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REVIEW

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The road to precision psychiatry: translating genetics into disease mechanisms

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Hundreds of genetic loci increasing risk for neuropsychiatric disorders have recently been identified. This success, perhaps paradoxically, has posed challenges for therapeutic development, which are amplified by the highly polygenic and pleiotropic nature of these genetic contributions. Success requires understanding the biological impact of single genetic variants and predicting their effects within an individual. Comprehensive functional genomic annotation of risk loci provides a framework for interpretation of neurobiological impact, requiring experimental validation with *in vivo* or *in vitro* model systems. Systems-level, integrative pathway analyses are beginning to elucidate the additive, polygenic contributions of risk variants on specific cellular, molecular, developmental, or circuit-level processes. Although most neuropsychiatric disease modeling has focused on genes disrupted by rare, large-effect-size mutations, common smaller-effect-size variants may also provide solid therapeutic targets to inform precision medicine approaches. Here we enumerate the promise and challenges of a genomics-driven approach to uncovering neuropsychiatric disease mechanisms and facilitating therapeutic development.

The high heritability of neuropsychiatric disorders (46.3% as a class)[1] is a tantalizing clue that genetics will finally provide a rigorous neurobiological framework for comprehending conditions that have evaded biological understanding for decades[2]. Heritability estimates indicate that inherited genetic variants contribute substantially to disease liability, often more so than early environmental influences or noninherited, *de novo* mutations, but clearly gene and environment usually contribute together (**Fig. 1**)[2–6]. Initial linkage and candidate gene studies of psychiatric disease often yielded inconsistent findings, as a result of limited power and difficulty accounting for systematic biases such as population stratification. When interpreting results from large-scale genomics studies, it is important to take statistical power into consideration[7]. So, in contrast to candidate gene studies, results from more recent, large-scale genome-wide studies have yielded much more robust results[3].

The genetic architecture of psychiatric disease has received much attention and is the subject of several recent reviews[2–6,8–10]. Genetic variants associated with neuropsychiatric disease take several forms based on detection methodology and study design (**Box 1** and **Table 1**)[4]. They can also be classified by effect size, which can be inferred from population genetics models that predict an inverse relationship between variant frequency and effect size[11].

Hundreds of causal genetic variants with varying effect sizes have been robustly associated with neuropsychiatric disorders, with thousands more likely involved[3,12–16]. An essential next step is deciphering the biological impact of these variants. Here we discuss biological interpretation of genetic variation, focusing on rare variants of moderate to large effect and common variants with small effect. This genetics-driven approach has several advantages. First, genetics accounts for a majority of disease liability for many neuropsychiatric disorders and is therefore expected to be a high-yield area of investigation (**Box 2** and **Fig. 1**). Second, genetic variants indicate biological causality. Third, human genetics is grounded in human biology, which is especially important for neuropsychiatric phenotypes that may not be fully conserved across species. Finally, next-generation sequencing technology provides a near-complete survey of the genetic search space in an unbiased fashion at genome-wide scale, circumventing many of the limitations in reproducibility that undermined earlier genetic approaches (**Table 1**).

Interpreting rare genetic variation

An early clue of the genetic contribution to major psychiatric conditions was their association with rare Mendelian syndromes, such as DiGeorge, Rett, or fragile X, each with characteristic morphologic, cognitive, and neuropsychiatric phenotypes. The advent of chromosomal microarrays enabled the detection of copy number variation (CNV), submicroscopic deletions or duplications in DNA. More recently, whole exome sequencing (WES) and whole genome sequencing (WGS) have enabled the large-scale detection of rare, unique and private single nucleotide variants (SNVs), small chromosomal rearrangements (<50 kb), indels, and inversions. Chromosomal microarrays and WES have such a high yield in identifying genetic variants underlying neurodevelopmental disorders that they are becoming the standard of care for children with autism spectrum disorder (ASD)[17].

Detection, association, and interpretation of disease-causing genetic variants have many challenges, largely driven by the relatively high number of potentially disease-causing rare variants in

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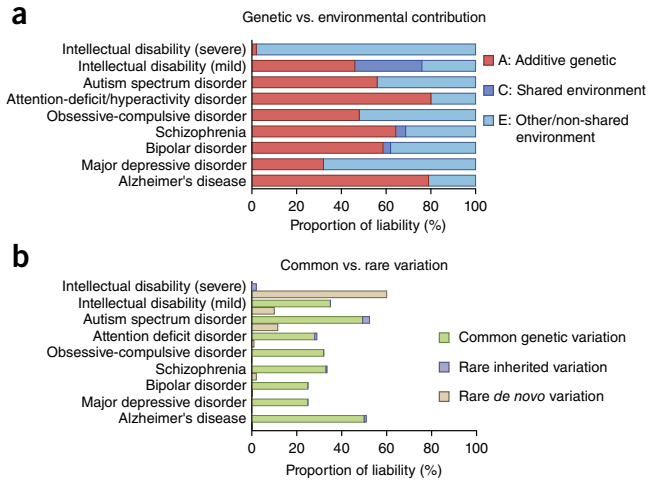


Figure 1 Genetic and environmental contribution to liability for neuropsychiatric disease. (**a**) ACE model liability estimates (see **Box 2**) are compiled for various neuropsychiatric disorders derived from large-scale twin and/or population-based studies. (**b**) Genetic contributions can be further partitioned by variant classes, including common, rare inherited, and rare *de novo* mutations. The contribution of *de novo* variants to disease liability is lower than their overall frequency in cases due to incomplete penetrance. Data are compiled from refs. 2,3,8–10,13,29,31,123,133–138.

every genome[18,19]. Sequencing studies are rarely sufficiently powered to detect disease association at a variant level, given the vast size of the genomic search space and potential number of ultra-rare or even private variants. To improve power, gene-based approaches are often applied, in which association testing is performed after variants are aggregated at the gene level[7]. Formal statistical significance should be assessed at genome-wide thresholds and statistical evidence of association should not be superseded by biological plausibility or 'functionality'.[20] The genome of a random individual will have on average 100 loss-of-function or likely gene-disrupting variants (nonsense, frameshift, and splice-site mutations), approximately one of which will be *de novo*. Furthermore, every individual carries on average 20 completely inactivated genes[19]. Synonymous variants are far more common and are therefore usually set aside, although there is now evidence that synonymous variation can have gene regulatory functions and can contribute to disease risk[21]. Individual rare genetic variants must therefore be interpreted in the context of the specific locus's or gene's tolerance for mutations, evolutionary or selective constraint[18], and population allele frequency[20]. Several bioinformatic tools exist that predict the deleteriousness of a given variant or tolerance for mutation at a gene level[18,22], although this remains an area of active development.

