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Delineating the Physiological Mechanism of Neurological Disorder using Next Generation Sequencing

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Abstract

An amalgamation of genetic status and environmental disruptions seem to be accountable for the outbreak of Major depressive disorder (MDD), Schizophrenic psychosis, and Bipolar disorder. Additionally, the pathological mechanism of the illness is hypothesized to be neurodevelopmental. Recent studies of the nervous system mechanisms powerfully counsel that no single method is answerable for driving an extremely complicated process. In a nutshell, these findings counsel that combinatorial genetic and environmental factors that disturb a standard biological process in the early phase of life lead to molecular and histogenic outcomes that contribute to different biological process pathways and the clinical constitution identified accounting schizophrenic psychosis. In this paper, we manifest about recent NGS (Next Generation Sequencing) technology, which is widely utilized in transcriptome and genome-wide studies and is known to have high precision in finding critical factors associated with the neurological disorder.

Keywords: *Next Generation Sequencing, Major depressive disorder, Schizophrenic, Bipolar disorder, Transcriptome, Neurodegenerative disorder.*

I. Introduction

The human brain, along with the spinal cord, makes up the central nervous system. Brain diseases comprise various neuropsychiatric disorders and brain infections, like inflammation, brain trauma, concussion, cerebral hemorrhage, and stroke, leading to convulsions, memory issues, lack of muscle management, and vision issues. The brain is the central processor, integrator, and decision-maker of the human nervous system. The brain communicates and conveys all information received from the sense organs to the rest of the body. Hence, any disfigurement of the mind will harm memory, sensation, temperament, and judgmental capabilities. Such issues can be a consequence of health problems, environmental factors, genetics, and traumatic injuries.

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1.1 Major depressive disorder

During the ancient Ramayana and the Mahabharata period (4th and 5th centuries BC), ancient Egypt (1500 BC), and the Old Testament Bible (1000 BC), talk about diseases such as depression were seen. The ancient Greeks considered depression as a medical condition called "melancholia" that arose from the imbalance of the abusive components, that is, the excess of "black bile"; In fact, "melancholia" is translated as "black bile."

Hippocrates (ca. 400 BC) described Melanocolia as long-term frustration, lack of appetite, and insomnia. These ancient details recall the same symptoms that are used today to diagnose major depression. Later, scholars challenged the spiritual basis of melancholia. Richard Burton's opposed the old theory of "The Anatomy of Melancholia" and suggested melancholia could be remedied by a healthy diet, sleep, meaningful work, and intellectual pursuits. With progressively unpredictable meanings of despondency, the term "depression" started to be utilized by the late 1800s, with depression saved for a subtype with psychosis.

In the late nineteenth and mid-twentieth century, Emil Kraepelin significantly impacted psychiatry by isolating manic-depressive from dementia praecox. In particular, manic-depressive displayed enhancement between episodes, while dementia praecox was persistent and crumbling.

Kraepelin's conceptualized Major Depressive Disorder and later Leonhard in 1957 coined the term "bipolar disorder" to recognize people who were diagnosed with both mania and depression (i.e., two poles) and those with just clinical depression (i.e., one pole, or "unipolar" depression). Specific reasons for sadness stay questionable. The current indicative systems depend on observational information that has distinguished ordinarily co-happening manifestations verifiably connected to depression. Thus, findings of real depression and related conditions depend on clinical evaluation of these side effects.

The two most broadly utilized criteria sets, to be specific, the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (5th edition; DSM-5) and the International Classification of Diseases (10th revision; ICD-10). Based on diagnosis symptoms of a minimum of four (ICD-10) or five (DSM-5) classifies the disease stage. Below are the symptoms:

Persistent feeling of sadness or bad mood.

- Anhedonia—loss of interest in delightful experiences
- Feelings of impracticality or low self-esteem
- Fatigue or being lethargic
- Excessive guilt or self-blame
- Notable changes in appetite and weight Anxiety
- Insomnia or hypersomnia
- Issues with concentration or indecisiveness

- Somatic problems such as back pain and headache
- Suicidal thoughts or behavior [1]

MDD is also known as clinical depression in medical terms. Those with this disorder may feel like having episodes for more extended periods. People with MDD have symptoms that arise every day, like feeling hopeless, sad, or numb, and decreased motivation for things that they used to like earlier [2]. All these symptoms will last for nearly two weeks. They will eventually lead to abnormal appetite resulting in either weight loss or gain, feeling fatigued, inability to concentrate, thoughts of death, self-harm, or even suicide.

