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Systems biology and gene networks in neurodevelopmental and neurodegenerative disorders

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Abstract

Genetic and genomic approaches have implicated hundreds of genetic loci in neurodevelopmental disorders and neurodegeneration, but mechanistic understanding continues to lag behind the pace of gene discovery. Understanding the role of specific genetic variants in the brain involves dissecting a functional hierarchy that encompasses molecular pathways, diverse cell types, neural

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Competing interests statement

The authors declare no competing interests.

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circuits and, ultimately, cognition and behaviour. With a focus on transcriptomics, this Review discusses how high-throughput molecular, integrative and network approaches inform disease biology by placing human genetics in a molecular systems and neurobiological context. We provide a framework for interpreting network biology studies and leveraging big genomics data sets in neurobiology.

Large-scale genetic association studies have begun to unravel the genetic architecture of neurodevelopmental and neurodegenerative disorders and have found that hundreds to thousands of genetic loci are involved in disease risk¹. To understand how genetic variants contribute to disease, neuroscientists are faced with the task of measuring and understanding phenotypes in the central nervous system (CNS), a hierarchically organized complex system (FIG. 1a). This leads to a reliance on models that only account for a few features of the CNS at a time, as is done in most laboratory experiments. Although this has been fruitful for some highly penetrant variants that yield clear phenotypes, it has been less successful for genetically complex diseases.

To understand how genes contribute to CNS phenotypes, it is necessary to adopt rigorous data-driven frameworks that operate at a systems or a network level^{2–4}. Methods have recently become available that permit the measurement of large-scale molecular^{4,5}, cellular⁶ and circuit-level³ phenotypes, and additional methods are currently in development⁷. One goal of these approaches is to connect genetic risk and mechanism by combining a molecular systems or integrative network approach with systems neuroscience to understand the molecular regulatory networks and pathways that underlie circuit function, behaviour and cognition in health and disease. Collaborative and consortium-level efforts have made substantial progress by mapping transcriptomic, epigenomic and proteomic landscapes in the brain^{8–10}. Recent important advances include the evaluation of spatial and temporal transcriptomes by the Allen Brain Institute and BrainSpan^{8,11–13}, the quantification of the epigenetic landscape in CNS tissue and cell types by the Roadmap Epigenomics Mapping Consortium¹⁴, and the integration of genetic variation with gene expression in the brain by the Genotype-Tissue Expression (GTEx) project¹⁵, as well as others^{16,17}. These efforts have provided the first systematic view of the immensely complex molecular landscape across brain development, between brain regions and among major cell types (FIG. 1b). However, the molecular signatures of specific cell types, finer-grained temporal dynamics and causal or reactive alterations in CNS diseases remain mostly uncharacterized (FIG. 1c). Nevertheless, these new resources serve as an important foundation and proof of the value of such tissue- and stage-specific profiling data.

Molecular profiling and network approaches in disease-relevant neuroscience research face several major challenges when applied to the CNS: the complexity of molecular phenotypes owing to cell type, spatial and temporal heterogeneity throughout nervous system development and maturation (BOX 1); a dearth of human tissue and model systems with definitive human relevance (the ‘translational’ and ‘evolutionary’ problems^{4,18,19}); and poor knowledge of appropriate intermediate phenotypes to measure. Although these challenges are not unique to studying the CNS, neuroscience has historically struggled with each of them owing to the extent that they affect the ability to link molecular function to behavior

and cognition. Foundational aspects of each point have not been agreed: the definition of a cell type in the brain remains controversial^{20,21}; the relationships of human disease phenotypes to developmental trajectories are relatively unknown; model systems in many neurobiological studies are often chosen on the basis of convenience and history; and most phenotypes are based on clinical and behavioural symptomatology rather than on biological mechanism or aetiology^{22–24}.

Box 1

The unique cytoarchitecture and development of the brain

Most neurodevelopmental and neurodegenerative disorders are defined by perturbations in specific cognitive and/or behavioural domains, pointing to a selective vulnerability of specific cells. Regional and cellular heterogeneity pose obstacles for transcriptomic studies in the central nervous system (CNS)^{100,200}, but whole-tissue investigations in post-mortem human brain tissue are essential for identifying human-relevant global changes. These changes can be compared across regions to identify the most vulnerable regions and time points for further investigation. In general, the value of whole-tissue profiling in post-mortem brain tissue depends on the disease. In neurodevelopmental disorders, the specific brain regions, cell types or time points that are most affected remain poorly defined and whole-tissue profiling still holds great value. By contrast, for many neurodegenerative diseases, the selective death of certain cellular populations and the infiltration of inflammatory cells is well characterized, so transcriptomic studies focusing on sorted cellular populations are now necessary to identify new associations with disease.

To maximize neurobiological understanding from whole-tissue profiles, global changes can be related to cell type-specific gene expression profiles^{30,32,149,163,201}, and targeted experiments can be carried out to identify novel insights, as highlighted by several recent studies^{202–205}. However, it will be impossible to study disease-affected cell types without a complete knowledge of cell identities in normal brain development and ageing. A priority is to develop a complete knowledge of the cellular identity and cytoarchitectural changes that occur over time. This will necessitate surveying the diversity of cellular types and deciphering their molecular identities using single-cell approaches^{206–208}.

Additionally, neuronal gene expression and epigenetic programmes also undergo changes at finer spatial and temporal scales, including changes induced by activity-dependent transcription in the nucleus and translation²⁰⁹ at the synapse. Locally regulated translation of these subcellular transcriptomes²¹⁰ has a crucial role in synaptic function and plasticity²¹¹. Deeper characterization of these events at a high spatiotemporal resolution in normal brains followed by integration with coarser profiles from specific diseases will identify cellular compartments and mechanisms for more targeted study that are currently missed. Network approaches are particularly useful for relating whole-tissue-level changes to data from these high-resolution experiments^{11–13,26} (FIG. 2a).

In this Review, we provide an overview of integrative genomics approaches that have been applied to understand the basis of CNS disorders, and we anchor this discussion around

transcriptomics (BOX 1). However, the themes discussed can be generalized to genomic, proteomic and epigenomic methods. We describe how large-scale molecular data sets and gene network approaches provide organizing principles that permit the development of testable hypotheses on a genome-wide scale. We discuss new insights into neurodevelopmental disorders and neurodegenerative diseases from these studies, highlight emerging themes and provide recommendations for designing and executing future molecular profiling studies.

Network biology and transcriptomics in the brain

Despite challenges in studying the CNS, dozens of informative transcriptional analyses of neurodevelopmental and neurodegenerative disorders have been carried out in the human brain. A major challenge, which has mostly been surmounted at the theoretical level and which now requires reduction to practice, has been measuring and identifying which genes are altered in disease in specific cells, circuits and regions. Differential gene expression analysis (DGE analysis) addresses this issue, albeit one gene at a time, but does not take into account the relationships between genes. This leads to additional challenges, including the interpretation of long lists of differentially expressed genes and integration of DGE sets with other data. Network methods (BOX 2) relate genes to each other using the measured or predicted relationships between them⁴ and provide an essential organizing framework that places each gene in the context of its molecular system. Gene network methods are now being applied to integrate genetics with transcriptomics, epigenomics and proteomics to identify causal molecular drivers of cellular, circuit-level and brain-wide pathology in disease. We review the principles of network analysis below and also delve into applications of molecular systems and integrative network approaches in neuropsychiatric and neurodegenerative disease.

Box 2

A framework for interpreting gene network analysis

Molecular profiling data can be modelled as a network in which molecules or gene products are nodes and their functional relationships with each other are edges. Gene network analysis can be summarized in five basic steps.

Node specification

Seeded (prior-based) networks have nodes that are selected using prior knowledge, such as genetic variants that are associated with a disorder, and unseeded (genome-wide) networks use all available measurements from the genome.

Edge specification

In order to define edges, studies need to include one or more of the following: experimentally observed pairwise statistical relationships^{25,212,213} evaluating shared patterns of molecular levels across experiments, such as co-expression; experimentally observed or literature-curated physical interactions, such as protein interactions from immunoprecipitation and yeast two-hybrid (Y2H) experiments; or computationally predicted relationships, such as transcription factor binding based on DNA motifs.

Notably, edges are susceptible to ascertainment biases^{52,214,215} and confounding factors that can induce spurious relationships¹⁷⁸ (FIG. 2b).

Module identification

Modules are identified from an adjacency matrix to simplify biological relationships at a higher-order level, identifying interacting or highly correlated gene products (FIG. 2c). Assessing node connectivity or position within the module can identify hubs and enables the comparison of changes between health and disease at the module level.

Annotation of modules or gene connectivity

There are several common approaches to annotate modules. External measures of gene importance (such as cell type specificity or genome-wide association study (GWAS) signals) can be related to module membership, intra-modular connectivity or network-wide gene connectivity. Module summary or hub gene measurements, such as module eigengenes or average expression levels, can be associated with biological traits. Any differential gene expression (DGE) test that can be applied at a single-gene level can be applied to module-level summaries, such as eigengenes. Module-level association reduces the problem of multiple comparisons, as there are far fewer modules than genes in a network. The preservation or changes in network connectivity for specific genes or modules can be assessed between health and disease. Data can be integrated at the edge level or the module level across biological levels, such as different cell types or brain regions, or different regulatory levels, such as gene expression and ChIP-seq signals.

