Rescuing the Fertility of adsl-1 Mutant C. elegans

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Abstract

Inborn errors of purine metabolism are a group of rare disorders that effect the way the body is able to synthesize purines and break them down into uric acid, one such disorder is adenylosuccinate lyase deficiency (ASLD), a metabolic disorder affecting humans. We use *C. elegans* as a model to study the mechanisms of the infertility seen in ADSL mutants. In this study I aim to investigate the reason for defects in fertility. We are going to do this by constructing a plasmid aimed at restoring *adsl-1* expression to its native location by using the lin-61/adsl-1 promoter in order to create transgenic animals whose progeny display the desired extrachromosomal array. The results show that the lin-61/adsl-1 promoter was able to rescue fertility 17.3% indicating that the low efficacy could be due to incomplete rescue. This means that further action can be taken to determine if the incomplete rescue is due to the animal not having a gonad or not having viable ovum. This experiment lays down the groundwork for future experiments like this using different promoters.

Introduction

Inborn errors of purine metabolism are a group of rare disorders that affect the way the body synthesizes purines and breaks them down into uric acid (1). These disorders present in a broad way that involves deterioration in neuromuscular, reproductive, and cognitive function. Disorders within the category of inborn errors of purine metabolism are the result of mutation leading to enzyme disfunction (2). The enzymes responsible are necessary for synthesis of nucleotides in RNA and DNA. Lower levels of these nucleotides or an increase in intermediates ultimately leads to the development of these disorders (3). The disorders often have immense negative effects on health, with there being few treatments available.

One such disorder is adenylosuccinate lyase deficiency (ASLD), a metabolic disorder affecting humans. In general, inborn errors of purine metabolism often go understudied and for ASLD this remains true with very few cases studied. This is partially due to the lack of awareness of the disorder. There are potentially more cases than reported because of the disorder's ability to disguise itself as other illnesses since its symptoms can mimic other disorders, such as autism spectrum disorder. The symptoms of ASLD include epilepsy, muscle ataxia, delays in development, and other autistic-like symptoms. Although the symptoms of ASLD are well documented, the biological mechanisms responsible for the phenotypes are still being discovered (2). Dysfunction in the adenylosuccinate lyase (ADSL) gene is to blame for

ASLD. ADSL is a gene that encodes for an enzyme of the same name. The enzyme ADSL plays a crucial role in as the catalyst for two steps in the *de novo* purine biosynthetic pathway, shown below in Figure 1.

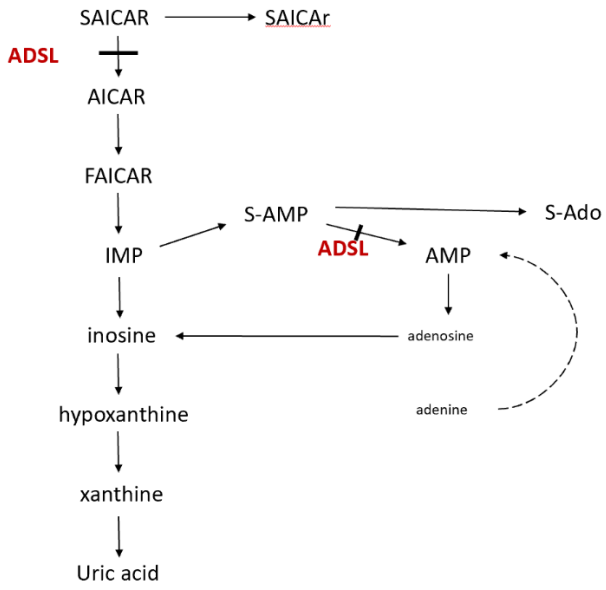

Figure 1: ADSL within the Purine Metabolism. ADSL is first used int eh conversion of SAICAR to AICAR. SAICAr would accumulate without the function of ADSL. The conversion of S-AMP to AMP also requires ADSL. Without this conversion, S-Ado would accumulate.

Abbreviations: R5P, ribose-5-phospate; SAICAR, succinylaminoimidazole carboxamide ribotide; SAICAr, succinylaminoimidazole carboxamide riboside; ADSL, adenylosuccinate lyase; AICAR, aminoimidazole carboxamide ribotide; IMP, inosine monophosphate; S-AMP, adenylosuccinate; S-Ado, succinyladenosine AMP, adenosine monophosphate; XMP, xanthine monophosphate; GMP, guanosine monophosphate.

