Molecular Diagnostics in Complex Disorders: Considerations for Schizophrenia

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New advances in biological sciences in particular, the fields of molecular biology, biochemistry and genetics over the past few decades has led to tremendous progress in medical sciences. It is expected that information about human genes and their regulatory processes would allow the development of new diagnostic tools, provide insights into treatment and management of many disorders which thus far remain unclear. The human genome project was a 3 billion dollar initiative started in 1990 which took almost 20 years to complete, with parallel efforts in the USA and UK. The first human genome was fully sequenced in 2001, with a full draft genome available in 2002 [1]. This revolutionized the field of medical genetics, providing enormous opportunity to understand the linkage between human disease and medical genetics. A complete human genome sequence was published in 2022 [2]. However, twenty years later, we still do not understand the drivers of complex genetic disorders especially, those that involve multiple genes. To understand how molecular based diagnostics may be developed and employed, it is essential to understand basic cellular and molecular processes. Foremost is understanding of the structure of DNA and its organization into chromosomes which are the essential components of hereditary material.

DNA and genetic variations

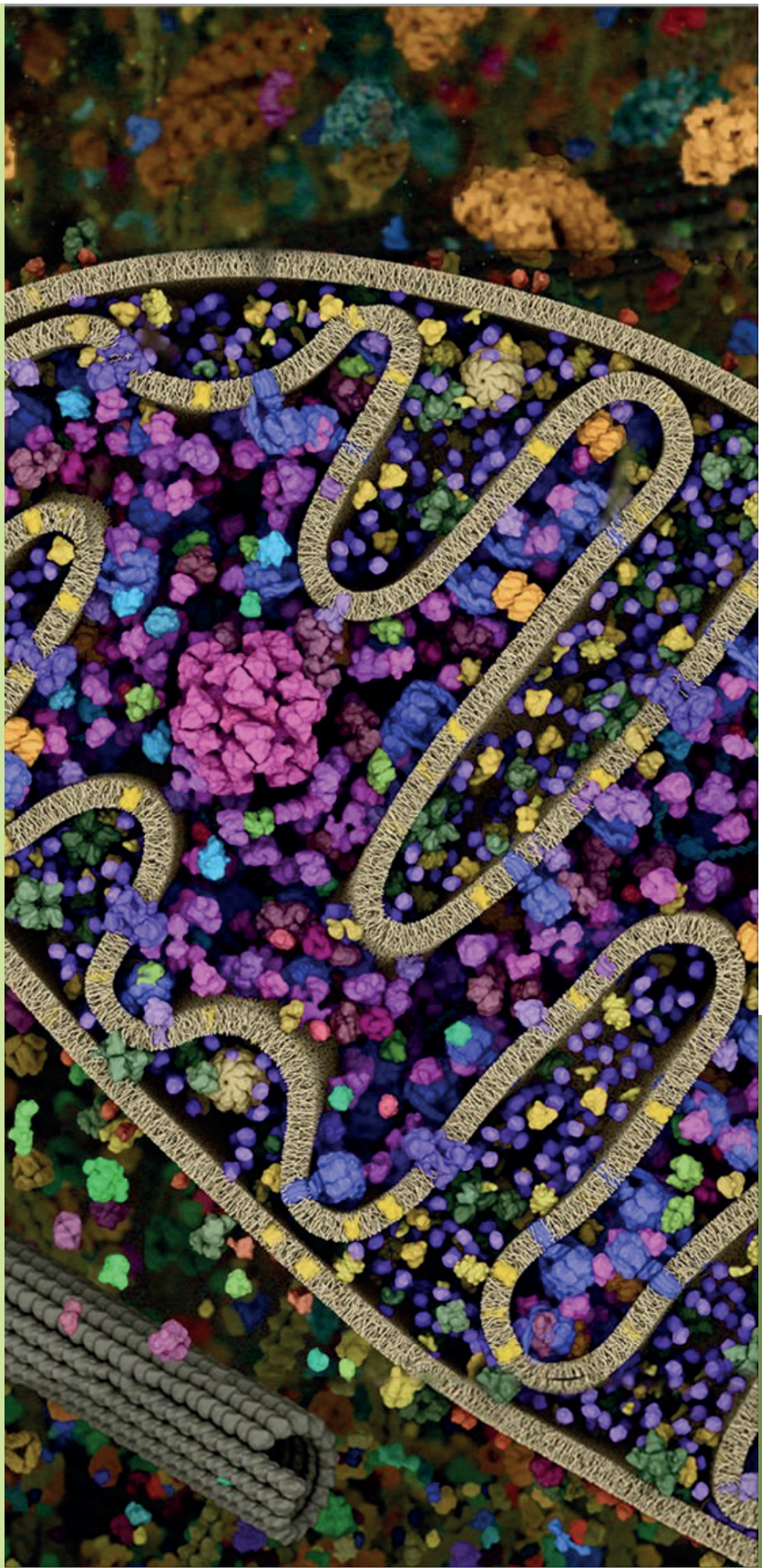
Cells have 46 chromosomes which contain 3 billion bases of DNA. Chromosomes are tightly packaged units of genetic material with genes kept under strict regulatory control, alternating between active and inactive states, leading to the switching 'on' and 'off' of genes. Each gene is a

