healx

Advancing treatments
for rare diseases

Fragile X Syndrome

Drug Repurposing Summary Report

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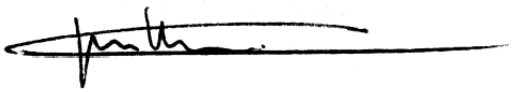
Executive Summary

Healx recommends 8 drugs for investigation as treatments for Fragile X Syndrome. This summary report includes a description of each recommended drug, including supporting evidence.

These drugs have been identified by the Healx Drug Repurposing Workflow, which combines in-depth biocuration, data analytics and expert review by drug repurposing experts. A description of this workflow is also included in this report.

We at Healx look forward to continuing to work with FRAXA to help find treatments for Fragile X Syndrome.



Dr Tim Guilliams, CEO

On behalf of the Healx Team

Content

Drug Repurposing Workflow

Overview

The Healx Drug Repurposing Workflow predicts which existing drugs may treat a particular rare disease. The workflow has four stages:

- Defining a treatment profile to address patients' unmet need.
- Predicting which existing drugs will be effective treatments using computational methods:
    - Drug-Gene Expression Matching (DGEM)
    - Prediction of Repurposed Indications with Similarity Matrices (PRISM)
- Combining the predictions from the computational methods with supporting evidence and literature mining.
- Expert drug discovery review of predictions to generate a list of top drug candidates recommended for further investigation.

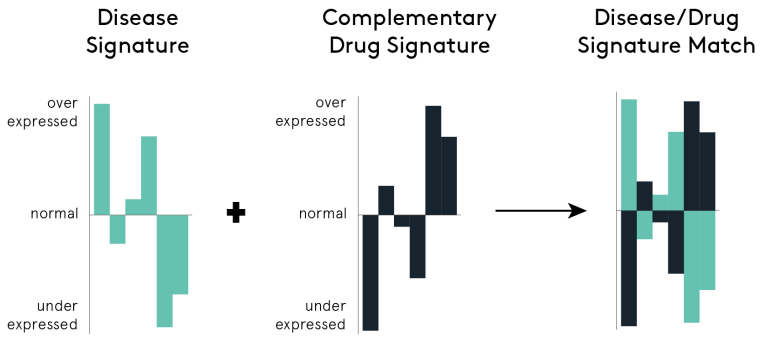
Treatment Profile

The treatment profile defines the key symptoms, affected genes and biological pathways associated with the phenotypes of the disease as well as the desired physical and biochemical properties of an ideal drug for repurposing. More importantly, it describes the desired therapeutic effects of a drug that may improve the patient's quality of life.

Symptomatic treatments for Fragile X Syndrome should increase cognition, alleviate seizures, and moderate mood and behaviour. Neurological pathways are relevant biological targets, such that an orally administered agent must penetrate the blood brain barrier and achieve a pharmacologically active dose.

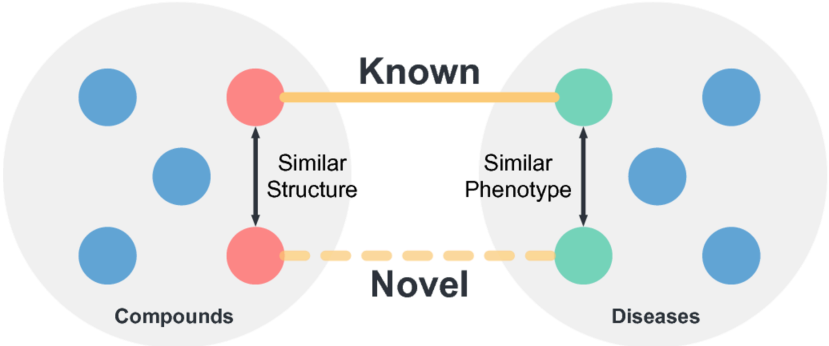
Drug-Gene Expression Matching (DGEM)

Drug-Gene Expression Matching (DGEM) compares the gene expression profile for a disease with the gene expression profiles from Healx's drug database. These profiles are curated from both public and proprietary datasets. We then predict which drugs would be effective treatments: for instance, a drug with the opposite profile to the disease would be a strong candidate (see figure below).