Validation

The crucial issue of reproducibility is addressed by validating network observations in independent data or experiments (BOX 3; TABLE 1). Biological validation may involve experimental testing of mechanistic predictions.

Box 3

Recommendations and general guidelines for transcriptomic studies

Experimental design

- Randomize or balance sample preparation and data collection over all known factors to reduce confounding variation from batch effects, which can introduce spurious correlations. For RNA-seq, we recommend barcoding and multiplexing samples (over eight per lane) to reduce batch effects²¹⁶.
- Evaluate the contribution of both biological and technical factors via unsupervised methods such as principal component analysis¹⁷⁸ and apply appropriate methods to remove unwanted variation from the data^{181,217}.
- RNA-seq studies with degraded RNA (RNA integrity number <9; essentially all post-mortem studies) should use ribosomal RNA depletion library preparation²¹⁸. Sequencing samples with a read length of 50 bp with 10 million unique reads (20 million paired-end reads) will detect most highly expressed genes. Deeper sequencing and longer read lengths may be required to accurately

and systematically detect noncoding RNAs, splicing or novel features, and pilot experiments are recommended for these scenarios.

DGE analysis

- In most experiments, biological variability is greater than technical variability, so biological replicates are of greater value than technical replicates^{174,175,219}.
- For well-controlled experiments with expected changes of >twofold in many genes, three or more independent samples per condition are recommended^{175,219}. For post-mortem samples, in which the detection of lower-fold changes may be important and variation may be greater owing to clinical heterogeneity and technical factors, at least 15 case and 15 control samples are recommended in an initial cohort.
- Appropriately transformed and normalized sequencing data can be treated similarly to microarray data as far as statistical modelling and multiple corrections are concerned^{175,220}. For differential gene expression (DGE), RNA-seq studies should observe existing analytical and statistical guidelines for microarrays²²¹ and, if possible, should carry out pilot experiments to estimate power²²².

Co-expression network analysis

- The power of network analysis is dependent on similar factors to DGE but is also dependent on the network features of interest. At currently available sample sizes, networks are most reproducible at a module level^{35,38,39}, then at the hub gene level^{41,223} and, last, at the level of precise gene connectivity rankings or precise module memberships of genes^{40,49}.
- To obtain module-level reproducibility, 20 independent samples are usually sufficient⁴⁰, but systematic and accurate reconstruction of specific edges, particularly for systematic regulatory relationship discovery, may require hundreds of samples⁴⁹. For studies comparing conditions, we recommend a minimum of 20 samples per condition. More samples may be necessary if many additional factors vary; for example, age, sex and different brain regions.
- Given the large number of parameters in network analysis, there is no 'one-size fits all' solution. The most rigorous approach is to apply the empirical reproducibility criteria discussed below.

Reproducibility and biological value

- Apply permutation analyses to ensure that gene network modules are significantly co-expressed (interconnected).
- To reduce over-fitting and to improve reproducibility, select the outcome of interest (fold change for each gene and gene membership in a module) and apply cross-validation or the bootstrap method²²⁴.
- Demonstrating reproducibility of major findings (for example, module definitions, top DGE genes and changes in gene network position between

conditions) is the most convincing form of validation of a particular analysis. Replication involves identifying the outcome of interest, applying the same analysis as in the original study but to independent data, and demonstrating statistical replication of the same finding.

- Generate hypotheses from the DGE and/or network analyses and test them bioinformatically or with wet-laboratory experiments to demonstrate predictive biological value.
- To allow other researchers to examine the data sets, raw data should be deposited in a public database (such as GEO, SRA or dbGaP).
- To allow for a comparison of analysis methods, always publish clear and usable code along with the publication reporting this analysis.

Networks organize biology

For gene expression studies, co-expression network analysis leverages the fact that gene expression reflects the state of the cellular or tissue system that is being analysed²⁵. A major advantage of network analysis over DGE analysis is that it can identify multiple levels of molecular organization within the hierarchy of brain region, cell type, organelle and molecular pathways using only transcriptional data, and can thus enable integration with other information, such as known pathway annotations, protein interactions and other molecular profiling data^{11,12,26,27} (BOX 1; FIG. 2a). Furthermore, when thousands of genes might be differential between conditions, network analysis can subdivide changes into smaller, more biologically coherent sets of modules for further experimental analyses.

Networks organize genome-wide molecular data by modelling molecules as nodes (typically genes or gene products) and the relationships between nodes as edges. Edges are not necessarily physical interactions — they may also reflect statistical similarity (for example, correlation or mutual information), computational inference or combinations of these edge types (FIG. 2b). Edges define the connectivity of nodes to each other in a network, and this connectivity can be used to organize and analyse the nodes. Many biological networks have a hierarchical structure such that their nodes can be organized into a relatively small collection of highly interconnected modules^{4,28,29} (FIG. 2c). Inter-modular connectivity reflects a higher-order structure of biological relationships in a gene network, and intra-modular connectivity can identify which genes are biological hubs within modules. In co-expression networks, hubs are highly connected genes; being a hub is an indication of the importance of a gene in the process of interest. Hubs can be key molecular drivers, such as transcriptional regulators that drive co-expression^{30,31}, or they may annotate a module by reflecting the predominant biological role of the module. For example, when evaluating co-expression across brain regions, hubs in modules that are associated with specific regions, such as the cerebellum, are usually markers for predominant cell types, such as granule cells^{11,12,26,32}.

Modularity is very useful, and although it provides a general organizing principle in biology, it need not be present in all constructed networks, and network biology provides many module-free analytical approaches; for example, nodes can be organized in relation to each

other by ranking direct and indirect connectivity. If two gene products share an edge, they are said to be neighbours in the network; the more highly interconnected, the closer the neighbours. Thus, gene products that are involved in an unknown cell type or biological process can be annotated on the basis of their proximity to marker genes of known function ('guilt by association')^{26,33,34}. Additionally, both modularity and connectivity rankings can be compared between studies to assess whether they are preserved³⁵, and how a module or the position of specific genes within a module change in health and disease can be evaluated to prioritize those that show the most significant changes for further evaluation^{35–37}.

Different approaches to gene co-expression

The most common workflow in gene co-expression network analysis in neuroscience involves the construction of co-expression relationships from microarray or RNA sequencing (RNA-seq) data, identifying modules and then annotating modules on the basis of the known function of module hubs, enrichment for gene sets and module-level association with biological factors such as disease (FIG. 2a). Discussion of the various options and the technical merits of specific network approaches is beyond the scope of this Review^{38–41}. Comparisons among methods have indicated several important points: weighted networks are more reproducible and powerful than binary networks⁴²; signed networks are more predictive of protein interactions and shared pathway relationships than unsigned networks^{38,42,43}; weighted networks constructed with the topological overlap of correlation (for example, by weighted gene co-expression network analysis (WGCNA)^{42,44}) have similar sensitivity and specificity for detecting true network structure for experiments involving monotonic relationships as do networks constructed with nonlinear association measures such as mutual information (for example, by the Algorithm for the Reconstruction of Accurate Cellular Networks (ARACNE)⁴⁵)^{38,39}; and edge relationships using mutual information or other association measures might be necessary to accurately detect modules in time-series data, which can be non-monotonic^{46–48}. Differential co-expression or connectivity methods^{36,37} are additional means for determining gene connectivity changes between conditions and can identify disruption or gain of function in pathways.

We provide guidelines in BOX 3 to aid co-expression network reproducibility regardless of the method used. Importantly, the replication of major conclusions in independent data and experimental validation lend the greatest confidence to a network analysis. There is a need for studies that rigorously compare network analysis in human CNS transcriptome data using experimental validation as a gold standard, similar to what has been done in the Dialogue on Reverse Engineering Assessment and Methods (DREAM) regulatory network inference challenge⁴⁹. The DREAM challenge identified that the integration of multiple network methods yields the most robust regulatory relationship predictions⁴⁹. This leveraged the availability of hundreds of gene expression profiles in single-cell organisms (bacteria and yeast) and compared regulatory predictions between methods with gold standard experimental validations. Building such regulatory networks in complex tissues such as the CNS is a step beyond current co-expression networks in the brain. Large amounts of data, ideally from homogeneous cellular populations, are necessary to systematically and accurately predict gene regulatory relationships in network studies.

Literature-curated data

There are many databases that aggregate experiments to construct genome-wide data sets that can be utilized for network construction (TABLE 1). Gene networks that are built on data that contain even a small fraction of literature-curated components can contain substantial bias. Furthermore, when data are from non-neuronal tissue, the database may contain relationships not found in neural tissues (TABLE 1). Although reliant on data from non-neuronal tissue, pathway databases such as the Gene Ontology (GO⁵⁰), the Kyoto Encyclopaedia of Genes and Genome Elements (KEGG⁵¹), Ingenuity Pathway Analyses and MetaCore are valuable for evaluating specific genes and pathways. However, networks with edges that are derived from shared pathway membership can reflect cellular states that might not be found in the CNS, and they will certainly lack many important CNS-specific relationships. In a worst-case scenario, hubs in these networks may be the most studied genes in other areas of biology, and therefore may not reflect neurobiological relevance. It is therefore important to distinguish between networks that are constructed using edges from pathway databases and those using edges derived from tissue-specific primary molecular profiling experiments.