single unit of inheritance, containing regions of regulation and expression. Genetic traits may be related to the 22 pairs of autosomes or the sex chromosomes, X or Y. Information passed on in a Mendelian manner results in transfer of information from parent to progeny in each generation. Whether or not a trait is evident in each generation depends on whether the mutation or change results in a recessive or dominant condition. DNA sequences are translated into messenger RNA which encode from proteins (amino acids). There may be several triplet codons for each amino acids, leading to a redundancy in coding, therefore, not all genetic variations result in a missense mutation – where the protein differs. The kind of nucleotide changes may vary from SNVs (single nucleotide variations), deletions, insertions, small or large structural changes or chromosomal aberrations such as, translocations or inversions or, copy number variations (CNV).

Impact of genetic variation

The impact of genetic changes depends on the location of the variation within the gene and also, the mode of inheritance associated with the particular trait. Genes may be located on autosomes or one of the sex chromosomes, X or Y. One of the first genes found associated with a particular disease strait was that from a single base pair mutation that leads to the sickle cell shape of hemoglobin, called sickle cell anemia, an autosomal recessive disease caused by a point mutation in the hemoglobin beta gene (HBB) found on chromosome 11p15.5. Hemaglobinopathies are common especially in Pakistan where there is a high occurrence of consanguineous marriages providing the opportunity for recessive disease orders to be manifested [3]. Mutations in HBB result in thalassemia which is 'major' if the change is on both alleles (homozygous, inherited from both parents), or 'minor' if the change is on one allele only (heterozygous, inherited from one parent).

In the case of linkage with genes on the X-chromosome, mutations result in expression in males whilst the same in a female lead to a carrier state but the



individual may be unaffected (due to the additional X chromosome that she carries). An example of such a disease is Duchene muscular dystrophy, caused by deletions or duplication mutations in the dystrophin gene [4].

Many medical conditions result from changes in polygenic loci such as, diabetes and cardiovascular diseases, where many genes have been associated with increasing disease risk. Mental health disorders are increasingly found to have genetic causes. Genetic changes have been associated triplet nucleotide repeat syndromes such as, Fragile-X and range of intellectual or developmental disabilities (IDD).