When the model organism *C. elegans* has ADSL knocked out it displays delays in cognitive development learning, neuromuscular dysfunction, as well as infertility. Here we will investigate the phenotype of fertility within our *C. elegans*. Our goal is to rescue fertility and our hypothesis is that if we restore ADSL to its native location in an ADSL mutant using the adsl-1 promoter, then there will be a rescue of fertility within these ADSL mutants. We used a constructed plasmid that includes the adsl-1 promoter , the ADSL gene, and GFP as a reporter, and injected it into the animals with the goal being to create a transgenic animal that will produce progeny with the extrachromosomal array that we will study.

Methods

C. elegans strains and maintenance

Strains were maintained under standard conditions at 20 °C (4). The strains used were tm3328/h2t (control), HV855, HV856, and HV873 (Table 1). To create the extrachromosomal arrays, hermaphrodites were injected with plasmid DNA at a concentration of 50 ng/µl (Table 1). Each week ten animals from each of the four strains were transferred to four new plates, separated by strain.

Table 1: The strains used in this study

Strain	Genotype	Extrachromosomal Array
HV855	adsl-1/h2T l; psEx305	*psEx305*[50 ng/µl pTG96 (sur-5p::GFP) + 50 ng/µl pBluescript]
HV856	adsl-1/h2T l; psEx306	*psEx306*[50 ng/µl pTG96 (sur-5p::GFP) + 50 ng/µl pBluescript]
HV873	adsl-1/h2T l; psEx324	*psEx324*[5 ng/µl pAA6 (lin-61p::*adsl-1*::GFP) +50 ng/µl pTG96 (sur-5p::GFP) + 45 ng/µl pBluescript*]
*Bluescript is an, empty vector		

Transgene construction

lin-61 (the adsl-1 promoter) and sur-5 promoters were then amplified using primers (HindIII lim-7p F and BamHI lim-7p R) and the N2 genomic DNA. Afterwards, the product of the amplification was purified by running 10 µl on gel to make sure the correct fragment was amplified, and a PCR cleanup kit was used to purify. The plasmid and the amplicon were digested separately using the same set of restriction enzymes (BamHI and HindIII). The reactions were run on electrophoresis gel and the bands that had the correct band sizes were isolated, purified via NEB® PCR cleanup kit, and had their concentrations measured using nanodrop. A ligation reaction was set up that had a 3:1 ratio of the backbone plasmid to insert molecular weight ratio (. 3x insert DNA mass:1x linearized vector plasmid). QIAprep Spin Miniprep Kit Cat No./ID: 27104. The newly constructed plasmid was transformed to E. coli bacteria (NEB® 5-alpha Competent E. coli (Subcloning Efficiency) Catalog C2988J) on Lysogeny broth (LB) with the corresponding antibiotic. A countable amount of colonies were present. Colony PCR was used to determine the presence of the plasmid in a couple of the colonies. The positive colonies were miniprepped using QIAprep Spin Miniprep Kit Cat No./ID: 27104. The plasmid was then sequenced for confirmation. Once confirmed, the animals were injected in the gonads with the mix. One of two promoters were used to create the original constructs, one was the lin-61/adsl-1 and the other was sur-5. The lin-61/adsl-1 promoter is expressed in the same location and acts as the promoter for *adsl-1*, while sur-5 is expressed everywhere within the *C. elegans* worms. The construction of the plasmid and injection was done by Abdulkareem AlShaheeb.

Balanced *adsl-1* knockout animals, (HV855, HV856, HV873) were injected with a constructed plasmid (Figure 2) to make the strains in Table 1. All strains were then observed under a GFP microscope and the adsl-1/adsl-1 progeny were taken to be studied. The plasmids used in this study are shown in Figure 2 below. The full list of the components of the strains are can be found I Table 1.

