Etiology and treatment of adrenoleukodystrophy: new insights from *Drosophila*

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ABSTRACT

Adrenoleukodystrophy (ALD) is a fatal progressive neurodegenerative disorder affecting brain white matter. The most common form of ALD is X-linked (X-ALD) and results from mutation of the *ABCD1*-encoded very-long-chain fatty acid (VLCFA) transporter. X-ALD is clinically heterogeneous, with the cerebral form being the most severe. Diagnosed in boys usually between the ages of 4 and 8 years, cerebral X-ALD symptoms progress rapidly (in as little as 2 years) through declines in cognition, learning and behavior, to paralysis and ultimately to a vegetative state and death. Currently, there are no good treatments for X-ALD. Here, we exploit the *Drosophila* bubblegum (*bgm*) double bubble (*dbb*) model of neurometabolic disease to expand diagnostic power and therapeutic potential for ALD. We show that loss of the *Drosophila* long-/very-long-chain acyl-CoA synthetase genes *bgm* and/or *dbb* is indistinguishable from loss of the *Drosophila* ABC transporter gene *ABCD1*. Shared loss-of-function phenotypes for synthetase and transporter mutants point to a lipid metabolic pathway association with ALD-like neurodegenerative disease in *Drosophila*; a pathway association that has yet to be established in humans. We also show that manipulation of environment increases the severity of neurodegeneration in *bgm* and *dbb* mutant flies, adding even further to a suite of new candidate ALD disease-causing genes and pathways in humans. Finally, we show that it is a lack of lipid metabolic pathway precursor that is causative of neurometabolic disease: addition of medium-chain fatty acids to the diet of *bgm* or *dbb* mutant flies prevents the onset of neurodegeneration. Taken together, our data provide new foundations both for diagnosing ALD and for designing effective, mechanism-based treatment protocols.

This article has an associated First Person interview with the first author of the paper.

KEY WORDS: Bubblegum (Bgm), Double bubble (Dbb), ABCD1, Fatty acid acyl-coA synthetase, Fatty acid transporter, Elongase, Neurodegeneration

INTRODUCTION

Adrenoleukodystrophy (ALD) is a neurodegenerative disorder associated with mutation of the peroxisomal ABC transporter protein ALDP (adrenoleukodystrophy protein) that is encoded by the X chromosome locus *ABCD1* (Mossor et al., 1993). The spectrum of clinical features associated with ALDP mutation is broad, ranging from adrenocortical insufficiency to slowly progressive myelopathy to cerebral demyelination (Raymond et al., 1999). ALDP is required for transport of very-long-chain fatty acids (VLCFAs), and ALDP deficits lead to VLCFA accumulation in plasma and tissue (Engelen et al., 2012; Raymond et al., 1999). With respect to disease etiology, it is thought that VLCFA accumulation is toxic to the adrenal gland and to the myelin sheath that surrounds the many nerve cells of the body (Engelen et al., 2012). However, several inconsistencies exist in patient studies that refute this model. First, while all individuals harboring disease-associated alleles of *ABCD1* exhibit VLCFA level increases, some never manifest neurodegenerative symptoms (Engelen et al., 2012; Raymond et al., 1999). Second, VLCFA levels do not correlate with patient neurological disabilities (Moser, 1997). Third, although the two recipients of hematopoietic stem cell therapy showed improvement in their neurological symptoms, plasma VLCFA concentrations remained high (Cartier et al., 2009). In addition to the documented role for the ABC transporter in VLCFA metabolism and ALD, a role for the acyl-CoA synthases (ACSs) that function immediately upstream of ABC transporters in fatty acid (FA) metabolism has long been contemplated, as decreased ACS activity is another biochemical hallmark of ALD (Hashmi et al., 1986; Lazo et al., 1988; Wanders et al., 1988). Consistent with this idea is the recent identification of a patient with ALD-like cerebral degeneration with a rare mutation in the *SLC27a6*-encoded ACS (Sivachenko et al., 2016). The cerebral form of ALD is severely progressive and, in the absence of cures and treatments, death is inevitable. It is also clear that our current understanding of ALD disease etiology is insufficient for the design of effective treatment protocols; in this regard, therapeutic manipulation of VLCFA levels does not impact disease progression (Engelen et al., 2012; Raymond et al., 1999). Animal models of neurometabolic disease, however, continue to enhance our understanding of ALD and yield new insights into ALD diagnosis and management. In particular, neurometabolic disease models in the mouse and fly are consistent with roles for ACSs in ALD (Heinzer et al., 2003; Min and Benzer, 1999; Sivachenko et al., 2016). These genetic platforms offer new opportunities for: (1) dissecting ALD-associated pathways enhancing early ALD diagnostic power and (2) identifying molecular targets suitable for therapeutic inhibition as well as alternative pathways that can potentially be boosted to alleviate degeneration.