Prediction of Repurposed Indications with Similarity Matrices (PRISM)

Prediction of Repurposed Indications with Similarity Matrices (PRISM) uses the principle that if a drug treats a disease, then a similar drug may treat a similar disease (next figure). To determine the similarity of drugs, PRISM considers target proteins, structural similarity and side effects. To determine the similarity of diseases, PRISM considers target genes, ontological structure and phenotypes. A machine-learning algorithm is then used to combine these similarities to predict novel treatment applications. Example, illustrated here:



Supporting Evidence, Expert Review and Literature Mining

The predictions from DGEM and PRISM are combined with drug and disease data from multiple sources, such as pharmacological properties of drugs, known treatments, clinical trials and scientific papers. This includes both manually curated data and the results of literature mining with natural language processing techniques on millions of scientific papers to find co-occurrences drugs with Fragile X Syndrome.

Healx's drug repurposing experts use these data and literature mining techniques to review each prediction. Eight strong candidates are recommended for further investigation, and are described in the following section.

Gene Expression Datasets

Nineteen gene expression datasets were found for Fragile X Syndrome. Following assessments of biological relevance and validity, and technical checks, four datasets below were selected for the final drug predictions.

A disease profile was generated from each dataset, which was then validated to ensure biological relevance. For instance, genes from relevant pathways, such as neurological signaling pathways, should be enriched in the disease profile. This profile was used to generate scores for each drug, providing a ranking by which the most promising candidates were identified.

Table 1: Characteristics of the gene expression data sources

Dataset	Publication title	Samples analysed	Comment
GSE62721	Molecular Mechanisms Regulating the Defects in Fragile X Syndrome Neurons Derived from Human Pluripotent Stem Cells	Neuronally differentiated iPSC fibroblast samples from 3 patients	Experiment is referenced in literature by Halevy, a key opinion leader.
GSE7329	Genome-wide expression profiling of lymphoblastoid cell lines distinguishes different forms of autism and reveals shared pathways	Lymphoblastoid cell lines from 8 males with FMR1 full mutation.	Although transformed cell lines, data is a sufficiently well-defined case / control study
GSE40630	Divergent dysregulation of gene expression in murine models of Fragile X Syndrome and tuberous sclerosis	Mouse model KO Fmr1. Data from 5 cerebellum and blood, only cerebellum derived samples analysed	Mouse model closely depicts human full mutation. This is a well-designed study
c-westmark	Unpublished feeding study (4 time points: Midnight, 6 AM, noon, 6PM)	Mouse model KO Fmr1. Data from cerebellum and blood, only cerebellum derived samples analysed	Well-designed study on Nimbelgen array. Matches a gene expression profile for an autistic spectrum disorder, with which Fragile X shares many phenotypes.

Drug Repurposing Recommendations

DGEM Predictions

Eight repurposing candidates that were shortlisted by expert review, together with the datasets the predictions resulted from, are summarised in the table 2 below. They are recommended for further *in vivo* investigation either as monotherapies and/or combined with existing medications

Themed mechanisms of action were identified. Gelatinase (MMP-9) inhibitors, PPAR gamma agonists, AMP kinase stimulators and beta-blockers featured in this report, the first two mechanisms represented by drug for which these are 'off-target' effects (disulfiram and sulindac).

