiated conditions (n=332), but some were abnormal only before (n=319) or after (n=404) differentiation (Supplemental Table 1).

Signal from canonical HGprt protein sequence was reduced by approximately 95% ($1-2^{-4.2} = 0.95$). Since 5% unlabeled signal is essentially equal to the unlabeled fraction of the heavy standard, this result implies an essentially complete loss of HGprt protein from the mutant lines. This finding of nearly complete loss of HGprt protein is consistent with prior studies showing nearly complete loss of HGprt enzymatic activity [28]. The majority of the remaining proteins identified as being abnormal in the mutant cell lines also were decreased, but some were increased. Before differentiation only 8 proteins were significantly increased in the mutants, and most were likely to be artifacts from tissue culture additives devoid of SILAC label (e.g. albumin, trypsin, keratins). After differentiation, 307 proteins were

significantly increased in the HGprt⁻ mutants. When considering only the proteins that were significantly increased, DAVID pathway analyses identified over-represented functions in the HGprt⁻ mutants to include mRNA splicing, RNA binding, and mitochondrial function (Table 1).

When considering the entire population of significantly altered proteins (both increased and decreased), DAVID pathway analysis revealed several partly overlapping functional categories to be abnormal in the HGprt⁻ mutant lines: 1) protein synthesis and metabolism, 2) mitochondrial function, 3) S-adenosyl methionine (SAM) and one-carbon metabolite generation via tetrahydrofolate, 4) monoamines and neurotransmission. Many of these fall into a broader umbrella category of proteins that bind purine nucleotides such as ATP or GTP (annotated in the tables as a superscripted 1). For example, there were a disproportionate number of protein translation elongation-associated factors significantly reduced in the mutant lines (EEF1G, EEF2, LOC498555, LOC684988, RPL6, RPL23A, RPL10A, RPL32, RPL35, RPS5, RPS15, RPS15A), many of which have GTPase activity (Table 2). Some of these showed recovery of expression after NGF differentiation, but many showed no significant recovery (Figure 2). The tRNA synthetases (YARS, KARS, DARS, WARS, MARS, SARS, QARS, VARS, IARS, RARS, GARS, AARS, TARS, NARS, LARS, CARS, and HARS) as a group were decreased significantly and consistently. All are ATP dependent. In addition, ribosome assembly factors such as BOP1 were decreased before differentiation. Also significantly reduced in the mutant cells were proteins involved in protein degradation, including peptidases (ACE, CPD, DNPEP, ERAP1, NPEPPS, RNPEP, XPNPEP1) and proteins related to the proteasome (PSMC2, PSME4, PSMD1, PSMD2, PSMD3, PSMD5, PSMD6, PSMD9, PSMD12, PSMD13, UBQLN1). Many of these proteins remained at significantly depressed levels after differentiation.

Many mitochondrial proteins were significantly increased or decreased in the HGprt⁻ lines (Table 3). This finding may not be surprising, because many of these proteins are involved in purine synthesis or bind purines or their derivatives. Some of these proteins recovered following NGF differentiation, whereas others did not (Figure 2).

SAM and folate are cofactors with roles as methyl group donors in multiple biosynthetic pathways, including methylation of protein or DNA. A significant over-representation of proteins in both of these pathways was evident in the HGprt⁻ mutant lines (Figure 3). Notably, methyl CpG binding protein 2 (MeCP2) was significantly elevated after differentiation, potentially balancing a significant decrease in de novo DNA methyltransferase (DNMT1) across the mutant lines.

The HGprt⁻ lines also showed multiple abnormalities in proteins involved in neurotransmission (Table 4). For example, tyrosine hydroxylase (TH) is the rate-limiting enzyme for dopamine synthesis and was significantly reduced in the mutant lines before and after differentiation. The vesicle-associated membrane proteins VAMP2 and VAMP3, which are involved in vesicular dopamine release, also were reduced in the mutants. The pheochromocytoma signature secretory vesicle proteins, SCG2 and SCG3, were elevated in the mutant lines after differentiation. Monoamine oxidase A, which degrades dopamine was reduced. Also affected were numerous small GTPases known to be involved in neuronal

differentiation and the establishment and formation of neurites and synapses such as 3C, 8A, 27A, RAB3A, RAC1, RHOA, RHOG, and others.

Discussion

Results from the current studies reveal that loss of HGprt-mediated purine recycling has an unexpectedly broad influence on protein expression in dopaminergic PC6-3 cells. The most significantly and consistently affected biological pathways included protein synthesis and metabolism, mitochondrial function, single methyl donor pathways involving SAM or folate, and monoamine neurotransmission.