One of the most common and significant symptoms in depressed patients is the recurring thoughts of suicide. Other symptoms of this disorder are called specifiers which can be classified into the following names-

1. Melancholic features: the tendency to get up early in the morning, feeling low or depressed in the morning sickness, feeling agitated, anorexia, depressed moods, and feeling of self-guilt.

2. Psychotic features: illusions, hallucinations, worthlessness, fatigue, etc.

3. The mental reaction is enhanced by events of positivity, increased food craving and weight, hypersomnia, and substantial feelings of being paralyzed in limbs [3].

1.2 Schizophrenia

Schizophrenia is a dangerous mental disorder that affects nearly 1% of the world population. At present, there is no established, effective drug to cure the same. The main reason for this limitation is that the mechanism involved has not been explored much on the molecular level [4]. Schizophrenia is a severe disorder that equally affects men and ladies and the more or less simple fraction of the world population. Many factors are found to be related to associate raised the risk to develop psychosis. In general, psychosis is a mental sickness with multiple factors contributing to the pathological process. The surge of psychosis is presumably set off by many turning points or “stressors” like infections, birth complications, drug abuse, urban background, or time of birth (higher prevalence for winter born people presumably because of infective agent triggers). However, the fundamental risk profile is especially looking on tributary genes, that is underpinned by the heritability of psychosis with up to eightieth in monozygotic twins. Different aspects like low economic standards and unmarried legal status have antecedently been attributed to a better risk of psychosis, which turns out to be an outcome of sickness and its connected negative consequences [5].

1.3 Bipolar disorder

It is an extreme mental disorder that can be differentiated by two episodes of depression, interpersonal problems, violence, and impulsiveness [6-8]. During manic episodes, affected patients require immediate hospitalization. It includes cyclothymic disorder, i.e., patients become creative and active due to enhanced mental and physical abilities. The presence of the manic or hypomanic condition in bipolar disorder distinguishes it from unipolar. Bipolar disorders can be classified into two subgroups: Bipolar 1 and Bipolar 2 disorder. In bipolar one, one or more

than one manic, mixed episodes are present [7]. While, in the case of bipolar two disorder patients, they experience hypomanic conditions as well as depression. The bipolar disorders usually occur in adolescence age and are almost equally distributed in males and females. Bipolar 2 is more common than bipolar 1 [9]. According to current studies, DSM-5 threw light on a new variety of some specific bipolar and their related disorder to leverage the hypomanic episodes of short tenure having insufficient symptoms, which does not make them fall in either bipolar one or bipolar 2 categories. Depression is seen in adolescence's tenure, usually have few of the mentioned features with probable chances of risk cancellation, which might be continued to the adult life [10-12]. Studies show and publish risk factors of depression as lower education status, poverty, rapid social change, insecurity, and hopelessness. Girls are more prone to such disorders as they often face certain societal situations [13, 14]. The 15-18 years of age may be a crucial time for analyzing vulnerability to depression, as the risk of depression is very high during this tenure. Young women are found more affected in adolescence period [15, 16].

1.4 Transcriptomics study for brain disorder

Charles Auffray coined the term “transcriptome” in 1996. It refers to the complete set of RNA transcripts obtained from the genome under particular circumstances; by using high-throughput sequencing methods. The science of the study of the transcriptome is referred to as “transcriptome analysis.” It is used to identify changes in a mutant phenotype and to discover pathways that respond to environmental stresses. RNA-Seq data find use in the identification of single-nucleotide polymorphisms, disease-associated gene fusions, and allele-specific expression. Also, the molecular characterization of organisms and tissues can be done at various stages of development by such an analysis method. The transcriptomic analysis helps in understanding the mechanisms of cellular differentiation and embryonic development. Hence, it is considered an excellent way of identifying targets for treating various diseases.