Table 2: Summary of Drug Predictions for Further Investigation

DGEM Dataset Prediction	Drug Prediction	Brief Comments
DGEM *Fmr KO mice (GSE40630)*	Topiramate	Anti-epileptic drug Autistic spectrum disorders
	Phenformin	Related to metformin which is linked to memory improvement in fruit fly model
	Quercetin	Antioxidant – possible links to anti-inflammatory effects
DGEM *Fmr KO mice c-westmark*	Penbutolol	Beta-blocker- related link to FXS is propanolol
	Zardaverine	Effective studies in FXS fruit fly model - phosphodiesterase inhibition.
DGEM *Human iPSC (GSE62721)*	Disulfiram	Inhibits MMP9- akin to minocycline mode of action
	Sulindac	Non-steroidal anti-inflammatory drug, also a PPAR agonist. PPARg suggested as FXS pathway.
	Metoprolol	Beta-blocker effect of a related compound, propranolol on autistic behaviours

PRISM Predictions

The results of PRISM are more likely to be symptom-relieving than disease-modifying. Particular symptoms that are addressed by the predictions include seizures, cognitive performance and inflammation (as in CNS inflammation, thought to play a role in Fragile X, particularly in microglial cells). These predicted compounds are detailed below.

Table 3: Summary of PRISM predictions

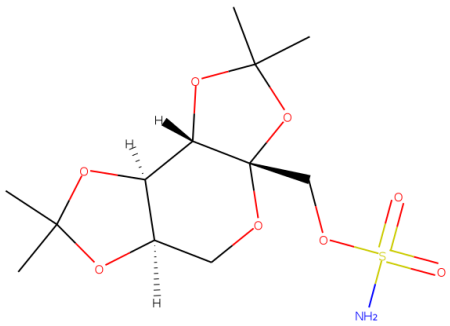
Symptom	Drug	Comments
Seizures	**Lamotrigine, Valproic Acid, Vigabatrin, Gabapentin, Carbamazepine, Topiramate, Levetiracetam, Progabide.**	Mainly antiepileptics, also activity against HDAC
Cognitive performance	**Memantine**	Approved for Alzheimer Disease, and other cognitive disorders such as Huntingdon.
	N-Acetyl Cysteine	Has been suggested to improve cognition as an adjunctive treatment in psychosis (PMID: 27894373). Also suggested for treatment of autistic conditions (although efficacy in autism is a concern).
Anti-Inflammatory	**Prednisone**	Not recommended for chronic use due to tolerability and effect on cognitive function.
	Lithium	Used therapeutically for bipolar disorder, and has inhibitory effect against GSK-3. Similar, but more selective compounds (e.g. Tideglusib) have been suggested for Alzheimer Disease, although to date without sufficient efficacy for approval.

Prediction Interpretations

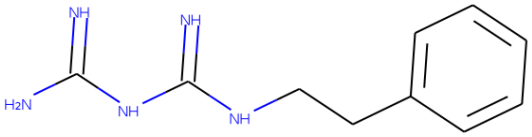
The following sections provide a commentary on the recommended drug predictions listed in Table 1 and the reasoning of the selection.

Drug predictions that stemmed from DGEM mouse *Fmr* KO dataset (GSE40630)

From this dataset listed in Table 1, three predictions emerge: these are **topiramate, phenformin and quercetin.**

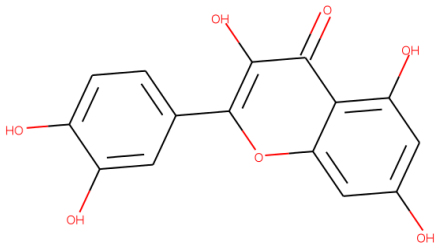


Topiramate is used to treat epilepsy in children and adults, and is indicated for Lennox-Gastaut syndrome in children; there is evidence of widespread off-label use of topiramate, and epilepsy is a phenotype of Fragile X Syndrome, though no specific reports of its use in Fragile X. Topiramate has pleiotropic effects against a number of pharmacological pathways. These include voltage-gated sodium channels; high-voltage-activated calcium channels; GABA-A receptors; AMPA/kainate receptors; and carbonic anhydrase isoenzymes. There is evidence that topiramate may alter the activity of its targets by modifying their phosphorylation state instead of by a direct action. There have been no studies of topiramate in the Fmr1 KO mouse, although this model is perhaps not of direct relevance to the profile of topiramate since Fmr1 KO mice have not been reported to display spontaneous seizures (they are however more prone to audiogenic seizures).



