PRPS-Associated Disorders and the Drosophila Model of Arts Syndrome

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Abstract: While a plethora of genetic techniques have been developed over the past century, modifying specific sequences of the fruit fly genome has been a difficult, if not impossible task. clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 truly redefined molecular genetics and provided new tools to model human diseases in Drosophila melanogaster. This is particularly true for genes whose protein sequences are highly conserved. Phosphoribosyl pyrophosphate synthetase (PRPS) is a rate-limiting enzyme in nucleotide metabolism whose missense mutations are found in several neurological disorders, including Arts syndrome. In addition, PRPS is deregulated in cancer, particularly those that become resistant to cancer therapy. Notably, Drosophila PRPS shares about 90% protein sequence identity with its human orthologs, making it an ideal gene to study via CRISPR/Cas9. In this review, we will summarize recent findings on PRPS mutations in human diseases including cancer and on the molecular mechanisms by which PRPS activity is regulated. We will also discuss potential applications of Drosophila CRISPR/Cas9 to model PRPS-dependent disorders and other metabolic diseases that are associated with nucleotide metabolism.

Keywords: Drosophila CRISPR; PRPS-associated disease; metabolic disorders; neurological disorders

1. Introduction

Inborn mutations causing metabolic disorders, particularly in purine and pyrimidine genes, often result in a variety of nervous system disorders [1–4]. However, the pathogenesis of their neurological anomalies is not well-understood, largely due to the extreme rarity of these diseases. While model organisms, like mice, could provide important insights into mechanisms of pathogenesis and treatment options, one has to consider the cost-benefit analysis of various approaches. For these reasons, Drosophila melanogaster is an excellent system to model rare diseases affecting the nervous system. These animals are relatively inexpensive and studies over the past decades demonstrated the validity of Drosophila as a model organism of neurological disorders [5–9]. Importantly, with the advent of clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 gene editing, patient-derived missense mutations can now be directly recapitulated in Drosophila. Obviously, the suitability of such an approach would depend on the degree of sequence and functional conservation between the human and Drosophila orthologs. One such metabolic gene is phosphoribosyl pyrophosphate synthetase (PRPS), the rate-limiting enzyme in nucleotide metabolism. The Drosophila PRPS ortholog is highly conserved to its human counterparts, showing about 90% protein sequence identity [10]. Among the three human orthologs, missense mutations of PRPS1 are found in a number of rare neurological disorders such as Arts syndrome. More recently, studies have also discovered that PRPS1 and PRPS2 are mutated in therapy-resistant relapsed ALL cancer patients. In this review, we will summarize the
role of PRPS mutations in human diseases, including cancer, and describe recent studies revealing the molecular mechanisms by which PRPS activity is regulated. We will then describe how Drosophila models helped to uncover unexpected physiological consequences of PRPS mutations and discuss the potential application of CRISPR/Cas9 genome editing in Drosophila.

2. Phosphoribosyl Pyrophosphate Synthetase (PRPS) Is a Rate-Limiting Enzyme in Nucleotide Metabolism

Nucleotides, composed of a nitrogenous base, a five-carbon sugar ribose, and a phosphate group, are essential for a myriad of fundamental molecular and cellular processes. For example, purine and pyrimidine nucleotides are the building blocks of DNA and RNA. Likewise, pyridine nucleotides are important co-factors for many important biochemical reactions. PRPS is the rate-limiting enzyme in nucleotide biosynthesis that is responsible for the transfer of the β-γ diphosphate of ATP to the C1 hydroxyl of ribose-5-phosphate (R5P), which is a compound produced by the pentose phosphate pathway [11]. This produces phosphoribosyl pyrophosphate (PRPP), a common precursor of the five-carbon sugar unit of all nucleotides. The following section briefly describes how PRPP is used during purine and pyrimidine nucleotide biogenesis.

