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Implications of *de novo* mutations in guiding drug discovery: A study of four neuropsychiatric disorders

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A B S T R A C T

Recent studies have suggested an important role of *de novo* mutations (DNMs) in neuropsychiatric disorders. As DNMs are not subject to elimination due to evolutionary pressure, they are likely to have greater disruptions on biological functions. While a number of sequencing studies have been performed on neuropsychiatric disorders, the implications of DNMs for drug discovery remain to be explored.

In this study, we employed a gene-set analysis approach to address this issue. Four neuropsychiatric disorders were studied, including schizophrenia (SCZ), autistic spectrum disorders (ASD), intellectual disability (ID) and epilepsy. We first identified gene-sets associated with different drugs, and analyzed whether the gene-set pertaining to *each* drug overlaps with DNMs more than expected by chance. We also assessed which medication classes are enriched among the prioritized drugs. We discovered that neuropsychiatric drug classes were indeed significantly enriched for DNMs of all four disorders; in particular, antipsychotics and antiepileptics were the *most* strongly enriched drug classes for SCZ and epilepsy respectively. Interestingly, we revealed enrichment of several unexpected drug classes, such as lipid-lowering agents for SCZ and anti-neoplastic agents. By inspecting individual hits, we also uncovered other interesting drug candidates or mechanisms (*e.g.* histone deacetylase inhibition and retinoid signaling) that might warrant further investigations. Taken together, this study provided evidence for the usefulness of DNMs in guiding drug discovery or repositioning.

1. Introduction

The past decade has witnessed rapid development in sequencing technologies. Whole-exome and whole-genome sequencing enables the discovery of many *de novo* mutations (DNM) (mutations present in the offspring but absent in either parent) in Mendelian as well as complex diseases. Recent studies have suggested an important role of DNM in neuropsychiatric disorders, such as schizophrenia (SCZ), autistic spectrum disorders (ASD), intellectual disability (ID) and epilepsy (Gauthier and Rouleau, 2012; Acuna-Hidalgo et al., 2016; Veltman and Brunner, 2012). De novo mutations are rare and unlike inherited variants, they are not subject to elimination due to evolutionary pressure. They are therefore likely to be have larger effect sizes on disease risk and more significant disruptions on biological functions (Veltman and Brunner, 2012). While a relatively large number of sequencing studies have been performed on neuropsychiatric disorders, their implications for the development for new therapies are rarely explored. Ideally, for affected individuals harboring DNM that are likely pathogenic, a "precision medicine" approach can be applied, such that the therapy will specifically target the mutations. This approach is however challenging and costly as hundreds of mutations have been identified for each of the abovementioned neuropsychiatric disorders.

In this study, we investigated another approach by considering the collection of DNM instead of focusing on a single mutation. We hypothesized that the *DNM* as a whole will reflect disease pathophysiology, and they might be associated with drugs known to treat or potentially useful for the diseases under study.

We focused on four neuropsychiatric disorders (SCZ, ASD, ID, epilepsy) here. Recent studies have shown genetic overlap between these four disorders (Li et al., 2016) and hence we will study them together. In terms of pharmacological treatment, a number of antipsychotics and antiepileptics have been developed for SCZ and epilepsy respectively. However, as a whole, different psychiatric medications are also commonly prescribed for these disorders, including for ASD and ID (Jobski et al., 2017; LeClerc and Easley, 2015; Tsiouris et al., 2013; Perr and Ettinger, 2011).

We are interested in this question: are gene-sets associated with neuropsychiatric drug classes over-represented among the DNMs? Specifically, we hypothesized that antipsychotic gene-sets may be over-represented among the DNMs of SCZ, and a similar relationship exists for antiepileptics and DNMs of epilepsy. We also expect enrichment of neuropsychiatric drug classes for ASD and ID due to shared genetic bases (Li et al., 2016; Owen, 2012) and clinical comorbidities (Belardinelli et al., 2016; Einfeld et al., 2011) with other neuropsychiatric diseases. If our hypothesis is true, the approach also serves as a way for drug discovery or repositioning based on DNMs:

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drugs whose gene-sets are significantly over-represented (but not indicated for the disorder) can serve as candidates for repositioning.

Using human genomic data to facilitate drug discovery and repositioning is gaining increased attention and will have very important impact on public health (Nelson et al., 2015; Stitziel and Kathiresan, 2017; Floris et al., 2018). For example, it has been shown that the proportion of drugs with genetic support from genome-wide association studies (GWAS) increased significantly along the development pipeline (Nelson et al., 2015), suggesting an importance role of GWAS signals in improving the efficacy in drug development. As sequencing cost continues to drop and more DNM studies are conducted, is intriguing to ask whether DNMs may also be useful to guide drug discovery, and if so, which drugs and drug categories may be prioritized using this approach. To our knowledge, this is the first study to address the above research question.

It is worth mentioning that here we adopted a "multi-target" paradigm for drug discovery. While conventional drug development focuses on single drug targets, many diseases involve complex interplay of multiple genes and pathways, and it has been argued that a multi-target approach may reveal drugs with better efficacies (Anighoro et al., 2014; Medina-Franco et al., 2013; Lu et al., 2012). Indeed many drugs that are effective and widely used in treating neuropsychiatric disorders are multi-target, such as valproate (Ximenes et al., 2012) and clozapine (Wong et al., 2010).

Our analyses can be broadly divided into two steps. Firstly, we identified gene-sets related to a variety of drugs, and analyzed whether the gene-set pertaining to *each* drug overlaps with DNM more than expected by chance. This is very similar to a pathway analysis performed on a set of candidate genes. A gene-set related to a drug can be viewed as a "pathway" in this case. The top-ranked drugs may then serve as repositioning candidates. Secondly, we further analyzed the prioritized drugs, and assessed which drug classes were enriched among the top results. As discussed above, we will test specifically if several neuropsychiatric drug classes are enriched. We also provided manual curations of the top repositioning candidates, which may serve as a useful reference for interested researchers. A brief overview of our analysis workflow is given in Fig. 1.

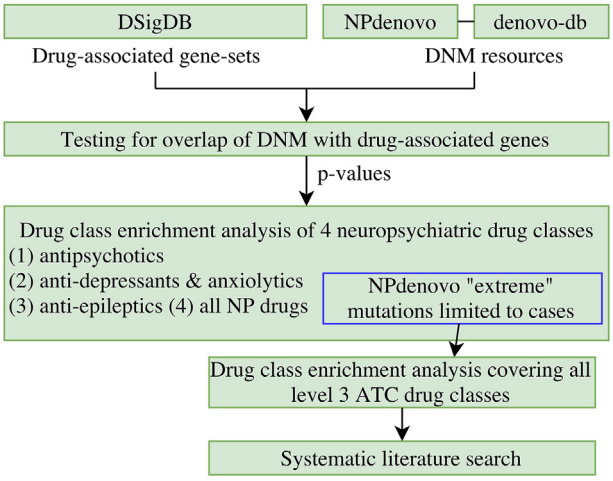

Fig. 1. An overview of our analysis workflow. We identified gene-sets related to a variety of drugs, and analyzed whether the gene-set pertaining to *each* drug overlaps with DNM more than expected by chance. DNM were extracted from two databases, NPdenovo and denov-db. The top-ranked drugs served as repositioning candidates. Next, we further analyzed the prioritized drugs, and assessed which drug classes were enriched among the top results. We specifically whether neuropsychiatric drug classes were enriched. As in general we found the strongest enrichment among NPdenovo "extreme" mutations that are limited to cases, we performed a more comprehensive analysis covering all level 3 ATC drug classes, and the top results were manually curated via a systematic literature search.(DNM: *de novo* mutations; NP, neuropsychiatric).

2. Methods

2.1. De novo mutation resources

We made use of two recently developed databases, NPdenovo (Li et al., 2016) and denovo-db (Turner et al., 2017), for the current analyses. NPdenovo (www.wzgenomics.cn/NPdenovo/) is a database dedicated to neurodevelopmental disorders, including SCZ, ASD, ID and epileptic encephalopathy (EE). Details of database construction and curation can be found in Li et al. (2016). Briefly, information of 3555 trios for the 4 aforementioned disorders together with unaffected siblings or controls were collected from whole-exome sequencing (WES) or whole-genome sequencing (WGS) studies. In total 17104 DNMs were extracted and annotated using various bioinformatics tools. NPdenovo also categorized some mutations with high estimated pathogenicity as "extreme" mutations. The authors first removed all DNMs with minor allele frequency > 0.1% in 1000 Genomes (dbSNP138) and ESP6500. Then several kinds of likely gene-disrupting (LGD) events including nonsense, splice-site and frameshift mutations are directly considered to be "extreme". A large proportion of DNMs are however missense mutations, for which pathogenicity are less clear. The NPdenovo database integrates functional predictions of the damaging ability of each variant from 12 computational tools, including SIFT, Polyphen2_hvar, Polyphen2_hdiv, MutationTaster, MutationAssessor, LRT, FATHMM, GERP++, PhyloP, SiPhy, RadialSVM and MetaLR. An aggregate "damaging score" is computed by summing up the number of tools that predict the variant to be deleterious, and any variant with a score ≥ 8 is considered an "extreme" mutation. Details of this procedure are described in Li et al. (2016). We also considered another database denovo-db that is slightly more recent; it is a more comprehensive resource covering all kinds of disorders. The database provides the Combined Annotation Dependent Depletion (CADD) score (Kircher et al., 2014) as a measure of likely pathogenicity, but did not categorize variant directly into "extreme" or "non-extreme" mutations. Readers are referred to Turner et al. (2017) for details of denovo-db.