Molecular diagnostics for clinical disorders

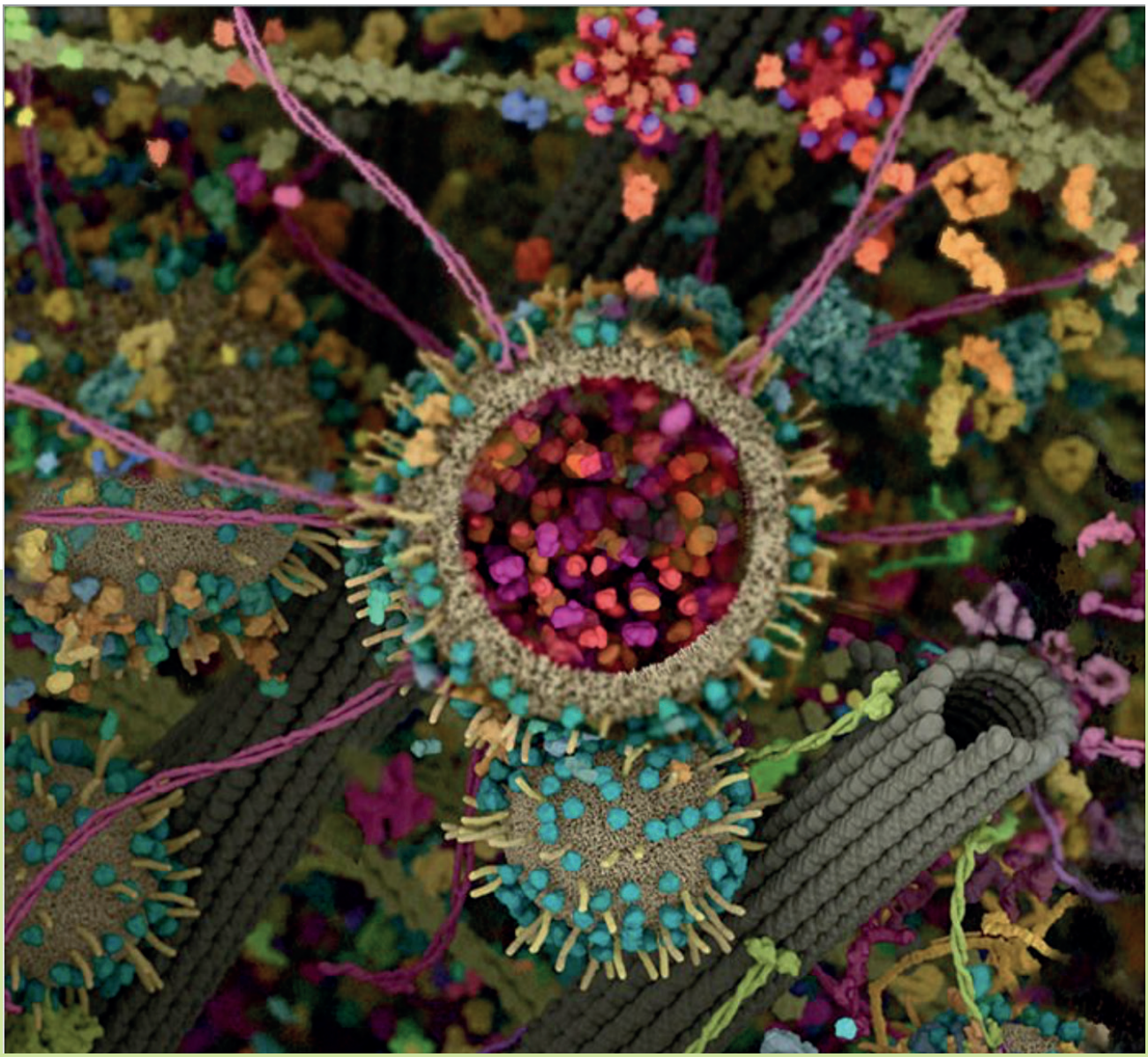
Our increasing knowledge about the workings of genes and their functions is enhanced by advances in molecular biology tools that lead to advances in diagnostics of genetics and genomics.