Even after taking into account inheritance and the predicted functional severity of a mutation, causal ambiguity often still exists even in cases of *de novo* protein truncating mutations, resulting in the assignment of 'variant of unknown significance'. Robustly identifying the most likely causal rare variants requires more complete genomic annotations (including the noncoding part of the genome) and extensive population allele frequency databases from several populations (**Fig. 2**). A rare allele in one population may actually be common in another and without strong phenotypic consequences, substantially changing the interpretation of pathogenicity[20]. To confront this, several large-scale efforts have been made to aggregate population-level genomic variation into searchable databases, including among others ExAC[23], DGV, and ClinVar. As an example, variants that are not found in the ExAC database, which includes WES results from over 60,000 unrelated adults without history of severe pediatric disease, are more likely to be deleterious[23,24]. Finally, the noncoding genome plays important regulatory roles, but is excluded from WES and has not been analyzed in the majority of published WGS papers. Having more comprehensive annotation of the noncoding genome in neural tissues is therefore a pressing goal of current research[25]. Standard pathway analyses should be applied only once variants have statistical support to avoid risk of false-positive results due to potential biases in these analyses, as well as inherent sensitivity to inclusion of spurious genes and population stratification (**Box 3**)[7,26,27].

The fact that *de novo* loss-of-function variants are predicted to have high impact[14,28] has made them attractive targets to study (see "Disease modeling" below). The vast majority of disease models have therefore been based on manipulation of genes harboring these alleles of large effect size[29]. However, most of these mutations are pleiotropic in nature, associated with variable but often severe abnormalities in multiple cognitive, medical, and behavioral domains. Understanding which molecular, anatomical, or physiological abnormalities relate to specific cognitive or behavioral phenotypes is difficult, and experiments that attempt to do so are rare. A notable recent example capitalized on an allelic series identified in *SHANK3*, in which two different loss-of-function variants have been associated with distinct clinical phenotypes in humans, namely schizophrenia and autism, albeit in only a few individuals[30]. Comparison of mice harboring orthologous mutations identified distinct neurobiological effects of the different variants, correlated with distinctive changes in prefrontal and striatal circuitry between models, a remarkable dichotomy[30]. Studying other alleles on different genetic backgrounds, and different genes showing similar phenotypic divergence in humans, as well as larger human cohorts with variable phenotypes associated with different alleles, will be necessary for appreciating the generalizability of these findings in mice to the observed divergence in disease mechanisms in humans.

Many high-penetrance rare mutations predispose to multiple clinically distinct disorders, including intellectual disability, epilepsy, autism, schizophrenia[28,31]. For example, about one-third of individuals with 22q11.2 deletions will have ASD and one-third schizophrenia[28,32]. As such, mice carrying a deletion syntenic to the human 22q11.2 locus should be viewed as a general model of neurodevelopmental disease, rather than a single disorder. Disease-associated CNVs have also been occasionally observed in apparently healthy carriers, for example in mothers with dup15q11–13 who pass the duplication to their affected children[33]. There is evidence that both genetic background and the environment can potentially have a large impact on the phenotypic outcome in these cases[32,34]. To account for this, it is prudent to conduct experimental manipulations at these loci on at least two genetic backgrounds. Furthermore, comprehensive clinical phenotyping of individuals with rare variants in the same locus will be essential to help decipher underlying neurobiological mechanisms[35]. Indeed, large-scale cognitive assessment of individuals carrying major-effect CNVs in the Icelandic population found substantially reduced performance in specific cognitive domains, even in carriers without a psychiatric diagnosis[36]. Neuroimaging has begun to elucidate the neuroanatomic and circuit-level impact of these rare variants, highlighting the promise of this bottom-up approach to mapping gene–brain–behavior relationships[37]. Furthermore, studying such people harboring the same mutation, but with different clinical outcomes, is likely to be high yield[36]. Finally, measuring other forms of genetic variation within rare-variant carriers, such as polygenic risk, may provide a potential explanation for underlying pleiotropy, as recently shown in schizophrenia[34].

Box 1 Large-scale genetic investigation of complex traits, such as neuropsychiatric disease

Technological advances now enable cost-effective, genome-wide interrogation of genetic variation in large cohorts, but they necessitate careful power analysis and study design to maximize variant discovery (**Table 1**)[7]. Microarray-based platforms can detect structural anomalies such as CNVs or genomic rearrangements. SNP microarrays provide a cost-effective platform for common trait GWAS. A genome-wide SNP backbone coupled with imputation to an ancestry-matched reference panel enables efficient genomic coverage. Population-specific platforms have been developed, such as the PsychChip, which has higher density in regions associated with psychiatric disease, including rare CNVs and exome variants. Despite this, coverage remains incomplete and generally limited to common or previously identified rare variants. Massively parallel, high-throughput sequencing platforms identify variants with single-base-pair resolution and can theoretically capture the full range of allele frequencies (for example, common, rare, private) and variant types (SNVs, indels, CNVs). In WES, the ~1% protein-coding portion of the genome is captured and then sequenced, to reduce cost and bolster interpretability of identified variants. WGS surveys the entire genomic space, although coverage is still often incomplete because of difficulties mapping repeat-dense regions. Sufficient depth is critical to overcoming potential sequencing errors and capturing heterozygous SNVs. Sanger sequencing is often performed as a confirmatory test.