2.2. Gene-set associated with each drug

We made use of the DSigDB database (Yoo et al., 2015) to extract gene-sets related to each drug. The whole database was downloaded from http://tanlab.ucdenver.edu/DSigDB. A total of 17839 unique compounds were included in this database. The gene-sets were compiled according to multiple sources: (1) bioassay results from PubChem (Kim et al., 2016) and ChEMBL (Gaulton et al., 2012); (2) kinase profiling assay from the literature and two kinase databases (Medical Research Council Kinase Inhibitor database and Harvard Medical School Library of Integrated Network-based Cellular Signatures database); (3) drug-induced differentially expressed genes (with > 2 fold-change compared to controls), as derived from the Connectivity Map (Lamb et al., 2006); and (4) manually curated and text mined drug targets from the Therapeutics Targets Database (Yang et al., 2016) and the Comparative Toxicogenomics Database (Mattingly et al., 2003).

2.3. Testing for overlap of de novo mutations with drug-related genes

We then tested for over-representation of drug-related gene-sets among the DNMs, with stratification by potential pathogenicity (see methods below). This is very similar to a pathway analysis performed on candidate genes, but here the "pathway" is a set of genes related to the drug action. We constructed for each drug a 2 × 2 contingency table, summarizing the total number of genes categorized by: (1) whether or not the gene belongs to a (specified type of) DNM; and (2) whether or not the gene is associated with the particular drug.

We tested for independence of the two categories by a one-tailed Fisher's exact test (we were testing for a *greater*-than-expected overlap,

hence one-tailed tests). We hypothesized that some drugs will target genes that overlap with DNM more than expected by chance; these drugs can be prioritized as repositioning candidates. To avoid results driven by too few genes and since we are focusing on a "multi-target" paradigm of drug discovery, we excluded drugs with less than 5 associated genes. A total of 5389 drugs were included in the final analyses.

2.4. Stratification of de novo mutations

As the functional significance and pathogenicity of DNMs may differ a lot, we performed analyses with different subgroups of DNMs. For mutations in NPdenvo, we repeated the over-representation analysis with all non-synonymous and then "extreme" mutations. For denovo-db, we first included mutations which are LGD (including stop-gained, stop-loss, frameshift, splice acceptor or donor mutations) as pathogenic variants. We then stratified missense mutations by their CADD scores. It should be noted that there is no consensus or strong theoretical basis for a particular cutoff of the score. Here we followed the suggestion by the authors (http://cadd.gs.washington.edu/info) and set 15 as a primary cutoff for pathogenic mutations; we also performed a subsidiary analyses with a much more stringent threshold of 30. In addition, for both databases, we stratified the mutations according to whether they are exclusively found in cases or also found in control subjects.

2.5. Testing for drug class enrichment

After we prioritized the drugs from the previous step, we tested for enrichment of individual drug classes. Briefly, we tested whether drugs of a particular class (e.g. anti-epileptics or antipsychotics) would have significantly lower p-values than drugs not belonging to that class. P-values were converted to z-statistics by a probit function [$z = \Phi^{-1}(p)$] and one-tailed t-tests were used to compare the means of drugs within or outside the specified drug class. (This is analogous to the principle of gene-set analysis described in ref. (de Leeuw et al., 2015; de Leeuw et al., 2016).)

As discussed earlier, we are specifically interested in the enrichment of neuropsychiatric drug classes, as they are most pertinent to the diseases under study. We specifically extracted drugs belonging to (1) antipsychotics; (2) antidepressants and anxiolytics; (3) antiepileptics; and (4) all psychiatric drugs, which include drugs for schizophrenia, bipolar disorder, depression, anxiety and phobic disorders, ASD, attention deficit hyperactivity disorder and dementia (including Alzheimer's disease). Drug classes were defined based on two sources, including the Anatomical Therapeutic Chemical (ATC) Classification System and the MEDI-HPS (MEDication Indication – High Precision Subset) (Wei et al., 2013). ATC is a classification system set by the World Health Organization for therapeutic agents, while MEDI is an ensemble resource of medication indication formed by integrating four commonly used medication resources (RxNorm, MedlinePlus, SIDER2 (Kuhn et al., 2010) and Wikipedia). MEDI-HPS is a subset of MEDI which only includes indications found in either RxNorm or at least 2 of the 3 other sources, with an estimated precision of 92% based on clinician review (Wei et al., 2013).

As will be discussed in the results section, we observed highly significant enrichment of neuropsychiatric drug classes for NPdenovo "extreme" mutations that are limited to cases. For this group of mutations, we also performed a more comprehensive enrichment test covering *all* level 3 ATC drug classes; the purpose is to examine drug classes (in addition to individual drugs) that may have potential for repositioning. Similar to our previous analyses, in order to avoid significant results driven by few drugs in a category, we only included pharmacological classes with at least 5 drugs (that had results from our previous drug-based analysis). Around 125 pharmacological classes were included for the final analysis of each disorder.

2.6. Literature search of prioritized drugs

For drugs prioritized by the analysis of NPdenovo extreme mutations (exclusive in cases), we also performed a systemic literature search in PubMed and Google Scholar for possible therapeutic relevance. The search strategy is as follows: (1) For SCZ: Drug_name AND (schizophrenia OR schizophrenic OR psychosis OR psychotic OR antipsychotic); (2) for ASD: Drug_name AND (autism OR autistic OR asperger); (3) for ID: Drug_name AND ("general learning disability" OR "intellectual disability" OR "mental retardation"); (4) for epilepsy: Drug_name AND (epilepsy OR epileptic OR seizure). Clinical studies were cited in preference to pre-clinical studies if available. Systematic review and meta-analysis were cited with higher priorities, generally following the hierarchy of evidence. Annotation was performed for the top 30 drugs for each disorder. A maximum of 3 references are included. The literature search was performed in Jul 2017. Due to the relatively large number of drug-disorder pairs, this is not meant to be a systematic review of current evidence but an approximate guide to the therapeutic relevance of each top-ranked drug, providing a reference for other researchers.

2.7. Multiple testing correction

We employed the false discovery rate (FDR) approach for multiple testing correction, which controls the expected proportion of false discoveries (Benjamini and Hochberg, 1995). FDR correction was performed by the Benjamini-Hochberg procedure implemented in the R function p.adjust. The corresponding "adjusted p-values", or q-values (Storey, 2003), were reported. Results with q-values less than 0.05 were regarded as significant associations, while results with $0.05 < q \leq 0.1$ were considered suggestive associations.

3. Results

3.1. Drug class enrichment results from NPdenovo

Table 1 shows the enrichment of neuropsychiatric drug classes for SCZ and ASD from analysis of the NPdenovo database (the FDR or q-values for all enrichment tests are included in Table S1). Antipsychotics were strongly enriched for SCZ DNM (lowest $p = 4.76E\text{-}9$ from four analyses of different subtypes of DNMs). The enrichment was highly significant regardless of the subtype of DNM we analyzed. Nevertheless, the enrichment was stronger for extreme DNMs, and also slightly stronger for DNMs found exclusively in cases. Interestingly, there was also enrichment for antiepileptics (lowest $p = 1.69E\text{-}4$), and as expected, for all psychiatric drugs (lowest $p = 1.31E\text{-}5$). There was a modest association with medications for depression and anxiety defined by MEDI-HPS (lowest $p = 2.37E\text{-}2$). For ASD, the strongest enrichment was for antiepileptics (lowest $p = 3.01E\text{-}11$), but we also detected an enrichment for MEDI-HPS drugs for depression and anxiety (lowest $p = 4.17E\text{-}4$), and for psychiatric medications in general.

Table 2 shows the enrichment of neuropsychiatric drug classes for ID and epilepsy from NPdenovo. For ID, a strong enrichment was observed for antiepileptics (lowest p = 2.67E-8). There was also evidence of enrichment for antipsychotics and antidepressants/anxiolytics, no matter the drug classes were defined by ATC or MEDI-HPS. Finally, for epilepsy, the most significant enrichment was for antiepileptics (lowest p = 8.69E-12), but there was also evidence that drugs for depression and anxiety were enriched (lowest p = 5.20E-3). Note that all the above-mentioned associations were significant at an FDR threshold of 0.05.

3.2. Drug class enrichment results from denovo-db

The enrichment test results from denovo-db were broadly similar to those obtained from analysis of NPdenovo. Results of analysis were

Table 1
Enrichment of neuropsychiatric drug classes based on *de novo* mutations in NPdenovo database (SCZ and ASD).

