STUDENT CORNER

Influence of Next-Generation Sequencing on Advancements in the Diagnosis of Major Psychiatric Diseases - A Review

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ABSTRACT

Rapid progress is being made in the development of next-generation sequencing (NGS) technologies, allowing repeated findings of new genes and a more in-depth analysis of genetic polymorphisms behind the pathogenesis of a disease. In a field such as psychiatry, characteristic of vague and highly variable somatic manifestations, these technologies have brought great advances towards diagnosing various psychiatric and mental disorders, identifying high-risk individuals and towards more effective corresponding treatment. Psychiatry has the difficult task of diagnosing and treating mental disorders without being able to invariably and definitively establish the properties of its illness. This calls for diagnostic technologies that go beyond the traditional ways of gene manipulation to more advanced methods mainly focusing on new gene polymorphism discoveries, one of them being NGS. This enables the identification of hundreds of common and rare genetic variations contributing to behavioral and psychological conditions. Clinical NGS has been useful to detect copy number and single nucleotide variants and to identify structural rearrangements that have been challenging for standard bioinformatics algorithms. The main objective of this article is to review the recent applications of NGS in the diagnosis of major psychiatric disorders, and hence gauge the extent of its impact in the field. A comprehensive PubMed search was conducted and papers published from 2013-2018 were included, using the keywords, "schizophrenia" or "bipolar disorder" or "depressive disorder" or "attention deficit disorder" or "autism spectrum disorder" and "next-generation sequencing"

Keywords: Gene Polymorphism; Next-Generation Sequencing; Genome Wide Association Study; Psychiatric Diagnosis.

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INTRODUCTION

The American Psychiatric Association as a disruption in cognition, emotion regulation, or behavior that reflects a dysfunction in psychological, biological, or developmental processes[1] characterizes a psychiatric disorder. Currently, various forms of mental disorders[2] affect 450 million people worldwide. Cases discussed in our study include schizophrenia, autism spectrum disorder (ASD), major depressive disorder (MDD), attention deficit hyperactivity disorder (ADHD) and bipolar disorder (BP).

Psychiatry has the difficult task of diagnosing and treating mental disorders without being able to invariably and definitively establish the properties of its illness. However, this calls for diagnostic technologies that go beyond the traditional ways of gene manipulation to more advanced methods mainly focusing on new gene polymorphism discoveries, one of them being next-generation sequencing (NGS). Because of its high throughput, reduced expenses, and fast speed, NGS is useful in various applications[3]. Patients with developmental delays showed a diagnostic yield of 28%[4]. Another study distinguished mitochondrial DNA variants in ASD and 84 damaging DNA irregularities in BP[5, 6].

Clinical NGS has been useful to detect copy number and single nucleotide variants and to

identify structural rearrangements that have been challenging for standard bioinformatics algorithms to manage[7,8]. Psychiatric genetics thus enables the identification of hundreds of common and rare genetic variations contributing to behavioral and psychological conditions. The main objective of this article is to review the recent applications of NGS in the diagnosis of major psychiatric disorders, and hence gauge the extent of its current impact in the field, as well as the potential for change in diagnostic approaches in the future.

For this narrative review study, PubMed search was performed, in the time range between 2013-2018. Keywords included "schizophrenia" or "bipolar disorder" or "depressive disorder" or "attention deficit disorder" or "autism spectrum disorder" and "next-generation sequencing". The search results were manually reviewed from 366 articles to select the ones most relevant to our study. Articles that made use of NGS to detect genes and mutations, and presented novel findings regarding the pathogenesis of the five major psychiatric diseases, were included. These study designs comprised of cohorts, cross-sectional, case controls and clinical trials. The related content of the 30 articles that met the inclusion criteria was reviewed and organized in tables.

DISCUSSION

SCHIZOPHRENIA

Schizophrenia has an inherent heterogeneity that has complicated its diagnosis and pathophysiology. A multifactorial disorder, identified by cognitive impairment, erratic speech, and behavior, delusions, and hallucinations; the greatest risk factor remains a positive family history[9]. In recent years, the use of RNA-Sequencing to profile the schizophrenia transcriptome has been extensive. Long non-coding RNAs (lncRNAs) contribute immensely through molecule functions such as decoys, scaffolds, and signals. The arrival of RNA-Sequencing has supplied the exact technology needed to study lncRNAs, as they are generally expressed at low levels making their detection and quantification challenging[10].

