“Mental Retardation: Study of cytogenetic etiology”

A Dissertation Project

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Dr.Sonal Bakshi

Contents

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Abstract

The present study was carried out in patients of mental retardation for phenotypic and genetic analysis. The medical history of 128 patients suffering from mental retardation was obtained by filling questionnaire, aided with Internet database query for phenotypic characters as guided by clinical assessment and chromosome analysis by Karyotyping.

The patients enrolled had following distribution of phenotypic characteristics:

Condition	Occurrence (%)
Developmental delay with convulsions	1.56
Down syndrome	14.06
Mild mental retardation	11.71
Moderate mental retardation	43.75
Severe mental retardation	10.93
Mental retardation (degree unknown)	6.25
Cerebral Palsy	7.81
Autism	3.9

Out of these, 5.46% patients had parents with consanguineous marriages, 69.53% patients had parents with non-consanguineous marriages, while of 25% patients, and the details about consanguinity were not known. Karyotyping was done in 6patients showing Non-syndromic Mental Retardation by standard technique of GTG Banding. The technique included Trypsinization and Giemsa staining of metaphase chromosomes in Phytohemaglutinin-M-stimulated lymphocytes cultured from peripheral blood. **All Karyotypes were normal** indicating the absence of any microscopic changes in the chromosome complement of the patients.

In addition, **phenotypic characterization** of patients was also done using POSSUM software. Abnormal features observed in the patients were entered in the POSSUM database, and according to the pre-set algorithm the threshold value was defined for each search. The database query resulted in list of possible syndromes in which combination of these anomalies are observed.

Only karyotyping is not sufficient in detecting submicroscopic changes in the chromosomes and hence clue regarding ruling out further anomalies is required. In addition to normal

karyotypes, phenotypic characterization in clinical terms can provide a clue to the possible underlying submicroscopic chromosomal anomalies like microdeletions and other rearrangements.

These cases are required to be considered for further analysis using whole genome scanning approach for precise conclusions. It is important to reach the diagnosis in non-syndromic patients of mental retardation by clinical assessment and genetic characterization with the available tools for data mining. The combined exercise can help in differential diagnosis, prognosis, selection of specific therapeutic &/or supportive treatment, and risk assessment in future progeny of parents as well as close relatives.

Introduction

1. Congenital disorders:

A congenital disorder is a condition existing at birth or that develops after birth during the first month of life (neonatal disease), regardless of causation. A congenital disorder may be the result of genetic abnormalities, the intrauterine environment, errors of morphogenesis, infection, or a chromosomal abnormality. The outcome of the disorder depends on the complex interactions between the prenatal deficit and the postnatal environment (Birth defects research, centres for disease control and prevention). Congenital disorders vary widely in causation and abnormalities.

2. Chromosomal Abnormalities:

A chromosome anomaly, abnormality or aberration reflects an atypical number of chromosomes or a structural abnormality in one or more chromosomes. Chromosomal abnormality can be structural or numerical. Chromosomal abnormalities affect at least 7.5% of all conceptions. Most of the foetuses with these abnormalities are spontaneously aborted and the frequency in live births is 0.6% (Connor and Ferguson-Smith, 1991). Around 3% of all births are associated with a major congenital malformation, mental retardation, or genetic disorder, a rate that doubles by seven to eight years of age, with later-appearing or later-diagnosed genetic disorders (Milunsky *et al*., 1992).

3. Mental retardation:

A great number of chromosomal disorders are associated with **mental retardation.** According to the World Health Organization, the prevalence rate of mental retardation in industrialized countries comes close to 3% (Roeleveld *et al*., 1997). The condition is present in 2 to 3 percent of the population, either as an isolated finding or as part of a syndrome or broader disorder. In at least 30 to 50 percent of cases, physicians are unable to determine etiology despite thorough evaluation. Diagnosis is highly dependent on a comprehensive personal and family medical history, a complete physical examination and a careful developmental assessment of the patient. These will guide appropriate

evaluations and referrals to provide genetic counselling, resources for the family and early intervention programs for the patient (Daily DK *et al.*, 2000).

Mental retardation (MR) is a congenital or early onset lifelong impairment of cognitive adaptive functioning or daily living skills. It is a serious and lifelong disability that places heavy demands on the society and the health system. The prevalence of visual and ocular disorders in children with MR is high, and can influence sensory-motor development and learning ability (Amira A. Abdel Azeem *et al.*, 2009). Around 1-3% of world population is suffering from mental retardation. Globally, at least 7.6 million children are born annually with severe genetic or congenital malformations (Anupam Kaur *et al.*, 2010) which has been defined as an Intelligence Quotient score under 70 (AAMR, 1992). Chromosomal abnormalities occur in 6% of all recognized congenital malformation. It also accounts for 30-40% of severe mental retardation, and 10% of mild mental retardation (Raynham *et al.*, 1996; Ahuja *et al.*, 2005).

Mental retardation is a subtype of intellectual disability and includes deficits that are too mild to properly qualify as mental retardation, too specific (as in learning disability), or acquired later in life, through acquired brain injuries or neurodegenerative diseases like dementia. This may appear at any age**. Syndromic mental retardation** is intellectual deficits associated with other medical and behavioural signs and symptoms. **Non-syndromic mental retardation** refers to intellectual deficits that appear without other abnormalities.

American Association on Intellectual and Developmental Disabilities (AAIDD, 2002) has defined MR as significant limitation both in intellectual functioning and in adaptive behaviour, which covers many everyday social and practical skills. This disability originates before the age of 18. Intellectual limitations refer to an Intelligence Quotient (IQ) which falls two standard deviations below the population mean of 100 (<70) (AAMR, 2000).

4. Mental retardation: Definition:

Mental retardation (MR) or intellectual disability (ID) is a descriptive term for sub average intelligence and impaired adaptive functioning arising in the developmental period (< 18 y). (http://emedicine.medscape.com/article/1180709-overview). It is the single largest neuropsychiatric disorder in every civilized society.

5. Mental retardation can be seen as:

Syndromic mental retardation

Non-syndromic mental retardation

5.1. Syndromic mental retardation is intellectual deficits associated with other medical and behavioural signs and symptoms. The genetic cause is known and the possible diagnosis is also known. The most prevalent genetic conditions include Down syndrome, Klinefelter's syndrome, Fragile X syndrome, Neurofibromatosis, congenital hypothyroidism, Williams's syndrome, Autism, Phenylketonuria (PKU), and Prader-Willi syndrome. Other genetic conditions include Phelan-McDermid syndrome (22q13del), Mowat-Wilson syndrome, genetic ciliopathy, and Siderius type X-linked mental retardation.

5.2. Non-syndromic mental retardation, also called **idiopathic mental retardation** or IMR, refers to individuals who show no evidence of gross chromosomal defects or single-gene anomalies. It is sometimes considered as representing the lower end of the IQ distribution. Its genetic causes are unknown. This type is of prime concern in our study**.** Idiopathic mental retardation is an etiologically heterogeneous group with some individuals showing retardation secondary to specific genetic causes, others because of environmental effects and the remainder due to multifactorial causes. (http://www.biology-online.org/articles / genetics-mental - retardation/idiopathic mental-retardation.html)

6. Levels of mental retardation:

Mental retardation is a spectrum disorder, with a wide range of severity. Mentally retarded individuals are typically classified into five different categories based on their level of functioning or severity as measured by IQ and adaptive functioning scores:

- **Profound mental retardation,** in which the IQ of the patient is below 20
- **Severe mental retardation,** in which IQ of the patient ranges from 20-34
- **Moderate mental retardation,** in which IQ of the patient is in the range of 35-49
- **Mild mental retardation,** in which the IQ of the patient is in the range of 50-69
- **Borderline mental retardation,** in which the IQ of the patient ranges from 70-84

6.1 Mild Mental Retardation:

This group constitutes the largest number. Persons in this group are considered "educable", and their intellectual levels as adults are comparable to that of the average 8 to 11 year old child. Their social adjustment often approximates that of the adolescent, although they tend to lack the normal adolescent's imagination, inventiveness, and judgement. Ordinarily they do not show the signs of brain pathology or other physical anomalies. Often they require some measure of supervision due to limited ability to foresee the consequences of their actions. With early diagnosis, parental assistance, and special educational programs, the great majority can adjust socially, master simple academic and occupational skills, and become self-supporting citizen.

6.2 Moderate Mental Retardation:

Individuals in this group fall in the educational category of "trainable". In adult life, individuals classified as moderately retarded attain intellectual levels similar to that of the average 4 to 7-year-old child. Some of the brighter ones can be taught to read and write a little, and some manage to achieve a fair command of spoken language, the rate of learning is relatively slow among members of this group. Physically, they suffer from bodily deformities and poor motor coordination. With early diagnosis, parental help, and adequate opportunities for training, most of the moderately

retarded can achieve partial independence in daily self-care, acceptable behaviour, and economic usefulness in a family or other sheltered environment.

6.3 Severe Mental Retardation:

Individuals in this group are sometimes referred to as "dependent retarded". Among these individuals, motor and speech development is severely retarded and sensory defects and motor handicaps are common. They can develop limited levels of personal hygiene and self-help skills, which somewhat lessen their dependence, but all their lives they will be dependent on others for care.

6.4 Profound Mental Retardation:

The term "life support" mental retardation is sometimes used in referring to individuals in this category. Most of these persons are severely deficient in adaptive behaviour and unable to master even the simple tasks. Useful speech is on the rudimentary level. Severe physical deformities, central nervous system pathology, and retarded growth are typical, and convulsive seizures, deafness, and other physical anomalies are common. These retardants must be maintained in custodial care all their lives.

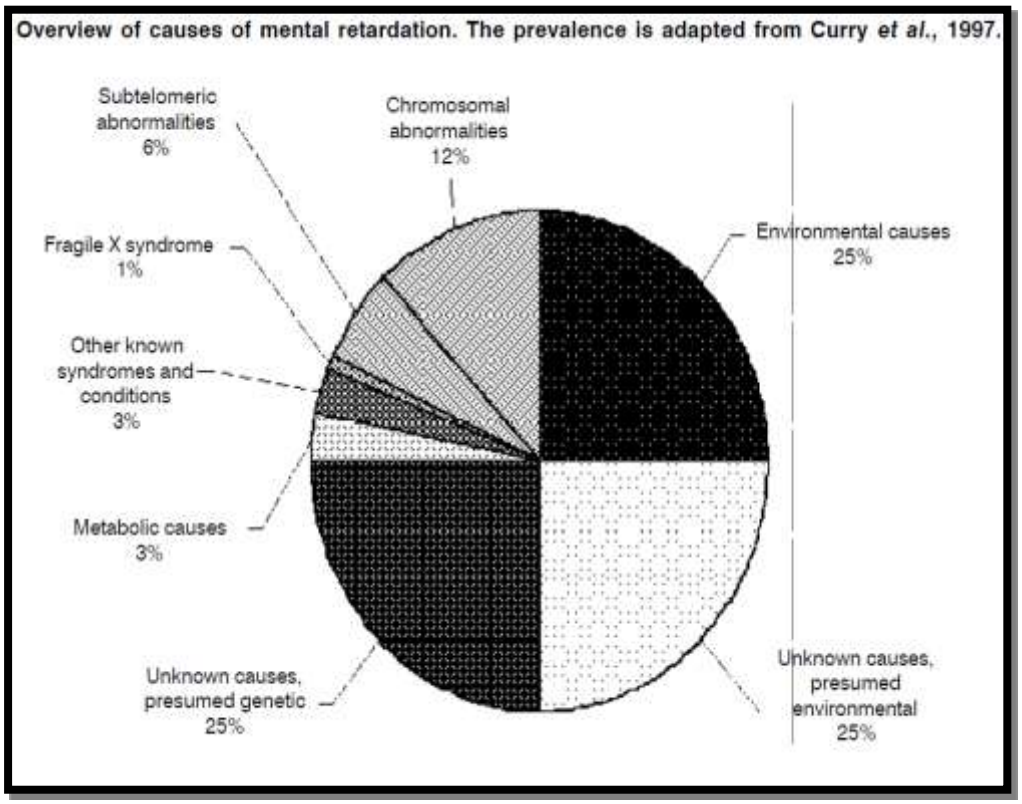
7. Signs and Symptoms:

Children with mental retardation have a slow growth and development of senses and adaptive behaviours e.g. they may learn to sit, crawl, talk, walk etc. little late than normal children.