	SCZ non-syn	SCZ extreme	ASD non-syn	ASD extreme
ATC classification				
Antipsychotics	**6.59E-06**	**1.33E-07**	9.43E-01	8.21E-01
Antidepressants or anxiolytics	8.37E-01	6.16E-01	1.76E-01	8.15E-01
All ATC psychiatric drugs	3.38E-01	**2.58E-02**	**2.46E-02**	*3.33E-02*
Antiepileptics	**1.38E-03**	**6.50E-04**	**3.50E-08**	**1.93E-08**
MEDI-HPS				
For Schizophrenia and bipolar	**6.17E-05**	**9.60E-06**	4.50E-01	2.48E-01
For MDD or anxiety disorders	4.10E-01	7.01E-02	**9.64E-04**	**1.39E-02**
All psychiatric drugs	**9.50E-03**	**4.74E-04**	**8.59E-03**	**1.65E-02**
Antiepileptics	**2.42E-02**	**4.84E-03**	**1.23E-09**	**4.33E-11**
Limited to mutations exclusively found in cases				
ATC classification				
Antipsychotics	**2.68E-06**	**4.76E-09**	8.86E-01	8.77E-01
Antidepressants or anxiolytics	6.24E-01	3.37E-01	1.00E-01	7.12E-01
All ATC psychiatric drugs	1.10E-01	**1.10E-03**	**1.30E-02**	**3.39E-02**
Antiepileptics	**1.70E-04**	**1.69E-04**	**1.68E-06**	**1.99E-08**
MEDI-HPS				
For Schizophrenia and bipolar	**1.53E-05**	**2.93E-07**	3.37E-01	2.27E-01
For MDD or anxiety disorders	1.76E-01	**2.37E-02**	**4.17E-04**	**7.36E-03**
All psychiatric drugs	**8.23E-04**	**1.31E-05**	**5.81E-03**	**1.11E-02**
Antiepileptics	**7.87E-03**	**3.52E-03**	**4.10E-08**	**3.01E-11**

SCZ, schizophrenia; ASD, autistic spectrum disorder.
Non-syn, all non-synonymous DNMs; extreme, extreme mutations as defined in the NPdenovo database.
Results with q-values ≤ 0.05 are in bold, and those with 0.05 < q ≤ 0.1 are in italics. Q-value may be considered as 'FDR-adjusted p-values', that are adjusted for multiple testing by the false discovery rate approach.

Table 2
Enrichment of neuropsychiatric drug classes based on *de novo* mutations in NPdenovo database (ID and epilepsy).

Disorder	ID non-syn	ID extreme	Epilepsy non-syn	Epilepsy extreme
ATC classification				
Antipsychotics	9.31E-02	*3.04E-02*	9.81E-01	9.68E-01
Antidepressants or anxiolytics	7.81E-02	2.39E-01	5.42E-01	1.19E-01
All ATC psychiatric drugs	**2.02E-04**	**1.72E-02**	*4.87E-02*	**4.20E-04**
Antiepileptics	**4.66E-06**	**2.17E-05**	**6.22E-09**	**1.92E-09**
MEDI-HPS				
For Schizophrenia and bipolar	1.36E-01	*3.18E-02*	8.80E-01	6.09E-01
For MDD or anxiety disorders	**1.01E-03**	**9.40E-03**	1.99E-01	**4.22E-03**
All psychiatric drugs	**6.59E-04**	**7.12E-04**	1.70E-01	**6.17E-03**
Antiepileptics	**4.93E-08**	**2.16E-05**	**7.55E-11**	**8.69E-12**
Limited to mutations exclusively found in cases				
ATC classification				
Antipsychotics	7.29E-02	**2.56E-02**	9.64E-01	9.69E-01
Antidepressants or anxiolytics	*4.70E-02*	2.18E-01	4.82E-01	1.31E-01
All ATC psychiatric drugs	**1.30E-04**	**1.27E-02**	*3.25E-02*	**4.97E-04**
Antiepileptics	**2.73E-06**	**2.14E-05**	**6.56E-09**	**2.46E-09**
MEDI-HPS				
For Schizophrenia and bipolar	9.87E-02	*3.13E-02*	8.22E-01	6.49E-01
For MDD or anxiety disorders	**5.19E-04**	**7.75E-03**	1.46E-01	**5.20E-03**
All psychiatric drugs	**1.46E-04**	**5.89E-04**	1.26E-01	**8.39E-03**
Antiepileptics	**2.67E-08**	**2.03E-05**	**5.20E-11**	**1.06E-11**

ID, intellectual disability.
Non-syn, all non-synonymous DNMs; extreme, extreme mutations as defined in the NPdenovo database.
Results with q-values ≤ 0.05 are in bold, and those with 0.05 < q ≤ 0.1 are in italics.

shown in Tables 3–4. Again we observed strong enrichment for antipsychotics in SCZ and antiepileptics in epilepsy. We also observed enrichment for medications against depression and anxiety (from MEDI-HPS) across all four disorders. Antiepileptics and the combined drug-set of psychiatric medications were also enriched across all four disorders. The enrichment appeared to be roughly similar for mutations limited to cases or not, although there was sometimes a slightly stronger enrichment for mutations exclusive in cases. The significance of enrichment was also not much different for the two CADD cutoffs, except for SCZ. Interestingly, in SCZ, enrichment for neuropsychiatric drug classes was no longer significant when we increased the CADD score threshold to 30, except for antiepileptics. One explanation is that there are fewer variants passing a threshold score of 30, which impairs the power of detecting associations; in addition, setting a very stringent CADD threshold at 30 may have stratified the SCZ patients into a distinct subgroup that is different from the more typical patients. It is worth noting that not all SCZ patients have DNMs and common genetic variants played a significant role in disease pathogenesis (Lee et al., 2013).

3.3. Additional analysis for antiepileptics

In our analysis we observe that antiepileptics are enriched for all four disorders under study. To further evaluate whether these findings are merely incidental, we performed additional enrichment analysis on mutations of presumably *lower* pathogenicity (which serves as a 'control' experiment). We expect weaker (or no) enrichment to be seen if we replace the more severe mutations with those having lower pathogenicity. We selected DNMs with CADD score < 15 and those that are synonymous or intronic (if available); we then tested their enrichment for antiepileptics (Table S7). For SCZ, we found that enrichment became non-significant when we tested the less pathogenic mutations. For ID and epilepsy, the level of statistical significance was much weakened, when compared to analyses using mutations with high CADD scores and gene-disrupting effects. For ASD, the number of genes with DNMs of lower pathogenicity (based on criteria above) is relatively large (~12000); for a fair comparison with DNMs of higher pathogenicity (N ~2000), we down-sampled the former gene-set to the same size, then performed enrichment tests. The random down-sampling procedure was repeated for 500 times, and the enrichment p-values averaged. Again, we observed much weakened statistical significance when the analysis was based on DNMs of lower pathogenic potential.

3.4. Enrichment for all ATC drug classes for extreme mutations limited to cases

As discussed earlier, we also performed a more comprehensive enrichment test covering all ATC (level 3) drug classes for NPdenovo extreme mutations limited to cases. The results are shown in Table 5 (a full list is available in Table S2; selected psychiatric drug categories with rankings is presented in Table S8). For schizophrenia, the most strongly enriched drug class is antipsychotics, while antiepileptics ranked fifth. Interestingly, lipid modifying agents ranked second with a

Table 3
Enrichment of neuropsychiatric drug classes based on *de novo* mutations listed in denovo-db (SCZ and ASD).

Disorder	SCZ_cadd15	SCZ_cadd30	ASD_cadd15	ASD_cadd30
ATC classification				
Antipsychotics	**1.11E-07**	1.38E-01	9.93E-01	8.49E-01
Antidepressants or anxiolytics	1.02E-01	9.73E-01	7.39E-01	8.05E-01
All ATC psychiatric drugs	**4.59E-03**	7.80E-01	2.09E-01	5.19E-01
Antiepileptics	**4.65E-04**	**8.89E-04**	**2.65E-09**	**2.87E-10**
MEDI-HPS				
For Schizophrenia and bipolar	**7.42E-08**	9.23E-02	8.88E-01	6.58E-01
For MDD or anxiety disorders	**1.99E-03**	1.36E-01	8.91E-02	**1.19E-02**
All psychiatric drugs	**5.10E-07**	1.01E-01	1.85E-01	2.36E-01
Antiepileptics	**3.44E-03**	**1.86E-03**	**1.71E-07**	**2.55E-12**
Limited to mutations exclusively found in cases				
ATC classification				
Antipsychotics	**1.28E-07**	1.42E-01	9.92E-01	8.94E-01
Antidepressants or anxiolytics	5.62E-02	9.72E-01	7.47E-01	5.31E-01
All ATC psychiatric drugs	**1.10E-03**	7.88E-01	3.08E-01	3.49E-01
Antiepileptics	**1.55E-04**	**8.79E-04**	**2.02E-09**	**5.03E-11**
MEDI-HPS				
For Schizophrenia and bipolar	**2.21E-08**	9.02E-02	8.91E-01	5.82E-01
For MDD or anxiety disorders	**6.34E-04**	1.34E-01	8.51E-02	**1.18E-03**
All psychiatric drugs	**4.79E-08**	9.82E-02	1.76E-01	1.27E-01
Antiepileptics	**1.17E-03**	**1.84E-03**	**1.86E-07**	**1.76E-13**

SCZ, schizophrenia; ASD, autistic spectrum disorder.
Cadd15, DNMs with CADD score > 15; Cadd30, DNMs with CADD score > 30. Note that we also included all likely gene disrupting (LGD) mutations, including stop-gained, stop-loss, frameshift, splice acceptor or donor mutations.
Results with q-values ≤ 0.05 are in bold, and those with 0.05 < q ≤ 0.1 are in italics.

slightly higher p-value than antipsychotics (p = 5.194E-9). Antimicrobial agents including anti-fungal drugs were also ranked high on the list. For ASD, the strongest enrichment was observed for antiepileptics; other groups of psychiatric medications, such as hypnotics/sedatives, anti-dementia drugs and anxiolytics were also ranked among the top with q-value < 0.05. Other top-ranked drug classes included anti-neoplastic drugs and antimetabolites, as well as calcium channel blockers. For ID, anti-neoplastic agents and antiepileptics were listed among the top results. As for epilepsy, agents known to treat seizures or raise seizure thresholds such as antiepileptics, hypnotics/sedatives and anxiolytics were all highly enriched.