De novo synthesis of purine nucleotides involves the assembly of the purine moiety directly onto PRPP in a 10-step reaction to form inosine monophosphate (IMP) [11]. IMP is then used to synthesize either adenosine monophosphate (AMP) or guanosine monophosphate (GMP). Purine nucleotides can also be synthesized through a less energy-demanding salvage pathway in which purine bases, adenine and guanine, are recycled to from AMP and GMP via phosphoribosyltransferases in a PRPP-dependent reaction. Similar to its role in purine nucleotide biosynthesis, PRPP is at the center of de novo pyrimidine nucleotide production. However, unlike purines, the pyrimidine ring is first synthesized with PRPP at a later step to produce uridine-5′-triphosphate (UTP) and orotidine monophosphate (OMP) de novo [12]. PRPP is also used in the pyrimidine salvage pathway in a similar manner as the purine salvage pathway. Notably, many metabolic enzymes involved in purine and pyrimidine metabolism are mutated in rare genetic disorders, and the inborn mutations affecting those enzymes can either activate or decrease the enzyme function [3,4]. In summary, PRPS is the rate-limiting enzyme in nucleotide metabolism as it produces PRPP, a critical molecule for nucleotide biogenesis.

3. PRPS Is Highly Conserved

Consistent with its essential role in nucleotide synthesis, PRPS is highly conserved across all domains of life (Figure S1 and [11]). There exists multiple PRPS orthologs among many eukaryotic organisms. Interestingly, while some yeast and plant species contain up to five PRPS orthologs, humans and rats have only three PRPS genes [11]. Both human PRPS1 and PRPS2, located on the X chromosome, are expressed in all tissues, while PRPS3 (PRPS1L1), located on chromosome 7, is restricted to the testes [13]. The structure of human PRPS1 has been solved and shown to be similar to PRPS from Bacillus subtilis (B. subtilis) despite limited sequence identity [14]. PRPS from both species form hexamers that are comprised of three homodimers arranged in a propeller-shaped tertiary structure. Interestingly, in addition to forming hexameric structures, a recent study has demonstrated that PRPS can form cytophilic filaments [15,16]. These are evolutionarily conserved intracellular structures, sometimes referred to as cytaphidia, which are thought to increase enzyme kinetics in response to various stressors (e.g., RNA stress granules, nutrient deprivation). Such structures are observed with other metabolic enzymes such as CTP synthase [16,17]. Strikingly, PRPS cytophilic filament formation is also conserved in a wide range of species including bacteria, zebrafish, rats, and humans, illustrating the functional importance of PRPS in all living species [16]. In humans, there are also PRPS-associated proteins (PAPs), PAP39 and PAP41, which share some sequence homology with PRPS (Figure S1 and [11]). PAPs were shown to physically interact with PRPS. However, PAPs lack a catalytic ATP binding site and do not directly participate in the phosphoribosyltransferase reaction but rather play a regulatory function [11,18].
Drosophila markedly has only one PRPS ortholog (dPRPS), coded by CG6767, which was recently characterized [10] and shares about 90% protein sequence identity with human PRPS1 [10]. Based on the high sequence similarity, the structure of dPRPS must resemble that of the human counterpart. Indeed, dPRPS cytophilic filament formation has been also observed in the Drosophila ovary [16]. In addition, Drosophila contains a single PAP ortholog CG2246, which is yet to be characterized. Although not as highly conserved as PRPS, CG2246 encodes a protein that is 57% and 63% identical to PAP39 and PAP41, respectively. The sequence similarity and the presence of the PAP ortholog make Drosophila an attractive genetic system to study the in vivo function of PRPS. Moreover, the fact that dPRPS and CG2246 are the only orthologs in Drosophila eliminates any issues of functional redundancy between family members, making genetic studies uncomplicated and straightforward.

4. PRPS Mutations in Neurological Disorder

Inborn mutations of PRPS1 are associated with a number of neurological disorders with a broad range of pathological symptoms [1,2]. This is not surprising given the ubiquitous nature of purine, pyrimidine, and pyridine nucleotides in basic molecular processes as fundamental as synthesizing DNA and RNA. At the same time, it is truly difficult to determine which metabolic pathway downstream of PRPS is responsible for the pathology of PRPS1-associated neurological disorders. In fact, inborn mutations in many other purine and pyrimidine genes downstream of PRPS1 also result in a variety of nervous system disorders [3,4]. Interestingly, PRPS1 mutations associated with neurological disorders can be either a loss-of-function or gain-of-function mutation. Regardless of their effect on enzymatic activity, a common symptom associated with most PRPS1 mutations is hearing impairment (either nonsyndromic or syndromic hearing loss). For some reason, sensory neurons in the auditory system appear highly susceptible to defects in nucleotide metabolism. The 29 PRPS1 mutations identified in neurological disorders are summarized in Table 1. Notably, approximately three-quarters are associated with loss-of-function mutations in X-linked neurological disorders. Gain-of-function mutations of this enzyme are associated with PRPS superactivity, with eight missense mutations reported thus far. Interestingly, a recent study identified a mutation, V142L, that may have both positive and negative effects on enzyme function, as it may affect the allosteric site as well as the ATP-binding site [19]. Whether the mutations in patients increase or decrease PRPS1 function, there is a paucity of treatment options and they only aim to slow disease progression and are not curative [20].