RESULTS

Loss of neuronal *Drosophila ABCD* transporter function causes neurodegeneration

Although neurodegeneration reminiscent of ALD has been successfully modeled in ACS loss-of-function flies (Min and...
Benzer, 1999; Sivachenko et al., 2016), the analysis of orthologs in the X-linked ALD (X-ALD) human disease gene (ABCD1) in animal models has remained elusive (Kobayashi et al., 1997) – in vertebrates likely due to gene duplication. This said, the recent development of comprehensive and bioinformatically validated RNAi libraries facilitating reverse genetic approaches has opened a pipeline for gene validation in the non-redundant (less highly duplicated) genome of the fly (Moulton and Letsou, 2016 for review).

Using reciprocal BLASTp algorithms, we identified duplicated genome of the fly (Moulton and Letsou, 2016 for review). Using reciprocal BLASTp algorithms, we identified CG2316 as the sole Drosophila homolog of human ABCD1. CG2316, a fourth chromosome gene henceforth designated dABCD (for Drosophila ABCD), is 53% identical and 71% similar to human ABCD1 (Fig. S1). Expression studies (Chintapalli et al., 2007) reveal moderate to high levels of dABCD in the adult head, consistent with a role for dABCD in the maintenance of CNS health in flies. As no genetically defined lesions for dABCD exist, we employed a short-hairpin microRNA from the VALIUM20 collection (dsRNA-HMS02382; confirmed bioinformatically to have no off-target effects) to target dABCD for studies of gene function. We found that dABCD<sup>dsRNA</sup> transgenics survive to adulthood, but suffer from neurodegeneration. Specifically, dABCD<sup>dsRNA</sup> flies exhibit a brain phenotype indistinguishable from that of animals homozygous for amorphic alleles of the bgm- and dbb-encoded Drosophila long/very-long-chain ACSs – that being an age-dependent retinal disorganization that is distinguished by retinal holes and pigment cell loss (Fig. 1A,B,F). Previous reports (Min and Benzer, 1999; Sivachenko et al., 2016) have validated both bgm and dbb as ALD-like models of neurodegeneration. However, the utility of these lines as disease models is now further bolstered by their shared loss-of-function phenotype with dABCD. Moreover, it is clear that ABCD1 should be added to the growing list of human neurodegenerative-disease-related genes with functional homologs in Drosophila (Chien et al., 2002).

Having established here and elsewhere (Sivachenko et al., 2016) that both neurons and glia die in Drosophila ABCD and ACS models of neurodegeneration, we next sought to determine the cell-type-specific requirements for the Drosophila-encoded FA transporter and synthetases. To this end, we used elav (neuronal), sim (embryonic midline glial and adult neuronal) and repo (glial) drivers (FlyBase, 2003) to mediate cell-type-specific expression of dsRNA targeting dABCD. Neuronal disruption of dABCD in dABCD<sup>elav</sup>-dsRNA and dABCD<sup>sim</sup>-dsRNA transgenics recapitulates retinal defects seen in dABCD<sup>bgm</sup>-dsRNA animals; in contrast, no defects result from glial-specific disruption in dABCD<sup>repo</sup>-dsRNA transgenics (Fig. 1C,E,K). Complementing this analysis is our study of the effects of cell-type-specific expression of a Drosophila bgm<sup>+</sup> transgene in a bgm<sup>-</sup> null mutant background. Both ubiquitous and neuronal bgm<sup>+</sup> expression rescue age-dependent neurodegeneration in bgm<sup>-</sup> mutants, although glial expression does not (Fig. 1F-J,L). Our observation of identical tissue-specific effects for dsRNA-HMS02382-mediated dABCD gene disruption and bgm<sup>+</sup> gene rescue is consistent with bioinformatic exclusion of off-target effects for dsRNA-HMS02382. Moreover, results from targeted disruption (dABCD) and rescue (bgm) studies show that the primary site of ABCD/ACS function in the Drosophila model of ALD-like neurodegeneration is the neuron and point to this cell type as the optimal target for prevention of neurodegeneration in ACS and ABCD fly models, and, by extension, for ALD therapy in humans.