Proteomic-metabolomic relationships

Prior studies of the PC6-3 model have shown that the loss of HGprt has a relatively small influence on total purine metabolite levels that varies according to differentiation status [28]. Among the major bioactive purines found in mammalian cells, the mutant cells showed only ~20% loss of AMP in the undifferentiated state and ~50% loss of IMP in the differentiated state. However, there were no apparent decrements in any downstream purine metabolites such as ATP or GTP. Despite the relatively small and circumscribed changes in purine pools, the current studies reveal widespread alterations in the proteome. The extent of the changes in protein expression is surprising, given the specific role of HGprt in recycling purine bases and the relatively minor changes in purine nucleotides in HGprt⁻ PC6-3 cells previously reported.

One potential explanation for the apparent discrepancy between the minor influence of HGprt deficiency on purine metabolites and the much broader impact on protein expression relates to the cell cycle. A relatively minor decrement of purine nucleotides may slow cell division, when there is a heavy demand for purine nucleotides for DNA replication and RNA transcription. This idea is consistent with a number of prior observations. First, cell proliferation is tightly linked with purine biosynthesis, and the demand for synthesis varies with the cell cycle,[56-59]. Second, protein synthesis is among the most sensitive of all cellular processes to ATP deficits [58, 59]. In fact, HGprt⁻ cells tend to grow more slowly than their HGprt-competent counterparts in culture,[25, 48] and prior transcriptomic studies of HGprt-deficient cells have pointed to abnormalities of cell cycling [60]. Thus when purines may be limiting during cell division, cells may down-regulate protein expression to conserve energy and purine nucleotides. This down-regulation of protein synthesis may then only partly recover in the few days it takes for PC6-3 cells to cease cell division during differentiation. This hypothesis could explain why many of the significantly reduced proteins in the current studies appear to recover at least partly after being exposed to NGF for 4 days, when most cells are no longer actively dividing.

An alternative explanation for the minor changes in purines versus broader changes in protein synthesis in the HGprt⁻ cells is that purines play so many essential roles in so many different pathways. Many of the pathways affected in the HGprt⁻ cells, including those involved in protein synthesis, are directly or indirectly dependent upon adenine or guanine nucleotides. For example, there is broad dysregulation of many pathways that depend on ATP to drive energy-dependent reactions, kinases that require ATP as the phosphate donor,

or proteins that utilize adenine-based cofactors such as NAD or cAMP. There is similar dysregulation of GTP-dependent pathways including those that involve small GTPases, guanine nucleotide exchange factors, or G-protein dependent signaling. Even release of nascent proteins from signal recognition particle to the endoplasmic reticulum is GTP-dependent [61]. Thus minor decrements in purine levels may have a very broad impact on protein synthesis, especially if purine-dependent regulatory proteins are affected, or if purine limitation is transiently exaggerated during a specific stage in the cell cycle.

Proteomic-transcriptomic relationships

There are no prior proteomic studies of HGprt-deficient cells for comparisons with the current studies, but there are several published transcriptomic studies involving mouse MN9D neuroblastoma cells [26], human NT2 neuroblastoma cells [43], human SH-SY5Y neuroblastoma cells [27], mouse ESD3 embryonic stem cells [30], and human fibroblasts [60]. A common finding in all of these studies was an unexpectedly broad array of gene expression abnormalities. The prominent disruption of SAM- and folate-related single carbon donor reactions may explain these prior findings. SAM contributes to the methylation of histones, a process that has a dramatic influence on chromatin structure and DNA transcription. Folate contributes to methylation of DNA CpG islands, a process central to silencing of DNA transcription.

Involvement of either the SAM or folate pathways could result in substantial and widespread changes in the expression of both mRNA and miRNA species. These changes, in turn, could have a major impact on cell division, when specific genes must be turned on or off in a carefully orchestrated series. They also could have a significant impact on the differentiation of specific cell types such as neurons, where highly regulated changes in gene expression must occur.

Proteomic-dopaminergic relationships

The current studies confirm a prominent influence of HGprt deficiency on the dopaminergic phenotype of PC6-3 cells. These findings are consistent with prior studies showing prominent abnormalities of dopamine neurons in human LND brains collected at autopsy [14-16], HGprt knockout mice [17-19], and other dopaminergic neural cells in culture [16, 20-22, 24, 25, 29].