Protein–protein interaction (PPI) databases, which compile known physical interactions between proteins, are another example of literature-curated data. PPI experiments may focus on a few proteins and evaluate interactions in a tissue-specific manner using co-immunoprecipitation followed by proteomics. Alternatively, most genome-wide PPI experiments use methods such as yeast two-hybrid (Y2H) screens or tandem affinity purification and are cell type agnostic. The genome-wide approaches yield many more interactions, so most databases typically combine both target-focused and genome-wide experiments⁵². Similar to pathway databases, these PPI data sets are biased to highly studied gene categories (for example, those implicated in cancer biology) and are still generally incomplete^{2,53} (TABLE 1). A particularly salient example of the utility of defining tissue-relevant networks is the power obtained by using PPIs derived from cardiac tissue to identify new human loci for long QT syndrome⁵⁴. To reduce bias and improve tissue specificity for genome-wide networks in the absence of tissue-specific PPIs, one approach is to intersect tissue-specific RNA expression or co-expression with literature-curated PPI data^{55,56}.

These considerations also apply to other physical interaction data, including CLIP–seq, ChIP–seq and miRNA binding data, unless they come from experiments using relevant tissues⁵⁷. Computational approaches to predict physical interactions can partly circumvent bias (TABLE 1), but they do not address tissue specificity, and there may be relatively low reproducibility across different methods^{58,59}. There is compelling evidence that using DNase hypersensitivity or ATAC–seq data to infer open chromatin, followed by combining transcription factor binding with open chromatin footprinting, can provide a powerful and comprehensive way to identify tissue-specific transcription factor regulation^{60,61}. The increasing availability of large amounts of relevant data sets within the public domain^{10,14} now permits the evaluation of network modules for complex regulatory relationships by combining network edges from statistical associations, time-series data, physical binding and computational predictions (FIG. 2b).

When combining multiple molecular levels in networks, it is important to recognize that transcriptomics, epigenomics and proteomics all query unique levels of cellular or tissue organization. For example, most proteins found only in mitochondria do not physically interact with most proteins found only in ribosomes or proteasomes, and these proteins would normally form distinct (but possibly connected) modules in PPI networks. However, in circumstances such as cellular stress or neurodegeneration, the genes encoding these organelle-specific proteins might be transcriptionally co-regulated and hence highly connected at a co-expression level. In this case, transcriptomics can provide a novel view of cellular mechanisms. In general, tissue-, time- or disease-specific data sets aid in conferring specificity to otherwise non-neuronal data. Until such data are available, we suggest beginning with genome-wide tissue-specific data such as transcriptomics, followed by combining literature-curated or non-tissue-specific evidence with gene co-expression modules.

Neurodevelopmental disorders

Neurodevelopmental disorders are characterized by abnormal behavioural or cognitive phenotypes originating either *in utero* or during early postnatal life, and can be accompanied by clinical features outside the CNS. Various genetic approaches have been successful in identifying the causes of more than 1,000 Mendelian, and fewer non-Mendelian, forms of neurodevelopmental disorders: prototypical examples are intellectual disability^{62–68}, autism spectrum disorder (ASD)^{69–77}, epilepsy^{78,79} and schizophrenia^{80–82}.

As more genetic risk variants for these disorders have been discovered, studies have found remarkable pleiotropy^{1,1,83,84}. Several rare, highly penetrant mutations in evolutionarily constrained fetal brain-expressed genes are associated with ASD, schizophrenia and intellectual disability, as well as epilepsy^{83,85–87}. We frame this issue using the concept of developmental canalization⁸⁸, whereby natural selection on developmental programmes in humans has led to robustness in a range of genetic or environmental perturbations^{89,90}: typical development occurs along a ‘track’ (FIG. 1c). Under this framework, the observed pleiotropy is consistent with the notion that disrupting highly evolutionarily constrained genes leads to the ‘derailment’ of typical development off this track, rather than setting the brain on a path to a specific clinically defined disorder (FIG. 1c). Thus, many severe mutations do not converge on one specific phenotype but instead seem to cause a range of clinical disorders^{74,76,80,81,84,87,91}. This formulation leads to several important questions that can be informed by integrative genomic studies, including whether diverse genetic lesions affect similar pathways and where disease specificity emerges. We provide examples below of gene network studies that use co-expression, PPIs and integrated networks to understand ASD and schizophrenia.

Dysregulated networks in the brains of individuals with ASD or schizophrenia

ASD is a phenotypically and aetiologically heterogeneous neurodevelopmental disorder that is defined by deficits in social communication and mental flexibility, with an onset in the first few years of life⁷⁵. More transcriptional studies of ASD post-mortem brains have been limited by the paucity of available tissue, which has made them underpowered to identify reproducible pathways with statistical rigour^{92–95}. Nevertheless, some themes have emerged

across studies, including the increased expression of immune-microglial genes and the decreased expression of synaptic genes in the cerebral cortex. The first ASD study to identify reproducible, genome-wide findings used WGCNA⁴² to identify two modules, one containing upregulated genes and another containing downregulated genes that defined coherent biological processes in ASD brains³⁰. This study used co-expression module eigengenes (the first principal component of the gene expression levels of each module) to identify modules associated with ASD and to ensure that they were unrelated to potential confounders such as RNA integrity, age or seizure history. This module-level association approach reduces the problem of multiple comparisons and highlights the advantages of using networks as an organizing framework⁹⁶. The integration of genetic data with co-expression modules showed that the downregulated neuronal signalling module has a potential causal role in ASD, and that the upregulated ASD module was probably a response, which is consistent with its enrichment in microglia and astrocyte genes³⁰. These results supported the findings of several previous smaller studies^{92,93}. Synaptic and microglial modules have been replicated in ASD cortex using RNA-seq in larger independent cohorts⁹⁷.

Schizophrenia is defined by prolonged or recurrent episodes of psychosis (characterized by hallucinations and delusions) as well as negative symptoms and deficits in cognitive function⁹⁸. Although diagnosis is usually made in late adolescence or early adulthood, extensive evidence indicates a neurodevelopmental origin⁹⁹. Transcriptional studies of schizophrenia have benefited from considerably larger sample sizes than those of ASD. However, patients with schizophrenia have greater comorbidity of confounders such as smoking, alcohol and substance abuse than those with ASD. Overcoming potential confounders requires careful matching of patient and control individuals and must take into account potential covariate effects when possible, as has been done in many studies^{100,101}. Despite variable results, consistent findings across studies can be identified, including dysregulation of GABAergic signalling¹⁰²; downregulation of oligodendrocyte- and myelination-related genes¹⁰³, mitochondrial function or energy metabolism¹⁰⁴, and synaptic genes¹⁰⁵; and upregulation of immune and inflammatory genes¹⁰⁶.

One of the first studies to put schizophrenia transcriptomics into a genome-wide co-expression network used mutual information and WGCNA¹⁰⁷. This study showed that, as in ASD, the overall transcriptomic structure that is observed in control brains is intact but that a neural differentiation module that is associated with schizophrenia does not follow the normal trajectory of downregulation with age. Another study confirmed that a dysregulated neuronal differentiation module was consistently observed in schizophrenia post-mortem brains and suggested that the same pathways were involved in bipolar disorder¹⁰⁸. Moreover, genome-wide association study (GWAS) signal enrichment analysis³⁰ found that common variants associated with schizophrenia and bipolar disorder were enriched in the neuronal differentiation module, suggesting that disorders sharing a genetic architecture⁸⁴ may also share functional transcriptional alterations: a hypothesis that warrants rigorous testing.

Mapping risk genes onto developmental networks

A shortcoming of studies using post-mortem brain tissue is that the tissue is usually obtained long after the disease-causing changes have occurred. Given that the human brain transcriptome has a reproducible structure^{12,26}, one useful way to explore how mutations in risk genes perturb typical brain development is to map risk genes onto transcriptional networks that represent normal brain structure or development (FIG. 1c). The first study to do this identified co-expression modules that had cell type-specific and region-specific expression patterns using nearly 1,000 adult brain regions^{12,109}, and identified neuronal gene-enriched modules containing ASD candidate genes and ASD GWAS signals. Moreover, this study found that genes in these modules have dynamic developmental trajectories, demonstrating a role for ASD risk genes in neural development.

The identification of genetic risk factors by whole-exome sequencing^{70–73} and the availability of transcriptome data spanning multiple brain regions and developmental stages^{13,17} created new opportunities to map disease risk genes onto developmental transcriptional networks. One network study defined robust co-expression modules that were reproducible in independent data and identified five developmentally regulated co-expression modules that were enriched for PPIs and ASD risk genes²⁷. By comparing these genes with genes that cause intellectual disability, this study identified molecular processes that are preferentially disrupted by ASD risk genes, including transcriptional regulation, chromatin regulation and synaptic development, and it identified disruption of specific pathways, such as BAF (SWI/ SNF) complex-mediated neuronal development^{30,110,111}. A complementary study identified developmental co-expression networks enriched for ASD risk genes seeded around nine genes with the highest ASD association signal from whole-exome sequencing¹¹². These investigators asked if, when and where ASD genetic risk converges during brain development by evaluating seeded co-expression networks. They started with the nine ‘high-confidence’ risk genes and expanded the network using combinations of spatial and temporal expression data from post-mortem brain tissue. They identified three spatiotemporal combinations that passed stringent correction for multiple testing: frontal cortical regions during the fetal period, and thalamic and cerebellar regions from birth to 6 years of postnatal age. Interestingly, there was no pathway or PPI enrichment in these modules, probably owing to the inclusion of both positive and negative correlations when computing co-expression relationships (unsigned networks), which is a method that is less sensitive to pathway and protein interaction detection^{38,43}.