**Gene-environment interactions modulate ALD penetrance and expressivity**

As is true for ALD patients, neurodegeneration in bgm, dbb and dABCD flies is incompletely penetrant and variably expressed (Sivachenko et al., 2016; see also Fig. 1). The origin of this variability is so far unknown, although multiple reports have pointed to an environmental interaction (Weller et al., 1992; Raymond, et al., 2010; Sivachenko et al., 2016). Thus, to examine environmental contributions to phenotype, we examined responses of bgm<sup>-</sup> and dbb<sup>-</sup> amorphs to environmental stress in the form of light manipulation (Johnson et al., 2002). In contrast to animals
raised in normal light/dark conditions (12 h light:12 h dark), animals raised in constant dark (24 h dark) exhibit significantly less neurodegeneration; in the case of dbb<sup>−</sup>−, neurodegeneration appears to be blocked entirely (Fig. 2A-FJ). In complementary constant light conditions, we observed a significant exacerbation of neurodegeneration in all backgrounds, including the wild type (Fig. 2G-J). Finally, in defining constant-light-induced neurodegeneration in wild-type animals as baseline, we determined that enhanced neurodegeneration in bgm<sup>−</sup>− and dbb<sup>−</sup>− animals is synergistic (Fig. 2J). Thus, environmental stress in the form of light modifies neurodegenerative phenotypes in bgm and dbb models of neurodegeneration, demonstrating that gene-environment interactions can modulate penetrance and expressivity of neurodegenerative phenotypes.

**Product insufficiency is causative of ALD**

Despite accumulation of VLCFAs (ACS and ABCD1 substrates) in ALD patients, it is still debated whether this marker of disease is also causative of disease, and whether accumulating VLCFAs should serve as a therapeutic target (Raymond et al., 1999). Here, we consider the alternative possibility – that it is the absence of activated VLCFAs and/or their metabolic products that is causative of disease. As a first step toward disease therapeutics and in our first direct test of the lack of product hypothesis for ALD, we fed bgm<sup>−</sup>− and dbb<sup>−</sup>− animals a diet high in medium-chain fatty acids [7% coconut oil (Birse et al., 2010)] from day 0 (d0) to day 20 (d20) post-eclosion, anticipating that bypass of the genetic block to activating long-chain FAs (LCFAs)/VLCFAs in bgm and dbb mutants via the elongase pathway might suppress neurodegeneration (Fig. 3A). Indeed, supplementation of bgm<sup>−</sup>− and dbb<sup>−</sup>− mutant diets with medium-chain FAs significantly reduces retinal defects observed at d20 post-eclosion in both bgm<sup>−</sup>− and dbb<sup>−</sup>− animals (Fig. 3B-D,H-J,Q). Second, in anticipation that increasing LCFAs/VLCFAs will enhance neurodegeneration if accumulating precursors are toxic (see Fig. 3A), we examined neurodegeneration in bgm<sup>−</sup>− and dbb<sup>−</sup>− animals fed a diet high in LCFAs (described in Carvalho et al., 2012). We found no changes in neurodegeneration in animals fed LCFA-enriched diets (Fig. 3E-G,P). That mutant neurodegenerative phenotypes were rescued by medium-chain-FA dietary supplementation and not exacerbated by LCFA dietary supplementation points to product loss as being causative of neurodegenerative disease.