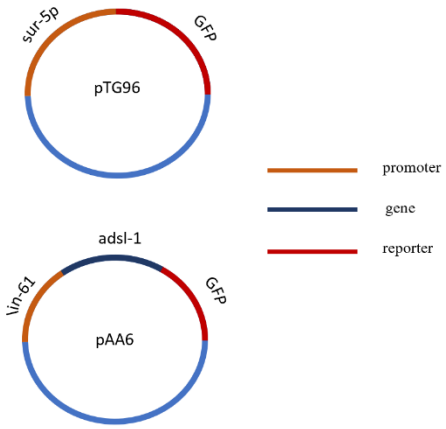

Figure 2: The constructed plasmids. There are three plasmids that underwent the process of construction. The middle of the constructs indicates the name of the plasmid(s) used. The orange curve shows the promoters used, while the dark blue for gene used, and red for reporter used.

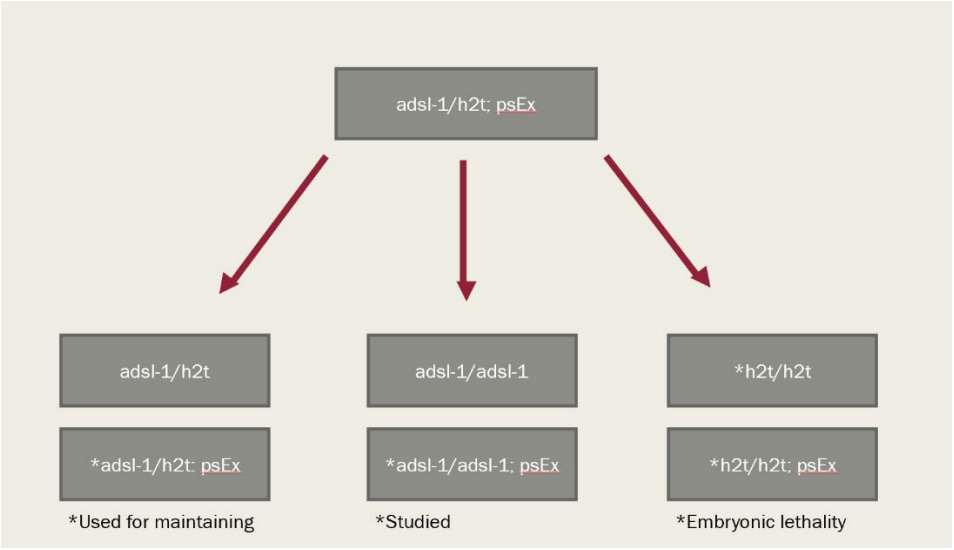

Figure 3: Possible progeny of each transgenic animal with the balancer strain. This figure shows all of the possible progeny for the heterozygous adsl-1/balancer animals. Both the h2t/h2t and h2t/h2t; psEx progeny are subject to embryonic lethality. The adsl-1/h2t; psEx was used to maintain the population and the adsl-1/adsl-1 psEx progeny were studied.

Fertility Assays

For the control strain, four animals were transferred, while for the HV855 strain, 15 animals were transferred, 14 for the HV5856 strain, and 11 for the HV873 strain. First, the animals were maintained. During the maintenance process, ten egg laying L3 to L4 (adsl-1/h2t for control; adsl-1/h2t: psEx for experimental) *C. elegans* were transferred onto an NGM plate spotted with op50 where they remained for the rest of the experiment. Then L3-L4 hermaphrodites were placed into individual plates, where they stayed for the remainder of the

experiment, starting three days after maintenance. They were incubated at 20°C for an additional two days before egg laying was documented for the following five days after. For the control, the protocol called for the transfer five animals into their own individual plates but instead only four were successfully picked. For the HV855, HV856, and HV873 strains, the protocol called for 20 of each strain to be picked but were unfortunately unsuccessful due to mistakes made during the initial picking process. The animals picked to be transferred were the adsl-1/adsl-1 progeny and they were picked using a GFP microscope. The GFP of the adsl-1/adsl-1 for the control animals is not expressed anywhere in the *C. elegans*, while the adsl-1/adsl-1; psEx animals used for the experimental groups expressed the GFP everywhere but the pharynx. More details on how animals were picked for to be transferred in included in Table 2 below.

Table 2: Imaging using GFP Microscope

Possible Progeny	**GFP Present**
adsl-1/h2t	GFP present only in pharynx
adsl-1/adsl-1	No GFP
h2t/h2t	Lethal (eggs do not hatch)
adsl-1/h2t; psEx	GFP in pharynx and everywhere
adsl-1/adsl-1; psEx	GFP everywhere but pharynx
h2t/h2t; psEx	Lethal (eggs do not hatch)

Results

Will restoring expression of ADSL to its original location rescue fertility?

