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ADDENDUM

A commentary on the utility of a new L-DOPA-responsive dystonia mouse model

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ABSTRACT
In a recent issue of Brain, we reported on the generation and characterization of a mouse model of the rare disease L-DOPA-responsive dystonia (DRD). Here, we discuss the utility of these mice for understanding broader disease processes and treatment strategies. Using specific experimental designs that either work “forward” from genetic etiology or “backward” from the symptomatic presentation, we discuss how our data and future work can be used to understand broader themes.

KEYWORDS
Basal ganglia; dopamine; neurogenetics; striatum; tyrosine hydroxylase

Background
L-DOPA-responsive dystonia (DRD) is a rare neurogenetic disorder caused by mutations in enzymes necessary for dopamine synthesis.1-3 Patients with DRD exhibit lower limb or generalized dystonia in early childhood. Those symptoms exhibit diurnal fluctuations and an excellent response to L-3,4-dihydroxyphenylalanine (L-DOPA)4,5. Loss of tyrosine hydroxylase (TH) protein content and striatal dopamine without markers of degeneration are considered the hallmark neuropsychopathies associated with DRD.6,7 DRD is most commonly associated with autosomal dominant mutations in GTP cyclohydrolase 1 (GCH1). GCH1 is necessary for the initial step in the synthesis of tetrahydrobiopterin, a cofactor for TH, the rate limiting enzyme in catecholamine synthesis.3,8,9 Autosomal recessive mutations in TH itself also cause DRD,2 or a more severe infantile parkinsonian syndrome depending on the mutation.10-12

In a recent edition of Brain, we reported on the generation and characterization of a new knockin mouse model of DRD.13 These mice carry a point mutation in mouse Th (c.1160C>A; p.382Q>K) homologous to the c.1141C>A; p.381Q>K mutation in TH described in a Dutch family.14 Mice homozygous for this mutation display many of the core features of DRD including reduced TH enzymatic activity and immunostaining without nigral degeneration, as well as dystonic movements, diurnal fluctuations in symptoms, and amelioration of symptoms with L-DOPA. Here we present thoughts on the utility of these mice, including an approach for understanding broader disease processes outside of the context of the rare and treatable DRD cases.

Animal models of rare diseases
The utility of an animal model typically relies on how well it satisfies 3 criteria: construct, face, and predictive validity.15 Construct validity is satisfied if the causative factor of the model mimics the etiology of the human disorder. Face validity refers to whether the animal displays the outward features that resemble those of the human disorder. An animal model has predictive validity if treatments developed with the model go on to show efficacy in humans. Often predictive validity is established by demonstrating that treatments known to be effective in humans are also effective in the model. For instance, the tail suspension and forced swim tests were established as predictive models of depression based on the fact that proven...
Experiments with this animal models with established construct validity. Though rarely discussed, an animal model of a rare disease must often satisfy a fourth criteria: the animal must be able to provide broader lessons that can be applied to different fields of study. Further, these lessons should translate into broadly applicable treatment strategies. This criteria is especially true for an animal model of DRD, for which L-DOPA already provides an excellent treatment option. In our work with the DRD mice, our goal was to satisfy this fourth criteria since a better understanding of DRD may provide broad insight into other dystonic disorders and disorders associated with low brain dopamine levels, such as Parkinson disease.

Experiments using animal models typically follow 2 different approaches. One approach uses etiological animal models with established construct validity. Experiments with this “forward” approach attempt to ascribe specific dysfunction caused by the etiology to the presentation of the disorder. Alternatively, experiments may use a symptomatic animal model and work “backward” to ascribe specific dysfunction to the symptomatic presentation. Both approaches have their strengths and limitations and are ultimately only as advantageous as their ability to generate knowledge of the underlying biology of disease processes and treatment strategies. Below, we use DRD mice to highlight both approaches and comment on how the results provide broadly applicable lessons.

**Forward approach**

The DRD knockin mice were designed to exhibit construct validity, making studies using the “forward” approach logical. Using biochemical and immunohistochemical techniques, we showed that the DRD mice exhibited reductions in TH protein content and activity. This was due to a deficit in stability of the p.382Q>K mutant, which was also observed in a study using the human homologous p.381Q>K mutation in a heterologous expression system.12 Our results expand on those in vitro findings to illustrate that these effects were specific to dendritic and axonal compartments of midbrain dopamine neurons. We observed a severe reduction in TH protein content and activity in the striatum which contain the axonal projections of midbrain dopamine neurons. The soma of the midbrain neurons themselves exhibited strong immunostaining for TH and TH activity in midbrain was only modestly reduced. TH instability and location-specific loss of TH immunostaining were also seen in another new mouse model of TH deficiency, which carry the p.223R>H TH mutation.17 These results illustrate the importance of combining animal model work with in vitro findings for fully elucidating disease processes and provide a broader lesson that can be applicable to other neurogenetic disorders in which the stability of mutant protein products is affected. If the protein of interest is required to be active in axons and dendrites, then pharmacological chaperones that promote the stable transport of the mutant protein to these regions could provide a valuable treatment option. Therapies in which pharmacological chaperones are used to stabilize mutant proteins are being discussed as potential treatments for lysosomal storage diseases,18 phenylketonuria,19 and Friedreich’s ataxia,20 to name a few neurogenetic disorders in which protein misfolding and instability are central pathologies. DRD mice not only provide another example of protein instability, but also provide an animal model to test novel pharmacological chaperones. Such compounds could be administered to DRD mice to test their ability to boost TH protein content in the axons and dendrites of midbrain dopamine neurons. Further, since the DRD mice have a measurable motor phenotype, the efficacy of these compounds in reversing symptoms without producing treatment-obscuring side effects could also be tested. Likewise, DRD mice may prove useful for testing gene therapy or gene repair approaches to treatment.