Interestingly, every PRPS1 mutation, both hypoactivating and hyperactivating PRPS1 mutations, are all missense mutations and, thus far, no nonsense mutation has been identified in the patients. This likely reflects the essential nature of PRPS1 in nucleotide metabolism for embryonic development and suggests that neither PRPS2 nor PRPS3 can compensate for PRPS1 function. This also indicates that the mutant form of PRPS1 in patients must retain some activity that allows affected individuals to complete embryonic and fetal development. Supporting this notion, structural analysis of mutant PRPS1 has revealed that, while most nonsense mutations affect either substrate binding, dimer interface, or secondary structure, none of them disrupt the overall structure of PRPS1 [21]. The spectrum of X-linked neurological disorders that are associated with decreased PRPS1 function includes Arts syndrome, Charcot-Marie-Tooth disease 5 (CMTX5), nonsyndromic sensorineural deafness 2 (DFN2), and more recently retinal dystrophy [22–24]. Notably, the severity of symptoms associated with these disorders likely reflects the extent to which the enzyme function is disrupted. Arts syndrome is characterized by the most severe disease presentation, such as mental retardation, early-onset hypotonia, ataxia, profound congenital sensorineural hearing impairment, and optic atrophy, as well as early-childhood death [23]. There have been excellent reviews delineating these disease phenotypes and will not be further discussed in this review [20,25]. PRPS1 overactivity is caused by missense mutations in the allosteric site that lead to reduced inhibition by its allosteric inhibitor, ADP, and increased stimulation by phosphate [25]. The physiological consequences of PRPS1 overactivity include neurosensory defects, hyperuricemia, and gout, caused by an accumulation of uric
acid, which is the end-product of purine catabolism. Gain-of-function mutations were first discovered in patients with gout presenting with high levels of uric acid [26].

Because PRPS1 is an X-linked gene, PRPS1-associated neurological disorders, caused by either loss-of-function or gain-of-function mutations, primarily affect males. Although less susceptible and frequent, females can also present with symptoms of PRPS1-associated disorders even if they are heterozygous for the mutation. A recent study found a female patient bearing a novel PRPS1 hyperactivating mutation [27]. Although both the patient and her mother carried a single copy of the mutant allele, the patient presented with symptoms associated with PRPS1 superactivity while her mother was largely unaffected. This finding demonstrated that heterozygous females can be affected by PRPS1 mutation if a skewed X-inactivation occurs during development. A similar observation was also made with PRPS1 loss-of-function mutations. Novel PRPS1 missense mutations were identified to cause retinal dystrophy in female patients, who were all heterozygous for the mutant alleles [28]. These findings also suggest that PRPS1-associated genetic disorders may be more prevalent than previously thought. Lastly, loss-of-function mutations in PRPS1 have been identified in cell lines of Hutchinson–Gilford progeria syndrome (HGPS), a rare disease characterized by accelerated aging [29]. This raises the possibility that PRPS1-associated disorders may not be limited to neurological disorders. Overall, PRPS1 mutations identified from human patients support the notion that PRPS1 is a critical enzyme required for nucleotide metabolism.