**A parallel route to VLCFA production (the elongase pathway) is required for CNS health and maintenance**

Given the deficiencies of Lorenzo’s oil in the treatment of ALD patients (Moser, 1999; van Geel et al., 1999; Zinkham et al., 1993) and as an extension of our medium-chain diet treatment results in bgm and dbb flies, we next tested whether disruption of the FA elongase pathway that uses activated medium-chain FAs to produce activated LCFAs is associated with neurodegeneration (see Fig. 3A). Using BLASTp, we identified four genes encoding *Drosophila* elongases [there are seven in humans (Jump, 2009)], but only one, CG2781 (44-56% identical to *ELOVL1*, 7 and 4 and henceforth identified as *dELOVL* for *Drosophila ELOVL*; Fig. S2), is expressed in a spatial and temporal manner analogous to *dABCD* and predictive of a role in neuronal health and maintenance (Chintapalli et al., 2007). There are no genetically defined *dELOVL* mutants; thus, as we did previously for *dABCD*, we used the binary UAS-GAL4 system in combination with RNAi-mediated gene disruption methods. We used two reagents to disrupt *ELOVL* gene function and thereby assess its function in CNS health and maintenance. The first, *dsRNA-HMC03112*, is from the VALIUM20 TRiP collection and has been confirmed bioinformatically to have no off-target effects. The second, *dsRNA-GD16713*, is from the Vienna *Drosophila* Resource Center. While ubiquitous *dELOVL* disruption (in *dELOVL<sup>dELOVLrepo>dsRNA-HMC03112</sup>* transgenics) leads to lethality before eclosion (data not shown) and points to an early essential role for *dELOVL*, specific neuronal knockdown of *dELOVL* (in *dELOVL<sup>dELOVLrepo>dsRNA-HMC03112</sup>* and *dELOVL<sup>dELOVLrepo>dsRNA-HMC03112</sup>* leads to neurodegeneration. Animals exhibit retinal defects, including holes and lost pigment cells, phenotypes replicating those that we have observed in bgm-, dbb- and *dABCD*-deficient animals. In an extension of the analysis, targeted disruption of *dELOVL* in glia (in *dELOVL<sup>dELOVLrepo>dsRNA-HMC03112</sup>* transgenics) does not produce degenerative phenotypes (Fig. 3K-O). Thus, cell-type specificity for all pathway components in VLCFA metabolism (ACSs, elongases and transporters) is neuronal. Despite a high background for leaky *dsRNA-GD16713* expression that causes death in the absence of a GAL4 driver, we reproduced all *dsRNA-HMC03112* qualitative results using *dsRNA-GD16713*.
Together, these data show that product loss is causative of disease and that mutations in genes encoding elongases and medium-chain FA acyl-CoA synthetases should be considered candidate ALD-causing disease genes, as well as targets for therapeutic options.

**DISCUSSION**

ALD is a progressive neurodegenerative disease, with the most severe form claiming the lives of school-age boys. Although* ABCD1*, the gene responsible for the most common form of ALD (X-ALD), has been cloned, the etiology of the disease has remained elusive and there are still no satisfactory treatments or cures (for review see Gordon et al., 2014). Using *in vivo* models of neurometabolic disease, we here provide new insights into human ALD. Our demonstration that mutations in long- and medium-chain FA metabolic pathways in *Drosophila* yield shared loss-of-function neurodegenerative phenotypes extends a single-gene association (*ABCD1*) for ALD to a pathway association (lipid metabolism). Importantly, this more expansive view of ALD offers a possibility of diagnosis to some of the 50% of leukodystrophy patients with undiagnosed conditions (Gordon et al., 2014). In addition, our demonstration that neurodegeneration in fly models of ALD does not result from a buildup of FA precursors, but instead is caused by a lack of activated FA product(s), shifts our understanding of ALD etiology and is expected to have profound effects on the design of effective therapeutics. Indeed, our data indicate that a diet high in medium-chain FAs provides a potential therapeutic approach for leukodystrophy patients with ACS mutations (Sivachenko et al., 2016). At the very least, our study validates the continued search for remedies other than Lorenzo’s oil for the treatment of X-ALD (Eichler et al., 2017; Kolata, 2017).

