MENTAL RETARDATION;
MOLECULAR CYTOGENETIC TECHNIQUES USED FOR DIAGNOSIS; A LITERATURE SURVEY

REVIEW
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ABSTRACT... Mental retardation, also termed as learning impairment or cognitive dysfunction, is a serious manifestation of nervous system. The defining features of mental retardation are low or subaverage intellectual functioning (Intelligence quotient<70), impairment in at least two of the adaptive skills (e.g communication ability, self care, self guidance, reading, writing ability, etc) before 18 year of age[1]. Molecular cytogenetics is the study of genetic disorders using advanced technologies combined with cytogenetic and molecular methodologies[2]. Molecular diagnosis has equal importance as clinical diagnosis in mental retardation and day by day new advancement in these methodologies are being introduced by molecular cytogeneticists. The promising achievement of molecular cytogenetic techniques is the genetic counseling of high risk pregnancies. The current mini-survey of literature discusses an overview of these techniques employed to investigate deletion, duplication, inversion and translocation of chromosomes associated with mental retardation.

Key words: Mental Retardation, Cytogenetics, IQ, karyotype.

INTRODUCTION

Mental retardation (MR) affects approximately 1-3 % of the general population in the United States (Higgins et al., 2004) but this figure would be higher in developing countries because of high rate of consanguinity. Prevalence of MR in male is higher (30% excess[3] than female with a 1.4-1.6:1 ratio of affected male to female and this is mainly because of X specific MR genes and presence of single X chromosome[4]. Intellectual or cognitive functioning of an individual can be assess by "Intelligence Quotient" (IQ) so on the basis of IQ score mental retardation is subcategorized as.

- Mild (IQ level: 50-55 to 70),
- Moderate (IQ level:35-40 to 50-55),
- Severe (IQ level:20-25[5] to 35-40)
- Profound (IQ level: below 20-25)

(American Psychiatric Association, fourth edition and ICD-10 classification[6].

Molecular cytogenetics is the combination of molecular biology and cytogenetics. Their utility has increased the resolution and diagnostic utility in the field of MR. A number of cytogenetic techniques and their advanced version are introduced every year. The prime and initial step of molecular diagnosis is karyotyping i.e analysis of the chromosomes of the probands by investigating chromosome numbers and or chromosomal abnormalities. If karyotype is normal then fragile X testing is performed. Afterward, on the basis of clinical evaluation, chromosome analysis and fragile X screening results, copy number analysis, microdeletions and duplications analysis, inversion and translocation mapping, homozygosity and linkage analysis for mutations in the responsible genes are carried out. There are varieties of cytogenetic techniques for screening of whole genome to look for the aberration. These include conventional and spectral karytyping, interphase FISH, comparative genomic hybridization (CGH), primed in situ labeling (PRINS), multiplex amplifiable probe hybridization (MAPH), chromosome microdissection, and microarray.

Conventional Karyotyping

This technique is used for assessing the number and appearance of chromosomes in a cell. In this methodology, size, position of centromere, differences between sex chromosomes and other characteristics are considered and the result is presented in the standard format termed as karyogram or ideogram. During karyotyping the chromosomes are visualized by different staining methodologies G-banding followed by digestion

of chromosome with trypsin. It produces dark bands (heterochromatic region) and light bands (euchromatic region) while R-banding is the reverse of G-banding where light bands represent heterochromatic region while dark bands depict euchromatic region. In C-banding uses Giemsa stain is only constitutive in heterochromatic region (centromere). Q-banding is used quinacrine for staining, the banding pattern is similar to G-banding method. T-banding gives idea of alizes telomere. Silver staining is most powerful to study the nuclealar organization region (NOR) associated protein to check the activity of rRNA in NOR.

Spectral Karyotyping

In this technique all chromosomes are visualized simultaneously by staining them with different dyes. Chromosome specific fluorescently labeled probes are generated which gives a specific spectrum. The spectrum is analysed by an interferometer attached to a fluorescence microscope. Then image is processed by using software which assign specific colour to each chromosome and in this way chromosomal aberrations are detected.

Interphase FISH

Interphase fluorescence in situ hybridization, (interphase FISH) developed by Christoph Lengauer, is used for detection and localization of specific DNA sequences on chromosomes. It can be used to screen large number of cells (preferentially uncultured cells), even when the chromosomal irregularity is present at a low-level mosaic state because chromatin in interphase nucleus are less coiled and are ideal materials for analysis of microdeletions and duplications. Interphase FISH using a chromosome12-specific alpha satellite probe, a whole chromosome paint, or a microdissection paint specific to a band on 12p has been used to detect isochromosome 12p i(12p) from fibroblast cells, uncultured lymphocytes, and buccal mucosal cells[7,8]; Ohashi et al[8]., suggested that buccal smear preparations generate significant number of positive cells as comparison to phyto-hemagglutinin-stimulated lymphocytes, harvested T and B cells and cultured skin fibroblasts as done in i(12p), the main feature of Pallister-Killian syndrome. Similar strategy is followed by Pipiras et al[9] while working on three new unbalanced structural aberrations on 13q22q33, 14q31qter, and 3p26pter loci. Advantages of Interphase FISH (on direct buccal smear preparations) include rapid, effective, and non-invasive confirmation of a chromosomal syndrome. Food and Drug Administration (FDA) has approved five sets of commercially available probes specific to chromosomes 21, 13, 18, X, and Y. A swift prenatal detection of common aneuploidies by employing interphase FISH on uncultured amniocytes or chorionic villus cells is a method of choice in many diagnostic labs and increasingly used in molecular cytogenetics for genetic counseling of high-risk pregnancies[10].