The mechanisms responsible for dysfunction of dopamine neurons in HGprt deficiency remain enigmatic. Previously we proposed that HGprt influences dopamine neurons via dysregulation of developmental pathways involving neuronal differentiation [26]. This proposal received support from microarray studies of HGprt⁻ MN9D neuroblastoma cells pointing to dysregulation of the engrailed genes En1 and En2 [26]. Later studies involving human NT2 neuroblastoma cultures in which HGprt was knocked down using short hairpin RNAs also revealed dysregulation of the developmental transcription factors Lmx1a and Msx1 [43]. Another study involving the shRNA knockdown strategy applied to human SH-SY5Y neuroblastoma cultures suggested abnormalities of the Wnt/beta-catenin pathways [27], and another showed changes in microRNAs that may influence levels of these transcription factors [40, 41]. A recent study of the transcriptome using the shRNA strategy

to knock down HGprt in ESD3 mouse embryonic stem cells differentiated towards the dopaminergic phenotype confirmed prominent dysregulation of the dopaminergic phenotype and associated developmental transcription factors [30].

The current proteomic studies provide some insights into the mechanisms that may underlie abnormal development and differentiation. Specifically, the HGprt⁻ PC6-3 lines showed a significant reduction in multiple small GTPases and related guanine-nucleotide exchange factors (GEFs). Some of these proteins are known to play a prominent role in neurite extension and synaptic remodeling [5, 6], and others play important roles as transcription factors during early development. Thus disruption of one or more of the GTPases involved in dopamine neuron development and differentiation, potentially combined with abnormalities of SAM- or folate-mediated regulatory processes that control gene expression, could explain the abnormalities associated with HGprt deficiency in dopamine neurons.

Study limitations

The current studies provide novel insights into the biology of HGprt deficiency, but they also have some limitations that should be acknowledged. The main limitation is that the PC6-3 cell line used in these studies was derived from a rodent pheochromocytoma. Whether the results found here can be extrapolated to human brain dopamine neurons remains to be established. Unfortunately, large-scale proteomic studies of brain dopamine neurons from LND brains are not technically feasible. Despite the potential species differences, it is interesting to note that some of the abnormalities detected using the PC6-3 cells are similar to those previously reported for immunostains of specific proteins in human autopsy material, such as reductions in TH [16].

Another limitation is that the unexpectedly broad abnormalities in protein synthesis and the bimodal distribution of changes in some of the HGprt⁻ lines required the application of a statistical strategy that might be overly conservative. This strategy means that we most likely revealed the most robust protein changes in the mutant PC6-3 cells. However, we may have inadvertently missed many more proteins that are significantly affected. The final limitation of our study is that we have not empirically validated the majority of the proteomic findings obtained from the PC6-3 cells. Instead, we relied on existing information from prior studies that have shown reductions in HGprt and TH in HGprt⁻ PC6-3 cells, human LND brains or the HGprt knockout mouse [16, 62, 63].

To address some of these limitations, proteomic studies of autopsy material from LND brains may be useful. Additionally, proteomic studies of differentiating neurons from induced pluripotent stem cells prepared from LND fibroblasts could also be useful. Such cells also could be useful for determining if correcting the presumed purinergic deficits with exogenously supplied purines might reverse some of the abnormalities found in recent proteomic or transcriptomic studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DAVID	Database for Annotation, Visualization and Integrated Discovery, and HGprt, hypoxanthine-guanine phosphoribosyltransferase
LC	liquid chromatography
LND	Lesch-Nyhan Disease
MS	mass spectrometry
N/A	not available
NGF	nerve growth factor
SILAC	stable isotopic labeling by amino acids in cell culture

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Highlights

- Loss of purine salvage causes a broad disruption of the proteome in cultured cells.
- Changes in individual proteins depend on the state of neuronal differentiation.
- Many protein changes may be explained by chronic, mild purine deficiency.

Figure 1.

Distribution of altered proteins. A histogram is shown of five biological replicate $\log_2(\text{mutant/control})$ quantified protein populations at baseline (black bars) and after 4 days NGF exposure (light bars). An additional quantitative population comparison of one mutant (b) versus another mutant (d) at baseline (blue bars) is provided as a synthetic null, which represents experimental noise level contributing to false positive quantitative differences in the actual experiment.

Figure 2. Altered proteins before and after differentiation. Log₂(mutant/control) average quantified protein amounts at 0 days (left) and 4 days NGF exposure (right) are shown for proteins which were significantly down-regulated in HGprt⁻ mutants compared to control cell line. Selected proteins which remained significantly downregulated at 4 days (blue lines) and HGprt itself (thick black line) are listed under the “No Recovery” heading and segregate from proteins which regain expression at 4 days NGF treatment (red lines), that are listed under the “Recovered” heading.

Figure 3.

Altered proteins involved in single-carbon transfer reactions. Each mutant line is shown separately (A, B, C, D) both before (0, open area) and 4 days after (4, shaded area) NGF-mediated differentiation. All of these proteins were significantly reduced in comparison to the normal controls, and virtually all showed significant recovery after differentiation.

