IN BRIEF

DRUG DISCOVERY

Using loss-of-function human mutations to evaluate drug targets

The identification of individuals with loss-of-function (LOF) variants in a gene of interest can provide information about gene function and disease biology, as well as the potential safety and phenotypic effects of therapeutic targeting. With the aim of guiding the interpretation of human LOF variation in drug development, Minikel et al. interrogated their Genome Aggregation Database (gnomAD) — a catalogue of 15,708 whole genomes and 125,748 exomes in 141,456 individuals, designed to capture the extent of genetic variation among a large group of individuals — and reported their key findings. Through comparison of constraint in human drug targets, they discovered that even a highly deleterious knockout phenotype may be compatible with a gene being a viable drug target. In addition, they report that given the rarity of LOF variants in most genes, identifying total knockout individuals will require 1,000-fold larger sample sizes. Finally, by manually curating gnomAD data and the scientific literature for six genes associated with gain-of-function neurodegenerative diseases, they demonstrated the value of manual curation of LOF variants in removing artefacts, assessing and interpreting the cumulative allele frequency of true LOF variants, and revealing important error modes or disease biology.

To investigate the value of such large-scale genomic databases in exploring the safety profile of drug targets, Whiffin et al. identified LOF variants in *LRRK2* and assessed associated phenotypic changes in three large cohorts of genetically characterized individuals: the 141,456 individuals sequenced in the gnomAD, 49,960 exome-sequenced individuals from the UK Biobank and more than four million participants in the 23andMe genotyped dataset. LRRK2 kinase inhibitors are currently in early clinical testing for Parkinson disease, although preclinical studies have raised toxicity concerns. Following manual curation of identified variants, 134 unique *LRKK2* LOF variants were identified across the three datasets. Heterozygous LOF variants in *LRRK2* resulted in a systemic lifelong decrease in LRRK2 protein levels, and, importantly, this partial inhibition had no discernible effect on survival or health.

ORIGINAL ARTICLES Minikel, E. et al. Evaluating drug targets through human loss-of-function genetic variation. *Nature* **581**, 459–464 (2020) | Whiffin, L. The effect of LRRK2 loss-of-function variants in humans. *Nat. Med.* https://doi.org/10.1038/s41591-020-0893-5 (2020)

RELATED ARTICLE Mullard, A. Calls grow to tap the gold mine of human genetic knockouts. *Nat. Rev. Drug Discov.* **16**, 515–518 (2017)

OBESITY

Reversing cerebrovascular dysfunction

The mechanisms underlying long-term obesity-associated cerebrovascular dysfunction remain unknown. Here, Shen et al. establish a prolonged diet-induced obesity (DIO) mouse model that suffers from basilar artery (BA) vascular dysfunction, and discover that microglial TAK1 is overactivated in the brainstem in these mice. Pharmacological blockade of TAK1 by injection of 5Z-7-oxozeaenol or genetic deletion of microglial *Tak1* improved BA dysfunction and protected against ischaemic stroke in prolonged DIO mice. Conversely, activation of TAK1 in brainstem microglia impaired BA function in chow-fed mice. Mechanistically, TAK1 activation in prolonged DIO mice increased the production of IL-18 in brainstem microglia. Antibody-mediated blockade of the IL-18 receptor-α improved BA vascular dysfunction in these mice.

ORIGINAL ARTICLE Shen, Q. et al. Reversal of prolonged obesity-associated cerebrovascular dysfunction by inhibiting microglial Tak1. *Nat. Neurosci.* https://doi.org/10.1038/s41593-020-0642-6 (2020)

AUTISM

Rebalancing protein synthesis in fragile X syndrome

There are currently no effective treatments for fragile X syndrome (FXS), the most prevalent inherited monogenic form of autism caused by transcriptional silencing of *FMR1,* the gene encoding fragile X mental retardation protein (FMRP). Writing in *Science Translational Medicine*, Bear, Wagner and colleagues now demonstrate that specific inhibition of glycogen synthase kinase 3α (GSK3α) safely and effectively corrects pathophysiology in a mouse model of FXS.

FMRP is an mRNA-binding protein that functions as a transcriptional repressor, the loss of which results in altered basal protein synthesis and protein synthesis-dependent synaptic plasticity. Rebalancing protein synthesis and synaptic function in FXS therefore represents a promising therapeutic approach.

Despite promising preclinical data, negative-allosteric modulators (NAMs) of metabotropic glutamate receptor 5 (mGluR5; an important regulator of protein synthesis at excitatory synapses) failed in the clinic. It is suspected that tachyphylaxis and dose limitations contributed to this outcome. Another treatment that rebalances protein synthesis, lithium, is also associated with numerous side effects that make it impractical for use in children. The beneficial effects of lithium in FXS are thought to be due to inhibition of GSK3 (which exhibits reduced inhibitory phosphorylation in a mouse model of FXS). However, more selective GSK3 inhibitors were found to stabilize β-catenin and activate gene expression, and therefore have the potential to stimulate malignant growth.

Given that previous studies have indicated that the loss of both GSK3 paralogues, GSK3α and GSK3β, is required to increase β-catenin levels, Bear and colleagues set out to investigate whether paralogue-selective inhibitors might circumvent this potential toxicity.

To do this, the authors treated the *Fmr1*$^{-/y}$ mouse model of FXS with their previously reported GSK3α and GSK3β selective inhibitors, BRD0705 and BRD3731 respectively, which were developed via exploitation of the Asp133 → Glu196 'switch' in the hinge-binding region between GSKα and GSKβ.

Following intraperitoneal dosing, the GSKα-selective and β-selective compounds penetrated the brain and inhibited direct phosphorylation of their respective GSK3 paralogue targets in *Fmr1*$^{-/y}$ mice.

Administration of BRD0705, but not BRD3731, reduced audiogenic seizure incidence in *Fmr1*$^{-/y}$ mice, reduced elevated protein synthesis and mGluR5-dependent long-term depression in *Fmr1*$^{-/y}$ hippocampal slices and corrected hyperexcitability in visual cortex slices. Furthermore, in an inhibitory avoidance learning task, only BRD0705 was demonstrated to reverse deficits in learning and memory in *Fmr1*$^{-/y}$ mice.

Importantly, unlike mGluR5 NAMs, there was no development of hyperlocomotion side effects and chronic administration of the GSK3α inhibitor did not induce tolerance.

In summary, these findings demonstrate the potential of selective inhibition of GSK3α to correct FXS phenotypes, without some of the side effects that have limited the use of other potential therapies. Efforts are underway to optimize this strategy for use in human clinical studies.

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ORIGINAL ARTICLE McCamphill, P. et al. Selective inhibition of glycogen synthase kinase 3α corrects pathophysiology in a mouse model of fragile X syndrome. *Sci. Transl Med.* **12**, eaam8572 (2020)

RELATED ARTICLE Berry-Kravis, E. M. et al. Drug development for neurodevelopmental disorders: lessons learned from fragile X syndrome. *Nat. Rev. Drug Discov.* **17**, 280–299 (2018)