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REVIEW

Analysis of Downs syndrome with molecular techniques for future diagnoses

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Abstract Down syndrome (DS) is a genetic disorder appeared due to the presence of trisomy in chromosome 21 in the G-group of the acrocentric region. DS is also known as non-Mendelian inheritance, due to the lack of Mendel's laws. The disorder in children is identified through clinical symptoms and chromosomal analysis and till now there are no biochemical and molecular analyses. Presently, whole exome sequencing (WES) has largely contributed in identifying the new disease-causing genes and represented a significant breakthrough in the field of human genetics and this technique uses high throughput sequencing technologies to determine the arrangement of DNA base pairs specifying the protein coding regions of an individual's genome. Apart from this next generation sequencing and whole genome sequencing also contribute for identifying the disease marker. From this review, the suggestion was to perform the WES is DS children to identify the marker region.

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1. Introduction

Down syndrome (DS) is an autosomal genetic disorder that causes Intellectual disability and increased risk of organic disorders caused by the trisomy 21 (~21q22 region), appearance of additional chromosome leading to birth defects (Mendioroz et al., 2015). Chromosomal aneuploidy is one of the main causes of developing trisomy 21 (Kamhieh-Milz et al., 2014). DS affects ~1 in 1000 in live born children throughout the globe. Earlier studies have reported the risk of DS when the maternal age is greater than 40 years, and increased age of the maternal grandmother may increase the risk of DS. Maternal age plays an important role in the frequency of DS (Ellaithi et al., 2008). The phenotypic characters are brachycephaly, flat facies, upward slanting palpebral fissures, epicanthus, and low-set round ears with abnormal folds, epicanthus, and unique transverse palmar crease, among others. Diagnosis is purely based on clinical features, and cytogenetic analysis (Garduno-Zarazúa et al., 2013). The clinical symptoms of DS are protruding tongue, small head, poor muscle tone (hypotonia), short height, flattened facial features, short hands and fingers (Patil et al., 2014). The initial identification of DS was based upon the clinical symptoms followed by karyotyping and fluorescent in situ hybridization analysis. There are no biochemical, histological, pathological and molecular tests to diagnose the DS. Unfortunately, the diagnosis of DS was performed with the chromosomal analysis. Cytogenetics is the time taking process (> 72 h) for the identification of the diagnosis. The development of extra chromosome is due to the error in cell division (chromosomal non-disjunction in meiosis 1). Trisomy 21 is an error in meiosis, i.e. failure of normal chromosomal pairing or premature unpairing and has a recurrence risk of about 1 in 100. The appearance of trisomy 21 is due to the improper development of egg/sperm cells during meiosis and subsidizes extra chromosome.

2. History

Escorel was the first person to describe the Down syndrome (DS) in children in 1838 (Weijerman, 2011). Later on, the DS was discovered by British physician Prof. John Langdon Haydon Down (John Down) as a mental disorder in 1866. From his research, he analyzed the children with DS has common physical characteristics, similar to Mongolian race and termed the disease as Mongolian Idiocy or Mongolism and patients were denoted as Mongoloids. Later on Lejeune, Turpin and Gautier identified the third chromosome 21 in patients with DS in 1959. From this turning point, discovery lead to an understanding of DS as trisomy of chromosome 21 (Weijerman, 2011). The accurate cause of DS was discovered in 1959 with the clinical symptoms. In 1974, Nebuhr suggested that the "Down syndrome phenotype" might be caused by the duplication of only a part of chromosome 21 band q22, which represents about one-half of the long arm (Desai, 1997). Turkel (1985) proposed Down syndrome as a biochemical disease. If the whole chromosome was present in triplicate, then each gene was also in triplicate. Considering one gene at a time could reduce one large problem to many smaller ones (Baggot and Baggot, 2014).