Study design is an important factor when considering large-scale genetic studies. Case/control is a standard design that compares allele frequencies across a diverse set of cases and controls *en masse*. However, subtle biases (for example, population stratification) must be rigorously accounted for and inheritance patterns cannot be determined. Family designs that include a proband and both parents ('trio') can account for population stratification and identify inheritance patterns but are more difficult and expensive to collect. Filtering for *de novo* variants in a proband with unaffected parents can facilitate interpretation of pathogenicity. However, 'unaffected' parents may harbor incompletely penetrant mutations, especially for complex traits.

Table 1 Platforms for large-scale, genome-wide interrogation of genetic variation

Technology	Outcome measure in individual	Outcome measure in population	Challenges to interpretation
Chromosomal microarray	CNV	Recurrence	(1) Pleiotropy (2) Incomplete penetrance (3) Pathogenic gene(s) not directly identified
Genome-wide SNP microarray	SNP Polygenic score	Genome-wide significant index SNP or haplotype	(1) Identifying causal variant (2) Identifying functional effect of variant (3) Function of noncoding regions often not well established, especially in CNS
Whole exome sequencing	(*De novo*) SNVs	Gene burden test	(1) Pathogenicity often difficult to establish unless multiple instances observed (2) Functional significance often unclear, especially for missense mutations
Whole genome sequencing	SNPs (*De novo*) SNVs, indels	Gene burden test or Recurrence	(1) Pathogenicity often difficult to establish unless multiple instances observed (2) Functional significance often unclear, especially for missense mutations (3) Function of noncoding regions often not well established, especially in CNS

Another approach to disentangling mechanisms is to study allelic series of variants with different effects on the phenotype in one locus[30]. Here one would expect to see concentration of phenotypes within specific subcategories of variants: for example, milder phenotypes in patients with heterozygous or missense mutations in genes known to cause severe recessive disorders. The application of WES and WGS in larger populations will enable us to answer this question in more detail and will be a boon to genotype–phenotype studies in humans.

Interpreting common genetic variation

Genome-wide association studies (GWAS) have successfully identified thousands of common genetic variants associated with complex diseases (http://www.ebi.ac.uk/gwas/), including several hundred loci for neuropsychiatric disorders[3,12,13,16,38,39]. Population-level screening for common genetic contributions to human phenotypes is on the near horizon. Despite these GWAS successes, the number of resolved psychiatric disease genes remains small due to the difficulty identifying the causal variant(s) and their functional impact.

GWAS does not identify a gene *per se*, but a region that is associated with disease status. When genome-wide significance is achieved (set at $P < 5 \times 10^{-8}$), the effective confidence interval surrounding a 'lead' or 'index' SNP (with the lowest *P*-value in a given locus) is set by the surrounding region of linkage disequilibrium (LD), which spans on average ~40 kb, but is highly variable throughout the genome. Identifying the underlying 'causal' variant(s) within a target region, and its biological effect, is typically an enormous challenge. In schizophrenia, for example, the strongest GWAS signal maps to the major histocompatibility complex (MHC) locus and spans several hundred genes[12]. Recent work elegantly dissects this locus to identify the likely causal variants within a few genes, including *C4A* (ref. 40), which we describe later in more detail.

A majority of common disease-associated genetic variation lies outside coding regions and is enriched in regulatory elements such as enhancers or promoters. Variants in these regulatory elements act to modulate the expression and splicing of distal gene targets, potentially with large effect. Regulatory elements also tend to act in a cell-type- and tissue-specific manner and can be inferred through evolutionary conservation, chromatin accessibility, and characteristic histone marks (**Box 3**)[41–44]. Projects such as ENCODE[45], the NIH Epigenetics Roadmap[46], PsychEncode[25] and GTEX[47] are building tissue-specific atlases of human gene regulation. However, these annotations are generally derived from only a few individuals and are far from complete, especially in neural tissues, directly limiting our ability to annotate genetic variants relevant to human brain disorders. There also is substantial evidence that gene regulation can occur at long intrachromosomal distances[48]. Consequently, identifying the gene targets of regulatory regions is a challenging problem and an area of active investigation using both computational[49] and experimental approaches, such as HiC[50]. Gene targets can also be inferred statistically, relying on expression quantitative trait loci (eQTL; see "Integrative approaches" below), which identifies variants that are associated with changes in gene expression in a given cell type or tissue. Although most (~80% of) variants acting as eQTLs occur within 100 kb of their target gene, many loci act on genes hundreds

Box 2 Genetic architecture of neuropsychiatric disease

A fundamental question for any complex human trait is the degree to which genetic or environmental factors influence phenotypic variance. Heritability (h^2) refers to the proportion of phenotypic variance due to genetic factors and in the narrow sense is also referred to as additive genetic variance (*A*). Environmental factors can be partitioned into the common, shared environment (*C*) and the residual, nonshared environmental variance (*E*). While the common, shared environment can be difficult to precisely pinpoint, it is often interpreted as *in utero* and early childhood factors. Classically, twin studies have been used to estimate these various components, although more sophisticated statistical methods have been developed (for example, generalized linear mixed models)[139]. Importantly, *de novo* genetic variation, which can contribute substantially to disorders such as ASD or intellectual disability, is generally not captured in heritability estimates. Disease-associated genetic variation can be further partitioned by allele frequency and inheritance patterns. Common variants (minor allele frequency >0.5%) generally have small effect sizes with odds ratios <1.3. Rare variants, including CNVs, have much larger effect sizes (odds ratios typically 2–60), and yet penetrance for specific clinically defined disorders can vary widely. Mutations of larger effect size have been constrained by natural selection because of negative effects on reproductive fitness and therefore tend to be both rare and *de novo*. The contribution of common genetic variation to overall disease liability (for example, SNP heritability) can be estimated using genome-wide complex trait analysis (GCTA)[140] or methods that partition heritability, such as LD-score regression (**Fig. 1**)[57]. Except for severe intellectual disability (IQ <50), current estimates indicate that rare variants contribute an order of magnitude less to overall disease liability than do common variants, although this varies across conditions.

of kilobases away[47,48,51]. Once a regulatory effect such as an eQTL or physical promoter–enhancer interaction is confirmed experimentally, further conclusive evidence can be derived from showing that such relationships exist in human brain and are altered in the disease-affected brain. Complementing such studies by investigating the effects of common disease-associated SNPs on human phenotypes, such as brain structure and function, can provide further insight into circuit mechanisms[52].