The adults and children with mental retardation show some characteristic symptoms like:

- Delay in oral language development
- Repressed memory skills
- Difficulty learning social rules
- Difficulty with logic and reasoning
- Delays in development of adaptive behaviours such as self-help or self-care skills

8. Causes of mental retardation:



(Source: Kooy *et al*., 2003)

The syndromic and idiopathic mental retardation can be caused due to variety of reasons and degree of risk will depend on the causes which are characterized as follows:

- Infections (present at birth or occurring after birth)
- Chromosomal abnormalities
- Environment
- Genetic abnormalities and inherited metabolic disorder
- Metabolic
- Nutritional
- Toxic
- Unexplained

In the etiology of mental retardation (MR), environmental factors may involve the prenatal, perinatal, or postnatal stages of development, while genome-attributable defects are conceptually prenatal, independent of the neurodevelopmental moment when

the cognitive defect manifests (Araceli Lantigua Cruz *et al.,* 2008). Thus in general, the origins of mental retardation can be classified as prenatal, perinatal, and postnatal and as independent category psychoses with mental retardation, describing as unclassifiable those cases with no criteria for discerning their etiology (Gustavson K H *et al.,* 1977).

8.1. Postnatal Causes:

- **Infections**: Bacterial and viral infections of the brain during childhood may cause meningitis and encephalitis and result in permanent damage.
- **Toxic substances:** Lead poisoning is still an important cause of mental retardation.
- **Psychosocial problems:** The developmental level of a growing individual depends on the integrity of the CNS and on environmental and psychological factors. Poverty predisposes the child to many developmental risks, such as teenage pregnancies, malnutrition, abuse, poor medical care, and deprivation. Mothers with severe and chronic illness might have difficulty providing adequate care and stimulation. Maternal depression during pregnancy has been shown to be associated with developmental delay in children at 18 months of age.

8.2. Perinatal Causes:

This period refers to 1 week before birth to 4 weeks after birth. During the neonatal period, the most important infection is herpes simplex type 2. The neonate is infected during the delivery and may develop microcephaly, profound mental retardation, and neurological deficits. Problems during delivery and low oxygen during birth are also the cause of mental retardation.

8.3. Prenatal Causes:

- **Maternal infections:** Viral infections in the mother can interfere with organogenesis, as exemplified by congenital rubella. Various systems are affected, and as a result, symptoms and impairments include mental retardation, microcephaly, hearing-vision impairment, congenital heart disease, and behaviour problems.
- **Toxic substances:** The most important of the teratogenic substances is ethanol, which is the cause of foetal alcohol syndrome (FAS) and causes abnormalities in 3 main categories:

  (1) Dysmorphic features, which originate during organogenesis

(2) Prenatal and postnatal growth retardation, including microcephaly

(3) CNS dysfunction, including mild-to-moderate mental retardation, delay in motor development, hyperactivity, and attention deficit.

8.4. Genetic Disorders

Congenital genetic disorders are characterized by changes in the genetic material, which may or may not have been inherited from the parents (Genetics of Mental Retardation, 2005). Over 7,000 genetic disorders have been identified and catalogued, with up to five new disorders being discovered every year (McKusick, 1994).

8.4.1. Disorders with autosomal-dominant inheritance: Tuberous sclerosis is an example of the disorders in this group, which might be associated with mental retardation. It is caused by a mutation in a gene affecting the formation of the ectodermal layer of the embryo, because the skin and the CNS develop from this layer, abnormalities are seen in both.

8.4.2. Disorders with autosomal-recessive inheritance: Most metabolic disorders belong to this category. They are caused by single mutated genes that disturb the metabolism by deficient enzyme activity. Phenylketonuria (PKU) is the best known and most common of the metabolic disorders.

8.4.3. X-linked mental retardation: Fragile X syndrome is the most common inherited form of mental retardation and, after Down syndrome, the most common genetic form. It is X linked, with dominant inheritance, and the penetrance is lower in females. Because of a constriction at the location Xq27.3, it appears as if the chromosome is fragile and a part of it is breaking off.

8.4.4. Chromosomal aberrations: - A chromosome aberration reflects an atypical number of chromosomes or a structural abnormality in one or more chromosomes. Chromosome anomalies usually occur when there is an error in cell division following meiosis or mitosis. Down syndrome is the best-known example of a prenatal genetic disorder. Down syndrome is caused by trisomy 21, in which the extra chromosome 21 in the egg or sperm cell results from the non-disjunction in the meiotic stage. In mosaicism, some cells have 47 chromosomes and others have 46 because of an error in one of the first cell divisions of the fertilized egg. For deletion best-known example is cri-du-chat

syndrome, which is characterized by a high-pitched voice and is caused by a 5p deletion in chromosome.

9. Management:

There is no cure for this established disability, though with appropriate support and teaching, most individuals can be trained to do certain tasks. There is no specific medication for mental retardation. Many patients with developmental disabilities have other medical complications for which they take several medications. For example autistic children with developmental delay may utilize anti-psychotics or mood stabilizers to help with behaviour. Use of psychotropic medications such as benzodiazepines in people with mental retardation is also reported in many studies.

10. Introduction: Cytogenetics and Karyotyping:

Cytogenetics is the study of chromosome structure, function, behavior and pathology. Chromosomal analysis is usually performed on white blood cell cultures. Other samples analysed on a routine basis include cultures of fibroblasts from skin biopsy samples, chorionic villi and amniocytes for prenatal diagnosis and actively dividing bone marrow cells. The cell cultures are treated to arrest growth during metaphase when the chromosomes are visible.

The chromosome constitution of a cell is referred to as its karyotype and there is an International System for Human Cytogenetic Nomenclature (ISCN) for describing abnormalities.

10.1 Identification of chromosomes: (Kuffel *et al*., 2007)

10.1.1 Description and Landmarks of G-banded A Group chromosomes:

Chromosome 1:

Chromosome 1 is the largest human chromosome and is metacentric.

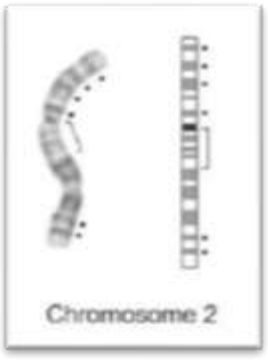
The most distinctive feature of chromosome 1 is the large, light-stain region on the distal half of the p-arm.

In the proximal half of the p-arm there are two distinct dark and (DB's).

Below the centromere on the q-arm is the qh region, which can vary in staining qualities: high-intensity dark stain, or dark and light. Varies in staining qualities more than the other qh regions.

The distal end of the q-arm has three evenly spaced dark bands; the most proximal one has the highest stain density of the three.

Chromosome 2:



Largest submetacentric chromosome.

The p-arm contains four distinct DB's that span the whole arm.

The q-arm starts with a light stain region with three low-stain density DB's.

The distal end of the q-arm has two evenly spaced DB's with equal stain density.

Chromosome 3:

Second largest metacentric chromosome

It has a distinct DB cap at the distal end of the p-arm.

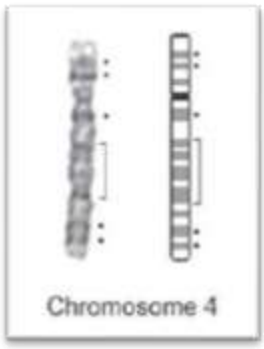
There are two light and (LB) "windows": one is centrally located in the p-arm, one is located proximal to center in the q-arm.

In the distal third of the q-arm there are 3- DB's (depending on

band resolution)

10.1.2Description and Landmarks of G-banded B Group Chromosomes:

Chromosome 4:



Submetacentric chromosome: p:q-arm length ratio of 1:3.

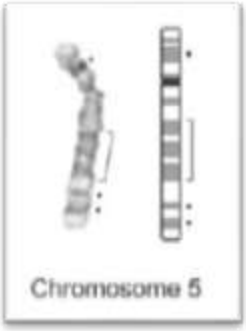
The p-arm has a road "pure" LB followed by two medium stain density DB's.

The proximal end of the q-arm contains a high density dark "shoulder" and.

In the central q-arm there are four closely spaced, medium-stain density DB's which may blend together.

The distal end of the q-arm contains two DB's of similar stain density.

Chromosome 5:



Submetacentric chromosome: p:q-arm length ratio of 1:3.

The p-arm has a distinct central DB.

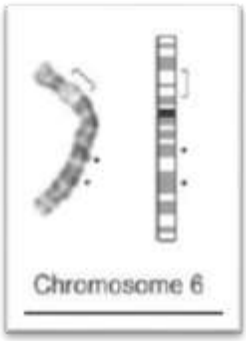
The proximal end of the q-arm contains a low-stain density "shoulder" DB.

In the central q-arm there are three closely-spaced, medium stain density DB's which may blend together.

The distal end of the q-arm contains two DB's of different stain density; the lower band of the two DB's is of higher stain density.

10.1.3Description and Landmarks of G-banded C Group Chromosomes:

Chromosome 6:



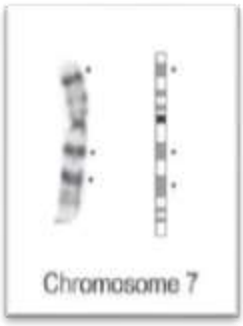
One of the three largest chromosomes in this group, the others being chromosomes 7 and X.

It has a p:q arm length ratio of 1:2.

There is a characteristic road LB "window" in the p-arm.

The q-arm has several DB's including two central high stain density DB's.

Chromosome 7:

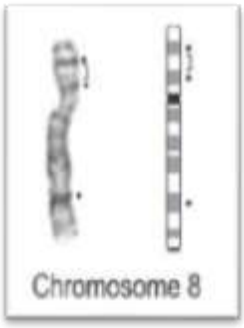


Comparable in size and p:q arm length ratio to chromosomes 6 and X.

The p-arm has a prominent high stain density DB near the distal end of the arm.

The q-arm has two prominent high stain density DB's one located 1/3 and one located 2/3 of the way down the arm.

Chromosome 8:

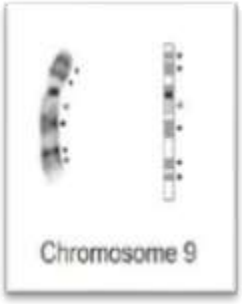


Similar in size and p:q arm length ratio to chromosome 10.

The p-arm has a small LB "window" with two low stain density DB's on either side of the "window."

The q-arm contains a prominent DB located about 2/3 of the way down the arm.

Chromosome 9:



Similar in size and p:q arm length ratio to chromosome 11.

The p-arm has two DB's which are located in the upper 1/2-2/3 of the arm.

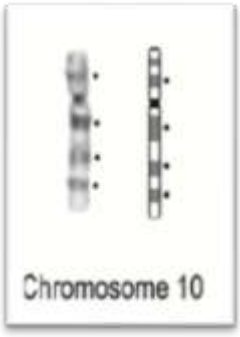
There is a qh region commonly located right below the centromere in the q-arm. This region varies more in location on chromosome 9 than it does on chromosomes 1, 16, and Y.

Alternative locations are as follows: 1) the qh may be split into two sections, one above and one below the centromere 2) the entire region may be located right above the centromere. The qh region stains with a light to medium grey stain coloration.

The q-arm has three distinct DB's: one DB is below the qh region followed by a broad LB; the other two DB's are distal to the broad LB.

The q-arm finishes with a road "pure" LB.

Chromosome 10:

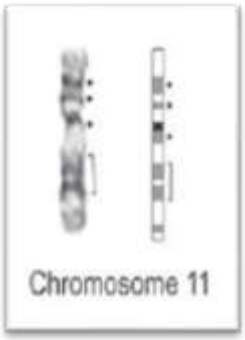


This is similar in size and p:q arm length ratio to chromosome 8.

The p-arm has a distinct central DB.

The q-arm has three evenly spaced DB's spread across the length of the arm; the first of the three has the highest stain density.

Chromosome 11:



Similar in size and p:q arm length ratio to chromosome 9.

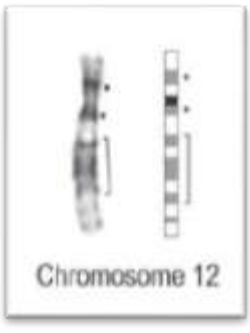
The p-arm has two distinct DB's located in the lower ½ of the arm.

The q-arm has a DB right below the centromere followed by a broad LB.

There are two distinct DB's centrally located in the q-arm followed by a large light stain region with a low density gray DB.

Distinctive features of chromosomes 11 and 12 are that they have broad light and dark staining regions in their q-arms.