Although hundreds of *ABCD1* alleles are associated with ALD, no genotype-phenotype correlations have emerged. Likewise, inheritance of the same allele within kindreds (or even an allele common to monozygotic twins) can lead to different disease phenotypes (Berger et al., 1994; Korenke et al., 1996). Thus, it...
seems clear that gene-environment interactions modulate ALD penetrance and expressivity. Gene-environment interactions extend to other players in the lipid metabolic pathway associated with ALD as well. In this regard, each of the young brothers in a recently described Utah leukodystrophy family harbors an allele pair associated with two incompletely penetrant conditions (PRT2/ childhood epilepsy and Scl27a6/leukodystrophy). Only the younger brother, however, exhibits both seizure and leukodystrophy phenotypes (Sivachenko et al., 2016).

Our finding that environmental stress in the form of light modifies neurodegenerative phenotypes in bgm and dbb models of neurodegeneration provides direct evidence for a gene-environment interaction that modulates penetrance and expressivity of neurodegenerative phenotypes (see Fig. 2). These data bolster our view that: (1) environmental stress in the form of seizure triggered neurodegeneration in a leukodystrophy patient with a predisposing ACS mutation (Sivachenko et al., 2016), and (2) traumatic brain injury triggered neurodegeneration in patients with catastrophic presentations of ALD (Raymond et al., 2010; Vawter-Lee et al., 2015; Weller et al., 1992). Moreover, as stress might precipitate a degenerative cellular phenotype when product generation is required to repair damaged or depleted cellular components, our bgm/dbb stress studies suggest that esterified LCFA and VLCSA products of Bgm and Dbb activity are required to prevent neurodegeneration, a hypothesis that contradicts the long-held view that precursor accumulation is causative of neurodegeneration in ALD patients.

In the Drosophila bgm/dbb model of neurodegeneration, inclusions in brain tissue and elevated levels of VLCSAs provide clear diagnostic markers of disease, and point to fly models as powerful in vivo models for testing the effects of FA dysregulation on neurodegeneration (Sivachenko et al., 2016). In humans, evidence for accumulating FAs as being causative of disease comes primarily from tissue culture studies, where accumulating FAs can lead to cell death (Hein et al., 2008; Reiser et al., 2006; Ulloth et al., 2003). Contradicting this view, however, are data from ABCD1 hemizygotes showing that, although all individuals harboring mutant alleles of ABCD1 exhibit similar significant increases in their circulating VLCSA levels, these correlate with neither the development of disease nor the timing of disease onset (Dubey et al., 2005; Raymond et al., 1999). Moreover, some recipients of hematopoietic stem cell gene therapy show improvement in neurological symptoms despite plasma VLCSA concentrations remaining significantly high (Cartier et al., 2009). Understanding disease etiology represents an essential first step in providing appropriate therapies. The current therapeutic option for the most severe ALD cases is bone marrow transplant; however, the procedure itself carries substantial risk and is not always successful (Berger et al., 2010; Gordon et al., 2014). Another touted therapy called Lorenzo’s oil is thought to target accumulated VLCSAs but remains a controversial option, with most agreeing that it is ineffective (Aubourg et al., 1993; Berger et al., 2010; Gordon et al., 2014).

Here, we demonstrate that bgm and dbb neurodegenerative phenotypes are rescued by medium-chain FA dietary supplementation, while being unaffected by LCFA (see Fig. 3). Together, data from these complementary studies identify product loss as causative of neurodegenerative disease. Indeed, end products of peroxisomal metabolism (including both glycerolipids and plasmalogens) constitute more than 80% of the phospholipid content of brain white matter (Schrader and Fahimi, 2008). Additionally, our results are the first to highlight potential for the activated medium-chain elongation pathway as an alternate route to production of missing VLCSA product(s) in ACS mutants. Of course, dietary supplementation is not necessarily expected to rescue ABCD Drosophila mutants (or to provide therapeutic value to X-ALD patients) because dABCD/dABCD functions at the intersection of the long-/very-long- and medium-chain lipid metabolic pathways (see Fig. 3A). This said, our prior association of the SLC27a6-encoded ACS with ALD (Sivachenko et al., 2016) suggests that there are forms of the disease that are likely to be alleviated by dietary supplementation with medium-chain FAs.