Transcriptome encompasses both the mRNA and non-coding RNA content in particular tissue cell types or organs. Along with the protein-coding mRNA, some non-coding microRNA regulates the expression due to their involvement in mRNA regulation. In specific disease conditions, the transcript for mRNA content and level of mRNA or transcript gets regulated. Multiple approaches are expertise for transcriptome study, microarray, and transcriptome sequencing.

Investigations of the transcriptome started a long time before its conceptualization. Large-scale surveys of gene expression in the murine thymus organ [17], the human cerebrum and liver [18], and human T cells came into the limelight since the mid-1990s. These autonomous gatherings utilized cDNA clones displayed on nylon films or glass slides to hybridize marked tissue-or cell-determined examples. These displayed cDNA clones spoke to the advanced microarrays models, presently used in transcriptome analysis [19].

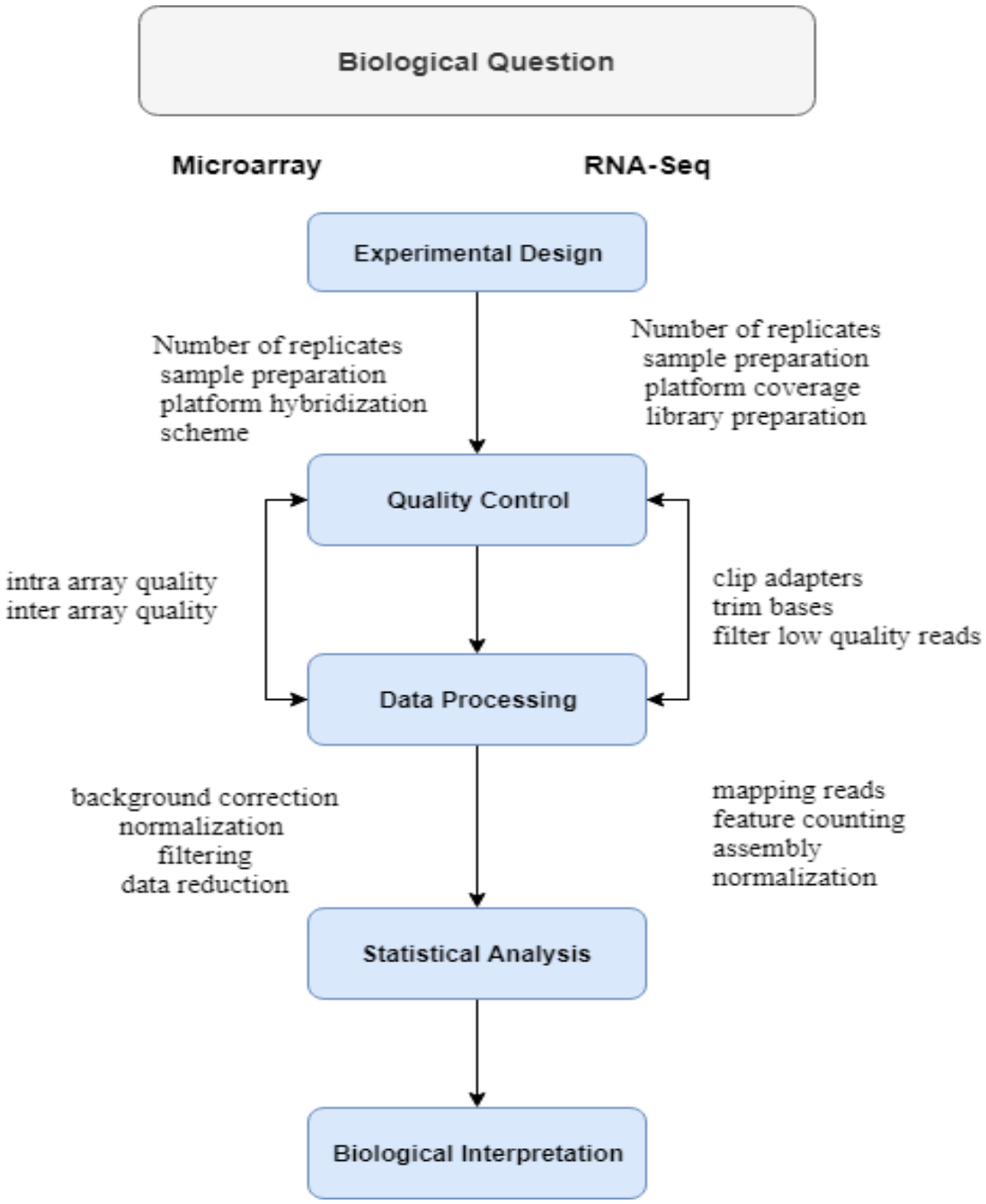

Fig:1 An overview of the steps in a typical gene expression microarray or RNA-Seq experiment [20]