Chromosome 12:



The p:q arm length ratio of 1:3.

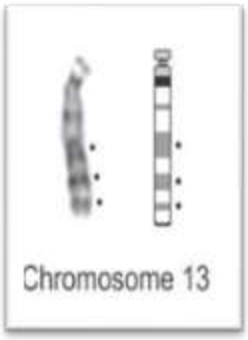
Smallest p-arm of any C-group chromosome.

The p-arm has a broad DB.

10.1.4 Description and Landmarks of G-banded D Group Chromosomes:

All chromosomes in this group are acrocentric and the p-arm/satellite region on these chromosomes is polymorphic. This group is made up of the three largest pairs of acrocentric chromosomes.

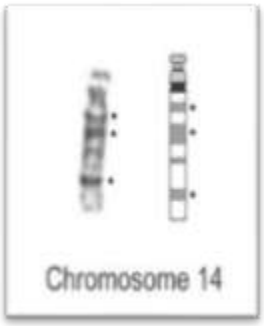
Chromosome 13:



The q-arm has its highest stain density DB's in the lower half of the arm.

There are three distinctive DB's in the lower half of the q-arm and one DB in the upper half.

Chromosome 14:



The highest stain density DB's are located high and low in the q-arm.

There are two DB's in the proximal end of the q-arm and one DB in the distal end of the q-arm.

Chromosome 15:

The highest stain density DB's are located in the upper half of the q-arm.

There are two distinctive DB's in the upper half of the q-arm. The lower half of the q-arm is light stained.

10.1.5 Description and Landmarks of G-banded E Group Chromosomes:

The chromosomes in this group are all submetacentric, but chromosome 16 may appear close to metacentric. The p-arm size decreases as go from chromosome 16 through chromosome 18.

Chromosome 16:

The p-arm has two low density DB's.

There is a qh region located right below the centromere in the q-arm. The qh region is a very high intensity dark staining area.

The q-arm has three evenly spaced DB's spread across the length of the arm, the first of the three (the qh) has the highest stain density.

Chromosome 17:

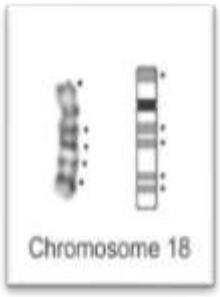
Chromosome 17 is lighter than chromosomes 16 or 18. Its highest stain density DB's are in a distal area of the q-arm.

The p-arm has a medium stain density central DB.

The q-arm has a medium stain density "shoulder" DB.

There are two high stain density DB's in the distal area of the q-arm followed y a road "pure" LB on the telomeric end.

Chromosome 18:



The p-arm has a DB cap. There is a small LB on the telomeric end of the p-arm, but often this LB does not resolve. Instead the LB gives the DB cap a fuzzy edge.

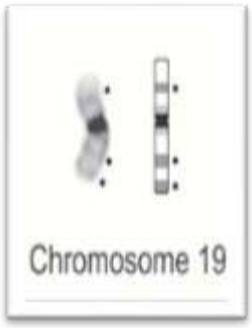
The p-arm is light after the DB cap and may give the arm an appearance similar to a satellite structure, but it is not.

The q-arm has four DB's at higher levels of and resolution: there are two DB's in the proximal end of the arm and two DB's in the distal end of the arm.

10.1.6 Description and Landmarks of G-banded F Group Chromosomes:

These chromosomes are the smallest metacentric chromosomes found in humans.

Chromosome 19:



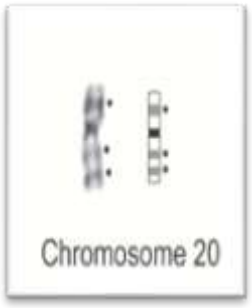
This chromosome, in overall appearance, is very light with a dark pericentric area.

The p-arm has a very low stain density central DB.

The q-arm has a very low stain density central DB and a similar DB on the telomeric end that is hard to see.

The p and q-arms at first glance look very similar, but the way to tell the difference between them is that the telomere of the p-arm fades into the background and the telomere of the q-arm has a distinctive edge to it.

Chromosome 20:



The p-arm has a broad, medium to high stain density DB in the middle to distal end (depending on resolution).

The q-arm has two DB's evenly spaced down the arm with a LB at the telomeric end.

10.1.7 Description and Landmarks of G-banded G Group Chromosomes:

The chromosomes in this group are the smallest human chromosomes and are acrocentric. The p-arm/satellite region of chromosomes 21 and 22 are polymorphic.

Chromosome 21:

This is the smallest human chromosome and it is acrocentric.

The q-arm has a broad, high stain density DB in the proximal end of the arm.

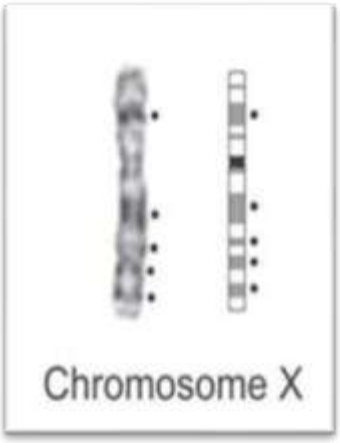
Chromosome 22:

This acrocentric chromosome, in overall appearance, is very light with a dark pericentric area.

The q-arm has a low stain density central DB.

10.1.8 Description and Landmarks of G-banded Sex Chromosomes:

Chromosome X:



A submetacentric chromosome.

This chromosome is comparable in size and centromere position to chromosomes 6 and 7.

The p-arm has a broad, high stain density mid-arm DB.

The q-arm also has a broad, high stain density DB located about equal distance from the centromere as the prominent DB in the p-arm. The prominent DB's in the p and q-arms are of similar stain density, but the q-arm DB is broader.

There are three DB's in the distal area of the q-arm and the third DB is the most distal high stain density DB of its size compared to the distal DB's of all other C group size chromosomes

Chromosome Y:

A submetacentric chromosome.

The p-arm has a medium stain density DB at the end of the arm.

The q-arm has a narrow low stain density "shoulder" DB.

There is a qh region located at the terminal end of the q-arm. The qh region is a very high stain density area typically either medium dark grey or very dark grey in coloration.

Type of human chromosomes:

Group	Size and type	Chromosome number
A	Largest Metacentric	1,2,3
B	Largest Submetacentric	4,5
C	Medium Submetacentric	6 to 12, X
D	Large Acrocentric	13,14,15
E	Small Submetacentric	16,17,18

F	SmallestMetacentric	19,20
G	Smallest Acrocentric	21,22,Y

11. Background:

The genetic and congenital mental disorder is the second most common cause of infant and childhood mortality and occurs with a prevalence of 25-60 per 1000 births. The higher prevalence of genetic diseases in a particular community may be due to some social or cultural factors. Such factors include tradition of consanguineous marriage, maternal age greater than 35 years, etc. Globally, at least 7.6 million children are born annually with severe genetic or congenital malformations (Anupam Kaur *et al.*, 2010); 90% of these are born in mid and low income countries. Several developments in the late 1950s and early 1960s led to the first introduction of cytogenetics as a clinical science. The use of Colchicine to block mitosis (Levan, 1938) and the accidental discovery of hypotonic shock (Hsu, 1952) resulted in the correct determination of the human chromosome number (Tijo and Levan, 1956) and to the identification of trisomy 21 in Down syndrome as the first known chromosomal abnormality (Lejeune *et al*., 1959). Improvements in cell-culture technology and the observation that phytohemaglutinin stimulates mitosis in leukocytes (Nowell, 1960; Moorehead *et al*., 1960) provided a reliable source of mitotic cells. This combination of improved culture and harvest techniques and consistent slide making methodology are the foundation upon which cytogenetics rests today (Hungerford, 1965). The introduction of Giemsa banding was another major advance in the field of cytogenetics to identify the chromosomes (Sumner *et al.*, 1971). The karyotype is determined by GTG banding (Caspersson *et al*., 1970). The Giemsa banding was improved by the pre-treatment of chromosome by trypsin for digestion (Seabright M, 1971; Wang HC, Federoff S, 1972). Chromosomal abnormalities are responsible for up to 28% of all mental retardation cases (Curry *et al*., 1997).

In a study by Luciani *et al*., 2003, telomeric 22q13 deletion occurred as a result of a ring chromosome, simple deletion and translocations. The deletion was shown to be highly

variable, ranging from 160kb to 9Mb. It was also shown that the parental origin of these deletions was more paternal (74%) than maternal (26%).

A study by Sismani *et al.,* 2001 detected an 8q subtelomeric deletion in an idiopathic mentally retarded subject by using FISH and Multiplex Amplifiable Probe Hybridisation (MAPH) Telomere Assay. A mild developmental delay and dysmorphism and very blue iris in a patient who showed 15(q24,q26.1) interstitial deletion was reported by Spruijt *et al.,* 2003.Idiopathic subjects also show chromosomal anomalies in the form of translocations.

A *de novo* balanced translocation between 17p13.3 and 20q13.33 was identified by Walter *et al.,* 2004. A study by Anderlid *et al.,* 2002, identified one de novo unbalanced translocation and three unbalanced translocation inherited in a patient suffering with idiopathic mental retardation. 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 the Multiplex FISH Assay.

Another unbalanced cryptic translocation between chromosomes 8 and 13: der(13)t(8; 13)(q24.3,q34) in two sisters was identified by Kleefsrta *et al.,* 2000. FISH studies in 84 families with idiopathic mental retardation (Ewa Bocian *et al.,* 2004) had revealed a large number of aberrations such as: 46,XY,t(7,10)(q36,q26); 46,XY,der(13)t(4,13)(p16,q34); 46,XY,der(2)t(2,7)(q37,q36); 46,XX,der(4)t(4,21)(p16,q22); 46,XY,der(6)t(4,6)(q35,p27); 46,XX,der(13)t(X,13)(q28,q34); 46,XY,der(10)t(10,19)(q26,p13.3); 46,XY, del(4)(p16.1,p16.3).

Cryptic unbalanced chromosomal rearrangements in telomeric bands of human chromosome constitute a significant cause of idiopathic mental retardation. This was supported by numerous investigations (Ghaffari *et al.,* 1998; Coco and Penchaszadeh, 1982; Flint *et al*. 1995; Arunkumar, 1998; Baker *et al*., 2002).

Cytogenetic investigations (karyotyping and FISH) done in 100 subjects with idiopathic mental retardation (Roy *et al.,* 2010) showed following chromosomal anomalies in the

subjects: del(15)(q11,q13); inv(X)(q13,q27); inv(X)(q13,q27); del(X)(pter→q21); r(15); r(22); t(1,7)(p21,q11.2); t(3,10)(q28.2,q21.1).

Various other anomalies identified were: -
2q23.1 microdeletion syndrome (de Vries *et al*, 2010; Pipiras *et al.*, 2009; Elsea *et al.*, 2009); 1p36microdeletion syndrome (Shaffer *et al.*, 2007; Battaglia *et al.*, 2005); 7q11.3 microduplication syndrome (Lupski *et al.*, 2008; Kooy *et al.*, 2009).

Thinking back to 1959 gives a sense of how recently the field has evolved. That was the year that Down syndrome ("Mongolism" in those days) was found to be caused by an extra chromosome, thus leading to our present understanding of this familiar disorder. It was also the year when the American Association on Mental Deficiency (AAMD) published the first definition of Mental Retardation (Heber, 1959). Guilford, 1956 described no less than 120 components of intellect and devised tests to measure most of them. Jane Mercer in 1974 put it as "Mental Retardation is neither a characteristic of the individual, nor a meaning inherent in the retarded person's behaviour, but a socially determined status, which he may occupy in some social systems and not in others, depending on their norms. It follows that a person may be mentally retarded in one system and not mentally retarded in another. He may change his role by changing his social group." He also argued that since one's status as a mentally retarded person is tied to a specific role in a specific social system, prevalence rates, in the traditional, epidemiologic sense, are meaningless.

More recently Gardener, 1983 proposed that there are separate "multiple intelligences" in the linguistic, musical, logico-mathematical, spatial, bodily-kinesthetic, and personal domains. Mental retardation refers to significantly subaverage intellectual functional resulting in or associated with impairments in adaptive behaviour and manifested during the developmental period (Grossman, 1983).