Capturing polygenicity

The biological effect of individual common variants (or loci) in most cases will be very small[4,53]. Since individual common variants account for such a small proportion of disease liability, how can they be of use? One major insight came from the work of Visscher, Wray, and colleagues, who used quantitative genetic reasoning to demonstrate that one could capture the aggregate effect of genetic variants (polygenicity), many of which fail to meet highly conservative genome-wide significance thresholds but nonetheless contribute to disease liability[54]. In schizophrenia, there are predicted to be over 8,000 disease-associated common variants[13]. A similar level of polygenicity is expected for virtually every major common neuropsychiatric disorder[3]. How this plays out in an individual patient is not yet known, and environmental factors (such as smoking[55] or cannabis use[56]) potentially contribute. Indeed, it is clear that genome-wide significant loci represent the tip of the iceberg in terms of the biological signal captured by GWAS[4,53,54]. New approaches such as LD score regression[57] can quantify the aggregate 'SNP heritability' captured by common variants within a given study, which can then be used to calculate genetic correlations across disorders or with other traits of interest[58], especially intermediate phenotypes[59]. An extension of this method can quantify the proportion of heritability attributed to SNPs within various functional categories (such as enhancers for specific cell types)[60]. Conceptually similar, polygenic risk scoring (PRS) quantifies within an individual the aggregate effect of common variants for a given trait, typically calculated as the sum of trait-associated alleles across the genome, weighted by effect size[61,62]. PRS can be used to identify high-risk individuals for closer clinical assessment, phenotyping before disorder onset to better understand disease trajectory, or to stratify for clinical trials, choosing or refining treatments on the basis of genetic signal. In addition, PRS provides a continuous, quantitative measure of genetic load that can be correlated with phenotypic or endophenotypic measures, such as structural or functional neuroimaging[63]. However, PRS is likely to be population-specific and is limited by the power of the initial GWAS. There is urgent need to expand such studies to more diverse populations of African, Hispanic, and Asian descent, so that individuals within these populations can benefit from the promise of genetic advances.

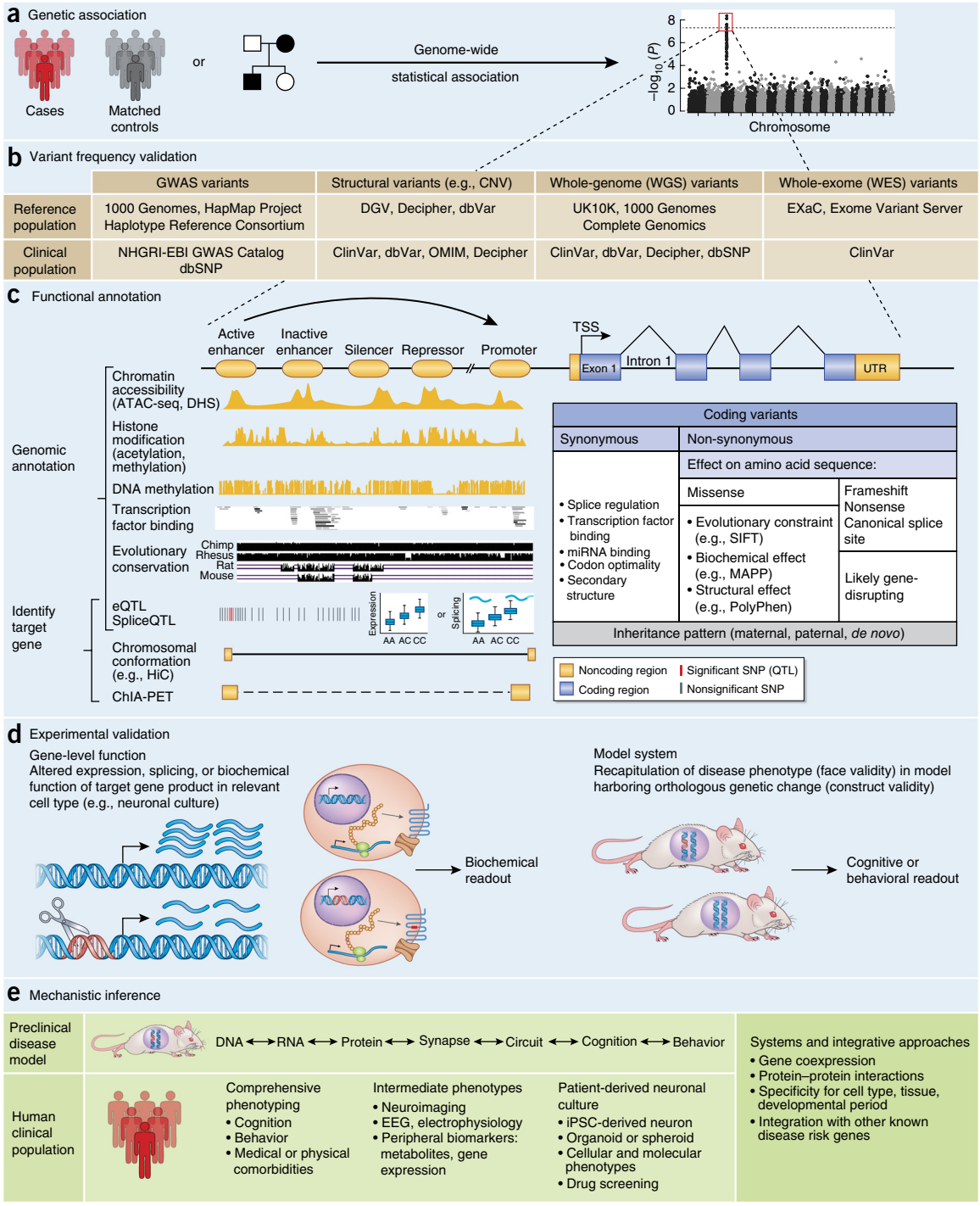
In schizophrenia, PRS can currently capture ~7% of variance in disease liability in independent populations of European ancestry[12]. While far from complete, this translates into odds ratios of 8–20 when comparing

