Next generation sequencing: window to a new era of molecular diagnostics

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The advent of next generation sequencing has changed the scenario of diagnostic methodology. This new technique has been used in various areas ranging from the identification of new genes for single gene disorders, diseases with locus/ phenotypic heterogeneity, discovery of cancer causing genomic variations and understanding the pathogenicity of various microorganisms in human diseases.

Using single cell sequencing data to model the evolutionary history of a tumor[1]

Routine sequencing methods detect average signals generated by different cells within a tumor tissue. Single cell sequencing is a recently developed technology that detects genetic variation within a single cell and sequencing of different cells from a tumor can identify the cellular heterogeneity within a tumor. The analysis of mutations detected in different cells can identify the earliest mutation that is present in all cells and those that are present in further sub – clones. This helps to understand the sequential series of mutational events that resulted in a heterogeneous tumor. The major drawback of this technology is the high error rate. Kim and Simon have proposed a new statistical method that determines the order of mutation between any two sites and also reduces the error rate. They also provide a method to estimate the proportion of time from the earliest mutation event of the tumor to the most recent common ancestor (MRCA) of the cells sequenced. Based on the pair-wise mutation orders of all sites, the authors have constructed a mutation tree using the minimal spanning tree algorithm. The data was obtained by single cell exome sequencing of 58 cells from a patient with essential thrombocythemia (ET) and two tissue sequencing data (one normal and one tumor tissue). The process of carcinogenesis involves multistage progression of tumor clones, so identifying the order of mutation in various genes helps in better understanding of the tumor progression.

Whole–Exome Sequencing Identified KCNJ11 as the Thirteenth MODY Gene[2]

"Maturity onset diabetes of the young" is a heterogeneous disorder characterized by autosomal dominant mode of inheritance, onset before 25 years of age and a primary defect in the pancreatic β-cell function. Till now, 12 different types of MODY caused due to mutation in different genes have been identified and together they account for around 70% of the MODY cases and the remaining 30% are categorized as MODY-X. Bonnefond et al have done whole exome sequencing of four individuals (3 affected and one unaffected) from a large French MODY-X family. After analysis of the data they identified 324 variants of interest (present in all 3 affected individuals, not present in the unaffected individual and not reported in the database dbSNP130). The authors then assessed the status of these variants in an additional 23 members of the family and in 406 controls aged above 47 years with normal fasting blood sugar levels. Among variants that were not present in 406 controls, only one variant (c.679G>A; p.Glu227Lys) located in the KCNJ11 gene, at a heterozygous state was found to be present in all eight relatives with overt non autoimmune diabetes. Linkage analysis also suggested that KCNJ11 p.Glu227Lys mutation is causal for MODY in the analyzed pedigree. This mutation in KCNJ11 is already known to be associated

with neonatal diabetes mellitus and screening is currently indicated by guidelines in all patients who present with diabetes diagnosed before 6 to 12 months of age. As affected carriers of this mutation can be treated with sulfonylureas instead of insulin, this study suggests that molecular diagnosis of MODY should include KCNJ11 testing.

Lifting the lid on unborn lethal Mendelian phenotypes[3]

Hanan et al have shown, for the first time, the use of exome sequencing in identification of the causative genetic variation in embryonic lethal disorders. They studied a consanguineous couple with history of two fetal losses due to non-immune fetal hydrops. Fetal karyotypes in both the pregnancies were normal. In the current pregnancy, fetal hydrops was detected at 19 weeks gestational age with an otherwise normal ultrasound. So, considering consanguinity, the causal mutation was considered to be most likely homozygous. Exome sequencing of the fetus identified 4400 homozygous variants and out of them 440 were present within the autozygome. Out of these 440, 2 (R254C in CHRNA1 and P825S in SAP130) were novel, i.e not present in dbSNP and 250 controls. The mutation in CHRNA1 was particularly significant as this gene had been previously associated with multiple pterygium and fetal akinesia syndrome. The other was considered unlikely to be contributory, as mice homozygous for a transposon insertion in this gene lack similar phenotype. Thus, this study highlights the role of NGS in identification of the genetic cause of unborn lethal phenotypes.

Actionable Diagnosis of Neuroleptospirosis by NGS[4]

Approximately 50% cases of meningoencephalitis remain undiagnosed despite extensive clinical and laboratory evaluation. Because more than 100 different infectious agents can cause meningoencephalits, conventional diagnostic methods such as culture, PCR and serological tests can miss the diagnosis. The authors have proven the use of NGS for the identification of the infectious etiology in a 14 year old boy having severe combined immunodeficiency who presented with fever, headache, hydrocephalus and status epilepticus with history of minor illnesses for last 1 year. The workup for infectious diseases was negative. MRI of the head showed persistent hyperintensities in the basal ganglia and basilar leptomeningitis extending into the cerebral hemispheres. Biopsy of the right frontal lobe was performed and histologic examination revealed inflamed leptomeninges with a granulomatous infiltrate. Immunohistochemical testing and electron microscopy did not identify fungi, bacteria, or viruses. NGS of the cerebrospinal fluid and serum was done and bioinformatics analysis was done for the detection of all known pathogens which identified 475 of 3,063,784 reads of leptospira infection in CSF but not in serum. He was started on targeted antimicrobial therapy and gradually improved. Identification of leptospira infection was later confirmed by targeted PCR and Sanger sequencing. So, next generation sequencing has proven helpful in many clinical situations and its role has expanded from the identification of genes for known as well as uncharacterized Mendelian disorders to understanding the molecular genetic basis of cancers, common disorders and microbial infections.

References

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