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Dietary rescue of *fumble*—a *Drosophila* model for pantothenate-kinase-associated neurodegeneration

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Summary: Hallervorden–Spatz syndrome (HSS) is a devastating neurological disease, characterized by iron accumulation in the globus pallidus in the basal ganglia. Most HSS cases are caused by mutations in one of the four human pantothenate kinases (*PANK2*). This *PANK2*-caused subgroup of HSS is sometimes referred as PKAN (pantothenate-kinase-associated neurodegeneration). No effective treatment for PKAN or HSS is currently available. *fumble*, a *Drosophila* mutant that carries a mutation in *Drosophila* Pank, has many features similar to those of PKAN patients. In this study, we used *fumble* as a model to evaluate various compounds or nutritional products for their possible therapeutic efficacy. While no product was found to dramatically improve the symptoms, GKE (containing *Ginkgo biloba* extract and flavone) and vitamin E showed statistically significant beneficial effects. Our studies indicate that pantothenate is of limited value in alleviating *fumble* phenotypes and also suggest that some compounds might have deleterious effects.

Hallervorden–Spatz syndrome (HSS), or pantothenate-kinase-associated neurodegeneration (PKAN), is a devastating neurological disease. Symptoms can begin at neonatal or adult stage, depending on the severity of the disease or the nature of the underlining mutation. Patients are characterized by motor abnormalities, dementia, dysarthria, dysphagia and retinal degeneration. A specific pattern on MRI of the brain, the 'eye-of-the-tiger sign', comprising hyperintensities within a hypointense medial globus pallidus on T2-weighted images, serves as a major diagnostic criterion of this disease (McKusick 234200).

Although heterogeneous by nature, the majority of HSS is caused by mutations in the pantothenate kinase 2 (*PANK2*) gene (Hayflick et al 2003; Zhou et al 2001). It is believed that *PANK2* defects may lead to shortage of CoA, a key and ubiquitous cellular metabolic intermediate. Because of Pank2 deficiency, another substrate for CoA synthesis—cysteine—is accumulated in the globus pallidus. This secondary cysteine accumulation might result

in the primary accumulation of iron in the basal ganglia. As well as PKAN, HARP (hypoprebetalipoproteinaemia, acanthocytosis, retinitis pigmentosa, and pallidal degeneration; OMIM #607263) disease is also caused by mutations in *PANK2* (Ching et al 2002; Houlden et al 2003). Thus PKAN and HARP are essentially two versions of the same disease.

The major isoform of Pank2, and possibly the one that is responsible for PKAN, has been shown to be located in mitochondria (Hortnagel et al 2002; Johnson 2004, Kotzbauer et al 2005). This characteristic/motif distinguishes Pank2 from the other Panks (Pank1, Pank3 and Pank4) in humans, which, on the basis of bioinformatic analyses, may all be cytosolic. It is thus inferred that PKAN may be a disease that affects metabolism in mitochondria. Considering its key cellular function, the observations that *PANK2* null mutations do not cause sudden death of the affected cells and that classical PKAN patients can survive up to their teens are intriguing. It is possible that other Panks (Pank1, 3 and 4) provide partial compensating functions for Pank2 defect.

There are four Panks in mice, as in humans. A *Pank2*-knockout mouse model unfortunately did not reproduce the characteristic neurological phenotype of PKAN patients: mutant mice do not display movement disorder (Kuo et al 2005), indicating that basal ganglia are probably not significantly affected in these mice. In contrast, *fumble* flies, a mutant caused by a transposon insertion near the dPank (*Drosophila* Pank) locus, display a movement coordination defect (Afshar et al 2001). The P-element insertion affects dPank activity. These flies normally die during the pupal stage or soon after eclosion and are infertile. From this, it seems that PKAN is better mimicked in *fumble* flies than in *Pank2*-knockout mice. Interestingly, in *Drosophila* there is only one Pank, with several isoforms—one of which is predicted to be targeted to mitochondria.

In this study we used *fumble* as a model for PKAN to study various candidate compounds for their rescuing effect. We analysed the potential efficacy of these compounds on several defects of *fumble* flies, including pupation and eclosion rates and adult survival time.

