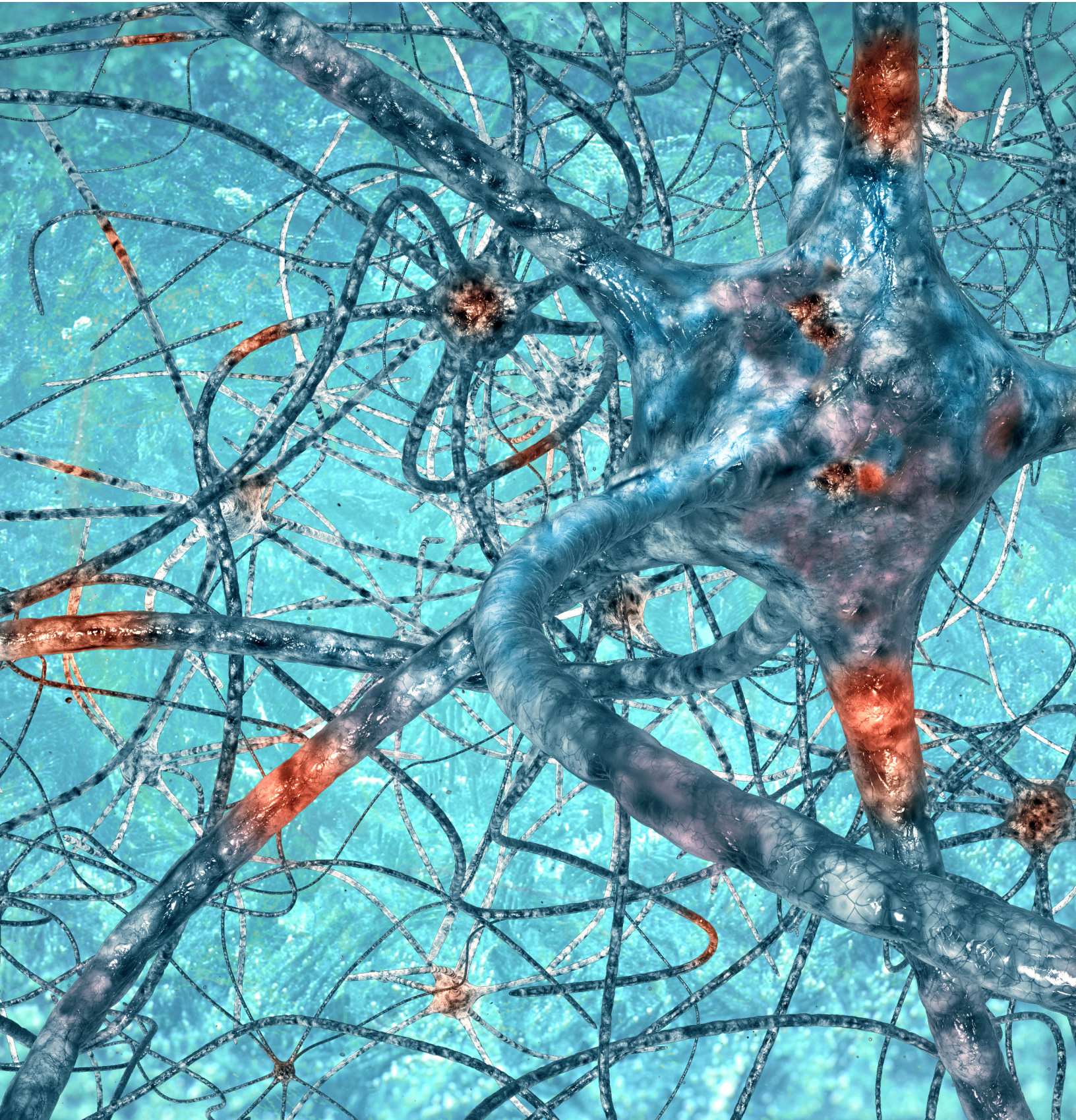


# Neurobiology Research Review

An Overview of Recent Publications Featuring Illumina Technology



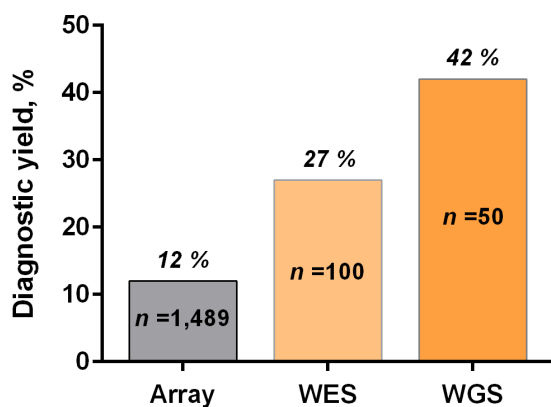
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# INTRODUCTION

Neurological disorders are complex diseases caused by a combination of genetic, environmental, and lifestyle factors. Most neurological diseases—such as schizophrenia, autism, and Alzheimer's and Parkinson's disorders—have been described many decades ago. However, it is only recently, with the use of next-generation sequencing (NGS), that their full complexity is being revealed.<sup>1,2</sup> There is an increasing awareness that disease development is driven through a complex interplay between somatic (non-inherited) mutations, inherited mutations, and epigenetic modifications.<sup>3,4,5,6</sup> This complexity results in extremely weak genotype-phenotype correlations, and the same disease can present with a variety of pathological phenotypes in different individuals. Not surprisingly, different neurological diseases present a spectrum of similar symptomatic profiles (such as dementia, e.g., Parkinson's disease and dementia with Lewy bodies).<sup>7</sup> These overlapping symptoms could indicate the involvement of the same underlying molecular mechanisms. A diagnosis based on these underlying molecular processes, as well as the observed phenotype, promises more objective and accurate diagnosis and treatment in the future.<sup>8</sup>

The increased information obtained from NGS, along with a variety of library preparation methods, offers an impressive armamentarium of tools to unravel the genomic and epigenomic aspects of psychiatric and neurodegenerative diseases. The improved understanding also could translate ultimately into the development of new, effective therapies. Additionally, whole-genome sequencing (WGS) potentially could detect predisposition to these disorders, to allow preventative care and early intervention. A recently announced massive 100,000 Genomes project, initiated by Genomics England, signifies the importance of genomic diagnostics for tomorrow's medicine.



Diagnostic yield for patients with severe intellectual disability (IQ < 50), specified by technology: genomic microarrays, whole-exome sequencing (WES), and WGS. Percentages indicate the number of patients in whom a conclusive cause was identified using the specified technique.<sup>9</sup>

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2. Koboldt D. C., Steinberg K. M., Larson D. E., Wilson R. K. and Mardis E. R. (2013) The next-generation sequencing revolution and its impact on genomics. *Cell* 155: 27-38
3. Yu T. W., Chahrouh M. H., Coulter M. E., Jiralerspong S., Okamura-Ikeda K., et al. (2013) Using whole-exome sequencing to identify inherited causes of autism. *Neuron* 77: 259-273
4. Fromer M., Pocklington A. J., Kavanagh D. H., Williams H. J., Dwyer S., et al. (2014) De novo mutations in schizophrenia implicate synaptic networks. *Nature* 506: 179-184
5. De Jager P. L., Srivastava G., Lunnon K., Burgess J., Schalkwyk L. C., et al. (2014) Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. *Nat Neurosci* 17: 1156-1163
6. Chung S. J., Armasu S. M., Anderson K. J., Biernacka J. M., Lesnick T. G., et al. (2013) Genetic susceptibility loci, environmental exposures, and Parkinson's disease: a case-control study of gene-environment interactions. *Parkinsonism Relat Disord* 19: 595-599
7. McCarthy S. E., Gillis J., Kramer M., Lihm J., Yoon S., et al. (2014) De novo mutations in schizophrenia implicate chromatin remodeling and support a genetic overlap with autism and intellectual disability. *Mol Psychiatry* 19: 652-658
8. Fromer M., Pocklington A. J., Kavanagh D. H., Williams H. J., Dwyer S., et al. (2014) De novo mutations in schizophrenia implicate synaptic networks. *Nature* 506: 179-184
9. Gillissen C., Hehir-Kwa J. Y., Thung D. T., van de Vorst M., van Bon B. W., et al. (2014) Genome sequencing identifies major causes of severe intellectual disability. *Nature* 511: 344-347

## Diseases

Neurological diseases represent a daunting spectrum of complex multifactorial pathologies, ranging from subtle to life-threatening. To illustrate the most recent research and the use of genomics, this review focuses on schizophrenia and autism as examples of complex neurodevelopmental disorders, and Alzheimer's and Parkinson's as examples of neurodegenerative diseases. The approaches and techniques used in these studies can be applied to a wide variety of neurological diseases.

## Schizophrenia

Schizophrenia is one of the most complex psychiatric diseases, and it affects as much as 1% of the global adult population.<sup>10</sup> The most common symptoms include irrational thinking, auditory hallucinations, false beliefs, and reduced social activity. The disease typically develops between 12 and 25 years of age and is a highly heritable, polygenic disorder. Recent genomic analysis studies have shown that schizophrenia is attributed to over a thousand gene loci, many of which appear in non-coding parts of the genome.<sup>11-15</sup> A significant subset of risk alleles for schizophrenia is also implicated across other diseases in this diagnostic category, such as bipolar disorder, autism, and depression.<sup>16,17,18</sup> All these diseases are truly spectrum disorders, which has complicated understanding of their genetic causes and the development of targeted therapies. However, the advent of high-throughput gene sequencing technology provides a tool for deeper analysis of the genetic basis of these diseases, and it holds promise for unraveling the complex interplay between multiple genetic and epigenetic modifications.

Genotype-phenotype correlations in schizophrenia are extremely weak, and the same disease can present with a variety of pathological phenotypes in different patients. Studies with twins revealed that over 80% of the risk of developing schizophrenia comes from genetic predisposition, but exposure to environmental risk factors can play a significant role.<sup>20</sup> The first genetic mutations detected in schizophrenia were rare variants, including copy-number variants (CNVs).<sup>21</sup> Altogether, these variants account for almost 20% of disease cases.<sup>22</sup> The remaining genetic hits are most likely represented by common variants.<sup>23</sup> Presumably, the effects of individual common variants are mild; however, in combination, they may be sufficient to trigger the onset of schizophrenia.<sup>24,25</sup>

“Schizophrenia liability is being mapped to hundreds, perhaps ultimately more than a thousand, genetic loci, each contributing a small increment of risk.” Hyman 2014

10. Elert E. (2014) Aetiology: Searching for schizophrenia's roots. *Nature* 508: S2-3
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13. Hyman S. E. (2014) Perspective: Revealing molecular secrets. *Nature* 508: S20
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19. Wright J. (2014) Genetics: Unravelling complexity. *Nature* 508: S6-7
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Along with determining the individual mutations leading to schizophrenia, the power of genetic analysis is expected to give answers to some other important questions, such as why the development of schizophrenia is associated with accelerated ageing<sup>26</sup> or why schizophrenic patients suffer from heart, lung, and metabolic diseases at young ages and at a much higher rate than the general population.<sup>29</sup> These effects could be due to additional lifestyle risks, such as substance abuse and smoking. Approximately 50% of patients with chronic schizophrenia have substance-use disorder, and their risk to develop this disorder is 4.6 times higher than for the general population. Over 80% of schizophrenic patients in the U.S. are also heavy smokers,<sup>30</sup> as nicotine serves as an agonist of the nicotine acetylcholine receptor and presumably can attenuate some cognitive impairment associated with schizophrenia.<sup>31</sup>



Smoking and substance abuse are common among schizophrenia patients. Over 80% of schizophrenic patients in the U.S. are heavy smokers,<sup>32</sup> as nicotine presumably can attenuate some of the cognitive impairment associated with schizophrenia.

Early, accurate, and objective diagnosis of the underlying molecular mechanisms of the disease will allow a better control of the disease symptoms with available therapies. In the more distant future, an improved understanding of the disease should lead to the development of more effective, targeted, and personalized therapies.

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**Fromer M., Pocklington A. J., Kavanagh D. H., Williams H. J., Dwyer S., et al. (2014) De novo mutations in schizophrenia implicate synaptic networks. *Nature* 506: 179-184**

Of the known risk alleles for schizophrenia, the only ones definitively shown to confer considerable increments in risk are rare chromosomal CNVs that involve deletion or duplication of thousands of bases of DNA. This study examined the effect of small *de novo* mutations affecting one or a few nucleotides. By Illumina HiSeq WES of 623 schizophrenia trios, the authors assessed *de novo* mutation rates and shared genetic etiology for schizophrenia, intellectual disability, and autism-spectrum disorders (ASDs). They found several insights to suggest a common etiological mechanism.

Illumina Technology: HiSeq for exome sequencing

**Karayannis T., Au E., Patel J. C., Kruglikov I., Markx S., et al. (2014) Cntnap4 differentially contributes to GABAergic and dopaminergic synaptic transmission. *Nature* 511: 236-240**

In an effort to understand the pathological development of neurological disorders, genetic effects have been studied in the context of proteins that are expressed in neural cells. In this study, the authors characterized the effect of CNTNAP4 knockouts on mouse behavior and development, and relate these results to the findings of CNVs in humans across a region including the CNTNAP2 gene. The authors found that CNTNAP4 is localized presynaptically, and its loss leads to a reduction in the output of cortical parvalbumin (PV)-positive GABAergic basket cells. In addition, CNTNAP4-mutant mice showed defects in these neuronal populations and exhibited sensory-motor gating and grooming endophenotypes.