3. Genetics and cytogenetics

The study of chromosomes is known as cytogenetics or chromosomal aberrations. Mendel's laws were rediscovered in 1900 and in 1903, the scientist Walter Sutton noted that chromosomes follow Mendel's laws and speculated that genes might be contained on chromosomes. In 1915, Morgan and colleagues published a synthesis of years of work entitled "The Mechanism of Mendelian Heredity", which made an almost incontrovertible case that genes are located on chromosomes. By 1920, the concept that chromosomes carry genes was widely accepted (Patterson and Costa, 2005). Finally in 1958, Tjio and Puck had confirmed that humans consist of 46 chromosomes (Tjio and Puck, 1958). James et al. (1999) were the first to observe an increased risk of chromosome non-disjunction due to abnormal folate metabolism, and this is responsible for abnormalities in the pattern of DNA methylation. Aneuploidy is defined as an abnormal number of copies of a genomic region and is the common cause for the development of genetic disorders. Aneuploidy was restricted to the presence of supernumerary copies (trisomy), or the absence of chromosomes (monosomy), but the definition includes deletions or duplications of subchromosomal regions (Antonarakis et al., 2004). DS are (i) non-disjunction, (ii) translocation and (iii) mosaicism. The 95% cases appear to be regular trisomy 21 (non-disjunction) in both males and females (47 XX or XY + 21). Robertsonian translocation is defined as the exchange of genetic material between chromosome 14 and 21/D and G groups (46 XX or XY rob (D or G; 21) (q10; q10), + 21. Mosaicism is termed as the presence of more than 2 different cell lines in the same individuals. Mosaicism is defined as the appearance of two or more populations of cells with different genotypes in one individual with a single fertilized egg or

Table 1 Frequencies of different modes of karyotypes.

Causes of DS	Mode of karyotype	Percentage
Non-disjunction	47 XX + 21/47XY + 21	95%
Robertsonian translocation	46 XX or XY rob (D or G; 21) (q10; q10), + 21	4%
Isochromosomes	46 XX/XY + 21, i (21) (q10)	
Mosaicism	47 XX/XY + 21 46 XX/XY	1–3%
Partial trisomy (21q22.3)	46 XX/XY, dup (21) (q22.3)	< 1%

occurrence of cells differ in their genetic component from other cells of the body. It can be germline mutation, somatic mutation, or a combination of both. DS is a somatic mutation that appears in the mosaicism of the non-Mendelian inheritance. Table 1 consists the mode of a type of DS, prevalence, and other details. DS is not a hereditary disease in trisomy 21 for non-disjunction and mosaicism. However, almost one-third of the cases are due to translocation which may be hereditary (1%).

4. Gene sequencing

Earlier studies by Hattori et al., 2000 sequenced the 21st chromosome and identified 329 genes, among them 165 are experimentally confirmed, 150 were based on expressed sequence taq databases, and 14 computer predictions. The actual fraction of chromosome 21 is transcribed into RNA might be an order of magnitude higher than the fraction occupied by gene coding sequences (Roizen and Patterson, 2003).

5. Gene identification

Gene identification is important for understanding the pathophysiology of disease and for improving diagnosis, prevention, and treatment (Khan et al., 2015). The sequence variation within a population is known as mutation and more than 1% of the population is known as polymorphism. Genetic variations take place in (i) simple base substitution (example: single nucleotide polymorphism) and (ii) insertion and deletion (example: variable number tandem repeat polymorphisms) (Barnes and Breen, 2009). The importance of gene polymorphism studies for human diseases is to identify single nucleotide polymorphisms (SNPs) in the coding region of genes. Almost, 90% of sequence variants in humans are differed by a single base in DNA sequences that code for the production of proteins. SNPs are common forms of genetic variations that can be used to search for and isolate the disease-causing genes. The importance of polymorphism in genetic studies are that (i) SNPs can be used to reconstruct the genome histories (ii) SNPs can be directly responsible for genetic diseases since they may alter the genetic sequence of gene or of a regulatory region, (iii) and SNPs may be utilized as markers to build high-density genetic maps needed to perform association studies, and to identify genes of functional importance. However, polymorphisms are not absolute indicators for the development of disease (e.g.: Alzheimer's disease and the ApoE gene) (Alharbi et al., 2015). Molecular sequencing tests were obtained for only single causative gene such as cystic fibrosis and sickle cell anemia. Sanger sequencing is the basic technique used in molecular diagnostics opting for a clinical testing method for genetic disorders in which both rare and common mutations make up a large percentage of causative variants (Rehm, 2013). Genome-wide association studies (GWAS) have identified large numbers of loci that contribute to the genetic basis of complex traits, whereas, linkage mapping and candidate gene loci underlying about one-half to one-third (~3000) of all known or suspected Mendelian disorders (Bamshad et al., 2011). Next-generation sequencing (NGS) or second generation sequencing now allows the rapid identification of causal mutations at single-nucleotide resolution even in complex genetic backgrounds. There is also ample evidence of modifying loci in Mendelian disorders, i.e. a mutation occurring in the gene leads to the disease in humans. NGS produces much larger quantities of data at less expense, but the individual raw sequence reads that are generated from individual amplified DNA-template sequences are shorter and have a lower quality (Lupski et al., 2010). Exome sequencing is a technique of sequencing all the protein-coding genes in a genome. It selects only the subset of DNA that encodes proteins and then sequences the DNA (Rabbani et al., 2014). Germline spontaneous mutations have the phenotypic consequences, affecting functionally relevant bases in the genome. Whole exome sequencing now permits to study the mutations and their role in disease in a systematic genome-wide manner. This approach has recently been used to identify causative genes in several rare syndromes (Vissers et al., 2015).