Phenformin is a more cell-permeable (and presumably CNS-permeable) version of metformin. It has been used for the treatment of type II diabetes, although it has been withdrawn since the late 1970s because of reports of life-threatening lactic acidosis. Relative to metformin, to which it is related, phenformin is associated with 10-20 times the rate of fatalities. Metformin is first-line treatment for type II diabetes.

Metformin has been shown to have effects in the Drosophila model of Fragile X, and is able to restore normal circadian behaviour and to rescue the memory deficits in this model. The investigators identified insulin misregulation as underlying the circadian and cognitive phenotypes displayed by the Drosophila Fragile X model, and metformin's effects on insulin sensitivity are presumably important in the observed effect of the drug. It is also worth reporting that insulin growth factor analogues (IGF-1) have been found to have a clinical benefit in related autistic spectrum disorders like Rett Syndrome (notably NNZ-2566 [trofinetide] from Neuren). No reports have been identified of the effect of metformin in the FMR1 mouse model.

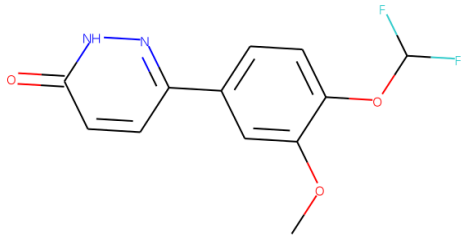


Quercetin is a naturally occurring polyphenol which shows antioxidant, anti-inflammatory, and antiallergic activities. Several recent clinical and preclinical findings suggest quercetin as a promising natural treatment for inflammatory skin diseases. It is an antioxidant and free radical scavenger, but has additional properties as a non-specific protein kinase inhibitor, and oestrogen mimic. It is not an approved drug but it is a plant pigment which occurs naturally in deeply coloured foods such as apples, citrus fruits, peppers, dark cherries, tomatoes, green tea, leafy green and cruciferous vegetables such as broccoli, cabbage and sprouts. There is some limited evidence of various antioxidants in inhibiting the mitochondrial defects in fibroblasts from individuals with Fragile X tremor/ataxia syndrome

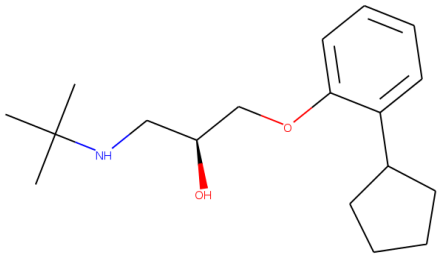
(FXTAS). This syndrome is observed in individuals with a 55-200 expansion of the CGG nucleotide repeat in the 5'-UTR of the FMR1 gene, as opposed to those with >200 repeats, who represent patients with Fragile X Syndrome. As such, it is a model of the 'pre-mutation'. The study showed various mitochondrial defects in FXTAS-affected individuals including increased mitochondrial reactive oxygen species (ROS) production, impaired Complex I activity and increased biomarkers of lipid and protein oxidative-nitrative damage. Quercetin did not normalise all of these markers. This represents relatively weak evidence for possible therapeutic efficacy.

Drug predictions that stemmed from the c-westmark *Fmr* KO dataset

Of the predictions from this set, tianeptine (AMPA stimulator), zardaverine (PDE3/4 inhibitor) and penbutolol have some evidence suggesting possible utility from an analysis of their biological profiles, prior art literature and analysis of the pathways affected. Of these, tianeptine (an atypical antidepressant) is quite promising, and this drug has also been considered by other investigators, such that a study of the drug has been undertaken in the FMR1 knockout mouse. Unfortunately, that study delivered negative results, even though there is no obvious flaw in the experimental design of the study that reached this conclusion.