Epidemiological studies on mental retardation (MR) were reviewed for data on prevalence, associated disorders, and etiology. Most studies yielded a prevalence of 3–4 per 1,000 for both mild and severe MR, although rates varied with age, gender, and method of ascertainment. Data derived from total population screening yielded higher prevalence rates than data obtained from cases registered and agency or professional

contacts. Several disorders, including epilepsy and cerebral palsy, were found to be associated with MR. The etiology of up to 50% of cases of mild (IQ 50–70) and severe (IQ < 50) retardation was found to be unknown. The remaining cases involved primarily prenatal etiologic factors (Mc Laren *et al*., 1087).

There is a general consensus that, for several reasons, people with mental retardation are at an increased risk of developing emotional disorders. Numerous research studies have examined the prevalence of psychiatric disorders among people with mental retardation, and a wide range of rates have been reported. Reasons for the variability in these results are discussed, including definitional and identification issues, and sampling issues. The need for updated epidemiological studies in this area is emphasized (Borthwick-Duffy *et al.,* 1994).

At least 209 different X linked mental retardation disorders have been described (Chiurazzi *et al.,* 2001). 140 forms of syndromic mental retardation are discerned and causative genes were identified for 27 of them. In addition, more than 87 forms of non-syndromic mental retardation have been described and causative genes have been identified in 11non-syndromic forms of mental retardation.

Frequency of mental retardation among the offspring of consanguineous parents was estimated to be about 0.2 %. According to Mendez *et al.* parental consanguinity increases the frequency of rare recessive disorder in inherited offspring. These reports suggest the role of rare recessive genes as the cause for mental retardation. A higher prevalence is seen in South India, where consanguineous marriages are strongly favoured and the coefficient of inbreeding is high. High rate of consanguinity among many communities further increases the prevalence of genetic disorders, while the lack of rehabilitative facilities escalates the burden of genetic disorders (C.P. Anitha Devi and D. Sudarsanam, 2011).

The incomplete development of mental capacities and associated behavioural abnormalities are referred to as mental retardation. It is single largest neuropsychiatric disorder in every civilized society affecting 2.5-3.0% of the total population. Chromosomal abnormalities are the important cause of mental retardation. Cytogenetic investigations were carried out on 143 mentally subnormal individuals that were referred

to the Centre for Genetic Disorders, Guru Nanak Dev University, Amritsar, India, during 1996 to 2002. These cases were referred mainly as suspected Down syndrome, delayed milestones, mental retardation, etc. The age group of the patients ranged from 1 month to 18 years. Interestingly, maximum number of patients i.e., 58/143 (40.5%) were the firstborns and the average maternal age was 27.6 years. Free trisomy 21 was found to be the most frequent autosomal aberration, both amongst males and females (45.4% males, 18.8% females). It was seen in 92/143 (64.3%) cases while translocations were seen in 2.7% cases. The latter included 45,XY,t(13;14); 46,XY,t(14;21); 45,XX,t (14;21) and 46,XX,t(14;21) karyotypes.

In India, the incidence of mental retardation is reported to be 2-3%. Of these, 30% cases of severe mental retardation are genetically determined. Out of these, 25% fall under the category of X-linked mental retardation (XLMR) disease, comprising over 100 varied types of retardations that can be associated with fragile- X chromosome, biochemical defects, neurological aberrations, bony dysplasia and a range of other disorders (Gracia, 1998). Monosomies and trisomies are reportedly the frequent cause of mental retardation.

Previous surveys have shown that the great majority of retards are of unknown etiology and about quarter of them have retarded siblings and/or parents and about 40% of those with retarded siblings have retarded parents. In 1938 Colchester's study showed that about 3.2% of all retardants had consanguineous parents, about double the consanguinity rate in the general population. Akesson's survey in South Sweden found first cousin parents in 3 of 60 families with undifferentiated retardation. Dcwey *et al.* found an increased rate of parental consanguinity among severe familial retardants in Wisconsin.

The etiology of mental retardation (MR) is unexplained in at least 50% of cases. Recently it was shown that subtle telomeric rearrangements may be a common cause of idiopathic mental retardation (IMR).84 families were studied with IMR and unspecific clinical features suggesting chromosomal aberration, including 59 patients with moderate to severe MR and 24 with mild MR. One healthy father of three deceased, severely MR children was also included. Fluorescence in situ hybridization (FISH) using 41 subtelomeric probes (the Chromoprobe Multiprobe--T System) was performed in all patients. Ten (11.9%) subtle chromosome rearrangements were identified. Nine (10.7%)

were subtelomeric abnormalities. Seven were familial, with six of paternal origin. All but one was product of parental balanced reciprocal translocation or inversion. Retrospective G-banding analysis showed that six of the nine rearrangements could be seen or suspected at the 450-550 band levels. Subtelomeric abnormalities were recognized in six patients with severe/moderate (including the father of children with severe MR) and in three with mild MR.

Jacobs *et al*., 2005 studied seven families with X-linked mental retardation clinically and cytogenetically. All affected males in six of the families were found to have a fragile site on Xq in a number of their peripheral lymphocytes. The fragile site was not seen in any of the affected males in the seventh family. The affected males in the six families with the fragile X had a syndrome characterized by a variable degree of MR, macro-orchidism, a characteristic repetitive, jocular speech, normal body proportions, and large jaws and ears. The fragile X chromosome could only be detected in a proportion of female carriers and its frequency in females was found to be correlated with their mental status and to be inversely correlated with their age.

Extensive study has been done on Mental Retardation, including all the levels and causes. Worldwide study gives the statistics of Mental Retardation being present in 3% of the population in the industrialized countries and the occurrence increased by 0.02% in consanguineous marriages. In this study, karyotyping and phenotypic characterization was done in 8 patients and the karyotypes obtained were normal. However, phenotypic characterization of the patients using POSSUM web gave important hints about the underlying genetic condition which may be submicroscopic.

Materials and methods

Materials

1. **Reagents**:

- **Chromic acid:** 10 % $K_2Cr_2O_7$ was added in 25 % of H_2SO_4 for glassware washing.

- **RPMI-1640 media:** RPMI stands for Roswell Park Memorial Institute, which is where it was developed. It is a growth medium consisting of vitamins, amino acids (Glutamine in highest amount), inorganic salts glucose, glutathione and a pH indicator (Phenol Red). The media also contains HEPES buffer, Penicillin (60mg/L), streptomycin (100mg/L), and 2% $NaHCO_3$.It needs to be supplemented with 5-20% foetal bovine serum, but is known to support growth of cells even in the absence of serum.

- **Phytohemaglutinin(1mg/mL):** 25mg of PHA was added to 25mL sterile distilled water and aliquots were dispensed.PHA is derived from extracts of *Phaseolus vulgaris* seeds and acts as a mitogen which stimulates division of lymphocytes in the culture.

- **Colchicine (1mg/mL):**10mg Colchicine was dissolved in 1000mL of sterile distilled water and aliquots were dispensed. Colchicine is a natural product obtained from the plant *Colchicum autumnale*, and does not allow polymerization of tubulin monomers to form microtubules and thus prevent formation of mitotic spindle. It is thus used to arrest dividing lymphocytes at metaphase stage. It also straightens chromosomes, crisps chromatid edges, and increases chromosome spreading as it releases them from the mitotic apparatus (Taylor EW, 1965; Waters K, 1995; Knight, 1980).

- **Hypotonic solution:** 0.56g of KCl powder was dissolved in 100mL of sterile distilled water. Hypotonic solution causes the cells to swell and thus facilitates proper spreading and separation of metaphase chromosomes. The prewarming of hypotonic solution to 37 ºC increases effectiveness by increasing water transport

across the cell membrane and by altering the permeability of the cytoplasmic membrane.

- **Carnoy's Fixative:** Methanol: Glacial acetic acid in the ratio 3:1 (Used chilled). Fixation removes water from the cells and preserves them, hardens membrane and chromatin and also prepares chromosomes for the banding procedure.

- **Trypsin-EDTA solution:**
  Phosphate buffered saline (PBS): 0.05g KCl, 2gNaCl, 0.36g $NaH_2PO_4$and 0.06g KH_2PO_4 were added in 250mL of sterile distilled water for obtaining pH 7.
  Stock Trypsin-EDTA solution: 0.01g of EDTA and 0.02g of trypsin were added to 50mL of PBS.
  Working Trypsin-EDTA solution: 25mL solution was taken from stock and 25mL of PBS was added to it.

- **Giemsa stain:** Giemsa stain is a complex mixture of dyes. The main component are the basic aminophenothiazin dye azure A, azure B, azure C, thionin and methylene blue and the acidic dye, eosin. The thiazin dyes vary in number of methyl groups attached to a core of two benzene rings bound together by nitrogen and sulphur atom.
  Sorenson's buffer: 0.345g of monobasic sodium phosphate (NaH_2PO_4) and 0.454g dibasic sodium phosphate (Na_2HPO_4) was added in 250mL of sterile distilled water for obtaining pH 7.
  Stock Giemsa stain: 1g Giemsa powder was added in 54mL glycerol mixed and kept at 60ºC water bath for overnight. 84mL of methanol is added and filtered and kept in dark bottle.
  Working Giemsa stain: 2mL of stock Giemsa stain was added in 25mL Sorenson's buffer having pH 7 and 25mL distilled water. Mixed well and prepared freshly whenever required.

- **DPX:** DPX was used to mount slides permanently.

- **Sodium hypochlorite:** It was used to dip glassware and syringes contaminated with blood before their disposal or washing.

2. Sterile glassware:

- Glass slides having 1 mm thickness
- Coverslips of 24×60 mm dimensions
- Centrifuge tubes having rounded bottom and rubber screw caps with liners
- Long glass droppers with rubber teats
- Glass beakers
- Pipettes- 1mL, 2mL,5mL and 10mL
- Blue cap bottles
- Amber coloured bottles
- Flasks

3. Other requirements:

- Micropipettes: 2-10 µg; 20-200 µg and 50-1000 µg with sterile tips
- Plastic beakers
- Coplin jars
- Gloves
- Forceps
- Spirit lamp
- Multisample needles with holder
- Aluminium foil
- Cotton
- Tissue paper
- Whatmann filter paper
- Sodium Heparinised vials

4. Instruments:

- CO_2 incubator
- Laminar air flow
- Centrifuge
- Microscope
- Hot plate
- Hot air oven
- Autoclave
- Water bath
- Digital pH meter
- Freezer
- Karyotype imaging system
- Cyclo mixer

Reagent	**Company**
Chromic acid	MERCK
RPMI-1640	Himedia
PHA	Invitrogen
Colchicin	Himedia
KCl	MERCK
Methanol	MERCK
Acetic acid	MERCK
Trypsin	Himedia
EDTA	Himedia
NaCl	MERCK
NaH_2PO_4	MERCK
KH_2PO_4	MERCK
Na_2HPO_4	MERCK
Glycerol	MERCK
Giemsa	Himedia
DPX	s.d.fine chemicals
Sodium hypochlorite	s.d.fine chemicals

Instrument	Company
Incubator	EIE instruments pvt. ltd.
Laminar air flow	MTronics
Centrifuge	REMI
Autoclave	Yorko
Hot air oven	EIE instruments pvt. Ltd
Water bath	Wiswo instruments
Digital pH meter	Lab-India
Freezer	Samsung
Microscope with Karyotype imaging system	Carl Zeiss, IKAROS software Metasystems

Methodology

Steps involved:

- Data collection
- Sample collection
- Culture setup
- Harvesting the cultured cells
- Slide preparation
- GTG banding
- Slide observation and scoring
- Karyotyping
- Karyotype analysis and reporting

1. **Data collection:**

- Medical history of 128 patients suffering from mental retardation was obtained using a questionnaire:

Institute of Science, Nirma University

Questionnaire for Study of Congenital Disorders

1. Proband (Name / Age / Sex):

2. Mother (Name / Age):

3. Father (Name / Age):

4. Contact details:

5. Consanguineous marriage: Yes/No

6. Obstetric history:

FTND (Year of mother's age/ Sex):

History of abortions if any (Gestation age, spontaneous or induced):

7. Family History:

Details (Age/Sex/Normal or Abnormal/not known/):

1.
2.
3.
4.