Illumina Technology: HumanHap550, HumanOmni1-Quad

**Purcell S. M., Moran J. L., Fromer M., Ruderfer D., Solovieff N., et al. (2014) A polygenic burden of rare disruptive mutations in schizophrenia. *Nature* 506: 185-190**

Identifying gene associations for complex genetic diseases remains challenging, with small sample sizes being a hindrance for finding significant effects. In this study of schizophrenia, the authors performed WES, based on Illumina technology, of 2,536 schizophrenia cases and 2,543 controls. They identified disruptive mutations distributed across many genes; however, no individual gene-based test for low frequency and moderately large effect achieves significance after correction for multiple testing.

Illumina Technology: HiSeq 2000, Genome Analyzer<sub>IIx</sub>

**Schizophrenia Working Group of the Psychiatric Genomics C. (2014) Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511: 421-427**

Schizophrenia is a highly heritable disorder, but the heritability is not found in a single gene effect. In this largest genome-wide association study (GWAS) for schizophrenia to date, the authors used single-nucleotide polymorphism (SNP) arrays for 36,989 cases and 113,075 controls to determine genetic risk factors for the disorder. The authors found that the significant genetic associations were not randomly spread across the genome, but enriched among genes expressed in brain and genes that have been associated with typical co-morbidity diagnoses, such as ASD and intellectual disability. Interestingly, links were also enriched within genes related to immunity, which fits the existing hypothesis of immune dysregulation in schizophrenia.

Illumina Technology: Human1M, HumanOmni2.5, HumanOmniExpress, HumanHap550, Human610, HumanHap650Y, HumanHap300

**Stefansson H., Meyer-Lindenberg A., Steinberg S., Magnusdottir B., Morgen K., et al. (2014) CNVs conferring risk of autism or schizophrenia affect cognition in controls. *Nature* 505: 361-366**

Certain CNVs contribute to the pathogenesis of schizophrenia and autism. In this study, the authors investigated the influence of these CNVs on phenotypes separate from those of the mentioned diseases. In a big population-wide study of nearly a third of the Icelandic population ( $n = 101,655$ ), the authors used Illumina SNP microarrays to test for associations of CNVs with cognitive deficits, dyslexia, dyscalculia, and brain structure changes. The authors found that the 15q11.2(BP1-BP2) deletion affects brain structure in a pattern consistent with first-episode psychosis in both schizophrenia and dyslexia.

Illumina Technology: HumanHap300, HumanCNV370-Duo, HumanHap650Y, Human1M, HumanOmni2.5, HumanOmniExpress, HumanOmni1S

Yoon K. J., Nguyen H. N., Ursini G., Zhang F., Kim N. S., et al. (2014) Modeling a genetic risk for schizophrenia in iPSCs and mice reveals neural stem cell deficits associated with adherens junctions and polarity. *Cell Stem Cell* 15: 79-91

Defects in brain development can contribute to the onset of neuropsychiatric disorders. This study set out to identify the functional role of the 15q11.2 deletion on neural development using induced pluripotent stem cell (iPSC)-derived human neural precursor cells (hNPCs) by RNA-Seq and SNP-genotyping arrays. They found that haploinsufficiency of CYFIP1, a gene within 15q11.2, affects radial glial cells, leading to their ectopic localization outside of the ventricular zone.

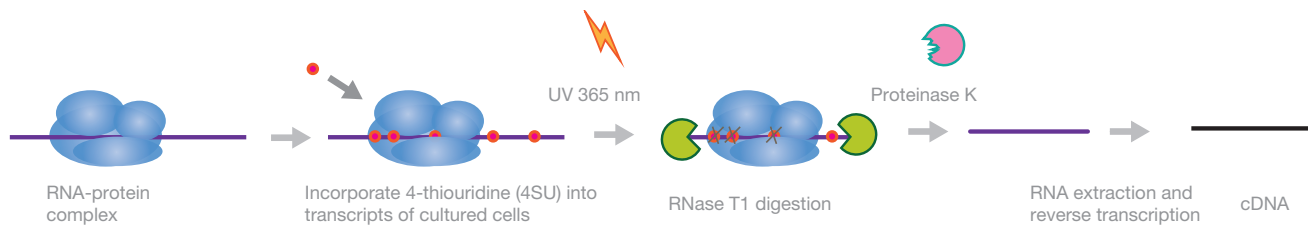
Illumina Technology: HumanOmni2.5S

Stoll G., Pietilainen O. P., Linder B., Suvisaari J., Brosi C., et al. (2013) Deletion of TOP3beta, a component of FMRP-containing mRNPs, contributes to neurodevelopmental disorders. *Nat Neurosci* 16: 1228-1237

Genetic studies, including studies of mRNA-binding proteins, have shed new light on the connection of mRNA metabolism to disease. In this study, the authors found that deletion of the TOP3b gene was associated with neurodevelopmental disorders in the Northern Finnish population. Combining the genotyping with photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP), the authors found that the recruitment of TOP3b to cytosolic messenger ribonucleoproteins (mRNPs) was coupled to the co-recruitment of FMRP, the disease gene involved in Fragile X syndrome.

Illumina Technology: Human Gene Expression, Human610-Quad, HumanHap300, HumanCNV370-Duo

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Photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) maps RNA-binding proteins (RBPs).<sup>39</sup> This approach is similar to high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) and cross-linking immunoprecipitation sequencing (CLIP-Seq), but uses much more efficient crosslinking to stabilize the protein-RNA complexes. The requirement for a photoactivatable ribonucleoside limits this approach to cell culture and in vitro systems. In this method, 4-thiouridine (4-SU) and 6-thioguanosine (6-SG) are incorporated into transcripts of cultured cells. Ultraviolet irradiation crosslinks 4-SU/6-SG-labeled transcripts to interacting RBPs. The targeted complexes are immunoprecipitated and digested with RNase T1, followed by Proteinase K, before RNA extraction. The RNA is reverse-transcribed to cDNA and sequenced. Deep sequencing of cDNA accurately maps RBPs interacting with labeled transcripts. (For more methods, see: <http://applications.illumina.com/applications/sequencing/ngs-library-prep/library-prep-methods.ilmn>)

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Todarello G., Feng N., Kolachana B. S., Li C., Vakkalanka R., et al. (2014) Incomplete penetrance of NRXN1 deletions in families with schizophrenia. *Schizophr Res* 155: 1-7

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# Autism Spectrum Disorder

Autism spectrum disorder (ASD) comprises a group of polygenic, multi-locus disorders,<sup>34</sup> often accompanied by symptoms of other disorders, such as developmental disability/intellectual disability (DD/ID; over 40% of ASD cases), attention deficit/hyperactivity disorder (ADHD; 59%–75%), obsessive-compulsive disorder (OCD; 60%), epilepsy (7%–46%), and other neurological and behavioral patterns.<sup>35,36</sup> Autism rates have been increasing rapidly, from 0.7% of the population in 2000 to 1.1% in early 2010.<sup>37</sup> In the U.S., 1 in 68 children has been diagnosed as autistic. This trend can be partially attributed to the improved diagnosis of the disease. Improved understanding of the genetic causes of ASD is anticipated to facilitate development of palliative or therapeutic care for affected individuals. It is also expected to be instrumental in providing a more accurate method to assess the mental condition of at-risk populations, such as criminals and individuals with other psychiatric pathologies.<sup>38,39,40</sup>

Autism is primarily a genetic disease, with 15–30 times increased risk of disease development in siblings of autistic children.<sup>41</sup> Heritability of this disease has been estimated as high as 90%–96%, suggesting yet unidentified non-genetic causes.<sup>42,43,44</sup> A more accurate, systematic approach is still needed to improve the distinction between essential autism and complex (syndromic, sporadic) autism (Table 1). Genomic approaches promise to be efficient and reliable tools for distinguishing between various types of autism.<sup>45</sup>

Table 1: Types of autism

Type of Disease	Percentage of Cases	Disease Characteristics
<b>Essential autism</b> <sup>46w</sup>	75%	Higher male to female ratio Lack of dysmorphic features Higher sibling recurrence risk Positive family history Common gene variants
<b>Complex (syndromic, sporadic) autism</b> <sup>47</sup>	25%	Large number of highly penetrant rare mutations

Similar to schizophrenia, ASD is a heterogenic disorder.<sup>48</sup> This heterogeneity may be observed not only across individuals, but even across different sections of brain. Gene expression may change based on the timing of the analysis, as shown in experiments with mice.<sup>49</sup>

“Two years from now, researchers will need a larger T-shirt to flaunt their findings.” Wright 2014

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CNVs were the first type of mutation associated with autism.<sup>50,51</sup> The *de novo* rate of CNVs is three to seven times higher than in controls. The gene groups most affected by autism-associated CNVs are GTPase/Ras, ubiquitin degradation genes, and genes involved in synapse development, axon targeting, and neuron motility.<sup>52,53,54</sup> Large CNVs are present in 5%–10% of ASD patients, primarily in those with a syndromic ASD phenotype.<sup>55,56,57</sup> Private CNVs in autistic individuals often affect genes encoding synaptic proteins and neuronal cell-adhesion proteins.<sup>58</sup> (see Copy-Number Variants)

Furthermore, ASD can also be caused by rare mutations, deletions, duplications,<sup>59</sup> and larger chromosomal abnormalities, which may be inherited or arise *de novo*.<sup>60</sup> Monogenic mutations known to date contribute to 2%–5% of syndromic cases, with Fragile X chromosome, PTEN macrocephaly, and tuberous sclerosis being the most common abnormalities.<sup>61,62</sup> PTEN mutations are also strongly associated with tumor syndromes.<sup>63</sup>



Twin studies have become a standard model in the research of psychiatric diseases. They allow assessment of the contribution of genes and the environment to disease risk.

Recent WES and WGS studies have identified multiple, high-confidence ASD genes.<sup>64</sup> Of two large-scale WGS projects, one was initiated by the U.K. government in collaboration with Illumina and the Wellcome Trust (100,000 Genomes project), and the second was initiated by Beijing Genomics Institute (BGI) in collaboration with Autism Speaks (Autism Genome 10K project). The pilot results of the latter study have been published by Jiang et al.<sup>65</sup>

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## Epigenetics

Susceptibility to ASD can arise at both the genetic and epigenetic levels.<sup>66</sup> Several groups have independently identified multiple differentially methylated regions (DMRs) in post-mortem samples of autistic individuals. These biologically diverse gene regions include DNase hypersensitive sites and an alternative transcript termination site.<sup>67,68,69</sup> These studies provide an additional level of evidence for the role of epigenetics in complex diseases, such as ASD.

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[Gaugler T., Klei L., Sanders S. J., Bodea C. A., Goldberg A. P., et al. \(2014\) Most genetic risk for autism resides with common variation. \*Nat Genet\* 46: 881-885](#)

Although ASDs have been studied widely, the proportion and nature of genetic heritability is uncertain. This study analyzed the largest autism cohort data to date, including 1.6 million Swedish families with at least two children and ~5,700 individuals with strict autism diagnoses. Using Illumina SNP arrays, the authors investigated the contribution of rare versus common genetic variants to the disease. They conclude that the heritability is ~52.4%, with common variation as the biggest contributor. Rare, *de novo* mutations contribute substantially to individual liability, yet their contribution to variance in liability is modest at 2.6%.

Illumina Technology: OmniExpress and Exome

[Lee H., Lin M. C., Kornblum H. I., Papazian D. M. and Nelson S. F. \(2014\) Exome sequencing identifies \*de novo\* gain of function missense mutation in KCND2 in identical twins with autism and seizures that slows potassium channel inactivation. \*Hum Mol Genet\* 23: 3481-3489](#)

Numerous studies have reported comorbidity of autism and epilepsy, but the relationship between the two disorders is unknown. In this study, identical twins, affected by both autism and severe intractable seizures, were studied using exome sequencing. A novel variant in the KCND2 gene was observed in both twins. The *de novo* mutation is located in the protein coding the Kv4.2 potassium channel, and the authors expressed the mutant protein in *Xenopus* oocytes to observe functional effects. Expression analysis showed that the mutation dominantly impairs the closed-state inactivation of the potassium channel, strongly supporting KCND2 as the causal gene for epilepsy in this family.

Illumina Technology: HiSeq 2000, Illumina Paired-End Sequencing Library Prep Protocol

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**King I. F., Yandava C. N., Mabb A. M., Hsiao J. S., Huang H. S., et al. (2013) Topoisomerases facilitate transcription of long genes linked to autism. *Nature* 501: 58-62**

Topoisomerases are expressed throughout the developing and adult brain, and are mutated in some individuals with ASD. However, the mechanism by which topoisomerases impact ASD is unknown. By transcriptome sequencing, in combination with genome-wide mapping of RNA polymerase II density in neurons, the authors found that expression of long genes was reduced after knockdown of topoisomerase in neurons. The authors noted that many high-confidence ASD candidate genes are exceptionally long and were reduced in expression after TOP1 inhibition. This observation suggests that defective topoisomerases could contribute to ASD.

Illumina Technology: HiSeq 2000, TruSeq RNA, TruSeq for ChIP-Seq

**Ladd-Acosta C., Hansen K. D., Briem E., Fallin M. D., Kaufmann W. E., et al. (2013) Common DNA methylation alterations in multiple brain regions in autism. *Mol Psychiatry***

Genetic as well as environmental factors are responsible for ASDs. In this study, the authors measured over 485,000 CpG loci and identified 4 genome-wide significantly different DMRs from 19 autism cases in human brain tissue. This study highlights a new selected set of affected genes.