6. Exome sequencing

Whole exome sequencing (WES) is the present methodology to sequence the exon regions in the genes with less expensive sequencing cost per genome/exome. These revolutionizing sequencing technologies, so-called NGS, are promising to be used in the clinic to improve human health, although their expensive costs, ethical issues related to the produced genetic data and the need for user-friendly software in the analysis of the raw sequence have to be addressed (Rabbani et al., 2014). WES is an advanced technique used to analyze the DNA sequence of the exome and there are many challenges in the clinical applications and for the diagnostic tool including, by definition, lack of coverage of non-coding regions of the genome and variable depth of coverage of coding regions. This technique has begun to show promise, specifically in the genetic work-up of patients who present with a challenging constellation of phenotypic features that has sent both clinicians and patients on a diagnostic odyssey (Volk et al., 2015). WES is a highly effective form of high-throughput genetic analysis, constitutes >90% of the coding DNA of an individual is sequenced. Exome capture technique will be implemented and specific DNA probes will be targeted for precise exons in the genome are used to extract the protein coding portion from the remainder of the genomic DNA. The targeted DNA is then sequenced using NGS technologies, and the patient's sequence is compared with the human reference genome for the identification of genetic variants (de Bruin and Dauber, 2015). This technique has largely contributed in identifying the new disease-causing genes and represents a significant breakthrough in the field of human genetics (Tetreault et al., 2015). The combination of WES with autozygosity mapping, a technique that identifies regions of the genome inherited from a common ancestor in a consanguineous family, and candidate gene screening were used to demonstrate homozygous loss-of-function mutations in the FEZF1 gene in four individuals with Kallmann syndrome from two independent families. Functional evidence for the involvement of FEZF1 was identified using mice, in which this gene was found to be involved in olfactory receptor neuron migration into the CNS (Kotan et al., 2014; Eckler et al., 2011).

7. Whole genome sequencing

Determining the sequence of the entire human genome is referred to as whole-genome sequencing (WGS) Bick and Dimmock, 2011. Whole genomic sequence allows the detection of rare sequence variants that range in effect from causing diseases to modifying complex disease risk variants that would recently either not have been observed or could not be tested for association with the disease on a sufficiently large scale (Gudbjartsson et al., 2015).

8. Differences between WES versus WGS

WES is generally accomplished using an array that captures the DNA containing the coding sequence from the patient's sample. This captured DNA is then sequenced. An exome is less costly to sequence than a whole genome because the exome represents only about 1% of the genome (Bick and Dimmock, 2011).

9. Relation between DS and WES

Malsegregation is the main cause to prone the DS, a chromosomal condition associated with intellectual disability, characteristic facial appearance, and hypotonia in infancy. All affected individuals experience cognitive delays, but the intellectual disability is usually mild to moderate. In DS, Heart defect is one of the birth defects faced by 50% children. DS is a complex of genetic and epigenetic origin with protean neurobiological consequences and several characteristic neurodevelopmental manifestations (Capone, 2001). Till now no biomedical treatments are available for the central nervous system impairment seen in DS children. There is no treatment for DS but this disorder can be managed normally with corrective surgery, physical and speech therapy. However, enriched environments significantly increase children's capacity to learn and lead meaningful lives.

10. Conclusion

Genetic variants play a major role in both Mendelian and non-Mendelian inheritance to rule out the disorders/diseases (Choi et al., 2009). WES is being used clinically to diagnose rare disorders when there is no specific test to analyze the disease. From this review, future studies should be implemented with exome sequencing analysis in the DS children, may be to rule out the disease and identify the disease marker because DS is the future risk for specific diseases such as cognitive decline, dementia, and Alzheimer's disease.

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