8. Pedigree chart:

9. Antenatal history:

- History of maternal illness (Fever, Rashes, Forg etc.)
- Exposure to Drug
- Microcephaly and hydrocephaly

10. Birth history:

- Apgar score:

  1 minute:

  5 minute:
- Neonatal septicemia:
- Hyperbilirubinemia:

11. Disability level:

- DQ (DSM IV)

12. I.Q.:

13. Neurobehavioral problems

ADHD (DSM IV):

Autism (CARS score):

14. Associated anomalies and Phenotypic Abnormalities:

Dysmorphic features:
Palpabral fold
Cleft palate
Epicanthal fold
Monoloid slant
Low set ears
Philthrum
Tapered fingers
Syndactyly
Polydactyly
Pes cavus
Height
Weight:
Head size:
Hand size:
Foot size:
Genitals:
Difficulty in walking:
Heart defects:
Growth Retardation:
Weak muscle development:
Any other:
Present behaviour / condition:

15. Developmental milestones:

Gross motor:

Fine motor:

Cognition:

Speech:

16. Prenatal & Post natal diagnostic work-up details:

Chromosome study:

Radiological investigations:

Biochemical:

Molecular:

Other tests with results if abnormal:

- Following information was obtained:

Condition	Occurrence (%)
Developmental delay with convulsions	1.56
Down syndrome	14.06
Mild mental retardation	11.71
Moderate mental retardation	43.75
Severe mental retardation	10.93
Mental retardation with unknown severity	6.25
Cerebral Palsy	7.81
Autism	3.9

- Out of these, 5.46% patients had parents with consanguineous marriages, 69.53% patients had parents with non-consanguineous marriages, while of 25% patients, the details about consanguinity were not known.

2. Sample collection:

- Subjects showing uncharacterized mental retardation were taken for study.
- Their clinical history was obtained and their blood samples were collected.
- Peripheral blood samples were collected in sterile sodium heparin vials by phlebotomy.

3. Culture setup:

- Short term culture of the collected blood samples was setup under strictly aseptic conditions.
- Complete RPMI-1640 culture tubes were taken and PHA was added at a final concentration of 1mg/mL.
- To each of the culture tubes, 1mL of whole blood was added.
- Culture tubes were allowed to incubate for 69hrs at 37 ºC in BOD or CO_2 incubator.

- T-cells are cultured by this method, and chromosomes are studied from these cells arrested at metaphase.

4. Harvesting the cultured cells:

- At the 69th hour, 3µL Colchicine (1mg/mL) was added to the culture for mitotic arrest and the culture was then incubated at 37 ºC in water bath for 2 hours.
- The culture was then centrifuged at 2000 rpm for 10 minutes, and the supernatant was discarded.
- 7mL of prewarmed hypotonic salt solution (0.56% KCl, 0.075M) was added to the residue with gentle agitation to resuspend the cells.
- The tube was then incubated for 20 minutes at 37 ºC.
- 4mL chilled fixative (methanol: acetic acid 3:1) was then added in the tube and gentle mixing was done, and the tube was then centrifuged for 10 minutes at 2000 rpm.
- The supernatant was removed and 7mL chilled fixative was added to the residue and the cells were resuspended by gentle mixing. The tube was then centrifuged at 2000 rpm for 10 minutes.
- The cells were given three more fixative washes for proper membrane hardening and dehydration, by repeating the above step three more times or till a clear pellet was obtained.
- After obtaining clear pellet, the cells were resuspended in fresh fixative and this suspension was then taken for slide preparation.

5. Slide preparation:

5.1 Slide preparation: Clean glass slides kept dipped in chilled water were used for making smear, so that the water film formed on the slides help in proper spreading of the chromosomes. Few drops of cell suspension were dropped on the slide held in a slanting position with forceps. The suspension was dropped

from a considerable distance to permit proper elongation of metaphase chromosomes.

5.2 Slide ageing: Slides were then allowed to age for 6 days at room temperature and then for half an hour at 60 ºC in hot air oven.

The aging process is very important as it gives better contrast and crispness to banded chromosomes because it drives off the water content and also makes the chromosomes more resistant to Trypsin. The major change in chromosome from aging may be the oxidation of protein sulfhydral groups (Evans HJ., 1977) and degradation of the chromosomal DNA (Mezzanotte R *et al.,* 1988).

6. Banding: GTG-banding (G-banding by Trypsin with Giemsa) uses the proteolytic enzyme trypsin for pre-treatment followed by staining with Giemsa (Seabright M, 1971; Wang HC, Federoff S, 1972).

G-banding is the most frequently used technique in clinical cytogenetic laboratories because of the permanence of the bands produced and the ease with which they can be photographed.

6.1 Trypsin treatment: The slides were dipped in the coplin jar filled with Trypsin-EDTA solution for 10-15 seconds.

6.2 Brief washes in distilled water: 2-3 washes in chilled distilled water were given to slides to stop Trypsin activity.

Trypsin is a serine protease which hydrolyzes the protein component of the chromatin, thereby allowing the Giemsa dye to react with the exposed DNA. Histones are present in extremely high concentration in the cell nucleus; their total mass is approximately same as that of DNA. The Histones are basic proteins with strong positive charge at neutral pH because they contain a high proportion of the positively charged amino acid lysine and arginine. Histones bind tightly to the negatively charged DNA (Al ert's *et al.,* 1994).

6.3 Staining: The slides were dipped in coplin jar filled with Geimsa stain for 20-25 minutes, followed by washes with distilled water to remove excess stain.

The standard cytogenetic analysis using G-Banding is not sensitive enough to detect subtle chromosomal rearrangements such as microdeletions, etc. and so, such cases should be considered for FISH. The selectivity of G-Banding is largely due to hydrophobic bonding, which is enhanced by the loss of hydrophilic Histones (Curtiz *et al.,* 1975; Curtiz *et al.,* 1982)

G bands have following specific properties (Bernardi *et al.*, 1989; Holmquist GP, 1992):

(1) Stain strongly with dyes that bind preferentially to AT-rich regions, such as Giemsa and Quinicrine.
(2) Comparatively AT-rich
(3) DNase insensitive
(4) Gene poor
(5) Condense early during cell cycle but replicate late
(6) Genes are large because exons are often separated by large introns.

7. **Slide observation and scoring:** The GTG banded slides were observed under microscope and metaphase plates showing proper banding and spreading were scored. Minimum 10 metaphase plates were scored per sample, and minimum 2 were selected for karyotyping analysis.

8. **Karyotyping and analysis:**

   Per patient around 10 cells were counted and around 5 metaphase plates were selected for image capture and analysis using Microscope attached with CCD camera and karyotyping software.

Phenotypic characterization

1. **Phenotypic characterization: Introduction and Importance:**

   The patients enrolled were subjected to detailed study of phenotypic characterization as collection of abnormal features when combined can give a clue to underlying genetic condition. There are numerous syndromes described with a range of phenotypic features and genetic alterations hence in addition to our chromosomal findings we carried out find-the-syndrome exercise with the help of a clinician specialist and POSSUM database.

2. **Introduction to POSSUM-web:**

   POSSUM-web is a computer-based system that helps clinicians to diagnose syndromes in their patients. It contains information on more than 3000 syndromes, including multiple malformation syndromes, chromosomal deletions and duplications, skeletal dysplasias and metabolic conditions with dysmorphic features. The comprehensive mediabase with extensive clinical photos for most conditions also includes x-rays, diagrams, and histopathology slides.

   Using POSSUM-web, clinicians can search for syndromes based on a patient's traits or by syndrome name to assist them in making a diagnosis or to learn about syndromes.

   Syndrome commentaries provide detailed information about clinical attributes, differential diagnoses, radiology and genetics. The extensive trait dictionary includes a searchable atlas to assist in choosing the most appropriate trait to describe the concerned patient.

   It has direct links to OMIM (Online Mendelian Inheritance in Man). One will need an active internet connection to use POSSUM-web. The database is updated continuously and the data uploaded every month. The images are updated with annual renewal of subscription.

   The POSSUM team is based at the Victorian Clinical Genetics Service and The Murdoch Children Research Institute Melbourne.

New information and new images are continuously being added to POSSUM-web. Since POSSUM-web became available, over 250 syndromes and more than 1000 new images have been added.

Results and Discussion

Patient #1

1. Clinical History:

1.1 Proband (Age / Sex): 14/M

1.2 Consanguineous marriage in parents: No

1.3 Obstetric history:

1.3.1 Mother's age at the birth of proband: 26yrs

1.3.2 History of abortions if any (Gestation age, spontaneous or induced): nil

1.4 Family History:

(Age/Sex/Normal or Abnormal or not known):

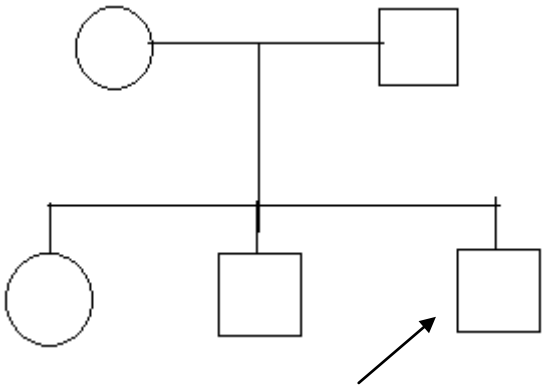
1.1.1.1. 15/F/Normal [sister]

1.1.1.2. 10/M/Normal [brother]

1.1.1.3. 35/F/Normal [mother]

1.1.1.4. 36/M/Normal [father]

1.5 Pedigree chart:



1.6 Birth history:

- Premature delivery- at 8th month
- Suffered from Meningitis at the age of 9months

1.7 DQ: 90%

1.8 I.Q.: 20-34

1.9 Neurobehavioral problems: Severe Mental Retardation

1.10 Associated anomalies and Phenotypic Abnormalities:

- Vision impairment in left eye since birth

- Spasticity of both legs

1.11 Height:150cm

1.12 Weight:37kg

1.13 Head size:49.6cm

1.14 Hand size: 16.5cm

1.15 Foot size: 21cm

1.16 Present behaviour/ condition: Speech impaired, shy, open mouth, protruding tongue

1.17 Developmental milestones:

1.17.1 Gross motor: Delayed

1.17.2 Fine motor: Poor and uncoordinated

1.17.3 Cognition: Severe to profound MR

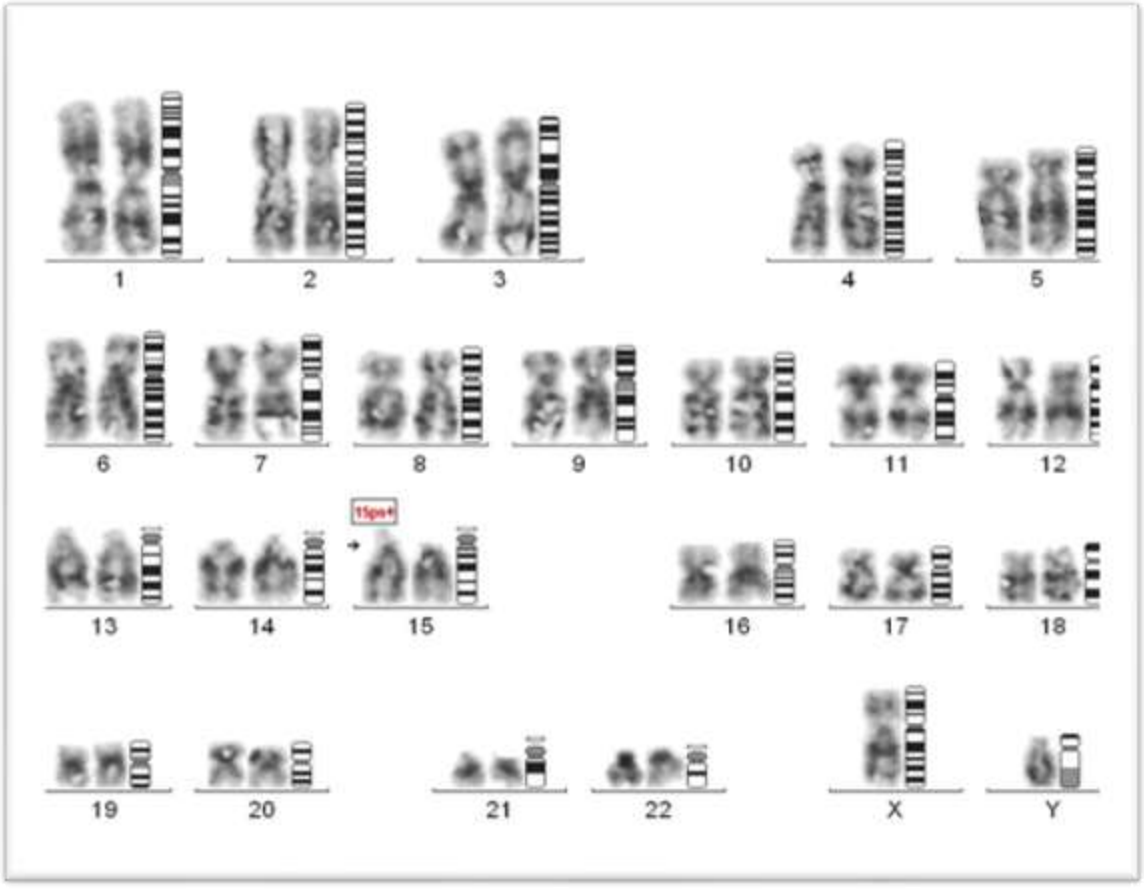
1.17.4 Speech: Impaired

1.18 Diagnosis: Severe Mental Retardation

1.19 Phenotypic characterization using POSSUM:

- The following parameters were added:
  1) Small deletion/duplication/Chromosomal variant
  2) Abnormal Posture/Gait
  3) Muscle weakness
  4) Speech delay/defect
  5) Muscular hypertonia/spasticity/rigidity/brisk reft
  6) Ataxia/in coordination
  7) Mental retardation- moderate to severe
  8) Visual loss/severe
  9) Protruding tongue
- Threshold value selected was 7 and all the added parameters were selected mandatory.
- Possum web result: 2 syndromes- Congenital disorder of glycosylation; Leigh syndrome.

