Article *NIMHANS Journal*

Newer Genetics in Mental Retardation - I: Fragile X Syndrome and Triplet Repeat Mutations

Volume: 14 Issue: 03 July 1996 Page: 201-207

~~Kallur P Suresh~~ .

Reprints request

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- *Registrar in Psychiatry, Roundhay Wing, ST. James University Hospital, Beckett Street, Leeds LS9 7TF, UK*

Satish R Girimaji &, - *Department of Psychiatry, National Institute of Mental Health & Neuro Sciences, Bangalore 560 029, India*

K R Manjunatha, - *Department of Human Genetics, National Institute of Mental Health & Neuro Sciences, Bangalore 560 029, India*

Abstract

In the last five years, there have been tremendous advances in the field of medical genetics. In a two-part article, we review the relevant literature with special reference to the genetics of mental retardation. Fragile X syndrome is instructive in its inheritance pattern, aetiology and as a condition with a specific genetic form of psychopathology. Triplet repeat mutations have been found to cause several neuropsychiatric diseases including fragile X syndrome, spino-bulbar muscular atrophy, myotonic dystrophy, spino-cerebellar ataxia type I and Huntington's disease.

Key words -

Mental retardation,
Fragile X syndrome,
Triplet repeats

Medical Genetics has come a long way from the time of Gregor Mendel, so much so that, it is increasingly recognised as a separate medical speciality in many countries. Ever since the publication of the structure of Deoxyribose Nucleic Acid (DNA) by Watson and Crick in 1953 [1], [2], newer developments in DNA technology and genetic engineering have had far-reaching impact on the practice of medicine. From the era of cytogentics, we have stepped into the era of molecular genetics and molecular medicine [3], [4], [5]. Cytogenetics studies the cellular aspects of heredity focusing on the chromosomes, which are large enough to be seen under the light microscope, whereas molecular genetics goes into the chemical and molecular basis of genes and the reactions that they undergo and control. The central dogma of molecular biology i.e., DNA (transcription) → RNA (translation) → Protein has been basic to all our understanding of heredity and variation. Newer techniques have been invented in molecular genetics, that have revolutionised genetics research. These include in-situ hybridisation, polymerase chain reaction for the amplification of DNA, the use of molecular probes of Restriction Fragment Length Polymorphisms (RFLPs). Recombinnt DNA technology [6] has given us new insights into the etiopathogenesis and treatment of many diseases.

Mental Retardation (MR) of varying degrees is an important manifestation of several genetically determined syndromes.

In the last five years, there have been several revolutionary advances in the genetics of MR. In the first of a two-part series, we review the recent advances in fragile X syndrome and triplet repeat mutations. The second part gives an overview of genomic imprinting, uniparental disomy, newer parental screening techniques and gene therapy.

Advances in molecular genetic techniques have demonstrated that, the phenotypes of many multisystem disorders are due to the involvement of genes related to each other by their physical proximity rather than by their function - which Schmickel called "continguous gene syndromes" or "microdeletion -microduplication syndromes" [7]. MIller-Dieker syndrome, Prader-Willi syndrome, Angelman syndrome, Langer-Giedion syndrome etc, are microdeletion syndromes while Pallister-Killian syndrome and Beckwith-Wiedemann syndrome are microduplication syndromes. Rubinstein-Taybi and Cornelia de Lange syndromes are possible candidates for the discovery of chromosomal abnormalities [8].

Fragile X syndrome

Fragile X syndrome is the most important X linked condition associated with MR and developmental disability [9]. It is the commonest known heritable cause of MR [10] and at least the second most common specific genetic form of MR in males and females [11]. It has an estimated prevalence of 1 in 2500 males and 1 in 200 females. The syndrome accounts for approximately 7 per cent of moderate and 4 per cent of mild MR among males and for approximately 2.5 per cent of moderate and 3 per cent of mild retardation in females [12]. The syndrome has many of the characteristics of the desired prototype condition for the biological investigation of a specific form of psychopathology. The opportunity to study individuals with a specific genetic cause of psychopathology makes this condition particularly interesting to biological psychiatrists [9].

Fragile sites are microscopically observable variations in the structure of the chromosome, that are detected in the karyotype preparation when cells (such as lymphocytes) are subjected to specific culture conditions. In the case of fragile X syndrome, culture conditions resulting in the depletion of folate and thymidine (e.g., folate deprivation, methotrexate, 2'-deoxy-5"-fluorouridine) are required to elicit the characteristic fragile site on the distal end of the long arm of the X chromosome (band q 27.3) [9]. The actual molecular deficit has only recently been elucidated - it is the expansion of trinucleotide repeats (vide infra) in the FMR-1 gene [13].