MATERIALS AND METHODS

Compounds: D-Pantothenate ((*R*)-*N*-[2,4-dihydroxy-3,3-dimethyl-L-oxobutyl]-β-alanine, hemicalcium salt), creatine and acetylcysteine were purchased from Sigma Chemical Co. (St. Louis, MO, USA) L-Carnitine 500 (L-carnitine L-tartrate, 500 mg/capsules) and NAC 600 (NAC600 and glutathione 100, each capsule contains 600 mg of *N*-acetyl-L-cysteine and 100 mg of L-glutathione) were from General Nutritional Company (Pittsburgh, PA, USA). Coenzyme A (CoA, 100 units/tube) and adenosine disodium triphosphate (ATP, 20 mg/tube) were from Shanghai No.1 Biochemical & Pharmaceutical Co. Inosine (0.2 g inosine per tablet) and ascorbic acid (minimum 99.7%) were from Beijing Yongkang Pharmaceutical Co. and Beijing Aoboxing Biotechnical Co., respectively. Ginkgo Biloba Capsules (GKE, each capsule contains 60 mg *Ginkgo biloba* extract and each 100 g contains 1.4 g of flavone) was a general nutritional product from Pharma-Rex, Inc. (Cerritos, CA, USA). Deferoxamine mesylate (DFO) desiccate for injection was from Novartis Pharma Stein AG (Stein, Switzerland). EDTA and $Fe_2(SO_4)_3$ (analytical grade) were reagents from the Beijing Chemical Plant. Vitamin E was a Kirkland product, distributed by CWC, Inc. (Seattle, WA, USA).

Flies and media: Heterozygous *fumble* flies (*fbl*/TM6B) were self-crossed and eggs were collected on grape juice plates topped with a thin layer of yeast. Collected eggs were incubated at 25°C and 70% humidity for 3–4 days until embryos hatched and larvae reached the second instar stage. *fumble* larvae (*fbl*/*fbl*) were distinguished by their normal body shape from those that containing TM6B, which display a tubby body. *fumble* larvae were picked and put into vials containing fly food with individual compounds added. The fly food was made of 35 g dry yeast, 15 g sugar, 10 g agar, 30 ml syrup and 10 ml propionic acid per litre of food. Thirty or 50 larvae were placed in each food vial and kept in an incubator (25°C and 70% humidity). Numbers of pupae and flies were counted 15 days later. Experiments were performed side by side with exactly the same conditions and altogether a minimum of 100 larvae were used for each group.

Analysis: Roughly equal numbers of *fumble* larvae were used side by side in each individual group. To increase statistical significance, data were pooled from several side-by-side experiments and chi-square analysis was utilized to compare whether the numbers were different from the control (without drug treatment) at significance levels of 0.01, 0.05 or 0.10.

RESULTS

The molecular defect of PKAN arises from mutations in *PANK2*, a gene intimately involved in CoA synthesis and metabolism. Pathophysiological studies show that PKAN patients accumulate iron and cysteine in the globus pallidus, and this could possibly lead to oxidative stress. We therefore hypothesized that compounds that boost energy metabolism or CoA synthesis, chelate ions, or work as general nourishment and antioxidants could have beneficial effects. Flies that are defective in Pank, or *fumble* flies, display lower pupation efficiency, eclosion rate and reduced adult survival. These easily discernible defects of *fumble* flies enabled us to set up a relatively straightforward approach to evaluate side by side different compounds for their rescue abilities. None of the compounds tested was able to increase significantly the survival of the eclosed *fumble* adults: all *fumble* flies eclosed, both in control or drug-added groups, died within 5 days of eclosion. For other parameters (pupation and eclosion), results varied from compound to compound. The compounds and their corresponding results are listed in Tables 1, 2 and 3, and are described individually below. Table 1 represents a parallel study for preliminary evaluation of the efficacy of various compounds. Several compounds that showed signs of beneficial effects in the preliminary studies (Table 1) were evaluated further in the second round of experiments; the results of both rounds of experiments were combined and are summarized in Table 2.