2. Result:



2.1 Karyotype: 46,XY

2.2 Inference: 15p+ normal variant

2.3 Remarks: Possibility of mosaicism cannot be ruled out as only one tissue is studied. Sub microscopic rearrangements and micro deletions cannot be ruled out with karyotyping alone. According to POSSUM web results, there is a possibility of Leigh syndrome or congenital disorder of glycosylation in the patient. Both of these cannot be detected by Karyotyping. For precise conclusions, genetic testing at molecular level is needed.

Patient #2

1. Clinical History:

1.1 Proband (Age / Sex):16/M

1.2. Consanguineous marriage in parents: Yes

1.3. Obstetric history:

1.3.1. Mother's age at the birth of proband: 19yrs

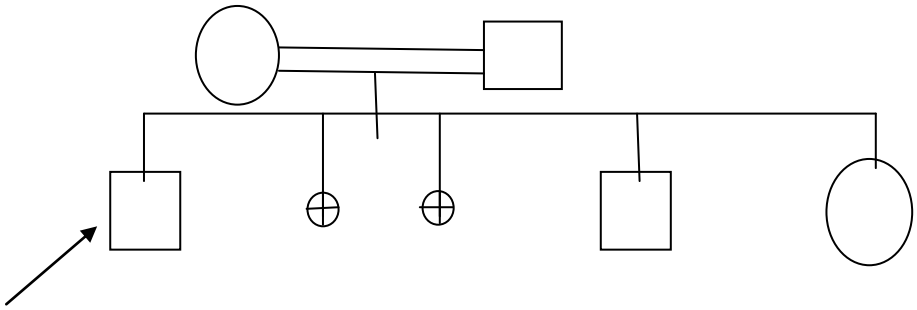
1.3.2. History of abortions if any (Gestation age, spontaneous or induced):nil

1.4. Family History:

(Age/Sex/Normal or Abnormal or not known):

1) M/Normal [younger brother]
2) F/Normal [younger sister]
3) M/Normal [father]
4) F/Normal [mother]
5) Two stillbirths after the birth of proband

1.5. Pedigree chart:



1.6. Birth history: Premature delivery, at 8th month

1.7. Post-natal details: At the age of 3yrs- convulsions, at the age of 5yrs cataract operation in both eyes**,** at the age of 8yrs- meningitis

1.8. DQ: 75%

1.9. I.Q.:40-45

1.10. Neurobehavioral problems: Moderate Mental Retardation

1.11. Associated anomalies and Phenotypic Abnormalities:

- Squint eye

- Far sightedness
- Weak eyesight
- Dysmorphic features
- Nystagmus
- Nasal septum larger
- Short stature

1.12 Height:148.6cm

1.13 Weight:39kg

1.14 Head size:48cm

1.15 Hand size:18.8cm

1.16 Foot size:23.3cm

1.17 Genitals: Normal

1.18 Growth Retardation: Yes, short stature

1.19 Present behaviour / condition:

- Lazy
- Talkative
- Looks upward and walks or talks

1.20 Developmental milestones:

1.20.1 Gross motor: sitting- 2yrs, walking- 3yrs

1.20.2 Fine motor: Poor

1.20.3 Cognition: Retarded

1.21 Diagnosis: Moderate Mental Retardation

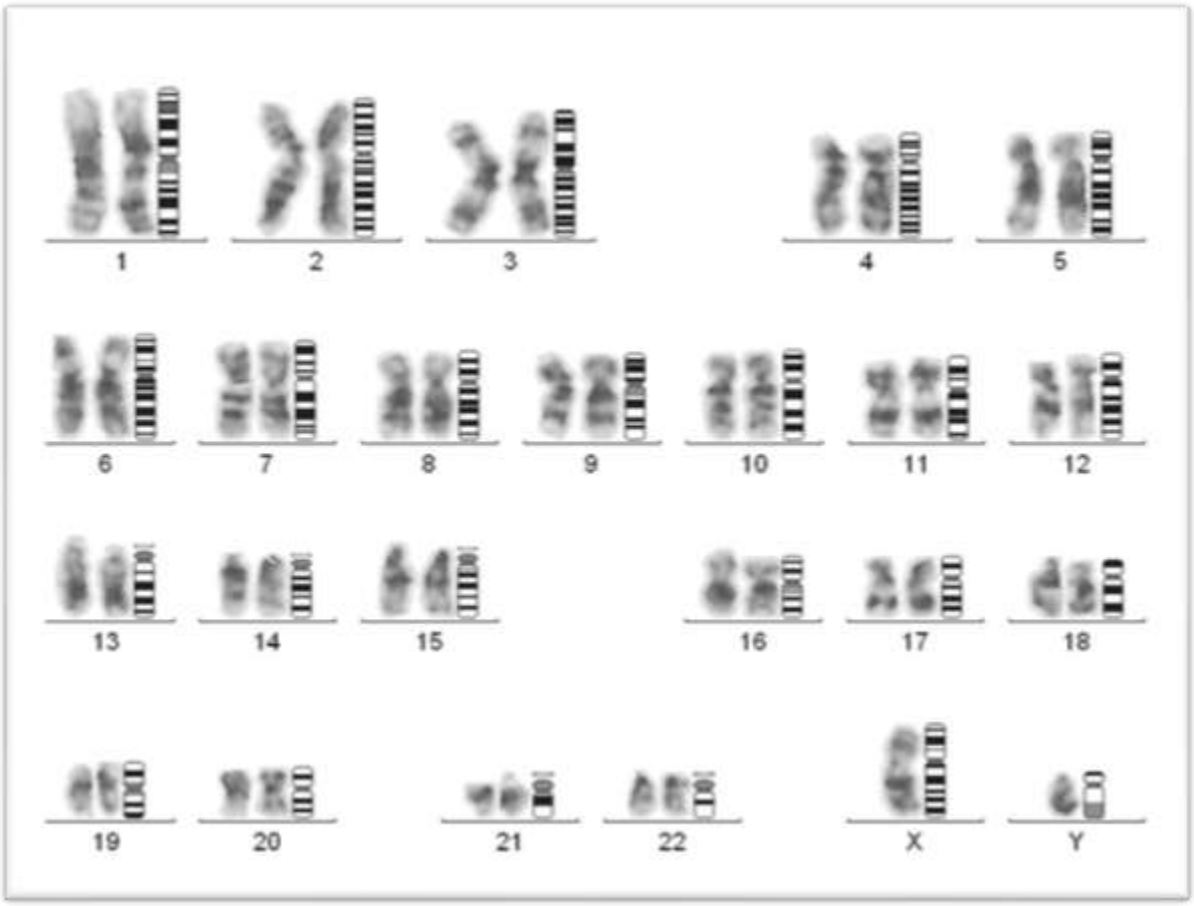
1.22 Phenotypic Characterization using POSSUM:

- Following parameters were added:
  1) Seizures of any type
  2) Moderate mental retardation
  3) Nystagmus
  4) Abnormal globe of the eye
  5) Short stature- Postnatal
  6) Abnormal nasal septum
  7) Ataxia/in coordination
  8) Preuricular tags/ear pits/ sinuses
  9) Squint/paresis of ocular muscles

10) Cataract

- All the parameters selected were mandatory.
- When the threshold value was selected 7: POSSUM result showed possibility of 9 syndromes
- When the threshold value was selected 8: POSSUM result showed possibility of 1 syndrome- Bronchiooculofacial Syndrome.

2. Result:



2.1. Karyotype: 46,XY

2.2. Inference: Normal

2.3. Remarks: From the results of POSSUM web, there is a possibility of the presence of Bronchiooculofacial syndrome, in which mutations occur in the gene *TFAP2A.* These mutations include small intragenic deletions/insertions and missense, nonsense, and splice site mutations as well as whole gene deletions and duplications.

Such mutations cannot be detected by karyotyping and this is justified by the normal karyotype of the patient.

Molecular genetic testing including methods like sequence analysis and deletion/duplication analysis should be carried out to further detect the disorder.

Patient #3

1. Clinical history:

1.1 **Proband (Age / Sex):**15/M

1.2 **Consanguineous marriage in parents:** No

1.3 **Obstetric history:**

1.3.1 **Mother's age at the birth of proband:**28yrs

1.3.2 **History of abortions if any (Gestation age, spontaneous or induced):**Not available

1.4 **Family History:** Not available

1.5 **Pedigree chart:** Not available

1.6 **Birth history:**

- Hyperbilirubinemia after 1 day of birth
- Forcep delivery
- Delayed birth cry

1.7 **DQ:**75%

1.8 **I.Q.:** 45

1.9 **Neurobehavioral problems:** Moderate Mental Retardation

1.10 **Associated anomalies and Phenotypic Abnormalities:**

- 2^{nd} finger of foot extra-long than others
- Flat foot
- Cubitus Valgus
- Speech problem

1.11 **Height:** 164.2cm

1.12 **Weight:** 55kg

1.13 **Head size**: 51cm

1.14 **Hand size:** 20cm

1.15 **Foot size:** 25cm

1.16 **Genitals**: Normal

1.17 **Present behaviour / condition:**

- Jumping gait

- Aphasia
- Screams when sits still
- Attention deficit
- Aggressive
- Cannot read or write
- Restless

1.18 Developmental milestones:

1.18.1 Gross motor: sitting- 4yrs, walking- 5yrs

1.18.2 Fine motor: ok

1.18.3 Cognition: poor

1.18.4 Speech: 7yrs [Aphasia]

1.19 Diagnosis: Moderate Mental Retardation

1.20 Phenotypic characterization using POSSUM:

- Following parameters were added:
  1) Irregular length or shape of toes
  2) Wide space between 1^{st} and 2^{nd} toes
  3) Cubitas Valgus
  4) Abnormal gait
  5) Flat foot/Pes Planus
  6) Mental Retardation: Moderate/Severe
- All parameters selected were mandatory and the threshold value was selected 5.
- POSSUM web result: 1 syndrome- De Grouchy Syndrome

2. Result:

2.1 Karyotype- Not done

2.2 Remarks: Distal 18q- is a genetic condition caused by a deletion of long arm of chromosome 18. Diagnosis can be done by methods like karyotyping and microarray analysis. In our study, although karyotyping could not be done, the patient showed

similar symptoms of this disorder. The condition can be confirmed by doing the karyotype analysis of the patient.

2.3 Karyotype: From the POSSUM web results, we could do the phenotypic characterization of the patient, and based on that we can say that the possible karyotype of the patient maybe 46, XY(18q-).