3.5. Literature search results of the top prioritized drugs

The full results of the literature search are listed in Tables S3–6. Some of the drugs with potential therapeutic relevance are highlighted in Table 6.

3.5.1. Top-ranked drugs across disorders

A few drugs are listed across disorders and are discussed here. Histone deacetylases inhibitors (HDAC) inhibitors such as trichostatin A, MS-275 and scriptaid were listed among the top for SCZ, ASD and ID. In fact the commonly used mood stabilizer valproate is also an HDAC inhibitor. HDAC is believed to play an important role in many complex diseases, including neuropsychiatric disorders (Kazantsev and Thompson, 2008; Qiu et al., 2017). HDAC removes acetyl groups from protein substrates on histones, and may lead to transcriptional silencing by allowing for chromatin compaction; HDAC inhibitors in turn block the deacetylation process (Kazantsev and Thompson, 2008). HDAC inhibitors have been suggested as new therapies for neuropsychiatric disorders (Kazantsev and Thompson, 2008; Qiu et al., 2017; Abe and Zukin, 2008; Grayson et al., 2010). For instance, HDAC inhibitor has been suggested to as a new therapy against positive, negative and cognitive symptoms in schizophrenia (Weiwer et al., 2013), mainly based on pre-clinical evidence. There is also evidence for HDAC inhibitors in the treatment of neurodevelopmental disorders such as ID. Fragile X syndrome (FXS) is the most common inherited cause of ID and the most common monogenetic cause of ASD (Lozano et al., 2016). Hypermethylation and increased histone deacetylation of the *FMR* gene has been shown to contribute to the disease (Chiurazzi et al., 1998, 1999). Treatment of lymphoblastoid cells derived from FXS patients with 5-aza-2-deoxycytidine (a de-methylating agent) together with HDAC inhibitors was reported to have synergistic effects in reactivating the *FMR1* gene (Chiurazzi et al., 1999). HDAC inhibitors were also implicated in the treatment of another syndromal cause of ID, Rubinstein-Taybi syndrome (Park et al., 2014). Decreased histone acetylation appears to be a common mechanism underlying many neurodevelopmental or other brain disorders (Qiu et al., 2017). However, current evidence was largely based on pre-clinical studies and further clinical investigations including randomized controlled trials (RCTs) are necessary to validate these findings.

Another drug, retinoic acid, was also listed among the top across SCZ, ASD and ID. Retinoic acid signaling plays an important part in the development of the central nervous system, for example in neural differentiation, axon outgrowth and neural patterning (Maden, 2007; Das et al., 2014). For schizophrenia, the retinoid signaling pathway was proposed as a novel therapeutic target (Lerner et al., 2016), and a retinoid X receptor (RXR) agonist (bexarotene) has been tested in two clinical trials (Lerner et al., 2008, 2013) with some evidence of benefit in SCZ as an add-on agent. As for the underlying mechanisms, it is worth noting that retinoid signaling and the dopaminergic pathway may be connected. It has been observed that single and compound null mutations in the genes for RXRβ and γ were associated with abnormalities in the mesolimbic dopaminergic signaling pathway, and the expression of DRD1 and DRD2 receptors in ventral striatum were decreased in mutant mice (Krezel et al., 1998). Moreover, the promoter of the D2 receptor contains a functional response element to retinoic acid (Samad et al., 1997). Retinoic acid was proposed as a potential therapy for ASD as well, based on its action of inducing CD38 transcription which in turn may be associated with improved autistic symptoms (Ebstein et al., 2011).

3.5.2. Top-ranked drugs for individual disorders

For SCZ, the top-ranked drug was valproic acid, a widely used mood stabilizer. Although the drug is mainly used for bipolar disorder, meta-analysis of mostly open RCTs showed that it may improve clinical symptoms of SCZ when used as an adjunct to antipsychotics, and may also be useful for aggression or irritability (Wang et al., 2016). Minocycline, which is a tetracycline with anti-inflammatory and neuroprotective properties, was ranked among the top and meta-analyses of RCTs have shown its efficacy for SCZ (Solmi et al., 2017; Xiang et al., 2017). Another drug estriadiol was demonstrated to be effective for treatment-resistant SCZ cases (in women aged 18 to 45) in a double-blind RCT (Kulkarni et al., 2015). Estrogens have been reported to be linked to signaling pathways of serotonin, glutamate and dopamine. For example, animal studies showed that estradiol treatment increased concentration of serotonin (Sanchez et al., 2013) and stimulated brain region specific metabotropic glutamate receptors (Meitzen and Mermelstein, 2011).

For ASD, valproic acid was ranked among the top. Antiepileptics such as valproic acid are frequently prescribed for the control of

Table 4
Enrichment of neuropsychiatric drug classes based on *de novo* mutations listed in denovo-db (ID and epilepsy).

	ID_cadd15	ID_cadd30	Epilepsy_cadd15	Epilepsy_cadd30
ATC classification				
Antipsychotics	6.52E-01	2.22E-01	9.44E-01	7.89E-01
Antidepressants or anxiolytics	1.51E-01	7.33E-01	5.62E-01	2.58E-01
All ATC psychiatric drugs	**2.46E-02**	*5.23E-02*	**6.91E-03**	**5.77E-03**
Antiepileptics	**9.25E-09**	**1.05E-07**	**2.34E-10**	**6.11E-10**
MEDI-HPS				
For Schizophrenia and bipolar	6.53E-01	1.21E-01	8.71E-01	5.55E-01
For MDD or anxiety disorders	**7.44E-04**	**1.18E-02**	**1.33E-02**	**3.36E-03**
All psychiatric drugs	**2.14E-02**	**4.88E-03**	6.31E-02	**3.65E-03**
Antiepileptics	**9.97E-11**	**2.53E-08**	**1.14E-13**	**2.79E-11**
Limited to mutations exclusively found in cases				
ATC classification				
Antipsychotics	6.50E-01	2.35E-01	9.41E-01	8.79E-01
Antidepressants or anxiolytics	2.33E-01	7.37E-01	5.48E-01	2.89E-01
All ATC psychiatric drugs	*3.24E-02*	6.53E-02	**6.25E-03**	**2.41E-02**
Antiepileptics	**7.97E-09**	**9.70E-08**	**2.50E-10**	**6.27E-10**
MEDI-HPS				
For Schizophrenia and bipolar	7.32E-01	1.33E-01	8.54E-01	7.35E-01
For MDD or anxiety disorders	**1.51E-03**	**1.12E-02**	**1.26E-02**	**3.41E-03**
All psychiatric drugs	*3.07E-02*	**6.40E-03**	*5.75E-02*	**1.71E-02**
Antiepileptics	**7.52E-11**	**2.34E-08**	**1.36E-13**	**2.42E-11**

ID, intellectual disability.
Cadd15, DNMs with CADD score > 15; Cadd30, DNMs with CADD score > 30. Note that we also included all likely gene disrupting (LGD) mutations, including stop-gained, stop-loss, frameshift, splice acceptor or donor mutations.
Results with q-values ≤ 0.05 are in bold, and those with 0.05 < q ≤ 0.1 are in italics.

irritability in patients (Fitzpatrick et al., 2016), although they are not directed for the core symptoms of the disorder. As discussed above, several HDAC inhibitors including trichostatin A and MS-275 were highly ranked.

For ID, several AMPA/kainate receptor antagonists including YM-90K, nbqx and dnqx were highly ranked. As abnormal excitatory glutamatergic transmission is implicated in many neurological and psychiatric disorders, these drugs are being developed for the treatment of a range of diseases, such as epilepsy, cerebral ischemia and Alzheimer disease (Catarzi et al., 2007; Swanson, 2009). Several HDAC inhibitors and two antiepileptics (which are also mood stabilizers) valproic acid and carbamazepine were also on the top list.

For epilepsy, many of the top hits are known anticonvulsants or hypnotics/sedatives that enhance GABA transmission. Manually curated descriptions of each drug and their potential therapeutic relevance are listed in Supplementary Tables 3–6.