Pantothenate: PKAN and *fumble* are defective in pantothenate kinase. An obvious question is whether excess pantothenate (vitamin B_5) can alleviate the associated defect. It was hoped that in the affected cells of both PKAN and *fumble* there might be minimal residual Pank activity—otherwise the cells might not even survive to the advanced developmental stage. In the case of PKAN, some mutations may not be null, so that partial Pank2 activity could be retained. Additionally, other Panks (Pank 1, 3, 4) might provide some activity in the affected cell. In the case of *fumble*, the mutation itself is not null, and maternal effect provides some other Pank activity. Thus the underlying rationale of pantothenate therapy was that with

Table 1 Preliminary studies for the possible effect of various compounds on survival of *fumble* flies[a]

Compound	*Dosage*	*No. of larvae*	*No. of pupae*	*No. of flies*	*Pupation rate*	*Eclosion rate*
	0.5	129	108	29*	0.837	0.221
GKE (mg/ml)	2.0	130	119	34*	0.915	0.258
Pantothenate	0.5	135	117	17	0.867	0.122
(mg/ml)	2.0	130	106	21	0.815	0.162
$Fe_2(SO_4)_3$	0.25	130	108	25	0.831	0.188
(mmol/L)	1	130	103	9	0.792	0.065
	0.2	130	100	24	0.769	0.181
DFO (mmol/L)	1	130	108	32*	0.831	0.246
	1.0	130	99	13	0.762	0.096
CoA (U/ml)	4.0	130	116	27	0.892	0.208
	0.2	130	121	15	0.931	0.115
ATP (mg/ml)	0.8	129	115	14	0.891	0.105
	0.2	130	109	18	0.838	0.135
EDTA (mmol/L)	1	130	97	19	0.746	0.146
Creatine	1.0	130	115	21	0.885	0.158
(mg/ml)	4.0	129	102	17	0.791	0.132
Carnitine	0.04	130	117	22	0.900	0.169
(capsule/vial)	0.12	130	103	9	0.792	0.065
Acetylcysteine	0.2	130	100	19	0.769	0.142
(mg/ml)	1.0	130	90	9	0.692	0.069
Inosine (mg/ml)	1.0	130	86	13	0.662	0.100
	5.0	130	83	12	0.638	0.088
NAC600	0.004	130	96	22	0.738	0.165
(capsule/vial)	0.02	130	91	14	0.700	0.104
Vitamin C	1.0	123	96	12	0.780	0.098
(mg/ml)	5.0	123	94	25	0.764	0.199
Normal food		128	109	17	0.852	0.129

[a]Thirteen compounds were tested individually, each with two dosages. Normal food without addition of any compound/drug was used as the control group

*Statistically significantly higher than control ($p < 0.05$)

increase in the concentration of the substrate (pantothenate), more CoA might be produced from the limited amount of residual Pank enzyme in these cells.

Our experiments did not show that pantothenate possesses any significant beneficial activity. Two dosages were used: 0.5 mg/ml and 2.0 mg/ml. As shown in Table 1, the pupation rate of the pantothenate groups (0.867 or 0.815) was not different from that of the normal food group (0.852). Similar results were obtained for the eclosion rate (0.122, 0.162 vs 0.129). This result was further confirmed when a bigger data set was used (Table 2). Although exact data varied slightly from experiment to experiment when they were performed at different times (one variable is the food used, which has to be prepared fresh each time), when they were performed at the same time side by side, no difference was observed between pantothenate-treated group and the untreated group in repeated experiments.

Table 2 Efficacy summary for effects of pantothenate, iron, GKE and DFO on *fumble* flies[a]

Group		*No. of larvae*	*No. of pupae*	*No. of flies*	*Pupation rate*	*Eclosion rate*
Normal food (control)		227	193	36	0.850	0.156
Pantothenate (mg/ml)	2.0	230	196	35	0.852	0.152
	6.0	234	198	39	0.846	0.165
Sum		464	394	74	0.849	0.158
$Fe_2(SO_4)_3$ (mmol/L)	0.25	230	188	43	0.817	0.185
	1.0	230	186	20^{--}	0.809	0.085
Sum		460	374	62	0.813	0.135
GKE (mg/ml)	0.5	229	203	68*	0.886	0.295
	2.0	230	197	60^{++}	0.857	0.259
Sum		459	400	128*	0.871	0.277
DFO (mmol/L)	0.2	230	186	39	0.809	0.167
	1.0	229	187	51	0.817	0.223
Sum		459	373	90	0.813	0.195

[a]Initial result suggested that GKE and possibly DFO had some beneficial effect on *fumble*. These two compounds, together with iron and pantothenate were tested further for efficacy. Results of both experiments are summarized in this table

'++' or '−−' indicates statistically significantly higher or lower than the control group