5. PRPS in Cancer

Recent studies have identified the role of PRPS in cancer, particularly in the context of gaining drug resistance to thiopurine and thioguanine cancer therapy in childhood acute lymphoblastic leukemia (ALL) (Table 2 and [30,31]). The PRPS1 mutation in ALL was first identified by whole exome-sequencing of samples from patients with pediatric relapsed ALL treated with thiopurine. Thiopurine is a key component of ALL chemotherapy, a prodrug that is converted by the purine salvage pathway to become a cytotoxic compound. It is proposed that cancer cells gain resistance to thiopurine by acquiring hyperactivating PRPS1 mutations that bypass ADP- or GDP-dependent allosteric inhibition. These mutations make cancer cells favor de novo nucleotide synthesis and become less sensitive to the drugs whose efficacy depends on the purine salvage pathway [30]. Interestingly, a study also showed that cell lines with a relapse-specific PRPS1 mutation become hypersensitive to 5-fluorouracil (5-FU), an antimetabolite drug that targets de novo pyrimidine biosynthesis [32]. This study supports the notion that the growth and survival of thiopurine-resistant cancer cells largely depend on de novo nucleotide biosynthesis. More recently, a large-scale whole-genome sequencing was performed to identify therapy-resistant specific mutations in relapsed ALL patients [31]. This study identified hyperactivating mutations in not only PRPS1, but also in PRPS2, demonstrating the crucial role of PRPS in nucleotide metabolism. The role of PRPS in ALL is further demonstrated by the observation that PRPS1 overexpression increases proliferation and inhibits apoptosis in B-ALL cell lines [33]. Of note, many of the hyperactivating PRPS1 mutations found in relapsed ALL occur in the same amino acid residues mutated in PRPS1 superactivity listed in Table 1.

In addition to its role in chemotherapy resistance in ALL, increased PRPS function may generally promote tumorigenesis. Indeed, gain-of-function mutations have also been identified in colorectal cancer and breast cancer [34]. Moreover, analysis using publicly available resources also supports this notion. The Catalogue Of Somatic Mutations In Cancer (http://cancer.sanger.ac.uk/cosmic) shows that PRPS1 is overexpressed in a number of cancers, such as lung squamous cell carcinoma, lung adenocarcinoma and colorectal adenocarcinoma. Furthermore, cBioPortal for Cancer Genomics (https://www.cbioportal.org/) indicates that all three PRPS orthologs are amplified in bladder/urinary tract and prostate cancers. Overall, recent findings clearly demonstrated the crucial role of PRPS in the proliferation and survival of cancer cells.
6. Post-Translational Modification of PRPS

In the past few years, several studies have identified post-translational modifications that regulate PRPS activity. Ketohexokinase (KHK) is a key enzyme that regulates fructose metabolism. KHK phosphorylates fructose into fructose-1-phosphate, which is then metabolized to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, two intermediate metabolites of glycolysis. Interestingly, there are two alternatively spliced forms of KHK, KHK-A, and KHK-C. While KHK-C efficiently phosphorylates fructose, KHK-A cannot. Surprisingly, KHK-A is a protein kinase that phosphorylates PRPS1 at Thr225, which increases PRPS1 function [35]. In hepatocellular carcinoma, it has been demonstrated that c-Myc-promotes expression of KHK-A over KHK-C and coordinately decreases fructose catabolism and increases de novo nucleotide synthesis. Importantly, KHK-dependent PRPS1 phosphorylation is shown to be a predictor of poor prognosis in hepatocellular cancers. Another kinase that couples oncogenic changes to PRPS1 function is cyclin-dependent kinase 1 (CDK1) [36]. CDK1-dependent phosphorylation at Ser103 activates PRPS1 and couples the cell cycle to nucleotide metabolism. Ser103 phosphorylation of PRPS1 is suggested to be required for maintaining increased PRPS1 activity in colorectal cancer cells. Notably, Ser103 is one of the amino acids that is frequently mutated in relapsed ALL patients, although they are not phosphomimetic mutations (Table 2). A recent study also found a phosphorylation event that inactivates PRPS. Upon glucose starvation, AMP-activated kinase (AMPK) phosphorylates Ser180 of PRPS1 and Ser183 of PRPS2 [37]. Unlike phosphorylation by KHK-A and CDK1, AMPK-dependent phosphorylation was shown to inhibit PRPS1/2 by preventing hexamer formation. This limits nucleotide synthesis under stressed conditions like nutrient deprivation. Apart from phosphorylation, PRPS2 has been shown to be arginylated by arginyltransferase ATE1 [38]. While ATE1 selectively arginylates and stabilizes PRPS2 and not PRPS1, the biological context in which the arginylation regulates nucleotide metabolism is yet to be determined. Overall, these findings highlight post-translational modifications of PRPS which couple nucleotide metabolism to various cellular signals and processes.