Table 1Significantly increased proteins in HGprt⁻ PC6-3 mutants after differentiation

Proteins involved in RNA splicing	
CDC5L	CDC5 cell division cycle 5-like (S. pombe)
DNAJC8	DnaJ (Hsp40) homolog, subfamily C, member 8
FUS	fusion, derived from t(12;16) malignant liposarcoma
GTF2F1	general transcription factor IIF, polypeptide 1
GTF2F2	general transcription factor IIF, polypeptide 2
HNRNPA1	heterogeneous nuclear ribonucleoprotein A1
HNRNPA2B1	heterogeneous nuclear ribonucleoprotein A2/B1
LOC687575	Splicing factor U2AF 35 kDa subunit
PTBP2	polypyrimidine tract binding protein 2
SF3A1	splicing factor 3a, subunit 1
SNRNP70	U1 small nuclear ribonucleoprotein polypeptide A
SNRPA	small nuclear ribonucleoprotein polypeptide A
SNRPB2	small nuclear ribonucleoprotein polypeptide B"
SNRPC	small nuclear ribonucleoprotein polypeptide C
SNRPD1	small nuclear ribonucleoprotein D1
SNRPD2	small nuclear ribonucleoprotein D2
SRRM1	serine/arginine repetitive matrix 1
YBX1	Y box binding protein 1
ZRANB2	zinc finger, RAN-binding domain containing 2
Proteins that bind RNA	
RPS15	40S ribosomal protein S15 (RIG protein)
RPL23A	60S ribosomal protein L23a
CALR	calreticulin
CNBP	CCHC-type zinc finger, nucleic acid binding protein
CDC5L	CDC5 cell division cycle 5-like
CPSF6	cleavage and polyadenylation specific factor 6
CIRBP	cold inducible RNA binding protein
CSDA	cold shock domain protein A
ELAVL2	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)
EIF3G	eukaryotic translation initiation factor 3, subunit G
EIF4H	eukaryotic translation initiation factor 4H
FUBP1	far upstream element (FUSE) binding protein 1
HNRNPA1	heterogeneous nuclear ribonucleoprotein A1
HNRNPA2B1	heterogeneous nuclear ribonucleoprotein A2/B1
KHDRBS1	KH domain containing, RNA binding, signal transduction associated 1
MECP2	methyl CpG binding protein 2
MRPL1	mitochondrial ribosomal protein L1
NXF1	nuclear RNA export factor 1
NOLC1	nucleolar and coiled-body phosphoprotein 1

Proteins involved in RNA splicing

NCL	nucleolin
NPM1	nucleophosmin (nucleolar phosphoprotein B23, numatrin)
PTBP2	polypyrimidine tract binding protein 2
PURB	purine rich element binding protein B
LOC684988	ribosomal protein S13
SARNP	SAP domain containing ribonucleoprotein
SERBP1	Serpine1 mRNA binding protein 1
SSB	Sjogren syndrome antigen B
SNRPC	small nuclear ribonucleoprotein polypeptide C
SF3A1	splicing factor 3a, subunit 1
LOC687575	Splicing factor U2AF 35 kDa subunit
SNRNP70	U1 small nuclear ribonucleoprotein polypeptide A
YBX1	Y box binding protein 1
ZRANB2	zinc finger, RAN-binding domain containing 2

Mitochondrial proteins

HSPD1	60 kDa heat shock protein 1 (chaperonin)
ABHD11	abhydrolase domain containing 11
ATP5H	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit d
ATP5I	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit E
ATP5B	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, beta polypeptide
ATP5D	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, delta subunit
ATP5C1	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, gamma polypeptide 1
ATPIF1	ATPase inhibitory factor 1; similar to ATPase inhibitor, mitochondrial precursor
ATP6V1E1	ATPase, H ⁺ transporting, lysosomal V1 subunit E1
BOLA1	bolA homolog 1 (E. coli)
CLU	clusterin
COQ9	coenzyme Q9 homolog (S. cerevisiae)
CHCHD3	coiled-coil-helix-coiled-coil-helix domain containing 3
C1QBP	complement component 1, q subcomponent binding protein
CYB5A	cytochrome b5 type A (microsomal)
COX5B	cytochrome c oxidase subunit Vb
COX5A	cytochrome c oxidase, subunit Va
LOC688869	cytochrome c oxidase, subunit VIb polypeptide 1
DIABLO	diablo homolog (Drosophila)
DBI	diazepam binding inhibitor (GABA receptor modulator)
FXN	frataxin
GCSH	glycine cleavage system protein H (aminomethyl carrier)
GM2A	GM2 ganglioside activator
GRPEL1	GrpE-like 1, mitochondrial
HSPE1	heat shock protein 1 (chaperonin 10)
HINT2	histidine triad nucleotide binding protein 2
HAGH	hydroxyacyl glutathione hydrolase