Patient #4

1. Clinical History:

1.1 **Proband (Age / Sex):**10/M

1.2 **Consanguineous marriage of parents:** No

1.3 **Obstetric history:**

1.3.1 **Mother's age at the birth of proband:**24yrs

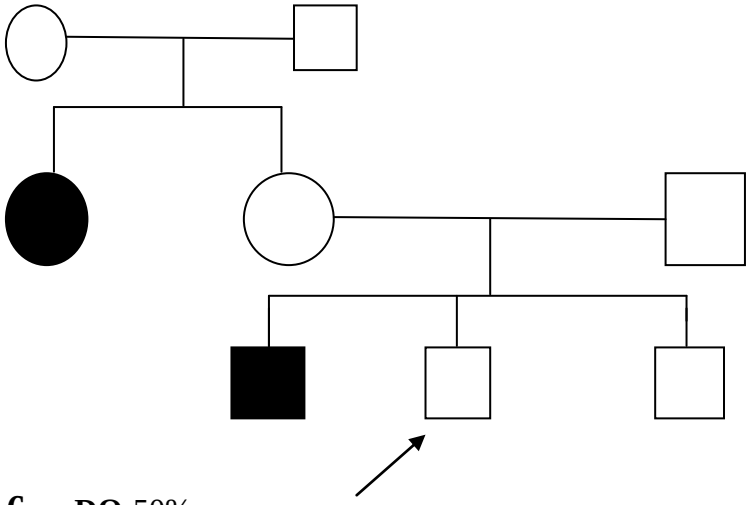
1.3.2 **History of abortions if any (Gestation age, spontaneous or induced):**nil

1.4 **Family History:**

(Age/Sex/Normal or Abnormal or not known):

1) 11/M/Mentally retarded [brother]
2) 5/M/Normal [brother]
3) M/Normal [father]
4) F/Normal [mother]
5) F/Mentally retarded [maternal aunt]

1.5 **Pedigree chart:**



1.6 **DQ:**50%

1.7 **I.Q.:**50-70

1.8 **Neurobehavioral problems:** Mild-Moderate Mental Retardation

1.9 **Associated anomalies and Phenotypic Abnormalities:**

- Short philtrum
- Prognathism

- Maloclusion
- Cobbler's chest
- Prominent forehead and Parital Bossing
- Big ears
- Big head
- Limited speech [Dyslalia]

1.10 **Height:**131.5cm

1.11 **Weight**: 23kg

1.12 **Head size:**49.6cm

1.13 **Hand size:**14.5cm

1.14 **Foot size:**23cm

1.15 **Genitals:** normal

1.16 **Present behaviour / condition:**

- Shy
- Needs help in daily activities
- Restlessness
- Normal gait

1.17 **Developmental milestones:**

Speech: impaired

1.18 **Diagnosis:** Mild-Moderate Mental Retardation

1.19 **Phenotypic characterization using POSSUM:**

- Following parameters were added:
  1) Mental Retardation- Borderline/Mild
  2) Short philtrum
  3) Prognathism
  4) Abnormal tooth position/Maloclusion/Open bite
  5) Pectusexcavatum
  6) Speech delay/defect
  7) Frontal bossing
  8) High forehead
- All parameters selected were mandatory and threshold value was selected 6.

- POSSUM web results: 10 syndromes

2. Result:

2.1 Karyotype: 46,XY

2.2 Inference: Normal

2.3 Remarks: Although the karyotype is normal, possibility of microdeletions, microduplications and other submicroscopic anomalies cannot be ruled out. Phenotypic characterization using POSSUM showed possibilities of 10 syndromes. These syndromes may not be detected by karyotyping method, and may require more sensitive molecular methods for detection.

Patient #5

1. Clinical History:

1.1 Proband (Age / Sex): 18/M

1.2 Consanguineous marriage in parents: No

1.3 Obstetric history:

1.3.1 Mother's age at the birth of proband: 20yrs

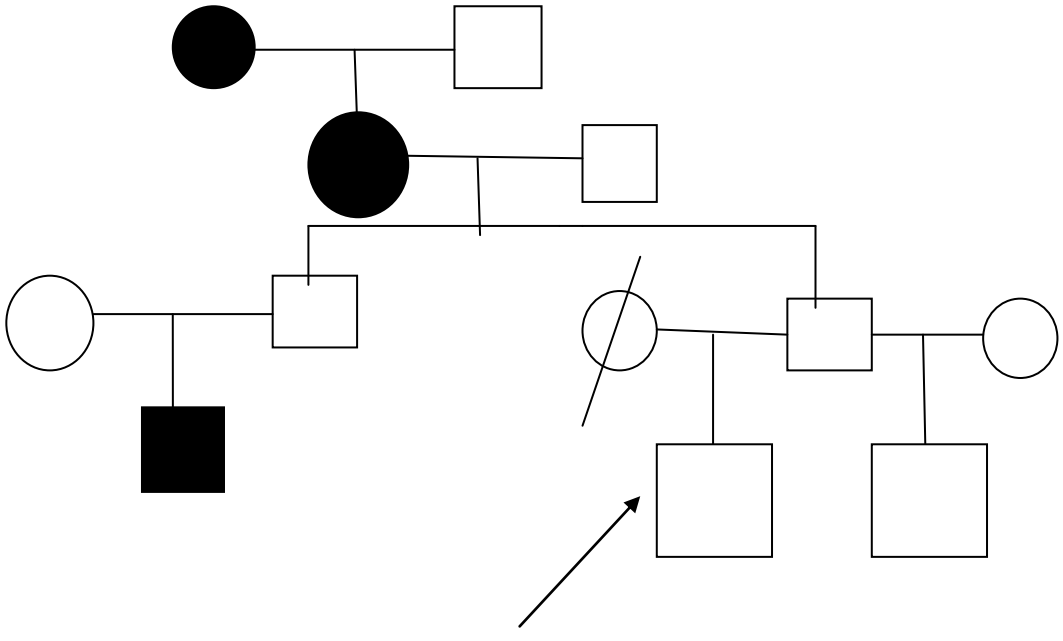
1.3.2 History of abortions if any (Gestation age, spontaneous or induced): nil

1.4 Family History:

(Age/Sex/Normal or Abnormal or not known):

1) M/ Normal [brother]
2) F/ Normal [step mother]
3) M/ Normal [father]
4) F/ Normal [real mother- dead]

1.5 Pedigree chart:



1.6 Birth history:

- Fever immediately after birth
- Convulsions at the age of 2yrs, for 2 minutes

1.7 I.Q.: 35-40

1.8 Neurobehavioral problems: Moderate Mental Retardation

1.9 Associated anomalies and Phenotypic Abnormalities:

- Hole behind right year
- Cubital angle of hand
- Dysphonia

1.10 Height: Not available

1.11 Weight:48.5kg

1.12 Head size:50.8cm

1.13 Hand size:20cm

1.14 Foot size:26cm

1.15 Genitals: Normal

1.16 Present behaviour / condition:

- Repeated speech when aggressive
- Dysphonia
- Restless, Naughty
- Stubborn
- Poor social behaviour
- Moody

1.17 Developmental milestones:

1.17.1 Gross motor: Walking- 1.5yrs, Standing- 1yr, Sitting- 7months

1.17.2 Fine motor: ok

1.17.3 Cognition: ok

1.17.4 Speech:3.5yrs

1.18 Diagnosis: Moderate Mental Retardation with speech problem

1.19 Phenotypic characterization using POSSUM:

- Following parameters were added:
  1) Speech delay/ Defect
  2) Wide carrying of elbow/ Cubitas Vulgas
  3) Mental Retardation- Moderate to Severe
  4) Preuricular tags/ Ear-pits/ Sinuses
- Threshold value was selected 4 and all the parameters were selectedto be mandatory.

- POSSUM web results: possibility of 1 of the 2 syndromes-
  Chromosome 13, deletion 13q
  Chromosome 6, partial duplication 6p

2. Result:

2.1 Karyotype: 46,XY

2.2 Inference: Normal

2.3 Remarks: Possibility of both the syndromes shown in POSSUM results may be there, but as the karyotype is normal, the possibility of their occurrence is very rare but cannot be ruled out as only one tissue is studied and the chances of mosaicism exist.

Patient #6

1. Clinical History:

1.1. Proband (Age / Sex): 19/M

1.2. Consanguineous marriage in parents: No

1.3. Obstetric history:

1.3.1. Mother's age at the birth of proband: 20yrs

1.3.2. History of abortions if any (Gestation age, spontaneous or induced): 1 spontaneous abortion at 3rd month, 1.5yrs before the birth of the Proband.

1.4. Family History:

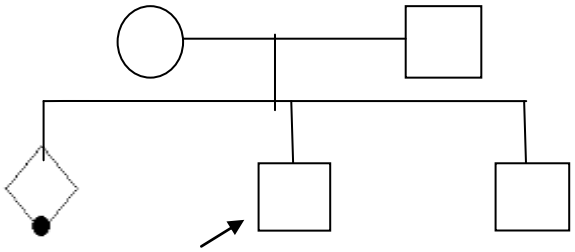
(Age/Sex/Normal or Abnormal or not known):

1) 16/M/Normal [brother]

2) M/Normal [father]

3) F/Normal [mother]

1.5. Pedigree chart:



1.6. DQ: 75%

1.7. I.Q.: 35-48

1.8. Neurobehavioral problems: Fits at the age of 4yrs, and after that the condition set in.

1.9. Associated anomalies and Phenotypic Abnormalities:

- Low set ears
- Short philtrum
- Malocclusion
- Café au lait spot
- Dyslalia

1.10. Height: 159.2cm

1.11. Weight:46kg

1.12. Head size:51.2cm

1.13. Hand size:17.2cm

1.14. Foot size:24cm

1.15. Genitals: Normal

1.16. Present behaviour / condition:

- Stubborn
- Medication for epilepsy going on
- Aggressive
- Repetition of words, actions
- Emotionally disturbed
- Depression due to epilepsy drugs
- Restless, Destructive, Naughty, Hyperactive
- Dyslalia
- Less understanding, copy writing
- Self-Mutilation

1.17. Developmental milestones:

1.17.1. Gross motor: Sitting- 7 to 8 months, Walking- 12 months, Monosyllables- 2yrs, Disyllables- 2.5yrs, Good Comprehension

1.17.2. Fine motor: ok

1.17.3. Cognition: ok

1.17.4. Speech: Limited

1.18. Diagnosis: Moderate Mental Retardation

1.19. Phenotypic Characterization using POSSUM:

- Following parameters were added:
  1) Mental Retardation- Moderate to severe
  2) Short philtrum
  3) Low set ears
  4) Café au lait
  5) Seizures of any type
  6) Speech delay/ defect

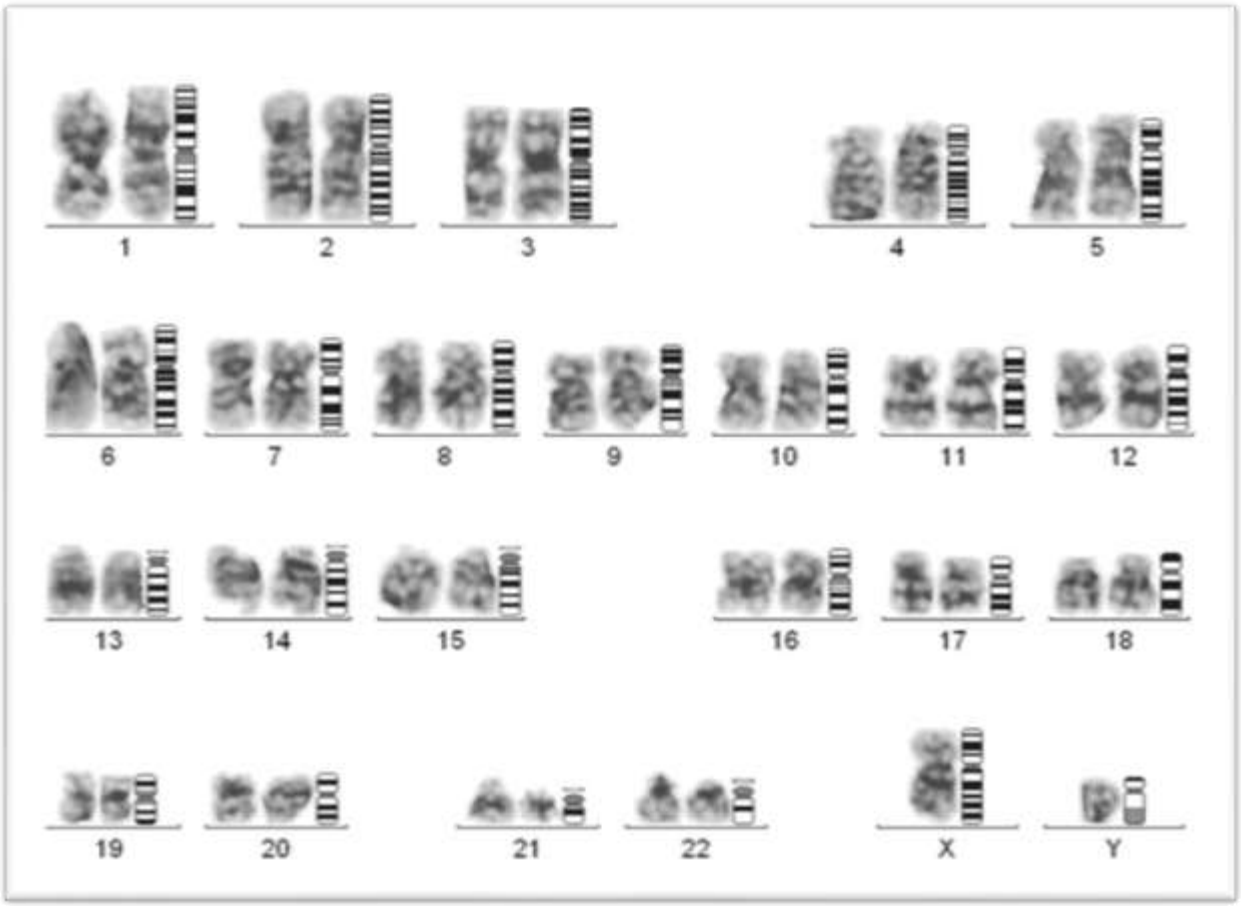
- Threshold value was selected 6 and all the characters were selected to be mandatory.
- POSSUM web results showed possibility of 3 syndromes-

  Del15q13

  Dup7(q11,q23)

  Partial dup9p

2. Result:



2.1 **Karyotype:**46,XY

2.2 **Inference:** Normal

2.3 **Remarks:** By the results of POSSUM, there exists a possibility of occurrence of 1 of the 3 conditions- del15q13; dup7(q11,q23); or partial dup9p.