Illumina Technology: HumanMethylation450

**Willsey A. J., Sanders S. J., Li M., Dong S., Tebbenkamp A. T., et al. (2013) Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism. *Cell* 155: 997-1007**

Recent studies employing WES and WGS have identified nine high-confidence ASD genes. This study examined the contribution of these nine genes to the common phenotype by combining Illumina WES and RNA-Seq data into co-expression networks. The authors explain how these networks will guide future ASD research by indicating which genes are most likely to have overlapping molecular, cellular or circuit-level phenotypes.

Illumina Technology: HiSeq 2000, Genome Analyzer

**Yu T. W., Chahrour M. H., Coulter M. E., Jiralerspong S., Okamura-Ikeda K., et al. (2013) Using whole-exome sequencing to identify inherited causes of autism. *Neuron* 77: 259-273**

Steel syndrome is a developmental structural disorder first described in 1993 in 23 Hispanic children from Puerto Rico. This paper presents the genomic analysis of a family with two affected siblings. The authors used whole-exome sequencing using the Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC) core design followed by sequencing of both affected siblings, parents, and an affected cousin and her unaffected parents. By filtering the detected genetic variants by segregation, the authors discovered a single homozygous missense variant segregating with the disorder. The variant disrupts the collagen gene COL27A1, which codes for a protein expressed in developing cartilage.

Illumina Technology: HiSeq 2000, HumanOmniExpress

Lionel A. C., Tammimies K., Vaags A. K., Rosenfeld J. A., Ahn J. W., et al. (2014) Disruption of the ASTN2/TRIM32 locus at 9q33.1 is a risk factor in males for autism spectrum disorders, ADHD and other neurodevelopmental phenotypes. *Hum Mol Genet* 23: 2752-2768

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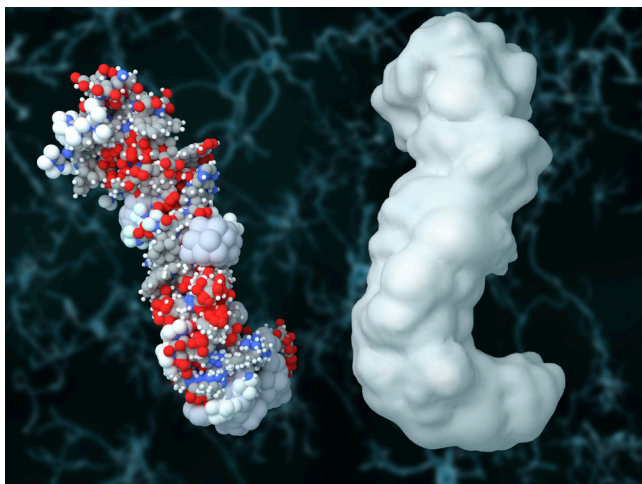
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## Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of dementia, affecting over 40 million people world-wide, and the incidence may double by the year 2050.<sup>70</sup> Eleven percent of people 65 or older and 32% of people 85 or older are affected by this fatal neurodegenerative disorder.<sup>71</sup> Early symptoms of AD include gradually worsening ability to memorize new information,<sup>72</sup> followed by confusion, irritability, trouble with language, and long-term memory loss. The disease destroys memory and cognitive skills through the accumulation of two types of abnormal insoluble aggregates in the brain: extracellular  $\beta$ -amyloid plaques and intracellular neurofibrillary tangles. The aggregates disrupt the intricate interplay between brain neurons, which eventually cause the death of the neurons with consequent significant shrinkage of the brain volume.<sup>73</sup>

Pathogenic  $\beta$ -amyloid protein belongs to a group of prion proteins, which kick-start a chain reaction of destructive processes by engaging new  $\beta$ -amyloid "seeds" into formation of insoluble oligomers and spreading across the brain. However, unlike "mad cow disease" (bovine spongiform encephalopathy or BSE) prions, AD-related amyloidosis is not infectious and cannot be transmitted between individuals.<sup>74</sup> Neurofibrillary tangles are formed intracellularly by oligomerization of the hyperphosphorylated form of Tau protein, which is normally abundant in axons and is responsible for maintaining the structure of microtubules. During the disease, tau protein is misfolded and mislocalized to the neuronal soma.<sup>75</sup>



Beta-amyloid protein is the major component of amyloid plaques.

Alzheimer's disease can be present in one of two forms: early-onset AD (EOAD, 30–60 years old) and late-onset AD (LOAD) (Table 2). EOAD is mostly a genetic disease, whereas LOAD is a sporadic disease, associated with a complex interplay among different mutations.

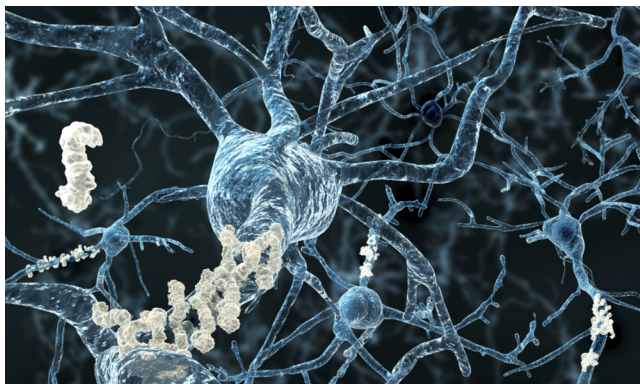
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Table 2: Types of AD

Type of Disease	Age of Onset	Percentage of Cases	Disease Characteristics
<b>Early-onset AD (EOAD)</b>	30–60 years	2%–5%	Mostly genetic <sup>76</sup>
<b>Late-onset AD (LOAD)</b>	>60 years	95%–98% <sup>77</sup>	Strong role for epigenetic markers, such as DNA methylation <sup>78</sup>

EOAD is associated with mutations in three genes: amyloid precursor protein (APP, integral Type I membrane glycoprotein) and presenelins PSEN1 and PSEN2. However, these mutations reportedly account for fewer than 2% of all AD cases.<sup>79,80</sup> Mutations in these genes deregulate the APP pathway and cause accumulation of plaques built of  $\beta$ -amyloid protein.<sup>81</sup> Interestingly, some mutations in APP can be protective against AD,<sup>82,83</sup> highlighting the importance of deep genetic analysis and correlative studies of this disease.

LOAD had, until recently, only one known genetic risk factor: the E4 variant of the apolipoprotein E gene (APOE).<sup>84</sup> The risk of developing LOAD in individuals with two copies of APOE4 (2% of population) is as high as 60% at 85 years old, and in individuals with one copy of this gene (25% of the population), it is 30%.<sup>85</sup> Each additional copy of APOE4 increases the risk of developing AD by a factor of three or more.<sup>86</sup> It is possible that low-frequency variants with large influence on LOAD pathogenesis may have been missed by traditional GWAS. Sequence-based association studies may be able to identify risk alleles in complex diseases, and it is anticipated that these studies will elucidate low-frequency variants with large effect sizes.<sup>87</sup>



Extracellular amyloid plaques stick to each other, as well as to the neuron. They disrupt neuronal networks, causing death of neurons and impairment of brain activity.

An interesting experimental approach proposes that WES be run on a thoroughly selected subgroup of individuals at increased risk of AD, and this analysis can be followed by a combination of genotyping and resequencing assays.<sup>88</sup>

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Aging and AD are associated with a spectrum of epigenetic changes, such as abnormal DNA methylation and histone modification.<sup>89</sup> These changes can occur under certain environmental conditions, such as stroke,<sup>90</sup> hypertension, type II diabetes, obesity,<sup>91</sup> exposure to heavy metals,<sup>92,93</sup> head injury, immunological proteins,<sup>94,95,96</sup> and maternal viral infections.<sup>97-100</sup> (see Epigenetic Modifications)

An analysis of the two largest GWAS available for AD has shown that there is a significant overlap between disease-associated genes in pathways associated with AD, cholesterol metabolism, and immune response.<sup>101</sup> Neurodegeneration is often accompanied by the accumulation of microglia and monocytes around amyloid plaques and dying neurons.<sup>102</sup> Furthermore, neurons express some molecules normally attributed to the immune system, thus hinting at an intricate interplay between neuronal and immune systems.<sup>103</sup> (see Biology: Immunity)

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“Twin studies support the notion that epigenetic mechanisms modulate AD risk.” [Huang and Mucke 2012](#)

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**Cruchaga C., Karch C. M., Jin S. C., Benitez B. A., Cai Y., et al. (2014) Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease. *Nature* 505: 550-554**

Research into LOAD has identified several genetic risk variants, but generally with small effects. To identify low-frequency coding variants with large effects, this study used WES on Illumina HiSeq in 14 large LOAD families and case-control data sets. The authors found a rare variant in PLD3 segregating with disease status in two independent families and doubling the risk for AD in seven independent case-control series. The authors conducted follow-up functional assays to determine the effect of PLD3 and found that it influences APP processing.

Illumina Technology: GoldenGate, HiSeq 2000

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**De Jager P. L., Srivastava G., Lunnon K., Burgess J., Schalkwyk L. C., et al. (2014) Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. *Nat Neurosci* 17: 1156-1163**

DNA methylation is a genetic mechanism that may affect gene expression and, as such, may be implicated in disease susceptibility. The epigenomic influence on AD onset and progression was examined in this study using Illumina HumanMethylation450 arrays and bisulfite sequencing on Illumina HiSeq 2000. The authors found several replicated, functionally validated associations between altered DNA methylation and the pre-symptomatic accumulation of AD pathology. They hypothesized that the observed DNA methylation changes may be involved in the onset of AD.

Illumina Technology: HumanMethylation450, HiSeq 2000

**Lunnon K., Smith R., Hannon E., De Jager P. L., Srivastava G., et al. (2014) Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. *Nat Neurosci* 17: 1164-1170**

In this study of AD methylomic variation, the authors used Illumina HumanMethylation450k arrays to characterize the genome-wide DNA methylation state across multiple tissues. Based on the results from 122 donor samples, the authors compared the methylation state in four brain regions and whole blood where available. The authors identified evidence for cortex-specific hypermethylation at CpG sites in the ANK1 gene associated with AD neuropathology.

Illumina Technology: HumanMethylation450, Human Gene Expression BeadArray

**Raj T., Ryan K. J., Replogle J. M., Chibnik L. B., Rosenkrantz L., et al. (2014) CD33: increased inclusion of exon 2 implicates the Ig V-set domain in Alzheimer's disease susceptibility. *Hum Mol Genet* 23: 2729-2736**

The identification of possible therapeutic targets for disease requires a functional identification follow-up to identified genetic disease variants. In this study, a previously identified risk allele for AD was scrutinized in detail using expression data on population cohorts stratified by their genetic risk variant identified by Illumina Infinium arrays. The authors found that the risk allele rs3865444(C) results in a higher surface density of CD33 on monocytes. The risk allele is strongly associated with greater expression of CD33 exon 2, which is likely to be the functional consequence of the risk variant.

Illumina Technology: Human OmniExpress

**Bai B., Hales C. M., Chen P. C., Gozal Y., Dammer E. B., et al. (2013) U1 small nuclear ribonucleoprotein complex and RNA splicing alterations in Alzheimer's disease. *Proc Natl Acad Sci U S A* 110: 16562-16567**

Many neurodegenerative diseases are characterized by deposition of insoluble protein aggregates. The universal presence of  $\beta$ -amyloid and tau proteins in AD has facilitated advancement of the amyloid cascade and tau hypotheses that have dominated AD pathogenesis research and therapeutic development. This study investigated the human brain-insoluble proteome in AD by mass spectrometry and transcriptome sequencing. The authors identified 36 proteins that accumulate in the disease and found similarities with protein aggregates in mild cognitive impairment.

Illumina Technology: HiSeq 2000 (mRNA sequencing)

**Cruchaga C., Kauwe J. S., Harari O., Jin S. C., Cai Y., et al. (2013) GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer's disease. *Neuron* 78: 256-268**

The progress of AD can be monitored by the tau phosphorylated threonine 181 (ptau) in cerebrospinal fluid (CSF). To identify the genetic mechanism associated with elevated ptau, the authors performed the largest GWAS to date, enrolling 1,269 participants. The participants were genotyped using Illumina OmniExpress arrays and tau/ptau levels measured. The authors identified three genome-wide significant loci for CSF tau and ptau; one of these showed a strong association with AD risk in independent data sets.

Illumina Technology: Human610-Quad, HumanOmniExpress

**Lambert J. C., Ibrahim-Verbaas C. A., Harold D., Naj A. C., Sims R., et al. (2013) Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 45: 1452-1458**

AD is a progressive neurological disorder, primarily affecting the elderly. Previous analyses have identified eleven genomic loci associated with LOAD. To search for additional risk loci, the authors conducted a large GWAS meta-analysis using published datasets of Illumina iSelect genotype data from ~17,000 AD cases and ~37,000 controls. The analysis resulted in 19 significant associated loci, of which 11 loci have not been associated previously with LOAD.

Illumina Technology: iSelect

**Zhang B., Gaiteri C., Bodea L. G., Wang Z., McElwee J., et al. (2013) Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. *Cell* 153: 707-720**

Despite decades of intensive research, the causal chain of mechanisms behind LOAD remains elusive. This study characterized the molecular systems associated with LOAD by RNA-Seq on Illumina HiSeq for both brain specimens and cell line samples. The authors built a rank-ordered molecular interaction network by LOAD pathology and identified an immune- and microglia-specific module that is dominated by genes involved in pathogen phagocytosis. The authors recommend the causal network structure as a useful predictor of response to gene perturbations and a framework to test models of disease mechanisms underlying LOAD.