Proteins involved in RNA splicing

HADH	hydroxyacyl-Coenzyme A dehydrogenase
ISCU	iron-sulfur cluster scaffold homolog (E. coli)
MRPL1	mitochondrial ribosomal protein L1
MRPL12	mitochondrial ribosomal protein L12
MRPL23	mitochondrial ribosomal protein L23
MRPL49	mitochondrial ribosomal protein L49
MRPS23	mitochondrial ribosomal protein S23
MRPS36	mitochondrial ribosomal protein S36
NDUFA2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2
NDUFAF4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 4
NDUFS8	NADH dehydrogenase (ubiquinone) Fe-S protein 8
PARK7	Parkinson disease (autosomal recessive, early onset) 7; similar to DJ-1
PRDX6	peroxiredoxin 6
PEBP1	phosphatidylethanolamine binding protein 1
PHB2	prohibitin 2
PSAP	prosaposin
QDPR	quinoid dihydropteridine reductase
PHB	similar to prohibitin; prohibitin
RGD1306917	similar to RIKEN cDNA 2900010M23; hypothetical protein LOC687308
STOML2	stomatin (Epb7.2)-like 2
SOD1	superoxide dismutase 1, soluble
SNCB	synuclein, beta
TXN2	thioredoxin 2
TFAM	transcription factor A, mitochondrial
TIMM9	translocase of inner mitochondrial membrane 9 homolog (yeast)
UQCRCF1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
VDAC1	voltage-dependent anion channel 1

Table 2Significant decreases in proteins involved in protein processing HGprt⁻ PC6-3 cells

tRNA Aminoacylation for protein translation	
AARS ^I	alanyl-tRNA synthetase
DARS ^I	aspartyl-tRNA synthetase
EPRS ^I	glutamyl-prolyl-tRNA synthetase
FARSB ^I	phenylalanyl-tRNA synthetase, beta subunit
GARS ^I	glycyl-tRNA synthetase
HARS ^I	histidyl-tRNA synthetase
IARS2	isoleucyl-tRNA synthetase 2, mitochondrial
KARS ^I	lysyl-tRNA synthetase
LARS ^I	leucyl-tRNA synthetase
LOC679383	similar to DNA segment, Chr 5, ERATO Doi 135, expressed
MARS ^I	methionine-tRNA synthetase
NARS ^I	asparaginyl-tRNA synthetase
QARS ^I	glutamyl-tRNA synthetase
RARS ^I	arginyl-tRNA synthetase
SARS ^I	seryl-tRNA synthetase
TARS ^I	threonyl-tRNA synthetase
VARS ^I	valyl-tRNA synthetase
WARS ^I	tryptophanyl-tRNA synthetase
YARS ^I	tyrosyl-tRNA synthetase
Translation initiation Factor Activity	
EEF1G	eukaryotic translation elongation factor 1 gamma
EEF2 ^I	similar to Elongation factor 2 (EF-2); eukaryotic translation elongation factor 2
EIF2B1 ^I	eukaryotic translation initiation factor 2B, subunit 1 alpha
EIF2B3	eukaryotic translation initiation factor 2B, subunit 3 gamma
EIF2B4	eukaryotic translation initiation factor 2B, subunit 4 delta
EIF2B5	eukaryotic translation initiation factor 2B, subunit 5 epsilon
EIF2S3X ^I	eukaryotic translation initiation factor 2, subunit 3, structural gene X-linked
EIF3C	eukaryotic translation initiation factor 3, subunit C
EIF3D	eukaryotic translation initiation factor 3, subunit D
EIF3S6IP	eukaryotic translation initiation factor 3, subunit 6 interacting protein
EIF4G2	similar to Eif4g2 protein; eukaryotic translation initiation factor 4, gamma 2
EIF5B ^I	eukaryotic translation initiation factor 5B
Proteasomal proteins	

tRNA Aminoacylation for protein translation

PSMD1	proteasome 26S subunit, non-ATPase, 1 (RPN2)
PSMD12	proteasome 26S subunit, non-ATPase, 12
PSMD13	proteasome 26S subunit, non-ATPase, 13
PSMD2	proteasome 26S subunit, non-ATPase, 2 (RPN1)
PSMD3	proteasome 26S subunit, non-ATPase, 3
PSMD5	proteasome 26S subunit, non-ATPase, 5
PSMD6	proteasome 26S subunit, non-ATPase, 6
PSMD8	proteasome 26S subunit, non-ATPase, 8
PSME4	proteasome activator subunit 4 (PA200)
PSMG1	proteasome assembly chaperone 1