Finally, over half of leukodystrophies remain undiagnosed. Our identification of the elongase pathway identifies new candidates for disease genes. At the biochemical level, fatty acyl-CoA chain elongation involves the addition of two carbon units to an existing fatty acyl-CoA, thereby bypassing a requirement for VLCSA activation by long- and very-long-chain ACSs (Beaudoin et al., 2002). Elongases have been implicated in ALD etiology, as enzyme levels are increased in induced pluripotent stem cell (IPSC)-derived brain cells from ALD patients (Baarine et al., 2015). Although increased elongase levels have been interpreted to mean that elongase function might exacerbate disease in patients, functional tests have yet to be undertaken and another interpretation of the data is that elongase levels are upregulated in ABCD1 mutants as an alternate route to the production of essential activated VLCSAs. Interestingly, in humans, the longest VLCSA species are found only in tissues expressing elongases, namely the retina, brain and testis, all three of which are key tissues affected in ALD (Akgana et al., 2010; Ahmad et al., 2009).

In summary, ALD in its most severe form results in acute and rapidly progressing degeneration of brain white matter and leads to death within a few years of diagnosis. The etiology of ALD has remained poorly understood, and there is still no treatment for this condition. ALD has long been thought to result from a build up of VLCSA in the brains of affected individuals. We used the Drosophila model of neurodegeneration to show that this long-held view is incorrect. While accumulating VLCSAs are indeed a marker of neurodegenerative disease in both flies and humans, we show that it is the absence of activated VLCSAs and/or their metabolic products that is causative of disease in the fly model. Our studies contribute to the fields of ALD and neurodegenerative disease in three major ways: (1) we show that activated VLCSAs and/or their products are necessary for neuronal health and maintenance; (2) we identify new candidate ALD-disease-causing genes, and (3) we show that a diet high in medium-chain FAs shows promise as a potential therapeutic approach for patients with neurometabolic degenerative disease.

**MATERIALS AND METHODS**

**Drosophila stocks**

Ga4 lines used to drive targeted transgene expression include repo-Ga4 (BL24715), elav-Ga4 (BL28760), sim-Ga4 (BL29150) and D.667-Ga4 (BL28171); tubulin-Ga4 (w1118; P[elav-Ga4]L7/TM3, P(Stel-GMR-nvYFP)3 Sb1) was the gift of Mark Metzstein (University of Utah, Salt Lake City, UT). The bgm1 and dbb1 mutants have been described (Min and Benzer, 1999; Sivachenko et al., 2016). dsRNA lines targeting dABC1 and deLOVL were obtained from the Transgenic RNAi Project at Harvard Medical School (#41984 and #50710, respectively); an additional dsRNA line targeting deLOVL was obtained from the Vienna Drosophila Resource Center (#50712). Unless otherwise noted, all flies were raised on a standard cornmeal diet at 25°C with 12-h light/dark cycles.

For tissue-specific expression of bgm, its coding sequence was inserted into pFLAG-CMV-3a (Sigma E7523) with primers 5′-ATAAAGCCTT- ATGTCCACAGTAGACCGCTC-3′ and 5′-CGGGTACCCGCAATATA- GTTTCCTGATC-3′. The tagged version of the gene was subsequently
inserted into the *Drosophila* expression vector pUAST using primers 5′-AATGGGCCGATACCGGCTGACCTACAGTAAGACAGCAAGGTGCTGAGG-3′ and 5′-AATCTCAGACTCGAGATAGGGCGGTAGGCGTGTACG-3′. qUAS-bgm-FLAG was sequenced verified and co-injected with transposase A2-3 into dechorionated *nox*ΔC31; +; *vk*27 embryos 1-2 h after egg lay. G0 injected animals were mated to *w1118* and progeny were screened for transformed germlines based on eye color. The *UAS-bgm-FLAG* line used for our studies is homozygous viable, with the transgene insertion on the second chromosome.