Illumina Technology: HiSeq 2000 (mRNA sequencing), HT-12 Expression BeadChip, HumanHap 650Y

Sherva R., Tripodis Y., Bennett D. A., Chibnik L. B., Crane P. K., et al. (2014) Genome-wide association study of the rate of cognitive decline in Alzheimer's disease. *Alzheimers Dement* 10: 45-52

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## Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder after AD.<sup>104</sup> Approximately 1 million people in the U.S., and over 4 million people world-wide, develop this pathological condition. The prevalence of PD in industrialized countries is estimated at 1%–2% in people over 60 years of age, and 3%–5% in people over 85 years old. Only 1% of PD cases are familial; the rest are sporadic.<sup>105</sup> Typical symptoms include muscle rigidity, bradykinesia (slow movement), tremors, and postural instability.<sup>106</sup> As the disease progresses, memory loss can occur and the symptoms may become very similar to those of AD.<sup>107</sup> Persons with PD may develop various neuropsychiatric disorders, such as anxiety, apathy, depression, hallucinations, and delusions.<sup>108</sup>

The diagnosis of PD is based on symptoms, and no molecular tests are used at this time.<sup>109</sup> A majority of neurons exhibit degenerative dysfunction or are lost before the onset of visible symptoms of PD,<sup>110</sup> so early detection could improve the prognosis substantially. Recently, a few groups have reported on the development of minimally invasive diagnostic systems for detection of AD<sup>111,112,113</sup> and PD<sup>114</sup> in whole peripheral blood, plasmacytoid bone marrow-derived cells (PBMCs), or CSF. Examples of the target molecules for diagnostics include eukaryotic initiation factor 2 (EIF2),<sup>115</sup> epidermal growth factor (EGF),<sup>116,117</sup> and amyloid  $\beta$ 1-42.<sup>118,119</sup>

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The biological causes of PD include progressive loss of substantia nigra dopaminergic neurons and striatal projections.<sup>120</sup> The major pathological indicator of PD is the accumulation of Lewy bodies, which are predominantly formed by self-assembling small protein  $\alpha$ -synuclein expressed in multiple brain segments.<sup>121</sup> Similar to plaques in AD, Lewy bodies spread to other compartments of brain (e.g., limbic and neocortical areas) as the disease progresses and cause neuronal death.<sup>122</sup>

Age is a major risk factor for development of PD<sup>123</sup>. Over 80% of PD patients will eventually develop dementia, a condition termed Parkinson's disease dementia (PDD). It is believed that the major cause of dementia of this kind is the spread of fibrillar  $\alpha$ -synuclein from the brain stem to limbic and neocortical structures.<sup>124</sup> Additionally, over 50% of PDD patients develop amyloid- $\beta$  plaques and neurofibrillary tangles, typical for AD.<sup>125</sup> Dual pathology of PDD and AD increases malignancy of the disease and significantly worsens prognosis.

Mutations in six genes listed in Table 3 have been associated with PD.<sup>126,127</sup>

Table 3: Gene mutations associated with PD

Gene Name	Protein Name	Functional Role
<b>SNCA</b>	$\alpha$ -synuclein	Essential for normal brain activity: involved in learning, development, cellular differentiation, neuronal plasticity, and regulation of dopamine uptake. <sup>128,129,130</sup> Risk factor both in familial and sporadic PD. <sup>131,132</sup>
<b>PARK2</b>	PARK2 (Parkin)	Core component of a complex to ubiquitinate cellular proteins for degradation. <sup>133</sup>
<b>PINK1</b>	PTEN-induced kinase protein 1	Mitochondrially targeted kinase. Protects cells from stress-induced mitochondrial dysfunction, oxidative stress, and apoptosis. <sup>134</sup>
<b>UCHL1</b>	Ubiquitin carboxyl-terminal hydrolase isozyme L1	Neuronal-specific ubiquitin C-terminal hydrolase. Recycles ubiquitin chains back to monomeric ubiquitin and adds ubiquitin to monoubiquitylated $\alpha$ -synuclein. <sup>135</sup>
<b>DJ1 (PARK7)</b>	PARK7	Modulates $\alpha$ -synuclein aggregation. <sup>136</sup>
<b>LRRK2</b>	Leucine-rich repeat serine/threonine protein kinase 2	LRRK2 may deregulate phosphorylation of $\alpha$ -synuclein, leading to initiation of PD pathogenesis. <sup>137</sup>

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The familial type of PD can be inherited in an autosomal dominant or autosomal recessive manner. The former is associated with  $\alpha$ -synuclein and LRRK2 and the latter with Parkin, PINK1, DJ1, and ATP13A2. Improved understanding of the role of these genes in PD will facilitate the development of genomic analysis as an early diagnostic tool for familial PD.<sup>138</sup>

Currently, the major approach to controlling clinical symptoms of patients with PD is pharmacological replacement of dopamine with L-DOPA, carbidopa, and monoamine oxidase-B inhibitors.<sup>139</sup> However, the effect of such treatment is transient, and patients develop resistance to these therapies, leaving no further options for treatment. Genomic analysis of PD is anticipated to improve treatment of PD. One of the new therapeutic approaches made possible by NGS is based on the use of mirtrons: miRNA relying on splicing, rather on Dicer and RNA-induced silencing complex (RISC) to generate precursors for targeting disease-specific mRNA via an RNA interference pathway.<sup>140</sup> With this approach, researchers attained up to 85% silencing of  $\alpha$ -synuclein and LRRK2.

Methylation of  $\alpha$ -synuclein was reduced in DNA from substantia nigra, cortex, and putamen of patients with sporadic PD.<sup>141</sup> Six risk loci have been associated with proximal gene expression or DNA methylation<sup>142</sup> (see Epigenetic Modifications).

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**Bras J., Guerreiro R., Darwent L., Parkkinen L., Ansorge O., et al. (2014) Genetic analysis implicates APOE, SNCA and suggests lysosomal dysfunction in the etiology of dementia with Lewy bodies. *Hum Mol Genet***

Clinical and neuropathological similarities between dementia with Lewy bodies (DLB), PD, and AD, suggest that these disorders may share the same etiology. To test this hypothesis, this study used Illumina NeuroX custom arrays to test for association of 54 genomic regions, previously implicated in PD or AD in a large cohort of DLB cases and controls. The authors identified APOE as a strong genetic risk factor for DLB, and several other potential risk loci. Overall, the results suggest the etiology of DLB is influenced by some of the same genetic risk factors as AD and PD, but that these loci may act in different manners.

Illumina Technology: Infinium HumanExome BeadChip

**Hollerhage M., Goebel J. N., de Andrade A., Hildebrandt T., Dolga A., et al. (2014) Trifluoperazine rescues human dopaminergic cells from wild-type alpha-synuclein-induced toxicity. *Neurobiol Aging* 35: 1700-1711**

PD is the most frequent neurodegenerative movement disorder. To study  $\alpha$ -synuclein-mediated toxicity in PD progression, the authors developed a new cell-line model in which moderate overexpression of wild-type  $\alpha$ -synuclein led to gradual death of human post-mitotic DA neurons. Using Illumina BeadArrays to monitor gene expression, the authors discovered that activating autophagy in human DA mid-brain neurons rescued them from  $\alpha$ -synuclein-induced cell death. The phenothiazine neuroleptic trifluoperazine, an activator of macroautophagy, may be a potential therapeutic target.

Illumina Technology: Human Gene Expression BeadArray

**Mencacci N. E., Isaia I. U., Reich M. M., Ganos C., Plagnol V., et al. (2014) Parkinson's disease in GTP cyclohydrolase 1 mutation carriers. *Brain* 137: 2480-2492**

GTP cyclohydrolase 1 (encoded by the GCH1 gene) is potentially associated with increased risk of PD. The frequency of GCH1 variants was evaluated in WES data of 1,318 cases with PD and 5,935 control subjects. Through their analysis, the authors identified 11 different heterozygous variants in GCH1. These include four previously reported pathogenic variants and seven variants of unknown clinical relevance. The frequency of GCH1 variants was significantly higher than in controls, indicating the clinical relevance of GTP cyclohydrolase 1 deficiency.

Illumina Technology: HiSeq 2000

**Nalls M. A., Pankratz N., Lill C. M., Do C. B., Hernandez D. G., et al. (2014) Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet* 46: 989-993**

Only a small fraction of genetic heritability has been discovered for PD to date. This study performed a meta-analysis of GWAS for PD in the search for new loci associated with the disease. Using Illumina genotyping arrays, the authors identified 24 loci that were both statistically significant and replicated across experiments. Six of the identified loci had not been previously reported associated with PD, and the authors estimated the cumulative risk of the loci to be substantial (odds ratio = 3.31).

Illumina Technology: ExomeChip, HumanOmniExpress, HumanHap550, Human610-Quad, Human660W-Quad; HumanMethylation27, Human Gene Expression BeadArray

**Chung S. J., Armasu S. M., Anderson K. J., Biernacka J. M., Lesnick T. G., et al. (2013) Genetic susceptibility loci, environmental exposures, and Parkinson's disease: a case-control study of gene-environment interactions. *Parkinsonism Relat Disord* 19: 595-599**

PD susceptibility is suspected to be a combination of genetic and environmental factors. This study examined the potential interactions between known genetic risk factors and environmental exposures (pesticide application, tobacco smoking, coffee drinking, and alcohol drinking). Using Illumina GoldenGate Genotyping arrays, the authors genotyped 1,098 PD cases and 1,098 controls, and performed an interaction analysis. They found limited evidence for pairwise interactions between the examined genotypes and environmental risk factors; however, larger sample sizes will be needed to confirm any effect on PD susceptibility.

Illumina Technology: BeadArray Reader, DNA Test Panel

Karlsson M. K., Sharma P., Aasly J., Toft M., Skogar O., et al. (2013) Found in transcription: accurate Parkinson's disease classification in peripheral blood. *J Parkinsons Dis* 3: 19-29

The prevalence of PD increases with age. In an effort to develop a blood-based test for early detection of PD, the authors performed a large-scale gene expression study using Illumina Gene Expression BeadArrays on a cohort of PD patients and controls to develop a prediction model and test its accuracy. Through cross-validation results, they showed that PD can be correctly classified from healthy controls with an accuracy of 88% compared to clinical diagnosis. These results suggest the potential for developing a blood-based gene expression test for PD.

Illumina Technology: Human Gene Expression BeadArray

Ryan S. D., Dolatabadi N., Chan S. F., Zhang X., Akhtar M. W., et al. (2013) Isogenic human iPSC Parkinson's model shows nitrosative stress-induced dysfunction in MEF2-PGC1 $\alpha$  transcription. *Cell* 155: 1351-1364

An association has been reported between PD and exposure to mitochondrial toxins. In this study, a stem cell model was used to characterize the response to toxins using Illumina BeadArray for gene expression analysis. The authors identified a pathway whereby basal and toxin-induced nitrosative/oxidative stress results in S-nitrosylation of transcription factor MEF2C. They reported the alteration contributing to mitochondrial dysfunction and apoptotic cell death, indicating a mechanism and potential therapeutic target for PD.

Illumina Technology: Human Gene Expression BeadArray

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Sibley C. R., Seow Y., Curtis H., Weinberg M. S. and Wood M. J. (2012) Silencing of Parkinson's disease-associated genes with artificial mirtron mimics of miR-1224. *Nucleic Acids Res* 40: 9863-9875

# GENETIC MECHANISMS

Schizophrenia, autism, PD, and AD are complex diseases driven by an intricate interplay between multiple genetic and environmental factors. The etiology of a complex disease is the sum of all these factors, including somatic (non-inherited) mutations, inherited mutations, epigenetic modifications, small RNAs, immunity, and many others. NGS provides the tools to measure most of these contributing factors. The future challenge will be to combine these measurements into a coherent view of these complex diseases.<sup>143</sup>

## Copy-Number Variants

CNVs are one of the most common mutations in psychiatric diseases. Some notable successes have been achieved with array-based approaches, particularly with mapping CNVs.<sup>144</sup> However, arrays cannot detect balanced translocations and fluorescence in situ hybridization (FISH) techniques have limited resolution. The true extent of balanced translocations in both healthy and diseased genomes was only discovered with the advent of NGS. Paired-end and mate-pair sequencing are particularly effective in mapping genomic rearrangements.<sup>145</sup> Two groups have estimated that the number of CNVs relevant to ASD range from 130 to 300 target loci.<sup>146,147</sup> CNVs also play a critical role in onset of schizophrenia and bipolar disorder.<sup>148-151</sup> A recent study of single cells and neurons in brain tissue found that most ( $\geq 95\%$ ) neurons in normal brain tissue are euploid. However, a patient with hemimegalencephaly (HMG) due to a somatic CNV of chromosome 1q had unexpected tetrasomy 1q in 20% of neurons. This observation suggests that different cells in the brain may have different mutations, and that CNVs in a minority of cells can cause widespread brain dysfunction.<sup>152</sup> This increased complexity can only be resolved with single-cell sequencing approaches (see Biology: Single Cells).