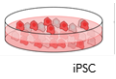


Of the other predictions, zardaverine is a mixed inhibitor of the PDE3 and PDE4 subtypes. Structurally, it possesses the right characteristics for penetration into the CNS. There is some evidence for the benefit of two other PDE4-selective drugs (rolipram and Ro-20-1724) in a Drosophila model of Fragile X Syndrome. This model measured mGluR-dependent LTD in the CA1 region of the hippocampus, which is one of the phenotypes of FXS. Although interesting, this evidence has not been repeated with mixed PDE3/4 inhibitors, nor has it been translated to mammalian models of FXS.

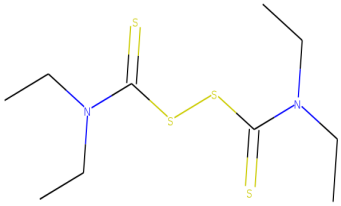


Finally, penbutolol is a beta-blocker (specifically it is a mixed beta-1/beta-2 antagonist, with additional 5-HT1A properties). There is a case report referring to the effect of another beta-blocker (propranolol) on stereotyped behaviours in a man with pervasive developmental disorder and Fragile X Syndrome. This report dates from 1991, and has not been replicated in larger studies, nor with any other

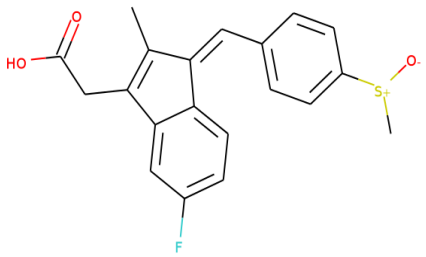
beta blocker. Propranolol is also a mixed beta-1/beta-2 blocker, but lacks the 5-HT1A effects of penbutolol. The beta-blocker class is quite heterogeneous, and includes drug with various agonist and antagonist effects against the beta-1 and beta-2 adrenergic receptors. We cannot conclude from this single report, whether penbutolol has a robust effect in Fragile X Syndrome, nor whether it possesses superior efficacy to other beta-blockers. This is especially the case since there is no report of the efficacy of any beta-blocker in the FMR1 knockout mouse model.



Drug predictions that stemmed from the FXS iPSC dataset (GSE62721)

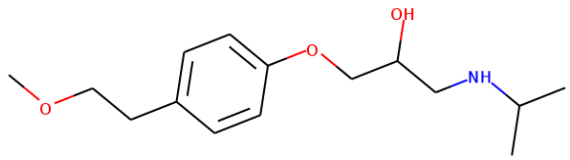


This dataset revealed three predictions of interest. Firstly, disulfiram, which is used for the treatment of alcohol addiction, also inhibits gelatinase (MMP-9). This is the presumed mode of action of minocycline, which has been studied both preclinically and clinically in FXS. Other evidence suggests disulfiram has anti-inflammatory effects via inhibition of NF-kB. The relevance of these pharmacological effects at concentrations achievable in the CNS after oral dosing at approved levels needs to be checked.



The second is sulindac, which although primarily recognised as an NSAID (non-selective inhibitor of cyclooxygenase), is also, like other drugs of this class, a PPAR-gamma agonist. PPAR-gamma has been suggested as a pathway in FXS. Preliminary results suggest that PPAR-gamma agonists such as pioglitazone and rosiglitazone interact with intracellular transduction signals (e.g. GSK3β, PI3K/Akt, Wnt/β-Catenin, Rac1 and MMP-9), and that interaction with these pathways can improve memory recognition in FXS animal models. As a CNS-penetrant anti-inflammatory drug, effects on microglial cells could be relevant in FXS. Note that both the PPAR-gamma agonist rosiglitazone and certain NSAIDs such as ibuprofen and diclofenac have been associated with excess cardiovascular risk; rosiglitazone is withdrawn in Europe and heavily monitored in its use in the USA for the treatment of type II diabetes. The restrictions on ibuprofen and diclofenac are much less stringent, but this is more because of the generally wider acceptance of the drugs for the treatment of inflammatory pain rather than because of any

inherently better side effect profile. The acceptable cardiovascular risk in a condition like FXS has not been established.