In a recent study, RNA-Sequence libraries were constructed to identify the functional pathways, which were most involved in the pathogenesis of schizophrenia, and analysis on each subtype of the gene set was performed. lncRNAs were identified as a result of functional modules being connected to synaptic transmission and immune responses[11].

Primary familial brain calcifications are caused by mutations in the SLC20A2 gene. NGS was utilized to conduct a pedigree study in three generations of a family, of whom three members presented with schizophrenia proband. NGS identified a novel nonsense heterozygous substitution of the SLC20A2 gene introduced a stop codon[12]. Hypofunction of N-methyl-d-aspartate receptors, through phosphorylation by CDK5, CSNK2A1, and EphB2, has been associated with schizophrenia. NGS was used to re-sequence all splice sites and coding regions of these genes and Sanger sequencing was used to remove variants for human control subjects and validate nonsynonymous and nonsense variants[13]. NGS was also used to select miRNAs for studying their diagnostic function with regard to schizophrenia. In the ultimate MiR-22-3p, miR-92a-3p, and miR-137 can also be useful as biomarkers for schizophrenia[14]. A repeated association has been established between neurodevelopmental disorders and CX3CR1, a G protein-coupled receptor that is manifested in microglia. NGS was used in a study to code exon targeted resequencing and the impact of different variants of CX3CR1 on these disorders was determined[15].

MAJOR DEPRESSIVE DISORDER (MDD)

MDD affects how one feels, thinks and behaves negatively, and is a leading cause of morbidity throughout the world. Strongly linked to neurotransmitter dysfunctions it can be a cause of various emotional and physical manifestations[16, 17]. In a recent study on Mexican-Americans, the sample showed significant differences between MDD cases and controls in 9 genes, when studying for single-nucleotide variant proportions (SNVP) across 46 genes. In an Australian sample, major outcomes were established for the MUC6 and TBC1D2B genes between MDD cases and controls. These findings indicate that the SNVP in some genes may be correlated with MDD[18].

In another study investigating common copy number polymorphisms (CNPs) modifying genes of the 5- hydroxytryptamine (5-HT) system, all known 5-HT related genes were examined using NGS for common structural variation[19]. A research study dating back to 2014, and applying high-throughput sequencing, suggested that a single-nucleotide polymorphism in the zinc finger protein gene showed a genome-wide association with MDD[20]. A significant network of limited variants that are over-represented in MDD has been identified, with the strongest association provided by the calcium channel 'adaptor' gene. This set consisted of twenty genes, 7 of which were expressed more frequently in cases compared with controls[21].

When RNA-seq of the frontal cortex was performed to ascertain the discrepancies in miRNA expression between depressed people, people who committed suicides and controls, cortical gene expression was shown to be altered in MDD, especially regarding glial cell functions, and suggested to influence suicidal tendencies in these patients[22]. A recent study implies that T cell phenotypes are skewed on several levels in patients with MDD, as blood drawn

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from these patients revealed a transformation in the CD4+ T compartment proceeding towards Treg cells[23].

ATTENTION DEFICIT HYPERACTIVITY DISORDER

A neurodevelopmental dysfunction marked by distraction, hyperactivity, and heightened impulsive behavior, childhood ADHD has been determined to be around 76%, suggesting a strong genetic association[24]. With the recent advancements in microarray and NGS technology, the genetic architecture regarding ADHD progression is starting to get uncovered[25]. One of the risk candidate genes involved in ADHD is the GTP-binding Ras-like protein 2 gene and prior studies (GWAS) on ADHD patients confirm this association. An experiment searching for rare variants in ADHD patients generated eight that were linked with the disease[24]. Rare copy number variations (CNVs) that affected ASTN2 or TRIM32 were the most significant findings in terms of shared risk factors between ASD and ADHD[25]. Rare CNVs at the ASTN2/TRIM32 locus were also found in most individuals with distinct neurodevelopmental diagnoses through other genome-wide scans. Single nucleotide polymorphisms within ASTN2 have been identified by recent GWAS in risk for ADHD and other neurodevelopmental disorders[25].