7. Model Systems of PRPS-Associated Disorder

7.1. Mouse Models of PRPS-Associated Disorders

Although knockout mice of PRPS1 and PRPS2 have been generated, neither were designed to interrogate PRPS-associated neurological disorders. PRPS1-knockout mice were generated as a part of a high-throughput screening searching for mouse genes important for skeletal phenotypes [39]. Like human PRPS1, murine PRPS1 is on the X chromosome and the study identified it as an X-linked gene that is required for animal viability and no further analysis was performed. PRPS2-knockout mice were generated in the context of a Myc-driven cancer model. In contrast to PRPS1-knockout mice, PRPS2-knockout mice are viable and fertile with no discernable developmental defects [40]. It is probable that PRPS1 mainly contributes to overall PRPS activity during development and that PRPS1 can compensate for the loss of PRPS2. Interestingly, while PRPS2 is nonessential for development, it was required for Myc-dependent cancer formation. Not only is PRPS2 expression regulated by Myc, but PRPS2-knockout mice are also resistant to Eµ–Myc-driven cancer development. This study suggests that at least in mice, PRPS2 is specifically targeted by Myc to promote tumorigenesis. Notably, neither of the PRPS-knockout mice were analyzed for any evidence of neurological symptoms. Next, we will summarize zebrafish and Drosophila models that were designed to investigate PRPS-associated neurological diseases.

7.2. Zebrafish Models of PRPS-Associated Disorders

There are two PRPS paralogs in zebrafish, prps1a and prps1b. A prps1a mutant was generated by retroviral vector insertion and prps1b was targeted by a zinc finger nuclease, creating a frameshift mutation in exon 2 [41]. Genetic studies using these mutants revealed that prps1a plays a predominant role during development. While prps1a showed defects in the eye and the neuromast hair cells,
the prps1b mutant displayed no discernable phenotype. However, prps1a;prps1b double mutants presented with more severe defects in the eye and the neuromast hair, indicating that prps1b does contribute to overall PRPS function during development. Importantly, prps1a;prps1b double mutants showed many features that are associated with PRPS1-associated neurological disorders such as defects in inner ear hair cells and motor neurons. A later study using morpholino oligonucleotides targeting prps1a and prps1b further validated zebrafish as a disease model, as they showed similar defects including significant sensorineural hearing loss [42]. One of the striking findings in the zebrafish studies is that many defects observed in prps1a and prps1a;prps1b mutants ameliorate with time. The authors proposed that this could be due to the fundamental difference in tissue development and homeostasis between zebrafish and humans. Interestingly, the study also revealed that while PRPP levels were severely reduced in prps1a;prps1b mutants, the levels of purine nucleotides, IMP, GTP, and AMP, were only reduced by about 25%. In fact, the levels of s-adenosylmethionine (SAM), a purine metabolite, were increased in prps1a;prps1b mutants. Perhaps, there is a metabolic reprogramming event in PRPS-deficient animals, which may contribute to their survival. Nevertheless, these studies have demonstrated that zebrafish are an excellent model of PRPS1-associated disorders, recapitulating many symptoms presented by the patients.

7.3. Drosophila Models of PRPS-Associated Disorders

Ever since it was first adapted for the use of genome engineering in Drosophila [43], the CRISPR/Cas9 system has been routinely used to generate precise and efficient edits to the fly genome. Moreover, the development of additional CRISPR-based tools to probe and interrogate the fly genome has been rapidly expanding in the Drosophila field. For example, with the combination of already existing tool kits such as the GAL4-UAS binary system, Drosophila geneticists can now carry out CRISPR/Cas9-mediated gene disruption in a tissue-specific manner. Moreover, libraries containing guide RNA (gRNA)-expressing plasmids and fly lines are being generated, which will allow genetic screening via the CRISPR/Cas9 system [44,45]. Notably, variant forms of Cas9 nucleases are also used in Drosophila that allow overexpression of a gene of interest from its endogenous promoter [46]. These techniques will further improve Drosophila as a model organism to probe and interrogate gene function.