Aminopeptidases

DNPEP	aspartyl aminopeptidase
DPP3	dipeptidylpeptidase 3
ERAP1	endoplasmic reticulum aminopeptidase 1
METAP2	methionyl aminopeptidase 2
NPEPPS	aminopeptidase puromycin sensitive
RNPEP	arginyl aminopeptidase (aminopeptidase B)
TPP2	tripeptidyl peptidase II
XPNPEP1	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble

Ribosomal proteins and ribosome biogenesis

BOP1	ribosome biogenesis protein BOP1
NOP56	nucleolar protein 56
NOP58	nucleolar protein 58
RPL10A	60S ribosomal protein L10a
RPS15A	40S ribosomal protein S15a
RPS5	40S ribosomal protein S5
RPS6KA3 ^I	ribosomal protein S6 kinase alpha-3

^I proteins that also bind purines

Table 3

Significant abnormalities of proteins involved in mitochondrial function

ABCB7 ¹	ATP-binding cassette, sub-family B (MDR/TAP), member 7
ABCD3 ¹	ATP-binding cassette, sub-family D (ALD), member 3
ABCE1	ATP-binding cassette, sub-family E (OABP), member 1
ABHD11 ²	abhydrolase domain containing 11
ACACA ¹	acetyl-coenzyme A carboxylase alpha
ACAD9 ¹	acyl-Coenzyme A dehydrogenase family, member 9
ACLY ¹	ATP citrate lyase
ACO2	aconitase 2, mitochondrial
ACSL1 ¹	acyl-CoA synthetase long-chain family member 1
ADSL	adenylosuccinate lyase
AGPS ¹	alkylglycerone phosphate synthase
ALDH3A2	aldehyde dehydrogenase 3 family, member A2
ARMC10	armadillo repeat containing 10
ASL	argininosuccinate lyase
ATAD1 ¹	ATPase family, AAA domain containing 1
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase
ATP5B ^{1,2}	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide
ATP5C1 ²	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1
ATP5D ²	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit
ATP5H ²	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d
ATP5I ²	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit E
ATP6V1E1 ²	ATPase, H+ transporting, lysosomal V1 subunit E1
ATPIF1 ²	ATPase inhibitory factor 1; similar to ATPase inhibitor, mitochondrial precursor
BAX	Bcl2-associated X protein
BCAT2	branched chain aminotransferase 2, mitochondrial
BOLA1 ²	bolA homolog 1 (E. coli)
C1QBP ²	complement component 1, q subcomponent binding protein
CCT7 ¹	chaperonin containing Tcp1, subunit 7 (eta)
CDS2	CDP-diacylglycerol synthase (phosphatidate cytidyltransferase) 2
CHCHD3 ²	coiled-coil-helix-coiled-coil-helix domain containing 3
CLIC4	chloride intracellular channel 4 (mitochondrial)
CLPB ¹	ClpB caseinolytic peptidase B homolog (E. coli)
CLTC	clathrin, heavy chain (Hc)
CLU ²	clusterin

COQ9 ²	coenzyme Q9 homolog (<i>S. cerevisiae</i>)
COX2	COXII
COX4I2	cytochrome c oxidase subunit IV isoform 2
COX5A	cytochrome c oxidase, subunit Va
COX5B ²	cytochrome c oxidase subunit Vb
CPT1A	carnitine palmitoyltransferase 1a, liver
CS	citrate synthase; similar to citrate synthase
CTPS2 ¹	CTP synthase II
CYB5A	cytochrome b5 type A (microsomal)
CYB5R1	cytochrome b5 reductase 1
CYB5R3 ¹	cytochrome b5 reductase 3
DBI ²	diazepam binding inhibitor (GABA receptor modulator)
DIABLO ²	diablo homolog (<i>Drosophila</i>)
DNM1L ¹	dynamamin 1-like
DRG2 ¹	developmentally regulated GTP binding protein 2
ETFDH	electron-transferring-flavoprotein dehydrogenase
FDPS	farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltranstransferase, geranyltranstransferase)
FKBP8	FK506 binding protein 8, 38kDa
FXN ²	frataxin
GARS ¹	glycyl-tRNA synthetase
GCSH ²	glycine cleavage system protein H (aminomethyl carrier)
GM2A ²	GM2 ganglioside activator
GOT2	glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)
GPD1	glycerol-3-phosphate dehydrogenase 1 (soluble)
GPD2	glycerol-3-phosphate dehydrogenase 2, mitochondrial
GRPEL1 ^{1, 2}	GrpE-like 1, mitochondrial
HADH ²	hydroxyacyl-Coenzyme A dehydrogenase
HADHA	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit
HAGH ²	hydroxyacyl glutathione hydrolase
HINT2 ²	histidine triad nucleotide binding protein 2
HK1 ¹	hexokinase 1
HK2 ¹	hexokinase 2
HSP90AB1 ¹	heat shock protein 90kDa alpha (cytosolic), class B member 1
HSPD1 ^{1, 2}	60 kDa heat shock protein 1 (chaperonin)
HSPE1 ^{1, 2}	heat shock protein 1 (chaperonin 10)
IARS2	isoleucyl-tRNA synthetase 2, mitochondrial