4. Discussion

In this study, we have explored the usefulness of DNMs in guiding drug discovery by looking for overlap of DNMs with drug-related gene-sets. We discovered that neuropsychiatric drug classes were indeed significantly enriched; in particular, antipsychotics and antiepileptics were the *most* strongly enriched drug classes (out of all level 3 ATC classes) for SCZ and epilepsy respectively. By inspecting individual hits, we also uncovered several interesting (although preliminary) drug candidates or mechanisms (e.g. HDAC inhibition and retinoid signaling) that might warrant further investigations.

To our knowledge, this is the first study to investigate the usefulness of DNM in guiding drug discovery for neuropsychiatric disorders. The majority of genetic studies in neuropsychiatry aimed at finding new susceptibility genes for specific disorders, however the translational potential of these findings in terms of drug discovery remains largely unexplored. Several related studies are worth mentioning here. A recent study by Ruderfer et al. (2016) found that gene-sets of antipsychotics were enriched for common and rare genetic variations of SCZ, which were derived from a genome-wide association study (GWAS) meta-analysis and a Swedish case-control exome-sequencing study respectively. Interestingly, they also found that agents against amoebiasis and other protozoal diseases was the most significantly enriched drug class (Ruderfer et al., 2016). Treatment resistant patients also had an excess of mutations in antipsychotics drug targets (Ruderfer et al., 2016). Another study by Gasper et al. (Gaspar and Breen, 2016) reported that as sample size increases, SCZ GWAS results were increasingly enriched for known psychiatric medications. We also recently revealed that GWAS results of depression and anxiety disorders (and related phenotypes) were enriched for psychiatric drug classes including antidepressants (So et al., 2018). Taken together, the current study adds to the mounting evidence that data from human genomic studies are useful in guiding drug development in neuropsychiatry.

Most DNM are rare and typically only few patients may share mutations in the same gene. An ultimate goal of drug discovery is to find an effective personalized therapy for *each* genetic subtype of the disorder. This is an important long-term goal but its success might be limited by the very high cost of drug development. An alternative approach is to consider these DNM genes as useful therapeutic targets in general. Here we followed this paradigm with a focus on multi-target (Medina-Franco et al., 2013) agents. Indeed we found that antipsychotics, which are generally effective for SCZ patients, is the most strongly enriched drug class from DNM. This provides a proof-of-concept of this approach in finding new therapeutic agents. Success stories of drug discovery in the field of cardiovascular medicine also support that rare genetic variations can lead to development of therapies useful for a wider population. For instance, PCSK9 inhibitors are now proven to reduce low-density lipoprotein (LDL) cholesterol and reduce cardiovascular events (Sabatine et al., 2017). The importance of PCSK9 in regulating lipid metabolism was first discovered from rare mutations in familial hypercholesterolemia (Abifadel et al., 2003, 2014). SGLT2 inhibitor is a new class of anti-diabetic agent (DeFronzo et al., 2017); notably rare mutations in the *SGLT2* gene cause familial renal

Table 5
Enrichment of all ATC (level 3) drug classes (only drug classes with q-value < 0.05 are shown).

Rank	ATC codes	Drug Class (Level 3)	pval	qval
Schizophrenia				
1	N05A	ANTIPSYCHOTICS	**4.76E-09**	**3.32E-07**
2	C10A	LIPID MODIFYING AGENTS, PLAIN	**5.19E-09**	**3.32E-07**
3	G01A	ANTIINFECTIVES AND ANTISEPTICS, EXCL. COMBINATIONS WITH CORTICOSTEROIDS	**2.91E-06**	**1.24E-04**
4	L01X	OTHER ANTINEOPLASTIC AGENTS	**2.96E-05**	**9.47E-04**
5	N03A	ANTIEPILEPTICS	**1.69E-04**	**4.33E-03**
6	A01A	STOMATOLOGICAL PREPARATIONS	**1.37E-03**	**2.92E-02**
7	D01A	ANTIFUNGALS FOR TOPICAL USE	**1.64E-03**	**3.00E-02**
8	D11A	OTHER DERMATOLOGICAL PREPARATIONS	**3.09E-03**	**4.94E-02**
Autistic Spectrum Disorders				
1	N03A	ANTIEPILEPTICS	**1.99E-08**	**2.59E-06**
2	L01X	OTHER ANTINEOPLASTIC AGENTS	**5.58E-07**	**3.63E-05**
3	N05C	HYPNOTICS AND SEDATIVES	**9.91E-07**	**4.29E-05**
4	N06D	ANTI-DEMENTIA DRUGS	**3.74E-06**	**1.22E-04**
5	N05B	ANXIOLYTICS	**5.86E-04**	**1.52E-02**
6	C08C	SELECTIVE CALCIUM CHANNEL BLOCKERS WITH MAINLY VASCULAR EFFECTS	**8.33E-04**	**1.80E-02**
7	L01B	ANTIMETABOLITES	**1.36E-03**	**2.33E-02**
8	S01H	LOCAL ANESTHETICS	**1.60E-03**	**2.33E-02**
9	C04A	PERIPHERAL VASODILATORS	**1.61E-03**	**2.33E-02**
10	N01B	ANESTHETICS, LOCAL	**2.03E-03**	**2.64E-02**
Intellectual Disability				
1	L01X	OTHER ANTINEOPLASTIC AGENTS	**5.34E-06**	**6.41E-04**
2	N03A	ANTIEPILEPTICS	**2.14E-05**	**1.28E-03**
3	G01A	ANTIINFECTIVES AND ANTISEPTICS, EXCL. COMBINATIONS WITH CORTICOSTEROIDS	**3.18E-04**	**1.27E-02**
Epilepsy				
1	N03A	ANTIEPILEPTICS	**2.46E-09**	**3.08E-07**
2	N05C	HYPNOTICS AND SEDATIVES	**3.79E-08**	**2.37E-06**
3	L01X	OTHER ANTINEOPLASTIC AGENTS	**9.98E-07**	**4.16E-05**
4	N01A	ANESTHETICS, GENERAL	**2.56E-06**	**8.00E-05**
5	N01B	ANESTHETICS, LOCAL	**3.54E-05**	**8.85E-04**
6	N05B	ANXIOLYTICS	**8.18E-05**	**1.70E-03**
7	R02A	THROAT PREPARATIONS	**2.91E-04**	**4.72E-03**
8	D08A	ANTISEPTICS AND DISINFECTANTS	**3.02E-04**	**4.72E-03**
9	L01C	PLANT ALKALOIDS AND OTHER NATURAL PRODUCTS	**8.23E-04**	**1.14E-02**
10	S01H	LOCAL ANESTHETICS	**3.74E-03**	**4.68E-02**

Rank, rank of the drug-group among all tested ATC drug categories; pval, p-values; qval, q-values (i.e. the corresponding false discovery rate).

glycosuria (Santer and Calado, 2010).

For all four neuropsychiatric disorders under study, there was significant enrichment for the set of combined psychiatric medications. For SCZ and epilepsy, the two disorders with well-established treatments, the strength of enrichment (*e.g.* for antipsychotics and antiepileptics) tended to be stronger when we focused on "extreme" mutations and mutations exclusively detected in cases. For ID and ASD, the difference was not obvious. One possible explanation is that ID and ASD have no established drug treatments; the therapeutic relevance of psychiatric medications may depend on the specific (comorbid) clinical symptoms of patients, instead of the severity of the mutations causing the disorder. For example, a patient with ID may have a DNM in a gene that also predisposes to depressive symptoms; antidepressants may be relevant to this patient, even if the DNM is not highly pathogenic for ID. On the other hand, more deleterious or "extreme" mutations for SCZ or epilepsy may indicate a more important role in disease pathogenesis, and hence higher potential as therapeutic targets.

A few drug classes with significant enrichment are worth mentioning. For SCZ, although antipsychotics ranked first, lipid-lowering agents were also highly significant (p = 5.19E-9). This observation is partially supported by clinical studies. For example statins have been shown to ameliorate SCZ symptoms in two small-scale clinical trials (Chaudhry et al., 2014; Vincenzi et al., 2014). In one study simvastatin as adjunctive treatment was tested and there was preliminary evidence for improving total symptoms scores, but the result was not statistically significant (Chaudhry et al., 2014). Another study investigated pravastatin also as an adjunctive therapy and found a significant reduction of positive symptoms at week 6, though the effect failed to maintain at week 12 (Vincenzi et al., 2014). Regarding the biological mechanisms, studies have shown that statins are anti-inflammatory and exhibit a variety of effects on cells of the innate and adaptive immune systems (Bu et al., 2011). On the other hand, immune dysfunction and inflammation have been implicated in SCZ (Khandaker et al., 2015). For example, cytokine alterations were observed in first-episode psychosis, acutely relapsed patients (Miller et al., 2011) and in first-degree relatives of SCZ subjects (Martinez-Gras et al., 2012). A recent report also reported shared genetic risk factors in immune and inflammatory pathways between SCZ and cardiometabolic traits, which may partially explain raised cardiovascular risks in SCZ patients (So et al., 2018). Taken together, the effects of statins on SCZ may be mediated by its anti-inflammatory actions, but the exact mechanisms require more in-depth studies.