In our group, we used CRISPR/Cas9 techniques to engineer two Drosophila alleles carrying equivalent mutations identified from Arts syndrome [10]. While null alleles were generated in other model organisms, the physiological consequence of disease-causing PRPS1 mutations has not been directly tested. We reasoned this was important since having no PRPS1 versus severely reduced PRPS1 may have different physiological consequences. Two mutant alleles carrying PRPS1 mutations identified from Arts syndrome, Q133P and R196W, were engineered in flies (Table 1). Initially, we were surprised by the fact that the two dPRPS alleles are viable and fertile with no discernable development defects, given its essential role in nucleotide metabolism. However, this also supported the notion that PRPS1 missense mutations from patients do not completely eliminate enzyme function. In fact, publicly available insertion mutant alleles of dPRPS, which are transcript null, are early larval lethal. One of the advantages of the Drosophila system is the ability to systematically examine a vast range of phenotypes ranging from cellular to behavioral defects owing to more than 100 years of research history. Despite the lack of morphological defects, we were able to quickly learn that dPRPS alleles carrying patient-derived mutations have clear signs of neurodegeneration. The adult flies had much shorter lifespans than controls and displayed locomotor defects, which are measured by a climbing assay [47]. In addition, using various molecular genetic tools, we were able to determine that mutant flies have profound defects in lipid mobilization, macroautophagy, and lysosome function, all of which are known to be associated with neurological disorders [10]. In fact, we were able to demonstrate that adult brains of dPRPS mutants accumulate ubiquitinated proteins suggesting that macroautophagy/lysosomal defects affect the nervous system. These cellular defects led to the discovery that, in addition to aging and locomotor defects, dPRPS-mutant flies are also hypersensitive to starvation and oxidative stress. Importantly, it has not yet been investigated whether patients with PRPS1 mutations have
autophagy/lysosomal defects nor are hypersensitive to starvation and oxidative stress. However, these Drosophila models will likely prove to be valuable in understanding the mechanism underlying PRPS1-related disorders. A clinical trial showed that therapeutic SAM treatment delays the onset of neurodegeneration in Arts syndrome patients [2]. Validating dPRPS mutant flies as a model of Arts syndrome, dietary supplementation of SAM can partially improve lysosome dysfunction and sensitivity to starvation in dPRPS-mutant flies [10]. Collectively, by engineering Drosophila carrying patient-derived mutations, we uncovered that patient-derived PRPS mutations are likely hypomorphic, maintaining a certain level of enzyme activity, and that cellular defects such as macroautophagy/lysosomal defects likely contribute to PRPS-associated neuropathology. Furthermore, these Drosophila models of Arts syndrome could potentially be used to test the efficacy of future treatments.

8. Investigating Inborn Error in Metabolism Using Drosophila CRISPR/Cas9

While different methods exist to model disease-associated mutations in Drosophila, several factors made PRPS1 mutations ideal candidates to model disease in Drosophila via CRISPR/Cas9. First, the dPRPS protein sequence is highly conserved from human PRPS1. This high conservation combined with the available structural data made certain that the patient-derived missense mutations would have the same molecular effects on the Drosophila PRPS. Second, every single PRPS1 inborn mutation identified from patients was a missense mutation and that the in vivo consequences of these mutations have not directly been studied. Third, there is only one PRPS ortholog in Drosophila, which shows high sequence and protein homology to human PRPS1. These three factors made the analysis and interpretation of the data in patient-derived Drosophila mutants straightforward.

A plethora of missense mutations associated with an inborn error in purine and pyrimidine metabolisms has been identified. Similar to PRPS1 mutations, different mutations in a single gene may result in a wide range of disease phenotypes [3,4]. With relatively low-cost and short generation time, Drosophila can be used to potentially model every disease-causing mutation. However, the practicality of this approach is dependent on the conservation between humans and Drosophila. For instance, our sequence analysis of the human and Drosophila orthologs of few genes in purine metabolism reveals limitations to this approach. Table 3 lists selected genes in purine metabolism whose deficiencies are associated with rare metabolic disorders [3]. ADSL (adenosylcysteinate lyase) and ATIC (5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase) are critical enzymes in de novo purine biosynthetic pathways and their deficiencies have been associated with a number of neurological features [3]. Similar to PRPS1, both ADLS and ATIC have clear Drosophila orthologs whose protein sequence shows a high degree of similarity, 78.7% and 83.3% respectively, making them ideal candidates. Interestingly, both enzymes are components of the purinosome, a multiprotein complex consisting of enzymes that carry out de novo purine biosynthesis [48]. Perhaps, the need to form a complex quaternary structure, similar to hexamer formation of PRPS, puts evolutionary pressure to conserve protein sequences among these enzymes. PNP (purine nucleoside phosphorylase), ADA (adenosine deaminase), APRT (adenine phosphoribosyltransferase) and HPRT1 (hypoxanthine phosphoribosyltransferase 1) are involved in purine catabolism and salvage pathway and their Drosophila orthologs have varying degrees of sequence similarity. PNP and APRT do have clear Drosophila orthologs but show relatively low sequence similarities, 55.6% and 62.7%, respectively. For such genes, the nature and location of the mutations from patients would determine whether such mutations are suitable to model in Drosophila via CRISPR/Cas9. If missense mutations are located in a well-conserved region and structural information of the enzymes are available, Drosophila mutants carrying patient-derived mutations would be informative. Surprisingly, HPRT1 (hypoxanthine phosphoribosyltransferase 1) and ADA (adenosine deaminase), whose deficiency is associated with Lesch–Nyhan disease and SCID (Severe combined immunodeficiency) respectively, have no clear sequence orthologs in Drosophila. This is striking given the crucial function that HPRT1 and ADA play in purine salvage and catabolic pathway. Perhaps other Drosophila proteins with similar enzymatic activities carry out their functions. It is also possible that there is a fundamental difference between
human and *Drosophila* purine metabolism that is yet to be discovered. Nevertheless, it would be difficult to use *Drosophila* to investigate inborn errors of such genes. In summary, the extent of sequence similarity, structural information, and mechanistic data will determine whether *Drosophila* is a suitable system to model a disease-associated mutation.