Importantly, both of these studies found convergence for rare *de novo* ASD-associated mutations during early fetal and mid-fetal development, with the greatest enrichment for risk in genes found in cortical glutamatergic neurons. Thus, despite the fact that the same gene is rarely hit recurrently by rare *de novo* variants in ASD, this class of variation preferentially disrupts projection neurons. Notably, the genome-wide approach²⁷ assessed both ASD and intellectual disability genes, and further suggested that the disruption of the upper neocortical layers (layers 2–4) is related to ASD-like phenotypes and not intellectual disability. Other studies have also found that fetal cortical development and glutamatergic neurons are affected by mutations in ASD, suggesting that it is a robust finding^{11,113,114}.

A seeded co-expression approach has also been used to identify risk convergence in schizophrenia, identifying fetal development of the prefrontal cortex as a point of convergence for *de novo* mutations¹¹⁵. This study did not extend the network to genes beyond the seed set, and it did not investigate cellular, laminar or regulatory relationships among these genes. As larger sets of risk genes are becoming available^{72,77,80,81}, a more refined view will emerge of how mutations in ASD, schizophrenia, intellectual disability and other psychiatric disorders overlap and diverge to affect cells and circuits.

Regulatory hubs in neurodevelopment and disease

Another promising approach to identify disease-associated networks is to experimentally construct a seed-based network for a candidate regulatory molecule. Using CLIP-seq, investigators identified the RNA binding targets of the translational regulator fragile X mental retardation protein (FMRP)¹¹⁶, and a subsequent analysis found that these targets are highly enriched for *de novo* mutations in ASD⁷². Both genome-wide²⁷ and seeded¹¹⁴ co-expression network analysis further connected FMRP targets with multiple forms of ASD genetic risk, including copy number variations (CNVs)¹¹⁴. Additionally, whole-exome sequencing studies of other neurodevelopmental disorders have found enrichment for FMRP targets in rare mutations in schizophrenia⁸¹, intellectual disability⁶⁸ and epilepsy⁷⁸. As many FMRP targets are highly conserved and are under purifying selection^{72,87,117}, FMRP-related activity-dependent regulation during fetal brain development might be particularly vulnerable to genetic perturbations, with severe mutations resulting in disruption of developmental canalization.

At the transcriptional regulation level, ChIP-seq in induced pluripotent stem cell-derived neurons has been used to define the network of genes regulated by chromodomain helicase DNA-binding protein 8 (*CHD8*)¹¹⁸, which is the gene most frequently affected by ASD-associated rare *de novo* variation^{72,119–121}. Integration of ChIP-seq, *CHD8* knockdown and gene co-expression suggested that CHD8 directly regulates co-expression modules that are enriched for rare *de novo* mutations and genes found in the proliferating layers of the fetal cortex²⁷. Another study applied a similar approach but evaluated CHD8 targets in the fetal brain *in vivo*⁵⁷. This study identified stronger enrichment for ASD mutations, suggesting that ChIP-seq in the human brain at the right time point identifies interactions that are more disease relevant⁵⁷. Given the emerging role of fetal brain-expressed transcriptional and chromatin regulators in ASD^{27,77,122}, integrating ChIP-seq of other transcriptional regulators with developmental co-expression networks may help to elucidate a shared, evolutionarily constrained regulatory network that is susceptible to disruption in brain development.

PPI networks define new interactions

Genetic investigations in ASD have constructed seed-based networks with literature-curated PPIs to identify the convergence of ASD risk genes^{71,73}. This approach was applied to identify a highly interconnected PPI subnetwork among rare *de novo* variants in ASD⁷¹. Genes in this subnetwork were evaluated in a larger cohort in a targeted sequencing study¹²⁰, which identified more risk variants compared with chance and demonstrated that PPI connectivity can be a predictor of ASD risk mutations. However, the biases inherent to

literature-curated data and the lack of tissue specificity in these PPI networks limit the identification of novel pathways or circuits with this approach (TABLE 1).

Recently, one study used global literature-curated PPI interactions in a genome-wide network analysis to identify modules that are enriched for ASD-associated genes¹²³. This identified a PPI module that is enriched for genes related to synaptic function and weakly enriched for mutations from individuals with ASD. Integration with transcriptomics annotated the module as highly expressed in oligodendrocytes and the corpus callosum, demonstrating that tissue-specific data are essential for a neurobiological interpretation of PPI modules¹²³. Given the biases inherent to global PPIs discussed above and in TABLE 1, these findings warrant replication with new PPI data. Understanding why these relationships are detected at the PPI level but not at the co-expression level will be valuable.

To evaluate whether ASD risk genes interact at the protein level in an unbiased manner, Sakai and colleagues¹²⁴ carried out a Y2H screen of 35 syndromic or candidate ASD genes and identified many novel PPIs. This study was the first of its kind in neurodevelopmental disorders and showed that the PPI network seeded around these 35 genes was indeed highly interconnected. Another Y2H study assessed a larger seed set of ASD candidate genes that corresponded to spliced isoforms identified by whole-brain RNA-seq¹²⁵, hypothesizing that isoform-level PPIs would allow for the discovery of tissue-specific PPI networks¹²⁶. The genes in the most interconnected component of the PPI network formed a module that was modestly enriched for gene co-expression, gene co-regulation and known ASD genes. These results further demonstrated convergence among known disease-relevant genes at the PPI level and also demonstrated that evaluating tissue-specific isoforms can be used to identify novel interactions. Both of these PPI studies used state-of-the-art quality control and validation, and identified many novel interactions. However, even with knowledge of isoform-specific interactions, the tissue environment for interaction cannot be efficiently recapitulated with current PPI approaches at a genome-wide scale (TABLE 1). This, and other recent work studying cardiac tissue⁵⁴, highlights how tissue-specific molecular data improve PPI analyses to identify or prioritize genetic variants that specifically function in that tissue, in this case causing cardiac arrhythmia.

Integrating multiple molecular levels

The idea that multiple lines of evidence may increase the power to detect disease-relevant interactions has motivated the integration of literature-curated, molecular and genetic evidence to support specific genes or pathways. The Network-Based Analysis of Genes (NETBAG)¹²⁷ approach combines multiple forms of literature-curated data using an integrated network approach that has been demonstrated to be effective for predicting gene essentiality in yeast¹²⁸. The goal of NETBAG is to construct a network in which highly interconnected genes are likely to participate in a similar phenotype. Edges in NETBAG are predominantly derived from multiple PPI databases, GO⁵⁰ and KEGG⁵¹, which are all literature-curated databases, and thus NETBAG is susceptible to the biases discussed above. The first study with NETBAG evaluated CNV-hit genes implicated in ASD and found a highly interconnected module related to synaptic function¹²⁹. Furthermore, genes in CNVs from females contributed more to the module connectivity than those from males,

suggesting that females are affected by more severe genetic hits in ASD, an observation that has been replicated in exome-sequencing studies^{76,117}. Another approach¹³⁰ has evaluated CNV duplications in addition to CNV deletions and also found an interconnected PPI network that was enriched for proteins involved in synaptic transmission, validating the observation that pathogenic CNVs affect similar gene networks¹²⁷. An extension of the NETBAG approach (dubbed NETBAG+) has also been applied to simultaneously evaluate large sets of single-nucleotide variants (SNVs) and CNVs in schizophrenia¹³¹ and ASD¹³², confirming the convergence of disease genes onto shared pathways.

An exciting approach is to simultaneously integrate PPIs, co-expression and mutational burden in neurodevelopmental disorders, as has been done by Merging Affected Genes into Integrated networks (MAGI¹³³). This approach begins with mutation-affected genes in their known pathways and then adds genes to these ‘seed pathways’ on the basis of high co-expression or PPI connectivity. The extent to which genes are added to make a module is determined by an objective function that maximizes pathogenic mutations from cases compared with controls in the module. MAGI identified modules containing functionally related genes enriched for deleterious mutations in ASD, many of which are under strong purifying selection, and are also found in epilepsy, schizophrenia and intellectual disability¹³³.

Neurodegenerative disease

Neurodegenerative diseases are characterized by a progressive loss of neural tissue that results in a decline in cognitive and behavioural function. Many of these diseases have known causes that involve mutations in ubiquitously expressed proteins¹³⁴, but they follow stereotyped patterns of degeneration that selectively affect certain cell subsets more severely, resulting in disease-specific spatial and temporal patterns of degeneration^{135–137} (BOX 1). Neuropathological investigations have identified protein-centric mechanisms that might be involved in disease pathogenesis, but causal mechanisms are difficult to pinpoint, as post-mortem samples reflect the consequence of years of ageing and disease progression. Important disease-associated molecular changes can be confounded by environmental and clinical factors. Additionally, although positional cloning has identified genes and pathways that are involved in many neurodegenerative diseases, pathological mechanisms, modulators of pathogenesis and disease biomarkers have remained elusive, suggesting that genome-wide approaches are needed. Transcriptional and PPI network studies have recently identified many new insights into these diseases. Below, we focus on representative transcriptomic studies of two genetically complex diseases (Alzheimer disease and frontotemporal dementia (FTD)) and PPI studies of two diseases for which causal genes are well defined (Huntington disease and inherited ataxia), but for which disease mechanisms are still poorly understood.