Males with fragile X syndrome carry the fragile X abnormality on the only X chromosome present in each cell (hemizygous state), which is maternal in origin. They show 5-50 per cent of affected cells in a karyotype preparation. Females carry the abnormality on one of the X chromosomes in each cell (heterozygous state) and hence show less percentage of affected cells (1-30 per cent) in the karyotype [9].

Clinical features

Fragile X males have more distinct clinical features as compared to females. Facial characteristics include a long face, large and/or prominent ears, and prominent jaw and forehead. Other common features include joint hypermobility, hypotonia, mitral valve prolapse and in post-pubertal males, macro-orchidism, the last few features implying connective tissue dysplasia [14]. The non-specificity of the features and the inconsistency with which they are observed in affected patients indicates that the diagnosis cannot be ruled in or ruled out on the basis of clinical features alone [9].

Neuropsychiatric features

Fragile X males

Males are almost always mentally retarded and more severely affected than females. The retardation is moderate to severe [9]. They tend to have a flat cognitive profile with deficits on tasks tapping visual spatial skills [15]. They have greatest difficulty processing novel, sequential information especially when short-term memory and flexibility in problem solving are required [16]. There is evidence to suggest a decline in IQ over middle childhood years because they do not acquire cognitive skills at a rate predicted by their initial IQ [17].

Global retardation of language development is also seen. Rapid rhythm of speech, preservation, impulsivity in speech, echolalia and low-pitched voice are characteristic [18]. Fragile X males show a greater frequency of social, communication, sensory and motor abnormalities than can be accounted for by their cognitive level alone. Some of the features such as stereotypies, poor eye contact and lack of reciprocal social interactions overlap with those of autistic disorder [19]. If a significant association is found, fragile X syndrome can be a prototypal condition for the study of pervasive developmental disorders and can have many genetic, neurobiological, educational and therapeutic implications, that can be applied to pervasive developmental disorders in the general population [9].

Fragile X females

Fragile X females are cognitively less affected with only one third being mentally retarded, most of them in the mild range. Adult fragile X females, show impairments associated with dyscalculia, right-left disorientation, attentional difficulties and constructional apraxia [20]. Reiss et al [21] found a strong association between the presence of fragile X chromosomes in female members of the family and a history of bipolar and schizoaffective disorders. In another study [22], a larger proportion of fragile X group than the control group met the Research Diagnostic Criteria for schizotypal features. Thus, in females, the dysfunction is in social interaction, thought processes and affective regulation. Depending on which of the two X chromosomes in the cell undergoes lyonisation (inactivation), the female will accordingly resemble or differ from the typical clinical and neuropsychiatric features.

Recently, Rousseau et al [23] have described a method of identifying carriers of fragile X mutation by direct DNA analysis using Southern blotting with a probe adjacent to the mutation target. This established the diagnosis unambiguously and is more powerful than cytogenetic testing or seggregation studies. They conclude, that it is a reliable primary test for the diagnosis of fragile X syndrome after birth, as well as for prenatal diagnosis and genetic counselling.

Preventive screening

One thousand nine hundred and seventy seven intellectually handicapped persons were screened for fragile X syndrome in Australia by Turner et al [24] by cytogenetic testing and 40 probands were found. Family studies identified 84 women as being at high risk. They were offered genetic counselling and prenatal diagnosis. Turner et al [24] recommend cytogenetic screening in all currently identified intellectually handicapped people, followed by routine screening of children newly identified as intelectually handicapped in schools. Sutherland et al [25] have reported a case in which prenatal diagnosis of fragile X syndrome was made by direct detection of the unstable DNA sequence [25].

More recently, Willemsen et al [26] have developed a rapid antibody to identify fragile X patients from a blood smear.This involves a non-invasive test and requires only one or two drops of blood. The lack of expression of the FMRP protein in the lymphocytes of fragile X patients is made use of in the test. It can be used for screening large groups of mentally retarded people and neonates for fragile X syndrome.

Triplet repeat mutations

For a long time the human genome was considered as a stable collection of genes which are inherited with little or no change in their structure or function. Diseases with genetic origin were thought to be caused or mediated by the 'rare' mutations, that would alter the gene structure by deleting a portion of it or changing its coding sequence [27].

But, recently a new form of human mutation - expansion of trinucleotide repeats - has been discovered to cause the diseases of fragile X syndrome [28], spinobulbar muscular atrophy (Kennedy's disease) [29], myotonic dystrophy [30], spinocerebellar ataxia type 1 [31] and most recently, Huntington's disease [32]. Thus, the human genome is populated by DNA sequences which are not all that static and vary with considerable frequency. Since the discovery of dramatic instability of DNA sequences, triplet repeat mutations have become one of the major tools for mapping hereditary defects to specific chromosomal locations and in some cases, they themselves cause a genetic disease [13], [27], [33].