IDE ¹	insulin degrading enzyme
ISCU ²	iron-sulfur cluster scaffold homolog (E. coli)
KRT5	keratin 5
LDHA	lactate dehydrogenase A
LETM1	leucine zipper-EF-hand containing transmembrane protein 1
LOC606294	hypothetical protein LOC606294
LOC688869 ²	cytochrome c oxidase, subunit VIb polypeptide 1
LONP1 ¹	lon peptidase 1, mitochondrial
LRPPRC	leucine-rich PPR-motif containing
LRRC59	leucine rich repeat containing 59
MAOA ¹	monoamine oxidase A
ME1	malic enzyme 1, NADP(+)-dependent, cytosolic
MGEA5	meningioma expressed antigen 5 (hyaluronidase)
MGST1	microsomal glutathione S-transferase 1
MRPL1 ²	mitochondrial ribosomal protein L1
MRPL12 ²	mitochondrial ribosomal protein L12
MRPL23 ²	mitochondrial ribosomal protein L23
MRPL49 ²	mitochondrial ribosomal protein L49
MRPS23 ²	mitochondrial ribosomal protein S23
MRPS36 ²	mitochondrial ribosomal protein S36
MTCH1	mitochondrial carrier homolog 1 (C. elegans)
MTCH2	mitochondrial carrier homolog 2 (C. elegans)
MTHFD1 ¹	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1, methylenetetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase
MTHFD1L ¹	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like
NARS ¹	asparaginyl-tRNA synthetase
NDUFA2 ²	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2
NDUFAF4 ²	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 4
NDUFS1	NADH dehydrogenase (ubiquinone) Fe-S protein 1
NDUFS2	NADH dehydrogenase (ubiquinone) Fe-S protein 2
NDUFS7	NADH dehydrogenase (ubiquinone) Fe-S protein 7
NDUFS8 ²	NADH dehydrogenase (ubiquinone) Fe-S protein 8
NDUFV1	NADH dehydrogenase (ubiquinone) flavoprotein 1
NLN	neurolysin (metallopeptidase M3 family)
NME3 ¹	non-metastatic cells 3, protein expressed in
NNT	nicotinamide nucleotide transhydrogenase
NRD1	nardilysin 1 (N-arginine dibasic convertase)
OAT	ornithine aminotransferase (gyrate atrophy)

OGDH	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)
OPA1 ¹	optic atrophy 1 homolog (human)
PARK7 ²	Parkinson disease (autosomal recessive, early onset) 7; similar to DJ-1
PC ¹	pyruvate carboxylase
PCK2 ¹	phosphoenolpyruvate carboxykinase 2 (mitochondrial)
PDE12	phosphodiesterase 12
PEBP1 ^{1,2}	phosphatidylethanolamine binding protein 1
PHB ²	prohibitin
PHB2 ²	prohibitin 2
PICK1	protein interacting with PRKCA 1
POR ¹	P450 (cytochrome) oxidoreductase
PPP1CC	protein phosphatase 1, catalytic subunit, gamma isoform
PRDX6 ²	peroxiredoxin 6
PRKACA ¹	protein kinase, cAMP-dependent, catalytic, alpha
PRKCD ¹	protein kinase C, delta
PSAP ²	prosaposin
PTCD3	Pentatricopeptide repeat domain 3
PTPN11	protein tyrosine phosphatase, non-receptor type 11
QARS ¹	glutamyl-tRNA synthetase
QDPR ²	quinoid dihydropteridine reductase
RAB11B ¹	RAB11B, member RAS oncogene family
RAB35 ¹	RAB35, member RAS oncogene family
RAB3D ¹	RAB3D, member RAS oncogene family
RARS ¹	arginyl-tRNA synthetase
RGD1306917 ²	similar to RIKEN cDNA 2900010M23; hypothetical protein LOC687308
RGD1309676	similar to RIKEN cDNA 5730469M10
RGD1309922	similar to 2610301G19Rik protein
RHOA ¹	ras homolog gene family, member A; ras homolog gene family, member C
RPL10A	ribosomal protein L10A; similar to ribosomal protein L10a
RPS15A	ribosomal protein S15a
SARS ¹	seryl-tRNA synthetase
SDHA ¹	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)
SFXN1	sideroflexin 1
SHMT1	serine hydroxymethyltransferase 1 (soluble)
SLC16A1	solute carrier family 16, member 1 (monocarboxylic acid transporter 1)
SLC25A1	solute carrier family 25 (mitochondrial carrier, citrate transporter), member 1

