Neurodegeneration enters the era of functional genomics

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Neurodegeneration enters the era of functional genomics

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Abstract

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Text

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The two decades since the first sequencing of a human genome have delivered substantial advances in our understanding of disease. Consequently, many areas of medicine are experiencing revolutionary changes. This is especially visible in “precision medicine,” which has been widely adopted in clinical oncology. Cancer patients are stratified according to the so-called “driver” mutations responsible for their disease and can be offered individualized therapies tailored to hit cancerous cells selectively. By comparison, the field of adult-onset neurodegenerative conditions, such as Alzheimer’s, Parkinson’s, and prion diseases, lags far behind—despite enormous research efforts. What accounts for the lackluster progress in therapeutics, and what are some possible ways out of the current impasse?

Much of the progress in our understanding of neurodegeneration comes from human genetics. Seminal findings arose from studying rare disease forms that seemed to mirror the more common “sporadic” AD and PD but were transmitted from one generation to another according to Mendel’s laws of inheritance. An early example was the discovery that inherited forms of prion disease are caused by point mutations in PRNP, the gene encoding the prion protein that causes transmissible spongiform encephalopathies. Other examples include the identification of mutations in genes encoding amyloid precursor protein and the presenilins in familial AD. Presenilins encode enzymes central to producing the main constituent of the neuritic plaques described by Alois Alzheimer a century ago.

The ubiquitous availability of low-cost DNA sequencing has resuscitated another approach to linking human genetics with the specific gene drivers of neurological disease—genome-wide association studies (GWASs). By comparing the genetic makeup of individuals suffering from a disease with that of nondiseased (“control”) persons, one can identify risk factors that increase the likelihood of developing the disease. GWASs led to the discovery of variations in specific genes, including TREM2 (triggering receptor expressed on myeloid cells 2) and GBA1 (glucocerebrosidase 1), as risk factors for non-Mendelian AD and PD, respectively. In some cases, GWAS results have highlighted previously underappreciated mechanisms contributing to pathogenesis. For example, genetic variants associated with AD risk are enriched in genes and enhancers (regions in the genome that control gene expression) acting in myeloid cells, likely microglia. This indicated the important role of innate immune cells in AD. Therefore, the enrichment of risk variants in specific biological pathways can deepen our mechanistic understanding of neurodegenerative diseases and may even point to new therapeutic targets.

However, the translation of GWAS results into new therapies for neurodegenerative diseases faces several challenges. First, variants with strong effects on disease risk are typically rare in human populations [variants in APOE (apolipoprotein E) are a prominent exception], whereas common variants typically have weak effects on disease risk—in the latter case, each variant makes a small contribution to a polygenic risk score. Therefore, even if common variants could be targeted by gene therapy, individual targeting of most variants would likely have negligible therapeutic benefits.

GWASs have uncovered important statistical associations between single-nucleotide polymorphisms (SNPs; the substitution of a single nucleotide at a specific position in the genome) and disease risk. However, SNPs do not necessarily point to the cause of disease. They may be physical neighbors of a gene that is proximally linked to disease. Thus, the formulation of mechanistic hypotheses from GWAS data necessitates a mapping of SNPs to causal SNPs to regulatory target genes and relevant cell types, and this has created a major bottleneck for the interpretation of GWAS studies.

Furthermore, crucial pathways of neurodegeneration may not be reflected in human genetic variation because such pathways may be so fundamental to cellular homeostasis that any mutations might lead to embryonic death, thereby eluding their detection by genome sequencing. Therefore, not all promising therapeutic targets can be revealed through studies of human genetics; orthogonal discovery approaches are needed to make them visible.

All of these challenges in identifying genetic factors that affect the risk of neurogenerative diseases can be addressed by functional genomics approaches. Over the past 10 years, large-scale genetic screens in human cells have been greatly facilitated by CRISPR-Cas gene editing technology. CRISPR-Cas enables precise perturbations of the human genome to interrogate functional consequences. Cas9 cleaves specific sequences in DNA, as dictated by a short guide RNA. Related systems enable the targeting of RNA molecules. Engineering of these naturally occurring enzymes has spawned a broad spectrum of applications. Although enzymatically “dead” versions of Cas9 don’t disrupt DNA, they can be used to recruit other proteins to specific genomic loci, including transcriptional repressors (CRISPRi), transcriptional activators (CRISPRa), base editors, and epigenetic methylators (CRISPRoff). Because the target genomic sequence of these tools is specified by a short guide RNA sequence, complex libraries of guide RNAs can be

developed for the high-throughput interrogation of many cellular functions.

The general concept of phenotypic screening is to probe the genome in a “hypothesis-free” manner. Every single gene of the genome is perturbed and relevant genes are identified by extracting the genetic elements from cells that have acquired the desired phenotype. Similar approaches have long been available in simpler organisms such as fruit flies and nematodes, where they catalyzed important discoveries in neurodegeneration. However, the application of this approach to mammals requires costly methods of chemical mutagenesis. RNA interference provided a targeted method for gene perturbation in model organisms and cultured human cells but was plagued by pervasive off-target effects that limited the robustness of large-scale screens. Now, gene editing with CRISPR-Cas9 has triggered an explosion of genetic screens in all areas of biology, including the neuroscience of disease. A major breakthrough in the application of CRISPR-Cas screening to neurodegenerative diseases has been screens of relevant human cell types, such as neurons, microglia, and astrocytes (the main cell types of the central nervous system) that were generated from induced pluripotent stem cells (iPSCs).

In a first proof of principle,SNPs linked to neurodegenerative diseases (based on GWAS studies) were targeted in a CRISPRi screen in both neurons and microglia, uncovering cell type–specific regulatory target genes that act as transcriptional enhancers. Because iPSCs can be derived from patients with mutations linked to familial neurodegenerative diseases, screens based on disease-relevant phenotypes have the potential to uncover both disease mechanisms and therapeutic targets. Once such targets will have been defined, mitigating the detrimental effect of the disease-causing mutations may become straightforward.

Next-generation sequencing enables the analysis of pooled CRISPR screens, which are scalable. Coupling pooled screens to single-cell RNA sequencing–based read-outs (for gene expression) provides a rich description of cellular phenotypes. A small number of pooled screens have already been implemented in vivo in healthy mouse brains, enabling the investigation of cell-autonomous phenotypes in a physiological organismal context.

A recent development vastly extends the territory of biological phenotypes that can be queried, including cell-nonautonomous phenotypes, time-resolved phenotypes such as neuronal activity and cellular motility, and phenotypes based on high-content images (which

maximize data capture) with subcellular resolution. Although some of these screens are currently being performed, the breadth of future possibilities is cause to be excited.

The era of functional genomics in neurodegenerative disease research has just begun There is no doubt that the integration of functional genomics with human genetics and single-cell profiling of human patient tissues will become a major engine for the discovery of disease mechanisms. The insights gained will help generate testable hypotheses and open new perspectives for urgently needed therapeutic strategies.

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