*Statistically significantly higher than the control group ($p < 0.01$)

Table 3 Effects of vitamin E and ATP on *fumble*[a]

Group		*No. of larvae*	*No. of pupae*	*No. of flies*	*Pupation rate*	*Eclosion rate*
Normal food		200	178	50	0.890	0.250
Vitamin E (U/ml)	10	400	350	161^{+++}	0.875	0.403
ATP (mg/ml)	0.2	199	150	43	0.754	0.216
	0.8	200	147*	36	0.735	0.180
Sum		399	297*	79	0.744	0.264

[a]In this separate experiment, the effect of vitamin E was evaluated side by side with that of the ATP and the normal food control

'+++' indicates results statistically significantly higher than normal (at $p < 0.01$ and $p < 0.05$ respectively);

*Lower than normal control at $p < 0.10$

CoA: A Pank defect will almost certainly result in CoA depletion inside the cell. Providing CoA to affected cells thus might alleviate their defects. However, it may be difficult for CoA to enter cells because the cell membrane could be impermeable to CoA. It was hoped that with an excess of CoA, a small amount might find its way inside the cell.

In the high-dosage CoA group (4.0 U/ml), from a total of 130 larvae we observed 116 pupae and 27 flies, compared to 109 pupae and 17 flies (from 128 larvae) with the normal food. These results indicate that CoA could have some benefit, but the results are not statistically significant ($P > 0.05$) within this data set. The numbers in the low-dosage group (1 U / ml) group were not greater than for control (Table 1). Combining the data from high and low dosages, we cannot conclude at this stage that CoA is beneficial.

ATP: CoA is required for metabolites to enter the TCA (tricarboxylic acid) cycle for ATP generation. In PKAN or *fumble* flies, affected cells die probably because of depletion of cellular energy. Supply of external ATP might thus rejuvenate the cells. Again the problem is that ATP might not be absorbed significantly by cells, and the brain barrier in humans might pose a further hurdle.

Average pupation rates of the ATP groups appear higher than that of the normal-food group (Table 1), but, the results do not reach statistical significance. Numbers of eclosed flies in treated and control groups are comparable. The effect of ATP was further tested in a separate experiment (Table 3). This experiment also failed to reveal any significant benefit of ATP on *fumble* flies.

Inosine: Inosine is a purine nucleoside that uses ribose in forming the building blocks of ATP, DNA and RNA. Inosine is considered, though has never been scientifically proved, by some to have a positive effect on overall physical strength and energy generation during exercise and is sometimes used in physical training.

To our surprise, inosine not only had no positive effect on *fumble* flies, it was actually toxic at high concentration. At the concentration of 50 mg/ml, virtually no flies survived on the food. Unsurprisingly, at lower dosages (5 mg/ml and 0.5 mg/ml), pupation rates appeared slightly lower than that of the control group (0.638 and 0.662 vs 0.852; Table 1). However, the numbers at these low concentrations were not large enough to demonstrate toxicity at 5% significance level. Taken together, these data at least indicate no helpful effect of inosine in *fumble* flies.

Carnitine: Carnitine plays an essential role in the transfer of long-chain fatty acids into mitochondria for β-oxidation. It also facilitates removal of short-chain organic acids from mitochondria, thereby freeing intramitochondrial CoA to participate in the β-oxidation and TCA cycle pathways.

Carnitine in our experiments did not demonstrate clear signs of benefit in *fumble* flies. The numbers of pupae from carnitine-fed groups (103 and 117 pupae out of 130 larvae) were not statistically different from that in the normal group (109 from 128 larvae). Of these, the eclosed flies in the carnitine groups did not show notably better results than the control (9 and 22 vs 17, Table 1).

NAC600: NAC600 is commonly available in nutritional stores. The NAC600 we used contained *N*-acetylcysteine (600 mg/capsule) and L-glutathione (100 mg/capsule), both of which are supposed to be antioxidants. One-tenth of a capsule of NAC600 per vial (10 ml food) killed all 30 *fumble* larvae, indicating that NAC600 can be detrimental at high concentration. Two groups fed with lower doses of NAC600 (1/50 capsule per 10 ml or 1/250 capsule per 10 ml food) both manifested pupation and eclosion rates comparable to that of the control (Table 1).