Even though common variant signals have been found in genome-wide association studies, these only explain some portion of the heritable nature of ADHD[26]. Furthermore, a large-scale NGS study of ADHD was performed on children and adolescent cases, controls, and associations were found between the genes coding for neurotrophic factors and ADHD[26]. Though most of the studies investigating ADHD have centered on the pathophysiology related to attention dysfunction, such as catecholamine and dopamine deregulation, copy number variation and GWAS have found many neurodevelopmental genes to be potential candidate genes contributing to ADHD[27].

AUTISM SPECTRUM DISORDER

ASDs are a range of neurodevelopmental disorders distinguished by impaired social ability, repetitive behavior, and very limited interests and activities. ASD begins to manifest itself during the earlier years of childhood, continues throughout life, and is highly heritable. A thorough study was conducted which aimed to construct a gene panel using pre-discovered SNVs and CNVs through NGS in order to aid diagnosis and treatment. While confirming the presence of a large number of previously detected genetic variations in their sample, it also identified 22 rare SNVs having a potential role in ASD[28].

Another research investigated the effects of stress on fetuses being carried by mothers possessing stress susceptible genes. It was concluded that the fetuses of stress-exposed mothers who possess the 5-HTTLPR polymorphism gene, were more likely to develop autism[29]. Even though many studies have been conducted to understand the etiology of ASD, the underlying complex transcriptomic circuitry of it is still unclear. A study was performed to conduct a transcriptomic analysis of the brains of ASD patients, which revealed the PPP1R3F regulator in the pathophysiology of ASD[30]. A recent study used NGS of the MBD5 gene of patients with ASD or schizophrenia in order to detect SNVs and CNVs in the gene, associated with these disorders[31].

Despite widespread research on the role of genetics in the development of ASD, most of them are focused on nuclear DNA. In a study of mitochondrial DNA (mtDNA) sequences obtained through NGS, an mtDNA variant in MT-ATP6 and a nuclear DNA variant in NDUFS4 were deduced[32]. Furthermore, the microorganisms residing in our diet and gut have been implicated as possible agents in ASD as well. The study uncovered a difference in the makeup of gut bacteria between ASD patients and controls and identified specific species that were closely related to the disease i.e., Bacteroides[33].

BIPOLAR DISORDER

BP is a serious neuropsychiatric illness in which people undergo severe mood changes often accompanied by sleep disturbances, changes in energy level and inability to think clearly. It mostly appears in genetically inclined people in their late teens and early adulthood. A thorough study was conducted on BP patients using NGS approach, rare variants with a missense of A to T mutation were isolated in patients and several other mutations were detected i.e., the splicing site of MTOR[34]. A case-control meta-analysis study was leading using NGS on lymphoblastic cell's DNA, which was extracted, from both cases and controls. A variant-level and gene-burden analysis provided confirmation for 19 of these genes that were found associated with BP disorder including the KDM5B gene, which is strongly implicated with an overlap of BP disorder and sporadic cases of ASD[35].

However, a couple of studies show evidence associating the mutated FKBP5 gene with suicidal behavior in BP disorder patients. The principal analysis focused on variants near or on the FKBP5 gene loci, with 5 of them signified separately in suicidal patients[36]. Genetic markers in gene ankyrin 3 (ANK) and the alpha 1C subunit of calcium channels (CACNA1C) were associated genes for BP disorder, identified using NGS[37]. Non-Parametric and parametric linkage analysis of the pedigree was also conducted using GWAS, which found a fraction of rare damaging variants in ANK3, ODZ2, ODZ4 and ITIH3 genes involved in BP disorder.

The extended linkage study of the pedigree on BP disorder reported positive findings with DNA markers around the HRAS1 and INS loci on chromosome 11, as well as three more genes ADAM18, INSL6, IFT8138.

Table 1: Recent applications of NGS on the study of Schizophrenia, MDD, ADHD, ASD and BP.