Post-mortem transcriptomic analysis in dementia

The major challenge in Alzheimer disease and FTD transcriptomics has been the identification of changes that are independent of alterations in cell type proportions, which accompany cell death and inflammation. Three major study design principles have been used to overcome this issue: transcriptomes in differentially vulnerable brain regions or

cellular populations can be compared to identify vulnerable or protected pathways^{138,139} (BOX 1); preclinical changes in at-risk individuals with a milder disease presentation can identify genes and pathways that might lead to disease¹⁴⁰; and cell type-specific markers can be used in combination with bioinformatic analyses to account for the effect of changes in cell proportion on the overall transcriptome^{141,142}.

Multiple transcriptomic studies of Alzheimer disease have been carried out in the human brain at varying spatial resolutions¹⁴³. Large studies using quantitative metrics of severity¹⁴⁰ and differentially vulnerable regions¹⁴⁴ have identified pathway-level changes in transcriptional regulation, apoptosis, cell proliferation, energy metabolism and synaptic transmission. One particularly powerful approach involved the use of the pattern of regional vulnerability to guide a microarray study that identified a defect in the retromer complex, which is responsible for endosome-mediated recycling of membrane proteins¹⁴⁵. The involvement of this pathway in Alzheimer disease was experimentally validated¹⁴⁶. The first large transcriptomic study (involving 188 controls and 176 individuals with Alzheimer disease)¹⁴⁷ connected genetic variation to expression changes by using expression quantitative trait locus analysis (eQTL analysis) in controls and Alzheimer disease, and further supported the pathway-level findings related to transcriptional regulation and energy metabolism in Alzheimer disease^{140,148}. Integration of eQTLs can identify causality in transcriptomic studies in the context of Alzheimer disease risk, adding a crucial mechanistic element to studies of post-mortem gene expression.

In FTD, transcriptional signatures related to differential regional vulnerability have helped to identify modulators of neurodegeneration. The first of two well-powered studies that applied this approach carried out transcriptomic analysis in a mouse model of FTD, identifying the gene *Npepps*¹³⁸. Cross-species analyses in flies and humans confirmed the expression pattern and neuroprotective effect of *NPEPPS*¹³⁸. The second study¹³⁹ compared post-mortem tissue from patients with FTD harbouring dominant mutations in the progranulin (*GRN*) gene, patients who had FTD but who did not have a known family history or mutations, and control individuals. This study also leveraged regional vulnerability by comparing transcriptome profiles in the frontal cortex, hippocampus and cerebellum, identifying a diminishing hierarchy of susceptibility to FTD. The findings demonstrated that *GRN*-positive individuals were a transcriptomically distinct group from those with sporadic FTD¹³⁹. Both of these studies in FTD demonstrate the value of using selective vulnerability and differential genetic risk in study design.

From individual genes to networks and mechanism

Most early post-mortem studies from individuals with Alzheimer disease or FTD generated long gene lists and were followed by analysis of GO or KEGG pathway enrichment^{139,140,147}. In an early network study, Miller and colleagues¹⁴⁹ applied network analysis to compare the transcriptome in normal ageing and Alzheimer disease, finding many shared features that were downregulated in Alzheimer disease and normal human ageing¹⁴⁹. They subsequently¹⁵⁰ incorporated more than 1,000 microarrays from mouse models of Alzheimer disease and human patients with Alzheimer disease from public databases to reproduce and extend these results, identifying additional co-expression

modules that are related to mitochondrial dysfunction and synaptic plasticity. This work also found major differences in dementia susceptibility genes between humans and mice, potentially identifying why some mouse models might not recapitulate human neuropathology. Another study used similar methods to identify overlap in transcriptional networks between vascular disease (a major risk factor for dementia) and Alzheimer disease, identifying potential molecular mechanisms that might underlie their co-occurrence¹⁵¹. Forabosco and colleagues¹⁵² used network analysis to explore the function of *TREM2* (triggering receptor expressed on myeloid cells 2), an Alzheimer disease risk gene, suggesting a role for microglial function and further implicating neuroinflammation in Alzheimer disease. In FTD, two studies re-analysed published transcriptome data¹³⁹ to discover a role for *WNT* signalling in *GRN*-mediated FTD^{153,154}. Both involved extensive bioinformatic analyses of expression data from *in vitro* neural progenitor models and identified transcriptomic changes shared across the post-mortem human brain, human neural cell lines and the mouse brain. Experimental validation of predictions from these networks showed that this cross-species approach can identify consistent, high-confidence perturbations in neurodegenerative disease^{46,153}. Additionally, the use of previously published human data in many of these studies highlights the value of policies supporting data sharing, especially from patient cohorts. Finally, studies of the regulatory networks and targets of specific miRNAs such as miR-339-5p in dementia are in their early stages^{155–157} but promise to reveal novel regulators of neurodegeneration.

Although transcriptomic studies have furthered our understanding of disease mechanisms beyond neuropathology and single genes, the effects of cell type loss have not been completely accounted for in most studies. Purifying cell populations or carrying out transcriptional analyses on single cells^{158–163} can identify important changes that are not apparent in whole-tissue transcriptomes^{141,142}. Combining bioinformatics approaches with single-cell sequencing will increase the resolution at which regional vulnerability can be assessed and will enhance the ability of gene co-expression networks to identify key changes associated with dementia.

Protein interaction networks with known disease genes

The causal mutations for Huntington disease and many inherited ataxias have been known for more than a decade, and thus the focus of molecular investigations has been on understanding disease mechanisms and modifiers. Lim and colleagues¹⁶⁴ used a seed-based approach based on a Y2H screen to identify interactors of the protein products of multiple causal and candidate genes in inherited ataxias. Analysis of the resultant PPI network identified an interconnected network of proteins related to inherited ataxias. Importantly, interactors in the network were potential modifiers of disease progression, and, in subsequent work, gain of function mediated by a newly identified protein complex was found to mediate disease pathogenesis¹⁶⁵. This Y2H approach has also been used to identify potential modulators of Huntington disease¹⁶⁶, in which it is thought that inter-actors of huntingtin (the causally mutated pathological protein) might modulate disease severity. Interestingly, *in vivo* PPI screening by large-scale co-immunoprecipitation and mass spectrometry provided tissue- and time-specific information that was not found by Y2H studies¹⁶⁷. WGCNA identified spatially and temporally specific modules associated with

mutant *Htt* (which encodes huntingtin); and proteins with high intra-modular connectivity (hub proteins) modulated neurodegeneration in flies. This work further emphasizes the importance of considering tissue context in the studies examining disease-relevant protein associations.

Integrating genetic variation and transcriptome networks

The most ambitious and exciting goal in systems biology is to elucidate the functional genetic architecture of diseases by systematically identifying causal effects using genome-wide variation to disambiguate primary and secondary changes that occur in disease^{168,169}. A recent study shows that this goal is possible in the CNS by using genetic variation as a causal anchor to define genetically driven network-level changes in Alzheimer disease and to provide experimental validation for network predictions¹⁷⁰. Zhang and colleagues¹⁷⁰ applied WGCNA to hundreds of post-mortem brain samples from individuals with Alzheimer disease, other neurodegenerative diseases and controls. They showed that multiple transcriptional modules were remodelled in Alzheimer disease: gain of connectivity was observed in immune and neurogenesis pathways, and loss of connectivity was predominant in pathways related to GABA signalling and myelination. An eQTL analysis followed by module-level genetic signal enrichment identified several modules in which genetic association signals were enriched. Given that gene expression changes are caused by genetic variation, this suggested these modules were causally involved¹⁷¹. The researchers then applied Bayesian network analysis to evaluate causal relationships in an Alzheimer disease-related microglial module, implicating *TYROBP* (TYRO protein tyrosine kinase-binding protein) as a regulatory hub. The role of *Tyrobp* was experimentally validated in mice¹⁷⁰, showing that network structure is predictive, as had previously been demonstrated with co-expression networks³². Overall, integrating genetics with co-expression networks using large sample sizes (with a minimum of 100 cases and controls) and establishing causality by evaluating genotype–phenotype relationships and eQTL is very promising.

Specificity and convergence across CNS disorders

Many of the most influential studies using gene networks to probe neuropsychiatric disease mechanisms integrate multiple data types (for example, RNA expression, GWAS signals and PPI) or data sets (for example, human post-mortem, mouse and *in vitro*), emphasizing the value of publicly available data sets. The further availability of raw molecular profiling data with necessary metadata amplifies the value of individual studies. In addition to generating new hypotheses, molecular systems approaches integrating data from diverse studies can reveal unexpected and distinct relationships that are common to different CNS disorders. FIGURE 3 describes an example of a network-based meta-analysis of brain transcriptional profiles from publicly available data in ASD, schizophrenia and Alzheimer disease, which identifies shared and distinct biological processes across disorders. Several modules are shared by two of the three disorders, including the red module (ASD and schizophrenia), which contains voltage-gated calcium channels, and the green module (ASD and Alzheimer disease), which contains microglial markers (FIG. 3b–d). This demonstrates how cross-disorder analyses can systematically reveal shared and distinct biological processes among disorders, even when the data are from different studies (see

Supplementary information S1 (box)). It will be fruitful to combine more CNS disorders and diseases and to integrate GWASs and rare mutations to identify which variants affect gene expression across diagnostic boundaries and which are more specific. Prioritizing the disease-specific genes for further investigation may also aid in clarifying the molecular processes that lead to behavioural and cognitive alterations that are specific to a particular disease.