Figure 2 Neurobiological framework for interpretation of individual disease-associated variants. (**a**) When considering a neurobiological framework for interpretation of disease-associated genetic variation, it is most important to begin with variants that meet genome-wide significance thresholds[20]. (**b**) Independent replication is also critical, which can be supported by prior reported associations in a clinical genetic database (for example, ClinVar) and by an appropriate observed frequency in large population reference databases (for example, ExAC). (**c**) Functional annotation differs for coding and noncoding variants, although some general principles apply to both (for example, inheritance, evolutionary conservation). For coding variants, the target gene is known and annotation is initially based on impact to the amino acid sequence. Synonymous mutations, often interpreted as neutral, can contribute to human disease risk by changing transcription factor or microRNA binding or by altering mRNA stability or secondary structure[21]. Nonsense, frameshift, and canonical splice site mutations are generally placed in the most deleterious, likely gene disrupting category, although their disease association must still be statistically supported. Interpretation of missense mutations is more difficult, relying typically on evolutionary constraint or by inferred disruption of protein structure or biochemical function[22]. Functional annotation of noncoding variants is a rapidly evolving area, but can be broadly conceptualized as (top) predicting a regulatory effect and (bottom) identifying target gene(s). Computational methods can predict the likelihood that noncoding regions act as enhancers, repressors, or insulators within a given tissue or cell line on the basis of epigenetic annotations[49]. Gene targets can be inferred through statistical frameworks such as eQTL or by mapping intrachromosomal physical binding interactions through chromosome conformation capture methods. (**d**) Predictions of the potential impact of a variant on the target gene should be experimentally validated. Gene-level disruption can be confirmed in a cell-based experimental system, as long as genomic and epigenetic context are considered. Model organisms with construct validity may also be useful. (**e**) Once the proximal biological effect of a disease-associated variant is determined, disease mechanisms can begin to be inferred through follow up investigation in preclinical or clinical settings. Performing comprehensive clinical and medical phenotyping of individuals harboring specific, known disease-associated variants will be especially important for mechanistic insight as well as future 'genotype-first' precision medicine approaches[35]. NHGRI, National Human Genome Research Institute; EBI, European Bioinformatics Institute; ATAC-seq, assay for transposase-accessible chromatin with sequencing; DHS, DNase I hypersensitivity sites; ChIA-PET, chromatin interaction analysis by paired-end tag sequencing; TSS, transcription start site; SIFT, sorting intolerant from tolerant; MAPP, multivariate analysis of protein polymorphism.

the highest vs. lowest decile groups, depending on population[12]. This finding was recently replicated in an independent UK population, in which PRS was found to account for 5.4% of variance in disease liability translating to an odds ratio of 7.7 between the highest and lowest deciles. As such, PRS is among the most strongly reproducible biological disease predictors to date[64].

PRS can also be a powerful tool for identifying patient subgroups. For example, polygenic risk for bipolar disorder predicts manic symptoms in schizophrenia, but not other clinical symptoms, suggesting a distinct mechanistic underpinning for this symptom domain[65]. A similar approach was recently taken in inflammatory bowel disease, in which PRS can distinguish ulcerative colitis from Crohn's disease and identify distinct subtypes of Crohn's disease[66]. In ASD, LD score regression was recently used to demonstrate that genetic risk for deficits in social function fall along a continuous, bell-shaped distribution within the general population[24], as previously predicted[67]. These studies demonstrate that quantification of polygenic risk coupled with systemic phenotypic assessment can facilitate new insights into disease biology.

However, a major challenge that remains is to understand the mechanisms by which multiple genetic risk factors of low individual effect size actually coalesce to increase disease risk. We emphasize the view



Box 3 Lessons in reproducibility

Psychiatric genetics is susceptible to false positive results, a problem amplified by frequent comorbidities, overlapping symptoms and limited biomarkers. The candidate gene era was fraught with false positives, which have been limited by genome-wide analyses[3]. However, in the era of whole exome and genome sequencing, nonpathogenic rare and private variants will be identified in every individual genome, so extra care must be taken to avoid overinterpretation of results[7,20].

Replication is critical; genotypes and phenotypes between discovery and replication sets should be comparable. For example, an early genetic finding in schizophrenia was a linkage peak including the *DTNBP1* locus. Replication studies measured different markers around *DTNBP1* without imputation to a common reference, each defining a different haplotype as the risk allele, with no concordance of findings[141]. And indeed, the largest schizophrenia GWAS to date has failed to find any association near the *DTNBP1* locus.

In case/control studies it is critical to account for all potential biological (for example, age, sex) and technical confounds, especially those related to experimental design, such as batch effects. For example, a study profiling gene expression in cell lines derived from subjects of European and Asian ancestry reported that 25% of genes were differentially expressed across ethnicities, which was claimed to reflect common genetic variation[142]. However, these results disappeared after accounting for a strong group × batch confound[143]. Similarly, a recent high-profile GWAS of longevity reported 33 genome-wide significant SNPs, which were able to predict lifespan in an independent cohort with a remarkable 77% accuracy[144]. This study was later retracted after it was determined that a batch effect likely accounted for the signal.