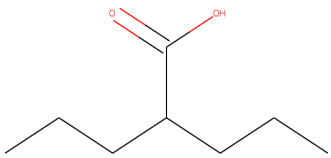
Lastly, metoprolol is another beta-blocker which is analogous to penbutolol (see above for a full analysis of this candidate). It has both beta-1 and beta-2 adrenergic blocking activity, but is more selective for the former than propranolol, which is the drug on which the case report is based.

PRISM Predictions

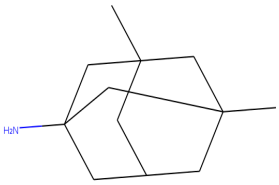
It is encouraging to see that several compounds with existing links in literature to Fragile X have been predicted despite not being part of the training set, such as valproic acid, lithium, lamotrigine and others. Some of the predictions, like anaesthetics, are not likely to be recommendable for chronic use in a condition such as Fragile X Syndrome.

The nature of the GBA method means that compounds closely related to the known associations for Fragile X used as training will be highly ranked. Within these results we see three additional statins and several other compounds similar to lovastatin. Several compounds, such as propofol, topiramate and progabide appear largely because they share a GABA target with arbaclofen and ganaxolone according to DrugBank curated target protein information.

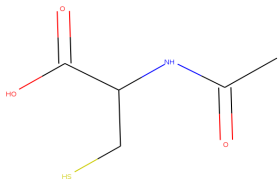
Other predictions were more strongly influenced by evidence of trials in related diseases, for example levetiracetam (which has been used to treat Tourette Syndrome), N-acetylcysteine (which has been studied in a phase 2 trial for Tourette Syndrome and several other rare diseases) and memantine (used to treat many diseases, the closest similarities to Fragile X being Huntington's disease and Down syndrome).

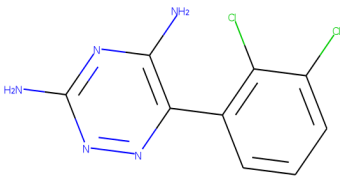


The results of this analysis are more likely to be symptom-relieving than disease-modifying. Particular symptoms that are addressed by the predictions include seizures, cognitive performance and inflammation (as in CNS inflammation, thought to play a role in Fragile X Syndrome, particularly in microglial cells). As far as seizures are concerned, the predictions include a number of antiepileptics (lamotrigine, **valproic acid**, vigabatrin, gabapentin, carbamazepine, topiramate, levetiracetam, progabide etc), even though from a mechanistic point of view these represent a heterogeneous set of drugs. Statins, from epidemiological studies, have also been suggested to have effects against post-stroke seizures (PMID:26203092), presumably an effect derived from off-target activity against HDAC rather than their classical activity as HMG-CoA inhibitors.



Regarding cognitive function, the list includes compounds such as **Memantine** (left), which is approved for Alzheimer Disease and has been proposed for other cognitive disorders such as Huntington. **N-Acetyl Cysteine** (right) is also on the list, and has been suggested to improve cognition as an adjunctive treatment in psychosis (PMID: 27894373); it has also been suggested for the treatment of autistic conditions, although recent findings regarding its efficacy in autism have been disappointing.





The final group of predictions seem to centre around anti-inflammatory compounds. This includes the corticosteroids, such as **Prednisone** (left), which would not be recommended for their chronic use because of tolerability issues, as well as negative consequences regarding cognitive function. It also includes **Lithium** which is used therapeutically for bipolar disorder and has, among its multiple targets, an inhibitory effect against GSK-3. Similar, but more selective compounds (such as tideglusib) have been suggested for Alzheimer disease, although to date without sufficient efficacy for approval.