We also found an enrichment of antimicrobial drugs (ATC class code G01A) and antifungal agents (D01A). This is consistent with Ruderfer et al. (2016) who found an enrichment of agents against amoebiasis and other protozoal diseases (P01A); we noted that many drugs such as quinoline derivatives and imidazole derivatives overlap. It should however be noted that this study focused on DNMs, which is different from Ruderfer et al. which investigated GWAS common variants and rare variants from a case-control exome study (Ruderfer et al., 2016). Another interesting observation is that antineoplastic agents were ranked among the top for the four neurodevelopmental disorders under study. Several reviews have commented on the possible link between autism and cancer (Crespi, 2011; Crawley et al., 2016). For example, a recent review by Crawley et al. (2016) pointed out several genes and signaling pathways that may be shared between autism and cancer, and suggested that anti-cancer drugs with reasonable safety profiles may be repositioned for ASD. Here we provided for the first time a quantitative assessment of this hypothesis, and indeed revealed enrichment of antineoplastic agents.

Another finding from the drug class enrichment analysis is that there is an enrichment of different types of medications (*e.g.* antidepressants/anxiolytics, antiepileptics) across diagnostic categories. Of note, the original paper describing the NPdenovo database (Li et al., 2016) demonstrated a significant overlap in DNMs among all four neurodevelopmental disorders, implying a shared pathophysiology. As a result, overlaps in enriched drugs or drug classes are expected.

Depression and anxiety are common comorbidities for all four disorders under study. There is good evidence to suggest increased rates of depression and anxiety disorders in patients with SCZ (Buckley et al., 2009) and epilepsy (Fiest et al., 2013; Scott et al., 2017). Depressive and anxiety symptoms also appeared to be prevalent in patients with ASD (Ghaziuddin et al., 2002; White et al., 2009) and ID (Borthwick-Duffy, 1994; Linna et al., 1999; Richards et al., 2001) in some studies, however further investigations are required.

A remarkable finding is the significant enrichment of antiepileptics across all four disorders under study. Antiepileptics such as valproate and lamotrigine are well-established treatments for bipolar disorder, which has a strong genetic correlation with SCZ (Cardno and Owen, 2014). However, there is also early evidence that antiepileptics such as valproate may improve symptoms of SCZ, when used as an adjunctive treatment to antipsychotics (Wang et al., 2016). Regarding the potential mechanisms, valproate enhances GABAergic transmission by binding to central benzodiazepine receptors and inhibition of GABA-transaminase (Owens and Nemeroff, 2003). It also has weak antagonistic effects on

Table 6
Selected drugs enriched for individual disorders (ranked within top 30) and therapeutic relevance.

Drug	Disorder	Drug Description	Therapeutic relevance
Valproic acid	SCZ, ASD, ID	Anticonvulsant and a mood stabilizer	Open RCTs showed that valproate may improve clinical response when added to antipsychotics and reduce aggression in patients; sometimes used in controlling irritability and comorbid epilepsy in ASD/ID.
Trichostatin A	SCZ, ASD, ID	Antifungal antibiotic and selectively inhibits class I and II mammalian histone deacetylase (HDAC).	HDAC may be involved in pathogenesis of SCZ and other neurodevelopmental disorders; trichostatin A was shown to have synergistic effects in FMR1 gene reactivation when combined with a de-methylating agent.
Retinoic acid	SCZ, ASD, ID	Metabolite of vitamin A.	Retinoid signaling may be implicated in SCZ; RCTs performed for bexarotene (Retinoid X receptor agonist) with positive results; also plays an important role in nervous system development
Minocycline	SCZ	A broad-spectrum tetracycline antibiotic	Meta-analysis of RCTs showed that adjunctive minocycline is efficacious and safe for SCZ
Estradiol	SCZ	A steroid, an estrogen and the primary female sex hormone	A large-scale RCT shows effectiveness for women with treatment-resistant SCZ
MS-275	ASD	HDAC1 and HDAC3 inhibitor, also known as entinostat.	HDAC may be involved in pathogenesis of neurodevelopmental disorders; showed antidepressant-like effects in mice
YM-90K	ID	AMPA/kainate receptor antagonist	Excessive glutamatergic transmission is implicated in many neurological disorders; shown to have neuroprotective activity and improve spatial memory in rats impaired by repeated ischemia
nbqx	ID	AMPA receptor (AMPAR) antagonist	Was shown in a mouse model to attenuate later-life seizures and autistic-like social deficits following seizures in the neonatal period
dnqx	ID, Epilepsy	AMPA and kainate receptor antagonist.	Shown to have anticonvulsant effects in animal models
Scriptaid	ID	Histone deacetylase (HDAC) inhibitor	Treatment of iPSC derived-neurons from patients with MECP2 duplication syndrome with scriptaid lead to a rescue of several aspects of neuronal morphology
Primidone	Epilepsy	Anticonvulsant of the barbiturate class	Known anticonvulsant
Topiramate	Epilepsy	Anticonvulsant	Known anticonvulsant
Felbamate	Epilepsy	Anticonvulsant	Known anticonvulsant
Acamprosate calcium	Epilepsy	Mainly used for maintenance of abstinence from alcohol	Was shown to attenuate handing-induced convulsions in mice during alcohol withdrawal

Please refer to Supplementary Tables 1–4 for references and more detailed descriptions. Only drugs ranked within top 30 for each disorder were included.

sodium channels (Stahl, 2004). In addition, it is an HDAC inhibitor, and dysregulation of HDAC has been implicated in many neuropsychiatric disorders (Chakravarty et al., 2014) (see discussions above). A combination of different mechanisms may contribute to the therapeutic potential of valproate and possibly other antiepileptics (Rosenberg, 2007; Yang and Tsai, 2017).

Epilepsy is a well-known comorbidity in ASD and ID (Bowley and Kerr, 2000; Jeste and Tuchman, 2015). The rate of epilepsy in autistic patients is ~20%, and the risk of epilepsy is higher in ASD patients with ID compared to those without. It has been suggested that shared pathophysiological mechanisms and genetics may contribute to the comorbidities (Jeste and Tuchman, 2015). Interestingly, antiepileptics have been tested in clinical trials in ASD patients. A recent meta-analysis did not reveal significant benefits of antiepileptics when used as monotherapy; however, Rezaei et al. (2010) reported that the addition of topiramate to risperidone led to a greater reduction in the Aberrant Behavior Checklist-Community (ABC-C) subscale scores for irritability, stereotypic behavior and hyperactivity, when compared with risperidone plus placebo. Further research is required to evaluate the effects of antiepileptics in patients with ASD or ID; it may also be worthwhile to study whether the effects differ by the status of DNMs in these patients.

There are a few limitations to this study. Firstly, here we presented a drug enrichment analysis based on related gene-sets and thus suggested a computational approach to guide drug discovery or repositioning leveraging *de novo* mutation data. However, the study itself did not provide confirmatory evidence for any of the repositioning candidates to be applied in clinical practice. Further pre-clinical and clinical studies, including RCTs, are necessary to verify the efficacy of the drug or drug classes highlighted in this report. Also, the pharmacological properties of each drug, particularly its safety and side-effects, need to be carefully considered.

In addition, it should be noted that the current gene-set analysis does not take into account the direction of effects. In other words, it is also possible that some of the top-listed drugs may be associated with an increased risk of neuropsychiatric side-effects. For example, many of top-listed drugs for epilepsy (see Table S6) have some literature support for therapeutic potential. However, a few drugs such as EBOB and procainamide could have adverse effects on the central nervous system [neurotoxic (Yagle et al., 2003) and may increase seizure risk respectively (DiMasi et al., 2016; Kim and Benowitz, 1990)]. This is a limitation for the current study; although viewed from another angle, one may also consider making use of a similar analytic framework to categorize drugs with higher potential for side-effects. To delineate the exact direction of drug effects, further work might be needed to characterize the functional impact of mutations as well as how each drug acts on different receptors and genes. Besides, the permeability of drugs through the blood-brain barrier might affect their effectiveness. These areas have not been investigated in this study. Finally, we mainly focused on DNMs in this work, but further integrative analyses with other sources of genomic data (e.g. GWAS) and drug-related information (e.g. chemical structure and properties), as well as other computational or experimental drug repositioning methods, might improve the chance of discovering effective therapeutics.

Finding a new therapy for a disease is a very complicated process and involves careful balance of the effectiveness and adverse effects. Here we wish to provide a proof-of-concept study to highlight that the potential of DNM in prioritizing candidates, and do not advocate the drugs highlighted can be immediately brought to trials. We recommend a combination of a wider range of computational and experimental methods for drug repositioning/discovery in practice. Nevertheless, we also believe that although most methods have their own limitations (be it experimental or *in silico*), and even an aggregate approach may not always yield successful candidates, given the ever-rising cost of drug development (up to ~$2558 million (DiMasi et al., 2016)/drug), any method that help to prioritize drug candidates even slightly better than current practice will result in large savings in absolute terms. In this regard, any source of information or method that may improve the effectiveness of drug development even by a minute amount might still be worth exploring.