The majority of *de novo* structural variations are attributed to new transposon insertions. Exome sequencing studies have shown that an increased level of ASD-causing single-nucleotide variants (SNVs) correlates positively with paternal age.<sup>153</sup> CNVs can also contribute to disease, in combination with other CNVs or other point mutations at different loci, but identifying these interactions is challenging.<sup>154</sup> To account for these multiple impacts, several groups have devised a “two-hit” hypothesis, analogous to that for cancer.<sup>155,156</sup>

“Recent advances in technology have allowed the interrogation of very large numbers of markers dispersed throughout the genome in a highly rapid and inexpensive manner.” Bras et al. 2012

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Scandinavian families have been used extensively as subjects for autism and schizophrenia GWAS. This selection is due to the ethnic uniformity of Scandinavian peoples, and the well-developed registry system of newborn blood samples (such as Danish Newborn Screening Biobank) and health records established in those countries.<sup>157</sup>

## Reviews

Cai X., Evrony G. D., Lehmann H. S., Elhosary P. C., Mehta B. K., et al. (2014) Single-Cell, Genome-wide Sequencing Identifies Clonal Somatic Copy-Number Variation in the Human Brain. *Cell Rep* 8: 1280-1289

Gilissen C., Hehir-Kwa J. Y., Thung D. T., van de Vorst M., van Bon B. W., et al. (2014) Genome sequencing identifies major causes of severe intellectual disability. *Nature* 511: 344-347

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Rosti R. O., Sadek A. A., Vaux K. K. and Gleeson J. G. (2014) The genetic landscape of autism spectrum disorders. *Dev Med Child Neurol* 56: 12-18

Malhotra D. and Sebat J. (2012) CNVs: harbingers of a rare variant revolution in psychiatric genetics. *Cell* 148: 1223-1241

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[Fromer M., Pocklington A. J., Kavanagh D. H., Williams H. J., Dwyer S., et al. \(2014\) De novo mutations in schizophrenia implicate synaptic networks. \*Nature\* 506: 179-184](#)

Of the known risk alleles for schizophrenia, the only ones definitively shown to confer considerable increments in risk are rare chromosomal CNVs that involve deletion or duplication of thousands of bases of DNA. This study examined the effect of small *de novo* mutations affecting one or a few nucleotides. By Illumina HiSeq WES of 623 schizophrenia trios, the authors assessed *de novo* mutation rates and shared genetic etiology for schizophrenia, intellectual disability, and ASDs. They found several insights to suggest a common etiological mechanism.

Illumina Technology: HiSeq (exome sequencing)

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**Gaugler T., Klei L., Sanders S. J., Bodea C. A., Goldberg A. P., et al. (2014) Most genetic risk for autism resides with common variation. *Nat Genet* 46: 881-885**

Although ASDs have been studied widely, the proportion and nature of genetic heritability is uncertain. This study analyzed the largest autism cohort data to date, including 1.6 million Swedish families with at least two children and ~5,700 individuals with strict autism diagnoses. Using Illumina SNP arrays, the authors investigated the contribution of rare versus common genetic variants to the disease. They conclude that the heritability is ~52.4%, with common variation as the biggest contributor. Rare, *de novo* mutations contribute substantially to individual liability, yet their contribution to variance in liability is modest at 2.6%.

Illumina Technology: OmniExpress

**Karayannis T., Au E., Patel J. C., Kruglikov I., Markx S., et al. (2014) *Cntnap4* differentially contributes to GABAergic and dopaminergic synaptic transmission. *Nature* 511: 236-240**

In an effort to understand the pathological development of neurological disorders, genetic effects have been studied in the context of proteins that are expressed in neural cells. In this study, the authors characterized the effect of CNTNAP4 knockouts on mouse behavior and development, and relate these results to the findings of CNVs in humans across a region including the CNTNAP2 gene. The authors found that CNTNAP4 is localized presynaptically, and its loss leads to a reduction in the output of cortical parvalbumin (PV)-positive GABAergic basket cells. In addition, CNTNAP4-mutant mice showed defects in these neuronal populations and exhibited sensory-motor gating and grooming endophenotypes.

Illumina Technology: HumanHap550, HumanOmni1-Quad

**Stefansson H., Meyer-Lindenberg A., Steinberg S., Magnusdottir B., Morgen K., et al. (2014) CNVs conferring risk of autism or schizophrenia affect cognition in controls. *Nature* 505: 361-366**

Certain CNVs contribute to the pathogenesis of schizophrenia and autism. In this study, the authors investigated the influence of these CNVs on phenotypes separate from those of the mentioned diseases. In a big population-wide study of nearly a third of the Icelandic population ( $n = 101,655$ ), the authors used Illumina SNP microarrays to test for associations of CNVs with cognitive deficits, dyslexia, dyscalculia, and brain structure changes. The authors found that the 15q11.2(BP1-BP2) deletion affects brain structure in a pattern consistent with first-episode psychosis in both schizophrenia and dyslexia.

Illumina Technology: HumanHap300, HumanCNV370-Duo, HumanHap650Y, Human1M, HumanOmni2.5, HumanOmniExpress, HumanOmni1S

**Chapman J., Rees E., Harold D., Ivanov D., Gerrish A., et al. (2013) A genome-wide study shows a limited contribution of rare copy number variants to Alzheimer's disease risk. *Hum Mol Genet* 22: 816-824**

AD is the most common form of dementia, and although genetically complex, it is also highly heritable. This study examined the prevalence of CNVs using Illumina Infinium arrays to determine whether rare CNVs play a role in AD susceptibility. Although the authors examined loci that had been highlighted in previous AD studies, they did not find a significant contribution of CNVs to the development of AD.

Illumina Technology: Human610-Quad

Lionel A. C., Tammimies K., Vaags A. K., Rosenfeld J. A., Ahn J. W., et al. (2014) Disruption of the ASTN2/TRIM32 locus at 9q33.1 is a risk factor in males for autism spectrum disorders, ADHD and other neurodevelopmental phenotypes. *Hum Mol Genet* 23: 2752-2768

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## Alternative Splicing

It has been established that up to 94% of multi-exon genes are alternatively spliced, and that incorrect alternative splicing can lead to at least 15% of disease cases in humans.<sup>158,159</sup> Alternative splicing is a mechanism by which exons of pre-mRNA can be grouped (spliced) into different arrangements to produce mature mRNA that codes structurally and functionally distinct protein variants. The advent of high-throughput genomic analysis tools, such as exon arrays and RNA-Seq, has allowed the identification of alternative splicing events which were undetectable previously by conventional microarrays.

Exon arrays can distinguish between different isoforms.<sup>160</sup> This technology also has some intrinsic limitations, such as the ability to detect only known splice variants of previously sequenced genomes, low signal-to-noise ratio, limited dynamic range, and cross-hybridization. The full power of this technology can be realized when combined with whole mRNA sequencing, which allows the identification of the exon and transcript boundaries at single-base resolution and detection of novel transcripts. In this approach, the mRNA is first converted into cDNA, which is further ligated to unique adapters and sequenced in a massively parallel fashion.<sup>161</sup>

Most genes commonly associated with AD have multiple splice variants, and some of those variants are pathogenic.<sup>162,163,164</sup> In PD, alternative splicing was detected for PARK2, SNCA, and SRRM2 genes. Although all three splice variants of PARK2 are believed to be non-pathogenic, it was hypothesized that the variable ratio of these three transcripts may determine disease susceptibility.<sup>165</sup> Finally, there is some evidence that unspliced mRNA corresponding to AD susceptibility genes can accumulate in brains of AD patients as a result of mutations in U1 small nuclear ribonucleoprotein (U1 snRNP), a component of the spliceosome complex. Multiple U1 snRNP subunits form cytoplasmic tangled agglomerations in AD.<sup>166</sup>

RNA samples from human brains are usually acquired post-mortem, which raises the problem of poor RNA quality for genetic analysis. However, correlative studies of RNA and protein expression in brain and in peripheral organs and tissues, such as blood, may provide the means for early, non-invasive diagnosis of neurodegenerative diseases, similar to the approach once established for the diagnosis of prostate cancer.<sup>167-170</sup>

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## Epigenetic Modifications

The extensive role of epigenetic changes in the onset and progression of psychiatric diseases has recently become apparent due to the development of microarray- and NGS-based protocols for detecting genome-wide epigenetic patterns. This role appeared to be especially strong in neurodegenerative diseases; however, some recently discovered epigenetic patterns in psychiatric disorders may be critical for better understanding the causes and patterns of these diseases. One of the complications of studying the epigenetics in neurological diseases is that the signature can only be detected post-mortem, and stability of samples and these modifications is often compromised.<sup>171</sup>

Genomic imprinting is an example of an epigenetic modification that occurs throughout life.<sup>172</sup> SHANK3, the first gene associated with ASD, has five CpG islands across the gene that display brain- and cell-type-specific DNA methylation patterns.<sup>173,174,175</sup> Similar specificity was observed for histone acetylation in this gene.<sup>176</sup> These modifications regulate the expression of the SHANK3 gene in an isoform-specific manner.<sup>177</sup> Several groups have independently identified other multiple differentially methylated regions (DMRs) in post-mortem samples of autistic patients representing biologically diverse gene regions, such as DNase-hypersensitive sites and an alternative transcript termination site.<sup>178,179,180</sup> These studies provide an additional level of evidence to unravel the mechanisms of complex diseases, such as ASD.

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Aging and AD—in particular, LOAD—are also associated with a spectrum of epigenetic changes, including abnormal DNA methylation and histone modifications.<sup>181,182</sup> These changes can be precipitated by physiological and environmental conditions, such as stroke,<sup>183</sup> hypertension, type II diabetes, obesity,<sup>184</sup> exposure to heavy metals,<sup>185,186</sup> and head injury.<sup>187</sup> Oxidative stress, for example, can cause an imbalance between methylation and demethylation of DNA in AD brains.<sup>188</sup> Changes in histone tail modifications (primarily decreased levels of H3 acetylation in the temporal lobe, and increased levels of histone deacetylases HDAC2 and HDAC6) have also been observed in post-mortem brains of AD patients.<sup>189,190,191</sup> Targeting these histone modifications by therapeutic agents is a potential strategy for the treatment of AD.<sup>192</sup> Experiments in mouse have shown that pharmacological inhibition of DNA methylation in the hippocampus of mice after a learning task impaired memory consolidation,<sup>193</sup> and promotion of histone acetylation had an opposite effect: it increased learning and memory through increased learning-related gene expression in aged mice.<sup>194,195</sup>

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Epigenetic modifications are also critical for the development of PD. Reduced methylation levels of the SNCA gene that encodes  $\alpha$ -synuclein protein were detected in the substantia nigra, cortex, and putamen regions of brain in patients with sporadic PD.<sup>196,197</sup> The protein  $\alpha$ -synuclein binds directly to histone H3 and inhibits histone acetylation.<sup>198</sup>

## Reviews

Coppede F. (2014) The potential of epigenetic therapies in neurodegenerative diseases. *Front Genet* 5: 220

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**De Jager P. L., Srivastava G., Lunnon K., Burgess J., Schalkwyk L. C., et al. (2014) Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. *Nat Neurosci* 17: 1156-1163**

DNA methylation is a genetic mechanism that may affect gene expression and, as such, may be implicated in disease susceptibility. The epigenomic influence on AD onset and progression was examined in this study using Illumina HumanMethylation450 arrays and bisulfite sequencing on Illumina HiSeq 2000. The authors found several replicated, functionally validated associations between altered DNA methylation and the pre-symptomatic accumulation of AD pathology. They hypothesized that the observed DNA methylation changes may be involved in the onset of AD.

Illumina Technology: HumanMethylation450, HiSeq 2000

**Hollerhage M., Goebel J. N., de Andrade A., Hildebrandt T., Dolga A., et al. (2014) Trifluoperazine rescues human dopaminergic cells from wild-type alpha-synuclein-induced toxicity. *Neurobiol Aging* 35: 1700-1711**

PD is the most frequent neurodegenerative movement disorder. To study  $\alpha$ -synuclein-mediated toxicity in PD progression, the authors developed a new cell-line model in which moderate overexpression of wild-type  $\alpha$ -synuclein led to gradual death of human post-mitotic dopaminergic neurons. Using Illumina BeadArrays to monitor gene expression, the authors discovered that activating autophagy in human dopaminergic mid-brain neurons rescued them from  $\alpha$ -synuclein-induced cell death. The phenothiazine neuroleptic trifluoperazine, an activator of macroautophagy, may be a potential therapeutic target.

Illumina Technology: Human Gene Expression BeadArray

**Ladd-Acosta C., Hansen K. D., Briem E., Fallin M. D., Kaufmann W. E., et al. (2014) Common DNA methylation alterations in multiple brain regions in autism. *Mol Psychiatry* 19: 862-871**

Genetic as well as environmental factors are responsible for ASDs. In this study, the authors measured over 485,000 CpG loci in human brain tissue and identified 4 genome-wide significantly different DMRs from 19 autism cases. This study highlights a new selected set of affected genes.

Illumina Technology: HumanMethylation450

**Lunnon K., Smith R., Hannon E., De Jager P. L., Srivastava G., et al. (2014) Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. *Nat Neurosci* 17: 1164-1170**

In this study of AD methylomic variation, the authors used Illumina HumanMethylation450k arrays to characterize the genome-wide DNA methylation state across multiple tissues. Based on the results from 122 donor samples, the authors compared the methylation state in four brain regions and whole blood where available. The authors identified evidence for cortex-specific hypermethylation at CpG sites in the ANK1 gene associated with AD neuropathology.