Subtle differences in allele frequencies between subpopulations within case and control groups (termed "population stratification") or (cryptic) relatedness among subjects can also introduce significant bias. A recent study claimed to predict a diagnosis of ASD with a remarkable ~70% accuracy using only 237 common SNPs, but did not properly account for population stratification, as claimed[145]. Rather, these SNPs were strongly associated with ethnicity differences between subjects, and did not predict ASD status[146]. Similarly, a recent paper claimed to identify eight genetically defined subtypes of schizophrenia in 4,196 patients and 3,827 controls, but did not account for population stratification[147]. One should be concerned that, without explicit correction, these results are driven by ancestry or other hidden confounds.

Finally, rare variants are present in every genome, can have a predicted functional effect without actually being pathogenic, can segregate with traits owing to hidden factors (for example, linkage disequilibrium), or can aggregate by chance in affected family members. A recent paper reported a new Mendelian form of multiple sclerosis caused by a rare mutation in *NR1H3*, identified in two multiplex families with a severe form of the disease[148]. The authors also show that the purported disease variant causes transcriptional dysregulation of *NR1H3* and its target genes. However, a study with 13-fold larger sample size found no such association. Rather, the results can be accounted for by a previously identified genome-wide significant common multiple sclerosis variant in moderate LD ~400 kb away[149]. Potential pathogenic variants should be assessed in large population-scale databases whenever possible, and evidence of a biological effect in a model system does not provide evidence for genetic association.

that systems biology and integrative approaches as described below are a necessary step in prioritizing potential disease mechanisms and drug targets for therapeutic development[3,68]. Such approaches provide platforms on which to understand convergence in disease and protective mechanisms from human population genetic data[3,68].

Systems genetics

Some of the same technological advances that have enabled large-scale genetic investigation of complex diseases have also enabled systematic characterization of epigenetic, molecular, cellular, and circuit-level landscapes of the human brain across typical development[25,69,70]. These resources now enable comprehensive pathway-based, systems-level approaches to articulating the neurobiological context in which genetic variation may exert its effects, as recently reviewed[71]. Perhaps most relevant for CNS disorders, disease relevant gene sets can be investigated for temporal, spatial, and cell-type specificity using large reference data sets. The BrainSpan[69] and BrainCloud[72] projects profiled gene expression in hundreds of human brain samples across the lifespan, beginning with early fetal timepoints. Spatial patterns are captured in exquisite anatomic detail in adult[73] and fetal[74] human brain samples, as well as primate[75], by the Allen Brain Institute. CNS cell-type-specific transcriptomes have been defined using single-cell RNA sequencing (RNA-seq) or cell sorting methods in primate[76], mouse[77], and now human[78]. Overlapping the growing list of reproduced genetic hits in psychiatric disease with more refined cell-type-specific profiles is likely to provide key circuit-level insight into disease[79].

Using these approaches, common genetic variation for schizophrenia, bipolar disorder, and depression has been suggested to converge on pathways for histone methylation, immune signaling, and neuronal signaling, although this must be viewed as preliminary owing to the small number of known loci in this analysis[80]. Gene coexpression networks can identify modules of genes with predicted functional relationships at specific spatiotemporal timepoints in brain. Intersecting these modules with risk genes can yield insights into disease biology[68,71,81,82]. Clustering genes on the basis of experimentally defined physical properties, such as protein–protein interactions, can identify sub-networks of convergent biological processes, such as chromatin remodeling and histone regulation in ASD[83–85]. Combining protein–protein interaction, gene expression, and other data into truly integrated networks reflecting CNS function will be critical to understanding pathway convergence of manifold genetic risk variants in these disorders.

Integrative approaches

Allele-specific expression and eQTL studies link genetic variation with altered transcript expression. Sample size and tissue specificity are critical limiting factors, as 10–45% and ~70% of eQTLs are predicted to be tissue and cell-type specific, respectively[47,86]. This has prompted several consortium-level efforts to generate eQTL databases of human brain, including GTEx[47], UKBEC[87], and CommonMind[88], among others. As current human brain eQTL studies contain at most a few hundred samples, they remain vastly underpowered given a large statistical search space relating a dense map of genetic variation to expression of ~20,000 genes. Furthermore, as eQTLs are often highly cell-type specific[86], tissue-level profiling of brain tissue homogenate likely obscures contributions from underlying individual cell types.

Nevertheless, psychiatric GWAS studies have found enrichment of brain-specific eQTL among disease-implicated SNPs as a class, suggesting that intersection with these regulatory data sets may provide important biological insights[39]. Critical steps moving forward will be to intersect GWAS-implicated disease variants with large-scale eQTL studies, followed by verification of the significance (and directionality) of predicted functional relationships through case-control transcriptome profiling. Recent innovative studies have begun to directly integrate GWAS and eQTL data to perform transcriptome-wide

association studies, which have the potential to provide powerful gene-centric insights into disease mechanisms[89,90]. On balance, however, we note that overlap of eQTL and disease association peaks does not provide evidence of a causal relationship to disease, since linkage disequilibrium acts on both signals and some degree of overlap is expected by chance alone. Furthermore, eQTL studies may be less statistically conservative in correcting for multiple comparisons than GWAS, leading to a higher propensity for false positive results[91].

Similar approaches exist for defining the landscape of epigenetic regulation of gene expression, which represents an additional layer of biological complexity[44]. Major psychiatric risk genes include *CHD8*, which encodes a chromatin remodeling enzyme associated with ASD and macrocephaly[35], and *SETD1A*, which encodes a histone methyltransferase and was recently associated with schizophrenia, developmental delay, intellectual disability, and epilepsy[92]. Common genetic variants for schizophrenia and bipolar disorder have also been linked to histone methylation, albeit less directly[80]. Recent, in-depth characterization of the spatial and developmental trajectory of methylation in human brain demonstrated that schizophrenia-associated variants strongly overlap with fetal brain methylation-QTL signals[70,93]. Similar approaches are being undertaken for histone acetylation QTL[94], for example, as part of PsychEncode[25].