Authors	Year	Phenotype	Study Design	Key Findings
Schizophrenia				
May J, et al[14]	2018	synaptic structure and function	miRNA sequencing	Micro RNA-22-3p, 92a-3p and 137
Yos hikawa A , et al[13]	2018	Hypofunction of *N*-methyl-d-aspartate receptors (NMDARs)	NGS (TruSeq Custom) Sanger Sequencing	CDK5
Ishizuka K[15]	2018	neurodevelopmental disorders including schizophrenia and autism	NGS (Ion Torrent: Proton / PGM sequencing)	CX3CR1
Major Depressive Disorder				
Patas K, et al[23]	2018	T Cell Phenotype and T Cell Receptors	NGS (Immunoseq)	CXCR3 CCR6
Yu C , et al[18]	2017	SNVP	WGS (whole - genome sequencing)	MUC6 TBC1D2B
Pantazatos SP , et al[22]	2016	altered glial, endothelial and ATPase activity	Paired-end, strand-specific sequencing for total RNA and single-end sequencing for microRNA (Illumina HiSeq 2500)	serpin peptidase inhibitor, MTRNRL8, (IL8), clade H SERPINH1, CCL4

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Attention Deficit Hyperactivity Disorder				
Lena G, et al[24]	2016	Association of DIRAS2 with promoter SNP rs1412005	NGS (Sequencing by Oligonucleotide Ligation and Detection)	rs1412005 in the DIRAS2 gene is in itself a causal variant
Park S, et al[39]	2015	Association between the gene for serotonin transporter (SLC6A4) methylation and ADHD	Quantitative DNA methylation analysis by NGS	SLC6A4 methylation seems to influence certain characteristics of ADHD
Anath C, et al[25]	2014	Disruption of the ASTN2/TRIM32	Screened exonic CNVs from clinical microarray data	ASTN2 variants
Autism Spectrum Disorder				
Calvin P , et al[29]	2017	Maternal stress susceptible gene leading to autistic behavior development in offspring	WGS and miRNA sequencing (Ion Proton System)	Maternal 5-HTTLPR polymorphism in SLC6A4
Doostparast Torshizi A, et al[30]	2018	Master regulators of transcriptional networks in ASD	Transcriptional network constructed using pre-processed RNA -Seq data	PPP1R3F
Alvarez-Mora MI, et al[28]	2016	Top ASD candidate genes	CMV analysis using targeted NGS (Illumina MiSeq)	Rare SNVs identified with amino acid changes, origin and allele frequencies

Bipolar Disorder				
Cruceanu, et al[34]	2013	Response to lithium monotherapy	Whole exome study (high-throughput DNA sequencing)	missense mutation of ZNF259
Marie E. Breen[36]	2016	Targeted sequencing of FKBP5 in suicide attempters with BD	SNP, gene-level and haplotype tests	Mutation in FKBP5 Sex specific variations
Benjamin Georgi, et al[41]	2014	haplotype and locus heterogeneity was observed	WGS (Microsatellite and SNP -array genotypes)	Association at the KCNH7 locus with BD

CONCLUSION

Implementation of NGS in clinical diagnosis is still new, but its use is likely to increase with advancements in technology and resources, increased patient education and reductions in costs. The studies included in this paper are indicative of the notable impact of NGS towards identifying new genetic polymorphisms and contributing to a greater understanding of the pathogenesis of clinical diseases. However, most of these are relatively small studies and interpreting the significance of CNVs and the contribution of SNPs, including accounting for de novo mutations, is still challenging. Further large-scale research is needed to identify gene variants affecting brain function, as well as the mechanisms through which they establish an effect, taking into account the overlap of different variants with many potential diagnoses. For the time being the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) remains the most utilized tool for psychiatric diagnosing.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article

AUTHORS' CONTRIBUTION

MN interpreted the data relating to schizophrenia, wrote that corresponding part of the manuscript, AS interpreted the data relating to bipolar disorder, and wrote that corresponding part of the manuscript. EMG interpreted the data relating to MDD, wrote that corresponding part of the manuscript AH interpreted the data relating to ASD, and wrote that corresponding part of the manuscript, DN interpreted the data relating to ADHD and wrote that corresponding part of the manuscript.

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