In summary, we demonstrated that DNMs may be useful in guiding drug discovery or repositioning. We presented a gene-set analysis

approach to achieve this aim and showed that the approach is able to pick up known drugs for the respective disorders; our analysis also highlights some preliminary repositioning candidates. We hope this work will open a new avenue of finding new therapeutics for neuropsychiatric disorders, and will stimulate further translational research with human genomics data.

Conflicts of interest

The author declares no conflict of interest.

Contributors

HCS conceived, designed and supervised the study. HCS undertook the analyses and wrote the first draft of the manuscript. YHW performed literature searches and manual curations of the prioritized drug candidates. All authors contributed to and have approved the final manuscript.

Role of funding source

The funders had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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Appendix A. Supplementary data

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References

Abe, T., Zukin, R.S., 2008. Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. Curr. Opin. Pharmacol. 8, 57–64.
Abifadel, M., et al., 2003. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat. Genet. 34, 154–156.
Abifadel, M., et al., 2014. Living the PCSK9 adventure: from the identification of a new gene in familial hypercholesterolemia towards a potential new class of anticholesterol drugs. Curr. Atheroscler. Rep. 16.
Acuna-Hidalgo, R., Veltman, J.A., Hoischen, A., 2016. New insights into the generation and role of de novo mutations in health and disease. Genome Biol. 17.
Anighoro, A., Bajorath, J., Rastelli, G., 2014. Polypharmacology: challenges and opportunities in drug discovery. J. Med. Chem. 57, 7874–7887.
Belardinelli, C., Raza, M., Taneli, T., 2016. Comorbid behavioral problems and psychiatric disorders in autism spectrum disorders. J. Child. Develop. Dis. 2, 11.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. J. Royal Statist. Soc. Ser. B-Methodol. 57, 289–300.
Borthwick-Duffy, S.A., 1994. Epidemiology and prevalence of psychopathology in people with mental retardation. J. Consult. Clin. Psychol. 62, 17–27.
Bowley, C., Kerr, M., 2000. Epilepsy and intellectual disability. J. Intellect. Disabil. Res. 44 (Pt 5), 529–543.
Bu, D.X., Griffin, G., Lichtman, A.H., 2011. Mechanisms for the anti-inflammatory effects of statins. Curr. Opin. Lipidol. 22, 165–170.
Buckley, P.F., Miller, B.J., Lehrer, D.S., Castle, D.J., 2009. Psychiatric comorbidities and schizophrenia. Schizophr. Bull. 35, 383–402.
Cardno, A.G., Owen, M.J., 2014. Genetic relationships between schizophrenia, bipolar disorder, and schizoaffective disorder. Schizophr. Bull. 40, 504–515.
Catarzi, D., Colotta, V., Varano, F., 2007. Competitive AMPA receptor antagonists. Med. Res. Rev. 27, 239–278.
Chakravarty, S., Bhat, U.A., Reddy, R.G., Gupta, P., Kumar, A., 2014. Histone deacetylase inhibitors and psychiatric disorders. In: Epigenetics in Psychiatry. Elsevier, pp. 515–544.
Chaudhry, I.B., et al., 2014. Add-on clinical effects of simvastatin and ondansetron in patients with schizophrenia stabilized on antipsychotic treatment: pilot study. Eur. Psychiatry 29.
Chiurazzi, P., Pomponi, M.G., Willemsen, R., Oostra, B.A., Neri, G., 1998. In vitro reactivation of the FMR1 gene involved in fragile X syndrome. Hum. Mol. Genet. 7, 109–113.
Chiurazzi, P., et al., 1999. Synergistic effect of histone hyperacetylation and DNA demethylation in the reactivation of the FMR1 gene. Hum. Mol. Genet. 8, 2317–2323.
Crawley, J.N., Heyer, W.D., LaSalle, J.M., 2016. Autism and cancer share risk genes, pathways, and drug targets. Trends Genet. 32, 139–146.
Crespi, B., 2011. Autism and cancer risk. Autism Res. 4, 302–310.
Das, B.C., et al., 2014. Retinoic acid signaling pathways in development and diseases. Bioorg. Med. Chem. 22, 673–683.
de Leeuw, C.A., Mooij, J.M., Heskes, T., Posthuma, D., 2015. MAGMA: generalized gene-set analysis of GWAS data. PLoS Comput. Biol. 11.
de Leeuw, C.A., Neale, B.M., Heskes, T., Posthuma, D., 2016. The statistical properties of gene-set analysis. Nat. Rev. Genet. 17, 353–364.
DeFronzo, R.A., Norton, L., Abdul-Ghani, M., 2017. Renal, metabolic and cardiovascular considerations of SGLT2 inhibition. Nat. Rev. Nephrol. 13, 11–26.
DiMasi, J.A., Grabowski, H.G., Hansen, R.W., 2016. Innovation in the pharmaceutical industry: new estimates of R&D costs. J. Health Econ. 47, 20–33.
Ebstein, R.P., Mankuta, D., Yirmiya, N., Malavasi, F., 2011. Are retinoids potential therapeutic agents in disorders of social cognition including autism? FEBS Lett. 585, 1529–1536.
Einfeld, S.L., Ellis, L.A., Emerson, E., 2011. Comorbidity of intellectual disability and mental disorder in children and adolescents: a systematic review. J. Intellect. Dev. Disabil. 36, 137–143.
Fiest, K.M., et al., 2013. Depression in epilepsy: a systematic review and meta-analysis. Neurology 80, 590–599.
Fitzpatrick, S.E., Srivorakiat, L., Wink, L.K., Pedapati, E.V., Erickson, C.A., 2016. Aggression in autism spectrum disorder: presentation and treatment options. Neuropsychiatric Dis. Treat. 12.
Floris, M., Olla, S., Schlessinger, D., Cucca, F., 2018. Genetic-driven druggable target identification and validation. Trends Genet. 34, 558–570.
Gaspar, H.A., Breen, G., 2016. Pathways Analyses of Schizophrenia GWAS Focusing on Known and Novel Drug Targets. bioRxiv, 091264.
Gaulton, A., et al., 2012. ChEMBL: a large-scale bioactivity database for drug discovery. Nucleic Acids Res. 40, D1100–D1107.
Gauthier, J., Rouleau, G.A., 2012. De novo mutations in neurological and psychiatric disorders: effects, diagnosis and prevention. Genome Med. 4.
Ghaziuddin, M., Ghaziuddin, N., Greden, J., 2002. Depression in persons with autism: implications for research and clinical care. J. Autism Dev. Disord. 32, 299–306.
Grayson, D.R., Kundakovic, M., Sharma, R.P., 2010. Is there a future for histone deacetylase inhibitors in the pharmacotherapy of psychiatric disorders? Mol. Pharmacol. 77, 126–135.
Jeste, S.S., Tuchman, R., 2015. Autism spectrum disorder and epilepsy: two sides of the same coin? J. Child Neurol. 30, 1963–1971.
Jobski, K., Hofer, J., Hoffmann, F., Bachmann, C., 2017. Use of psychotropic drugs in patients with autism spectrum disorders: a systematic review. Acta Psychiatr. Scand. 135, 8–28.
Kazantsev, A.G., Thompson, L.M., 2008. Therapeutic application of histone deacetylase inhibitors for central nervous system disorders. Nat. Rev. Drug Discov. 7, 854–868.
Khandaker, G.M., et al., 2015. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. Lancet Psychiatry 2, 258–270.
Kim, S.Y., Benowitz, N.L., 1990. Poisoning due to class IA antiarrhythmic drugs. Quinidine, procainamide and disopyramide. Drug Saf. 5, 393–420.
Kim, S., et al., 2016. PubChem substance and compound databases. Nucleic Acids Res. 44, D1202–D1213.
Kircher, M., et al., 2014. A general framework for estimating the relative pathogenicity of human genetic variants. Nat. Genet. 46 310-+.
Krezel, W., et al., 1998. Impaired locomotion and dopamine signaling in retinoid receptor mutant mice. Science 279, 863–867.
Kuhn, M., Campillos, M., Letunic, I., Jensen, L.J., Bork, P., 2010. A side effect resource to capture phenotypic effects of drugs. Mol. Syst. Biol. 6.
Kulkarni, J., et al., 2015. Estradiol for treatment-resistant schizophrenia: a large-scale randomized-controlled trial in women of child-bearing age. Mol. Psychiatr. 20, 695–702.
Lamb, J., et al., 2006. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science 313, 1929–1935.
LeClerc, S., Easley, D., 2015. Pharmacological therapies for autism spectrum disorder: a review. P T 40, 389–397.
Lee, S.H., et al., 2013. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat. Genet. 45 984-+.
Lerner, V., et al., 2008. Bexarotene as add-on to antipsychotic treatment in schizophrenia patients: a pilot open-label trial. Clin. Neuropharmacol. 31, 25–33.
Lerner, V., et al., 2013. The retinoid X receptor agonist bexarotene relieves positive symptoms of schizophrenia: a 6-week, randomized, double-blind, placebo-controlled multicenter trial. J. Clin. Psychiatr. 74, 1224–1232.
Lerner, V., McCaffery, P.J.A., Ritsner, M.S., 2016. Targeting retinoid receptors to treat schizophrenia: rationale and progress to date. CNS Drugs 30, 269–280.
Li, J.C., et al., 2016. Genes with de novo mutations are shared by four neuropsychiatric disorders discovered from NPdenovo database (vol 21, pg 290, 2016). Mol. Psychiatr. 21 298-298.
Linna, S.L., et al., 1999. Psychiatric symptoms in children with intellectual disability. Eur. Child Adolesc. Psychiatry 8 (Suppl. 4), 77–82.
Lozano, R., Azarang, A., Wilaisakditipakorn, T., Hagerman, R.J., 2016. Fragile X syndrome: a review of clinical management. Intractable Rare Dis. Res. 5, 145–157.
Lu, J.J., Pan, W., Hu, Y.J., Wang, Y.T., 2012. Multi-target drugs: the trend of drug