Guidelines for transcriptomic and network studies

Given the promise of molecular systems and integrative network approaches, it is perhaps surprising that there are few universally agreed on metrics, power analysis tools or methodological comparisons to guide experimental design, execution and analysis, such as there are for genetic association studies¹⁷². For DGE and network analysis, there are many studies with guidelines that are based on theoretical models and empirical assessments^{40,173–176}, but most studies use data from experiments that do not have the spatial, temporal or disease-relevant complexities that occur in studies of the CNS or post-mortem tissue. There is no experimental design that suits all aims, but we suggest criteria for initial experimental design, ensuring reproducibility and improving biological interpretability for transcriptomic analyses in BOX 3.

In general, it is helpful to think of all variation in gene expression or other molecular profiling data as a consequence of technical, biological and unmeasured factors¹⁷⁷, rather than assuming that differences are due to experimental interventions or disease status⁴¹. Optimal methodological choices and study designs ensure that the biological signals from the main factors are not confounded by variation from unwanted factors¹⁷⁸. Notably, molecular profiles in post-mortem gene expression studies are affected by RNA degradation and post-mortem intervals¹⁷⁹, but other technical factors including library preparation and sequencing depth in RNA-seq analysis should also be carefully evaluated^{180,181}.

Additionally, we note two important points about studies that construct predictive models and studies that make causal claims. For studies that develop predictive models, such as disease classifiers, experimental design should include the estimation of a model on initial data followed by evaluation of accuracy in held out or, preferably, independent data¹⁸². As far as causality is concerned, most molecular profiling studies, especially those using post-mortem tissue, cannot show causality without follow-up controlled experiments or genetic evidence^{169–171}. We also strongly suggest the experimental validation of key network predictions, as this provides avenues for refinement and biological grounding of the network^{30,32,153,170}.

Gene set enrichment with networks

As shown by multiple studies, gene network analyses can aid in understanding genetic association studies. Grouping genetic findings using disease- and tissue-relevant modules can increase the power to detect genetic associations with disease by combining signals that reflect similar underlying biology while simultaneously informing biological mechanism by functionally annotating genetic findings^{30,109,122,183}. Enrichment for genetic variants in a module can be evaluated using gene set enrichment methods, which rely on comparing

enrichment in a module relative to a control gene set. However, studies have demonstrated biases in gene length, gene mutability and other factors that can drive gene set enrichment instead of a true biological signal. For example, longer genes are more likely to be implicated by CNVs and SNVs^{87,184,185}, and genes highly expressed in the brain, particularly those involved in synaptic function, are longer on average than other genes¹⁸⁶. These biases inflate enrichment results and can result in false positives, so it is important to identify appropriate control sets or to apply the correct statistical methods (permutation tests or covariate modelling¹⁸⁷). We note that each of the points discussed here and in BOX 3 is applicable to many other types of high-throughput data and that there are many valid variations to FIG. 2a.

Future directions

In this Review, we have discussed how transcriptomic and integrative network approaches have been applied to provide a systems-level understanding of CNS disorders in an unbiased and reproducible manner. Mapping genetic variants to gene expression and PPI networks has been fruitful, but most disease-associated variation in complex diseases lies in the noncoding regulatory regions of the genome¹⁸⁸. The next crucial step for high-throughput molecular studies in the brain will be to understand regulatory alterations and interactions during development with histone mark profiling and chromosome conformation capture approaches^{189,190}. Additionally, understanding transcriptomic and epigenetic changes in more homogeneous cellular populations, or at a single-cell resolution, will greatly improve our mechanistic understanding of normal human brain development. Initial maps of these neurobiologically relevant epigenetic landscapes and cell type differences, mostly at the tissue level, are under construction by the PsychENCODE consortium.

As noted throughout this Review, studies of proteomic data are highly complementary to co-expression data and have revealed a crucial level of organization and regulation at the translational and post-translational levels. A particularly salient example is the synaptic signalling apparatus, more specifically the postsynaptic density (PSD), which has been extensively characterized at the protein level in humans and mice, showing key areas of overlap and divergence^{191,192}. However, developmental and cell subtype differences in the PSD are not well understood, so obtaining PSD co-expression and PPI networks in relevant neural tissue and time points, similar to what BrainSpan has done for gene expression, will be invaluable. Currently, high-throughput, high dynamic range spatial and temporal data from minute sample quantities with proteomics are not available, so creative integration of cell type-specific transcriptional data with more generic PPI data may provide an approximation of the regional or cellular differences in synaptic structure in the near future. The development of methods, including benchmarking and refining methods for the integration of different forms of data (for example, PPI and gene expression data), developing tools for exploring network structure at a more fine-grained level, and empirically defining the most sensitive and robust network approaches, will also be crucial.

One of the greatest challenges is to systematically infer causality in molecular networks with a systems genetics approach^{168,169}. This will necessitate more comprehensive eQTL studies, particularly in early brain development and disease¹⁷⁰. Core molecular pathways that are

confirmed to be perturbed in disease can then be interrogated with drug or environment perturbation data^{193,194} to identify interventions that will perturb networks from a disease state into a healthy state. This area of *in silico* drug screening based on DGE or network modules has barely been explored in the CNS, but it has considerable promise¹⁹³.

Additionally, with the advent of mandatory electronic medical records, population-level studies, including longitudinal data for many simple phenotypes, coupled with biobanking, can provide the scale needed to more fully understand genetic contributions to disease risk as well as disease relationships across the lifespan^{195–197}.

Even once we have the information from thousands of genomes, biological insights into the CNS require the assessment of relevant behavioural and cognitive phenotypes, which are not well defined for most neuropsychiatric diseases^{198,199} and are rarely collected in large populations. Genome-wide transcriptomic approaches provide a quantitative endophenotype, or a biomarker, that genetic association studies can use to further refine the measurement of disease states. Transcriptomic and other molecular systems measurements can also be correlated with systems neuroscience phenotypes, such as MRI and functional MRI measurements, or behavioural phenotypes to identify non-invasive indicators of disease state⁴ (FIG. 1a).

Conclusions

Currently, much basic and translational neuroscience research is still focused on candidate genes and candidate hypotheses, so sceptics may question the value of measuring entire systems. However, biological complexity cannot be ignored; genome-wide measurements, in conjunction with studying individual genes and pathways, are essential to address the true underlying mechanisms of neurodevelopmental and neurodegenerative disorders. Well-designed, reproducible molecular profiling studies allow biologists to simultaneously evaluate hypotheses in an unbiased manner and to generate new hypotheses. Although certainly vast and seemingly complex, gene networks provide an organizational framework that simplifies the process of hypothesis generation and testing. The general paradigm of using correlational and physical interaction molecular networks in neurobiology to understand molecular systems changes can be applied across methodologies and enables the investigation of relationships that span multiple levels of analysis. The results of high-quality genome-wide studies will be essential to develop and test hypotheses that look beyond where our current knowledge ends to develop a more encompassing view of the problems posed by neurodevelopmental and neurodegenerative disorders, and their potential solutions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Genetic architecture	For genetic variants, the relationship among allele frequency, effect size, number of contributing variants and how they quantitatively influence a given trait
Molecular systems or integrative network approach	Systems biology methods that use high-throughput quantification, analysis and interpretation of the molecular relationships within and across molecular levels, including the genome, transcriptome, epigenome, proteome and other 'omes'
Systems neuroscience	An area of neuroscience that focuses on short- and long-range circuits that are usually related to specific behavioral or cognitive functions (vision, motor function, attention and so on)
Gene network	A graph consisting of genes as nodes connected by edges that represent relationships between genes
Differential gene expression analysis (DGE analysis)	An approach commonly used in transcriptomic studies that serially compares thousands of genes between groups (for example, disease and controls) to evaluate the mean difference and its significance for each gene independently
Modules	Also known as clusters, cliques and communities. Highly interconnected subsets of genes in a gene network; for example, genes in a transcriptomic network sharing highly similar patterns of gene expression
Nodes	Molecular entities that constitute a network; for example, genes in a gene network or proteins in a protein interaction network
Edges	The relationships between nodes in a network delineating some measure of shared function; for example, correlations or physical interactions
Mutual information	A measure of dependence between two variables that can capture complex relationships, including nonlinear and nonmonotonic patterns, that could be missed by linear correlation measures
Hubs	Genes in a network or module that are highly connected; that is, they have a relatively high number of edges compared with other genes
RNA sequencing (RNA-seq)	An assay for measuring RNA transcript levels in a genome-wide manner that involves the extraction of RNA followed by construction of cDNA libraries that undergo high-throughput sequencing

Weighted networks	Networks in which the edges have continuous values, with higher values reflecting an increased strength or probability of connectivity
Binary networks	Networks in which the edges are all or nothing, either because this is inherent to the edge measurement (for example, physically interacting or not) or because a cut-off or threshold has been applied to a continuous measurement (for example, by applying a rule that all correlation values ≥ 0.7 are 1, all others are 0)
Signed networks	Networks in which the direction of association is taken into consideration in addition to the magnitude of the correlation; for example, in a signed correlation network, high positive correlations are assigned high edge values, but high negative correlations are assigned low edge values
Unsigned networks	Networks in which any high magnitude association is assigned a high edge value regardless of the direction of the association
Topological overlap	A computation on direct edge relationships in a network that transforms them into indirect edge values that reflect the sharing of neighbourhoods between genes
Seeded (prior-based) networks	Network analysis approaches in which edges are ‘grown’ around ‘seed’ genes that are selected on the basis of previous experiments or prior hypotheses, and the network structure is dependent on these seed genes
Unseeded (genome-wide) networks	Network analysis approaches in which edges are evaluated in a genome-wide manner, and network structure is not dependent on prior knowledge of a particular set of genes
Adjacency matrix	A matrix of pairwise node–node relationships that quantifies all possible edges in a network. Edge relationships may be determined from one data type or by weighting the contribution from multiple types of data
CLIP-seq	An assay for measuring the binding sites of a protein on RNA transcripts in a genome-wide manner that involves crosslinking immunoprecipitation followed by high-throughput sequencing
ChIP-seq	An assay for measuring the binding sites of a protein on DNA across the genome that involves chromatin immunoprecipitation followed by high-throughput sequencing
DNase hypersensitivity or ATAC-seq	Sequencing methods that infer regions of the genome in a particular cell or tissue with open chromatin by exploiting the fact that these regions are preferentially accessible to the DNase I enzyme or a transposase