II. Literature Review

Fiori and Turecki (2010) examined microarray studies that are accustomed to establishing genes displaying alteration of expression in suicide completers and focusses on a number of the essential method concerns and metabolic trajectories arisen from these experimentations

Schmidt et al.(2010) investigated hypocretin (hcrt) levels in patients with a frenzied episode and compared with age-matched patients with MDD and controls

Kang et al. (2012) detected the diminished expression of conjunction function-related genes in the dlPFC of MDD subjects and a corresponding reduction within the synapses range. Foreseen that the appearance of transcriptional represser is augmented in MDD and also decreases the expression of synapse-related genes, causes loss of spines and dendrites, and turns out depression

Kohen et al. (2014) found that miR-182 by microRNA target analysis. Since miR-182 has a discontinuous sign in mental disease patients, it is determined that there's a distinction in miR-182 in healthy subjects and people with affective disorder

Pantazatos et al. (2017) examined cluster variations in brain factor and miRNA expression between depressed suicides, depressed non-suicides, and overtime non-psychiatric controls. Differential expression factors are administered to spot the necessarily associated pathway for brain expression identification

Lisa M Chung et al. (2013) found that RNA-Seq technology was used to measure the transcript abundances by the generation of sequencing reads and to analyze their frequency count in various biological conditions. He also inferred that for identifying differentially expressed genes between two conditions, it is essential to consider both the experimental design and the distributional property of the data. With the help of the Bayesian hierarchical mixture model for RNA-Seq data, they accounted for the variability within and between individuals from paired data structures. They also performed identification of transcripts with prolonged treatment effects but with low expression levels. They concluded that the Bayesian model has better power for detecting differential gene expression than any other approach

Dillman et al. (2017) examined organic phenomena within the human dorsolateral cortical area victimization RNA- Seq to colonize a full factor analysis of the co-expression network. It results show that modules of enrichment of co-expressed genes for the cryptography conjunction proteins contribute to measuring the vulnerability to modification with age. Conjointly, the extent to which cellular composition changes account for age associations is calculable and located that their square measure freelance signals for physiological state and aging

Magdalena Sowa-Kućma et al. (2018) found that depression is related to inflated IL-6 trans-signaling and supermolecule peroxidation. The severity of depression, range of episodes, and dangerous makes an attempt area unit related to activated Immune pathways

III. Analysis of Expression Data

Analysis of transcriptome includes microarray and RNA-Seq data. Genome-scale transcriptomics of eukaryotes (for say humans) produces a large amount of data. Microarray data can be analyzed using open-source software, which is freely available, while NGS RNA-Seq data requires bioinformatics expertise. RNA-Seq data

analysis is computationally intensive, which requires high-end server and storage space. Details for the steps involved in RNA-Seq data analysis has been shown below:

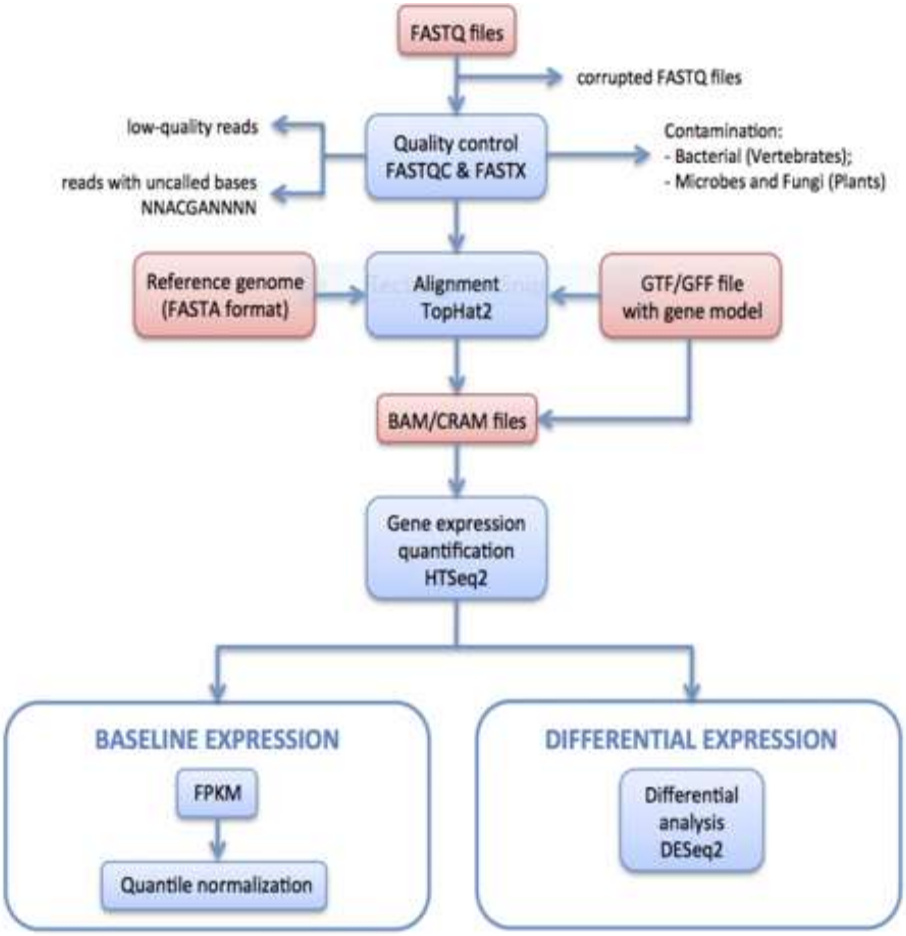

Fig. 2: RNA-Seq processing pipeline used to generate gene expression data [29]

The raw FASTQ files are first pre-processed to eliminate all undesired quality, such as corrupted FASTQ files, low-quality reads, reads with undesired bases, and those with any contamination. The quality control tool used for this is FASTQC or FASTX. This step is then followed by alignment of each of the sequences against the reference genome GTF (Gene Transfer Format) or GFF (General Feature Format) file to give rise to SAM (Sequence Alignment Map) or BAM (Binary Alignment Map) with the help of different alignment tools such as TopHat2, HISAT2, etc. The alignment results are used to quantify gene expression in the later stages, such as in baseline expression analysis or in the study of differentially expressed genes (DEGs).

3.1 Identification of significant DGE and disease associated markers

The essential objective of gene expression is to distinguish genes that are differentially communicated between RNA tests from two kinds of sample conditions. Differential gene expression can give bits of knowledge into natural systems or pathways and shape the reason for further trials by deciding the example and quality likeness utilizing clustering analysis or testing a gene set for enrichment.

Differential expression analysis scans the genes whose quantity has been modified substantially over the sample conditions. This implies taking the evaluated and standardized gene expression for every library and performing measurable testing between sample groups. In principle, the transcript count of the mRNA is associated with the number of reads sequenced for particular mRNA, which decide the expression level [29].

Based on the biological insight and significantly differentially expressed genes, we obtain the Molecular markers. Specific methods are present for testing of the gene set, inference of network and knowledge databases have been developed for analysis and understanding of lists of differentially expressed genes (DEGs).

Gene set enrichment analysis (GSEA) for the DEGs include a combination of functional themes, such as those defined by the Gene Ontology consortium [30], and signaling and metabolic pathways, such as KEGG pathways. Routes that are significant and overrepresented based on the DEG test for the set of genes would be able to identify associated markers for neurological disease.

IV. Conclusion

Dysregulation of specific genes, either up-regulated or down-regulated, are a possible cause for the neurological study. With NGS RNA-Seq pipeline, taken as the basis for identifying the essential associated gene, the genes of higher significance can be used as a marker for the diseased neurological condition. Improving technologies and computational devices reduces time and cost and enables researchers to understand complex problems better.

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