Illumina Technology: HumanMethylation450k, Human Gene Expression BeadArray

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Nalls M. A., Pankratz N., Lill C. M., Do C. B., Hernandez D. G., et al. (2014) Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease.

**Nat Genet 46: 989-993**

Only a small fraction of genetic heritability has been discovered for PD to date. This study performed a meta-analysis of GWAS for PD in the search for new loci associated with the disease. Using Illumina genotyping arrays, the authors identified 24 loci that were both statistically significant and replicated across experiments. Six of the identified loci had not been previously reported associated with PD, and the authors estimated the cumulative risk of the loci to be substantial (odds ratio = 3.31).

Illumina Technology: ExomeChip, HumanOmniExpress, HumanHap550, Human610-Quad, Human660W-Quad; HumanMethylation27, Human Gene Expression BeadArray

Wong C. C., Meaburn E. L., Ronald A., Price T. S., Jeffries A. R., et al. (2014) Methyloomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. *Mol Psychiatry* 19: 495-503

## Small RNAs

MicroRNA (miRNA) is enriched in the brain, and neuronal-specific miRNA controls neuronal differentiation, excitability, and function.<sup>199</sup> Other RNAs, such as non-coding RNA (ncRNA), appear to play a role in neurodevelopment.

A number of miRNAs are associated with AD and PD, and were detected not only in brains, but also in peripheral tissues of affected individuals.<sup>200,201</sup> This observation suggests that minimally invasive diagnostic tools could be developed for early prediction of neurodegenerative disorders. miRNAs have also been considered as therapeutic agents against AD pathogens, such as APP<sup>202</sup>. miRNA molecules can be conjugated to—or otherwise associated with—aptamers, monoclonal antibodies, peptides, or exosomes for delivery *in vivo* to specific cell types and tissues.<sup>203</sup>

### Reviews

Sekiyama K., Takamatsu Y., Waragai M. and Hashimoto M. (2014) Role of genomics in translational research for Parkinson's disease. *Biochem Biophys Res Commun* 452: 226-235

Maciotta S., Meregalli M. and Torrente Y. (2013) The involvement of microRNAs in neurodegenerative diseases. *Front Cell Neurosci* 7: 265

Mills J. D. and Janitz M. (2012) Alternative splicing of mRNA in the molecular pathology of neurodegenerative diseases. *Neurobiol Aging* 33: 1012 e1011-1024

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Raj T., Ryan K. J., Replogle J. M., Chibnik L. B., Rosenkrantz L., et al. (2014) CD33: increased inclusion of exon 2 implicates the Ig V-set domain in Alzheimer's disease susceptibility. *Hum Mol Genet* 23: 2729-2736

The identification of possible therapeutic targets requires a functional identification follow-up to known genetic disease variants. In this study, a previously identified risk allele for AD was examined in detail. The authors used expression data in population cohorts stratified by their genetic risk variant, identified by Illumina Infinium arrays. They found that the risk allele rs3865444(C) results in a higher surface density of CD33 on monocytes. The risk allele is strongly associated with greater expression of CD33 exon 2, which is likely to be the functional consequence of the risk variant.

Illumina Technology: OmniExpress

Bai B., Hales C. M., Chen P. C., Gozal Y., Dammer E. B., et al. (2013) U1 small nuclear ribonucleoprotein complex and RNA splicing alterations in Alzheimer's disease. *Proc Natl Acad Sci U S A* 110: 16562-16567

Many neurodegenerative diseases are characterized by deposition of insoluble protein aggregates. The universal presence of  $\beta$ -amyloid and tau proteins in AD has facilitated advancement of the amyloid cascade and tau hypotheses that have dominated AD pathogenesis research and therapeutic development. This study investigated the human brain-insoluble proteome in AD by mass spectrometry and transcriptome sequencing. The authors identified 36 proteins that accumulate in the disease and found similarities with protein aggregates in mild cognitive impairment.

Illumina Technology: HiSeq 2000 (mRNA sequencing)

Li M. M., Jiang T., Sun Z., Zhang Q., Tan C. C., et al. (2014) Genome-wide microRNA expression profiles in hippocampus of rats with chronic temporal lobe epilepsy. *Sci Rep* 4: 4734

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# Genetic Variants

## Genome Wide Association Studies

Genome-wide association studies (GWAS) are conducted to identify common disease susceptibility alleles by comparing allele frequencies across the genome in large groups of cases and controls.<sup>204,205</sup> This approach has yielded an unprecedented amount of clinically relevant data, including the majority of mutations and variants associated with AD and PD.<sup>207-211</sup> However, in spite of over 9,000 GWAS published to date, this approach has uncovered only a fraction of the true heritability. Additionally, GWAS can miss epigenetic patterns, such as methylation, and it can mistakenly identify genes in the vicinity of pathogenic<sup>213</sup> SNVs as pathogenic. The sequencing of entire genomes in large cohorts at affordable prices is likely to generate additional genes, pathways, and biological insights, as well as the potential to identify causal mutations.<sup>214</sup> NGS, both alone or in combination with microarrays, can address most of these limitations and significantly improve the results from these studies.<sup>215</sup>

## Reviews

Sharma M., Kruger R. and Gasser T. (2014) From genome-wide association studies to next-generation sequencing: lessons from the past and planning for the future. *JAMA Neurol* 71: 5-6

Keogh M. J. and Chinnery P. F. (2013) Next generation sequencing for neurological diseases: new hope or new hype? *Clin Neurol Neurosurg* 115: 948-953e>

Koboldt D. C., Steinberg K. M., Larson D. E., Wilson R. K. and Mardis E. R. (2013) The next-generation sequencing revolution and its impact on genomics. *Cell* 155: 27-38

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[Karayannis T., Au E., Patel J. C., Patel J. C., Kruglikov I., Marx S., et al. \(2014\) Cntnap4 differentially contributes to GABAergic and dopaminergic synaptic transmission. \*Nature\* 511: 236-240](#)

In an effort to understand the pathological development of neurological disorders, genetic effects have been studied in the context of proteins that are expressed in neural cells. In this study, the authors characterized the effect of CNTNAP4 knockouts on mouse behavior and development, and relate these results to the findings of CNVs in humans across a region including the CNTNAP2 gene. The authors found that Cntnap4 is localized presynaptically, and its loss leads to a reduction in the output of cortical parvalbumin (PV)-positive GABAergic basket cells. In addition, CNTNAP4-mutant mice showed defects in these neuronal populations and exhibited sensory-motor gating and grooming endophenotypes.

Illumina Technology: HumanHap550, HumanOmni1

[Nalls M. A., Pankratz N., Lill C. M., Do C. B., Hernandez D. G., et al. \(2014\) Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. \*Nat Genet\* 46: 989-993](#)

Only a small fraction of genetic heritability has been discovered for PD to date. This study performed a meta-analysis of GWAS for PD in the search for new loci associated with the disease. Using Illumina genotyping arrays, the authors identified 24 loci that were both statistically significant and replicated across experiments. Six of the identified loci had not been previously reported associated with PD, and the authors estimated the cumulative risk of the loci to be substantial (odds ratio = 3.31).

Illumina Technology: ExomeChip, HumanOmniExpress, HumanHap550, Human610-Quad, Human660W-Quad; HumanMethylation27, Human Gene Expression BeadArray

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Cruchaga C., Kauwe J. S., Harari O., Jin S. C., Cai Y., et al. (2013) GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer's disease. *Neuron* 78: 256-268

The progress of AD can be monitored by the tau phosphorylated threonine 181 (ptau) in CSF. To identify the genetic mechanism associated with elevated ptau, the authors performed the largest GWAS to date, enrolling 1,269 participants. The participants were genotyped using Illumina OmniExpress arrays and tau/ptau levels measured. The authors identified three genome-wide significant loci for CSF tau and ptau; one of these showed a strong association with AD risk in independent data sets.

Illumina Technology: Human610-Quad, HumanOmniExpress

## Next-Generation Sequencing

Next-generation sequencing for the analysis of eukaryotic DNA genome consists of two modalities: WGS and WES.<sup>216</sup> A combination of WES with custom-designed microarrays is the method of choice for large sample sizes.<sup>217</sup> This combinatory approach allows the effective resolution of such common genetic analysis problems as pseudogenes, repeated exons, and a failure to detect rare and/or novel mutations.<sup>218</sup> Additionally, WES per se is not very adequate currently for addressing CNVs, because sample preparation relies on non-quantitative PCR amplification.<sup>219</sup> However, an approach that combines WES with genotyping resolves this complication.<sup>220</sup>

A multi-pronged approach—supplementing WES with WGS, proteomics, and epigenomics—is anticipated to deliver full understanding of the effects of newly discovered genetic variability. Rare mutations often have significantly stronger effects on disease pathology than common mutations, and that effect has been especially remarkable in the field of complex diseases. The power of NGS was further proved in the discovery of *de novo* mutations, which arise in individuals during their life and are more likely to have functional roles in rare diseases. Although the appearance of such mutations seems stochastic, the mutation rate and its dependence on parental age<sup>222</sup> and other environmental factors are only some of the important results of using this technology.<sup>223</sup>

Sequencing is becoming indispensable for diagnosing disease by analyzing circulating tumor DNA. Not only the sequence of that DNA, but also fluctuations in cell count, often correlate with the disease pathology and state. The “liquid biopsy approach” allows the detection of somatic mutations for a specific tumor type in plasma or liquid Papanicolaou (Pap) smears.<sup>224,225</sup> This approach is currently being developed to detect PD<sup>226</sup> and AD<sup>227,228</sup> in peripheral blood. The recent discovery that the whole fetal genome is present in maternal plasma during pregnancy<sup>230</sup> has launched the era of pre-natal non-invasive genetic diagnostics.

“Unlike GWAS which examines common mutations, sequencing facilitates the discovery of rare mutations which often associate with complex phenotypes.”

Koboldt et al. 2013

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[De Jager P. L., Srivastava G., Lunnon K., Burgess J., Schalkwyk L. C., et al. \(2014\) Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. \*Nat Neurosci\* 17: 1156-1163](#)

DNA methylation is a genetic mechanism that may affect gene expression and, as such, may be implicated in disease susceptibility. The epigenomic influence on AD onset and progression was examined in this study using Illumina HumanMethylation450 arrays and bisulfite sequencing on Illumina HiSeq 2000. The authors found several replicated, functionally validated associations between altered DNA methylation and the pre-symptomatic accumulation of AD pathology. They hypothesized that the observed DNA methylation changes may be involved in the onset of AD.

Illumina Technology: HumanMethylation450, HiSeq 2000

[Fromer M., Pocklington A. J., Kavanagh D. H., Williams H. J., Dwyer S., et al. \(2014\) De novo mutations in schizophrenia implicate synaptic networks. \*Nature\* 506: 179-184](#)

Of the known risk alleles for schizophrenia, the only ones definitively shown to confer considerable increments in risk are rare chromosomal CNVs that involve deletion or duplication of thousands of bases of DNA. This study examined the effect of small *de novo* mutations affecting one or a few nucleotides. By Illumina HiSeq WES of 623 schizophrenia trios, the authors assessed *de novo* mutation rates and shared genetic etiology for schizophrenia, intellectual disability, and ASDs. They found several insights to suggest a common etiological mechanism.

Illumina Technology: HiSeq (exome sequencing)

[Lee H., Lin M. C., Kornblum H. I., Papazian D. M. and Nelson S. F. \(2014\) Exome sequencing identifies \*de novo\* gain of function missense mutation in KCND2 in identical twins with autism and seizures that slows potassium channel inactivation. \*Hum Mol Genet\* 23: 3481-3489](#)

Numerous studies have reported comorbidity of autism and epilepsy, but the relationship between the two disorders is unknown. In this study, identical twins, affected by both autism and severe intractable seizures, were studied using exome sequencing. A novel variant in the KCND2 gene was observed in both twins. The *de novo* mutation is located in the protein coding the Kv4.2 potassium channel, and the authors expressed the mutant protein in *Xenopus* oocytes to observe functional effects. Expression analysis showed that the mutation dominantly impairs the closed-state inactivation of the potassium channel, strongly supporting KCND2 as the causal gene for epilepsy in this family.

Illumina Technology: HiSeq 2000, Illumina Paired-End Sequencing Library Prep Protocol

[King I. F., Yandava C. N., Mabb A. M., Hsiao J. S., Huang H. S., et al. \(2013\) Topoisomerases facilitate transcription of long genes linked to autism. \*Nature\* 501: 58-62](#)

Topoisomerases are expressed throughout the developing and adult brain, and are mutated in some individuals with ASD. However, the mechanism by which topoisomerases impact ASD is unknown. By transcriptome sequencing, in combination with genome-wide mapping of RNA polymerase II density in neurons, the authors found that expression of long genes was reduced after knockdown of topoisomerase in neurons. The authors noted that many high-confidence ASD candidate genes are exceptionally long and were reduced in expression after TOP1 inhibition. This observation suggests that topoisomerases could contribute commonly to ASD.

Illumina Technology: HiSeq 2000, TruSeq RNA, TruSeq for ChIP-Seq

**Willsey A. J., Sanders S. J., Li M., Dong S., Tebbenkamp A. T., et al. (2013) Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism. *Cell* 155: 997-1007**

Recent studies employing WES and WGS have identified nine high-confidence ASD genes. This study examined the contribution of these nine genes to the common phenotype by combining Illumina WES and RNA-Seq data into co-expression networks. The authors explain how these networks will guide future ASD research by indicating which genes are most likely to have overlapping molecular, cellular or circuit-level phenotypes.