Eigengenes	Module-level summaries of expression utilized in co-expression networks calculated by taking the first principal component of the expression levels of genes in a module
Psychosis	A mental state defined by a loss of contact with reality and characterized by exaggerations or distortions of normal perception
Negative symptoms	Symptoms involving a loss of normal emotional responses, including a lack of motivation, an inability to experience pleasure and reduced expression through speech
Unsupervised methods	Analysis approaches that use the intrinsic variation in data to define shared patterns without explicit prior knowledge of how the data should be grouped (for example, hierarchical clustering). This can identify novel clusters or groupings of data points
Expression quantitative trait locus analysis (eQTL analysis)	A specific case of genotype-to-phenotype association that uses RNA transcript levels as the phenotype in order to identify genetic loci that regulate RNA levels
Selective vulnerability	The relative susceptibility of specific brain regions, cell populations or time points to genetic or environmental insults that result in disease
Causal anchor	A causal factor, such as genetic variation, that can be used to orient edges to transform an undirected correlational network to a directed causal network
Gene set enrichment	An analysis approach that assesses the statistical significance of the overlap between two gene sets, one set is usually an annotated reference set, and the other is an unannotated set of interest

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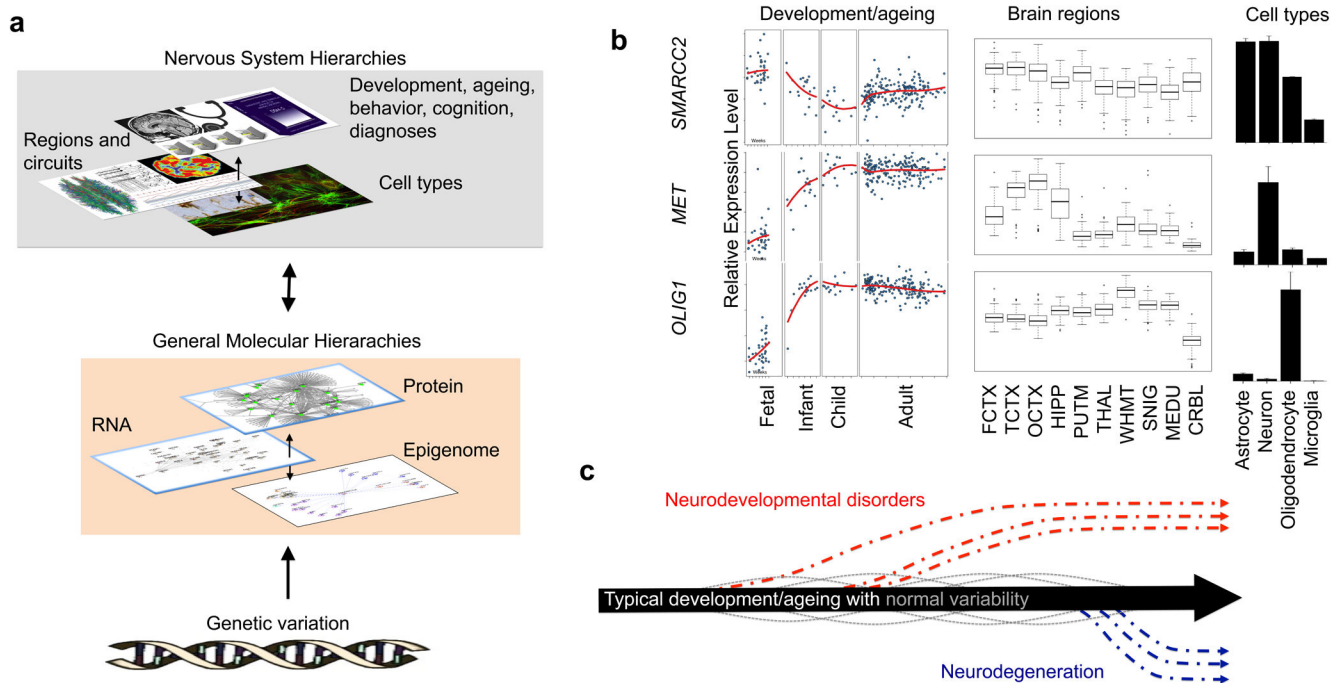


Figure 1. Molecular systems and the neurobiological hierarchy

a | Genetic variants exert their effects on cognitive and behavioural phenotypes associated with neurodevelopmental or neurodegenerative disease through a neurobiological hierarchy that includes multiple molecular levels (transcriptomic, proteomic and epigenomic) that can be modelled as networks on the basis of physical interactions and correlations within and across multiple molecular levels (BOX 2). These molecular levels of organization can vary at multiple neurobiological phenotypic levels (cells, circuits, and cognition and behaviour) across the lifespan. **b** | Gene expression levels vary dramatically across development and ageing, brain regions and cell types, as illustrated by three genes: *SMARCC2*, which is a pan-regional neurodevelopmental gene; *MET*, a regionally patterned adult neuronal gene; and *OLIG1*, a gene most highly expressed in white matter and oligodendrocytes. Development and ageing data are from BrainCloud¹⁷, regional data are from Braineac¹⁶ and cell type expression data are from fluorescent-activated cell sorted transcriptomes from mouse cortex¹⁶² (<http://web.stanford.edu/group/barreslab/brainrnaseq.html>). **c** | Both the molecular and phenotypic levels exhibit a typical trajectory with normal variation during development and ageing that can be altered in disease, resulting in abnormal temporal trajectories. The *x* axis on this plot reflects the progression of time, and the *y* axis reflects theoretical deviation from the normal trajectory for any molecular or phenotypic measurement. CPi, inner cortical plate; CPo, outer cortical plate; CRBL, cerebellum; FCTX, frontal cortex; HIPP, hippocampus; ISVZ, inner subventricular zone; IZ, intermediate zone; lncRNA, long noncoding RNA; MEDU, brainstem medulla; miRNA, microRNA; OCTX, occipital cortex; OSVZ, outer subventricular zone; PUTM, putamen; SNIG, substantia nigra; SP, subplate; TCTX, temporal cortex; THAL, thalamus; VZ, ventricular zone; WHMT, subcortical white matter.

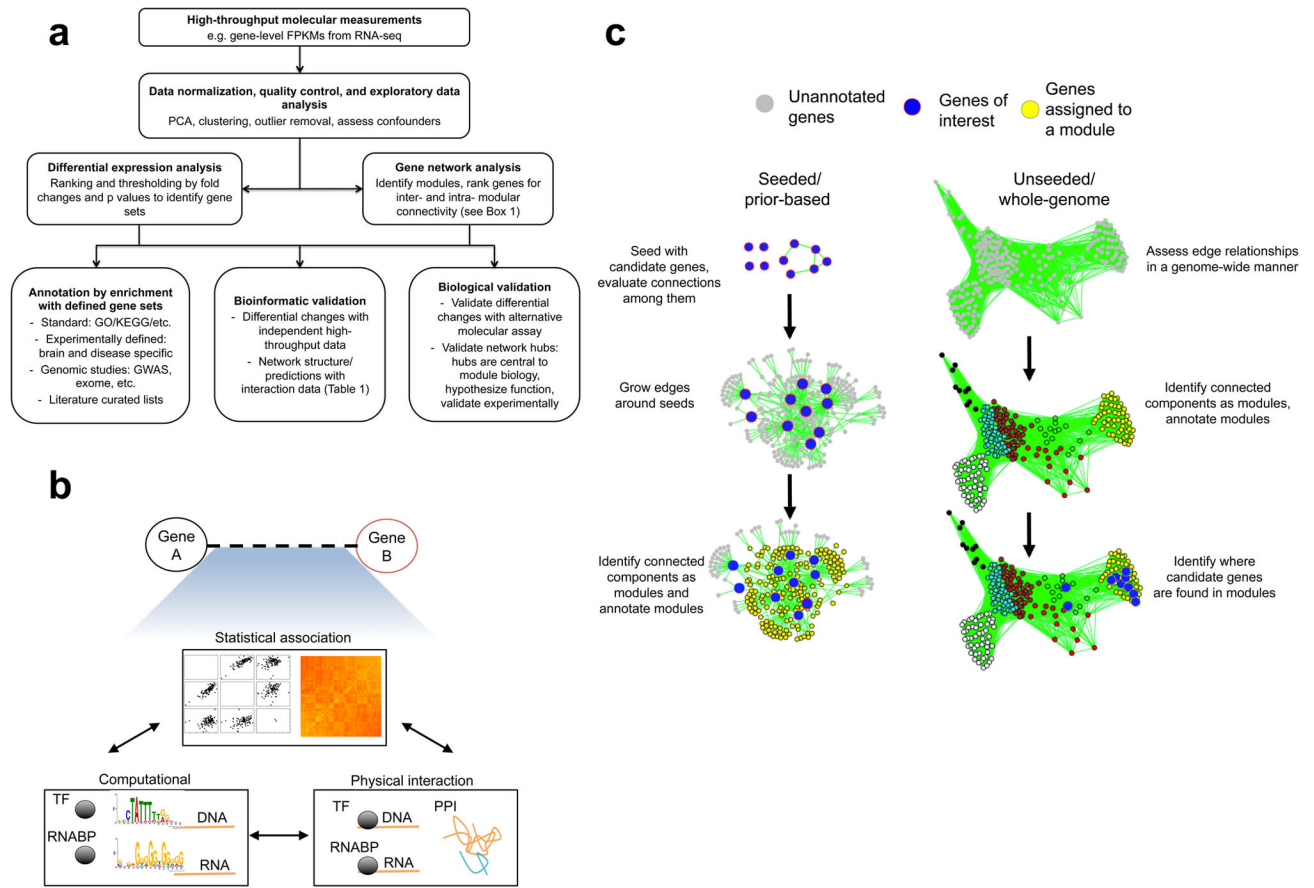


Figure 2. Flowchart of transcriptomic analysis and illustration of seeded and genome-wide approaches to network analysis

