

Genetic Analysis of Mental Retardation: The Genes and the Means

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ABSTRACT

The past few decades have seen a dramatic increase in the field of research in mental retardation. Significant work has been done on the genes involved in the various mental disorders. Cytogenetic assessments of such disorders analyze the association between the occurrence of chromosomal aberrations and specific syndromes. Recent techniques include the study of fragile sites, high resolution banding to identify subtle chromosomal aberrations and more recent techniques such as Fluorescent in Situ Hybridisation (FISH). These techniques, in addition to the various other molecular diagnostic assays, are valuable in the diagnosis, prevention and management of mental retardation. In addition, they are useful aids for appropriate Genetic Counselling for the affected and their families. New avenues of Gene therapy are available for treatment of these disorders.

Key Words: Mental Retardation, Genes, Cytogenetics, Fragile sites, High Resolution Banding, FISH, Genetic, Counselling, Gene Therapy

Introduction:

Mental Retardation has been defined in terms of social competence. The term mental retardation (MR) covers a broad range of disorders that affect an individual's mental capacity in terms of cognition, motor skills and response behavior. Guthrie (1961) estimated that 40% of severe mental retardation could be prevented. mental retardation was estimated to be twice in males than in females (Jacobs, 1978).The American Psychiatric Association defines MR as the combination of (Landgren et al, 1996):

- (1) Tested IQ at or under 70
- (2) Problems with learning and social adaptation
- (3) Symptoms that begin before 18 years of age

The degree of mental retardation (which ranges from mild through moderate to severe) and its manner of manifestation greatly varies. Therefore it is critical to recognize the type of disorder clearly in order to establish phenotype genotype correlations accurately. More often than not, such disorders occur through complex interactions between the

environment and the Genes. Etiology based classification of Mental Retardation has been detailed in the Atlas by Holmes et al. (1972).

The past decade has seen tremendous advancements in the diagnosis of mental retardation, none more so than in the field of understanding the genetic causes of these disorders. Cytogenetics involves the study of heredity from a cellular point of view, focusing mainly on the chromosomal aspects. However, the era of molecular genetics heralded the arrival of many new diagnostic tools for the investigator in this field. Nowadays the classical genetics studies are well complimented by these modern techniques.

Genetic disorders are classified on the basis of the number of genes and mode of inheritance involved into:

1. Single Gene Disorders i.e. Monogenic. Eg. PKU, Tay Sach's Disease.
2. Polygenic Disorders. Eg. Microcephaly.
3. Chromosomal Aberration- structural and numerical. Eg. Down's Syndrome
4. Sex linked disorders. Eg. Fragile X Syndrome.
5. Somatic Mutations. Eg. Neoplasms and Cancers.
6. Mitochondrial Inheritance. Eg. Myopathies.

Cytogenetic Analysis of Mental Disorders:

Analysis of these disorders is carried out in a systematic manner. The patient's family history and pedigree are taken, clinical examination, dermatoglyphic analysis, buccal smear analysis, biochemical assays, HLA tissue-antigen typing and karyotyping are done. A karyotype is defined as a set of chromosomes prepared from a photomicrograph of a metaphase spread and its diagrammatic representation is called an Idiogram. Karyotypes are usually made from easily obtainable tissue such as peripheral blood lymphocytes, bone marrow and skin. In case of prenatal diagnosis, amniotic fluid, chorionic villi samples and fetal blood is used.

Routinely 25 to 30 metaphases (chromosomes are most condensed at this stage) are analyzed. Chromosomes in each spread are counted to identify the presence of any numerical abnormality. Four or more of these spreads are photographed and at least five figures are drawn and all the chromosomal complements identified. Karyotypes are made from these photographs. Normal metaphase banding resolution is between 450-500 bands. All the karyotype nomenclature is done in accordance with the guidelines of the International Society for Chromosome Nomenclature (ISCN, 1981)

The technique of High Resolution Banding (HRB) is used to study more subtle chromosomal variations that may contribute to these disorders. It can give upto 1000 bands. Here the chromosomal analysis is done at the prometaphase stage, where the chromosomes are more elongated. There are various methods to obtain HRB. Some of the more commonly used chemicals are 2 Mercaptoethanol (2ME), Ethidium Bromide

(EtBr), Methotrexate (MTX), Actinomycin D and Bromo deoxyUridine (BdUr). The technique of HRB has been used to identify numerous subtle chromosomal anomalies (Yunis et al 1978 and Yunis, 1976) such as:

1. del 15q 11-13 (Prader Willi Syndrome)
2. del 16p 13.3 (Rubenstein--Taybi Syndrome)
3. del 22q 13 (Di George Syndrome)
4. del 5p 15.2 (Cri-du-chat Syndrome)
5. del 17p 13.3 (Miller-Dieker Syndrome)

The Fluorescent in Situ Hybridization (FISH) is a molecular Cytogenetic technique. This method of analysis utilizes labelled oligonucleotide probes. These probes are hybridized with the sample and then viewed under fluorescence microscope to obtain the appropriate probe signals. Newer and more specific probes that are commercially available reduce the risk of non-specific hybridization. As hybridization can be done at both interphase and metaphase, this technique provides a simple mean to detect aberrations such as translocation, deletions, inversions etc and even numerical aberrations. Other techniques based in this are, Whole Chromosome Painting (WCP), Comparative Genomic Hybridization (CGH) and Spectral Karyotyping (SKY).

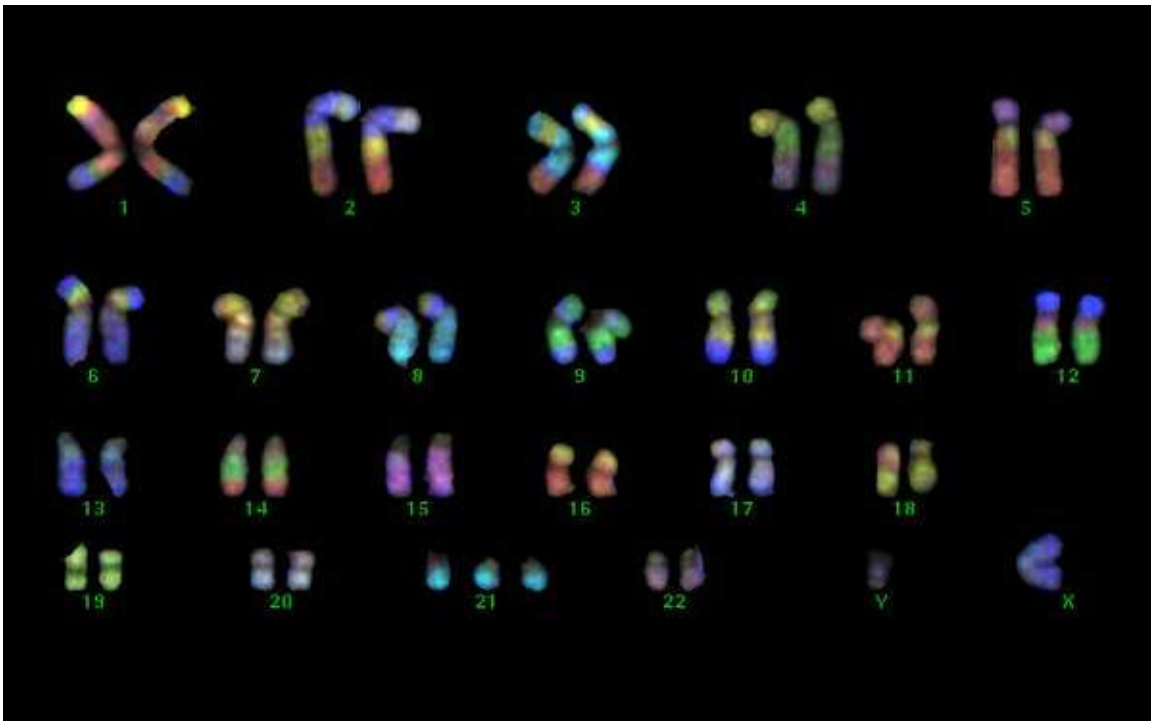


Image1: Shows the Karyotype (Multiplex FISH) of a patient with Trisomy 21 (47 XY, 21+)

Genetic Causes for Mental Retardation

The most common genetic disorders that have been shown to cause MR are Trisomies. The common ones are trisomy 21(Down's syndrome), trisomy 13 (Edward's Syndrome) and trisomy 18 (Patau's Syndrome). In trisomy 21, which causes Down's syndrome, the

affected child inherits three chromosome 21's, often due to non-disjunction of chromosomes during meiosis. Sex linked chromosomal aberration are seen in Klinefelter's Syndrome (47, XXY; extra copy of X chromosome) and Turner's syndrome (45, X 0; no X or Y chromosome).