SLC25A11	solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11
SLC25A20	solute carrier family 25 (carnitine/acylcarnitine translocase), member 20
SLC25A3	solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 3
SLC25A4	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4
SLC25A5	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5
SNCB ²	synuclein, beta
SND1	staphylococcal nuclease and tudor domain containing 1
SOD1 ²	superoxide dismutase 1, soluble
STOML2 ²	stomatin (Epb7.2)-like 2
STXBP1	syntaxin binding protein 1
SUCLA2 ¹	succinate-CoA ligase, ADP-forming, beta subunit
SURF4	surfeit 1; surfeit 4
SYNJ2BP	synaptojanin 2 binding protein
TBC1D15	TBC1 domain family, member 15
TFAM ²	transcription factor A, mitochondrial
TFRC	transferrin receptor
TH	tyrosine hydroxylase
TIMM9 ²	translocase of inner mitochondrial membrane 9 homolog (yeast)
TOMM70A	translocase of outer mitochondrial membrane 70 homolog A (<i>S. cerevisiae</i>)
TST	thiosulfate sulfurtransferase, mitochondrial
TXN2 ²	thioredoxin 2
UQCRCF1 ²	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
VARS ¹	valyl-tRNA synthetase 2, mitochondrial (putative); valyl-tRNA synthetase
VDAC1 ²	voltage-dependent anion channel 1
VDAC3	voltage-dependent anion channel 3

¹Proteins that also bind purines.

²Proteins that were significantly increased; all of the rest were decreased.

Table 4Significant decreases involved in proteins for neural transmission in HGprt⁻ PC6-3 cells

Amine biosynthesis	
ASL ^I	argininosuccinate lyase
ASNS	asparagine synthetase
GOT2	glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)
MRI1	methylthioribose-1-phosphate isomerase homolog (<i>S. cerevisiae</i>)
MTHFD1 ^I	methylenetetrahydrofolate dehydrogenase (NADP ⁺ dependent) 1, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase
PHGDH	3-phosphoglycerate dehydrogenase
PSAT1	phosphoserine aminotransferase 1
SHMT1	serine hydroxymethyltransferase 1 (soluble)
TH	tyrosine hydroxylase
Amine binding	
AARS ^I	alanyl-tRNA synthetase
FASN	fatty acid synthase
GCLC ^I	glutamate-cysteine ligase, catalytic subunit
GOT2	glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)
KARS ^I	lysyl-tRNA synthetase
MAOA ^I	monoamine oxidase A
NEDD4	neural precursor cell expressed, developmentally down-regulated gene 4
RARS ^I	arginyl-tRNA synthetase
SHMT1	serine hydroxymethyltransferase 1 (soluble)
SLC1A5	solute carrier family 1 (neutral amino acid transporter), member 5
TH	tyrosine hydroxylase
Neurotransmitter transport	
NSF ^I	N-ethylmaleimide-sensitive factor
RAB14 ^I	RAB14, member RAS oncogene family
RAB3C ^I	RAB3C, member RAS oncogene family
SLC25A4	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4
STX1A	syntaxin 1A (brain)
STX1B	syntaxin 1B
STX7	syntaxin 7
SV2A	synaptic vesicle glycoprotein 2a
SYT1	synaptobrevin 1
VAMP2	vesicle-associated membrane protein 2
Neurotransmitter regulation	
MAOA ^I	monoamine oxidase A
NSF ^I	N-ethylmaleimide-sensitive factor

RAB14 ^I	RAB14, member RAS oncogene family
RAB3C ^I	RAB3C, member RAS oncogene family
SLC25A4	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4
STX1A	syntaxin 1A (brain)
STX7	syntaxin 7
SYT1	synaptotagmin I
TH	tyrosine hydroxylase
VAMP2	vesicle-associated membrane protein 2
Neurotransmitter secretion/release	
NSF ^I	N-ethylmaleimide-sensitive factor
RAB14 ^I	RAB14, member RAS oncogene family
RAB3C ^I	RAB3C, member RAS oncogene family
STX1A	syntaxin 1A (brain)
STX7	syntaxin 7
SYT1	synaptotagmin I
VAMP2	vesicle-associated membrane protein 2
CADPS	calcium-dependent secretion activator 1; required for Ca ⁺⁺ -dependent exocytosis of synaptic vesicles
SCAMP3	secretory carrier-associated membrane protein 3

^IProteins that also bind purines.