Partitioning the GWAS SNP heritability from schizophrenia and bipolar disorder on the basis of functional categories defined by these epigenetic signatures identified strong CNS enrichment for common genetic variation in both disorders and fetal brain, specifically in schizophrenia[60,95]. Concordantly, genetic variants conferring risk for schizophrenia so far seem enriched in fetal prefrontal cortex gene coexpression networks[81,82]. These results suggest that fetal brain development represents one critical window during which genetic risk factors for certain specific neuropsychiatric disorders exert their effects.

Finally, the most powerful approaches will integrate multiple orthogonal data sets to assess differing levels of genetic, epigenetic, and neurobiological regulation. An exemplary recent example of this type of approach was the investigation of the top genome-wide significant locus in schizophrenia, spanning the highly complex MHC region[40]. This work combined fine mapping of this locus in schizophrenia with a newly generated reference of structural haplotypes to predict that disease-associated variants function by increasing expression of the complement component 4A gene (*C4A*) in brain. The role of *C4A* was verified using gene expression profiling in schizophrenia brain samples, and the C4 protein was shown to regulate synaptic pruning in a rodent model, identifying one of the causal neurobiological mechanisms contributing to disease risk. Integrative approaches have also been undertaken to characterize other GWAS loci in schizophrenia—for example, identifying risk variants that function as eQTL and map to enhancer regions encoding the L-type calcium channel *CACNA1C*[95].

Disease modeling

Many powerful basic research tools now exist that can guide mechanistic insight into disease-associated genetic variation, ranging from *in vivo* animal models to *in vitro* culture systems of human fetal neuron progenitor cells, adult induced pluripotent stem cell–derived neurons, and cerebral organoids[96,97], each with advantages and limitations[29]. Caution is always warranted, as insights from behavioral and circuit-level analyses related to human higher cognition and behavior are limited by evolutionary divergence. Even at a molecular level, some genes and signaling pathways are not well conserved between humans and rodent models[98,99]. In addition, genetic risk alleles for psychiatric disease may converge on human-specific transcriptional processes, or pathways that are not well preserved in lower organisms[100–102].

Classic model organisms used for molecular genetics have predominantly consisted of fruit fly (*Drosophila melanogaster*), zebrafish (*Danio rerio*), and mouse (*Mus musculus*), owing to the relative ease of genetic manipulation and potential for high throughput investigation. Recent advances in genome engineering have facilitated the creation of transgenic rat[103] and primate[104] models of neuropsychiatric disease, limiting throughput but enabling investigation of more complex neural circuitry[105]. Model organisms have historically been used to investigate the effect of rare, deleterious variants or Mendelian syndromes associated with neuropsychiatric disease. Common genetic variants are much more difficult to model in animals as most lie in regulatory regions poorly conserved across species. Transgenic mice have been used to model major effect forms of autism (including mutations in *FMR1*, *TSC1*, *TSC2*, *CNTNAP2*, and *MeCP2*), as well as copy-number variation (16p11.2, 22q11.2, and dup15q11), as recently reviewed[29]. Adult rescue of phenotypic deficits has been demonstrated in major gene mouse models of neurodevelopmental disorders, such as fragile X syndrome, tuberous sclerosis and Rett syndrome, providing hope for treatment. However, analogous treatments in the human clinical populations have largely failed, for largely unknown reasons[106]. Similar models of rare variants have been investigated in flies, including loss-of-function mutations in the *FMR1* homolog *dmfr1* (ref. 107), and zebrafish, such as *cntnap2* mutants[108]. Notwithstanding the above caveats, major advantages of *in vivo* models include the ability to directly interrogate complex circuit-level alterations, to assess basic cognitive phenotypes, to measure and manipulate neurodevelopmental processes, and to perform large-scale genetic or pharmacologic screens, among others. Modeling of 16p11.2 deletion syndrome in mice, for example, has enabled circuit-level phenotypic dissection, identifying a number of abnormalities in the physiology and function of the basal ganglia[109]. Molecular genetic dissection of this locus in zebrafish implicated a single gene in this region, *KCTD13*, as mediating the underlying neuroanatomic phenotype[110]. However, the region is complex and it is likely that other genes in this region contribute to the broader cognitive and behavioral phenotypes.

Recent developments in stem cell biology have enabled the *in vitro* generation of human neurons, providing a greatly needed experimental platform for phenotypic characterization and drug screening[97]. Much of the excitement centers on the potential for creating patient-derived 'virtual biopsies' for a tissue is inaccessible to direct investigation. Characterizing neurons derived from human induced pluripotent stem cells from subjects with known penetrant mutations[111] and those without established genetic causes of disease both have value. In the latter, the likely causal heterogeneity requires higher numbers than are typically studied to yield generalizable results[112]. Advantages of this approach include the ability to capture polygenicity, incorporation of genetic background, ability to investigate human-specific biological processes, and potential for high throughput assays[113]. Pharmacologic screening is thereby possible for patient-derived mutations[114], presaging future precision medicine approaches. One limitation is that until we are able to develop mechanistic knowledge based on our genetic findings, it is not clear what relevant cellular or molecular phenotypes should be screened for *in vitro*[29]. Systematic approaches, such as gene expression profiling, are likely a good starting point and, critically, can be used to quantify the relative maturity, variability, cellular, and regional identity more rigorously than individual markers[115]. Other technical hurdles include line-to-line heterogeneity, a limited number of neuronal cell types that can be differentiated, and an inability to form complex circuits. More sophisticated approaches have recently

been undertaken to address some of these limitations, including the development of cerebral organoids[112] and human cortical spheroids[96], which exhibit a cytoarchitectural structure with cortical lamination, incorporate neuronal and glial cell types, form functional synapses, and display spontaneous electrical activity. Considering genetic background effects, a final critical factor is sample size, which can be partially mitigated using either unaffected family members as controls or isogenic lines in which the genetic risk alleles have been corrected.