Trinucleotide repeats consist of tandemly organised stretches of the same nucleotide sequences over and over. The core repeating unit can vary in size from as few as 2 bases to as many as 100 or more. These undergo mutation occasionally so that the number of repeats is increased or decreased. As a result of this, varying lengths of the same repeat can be found on different chromosomes. Simpler types of repeats are called microsatellites, where a small core unit is repeated several dozen times [34]. More complex ones are termed minisatellites with a core repeating unit of 10 to 100 bases [35], [36]. These repeats are extremely polymorphic in humans, unevenly distributed in the genome and are most often found near the ends of chromosomes. Because of their high polymorphism, no two human beings (except identical twins) are likely to have the same pattern of repeats at all chromosomal locations. The repeats also serve as excellent genetic makers for inheritance studies as they can be tracked through several generations of a family with a genetic disease, in order to search for correlation with the inheritance of the disease [37].

The genetic basis of fragile X syndrome is the expansion of a CCG repeat in a gene called FMR-1 gene located on the X chromosome [33]. The repeat is a 4.8 kb mRNA. In the normal population, the mRNA of the FMR-1 gene is expressed in highest levels in the brain and the testes-two of the major organs affected in fragile X syndrome. The function of the FMR-1 gene is unknown at present. Within the 5' untranslated portion of the message is a region of multiple repeats of CCG. As described above, the CCG repeat is highly polymorphic in the normal population, the length of the repeat ranging between 6 and 42 triplets. In pedigress with fragile X syndrome, the length of the repeat expands from one generation to the next. Unaffected members of the family may have between 50 and 200 repeats. This is the stage of "premutation" which is unstable and expands further to cause clinical disease in subsequent generations. Affected members may have expansions of upto several thousand repeats of CCG in the said region. The size of the premutation in women correlates with their likelihood of bearing affected children [13].

Thus, the inheritance pattern of fragile X syndrome shows a form of genetic "anticipation". It means increasing severity and decreasing age at onset of the disease in succeeding generations [38]. The mechanism of anticipation is not yet known. Anticipation is seen in other neuropsychiatric disease like spino-bulbar muscular atrophy (Kennedy's disease), myotonic dystrophy, spino-cerebelar ataxia type 1 and Huntington's disease [13].

More importantly, there is evidence to suggest, that several other disorders exhibit features of genetic anticipation. The most striking of them is bipolar affective disorder, which is much commoner than the above diseases. In a study of pedigress with unilineal transmission of bipolar disorder [39], marked anticipation was found, with age of onset about 11 years earlier and episode frequency about twice as great in the second generation compared with the first. Schizophrenia, also has inheritance patterns consistent with anticipation in some pedigrees [40].

The exact mechanism of the origin of triplet repeat mutations is unknown. New alleles may result from "slippage" or "stuttering" of the DNA replication machinery during DNA synthesis [27]. There is a good chance that repetititve elements will misbehave during DNA transactions. This may involve unequal crossing over between repeats or DNA strand misalignment during replication or repair [41].

How do these mutations expand in succeeding generations? Laird has suggested a hypothesis which involves a two-step mutational event [42]. The first step consists of an event at the fragile X locus which by itself is not sufficient to cause phenotypic expression of the clinical syndrome. The second step is the process of inactivation-incomplete reactivation in a female embryonic cell i.e., pre-oogonial cell. Passage through a female increases the risk in the next generation for children to be affected. This is known as the Sherman paradox i.e., the male grandchildren of transmitting males have greater chance of being affected than the children of such males [43].

The premutation renders the DNA sequence unstable and it can expand to much greater lengths during female meiosis. In fragile X syndrome, there appears to be a relationship between methylation of a CpG island in a region 5' to the FMR-1 gene and expansion of the repeat [13]. Once the expansion has taken place, it becomes more and more unstable and continues to expand in subsequent generations, with the cycle presumably ending, when the disease becomes so severe as to prevent reproduction. Rarely, contraction of an expanded repeat can occur back to normal length and this has been described in myotonic dystrophy [44].

It is uncertain how expanded triplet repeats cause the disease. A study using magnetic resonance imaging found selective atrophy of the cerebellar vermis in fragile X patients compared to either normals of patients with other developmental disabilities [45]. It is not clear how this, relatively selective effect results from a mutation in a gene, that is so widely expressed in the brain.

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