Comparative Genomic Hybridization (CGH)

Comparative genomic hybridization (CGH) or chromosomal microarray analysis (CMA) is an advanced molecular cytogenetic technique in which the whole genome is screened for chromosomal imbalance on the basis of copy number changes in a single hybridization experiment and does not requiring the mitotically active sample material[11]. CGH can be used in constitutional karyotyping of prenatal[12];[13], postnatal[14], and pre-implantational[15] cases. CGH can only detect loss or gain of DNA sequence in terms of copy number, while structural changes like translocation, inversion cannot be determined. CGH has detection power of 2–10 Mb[16]. Array CGH is the most advanced modification of microarray based CGH methodology.

Primed in situ labeling (PRINS)

PRINS is a cytogenetic method which is alternative to FISH for chromosome anomalies analysis because of its less time consumption (1–4 hr), cost effectiveness and high specificity. This technique can be used for detection of repetitive DNA sequences, like telomere repeats[17], centromeric alpha satellite sequences[18], Alu sequences[19], and single-copy genes[20-22] Koch et al[23]., first develop this method since then numbers of changes in its methodology have been made such as Single-, double-, and multicolor PRINS[24]. Same group[18] developed a panel of primer sets specific to every human chromosome (except 6, 19, and 20) used efficiently for detection of aneuploidies (e.g., Down syndrome). This technique can be employed for detection of subtelomeric deletion by using[17] telomere specific TTAGGG repeats as the primer.

On the basis new advancements, PRINS can also be used for screening of microdeletions associated with the Prader Willi/ Angelman and DiGeorge/Velocardiofacial syndromes[22].

Multiplex amplifiable probe hybridization (MAPH)

Different versions of microarray-based approaches, e.g comparative genomic hybridization (array-CGH), have been introduced in molecular cytogenetics, one such technique is termed as array-MAPH which is advancement of conventional MAPH method. Array-MAPH is considered as unique technique because of low test DNA complexity prior to hybridization, similar to target sequences on the microarray, yielding a mixture of specific probes, and increased hybridization specificity. In this technique, 400-600 bp long amplifiable fragments are designed for any genomic region under observation. After PCR amplification, products are spotted onto arrays as targets and are mixture and hybridized to mixture and genomic DNA immobilized on a membrane. At the end recovery of bound probes are done by quantitative PCR and hybridized. This methodology can also be employed for determination of small scale copy-number changes as well[25,26].

Chromosome Microdissection

This methodology is based on the removal of a large segment of DNA from a complete chromosome. In this method, least portion of DNA that can be removed ranges up to 10 million base pairs, hundreds or thousands of individual genes. This technique is used to generate DNA sequences specific for a chromosome, an arm, or a region and used for various purposes, including genomic mapping and gene isolation[27] and generation of chromosome, arm, or region specific paints for FISH analysis and multicolor FISH banding[28]. Microdissection analysis, in case of MR, has been used in characterization of markers, rings, deletions/duplication, and complex rearrangements[29]. Microdissection can also be used in prenatal[30] and postnatal diagnosis. Microdissection in association with SKY and FISH, an extra ring chromosome consisting of the entire 10p was identified in a patient with MR and other multiple congenital anomalies. This case represents another new cytogenetic mechanism leading to the formation of pure complete trisomy 10p. Diagnosis can be made by this technique even on long-term stored specimens as verified by Chinen et al[31,32]. by identifying two different chromosome abnormalities[10].

Microarray

Microarray is the most recent, advanced, elaborative and precise molecular cytogenetic technique which analyses whole genome for copy number changes and gene expression and single nucleotide polymorphism analysis (SNP analysis). This method can potentially diagnose deletions/duplications of a complete chromosome (e.g., Down syndrome), a chromosomal segment, or disease-specific sequences (e.g., deletion of 22q11 in DiGeorge syndrome) or a mutation by linkage analysis.

CONCLUSIONS

Mental retardation is a heterogeneous disorder with no set clinical criteria associated with mental retardation that's why scientists are more emphasizing on molecular cytogenetic techniques as compared to clinical techniques and are seeking to introduce new cytogenetic techniques in the field of molecular neurology or making advancements and improvements in the old techniques. The current review has addressed some of the frequently used techniques in MR and their applications; in near future many more are expected to be launched by cytogeneticist that will aid in the prognosis, diagnosis and genetic counseling of inherited mental retardation disorders.

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