GKE: GKE (*Ginkgo biloba* extract + flavone) is a commonly used nutritional product for general nutrition. GKE is commonly considered to have various antioxidant activities. Some studies have suggested its potential application as a drug for improving neurological dysfunction (Hofferberth 1989; Polich and Gloria 2001; Quaranta et al 2003).

Increased eclosion rate was observed in GKE groups, suggesting that it might help *fumble* flies (Table 1). Repeated experiments have confirmed this result. In two experiments we used

altogether about 230 larvae, and the result is summarized in Table 2. GKE-treated *fumble* larvae showed >50% better eclosion than those grown in normal food (0.295 and 0.259 vs 0.156). Nevertheless, these eclosed flies did not live much longer than the controls: all died within 5 days of eclosion. Nor did pupation rates increase obviously (pupation rates were 0.886 and 0.857, respectively, vs 0.850 for controls). We cannot exclude the possibility that GKE increases pupation rate, because the control already has high pupation rate (0.850) and it might be hard to detect significant improvement in a limited data set.

$Fe_2(SO_4)_3$: A cardinal feature of PKAN is iron accumulation in the basal ganglia. Iron is a potent catalyst for the Fenton reaction. The presence of additional iron will almost certainly result in more oxidative stress. However, it is also possible that under oxidative stress the crucial Fe–S linkage will be damaged and additional iron might help mitochondrial function. Does this iron-accumulation alleviate or aggravate the development of the disease? Alternatively, would external iron help *fumble* flies?

Preliminary results for iron (Table 1) did not show significant benefit or damage. This was tested in a second round of experiments (Table 2). The result for the 0.25 mmol/L $Fe_2(SO_4)_3$ group did not deviate significantly from that in the control group. However, at higher concentration (1 mmol/L), evidence of a harmful effect of iron was noted on *fumble* eclosion: just 20 flies eclosed from 230 larvae, vs 36 adults from 227 larvae in the control group (Table 2, >95% significance level). It is noteworthy that this level of iron (1 mmol/L) is much higher than physiological iron concentrations in humans.

DFO: DFO (deferoxamine mesylate) is an iron chelator approved for human use to reduce iron overload. In the preliminary experiment, the 0.2 mmol/L DFO group did not differ significantly from the normal group, but the 1 mmol/L DFO group showed higher eclosion rate (0.246 vs 0.129, $p < 0.05$). Further experiments failed to confirm this result. Taking both rounds of experiments (Table 2), the data set obtained for the eclosion rates in the 1 mmol/L DFO group and the control is not big enough to allow a definitiue conclusion (0.223 vs 0.156, Table 2), although a larger data set might reveal significance.

EDTA: EDTA is a general metal ion chelator. It effectively chelates various heavy metals, including zinc. Because many metals, including iron, copper, zinc and manganese, have been shown to be involved in neurodegeneration, EDTA might have some effect on *fumble*.

At the indicated dosages (0.2 and 1 mmol/L), EDTA had no significant effect on *fumble* flies. The high-dosage EDTA group (1 mmol/L) had 97 pupae from 130 larvae vs control 109 from 128 larvae. At even higher dosage, however, some toxic effect was observed, without any benefit.

Acetylcysteine: Cysteine is another substrate for the synthesis of CoA. Pank defects lead to cysteine accumulation. External addition of cysteine might thus be justified as it might drive more CoA synthesis. In contrast, if cysteine induces iron accumulation and creates oxidative stress, additional cysteine would not then be beneficial. Acetylcysteine is absorbed more efficiently and is converted to cysteine intracellularly.

For *fumble* larvae, we found that addition of 10 mg acetylcysteine to a vial with 10 ml of food (1.0 mg/ml) produced slightly lower pupation and elcosion rates (0.692 and 0.069 vs 0.852 and 0.129, Table 1), while 0.2 mg/ml acetylcysteine had a milder effect. However, the data set is not large enough for statistical significance, but the data at least suggest no

benefit from application of this compound at these levels. Higher dosage of acetylcysteine (4 mg/ml) causes severe reduction of viability, indicating serious toxicity.

Creatine: In the human body creatine is changed into phosphocreatine, which serves as a reservoir for rapidly accessible energy. Protective effects of creatine in some neurodegeneration diseases, including ALS, have been reported (for review, see Tarnopolsky and Beal 2001).