Another type of aberration is one which involves parental genomic imprinting (Hall, 1991; Hall, 1992). Such is the case in two related syndromes Prader-Willi and Angelman's Syndrome. Both have distinct phenotypes but occur due to a deletion of the 15th Chromosome at q13 region. Prader-Willi manifests itself when the paternal copy gets deleted and Angelman's when it occurs in the maternal copy. Another interesting aspect in these two syndromes is that they can occur as a result uniparental disomy, wherein both of the chromosomes (in this case the 15th) are inherited from one parent. Mascari et al (1992) demonstrated uniparental disomy in 20 percent of the thirty cases of Prader-Willi Syndrome studied by them. If both the chromosomes are identical then it is known as Isodisomy (Voss et al, 1989). Such a condition can lead to expression of recessive disease traits. In addition to the 15th Chromosome, uniparental disomy is also seen in chromosomes 4, 6, 7, 11, 14, 16 and 21.

The predominant heritable form of X linked mental retardation is that of the Fragile X syndrome. This occurs at the Xq 27 region in the FMR-1 Gene. Here the expansion in the number of trinucleotide repeats (CGG), beyond a certain number, causes inactivation of the gene via methylation. The resultant loss of the protein FMRP causes mental retardation. Males carry the abnormality on only the X chromosome present in each cell (in the hemizygous state). They show 5-50 percent of affected cells in karyotype preparations. However, females carrying only one affected copy show 1-30 percent of affected cells in the karyotypes. Hence the females are less affected in comparison to the males. Recently it has been shown that the Loss of function mutation of FMR-1 indirectly affects the translational regulation of miRNA- targeted mRNAs that are also indirectly regulated by FMRP. The first case of Fragile X in the Indian population was reported by Manjunatha et al (1988).

Weatherall (1983) studied 100 children with severe mental retardation to determine the cause of mental retardation. In 34 (one-third) children the cause could not be ascertained (Table 1). This type of mental retardation is known as Idiopathic Mental Retardation.

Recent advances in molecular genetics have shown that idiopathic mental retardation has a genetic component. Cryptic chromosomal rearrangements are seen in most cases of this type of retardation. A study by Slavotenik et al (1999) showed that dysmorphic features of idiopathic mental retardation were due to 18q deletion of 5.6 Mb (Mega Bases) and 1p 4 Mb deletion. Granzow et al (2000) identified an unbalanced cryptic translocation der (5) t(3;5) (q27-p15.3) in a family with three cases of unexplained mental retardation and dysmorphic features using Multiplex FISH assay.



Image 2: Photograph of The Metaphase of a Patient with Fragile X chromosome (Arrowhead shows the Fragile Site)

Table 1

Different categories of severe mental retardation (Weatherall, 1983)

Category	%
KNOWN	
1. Pre-fertilization	
Chromosomal	
(i) Down's Syndrome	32

(ii) Other Autosomal	2
(iii) Sex chromosomal (Average of both sexes)	6
Monogenic	15
2. Intrauterine (Environmental (maternal infection))	2
3. Perinatal (Asphyxia / cerebral hemorrhage)	7
4. Postnatal (Meningitis / Encephalitis / Trauma / Hypoglycemia)	2
Total Known	66 (2/3)
UNKNOWN	
With congenital defect/ dysmorphic features	14
With additional evidence of brain damage	10
Without other abnormalities	10
Total Unknown	34 (1/3)
Grand total	100

Discussion: What the future holds

With the rapid development of numerous diagnostic tools available for molecular cytogenetic studies of mental retardation the scope for discovering new genes is tremendous. Also, the detection of serious birth defects can be genetically detected by prenatal genetic studies. These complications threaten the lives of 3% of live births and

account for one-fifth of neonatal deaths. Some of the techniques used in prenatal Genetics are amniocentesis, chorionic villi sampling, foetal biopsy and ultrasonography. In vitro fertilization has ushered in the era of preimplantation genetics. Three general approaches in preimplantation genetics are polar body biopsy, trophoctodermal analysis and blastomere analysis. The last of these techniques, Blastomere Analysis Before Implantation (BABI) was pioneered by Handyside et al (1992).

Another important aspect that is closely linked to diagnosis is Genetic counselling. Accurate counseling is vital, as it not only provides the family with critical information as to the nature of the patient's disorder, but also about the care necessary for him / herself. Usually counselling is done for the families who are at high risk for a child with a genetic disorder (Fig. 1). Consensus is taken from clinicians, sonologists, and laboratory diagnosis. A probability is then estimated from the family history, cytogenetics, investigations, relevant literature and Bayesian calculations. Following the counselling, the parents can decide whether they want to go ahead with the conception or not. In either case the decisions may need referral to other specialists. If the 'go ahead' decision by them results in recurrence, further counselling maybe needed.

