Advances in genetic sequencing and genomics in the detection and analyses of genetic variants in neurological disorders: A review

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Abstract

With recent advances in genetics and genomic sequencing, it has become possible to screen for genetic variants and polymorphisms in the human genome that contribute to heritable forms of neurological diseases. With an increasing proportion of the population aged 55 years and older, there will be an increased incidence of neurological disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD). That will mean an increasing burden on our healthcare system and an increasing need for resources and expertise to treat and manage these diseases. In addition, multiple sclerosis (MS) is the most common neurological disorder in young adults, and the prevalence of MS has increased over the last few decades, with MS incidence and prevalence in British Columbia among the highest worldwide. This review will: 1) Highlight recent advances in genetic sequencing and genomics that have allowed for improved detection, characterization, and analyses of genetic variants linked to cases of AD, PD, and MS and 2) Address some considerations when using genetic testing to detect and analyze genetic variants linked to cases of neurological diseases.

Introduction

Improvements in genetic sequencing and genomics have allowed researchers and geneticists to identify genetic variants in the human genome that are associated with an increased risk of developing neurological disorders such as AD, PD, and MS. Genetic variants and polymorphisms associated with an increased risk of developing neurological disorders tend to be more prevalent in families with a history of multiple relatives suffering from a disease. Studying families with rare genetic variants that increase the risk of neurological disorders allows researchers to investigate the effects of genetic variants on the likelihood of developing a disease as compared to the general population. In the past few years, the invention of massively parallel sequencing technologies have allowed scientists to study the causes of a disease at a molecular level while using advanced computational techniques to filter and analyze large amounts of genomic data to find genetic information that relates to an individual's health and the pathophysiology of disease processes.[1] Researchers have dubbed massively parallel sequencing techniques as "-omics" technologies, which have allowed researchers to study the structure, function, and interactions of biomolecules making up cells.[1] Examples of massively parallel sequencing techniques include next generation sequencing (NGS) for detecting DNA variants involved in the development of disease and RNA sequencing for transcriptome analysis and studying the roles of non–coding RNA in disease pathogenesis.[1] The full list of sequencing technologies available for studying the molecular mechanisms of disease is extensive, but it is important to note that NGS is already being used to develop more sensitive diagnostic tools and to identify new molecular targets that could potentially be used in the development of therapeutics.

Advances in genomics for diagnosing early onset Alzheimer's disease

Currently, AD can be confirmed only with a post–mortem autopsy and is characterized by neuropathology, such as extracellular β–amyloid plaques formed by insoluble Aβ-42 peptides and intracellular Neurofibrillary Tangles (NFTs) that are composed of hyper–phosphorylated Tau proteins.[2,3] During the course of AD, neurodegeneration triggered by Aβ-42 peptides and NFTs occurs with extensive loss of grey matter in the hippocampus, the temporal lobes, and the neocortex.[2,3] These pathological processes lead to the characteristic behavioural symptoms seen in AD, which include cognitive decline, memory loss, and impairments in consolidation of new memories.[2,3] In a recent study of individuals with early onset AD (family history of AD at <65 years) or very early onset AD (≤55 years), researchers used exome sequencing to study all coding regions of the genome to look for genetic variants associated with an increased risk of AD.[4] Twenty–nine genetic variants potentially involved in the development of early onset AD were identified, while only one gene, Protein Tyrosine Kinase Binding Protein (TYROBP), was selected for genetic and functional validation.[4]

TYROBP was further studied because it is known to be upregulated in AD brain tissue and was shown to be involved in the pathogenesis of late onset AD.[5,6] TYROBP is a binding partner of Triggering Receptor Expressed On Myeloid Cells 2 (TREM2), which is itself a genetic risk factor for AD.[7,8] In the central nervous system (CNS), TYROBP is expressed in microglia, where it binds to ligands such as TREM2, which leads to a cell signaling cascade triggering proinflammatory responses and phagocytosis of cellular debris by microglia.[9,10] TYROBP is also thought to be involved in Amyloid-β (Aβ) turnover, which is significant because TYROBP genetic variants implicated in AD could alter normal microglia functions, such as the clearance and phagocytosis of abnormal proteins in the maintenance of tissue homeostasis in the CNS.[6] Studies using advanced genetic sequencing techniques have allowed researchers to understand more about the neuropathology and pathogenesis of AD, as well as to identify genetic variants linked to rare forms of early onset AD.[4,6]