Pathways to precision health

Moving forward, how can we translate genetic hits into mechanistic insight to reinvigorate a stalled CNS drug development pipeline[116]? The genomics era has instilled much optimism in this regard[117], having recently identified new causal pathways in schizophrenia[40], new genetic predictors of treatment response in bipolar disorder[118], and genetic risk factors for serious side effects of psychotropic medication[119], among others. It is notable that most of these advances are the product of large-scale collaborative approaches[120].

A related question that remains is how to prioritize genetically identified biological targets for development of new medicines. To date, such efforts have disproportionately focused on the mutations with the largest effect sizes, which are easier to identify, interpret, and model in preclinical settings. However, there is evidence that small effect-size (typically common, inherited, polygenic) and large-effect size (typically rare, noninherited) variants converge on distinct biological processes. In ASD, for example, inherited variants converge largely on postnatal synaptic processes, whereas *de novo* loss-of-function variants are enriched for developmental regulation and chromatin modification pathways[15,71]. A potential interpretation is that more highly penetrant mutations disproportionately disrupt the robustness of the neurodevelopmental trajectory to an environmental or genetic perturbation ('canalization')[121]. This would explain the association of rare variation with more severe and pleiotropic syndromes including intellectual disability, epilepsy, and ASD. This would also predict that clinical disease specificity is guided by distinct factors, such as environmental or common variants, in accordance with recent evidence[34].

We propose that genes and pathways affected by common variants may be at least equally, if not more, amenable to therapeutic intervention than those disrupted by high penetrance mutations (**Box 4**). First, the small effect size of common variants suggests that disease risk is inherently modifiable and that 'protective' environmental exposures in the form of biological intervention could prevent disease or reduce risk. Second, common variation by definition is present in a larger proportion of the population and therefore is likely more generalizable. Third, for most neuropsychiatric disorders, common variation is predicted to contribute more substantially to disease liability than highly penetrant mutations, often by an order of magnitude[31,122,123]. Finally, in other complex disorders, successful new drug targets can often be retrospectively substantiated by genome-wide significant variants (**Table 2**)[124]. In hyperlipidemia, for example, targets of statins (*HMGCR*) and the new class of lipid-lowering PCSK9-inhibitors (*PCSK9)* are among the top GWAS-identified risk variants[125], although these targets were discovered before the GWAS era. There are enormous challenges to targeting common variants using traditional methods. First, we need to better characterize composite genetic risk in individuals—what common and/or rare risk variants are necessary and sufficient to cause disease in an individual. Individual genetic subtypes of a disorder could be identified on the basis of convergent risk profiles defined by population scale WGS, thus stratifying patients by their underlying biology[65,66].

Box 4 FDA-approved medications supported by common-variant association

Nearly all classes of medications currently used to treat neuropsychiatric disease were discovered by serendipity and target the same molecular pathways as their prototypes, developed decades ago[116]. Novel therapeutic targets are greatly needed and genetics provides an avenue for their identification[117]. Preclinical drug development has historically favored targets based on rare, moderately penetrant genetic variants, which are easier to identify, interpret, and investigate in model organisms. Although this has been successful in some cases, the recent dismal approval rate of candidate drugs entering clinical trials for neuropsychiatric disorders suggests that alternative approaches may be needed[106]. We argue that pathways enriched for common genetic variation should receive more attention for drug development. In support of this, we have surveyed the literature for examples of FDA-approved medications that are supported by GWAS-identified targets (**Table 2**). While most of these drugs were developed before the GWAS-era, their targets can be retrospectively validated by genome-wide significant loci associated with disease risk. One can extrapolate from these successes to predict that additional pathways enriched for common variation from disease GWAS can identify future efficacious drug targets[131]. We note that this is neither prospective nor a formal statistical analysis assessing enrichment of approved drugs acting on GWAS-identified targets. However, others have estimated that genetic evidence as a whole could double the success rate of clinical drug development[124].

Table 2 FDA-approved medications supported by GWAS variants

Disease	Lead GWAS SNP	Genetic locus	FDA-approved medication Drug class
Psoriasis	rs9988642	*IL12R–IL23R*	Ustekinumab Biologic
Hyperlipidemia	rs12916	*HMGCR*	Many Statin
Hyperlipidemia	rs2479409	*PCSK9*	Alirocumab Biologic
Type 2 diabetes	rs1801282	*PPARG*	Many Thiazolidinediones
Type 2 diabetes	rs5219	*KCNJ11*	Many Sulfonylurea
Osteoporosis	rs9533090	*TNFSF11* (*RANKL*)	Denosumab Biologic
Osteoporosis	rs7751941	*ESR1*	Many Selective estrogen receptor modulator
Schizophrenia	rs2514218	*DRD2*	Many Antipsychotic
Rheumatoid arthritis	rs2228145	*IL6R*	Tocilizumab Biologic
Rheumatoid arthritis	rs3087243	*CTLA4*	Abatacept Biologic

See **Supplementary Table 1** for further details.

High-throughput precision health approaches are gaining traction and may provide an additional platform through which to validate potential drug targets. Phenome-wide association studies, which integrate clinical and genomic data to identify genotype–phenotype relationships on the basis of electronic medical records, offer great promise[126,127] as evidenced by pharmacogenomic-based predictors of drug efficacy[128]. Other powerful new approaches include computational drug repositioning[129,130], integrating, for example, a database of known drug targets with GWAS-implicated disease loci[131]

or with the transcriptomic profile of a drug from resources such as the Connectivity Map[132]. With large enough samples, the goal is that phenome-wide association studies will allow dissection of genetic contributions to specific phenotypes that, when combined, produce a specific clinical syndrome. Finally, the importance of environmental factors (such as gut microbiota) is becoming increasingly realized. Once genetic risk factors and pathways are accounted for, it will become possible to more systematically query the impact of the environment and its interaction with genetics. This approach has shown recent success in dissecting the role of smoking[55] and cannabis[56] use on risk of schizophrenia. As such, the knowledge imparted by understanding genetic contributions to disease risk can serve as a causal anchor, magnifying the power of follow-up studies and providing a strong foundation for finally unraveling the complex brain–behavior relationships underlying neuropsychiatric disease.

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