The basic concept behind Gene therapy is the introduction of a functional copy of a gene to replace or supplement the activity of the resident defective gene. This is more applicable to well defined single gene disorders associated with mental retardation such as PKU and Lysosomal Storage Diseases. Commonly for human disorders only somatic cell based gene therapy is possible. The most common method for introduction of the appropriate gene is by using vectors. Many viral vectors are currently available including numerous retroviral and adenoviral constructs. One such strategy consists of the insertion of cDNA among two viral Long Term Repeats (LTRs) in a plasmid vector. These constructs are then introduced into cultured cell lines that provide the necessary packing protein for the pseudo viral assembly. The target cells can be removed purified and infected with these viruses and repopulated in the organ from which they were derived (Beaudet et al, 1994). Systemic delivery is easier than to other tissues. The first gene therapy trial was done for the Adenosine Deaminase Gene, the deficiency of which results in Severe Combined Immune Deficiency or SCID (Miller, 1992; Anderson, 1992). The major drawbacks of this type of therapy are stable long term gene expression, proper gene integration and unwanted gene activation and inactivation.

Stem cells provide another promising avenue for gene therapy. These cells are pluripotent or multipotent (have not yet undergone complete differentiation). A common source for the stem cells is embryos i.e. Embryonic Stem cells (ES). Adult stem cells such as the Mesenchymal Adult Progenitor Cells (MAPCs) are also used. These cells can be used to replace cells which are lost due to some genetic defect. In July 2002, a study by Kim Hoon J. et al, showed that ES can be induced to form Dopaminergic neurons, similar to those lost in Parkinson's Disease. There are numerous ethical issues on harvesting and usage of these stem cells in therapy and this is still a very expensive method. The newest arrival in the field is the use of the mechanism of RNA interference (RNAi). This is a naturally occurring process in cells wherein double stranded RNA