**Diet and light manipulations**

Medium- and long-chain diets were prepared as previously described (Birse et al., 2010; Carvalho et al., 2012). Adult males were collected within 24 h of eclosion and maintained on prescribed diets until sacrifice 20-22 days post-eclosion. For light manipulations, males were isolated within 48 h of eclosion and habituated in 24 h light, 12 h light/dark, or 24 h dark cycles in a temperature- and humidity-controlled room until sacrifice at 20-22 days post-eclosion.

**Aging and histology**

Heads from adult *Drosophila* males were prepared, sectioned and imaged as previously described (Sivachenko et al., 2016). Samples were scored blindly in three to five serial sections for each animal. The degree of retinal degeneration was scored qualitatively as 0 for normal appearance, 1 for mild tissue loss, 2 for moderate degeneration and 3 for severe degeneration (Cao et al., 2013). Data were analyzed by ANOVA and Welch two-sample t-tests. Data were analyzed using GraphPad Prism software (GraphPad Software).

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**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**


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**Supplementary information**

Supplementary information available online at http://dmm.biologists.org/lookup/doi/10.1242/dmm.031286.supplemental

**References**


**Figure S1.** Amino acid alignment of human ABCD1 and Drosophila CG2316 shows high levels of conservation. Sequences were aligned using COBALT software through NCBI.
**Figure S2.** Amino acid alignments of *Drosophila* elongase CG2781 and three human elongases (ELOVL1, 4, and 7). Sequences were aligned using COBALT software through NCBI.
First Person is a series of interviews with the first authors of papers published in Disease Models & Mechanisms, helping early-career researchers promote themselves alongside their papers. Hannah Gordon is first author on ‘Etiology and treatment of adrenoleukodystrophy: new insights from Drosophila’, published in DMM. Hannah conducted the research in this article while a PhD student in the lab of Anthea Letsou at the University of Utah, Salt Lake City, USA, but is now a postdoc in Kristen Kwan’s lab at the same institute, investigating signaling pathways and morphogenesis in eye development.

How would you explain the main findings of your paper to non-scientific family and friends?
This paper describes how disruption of a specific kind of fat metabolism results in neurodegeneration. Disruption of this metabolic pathway causes a human disease called adrenoleukodystrophy (ALD), which itself is fairly rare. However, the disease is unique in that it informs us on how the nervous system relies on certain fats to be healthy and maintain proper function, which can also apply to humans without the disease. In this paper, we used a fly model we previously developed (Sivachenko et al., 2016) to answer outstanding questions in the ALD field, namely, why does a block in this specific kind of fat metabolism make neurons die? For me, this was the most important and most interesting question we could use our fruit fly model to address. In humans and flies (and most living cells), fatty acids (a single unit of fat) need to be ‘activated’ by an enzyme inside of a cell before those fats can be modified by other enzymes and used for purposes such as building blocks for membranes or as fuel for energy. Only a few enzymes perform this activation function and in humans and flies with ALD, the function of one of these enzymes is disrupted. With this enzyme activity reduced (or completely off), two things happen in the cell: 1) an accumulation of fatty acid precursor and 2) a lack of activated fatty acid product. We wanted to know which of these two consequences of enzyme disruption resulted in neurodegeneration. We tested this by supplying mutant flies with fatty acids from which they could produce a missing product, and this alleviated their neurodegeneration. We also found that if we blocked a different pathway that produces the same activated fatty acid product, in an otherwise healthy fly we could cause neurodegeneration. In addition to suggesting a much-needed potential therapy for ALD patients, this paper also identifies additional genes that might be causative of other undiagnosed leukodystrophies and sheds light on the inner workings of the nervous system and its dependence on (or sensitivity to) specific kinds of fats for overall health.