**Backward approach**

We also illustrated that experiments can be designed with the DRD mice using the “backward” approach to uncover the neural mechanisms of dystonic movements. Dystonia is a heterogeneous disorder with a long list of both inherited and acquired causes.21 Thus, by probing mechanisms directly related to phenotype, the “backward” approach can be used to uncover mechanisms central to dystonia, arising from several different etiologies. With this approach we delineated the dopamine receptor mechanisms of the dystonic movement in the DRD mice. We showed that administration of either D1-type or D2-type dopamine receptor antagonists exacerbated the dystonic movements. Thus, both D1-type and D2-type dopamine receptors play a role in dystonic
movements. Further, by examining the behavioral response to dopamine receptor agonists, we showed that signaling through both dopamine receptor subtypes was abnormal. Specifically, signaling through D1-type dopamine receptors was supersensitive while signaling through D2-type dopamine receptors was blunted or altered in valence. These findings argue that maladaptive changes to both the D1-type or D2-type dopamine receptors may play a role in many different types of dystonic movement. A similar “backward” approach will be useful to understand downstream signaling pathways, developmental processes, and the role of other transmitters.

**Predictive studies with DRD Mice**

Similar to the “backward” approach, predictive studies with the DRD mice aim to manipulate the phenotypic presentation of the mice. The difference between the 2 approaches is that predictive studies ask whether known treatments for humans are also efficacious in mice. We showed that both trihexyphenidyl and L-DOPA reduce dystonic movements in DRD mice. Trihexyphenidyl, a nonselective muscarinic receptor antagonist, is effective in many different forms of dystonia, including DRD. Trihexyphenidyl, also causes untoward side effects that limit its utility. Thus, the efficacy of trihexyphenidyl in DRD mice opens a logical line of predictive studies testing subtype-selective muscarinic receptor antagonists for efficacy and side effect profiles in DRD mice. L-DOPA, on the other hand, is effective in reducing dystonia in DRD patients, but rarely effective in other forms of dystonia. Thus, investigators might be misled into concluding that the L-DOPA response in DRD mice does not provide insight. Instead, one should consider why L-DOPA is not effective in patients who do not carry mutations in GCH1 or TH. L-DOPA requires several processes to occur in order to be effectively packaged and released as dopamine. Once in the brain, L-DOPA must be transported into the dopamine terminal, converted to dopamine by aromatic acid decarboxylase, and packaged into synaptic vesicles by the vesicular monoamine transporter 2. When dopamine synthesis is not grossly affected, as is likely the case for most dystonias, therapeutic L-DOPA is in competition with endogenously synthesized dopamine for these processes, limiting is efficacy. Although dopamine receptors may be dysfunctional in these patients, as they are in DRD mice, L-DOPA is not likely to solve those postsynaptic problems.

Direct dopamine receptor agonists might prove to be far more useful than L-DOPA for reversing the types of dopamine receptor dysfunction we observed in the DRD mice. Indeed, direct D2 dopamine receptor agonists have been effective in reducing dystonia in a handful of small-scale studies with various types of dystonia, though many patients in these studies showed no benefit. These studies show that dopamine receptor agonism has promise as a treatment strategy for dystonia, but needs improvement. Isolating specific signaling cascades downstream of dopamine receptors is one way to isolate a specific anti-dystonic effect while limiting off-target effects. Recently, several ligands that promote such biased signaling downstream of the D2 dopamine receptor have been synthesized. Further, the biased properties of several clinically approved D2 dopamine receptor agonists have been recently characterized. Predictive studies with biased ligands could be employed in the DRD mice to better understand the signaling systems involved in dystonia as well as guide better treatment strategies for the disorder.

**Concluding thoughts**

The DRD mice present an excellent model of the rare disease caused by the p.381Q>K TH mutation in that they satisfy construct, face, and predictive validity. By carefully selecting the research questions, DRD mice are likely to provide broader lessons about disease processes as well as treatment strategies that may be effective in many forms of dystonia.

**Abbreviations**

- L-DOPA  L-3,4-dihydroxyphenylalanine
- DRD  L-DOPA-responsive dystonia
- GCH1  GTP cyclohydrolase 1
- TH  tyrosine hydroxylase

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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