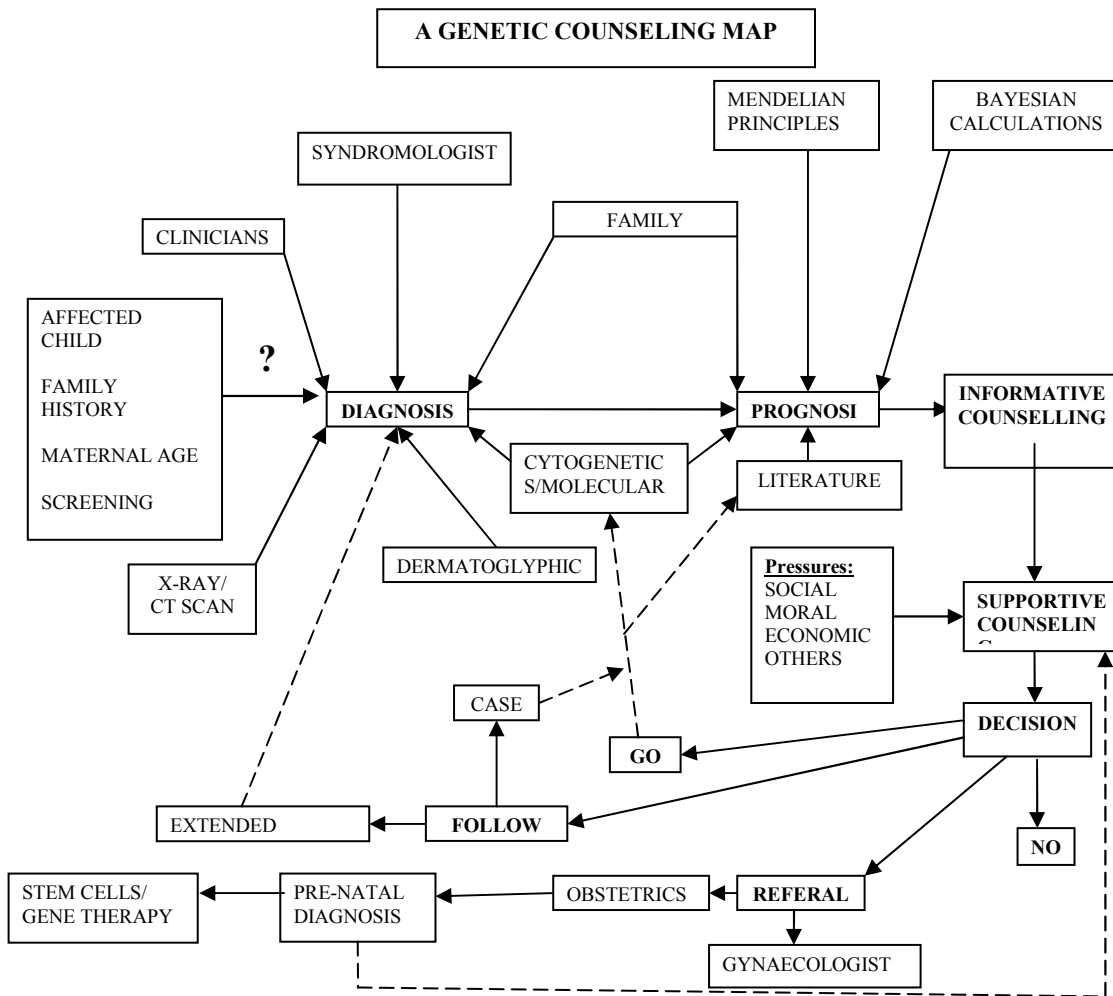


Fig. 1

Map of the Genetic counselling process. It begins with a question, raised by the advent of a child with a ? Genetic disorder, other aspects of the family history, etc. There must be a diagnosis which may depend on information from clinicians, syndromologists, laboratory tests, cytogenetics, etc. To answer the question usually requires estimating a probability, using information from the family history, cytogenetics, literature, etc. Following the two phases of counselling namely Informative and Supportive, the counselee may reach a decision to either refrain from conception (NO) or to go ahead (GO). If the GO decision results in recurrence, further counselling may be required.

induces translation silencing of the corresponding mRNA and its degradation, thereby effectively silencing the gene. This technique is still very new and is more in use for identification of genes and their functions than in therapeutics. Systemic delivery of these RNAs invokes a strong Interleukin 1 (IL 1) response. However, in 2004, Southshek et al achieved silencing of endogenous gene by systemic administration of modified siRNAs (Short Interfering RNAs).

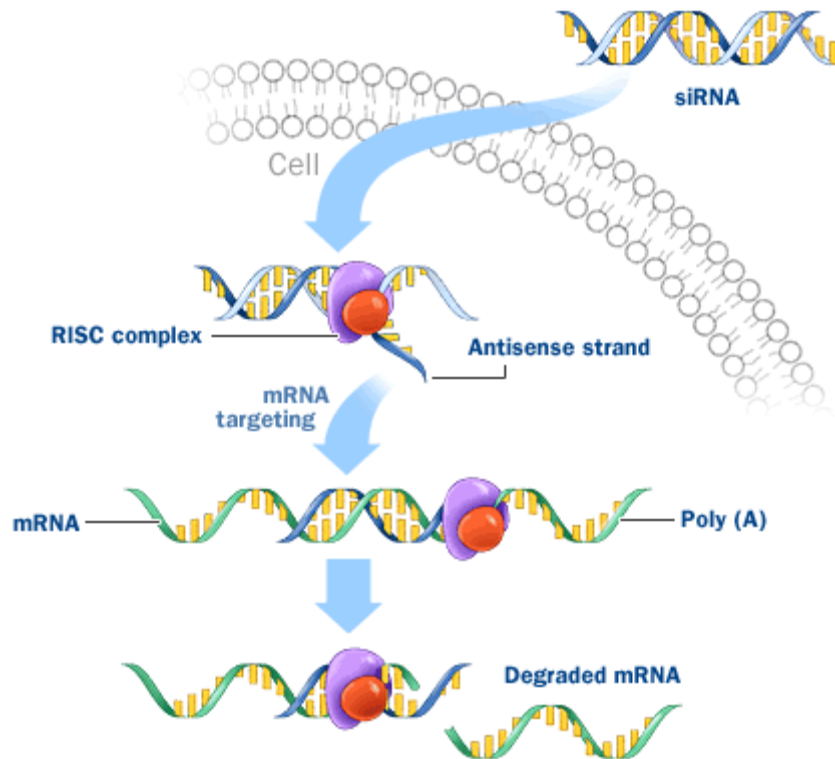


Fig. 2
The RNA Interference Pathway

Conclusion

The evolution of both diagnostic and therapeutic tools is far from complete. Thanks to the numerous advancements, few of which are mentioned above, it is now possible to provide early accurate diagnosis of the genetic mechanisms involved in the various mental disorders. Effective counselling of the affected individuals and their family members can be done. Thus better prevention, management, monitoring and indeed treatment of these disorders is possible.

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