“Validating our fly model of ALD in our previous DMM paper provided a platform to take this model one step further”

What are the potential implications of these results for your field of research?
Before this paper, the main hypothesis in the field was that when you block this step in fat metabolism, a build-up of fat precursor occurred and that this build-up was toxic to the cells, resulting in neuron death. Multiple observations over decades, however, suggested this wasn’t the whole story, but there was no in vivo system available that allowed an uncoupling of the build-up of precursor from the lack of activated fatty acid product. Validating our fly model of ALD in our previous DMM paper provided a platform to take this model one step further and with various methods, uncouple the fatty acid precursor and product so they could be tested independently. I think gaining an understanding of how specific kinds of fats are used by the nervous system is fascinating on its own, but pinning down the cause of death for neurons is crucial when we think about therapeutic approaches. For example, if the build-up of precursor is toxic, our therapeutic approach would be to find ways to prevent or remove that build-up (which has been attempted in the past and failed). However, if the lack of activated product is causing neuron death, our therapeutic approach would be quite the opposite, where we try to find ways to supply the cells with the fatty acids they need. I think this fundamental shift in therapeutic approach is the biggest impact of our results.

What are the main advantages and drawbacks of the model system you have used as it relates to the disease you are investigating?
Having a fruit fly model of this disease is really a scientist’s dream come true. Flies have a nervous system that is much simpler than our nervous system but if you zoom in to the cellular level, a fly neuron functions almost indistinguishably from that of a human neuron. Zoom out, and that fly neuron resides in a much simpler organism that is easier to study than humans. Flies also offer so many genetic tools to test complex ideas about how the nervous system functions (and more of these tools are being generated every month). Probably the biggest drawback is that it’s sometimes hard to convince people
of the strengths of this model organism (and other model organisms, for that matter) and thus it can be difficult to obtain funding for such productive research, despite these model organisms proving their value many, many times over.

"Probably the biggest drawback is that it’s sometimes hard to convince people of the strengths of this model organism (and other model organisms, for that matter)"

What has surprised you the most while conducting your research?
The rescue with medium-chain fatty acids really surprised me! This idea that neurodegeneration could also be caused by a lack of fatty acid product really stemmed from looking at multiple lines of evidence that, despite a lot of work from very dedicated people, we didn’t have the whole story right. It was a fundamentally simple hypothesis, so we thought that if we could do it right, why not test it?

Describe what you think is the most significant challenge impacting your research at this time and how will this be addressed over the next 10 years?
One main conclusion of our paper is that a particular kind of fatty acid might be necessary for maintaining a healthy nervous system. If we think of fatty acids as links on a chain, they can be separated into classes based on chain length and the ones we think might be required for a healthy nervous system are the longest of these chains. That kind of fatty acid is fairly rare in the body and is most abundant in the testes, brain and retina. Because of their rarity, many studies that look at fatty acids gloss over this class, so we know less about what they are doing in the cell than the other classes of fatty acids. Biochemical techniques are changing, however, and many more researchers are using lipidomics to study all sorts of qualities of fatty acids. Ultimately, I’d love to know what that very-long-chain fatty acid is being used for in the cell and to answer this I’d love to see the development of more tools to study very-long-chain fatty acid biology.

What changes do you think could improve the professional lives of early-career scientists?
I would love to see the standard implementation of amenities like high-quality health insurance and a retirement plan at all universities, which might be necessary to retain the most talented postdoctoral scientists. Being a postdoc has traditionally been thought of as a transition period, which may be why those amenities were less emphasized as part of the postdoc position. However, competition for limited faculty positions is growing, requiring more time as a postdoc to meet the conditions for these positions. From what I’ve observed, one major determining factor for whether talented individuals stay in academia is whether they can afford to spend years working well below their post-degree earning potential, which can be heavily influenced by socioeconomic background. On top of this is that an individual’s 20s and 30s (the age range of most early career scientists) represent a critical earning period in their lives. I was introduced to academia as a place for anyone seeking the pure endeavors of knowledge, leading to a growing community of diverse and passionate individuals of all ethnicities. I’d love to see academia challenge itself to continue broadening this acceptance through support of different socioeconomic backgrounds at the postdoctoral level. I think the positive effects of these efforts could be socially far-reaching in 1) making science more accessible for more people and 2) casting aside the all-too-common association of science as an endeavor only of the elite.

What’s next for you?
Since finishing my PhD I have moved to a completely new organism and field and I am so far enjoying all of the new adventures my research has brought me. I am working hard to develop zebrafish as a model organism for a new field of research. With its ever-growing genetic toolkit and optical transparency, I’m really excited about the contributions this tiny vertebrate can add to understanding fundamental biology as well as human health and disease.

References