A flowchart demonstrating the general approach to a transcriptomic study that uses differential gene expression (DGE) and network analysis (part a). Network-level features, such as connectivity ranking and module-level enrichment, allow the integration of many external data sources and experiments. Network analysis involves first (part b) connecting genetic or molecular nodes with information about pairwise relationships, which may be one or more of the following: statistical associations relating molecular patterns measured across experiments, such as variation in gene expression levels across brain regions; physical interaction data from experiments or curated from the literature such as transcription factor (TF) or RNA-binding protein (RNABP) binding or protein–protein interactions (PPIs); or computational predictions about TF or RNABP binding using motif enrichment analysis (here, U on the RNA motif is depicted as T). Next, the structure of the network is used to (part c) define modules using a seed-based or genome-wide approach, which groups together the genes that share similar edge-level properties. The seeded (prior-based) approach is shown on the left-hand side, and the unseeded (genome-wide) approach on the right-hand side. The seeded approach involves starting with genes of interest, expanding edges to bring in additional (unannotated) genes and identifying highly connected components as modules. The unseeded approach (right-hand side) involves starting with unannotated genes, using edges to identify interconnected components as modules and then evaluating where genes of

interest fall in the resultant network structure. Modules from either approach can be further annotated with external information such as genetic associations and known pathways, integrated with additional data or used to prioritize targets for experimental validation (see BOX 2 and TABLE 1 for more details). Alternative depictions of the network analysis process are also available elsewhere^{28,41,169}. GO, Gene Ontology; KEGG, Kyoto Encyclopaedia of Genes and Genome Elements.

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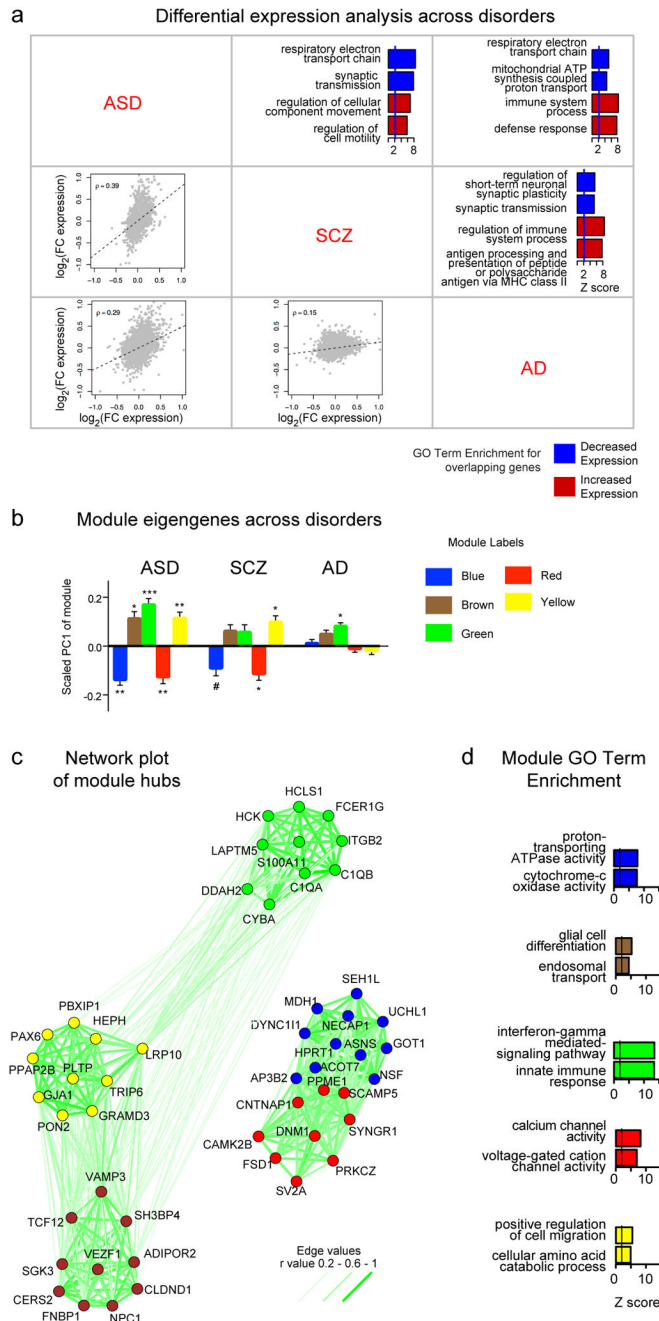


Figure 3. Transcriptomic convergence and divergence across central nervous system disorders
 Transcriptomics can systematically compare genes and pathways across neurobiological disorders. To provide a simple example, we compare genome-wide expression patterns in the cerebral cortex across published microarray studies of autism spectrum disorder (ASD)³⁰, schizophrenia (SCZ)²²⁵ and Alzheimer disease (AD)²²⁶ (part **a**). We applied differential gene expression (DGE) analysis across these disorders in a pairwise manner and performed a meta-analysis with weighted gene co-expression network analysis (WGCNA). Please see Supplementary information S1 (box) for details. The bottom-left half of the

comparisons shows pairwise comparison of DGE across conditions. ASD–SCZ and ASD–AD are significantly correlated in DGE changes, as demonstrated by Spearman correlations (ρ values) between genome-wide DGE effect sizes in each disorder. On the upper-right half of the comparisons, Gene Ontology (GO) term enrichment of pairwise shared upregulated and downregulated changes demonstrates that upregulated inflammation and downregulated synaptic function and oxidative phosphorylation are common to all three disorders. Results are shown as enrichment Z scores for pathway enrichment, $Z > 2$ suggests enrichment²²⁷. WGCNA across these three conditions identified five modules (labelled with different colours) that are perturbed across at least one condition, as demonstrated by differences in eigengene expression ($*p < 0.05, **p < 0.01, ***p < 0.001$, two-tailed t -test) (part **b**). The top ten interconnected (hub) genes in each module with edges reflecting the strength of correlation between genes reveals (part **c**) and GO term enrichment for each module (part **d**). MHC, major histocompatibility complex.

Table 1

Different edge types in gene networks: practical and theoretical considerations.

	Gene co-expression	Protein-protein interaction	Motif enrichment for transcription factors
Edge relationships	Statistical association (correlation or mutual information)	Physical binding (interacting or not interacting)	Computational inference (motif binding scores)
Main advantages	Indirectly predicts co-regulation, physical interactions and cell type specificity; easiest to measure from tissue of interest	Based on direct physical interactions; predicts protein complexes and signalling pathways	Identifies putative co-regulatory relationships without needing to carry out new experiments
Completeness of data across the genome	Most genes are similarly covered genome-wide	Incomplete assessment for most interactions; biased towards most well-studied molecules	Predictions restricted to availability and accuracy of available motif information
Tissue specificity	Primary data are often tissue specific	Primary data are rarely tissue specific	Primary data not usually tissue specific
Module-level interpretation	Reflects cell types and transcriptionally co-regulated biological processes	Protein complexes; signalling cascades; subcellular structures	Groups of transcriptionally co-regulated genes
Interpretation of hubs	Cell type-specific markers; molecular regulators such as transcription factors or RNA-binding proteins	Key proteins in complexes; converging points of signalling cascades	Gene to which many transcription factors bind, perhaps under more complex regulation
Sources of bias	Technical artefacts (RNA quality and batch effects); biological confounders (age and sex); post-mortem artefacts (cause of death)	Literature-curated data contain biases towards more well-studied interactions, which tend to be non-neuronal	Unlikely to reflect tissue-specific interactions or regulation without additional data
Examples of bioinformatic validation	Preservation of co-expression in independent data; enrichment of physical interactions in modules	Enrichment of co-expression from independent data	Enrichment of predicted binding sites from independent ChIP-seq data
Examples of experimental validation	Showing cell type specificity of hubs by <i>in situ</i> hybridization; demonstrating regulatory potential of hubs by hub gene knockdown	Co-immunoprecipitation of proteins of interest; disruption of protein complexes when hubs are targeted	Showing changes in transcription of targets on perturbation of regulators