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Genetic sequencing and hereditary forms of Parkinson's disease

Discovery of genetic variants conferring an increased risk of developing PD has come from over 25 years of research on the etiology and pathogenesis of the disease. In a paper published in 1997, a genetic mutation in the α-synuclein gene that caused an autosomal dominant pattern of parkinsonism was identified in a large family of Italian descent.[11-13] Alpha–synuclein is a presynaptic protein found in CNS neurons that is a major constituent of Lewy bodies (the major neuropathological hallmark of PD and dementia with Lewy bodies) and can be found in dopaminergic neurons of the substantia nigra pars compacta, which undergo neurodegeneration in cases of idiopathic PD.[14] Family members who possessed the α–synuclein mutation manifested the disease 85% of the time and presented with typical clinical symptoms of PD, but had an earlier age of disease onset as compared with idiopathic PD.[11,12]

More recently, new genetic variants have been identified as causes of familial PD, with signs and symptoms similar to idiopathic PD. Leucine–rich repeat kinase 2 (LRRK2) gene mutations were linked to autosomal dominant parkinsonism that presented with α–synuclein neuropathology.[15,16] In a 2008 study, it was determined that six genetic mutations in LRRK2 were pathogenic in the development of PD.[14] Of these, the frequency of the more common Gly2019Ser mutation was estimated to be present in 1% of idiopathic PD patients, as compared with 4% of patients with a heritable form of PD.[15] The risk of developing PD for those who are carriers of the LRRK2 Gly2019Ser mutation increases steadily with age.[15] Patients with mutations in LRRK2 on average had a shorter disease duration and slightly earlier onset of PD symptoms as compared with patients with idiopathic PD.[15]

In addition to improving the ability to discover hereditary forms of PD, which are estimated to contribute to 15% of total PD cases, genetic sequencing has shown that LRRK2 Gly2019Ser mutation carriers have a unique clinical presentation that could aid in the diagnosis and management of this subtype of parkinsonism.[15,17] In a large study of PD patients with LRRK2 Gly2019Ser mutations, subjects presented with bradykinesia, asymmetrical tremors, and muscle rigidity, which responded to dopamine replacement therapy and functional neurosurgery, with tremor symptoms and abduction–adduction leg tremor being more common in comparison to idiopathic PD subjects.[15] Interestingly, those PD patients with LRRK2 Gly2019Ser mutations had a lower risk of developing non–motor symptoms, such as cognitive impairment and hyposmia, as compared with patients with idiopathic PD.[15]

In a clinical genetics study published in 2016, researchers found that genetic variability in the dynamin-3 (DNM3) gene modified the age of onset for LRRK2 Gly2019Ser parkinsonism.[18] Using genome-wide linkage analysis and whole genome sequencing (WGS), researchers were able to identify genetic associations that can affect the genetic penetrance and clinical phenotypes of a hereditary form of PD.[18]

Identifying genetic variants associated with multiple sclerosis

In recent years, it has become apparent that genetic predisposition plays an important role in the risk of developing MS as well. MS is a progressive inflammatory and demyelinating disease of the CNS and is the most common neurological disorder in young adults, affecting 100,000 Canadians and 2.5 million people worldwide.[19] MS can be separated into several subtypes based on clinical features and disease phenotypes. The most common pathological feature of MS is the presence of focal lesions or plaques in the white matter (WM) of the CNS that can be visualized and characterized by histology or MRI techniques.[20,21]

Recent studies have identified both genetic variants and biomarkers in the cerebrospinal fluid (CSF) of MS patients that could be used to predict symptom severity and clinical course in the progression of MS.[22] In one study, researchers recruited a cohort of 127 patients who had recently experienced their first demyelinating episode, and subjects were genotyped to determine if they possessed MS–associated single nucleotide polymorphisms (SNPs) that could predict the clinical course of disease.[23] Nine SNPs were identified that correlated with conversion to MS and/or episodes of MS relapse; two SNPs were located in Human Leukocyte Antigen (HLA) genes, and seven SNPs were in non–HLA genes.[23]