9. Concluding Remarks

With sophisticated genetic tools such as the Gal4/UAS binary system and the FRT/FLP recombination system, *Drosophila* has thrived for many years as a simple yet elegant genetic system [49]. However, an inability to modify the genome in a sequence-specific manner had been the Achilles heel for *Drosophila* geneticists and other model systems such as yeast and mice had advantages over the fruit fly in this respect. The introduction of CRISPR/Cas9 in the past few years has leveled the playing field and changed the way *Drosophila* scientists plan and design their experiments. For example, in the future, we can engineer dPRPS alleles carrying patient-derived gain-of-function mutations or directly determine the physiological significance of the PRPS phosphorylation sites described above. With improved sequencing technology, an increasing number of disease-associated mutations are being identified. In addition, novel CRISPR/Cas9 technology is constantly being developed, thus enhancing our ability to edit the genome. For example, a modified Cas9 endonuclease fused with a reverse transcriptase has recently been engineered [50]. This variant form of Cas9 together with a modified guide RNA allows precise genome editing without introducing double-stranded breaks on DNA nor requiring any donor template DNA. Overall, CRISPR-based techniques together with already existing elegant genetic tools make *Drosophila* an attractive organism to study and interrogate the effect and mechanisms underlying patient-derived mutations.

### Table 1. Missense mutations identified in phosphoribosyl pyrophosphate synthetase 1 (*PRPS1*) associated with *PRPS1*-associated neurological disorders.

| Disorder                                      | Effect on *PRPS1* Function | Mutation | Amino Acid Change |
|-----------------------------------------------|----------------------------|----------|------------------|
| PRPS-1 Superactivity                          | Gain of Function           | 154G > C [51] | D52H                  |
|                                               |                            | 341A > G [52] | N114S            |
|                                               |                            | 385C > A [51] | L129I           |
|                                               |                            | 521G > T [27] | G174V           |
|                                               |                            | 547G > C [52] | D183H           |
|                                               |                            | 569C > T [53] | A190V           |
|                                               |                            | 578A > T [54] | H192L           |
|                                               |                            | 579C > G [51] | H193Q           |
| Nonsyndromic X-linked sensorineural deafness (DFN2) | Loss of Function           | 193G > A [55] | D65N           |
|                                               |                            | 259G > A [55] | A87T           |
|                                               |                            | 869T > C [55] | I290T           |
|                                               |                            | 916C > A [55] | G306R           |
| Charcot-Marie-Tooth neuropathy type 5 (CMTX5) | Loss of Function           | 129A > C [56] | E43D           |
|                                               |                            | 334G > C [24] | V112L           |
|                                               |                            | 344T > C [56] | M115T           |
| Arts syndrome                                 | Loss of Function           | 398A > C [23] | Q133P           |
|                                               |                            | 455T > C [23] | L152P           |
|                                               |                            | 856C > T [22] | R196W           |
| PRPS-1 Superactivity and Arts syndrome         | Loss of Function           | 424G > C [19] | V142L           |
Table 1. Cont.