Illumina Technology: HiSeq 2000, Genome Analyzer

**Yu T. W., Chahrour M. H., Coulter M. E., Jiralerspong S., Okamura-Ikeda K., et al. (2013) Using whole-exome sequencing to identify inherited causes of autism. *Neuron* 77: 259-273**

Steel syndrome is a developmental structural disorder first described in 1993 in 23 Hispanic children from Puerto Rico. This paper presents the genomic analysis of a family with two affected siblings. The authors used whole-exome sequencing using the Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC) core design followed by sequencing of both affected siblings, parents, and an affected cousin and her unaffected parents. By filtering the detected genetic variants by segregation, the authors discovered a single homozygous missense variant segregating with the disorder. The variant disrupts the collagen gene COL27A1, which codes for a protein expressed in developing cartilage.

Illumina Technology: HiSeq 2000, HumanOmniExpress

# MODEL SYSTEMS

Modeling of neurological diseases has been challenging for two primary reasons: extremely limited access to the primary brain tissue of individuals affected by these diseases, and polygenicity of these disorders. Traditional knockout models reiterate only a fraction of the disease phenotype, leaving room for speculation about the relevance of research results to real human diseases. The introduction of triple-knockout mice, transgenic rats, and stem cells as in vitro models has significantly broadened the arsenal of tools available to researchers in the field and moved closer to the “hypothetical” ideal disease model.<sup>231</sup> The development of adequate model systems is essential for developing accurate diagnostic and therapeutic strategies.

## Animal Models

The development of animal models for schizophrenia and ASD has been challenging, because over 90% of these diseases are polygenic. Therefore, a standard single-knockout mouse model can only partially mimic the disease phenotype. Introduced mutations are only tangentially relevant to the disease, and symptoms observed in animals may represent other diseases in this spectrum.<sup>232</sup> Another complication is associated with the recognition and quantification of the symptoms: animal behavioral patterns are different from those of a human; therefore, interpretation of behavioral changes, feelings, and intentions of animals may be highly subjective.

“Given the low penetrance of schizophrenia-associated alleles, and their ability to contribute to different diseases, inserting one or even several into an animal model may yield a phenotype that is ambiguous with respect to human disease — or no phenotype at all.” Hyman 2014

## Schizophrenia

Rodents have been the most commonly used models for studying schizophrenia and ASD. Until recently, these models were limited to mice, but now some rat knockouts have also become available.<sup>233</sup> Rats may be beneficial over mice, as they are highly social animals and possess a rich acoustic communication system (including frequencies in the ultrasonic range) and have a closer resemblance to human neural processes.<sup>234</sup> Additionally, pre-clinical toxicology studies are normally carried out in rats; therefore, use of these animals as a research model can significantly streamline the drug development process.<sup>235</sup>

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Several genetic mouse models of schizophrenia have been developed that use one of the three traditional methods: conventional gene targeting, conditional gene targeting, or point mutation by chemical mutagens.<sup>236</sup> However, these techniques allow for generation of phenotypes only scarcely similar to schizophrenia. For that reason, Cre/loxP-based chromosome engineering technique was used to generate models reflecting complex genomic rearrangements, such as large deletions, inversions, and duplications.<sup>237</sup>

One of the oldest models of schizophrenia is the dominant-negative disrupted in schizophrenia 1 (DISC1) gene. DISC1 mice are a good model for studying not only schizophrenia, but also a dual diagnosis of schizophrenia and substance-abuse disorder.<sup>238</sup> The effects of this mutation on brain structure or function remain to be studied.<sup>239</sup>

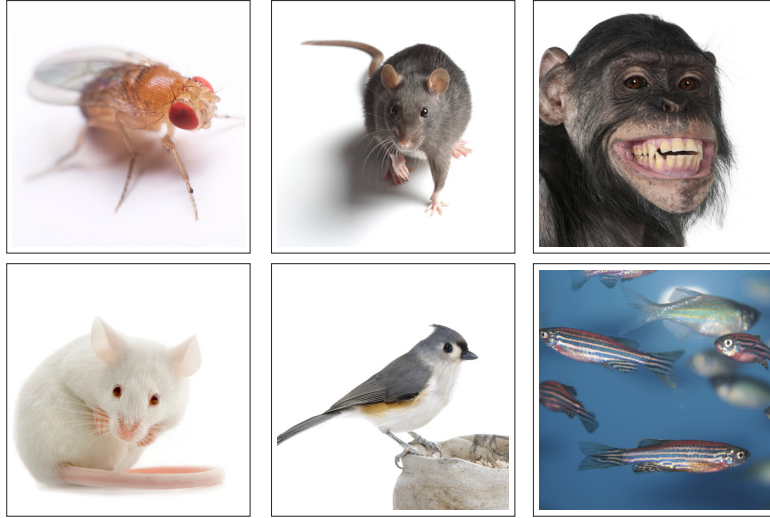
### Autism Spectrum Disorder

Mouse models have allowed for the demonstration of fundamental principles of ASD diseases. Mouse knockout models, with mutations in a range of genes, are making a significant contribution to understanding disease onset. These genes include SHANK3 (Phelan-McDermid syndrome, idiopathic ASD), MeCP2 (Rett syndrome), Fragile X chromosome (FMR1), PTEN (autism), and others.<sup>240,241</sup> SHANK3 is a good example of the importance of reproducing the exact type and point of mutation of the gene: some mutations in this gene are also associated with other diseases, including schizophrenia and intellectual disability.<sup>242,243</sup> Microduplications of SHANK3 were also associated with developmental delay and dysmorphic features in children.<sup>244</sup> This phenomenon underpins the need to use genomic analysis tools for accurate identification of mutations in spectrum disorders, and for the verification of their accurate reproduction in animal models.

Additional models of ASD include non-human primates, songbirds, zebrafish, *Drosophila*, and *C. elegans*. Non-human primates have been instrumental in studying the behavioral patterns in this disorder, as the anatomy of their neural circuits responsible for mediating social behavior is very similar to that of humans.<sup>245</sup> Like humans, they possess mirror neurons—cells responsible for repeating the actions of others, commonly damaged in autism. For ethical reasons, introduction of genetic mutations into primates is currently not feasible.

Songbirds have been used as a model due to their well-developed vocal machinery. As in humans, vocal learning is an important element of language in this species, and its impairment is commonly associated with ASD.<sup>246</sup> Finally, zebrafish, *Drosophila*, and *C. elegans* have been used extensively to study the genetic fundamentals of psychiatric diseases.<sup>247</sup>

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Animal models used in research of psychiatric and neurodegenerative diseases: mice, rats, non-human primates, *Drosophila*, songbirds, and zebrafish.

## Alzheimer's Disease

Animal models of AD are challenging to develop, as spontaneous amyloidosis is not common in laboratory animals. Aged non-human primates can develop  $\beta$ -amyloidosis and tau fibrillary inclusions; however, these animals do not develop the clinical signs of human AD.<sup>248</sup> Currently used animal models of AD are mostly limited to genetically engineered mice.<sup>249</sup> These models allowed for the successful mimicking of most human cerebral amyloidosis, including  $\beta$ -amyloidosis and tauopathies.<sup>250</sup> In all cases, overexpression of human amyloidogenic protein was required.<sup>251</sup>

A mouse model overexpressing  $\beta$ -amyloid protein has also been established. Although neurofibrillary tangles are not produced in brains of these mice, tau pathology is still observed, because  $\beta$ -amyloid pathology activates kinases, down-regulates phosphatases, and impairs tau degradation.<sup>252</sup> Mice expressing both mutated APP and tau demonstrate greater neurofibrillary tangle pathology than mutated tau mice, thus suggesting a role of  $\beta$ -amyloid accumulation in the development of tau pathology.<sup>253</sup>

Apart from the previously mentioned models, knockout models for genes involved in APP processing—such as presenilin-1, presenilin-2, and b-secretase enzyme (BACE1) are now available. Unfortunately, none of them accurately mimics all the key symptoms and molecular signatures of this disease. Specifically, increased neuronal death has been one of such symptoms, and it seems to be necessary for selecting and testing drugs against AD. A more advanced model was obtained by crossing APP-overexpressing mice with transgenic animals expressing mutated presenilin-1

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or presenelin-2, with the third transgene (mutated tau) added to the system. These mice featured accelerated  $\beta$ -amyloid pathology, formation of neurofibrillary tangles, neuronal loss and cognitive decline, and tau pathology.<sup>254</sup>

Although significant progress has been made in the development of mouse models of AD, the available models do not take into account genetic and epigenetic variability or the immunological factors common in LOAD. Recent advances in NGS technology are anticipated to intensify building of these models, which are essential to develop disease-modifying drugs for AD.<sup>255</sup>

### Parkinson's disease

The etiopathogenesis of PD is not yet clarified, and existing animal models have many limitations. Nevertheless, they allow for unraveling some fundamental mechanisms underlying the molecular and cellular basis of this neurodegenerative disorder. Most animal models of PD developed to date are toxic, rather than genetic, models. Toxic (also known as pharmacological) models—in particular, neurotoxin-based models—were most effective in reproducing dopaminergic neuron death and striatal dopamine deficit in non-human primates and rodents. The additional advantage of toxic models is the feasibility of their use in non-human primates, whose motor symptoms and neuronal structure are very similar to those of a human. The only limitation is the lack of formation of classic Lewy bodies in primate brains.

Genetic models of PD are very limited. The reason for this limited availability is the low contribution of the genetic component to this disorder: only 5% to 10% of all PD cases are inherited. The most common mutations in this form of PD are those in LRRK2 (which encodes an enzyme that may be involved in the deregulation of  $\alpha$ -synuclein phosphorylation),<sup>256</sup> PINK1 (PTEN-induced putative kinase 1), and Parkin (which participates in the ubiquitin proteasome system). Transgenic mice with knockouts in one of these genes exhibit only part of the PD phenotype, such as motility abnormalities, mitochondrial and nigrostriatal neurotransmission deficits, and others.<sup>257</sup>

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None of the single-gene transgenic mouse models featured substantial nigrostriatal degeneration.<sup>258</sup> Multi-gene transgenic mouse models are also available (with  $\alpha$ -synuclein and parkin or DJ-1 knockouts, or simultaneous silencing of PINK-1, DJ-1, and parkin), but they too have only limited relevance to the symptomatic and phenotypical spectrum of PD.<sup>259</sup>

Recently developed rat models with monogenic PD mutations are believed to be advantageous over mouse models. Rat neuronal circuitry is closer to that of a human, and they are less prone to anxiety than mice, which is important for the evaluation of behavioral patterns. Transgenic rats with mutated  $\alpha$ -synuclein do not have major motor deficits, but do exhibit significant olfactory deficits.<sup>260</sup> Rats with a neuron-specific mutation in LRRK2, driven by adenoviral vectors, exhibit progressive degeneration of nigral dopaminergic neurons.<sup>261,262</sup>

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# STEM-CELL MODELS

One of the most promising models for studying neurological diseases is patient-derived iPSCs. These cells would allow testing of new therapeutic approaches directly on human neural tissue that contains molecular alterations typical for ASD.<sup>263</sup> The use of iPSCs in animal models has already provided a tool to correct abnormal synaptic morphology and physiology, and to reverse behavioral alterations, even in symptomatic animals.<sup>264</sup>

Human iPSC have been used as a model for studying PD.<sup>265</sup> Because only a limited population of dopaminergic neurons located in the midbrain is most prone to degeneration, the engrafted stem cells must be matched to those affected by degeneration.<sup>266</sup>

Another stem-cell model has been used for the treatment of ASD in clinical trials—mesenchymal stem cells (MSCs).<sup>267</sup> Reportedly, they overturn ASD symptoms by restoring integration into the neural network, facilitating the recovery of synaptic plasticity, and releasing anti-inflammatory cytokines.<sup>268</sup>

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Defects in brain development can contribute to the onset of neuropsychiatric disorders. This study set out to identify the functional role of the 15q11.2 deletion on neural development using induced pluripotent stem cell (iPSC)-derived human neural precursor cells (hNPCs) by RNA-Seq and SNP-genotyping arrays. They found that haploinsufficiency of CYFIP1, a gene within 15q11.2, affects radial glial cells, leading to their ectopic localization outside of the ventricular zone.

Illumina Technology: HumanOmni2.5S

**Ryan S. D., Dolatabadi N., Chan S. F., Zhang X., Akhtar M. W., et al. (2013) Isogenic human iPSC Parkinson's model shows nitrosative stress-induced dysfunction in MEF2-PGC1alpha transcription. *Cell* 155: 1351-1364**

An association has been reported between PD and exposure to mitochondrial toxins. In this study, a stem cell model was used to characterize the response to toxins using Illumina BeadArray for gene expression analysis. The authors identified a pathway whereby basal and toxin-induced nitrosative/oxidative stress results in S-nitrosylation of transcription factor MEF2C. They reported the alteration contributing to mitochondrial dysfunction and apoptotic cell death, indicating a mechanism and potential therapeutic target for PD.

Illumina Technology: Human Gene Expression BeadArray

Zhang Y., Schulz V. P., Reed B. D., Wang Z., Pan X., et al. (2013) Functional genomic screen of human stem cell differentiation reveals pathways involved in neurodevelopment and neurodegeneration. *Proc Natl Acad Sci U S A* 110: 12361-12366

# BIOLOGY

Neurological and neurodegenerative diseases occur and develop as a result of genetic and epigenetic mutations. However, many of these mutations are somatic (non-inherited) and occur under the influence of biological factors, such as immune activity, gut microbiome activity, and environmental factors. The contribution of these factors at a multi-cell and single-cell level has long remained hypothetical and controversial. However, the advent of high-resolution sequencing techniques now allows the uncovering of these mechanisms and the significance of their contribution to disease.