research and development. PLoS One 7.
Maden, M., 2007. Retinoic acid in the development, regeneration and maintenance of the nervous system. Nat. Rev. Neurosci. 8, 755–765.
Martinez-Gras, I., et al., 2012. Altered immune function in unaffected first-degree biological relatives of schizophrenia patients. Psychiatr. Res. 200, 1022–1025.
Mattingly, C.J., Colby, G.T., Forrest, J.N., Boyer, J.L., 2003. The comparative Toxicogenomics database (CTD). Environ. Health Perspect. 111, 793–795.
Medina-Franco, J.L., Giulianotti, M.A., Welmaker, G.S., Houghten, R.A., 2013. Shifting from the single to the multitarget paradigm in drug discovery. Drug Discov. Today 18, 495–501.
Meitzen, J., Mermelstein, P.G., 2011. Estrogen receptors stimulate brain region specific metabotropic glutamate receptors to rapidly initiate signal transduction pathways. J. Chem. Neuroanat. 42, 236–241.
Miller, B.J., Buckley, P., Seabolt, W., Mellor, A., Kirkpatrick, B., 2011. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol. Psychiatry 70, 663–671.
Nelson, M.R., et al., 2015. The support of human genetic evidence for approved drug indications. Nat. Genet. 47, 856–860.
Owen, M.J., 2012. Intellectual disability and major psychiatric disorders: a continuum of neurodevelopmental causality. Br. J. Psychiatry 200, 268–269.
Owens, M.J., Nemeroff, C.B., 2003. Pharmacology of valproate. Psychopharmacol. Bull. 37 (Suppl. 2), 17–24.
Park, E., et al., 2014. Epigenetic mechanisms of rubinstein-taybi syndrome. NeuroMolecular Med. 16, 16–24.
Perr, J.V., Ettinger, A.B., 2011. Psychiatric illness and psychotropic medication use in epilepsy. In: The Neuropsychiatry of Epilepsy, vol. 16. pp. 165–195.
Qiu, X.Y., Xiao, X., Li, N., Li, Y.M., 2017. Histone deacetylases inhibitors (HDACis) as novel therapeutic application in various clinical diseases. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 72, 60–72.
Rezaei, V., et al., 2010. Double-blind, placebo-controlled trial of risperidone plus topiramate in children with autistic disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 34, 1269–1272.
Richards, M., et al., 2001. Long-term affective disorder in people with mild learning disability. Br. J. Psychiatry 179, 523–527.
Rosenberg, G., 2007. The mechanisms of action of valproate in neuropsychiatric disorders: can we see the forest for the trees? Cell. Mol. Life Sci. 64, 2090–2103.
Ruderfer, D.M., et al., 2016. Polygenic overlap between schizophrenia risk and antipsychotic response: a genomic medicine approach. Lancet Psychiatry 3, 350–357.
Sabatine, M.S., et al., 2017. Evolocumab and clinical outcomes in patients with cardiovascular disease. N. Engl. J. Med. 376, 1713–1722.
Samad, T.A., Krezel, W., Chambon, P., Borrelli, E., 1997. Regulation of dopaminergic pathways by retinoids: activation of the D2 receptor promoter by members of the retinoic acid receptor-retinoid X receptor family. Proc. Natl. Acad. Sci. U. S. A. 94, 14349–14354.
Sanchez, M.G., Morissette, M., Di Paolo, T., 2013. Oestradiol modulation of serotonin reuptake transporter and serotonin metabolism in the brain of monkeys. J. Neuroendocrinol. 25, 560–569.
Santer, R., Calado, J., 2010. Familial renal glucosuria and SGLT2: from a mendelian trait to a therapeutic target. Clin. J. Am. Soc. Nephrol. 5, 133–141.
Scott, A.J., Sharpe, L., Hunt, C., Gandy, M., 2017. Anxiety and depressive disorders in people with epilepsy: a meta-analysis. Epilepsia 58, 973–982.
So, H.C., et al., 2018. Translating GWAS findings into therapies for depression and anxiety disorders: gene-set analyses reveal enrichment of psychiatric drug classes and implications for drug repositioning. Psychol. Med. 1–17. https://doi.org/10.1017/S0033291718003641.
So, H.C., Chau, K.L., Ao, F.K., Mo, C.H., Sham, P.C., 2018. Exploring shared genetic bases and causal relationships of schizophrenia and bipolar disorder with 28 cardiovascular and metabolic traits. Psychol. Med. 1–13.
Solmi, M., et al., 2017. Systematic review and meta-analysis of the efficacy and safety of minocycline in schizophrenia. CNS Spectr. 1–12.
Stahl, S.M., 2004. Anticonvulsants as mood stabilizers and adjuncts to antipsychotics: valproate, lamotrigine, carbamazepine, and oxcarbazepine and actions at voltage-gated sodium channels. J. Clin. Psychiatr. 65, 738–739.
Stitziel, N.O., Kathiresan, S., 2017. Leveraging human genetics to guide drug target discovery. Trends Cardiovasc. Med. 27, 352–359.
Storey, J.D., 2003. The positive false discovery rate: a Bayesian interpretation and the q-value. Ann. Stat. 31, 2013–2035.
Swanson, G.T., 2009. Targeting AMPA and kainate receptors in neurological disease: therapies on the horizon? Neuropsychopharmacology 34, 249–250.
Tsiouris, J.A., Kim, S.Y., Brown, W.T., Pettinger, J., Cohen, I.L., 2013. Prevalence of psychotropic drug use in adults with intellectual disability: positive and negative findings from a large scale study. J. Autism Dev. Disord. 43, 719–731.
Turner, T.N., et al., 2017. denovo-db: a compendium of human de novo variants. Nucleic Acids Res. 45, D804–D811.
Veltman, J.A., Brunner, H.G., 2012. Applications of next-generation sequencing de novo mutations in human genetic disease. Nat. Rev. Genet. 13, 565–575.
Vincenzi, B., et al., 2014. A randomized placebo-controlled pilot study of pravastatin as an adjunctive therapy in schizophrenia patients: effect on inflammation, psychopathology, cognition and lipid metabolism. Schizophr. Res. 159, 395–403.
Wang, Y.J., Xia, J., Helfer, B., Li, C., Leucht, S., 2016. Valproate for schizophrenia. Cochrane Database Syst. Rev. 3.
Wei, W.Q., et al., 2013. Development and evaluation of an ensemble resource linking medications to their indications. J. Am. Med. Inf. Assoc. 20, 954–961.
Weiwer, M., Lewis, M.C., Wagner, F.F., Holson, E.B., 2013. Therapeutic potential of isoform selective HDAC inhibitors for the treatment of schizophrenia. Future Med. Chem. 5, 1491–1508.
White, S.W., Oswald, D., Ollendick, T., Scahill, L., 2009. Anxiety in children and adolescents with autism spectrum disorders. Clin. Psychol. Rev. 29, 216–229.
Wong, E.H.F., Yocca, F., Smith, M.A., Lee, C.M., 2010. Challenges and opportunities for drug discovery in psychiatric disorders: the drug hunters' perspective. Int. J. Neuropsychopharmacol. 13, 1269–1284.
Xiang, Y.Q., et al., 2017. Adjunctive minocycline for schizophrenia: a meta-analysis of randomized controlled trials. Eur. Neuropsychopharmacol. 27, 8–18.
Ximenes, J.C.M., Verde, E.C.L., da Graça Naffah-Mazzacoratti, M., de Barros Viana, G.S., 2012. Valproic acid, a drug with multiple molecular targets related to its potential neuroprotective action. Neurosci. Med. 3, 107.
Yagle, M.A., et al., 2003. [3H]Ethynylbicycloorthobenzoate ([3H]EBOB) binding in recombinant GABAA receptors. Neurotoxicology 24, 817–824.
Yang, A.C., Tsai, S.J., 2017. New targets for schizophrenia treatment beyond the dopamine hypothesis. Int. J. Mol. Sci. 18.
Yang, H., et al., 2016. Therapeutic target database update 2016: enriched resource for bench to clinical drug target and targeted pathway information. Nucleic Acids Res. 44, D1069–D1074.
Yoo, M., et al., 2015. DSigDB: drug signatures database for gene set analysis. Bioinformatics 31, 3069–3071.