| Disorder                      | Effect on PRPS1 Function | Mutation | Amino Acid Change |
|-------------------------------|--------------------------|----------|------------------|
| CMTX5 and Arts syndrome       | Loss of Function         | 830A > C [57] | Q277P            |
| DFN2 and CMTX5                | Loss of Function         | 337G > T [58] | All3S            |
| DFN2 and CMTX6                | Loss of Function         | 343A > G [58] | M115V            |
| DFN2 and CMTX7                | Loss of Function         | 925G > T [58] | V309F            |
| DFN2 and CMTX8                | Loss of Function         | 62C > G [59] | A121G            |

| Retinal Dystrophy             | Loss of Function         | 46T > C [60] | S16P             |
|                               |                          | 47C > T [28] | S16F             |
|                               |                          | 586C > T [28] | R196W            |
|                               |                          | 640C > T [28] | R214W            |
|                               |                          | 641G > C [28] | R214P            |

Amino acid number adjusted based on UNIPROT data.

Table 2. Missense mutations of PRPS identified in human cancer.

| Cancer Type                  | Gene  | Amino Acid Change |
|------------------------------|-------|-------------------|
| Relapse-specific ALL         | PRPS1 |                   |
|                              |       | V53A [30]         |
|                              |       | I72V [30]         |
|                              |       | C77S [30]         |
|                              |       | S103I [30]        |
|                              |       | S103N [30]        |
|                              |       | S103T [30]        |
|                              |       | S103R [31]        |
|                              |       | N114D [30]        |
|                              |       | D139G [30]        |
|                              |       | N144S [30]        |
|                              |       | G174E [30]        |
|                              |       | K176N [30]        |
|                              |       | R177S [31]        |
|                              |       | D183E [30]        |
|                              |       | A190V [30]        |
|                              |       | A190T [30]        |
|                              |       | L191F [30]        |
|                              |       | T303S [30]        |
|                              |       | Y311C [30]        |
|                              |       | V316L [31]        |
|                              | PRPS2 | V48M [31]         |
|                              |       | S120S [31]        |
|                              |       | A134T [31]        |
|                              |       | P173Y [31]        |
|                              |       | A177T [31]        |
| Breast Cancer                | PRPS1 | D203H [34]        |
| Colorectal Cancer            | PRPS1 | H231D [34]        |
Table 3. Genes causing inborn errors in purine metabolism.

| Gene   | ensID          | Gene Name                                           | Drosophila Homologues | FB ID               | Homology between Human and Fruit Fly Orthologs |
|--------|----------------|-----------------------------------------------------|-----------------------|---------------------|-----------------------------------------------|
| ADSL   | ENSG00000299900 | adenylosuccinate lyase (de Novo)                     | AdSL                  | FBgn0038467         | Identity: 65.6% 
Similarity: 78.7%                           |
| ATIC   | ENSG0000138363  | 5-aminimidazole-4-carboxamide ribonucleotide formyltransferase (IMP cyclohydrolase (de Novo)) | CG11089               | FBgn0039241         | Identity: 70.6% 
Similarity: 83.3%                           |
| PNP    | ENSG0000198805  | purine nucleoside phosphorylase (Catabolism)        | CG16758               | FBgn0035348         | Identity: 44.6% 
Similarity: 59.8%                           |
| ADA    | ENSG0000196639  | adenosine deaminase (Catabolism)                    | ADA                   | FBgn0037661         | Identity: 23.7% 
Similarity: 38.8%                           |
| APRT   | ENSG0000198931  | adenine phosphoribosyltransferase (Salvage)         | APRT                  | FBgn0000109         | Identity: 44.3% 
Similarity: 62.7%                           |
| HPRT1  | ENSG00000165704 | hypoxanthine phosphoribosyltransferase 1 (Salvage)  | Unknown               | -                   |                                               |

Selected genes associated with inborn error in purine metabolism and their Drosophila orthologs are shown. Protein sequence homology is determined by an alignment tool from EMBL-EBI (www.ebi.ac.uk/Tools/psa/).

Supplementary Materials: The following are available online at http://www.mdpi.com/1422-0067/21/14/4824/s1, Figure S1: Phylogenetic tree of PRPS and PRPS-binding proteins (PAP) across species. Phylogenetic relationships of PRPS homologs across species were estimated using PhylomeDB (http://phylomedb.org/). Red indicates duplication events. Blue indicates speciation events. Green dot marks the target sequence, human PRPS1. Q9VT33 is the only Drosophila PRPS ortholog. PRPS-associated protein 1 and 2 (PRPPSAP1 and PRPPSAP2) are orthologues of human PAP39 and PAP41, respectively. Conserved domains of PRPS-family are shown in the right panel.

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