## Immunity

The immune system, which was long believed to be independent from the central nervous system (CNS), is now acknowledged to have an important contribution to normal CNS function as well as to multiple neurological disorders.<sup>269,270</sup> For example, in PD, elevated levels of inflammatory cytokines are associated with more severe forms of the disease, such as PD with dementia.<sup>271</sup> In AD, an analysis of the two largest GWAS has shown a significant overlap between disease-associated genes in pathways associated with AD, cholesterol metabolism, and immune response.<sup>272</sup> In schizophrenia, a number of immune genes have been identified as genetic risk factors associated with this disease.<sup>273,274,275</sup> In autism, ongoing inflammation has been determined as one of the common components of the disease. Interestingly, fever in some autistic children was associated with improvement of their social behavior, underpinning the involvement of inflammation in the symptomatic profile.<sup>276</sup>

“Both inflammation and oxidative stress tend to increase with age and are associated with a variety of chronic diseases. They have also been linked to neurodegeneration and proposed as factors that might contribute to schizophrenia.” *Anthes* 2014

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The blood-brain barrier prevents penetration of many types of immune cells into the brain; however, a very limited number of certain immune cell populations, such as dendritic cells and microglia, reside in the brain and facilitate the removal of dead neurons.<sup>277,278</sup> Neurodegeneration is most often accompanied by the accumulation of microglia and monocytes around amyloid plaques and dying neurons.<sup>279</sup> According to one hypothesis of PD origin, the death of dopaminergic neurons in PD per se may be facilitated by neuroinflammation.<sup>280</sup> Infection of neurons and neighboring glial cells with viruses, such as Japanese encephalitis virus (JIV), can increase the vulnerability of neurons to factors such as aging, oxidative stress, environmental stress, and genetic predisposition.<sup>281</sup> Furthermore, neurons express some molecules normally attributed to the immune system, thus uncovering an intricate interplay between neuronal and immune systems.<sup>282</sup>

Immunotherapy is considered one of the promising approaches to treating neurological diseases. Examples of such treatment include celecoxib, an inhibitor of cyclooxygenase-2, which has shown some improvement of schizophrenia symptoms in four studies<sup>283</sup>, and anti- $\beta$ -amyloid antibody, used in multiple trials up to phase III as an agent to remove pathogenic  $\beta$ -amyloid plaques.<sup>284,285</sup>

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**Schizophrenia Working Group of the Psychiatric Genomics C. (2014) Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511: 421-427**

Schizophrenia is a highly heritable disorder, but the heritability is not found in a single gene effect. In this largest GWAS for schizophrenia to date, the authors used SNP arrays for 36,989 cases and 113,075 controls to determine genetic risk factors for the disorder. The authors found that the significant genetic associations were not randomly spread across the genome, but enriched among genes expressed in brain and genes that have been associated with typical co-morbidity diagnoses, such as ASD and intellectual disability. Interestingly, links were also enriched within genes related to immunity, which fits the existing hypothesis of immune dysregulation in schizophrenia.

llumina Technology: Human1M, HumanOmni2.5, HumanOmniExpress, HumanHap550, Human610, HumanHap650Y, HumanHap300

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# METAGENOMICS

Metagenomics refers to the study of genomic DNA obtained from multiple micro-organisms that often cannot be cultured in the laboratory. Humans carry 10 times more bacterial cells than human cells, and 100 times more bacterial genes than the human genome.<sup>286</sup> The new generation of sequencing technology, with its ability to sequence thousands of organisms in parallel, has proved to be uniquely suited to this application. Recent technical improvements allow nearly complete genome assembly from individual microbes directly from environmental samples or clinical specimens, without the need for cultivation methods.<sup>287</sup> This accumulation of sequence information has greatly expanded the appreciation of the dynamic nature of microbial populations, as well as their impact on the environment and human health. With this extraordinary and powerful set of sequencing tools now available, it is no surprise that metagenomics has become one of the fastest-growing scientific disciplines.

The concept of a gut-brain axis has been developed recently, to highlight the important influence of the metagenome on mental processes. It has been shown that the gut microbiome plays an unexpectedly important role in the development of depression, anxiety, irritable bowel syndrome, and neurological diseases, such as autism<sup>289,290</sup> and schizophrenia.<sup>291</sup> The gut-microbial products may impose their effect on the brain through chromatin plasticity, which causes changes in neuronal transcription.<sup>292</sup> Hsiao et al. proposed the use of microbiome-mediated therapies for treating neurodevelopmental disorders.<sup>293</sup>

Interestingly, some gut bacteria (defined as psychobiotics) have a positive effect on mental health and foster brain activity.<sup>294</sup> These living organisms produce such neuroactive substances as g-aminobutyric acid and serotonin, and are beneficial not only for healthy individuals, but also for those with psychiatric disorders.<sup>295</sup>

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Markle J. G., Frank D. N., Mortin-Toth S., Robertson C. E., Feazel L. M., et al. (2013) Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* 339: 1084-1088

Microbial exposure and sex hormones exert potent effects on autoimmune diseases. This study examined the effect of early-life microbial exposure on sex hormone levels and autoimmune disease in a non-obese diabetic (NOD) mouse model. The microbiome was characterized using 16S rRNA Illumina sequencing. Comparing the effects across both male and female mice, the results indicate that alteration of the gut microbiome in early life potentially suppresses autoimmunity in animals at high genetic risk for disease.

Illumina Technology: MiSeq

Maurice C. F., Haiser H. J. and Turnbaugh P. J. (2013) Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell* 152: 39-50

The microorganisms in the human gut microbiome are known to impact digestive health, but their influence on health by the metabolism of xenobiotics, including antibiotics and drugs, is still unclear. In this metagenomics study, xenobiotic-responsive genes were found across multiple bacterial phyla involved in various metabolic and stress-response pathways. The results suggest that xenobiotics may have important implications for the human gut microbiome.

Illumina Technology: HiSeq to sequence the V4 region of the 16S rRNA gene

Schloissnig S., Arumugam M., Sunagawa S., Mitreva M., Tap J., et al. (2013) Genomic variation landscape of the human gut microbiome. *Nature* 493: 45-50

Subjects sampled at varying time intervals exhibited individuality and temporal stability of SNV patterns, despite considerable composition changes of their gut microbiota. This observation indicates that individual-specific strains are not easily replaced, and that an individual might have a unique metagenomic genotype, which may be exploitable for personalized diet or drug intake.

Illumina Technology: Illumina reads obtained from European MetaHIT study<sup>296</sup> and U.S. Human Microbiome Project<sup>297</sup>

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# ENVIRONMENTAL FACTORS

Exposure to microbes<sup>298,299</sup>, as well as various industrial and agricultural chemicals (especially pesticides), has been implicated as conferring risk for multiple diseases, including neurodegenerative disorders. Environmental pesticides serve as mitochondrial toxins and induce nitrosative stress that inhibits activity of the myocyte-specific enhancer factor 2C (MEF2C). This factor is involved in cardiac morphogenesis, myogenesis, and vascular development. MEF2C, in turn, inhibits the expression of peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1  $\alpha$ ), and hence suppresses the neuroprotective function of this transcriptional co-activator.<sup>302</sup>

Neurodegenerative disorders, including PD, often co-exist with metabolic diseases. For example, type 2 diabetes may stimulate the development of PD.<sup>303</sup> Sekiyama et al. suggest that genomic studies should contribute to better understanding of the mechanism of this interaction, and help to develop strategies for new therapeutic approaches.<sup>304</sup>

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# SINGLE CELLS

Each cell type has a distinct lineage and function, which contributes to the functioning of the tissue, the organ, and—ultimately—the organism. The lineage and developmental stage of each cell determine how they respond to each other and to their environment. While the ultimate goal of an exhaustive understanding of tissues at their cellular level is still elusive, recent progress in single-cell analysis is offering a glimpse at the future.

A recent study of single cells and neurons in brain tissue found that most ( $\geq 95\%$ ) neurons in normal brain tissue are euploid. However, a patient with hemimegalencephaly (HMG) due to a somatic CNV of chromosome 1q had unexpected tetrasomy 1q in 20% of neurons. This observation suggests that CNVs in a minority of cells can cause widespread brain dysfunction.<sup>305</sup> This complexity can only be resolved with single-cell sequencing approaches.

Recent advances in research have highlighted the mosaic genomes of individual neurons, exhibiting CNVs even among cells that make up a specific region of the brain.<sup>306</sup> Even though genetic variations in the brain arise during fetal development,<sup>307</sup> the functional relevance of this mosaicism is unclear at present. It will be of interest not only to discover the significance of mosaicism in the normal brain, but also to study its role in neurological diseases and psychological disorders.<sup>308-311</sup>

Research has just begun to shed light on mosaicism, where heterogeneity among cells is notable at the genome level. If mosaicism exists in the genetic code among single cells,<sup>312</sup> there are likely also variations in protein expression, epigenetic changes,<sup>313</sup> and RNA isoforms.<sup>314</sup> Sequencing single cells provides a larger integrated image of the collected data, helping to account for the effects of mosaicism on individual cellular phenotypes within a given region of the brain.<sup>315,316,317</sup>

The high accuracy and specificity of NGS lends itself well to single-cell and low-level DNA/RNA sequencing. The growing armamentarium of published techniques includes the detection of DNA mutations, CNVs, DNA-protein binding, RNA splicing, and the measurement of RNA expression values.

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RNA sequencing methods that rely on RNA extracted from cell mixtures do not convey the individual variability in expression among cells of the same tissue. In this paper, the authors present a transcriptome *in vivo* analysis (TIVA), which is applicable to single-cell studies. In combination with Illumina sequencing technology, the authors capture and analyze the transcriptome variance across single neurons both in culture and *in vivo*. This method is furthermore non-invasive and may be applied to intact tissue. It will enable detailed studies of cell heterogeneity in complex tissues that have been intractable previously, and it opens up the possibility of use in conjunction with *in vivo* live functional imaging.

Illumina Technology: 670k BeadChip Array

Young G. T., Gutteridge A., Fox H. D., Wilbrey A. L., Cao L., et al. (2014) Characterizing Human Stem Cell-derived Sensory Neurons at the Single-cell Level Reveals Their Ion Channel Expression and Utility in Pain Research. *Mol Ther* 22: 1530-1543

Research into the biology of pain is commonly performed on animal models due to the lack of sensory neuronal cell lines. In this paper, the authors present human stem-cell derived sensory neurons and use a combination of population and single-cell techniques to perform detailed molecular, electrophysiological, and pharmacological phenotyping. The directed differentiation was monitored over 6 weeks and the gene expression characterized using Illumina BeadArrays. The authors show the derived neurons are both molecularly and functionally comparable to human sensory neurons derived from mature dorsal root ganglia.

Illumina Technology: BeadArrays

Lister R., Mukamel E. A., Nery J. R., Urich M., Puddifoot C. A., et al. (2013) Global epigenomic reconfiguration during mammalian brain development. *Science* 341: 1237-1245

DNA methylation is implicated in mammalian brain development and plasticity underlying learning and memory. This paper reports the genome-wide composition, patterning, cell specificity and dynamics of DNA methylation at single-base resolution in human and mouse frontal cortex throughout their lifespan. The extensive methylome profiling was performed with ChIP-Seq on Illumina HiSeq, revealing methylation profiles at single-base resolution.

Illumina Technology: TruSeq RNA, TruSeq DNA, HiSeq

McConnell M. J., Lindberg M. R., Brennand K. J., Piper J. C., Voet T., et al. (2013) Mosaic copy number variation in human neurons. *Science* 342: 632-637

The genome of a species varies not only between individuals, but also between mother and daughter cells as a result of errors and uneven distribution of DNA material after cell division. The extent of such genetic variation between somatic cells of the same individual has been hitherto unknown. With the advent of single-cell sequencing, it is now possible to examine variations within cells of the same tissue. In this study, the CNVs in individual neuron cells were studied using Illumina genome-wide sequencing. The authors discovered that CNVs are abundant even between neuronal cells from the same tissue.

Illumina Technology: Genome Analyzer<sub>IIx</sub>, MiSeq, Nextera DNA Sample Prep Kit

Pan X., Durrett R. E., Zhu H., Tanaka Y., Li Y., et al. (2013) Two methods for full-length RNA sequencing for low quantities of cells and single cells. *Proc Natl Acad Sci U S A* 110: 594-599

Gene expression profiling by RNA-Seq is a powerful tool for understanding the molecular activity of specific tissues. However, the heterogeneity of gene expression within a tissue requires RNA-Seq technology that can manage single-cell amounts of input RNA. This study presents two methods: Phi29 DNA polymerase-based mRNA transcriptome amplification (PMA), and semi-random-primed PCR-based mRNA transcriptome amplification (SMA). Both techniques are coupled with Illumina sequencing for expression detection from low RNA input amounts. Both protocols produced satisfactory detection/coverage of abundant mRNAs, even from a single cell.

Illumina Technology: HiSeq

Binder V., Bartenhagen C., Okpanyi V., Gombert M., Moehle B., et al. (2014) A New Workflow for Whole-Genome Sequencing of Single Human Cells. *Hum Mutat* 35: 1260-1270

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