Journal Club

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Mad Fly Disease

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Review of Gavin et al. (http://www.jneurosci.org/cgi/content/full/26/48/12408)

The prion disorders are a rare and intriguing group of neurodegenerative diseases characterized by spongiform degeneration of the brain, accumulation of a misfolded form of a host protein [the prion protein (PrP) or, in its misfolded form, PrP^{Sc}] and, uniquely, an etiology that can be inherited, sporadic or transmitted from host to host (Prusiner, 1998). Although the details of the mechanism whereby these diseases propagate remains unclear, the use of many different animal and cellular models over the past 50 years, ranging from primates to unicellular yeast, have greatly increased our understanding of their molecular basis (Collinge, 2005). Recently, Gavin et al. (2006) have added to the list of species used to model these disorders with the development of a *Drosophila melanogaster* model of Gerstmann–Sträussler–Scheinker syndrome (GSS), an inherited form of prion disease. *Drosophila* presents an attractive species in which to develop a prion disease model, with low generation time, low maintenance costs, a well studied system for quantitative trait loci analysis and malleability to genetic manipulation.

After an attempt to generate a fly prion disease model by expression of a murine PrP transgene containing an octopeptide repeat mutation causative for familial Creutzfeldt-Jakob disease (CJD), Gavin et al. (2006) used a mouse prion protein transgene containing a proline to leucine mutation at codon 101 (P101L), homologous to a human mutation (P102L) which causes GSS. A similar transgene was used in the generation of the first mouse model of familial prion disease, which was no doubt part of the reason why this construct was chosen for the *Drosophila* experiments (Hsiao et al., 1990). The authors opted to cross wild-type or mutant PrP transgenic flies ("target") with transgenic flies containing transcriptionally active ("driver") promoters preferentially targeting either cholinergic (WT^{CH}/$P101L^{CH}$) or dopaminergic (WT^{DA}/$P101L^{DA}$) neuronal populations [Gavin et al. (2006), their Fig. 1*A–C* (http://www.jneurosci.org/cgi/content/full/26/48/12408/F1)]. Expression of mutant P101L PrP induced developmental defects that were absent in wild-type (WT) flies: nearly half of the larvae expressing $P101L^{CH}$ failed to emerge from the pupa, whereas nearly 30% failed when expressing $P101L^{DA}$ [Gavin et al. (2006), their Table 1 (http://www.jneurosci.org/cgi/content/full/26/48/12408/T1)]. Larvae expressing either WT^{CH} or WT^{DA} with a similar level of PrP protein as corresponding mutants were able to eclose normally. Surviving mutant $P101L^{CH}$ flies exhibited varying degrees of locomotor dysfunction through adulthood, and died by 3–4 weeks instead of the 8–10 week lifespan of the wild-types [Gavin et al. (2006), their Figs. 3 (http://www.jneurosci.org/cgi/content/full/26/48/12408/F3) and 4 (http://www.jneurosci.org/cgi/content/full/26/48/12408/F4)]. Interestingly, the mutant $P101L^{DA}$ flies surviving the larval stage displayed only mild and statistically insignificant coordination defects, and yet 20–90% of the time died within the first 24 h after eclosion. Although this suggests that any general neuronal expression of P101L may have deleterious effects in *Drosophila*, it does not clarify the reason for the marked response displayed by $P101L^{DA}$ flies. The authors are currently investigating the heterogeneous lethality observed in these $P101L^{DA}$ flies.

The neuropathology of larval and adult transgenic flies suggests a specific deficit caused by expression of $P101L^{CH}$. Using a green fluorescent protein-encoded transgene available on one of the "driver" lines of flies, Gavin et al. (2006) were able to visualize the cholinergic neurons in the larvae [Gavin et al. (2006), their Fig. 2*A* (http://www.jneurosci.org/cgi/content/full/26/48/12408/F2)]. Prion protein expression appeared in intracellular and extracellular aggregates in the $P101L^{CH}$ larvae instead of the expected cytoplasmic localization of PrP in WT^{CH} flies [Gavin et al. (2006), their Fig. 5 (http://www.jneurosci.org/cgi/content/full/26/48/12408/F5)]. The surviving mutant $P101L^{CH}$ flies that escaped the eclosion deficits showed a progressive increase in vacuolar pathology and PrP inclusion bodies compared with WT^{CH} flies. This mirrors vacuolar pathology seen in the P101L over-expressing mouse, although it should be noted that the human form of this disease does not exhibit spongiform degeneration (Table 1).

Received Dec. 15, 2006; revised Jan. 3, 2007; accepted Jan. 4, 2007.

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DOI:10.1523/JNEUROSCI.5427-06.2007

 0270-6474/07/270971-02$15.00/0

Table 1. Characteristics of human GSS P102L cases and corresponding model systems

	Human	Mouse		Fly
Mutation (background)	P102L (huPrP)	P101L (moPrP, overexpressed)	P101L (moPrP, knock-in)	P101L (moPrP, overexpressed)
Phenotype	Ataxia	Ataxia, rigidity, lethargy	None	Developmental, locomotor
Pathology	PrP amyloid deposits	Vacuoles, gliosis	None	Vacuoles, inclusion bodies
Protease resistance	Yes	Yes, but low	No	No
Transmissibility	*circa* 1/3 cases	No	No	No

huPrP, Human PrP; moPrP, mouse PrP.

PrP^{Sc} as an infectious entity was originally defined by its biochemical properties, notably insolubility and protease resistance, and, although the nature of the infectious agent in the prion diseases remains a subject of debate, these two characteristics are frequently used as markers for disease (McKinley et al., 1983). Based on this, Gavin et al. (2006) investigated the proteinase K resistance and Sarkosyl solubility of PrP expressed in *Drosophila* [Gavin et al. (2006), their Fig. 6*C*,*D* (http://www.jneurosci.org/cgi/content/full/26/48/12408/F6)]. Surprisingly, there was no increase in protease resistance or solubility associated with the presence of the P101L mutation. The altered biochemical properties of PrP^{Sc} are thought to reflect a change in protein conformation away from the normal cellular fold. The authors investigated this concept using a commercially available conformation-sensitive immunoassay for misfolded, disease-associated PrP. In contrast to the gross biochemical analysis, the P101L mutant was differentially recognized in this assay, suggesting that there is a conformational divergence from the wild-type protein. Thus, the "mutant" flies generate PrP that lacks protease resistance, but possess aberrantly folded PrP. Interestingly, there are well characterized examples of protease resistance in the absence of prion disease and prion disease in the absence of protease resistance, indicating that there is a complex relationship between conformational alterations in PrP and disease (Lasmezas et al., 1997).

Although P102L is the most common PrP mutation linked to GSS, other amino acid alterations covering a wide phenotypic spectrum have been described. GSS is also an atypical prion disease in that it, unlike CJD, presents predominantly as an amyloidopathy. This may mean that, although this model is useful with regard to understanding the pathobiology of P102L GSS cases, extrapolating these findings to other GSS mutations or other forms of prion disease might not be straightforward. This model, with an easily measurable phenotype does, however, represent a powerful tool for the delineation of genetic factors modifying prion disease progression (Table 1). One need only look at the impressive results gained with a *Caenorhabditis elegans* model of amyloid β toxicity to see how useful such reductionist models of disease can be (Cohen et al., 2006). One potentially interesting set of experiments would be to investigate the transmissibility of this phenotype, by inoculation into either transgenic flies or mice. Although it is unlikely that detectable infectivity would be generated in this model system, this is one aspect of prion biology not addressed in the current paper and, as a glance at the history of prion diseases will emphasize, stranger things have happened.

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