With increasing information available on human genomes from Genome Wide Association Studies (GWAS), we learn more about the effect of changes in the genetic code and any pathogenic effect.
The first full human reference genome published in 2022 provides a guide for CNVs. It is the projects such as the International HapMap Project and the 1000 Genomes Project where identification and mapping of millions of common single nucleotide polymorphisms (SNPs), have been instrumental for the GWAS approach. GWAS are based on understanding linkages between genes – the association of changes in alleles at two or more loci. Recent technological advances such as microarrays and chips have made it possible to quickly and inexpensively scan a million SNPs genome-wide.

The challenge is to fully understand the impact of genetic variation; to identify sequence modification (from reference) using particular methodologies and then to interpret the information appropriately in order for it to have an application. The field of Molecular Pathology is a branch of Pathology that includes, cytogenetics, human genetics and other molecular based diagnostics such as for, oncology or infectious pathogens. Interpretation of genetic data needs to be done with care and according to guidelines provided such as, by the American College of Medical Genetics (ACMG). Genetic changes can result in a 'Pathogenic mutation', or a 'likely Pathogenic mutation, alternatively they may be 'Benign' or a 'variant of unknown significance, VOUS' [5].

Genes related to mental health disorders

Increasing information gained through genome sequencing, GWAS studies and chromosomal microarray analysis have revealed micro-deletion, micro-duplication and CNVs associated with IDD [6, 7]. The role of environmental factors and epigenetic effects on mental disorders is also well known. Schizophrenia has been associated with



a strong genetic component. It is now established as polygenic, with risk alleles in many genes contributing the disease spectrum. Further, schizophrenia shares risk is also found to share risk alleles with other neuropsychiatric phenotypes, such as bipolar disorder, major depressive disorder, autism spectrum disorder, intellectual disability and attention-deficit hyperactivity disorder (ADHD) [8]. In particular, small microdeletion in a region of chromosome 22 called 22q11, including deletions on chromosome 1q21.1, 3q29, 15q13.3 and 22q11.2, and duplications on chromosome 16p11.2 and 16p13.11 all increase the risk of developing schizophrenia. A study from University College London has identified 10 genes associated with increased risk of the disease [9]. Genes associated with

schizophrenia include the dopamine receptor D2, and genetic markers across the Major Histocompatibility Complex (MHC) locus on chromosome 6 (25–34 Mb).

Molecular Pathology and the way forward

Diagnosis of genes associated with clinical disorders needs to be conducted and interpreted under international guidelines. Diagnosis of specific disorders may be conducted for screening, diagnostic confirmation, treatment and management of disease. Further, molecular diagnosis may be conducted in pre-natal specimens such as, amniotic fluid or chorionic villus sampling. These require complex laboratory settings, high level infrastructure and well trained staff and pathologists to give correct diagnostic reports.

Ethical considerations are important when interpreting molecular diagnostic reports. Patients must be counseled as to what the consequence of their test would be. Test interpretations may have impact on their immediate or subsequent generations. Therefore, genetic counseling is important. There is a very limited information on this in Pakistan. There are international standards for the operation of laboratories that conduct Molecular Pathology testing which include the College of American Pathologists, USA, and perform routine proficiency testing of their samples to keep with validation requirements. Aga Khan University Hospital has CAP accreditation since 2018 and also trained Residents in Molecular Pathology. The Pakistan Society of Medical Genetics is an interest group that is providing training for counseling of medical genetics and disorders [10]. This is essential in training a community of individuals who can accurately interpret and guide on genetic tests Further, it is essential that Molecular pathology training be made available at post-graduate medical centers and that it be part of Pathology residency curricula [11]. There are ongoing efforts for the introduction of a Fellowship for Molecular Pathology so that formal training for this important discipline is provided in Pakistan. Overall, it will be the combined efforts of establishment of advanced laboratory infrastructure and training of laboratory scientists, pathologists and genetic counselors which would be required for the development of Molecular Pathology services in Pakistan.

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