In order to determine if restoring *adsl-1* to its original location will result in rescue of fertility, we used the lin-61/adsl-1 promoter when constructing the plasmids to restore expression of *adsl-1* in the mutants. We isolated and observed the adsl-1/adsl-1; psEx progeny of the adsl-1/h2t psEx strain in the experimental strain (HV873). For the control consisting of mutants without any injected plasmids, we isolated the adsl-1/adsl-1 progeny of the adsl-1/h2t. For the control, none of the four samples laid any eggs. As for the two other control strains (HV855 and HV856), neither had any animals that laid any eggs, of the 15 HV855 collected and 14 HV856 collected. For the experimental (HV873), of the 11 animals observed, only three lad any eggs. the number of eggs each animal laid were recorded (Table 3).

Table 3: The number of eggs laid for each worm for each strain for five days

Strain	Control					HV **855**					HV **856**					HV **873**				
Day / Worm	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5						0	0	0	0	0	0	0	0	0	0	0	0	0	18	43
6						0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7						0	0	0	0	0	0	0	0	0	0	0	0	0	0	23
8						0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9						0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10						0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11						0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12						0	0	0	0	0	0	0	0	0	0					
13						0	0	0	0	0	0	0	0	0	0					
14						0	0	0	0	0	0	0	0	0	0					
15						0	0	0	0	0										

It was expected that the control animals would not lay eggs, this was seen in all four animals of the control. This is because the control strain is simply the mutant *adsl-1* animals without input of any DNA fragments (Table 3). I also found that the strains where the sur-5p::GFP plasmid was injected (HV855 and HV856) resulted in no eggs laid in any case (Table 3). Lastly, the experimental strain containing lin-6::*adsl-1*::GFP, was expected to result in cases of eggs being laid and this was the case in three of the 11 observed animals. The number of eggs laid were counted and recorded each day after they were transferred. Only on day four were there any eggs laid and it was only by one animal. On the last day, three animals had laid eggs. The sample size for the experimental strain (HV873) was 11 and of those 11, three laid eggs. These results indicate that 27.3% of the experimental transgenic animals were fertile compared to 0% of the controls.

Discussion

The results of this experiment support our original hypothesis that restoring expression of *adsl-1* to its native location will lead to the rescue of fertility. With that being said, a vast majority of *C. elegans* with this extrachromosomal array were not able to lay eggs, which brings in to question why? If we were to restore *adsl-1* to its native location using the lin-61/adsl-1 promoter, we should be able to see high efficacy but from this we are able to see that only 27.3% of the experimental animals showed rescue in fertility. We speculate that the injection of the constructs led to an incomplete rescue. In order for reproduction to be restored back to the *C. elegans* both the gonad and ovum need to be rescued. We believe that a partial rescue occurred where either the gonad or ovum were rescued but not both leaving the *C. elegans* unable to reproduce. In order to see if this hold up, first, we would look to see if any have a gonad using higher power magnification. Then we can investigate whether or not the ovum was rescued using

PCR or a western blot. Another reason for this low efficacy, we speculate that maybe the extrachromosomal array is not expressed in the same way in every animal and that it does not rescue the same way for all animals.

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References

1. Balasubramaniam S., Duley J.A., and Christodoulou J. (2014) Inborn errors of purine metabolism: clinical update and therapies. *J Inherit Metab Dis 37*:669–686 https://doi.org/10.1007/s10545-014-9731-6

2. Fenton A.R., N. Janowitz, M.R. McReynolds, W. Wang and W. Hanna-Rose. *Caenorhabditis elegans* model of adenylosuccinate lyase deficiency reveals neuromuscular and reproductive phenotypes of distinct etiology. *PLOS Genetics* In revision. *BioRxiv* https://doi.org/10.1101/181719

3. Crook, M., McReynolds, M. R., Wang, W., & Hanna-Rose, W. (2014). An NAD(+) biosynthetic pathway enzyme functions cell non-autonomously in *C. elegans* development. *Dev. Dyn. 243*(8), 965-76. 10.1002/dvdy.24139

4. Brenner S. The genetics of *Caenorhabditis elegans*. Genetics. 1974;77:71–94.