In all three aspects studied (pupation, elosion and adult survival) in *fumble* flies, there were no statistical differences between the creatine-fed group and the normal group.

Vitamin C: Addition of 10 mg or 50 mg vitamin C to each vial (1 or 5 mg/ml) did not result in significantly more pupae or adults, indicating that the effect of vitamin C on *fumble* flies is limited. At high dosage (5 mg/ml), there were some sign of increased eclosion rate, accompanied by reduced rate of pupal formation. However, these results did not show statistical significance at $p < 0.05$, and the inconsistent beneficial effects on pupation and eclosion suggest little overall value for vitamin C in treating *fumble* flies.

Vitamin E: Because the experiment described above suggested that antioxidants appear to be helpful for treating *fumble* flies, we also examined vitamin E for its rescue ability in the fly model. Vitamin E was independently tested in another experiment, side by side with ATP and controls (Table 3). We observed 25% rate of eclosion for the control group, and 40.3% for vitamin E-treated group, suggesting that vitamin E is beneficial to *fumble* (significance $>99\%$).

DISCUSSION

Positional cloning of PKAN made possible rational design of therapies to slow the process of disease development. Using *fumble* as a model for PKAN, we tested a number of compounds for their possible efficacy. Because PKAN is due to a defect that is central to the cellular metabolism and the cell membrane is not permeable to the products used, complete rescue is expected to be difficult with dietary measures. Unsurprisingly, none of our tested compounds dramatically saved the flies from early lethality. However, GKE, and possibly vitamin E, showed beneficial effects on the survival of *fumble* larvae, as reflected by statistically significant higher eclosion rates. Results with iron therapy were unconvincing. DFO intake appears to have a minor effect, but the data did not reach adequate statistical significance for a definite conclusion. Pantothenate, a natural candidate for the therapy, is seen to be not helpful at all.

It is interesting that vitamin C has no beneficial effect whereas GKE and VE do, given that all three compounds are antioxidants. We suspect that only hydrophobic or lipid-soluble antioxidants have a positive influence on *fumble* eclosion. The GKE compound we used is a mixture of *Ginkgo biloba* extract (itself a complex mixture of many components) and flavone in which we are not sure precisely which component or combination of components generates the beneficial effect. We speculate that the beneficial effect seen arises through a general protective effect of these compounds on the oxidative damage produced in Pank deficiency, or through reduction of the lipid turnover rate so that more CoA can be channelled to other essential metabolic pathways.

Eclosion rates of controls differed slightly at different times. This might be due to the fact that growth conditions were slightly different. First, food had to be made afresh each time. It is known that food condition—for example, addition of propionic acid or the freshness or dryness of the food—can cause pupation and eclosion rates to differ. There are two foods involved here; one is grape plate for egg collection, where the eggs hatch and grow until second instar stage, when they are big enough to be accurately picked by shape and transferred to control food or food with compounds added. External temperature and humidity might be another variable. Nevertheless, when experiments were performed side by side we observed minimal variations; thus, conclusive results can be and have to be drawn from parallel experiments performed side by side.

Our results imply the necessity, few caution when treating PKAN patients with several compounds. While two antioxidants, GKE and vitamin E are helpful for *fumble* larvae, acetylcysteine and NAC-600, also antioxidant compounds, could well have the opposite effect. NAC-600 also contains acetylcysteine, suggesting that its toxic effect on *fumble* may derive from its acetylcysteine content. This also indicates that the cysteine accumulation observed in PKAN may be harmful, and suggests that reduction of cysteine accumulation may be a beneficial therapy for PKAN or HSS patients.

Previously, *bubblegum*, a potential fly model for adrenoleukodystrophy (ALD) has been successfully used to study the effect of 'Lorenzo's oil', a mixture of unsaturated fatty acids, in lowering elevated levels of very long-chain fatty acids (Min and Benzer 1999). *bubblegum* and ALD, though displaying some similar features, are in fact caused by different molecular defects. We consider that *fumble* could serve as a good model for dietary or other pharmacological rescue studies of PKAN, because it not only manifests similar phenotypes to that in human PKAN patients but also arises from the same genetic defect. Furthermore, the affected pathway is highly conserved in evolution: the basic mechanism of CoA metabolism is virtually the same between *Drosophila* and humans. This conservation of disease mechanism implies that results obtained from the flies could be applied to humans.

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