A recent paper also identified the first potential pathogenic mutation for MS in the Nuclear Receptor NR1H3 gene in seven patients with MS from two unrelated families with multiple cases of MS diagnosed in family members.[24] Individuals from the two families presenting with MS had severe and progressive forms of the disease, with an average age of onset of 34 years.[24] Exome sequencing of the two families discovered the presence of a p.Arg415Gln amino acid substitution in the NR1H3 gene, with the mutant form of NR1H3 inhibiting the heterodimerization of LXRA (the gene product of NR1H3; Liver X receptor-α), leading to alterations in gene transcription, which could be involved in the pathogenesis of some forms of MS.[24]

Considerations and conclusion

While genetic sequencing and genomics have improved the detection of rare genetic variants linked to neurological disorders, there are drawbacks to the use of whole genome sequencing (WGS) in identifying and characterizing diseases such as AD, PD, and MS. Drawbacks include detection of false positive genetic variants and increased false discovery rates (FDR) of genetic variants when using WGS. In a recent Japanese study, geneticists used WGS to identify de novo mutations in the human genome.[25] Discovery of false positive results and the FDR for genetic variants is affected by the choice of genomics platform and the statistical analyses used to determine the significance threshold in the detection of genetic variants potentially linked to a disease.[25] When identifying novel SNPs using whole genome sequencing (WGS) in the human genome with the error rate set at 0.001%, researchers found 30,000 false positive results across the entire human genome.[25]

More recently, a genetic study has demonstrated the use of WGS for increasing diagnostic utility and improving clinical management of pediatric patients diagnosed with neurological disorders or congenital abnormalities.[26] In the study, authors compared the use of WGS and chromosome microarray analysis (current standard genetic testing) in identifying disease-causing genetic variants in 100 children referred to the pediatrics genetic service.[26] The authors found that WGS identified genetic variants meeting clinical diagnostic criteria in 34% of cases, as compared with 8% of cases using standard genetic testing.[26] Other studies showed that WGS identified genetic variants meeting clinical diagnostic criteria in 25% of patients with neurological and congenital disorders.[27,28] However, the percentage of cases in which WGS can identify a disease-causing genetic variant is still fairly low, and the cost of sequencing a single patient's genome is expensive (estimated to be $3,000 per patient in some recent studies).[26] It is important to highlight

the fact that many neurological diseases are caused by the interaction of environmental factors with genetic variants at multiple different genetic loci and that each gene variant usually conveys only a small risk in the development of a neurological disease.[29] Therefore, genetic testing might currently only be useful in those patients with de novo mutations leading to development of disease or in those individuals with a strong family history of a neurological disease.

This review provides a snapshot of emerging clinical research using genetic sequencing and genomics techniques to identify pathogenic gene variants and mutations in neurological diseases such as AD, PD, and MS. While genetic sequencing has provided numerous insights into the etiology, pathogenesis, and molecular mechanisms of neurological disorders, there are several considerations one must take into account when using genomics to detect pathogenic genetic variants in patients. All patients should be offered pre-test and post-test genetic counseling about the risks and benefits of receiving testing for detecting genetic variants in determining risk and diagnosis of neurological diseases. Physicians should clearly explain the sensitivity and specificity of the genetic techniques and explain the relative risk of developing a disease if the patient is a carrier for a given gene variant. Implications of genetic testing should also be considered for a patient's family members, as pathogenic gene variants tend to cluster in families and family members of an individual identified as a carrier. A patient's family members should also be offered genetic counseling and genetic testing to determine if they are carriers.

Finally, the decision to conduct genetic testing for a patient should take into account the frequency of the mutation in the population and whether the patient is in a high–risk group for carrying a particular pathogenic mutation.[15] Presymptomatic patients and patients with a neurological disease should be advised that the main benefits of testing are to improve the accuracy of a diagnosis or to determine the relative risk of developing a disease if an individual is a mutation carrier.[15] In many cases, testing will not influence therapeutic options for a patient, but it can influence patient-centered care for the management of a disease, which is important in allowing individuals to make informed choices about their healthcare. For these reasons, it is important for researchers, healthcare providers, and ethicists to develop best practice guidelines for how personalized genetic information should be used to maximize the benefits and minimize the risks for the physical and mental wellbeing of patients and their families.

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