All these 3 conditions cannot be detected by karyotyping, and require studies at molecular level. Thus the normal karyotype of the patient is justified.

Also, the history of the patient showed that the onset of the condition was after he got epileptic fits at the age of 4 yrs. Thus there also exists a possibility that Mental retardation may be due to epilepsy.

Patient #7

1. Clinical History:

1.1. Proband (Age / Sex):19/M

1.2. Consanguineous marriage in parents : No

1.3. Obstetric history:

1.3.1. Mother's age at the birth of proband:21yrs

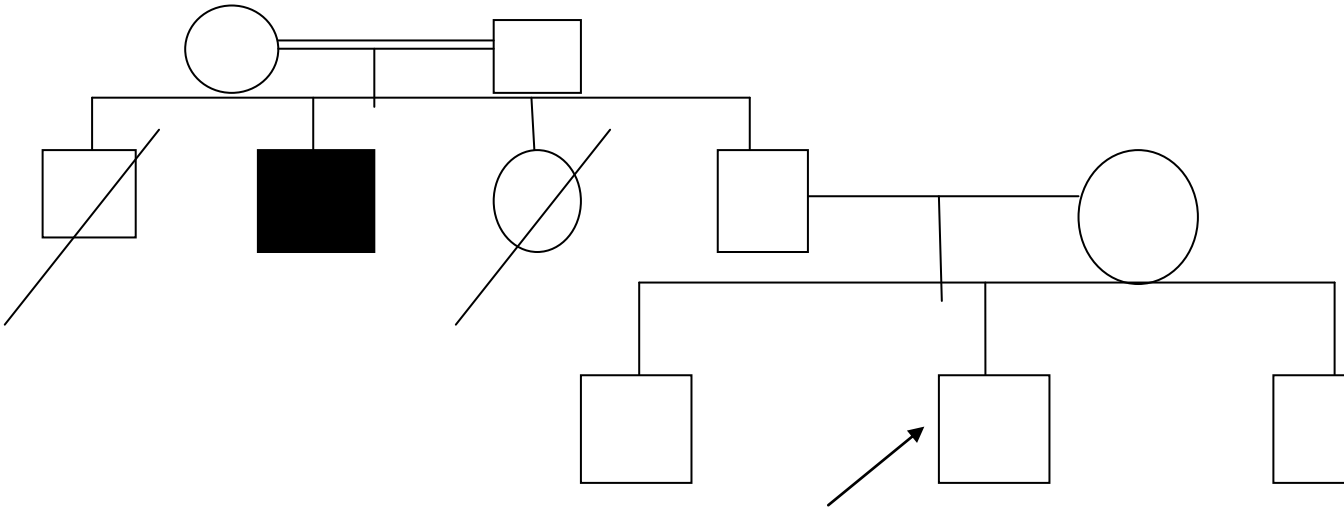
1.3.2. History of abortions if any (Gestation age, spontaneous or induced):nil

1.4. Family History:

(Age/Sex/Normal or Abnormal or not known):

1) 21/M/Normal [brother]
2) 17/M/Normal [brother]
3) M/Normal [father]
4) F/Normal[mother]
5) 49/M/Mentally Retarded [uncle]

1.5 Pedigree chart:



1.6 Birth history:

- Premature delivery, at 8th month
- Cyanosis at birth
- Suffered from seizures at the age of 1yr
- History of ascetic effusion at 1yr age

1.7 DQ: 50%

1.8 I.Q.:55-60

1.9 Neurobehavioral problems: Mild Mental Retardation

1.10 Associated anomalies and Phenotypic Abnormalities:

- Short philtrum
- Saddle back [Lumbar Lordosis]
- Protruded lower lip
- Skin scar
- Height and weight more according to age
- Mongoloid slant
- Fetal finger pads

1.11 Height: 174.7cm

1.12 Weight:80kg

1.13 Head size:55.5cm

1.14 Hand size:19.5cm

1.15 Foot size: 26.4cm

1.16 Genitals: Normal

1.17 Present behaviour / condition:

- Obsessive behaviours
- Aggressive
- Self -Mutilation
- Convulsions
- Communication fear
- Stubborn, Restless

1.18 Developmental milestones:

1.18.1 Gross motor: Walking- 1yr, Sitting- 7 to 8months

1.18.2 Fine motor: Ok

1.18.3 Cognition: Poor

1.18.4 Speech: 2yrs

1.19 Diagnosis: Mild Mental Retardation

1.20 Phenotypic characterization:

- Following parameters were added:
  1) Mental Retardation- Borderline/Mild
  2) Seizures of any type

3) Fetal finger pads
4) Accentuated Lumbar Lordosis
5) Drooping lower lip
6) Speech delay/defect

- Threshold value was selected 6 and all the parameters were selected to be mandatory
- POSSUM web result: 1 syndrome- "Mental Retardation, Dysmorphic facies, Acromicria Hypogonadism"
- POSSUM web result when the threshold was kept 5:
  2 syndromes possible-
  "X-linked Mental retardation, Hypotonic facies syndrome"
  "Mental Retardation, Dysmorphic facies, Acromicria Hypogonadism"

2. Results:

2.1 Karyotype: 46,XY

2.2 Inference: Normal

2.3 Remarks: Eventhough the karyotype is normal, according to the POSSUM web results, possibility of occurrence of X-linked Mental retardation or hypotonic facies syndrome is there. Both these conditions cannot be detected by karyotyping, and more sensitive molecular methods are required to be done to confirm the presence of any of these 2 syndromes.

Discussion

The etiological study of mental retardation does not ensure the corresponding treatment to be completely fruitful. However it is worth making an effort as it can help managing the disease and creating awareness in the society and bridging the communication gaps. The sub-microscopic mutations in the chromosome cannot be detected by only karyotyping. However, phenotypic characterization with the help of a clinician and an online software POSSUM can give a list of possible disease conditions and syndromes that can provide further indications for laboratory testing. The further data query using OMIM can help clinicians, medical geneticists, and researchers to narrow down the possible diagnosis from an array of clinical phenotypes. The diagnosis of a clinical condition is important to select the most relevant treatment, supportive therapy, risk assessment for the affected individual as well as siblings and future progeny.

The parameters considered for query into POSSUM database software for patients resulted in list of possible corresponding syndromes. When the OMIM search was done for the suggested syndromes, the phenotypic characters could be correlated in index patient as entered in POSSUM.

The query for patient-1 (14yrs/M) yielded around 163 possible syndromes corresponding to the phenotypic characters entered in POSSUM. These syndromes did not have all the parameters added however, hence the search was repeated with increased number of threshold for mandatory features which resulted in following two syndromes with maximum listed parameters.

2.3.1.1.1.1.1. Leigh Syndrome: The MIM number of Leigh Syndrome was #256000. A number sign (#) is used with this entry because of extensive genetic heterogeneity in Leigh syndrome. Mutations have been identified in both nuclear- and mitochondrial-encoded genes involved in energy metabolism, including mitochondrial respiratory chain complexes I, II, III, IV, and V, which are involved in oxidative phosphorylation and the generation of ATP, and components of the pyruvate dehydrogenase complex. It is an early-onset progressive neurodegenerative disorder with a characteristic neuropathology consisting of focal, bilateral lesions in one or more areas of the central nervous system, including the brainstem,

thalamus, basal ganglia, cerebellum, and spinal cord. The lesions are areas of demyelination, gliosis, necrosis, spongiosis, or capillary proliferation. Clinical symptoms depend on which areas of the central nervous system are involved. The most common underlying cause is a defect in oxidative phosphorylation (Dahl, 1998).Leigh syndrome may be a feature of a deficiency of any of the mitochondrial respiratory chain complexes: complex I deficiency (252010), complex II deficiency (252011), complex III deficiency (124000), and complex IV deficiency (cytochrome c oxidase; 220110), or complex V deficiency (604273).

2.3.1.1.1.1.2. Congenital Disorder of Glycosylation whose MIM number of was #212065ICD+. A number sign (#) is used with this entry because congenital disorder of glycosylation type Ia (CDG Ia, CDG1A) is caused by mutation in the gene encoding phosphomannomutase-2 (PMM2; 601785).Congenital disorders of glycosylation (CDGs) are a genetically heterogeneous group of autosomal recessive disorders caused by enzymatic defects in the synthesis and processing of asparagine (N)-linked glycans or oligosaccharides on glycoproteins. These glycoconjugates play critical roles in metabolism, cell recognition and adhesion, cell migration, protease resistance, host defence, and antigenicity, among others.

The query for patient-2 (16yrs/M) resulted in one syndrome that corresponded to all the parameters we entered in POSSUM. It was Bronchiooculofacial syndrome (BOFS) and its MIM number was #113620.A number sign (#) is used with this entry because of evidence that branchiooculofacial syndrome is caused by heterozygous mutation in the *TFAP2A*gene*(*Activating enhancer binding Protein 2 alpha) (107580).The AP-2 alpha protein acts as a sequence specific DNA-binding transcription factor recognizing and binding to the specific DNA sequence and recruiting transcription machinery. Its binding site is a GC-rich sequence that is present in the cis-regulatory regions of several viral and cellular genes. Branchiooculofacial syndrome (BOFS) is characterized by branchial cleft sinus defects, ocular anomalies such as microphthalmia and lacrimal duct obstruction, a dysmorphic facial appearance including cleft or pseudo cleft lip/palate, and autosomal dominant inheritance. Although anomalies of the external and middle ear frequently cause conductive hearing loss in BOFS, severe to profound sensorineural hearing loss due to inner ear anomalies has rarely been reported (Tekin *et al.,* 2009).

The queries entered for patient-3 (15yrs/M) gave no syndromes matching with all the parameters entered in POSSUM. We filtered the search and compromising on a parameter, one syndrome was shown. It was De Grouchy Syndrome and its MIM number was

#146390ICD+, del18p Syndrome. A number sign (#) is used with this entry because it represents a contiguous gene deletion syndrome. The 18p- syndrome was first described in 1963 by De Grouchy *et al.* The main clinical manifestations are mental retardation, growth retardation, craniofacial dysmorphism including round face, dysplastic ears, wide mouth and dental anomalies, and abnormalities of the limbs, genitalia, brain, eyes, and heart. Tsukahara *et al.*, 2001 noted that the round face characteristic in the neonatal period and childhood may change to a long face with linear growth of the height of the face.

The queries for patient-4 (10yrs/M) resulted in a set of 10 syndromes relating to the parameters given in POSSUM. They were 3M syndrome, Marshall-Smith Syndrome, subtelomeric del19p, partial del9q, etc. There are three 3M Syndromes naming 3M Syndrome 1, 2 and 3 and there phenotypic MIM numbers are #273750, #612921 and #614205 respectively. There phenotypic characters and clinical features are related. The 3M syndrome is an autosomal recessive disorder characterized by distinctive facial features, severe prenatal and postnatal growth retardation, and normal mental development. The main skeletal anomalies are long, slender tubular bones, reduced anteroposterior diameter of the vertebral bodies, and delayed bone age. Other skeletal manifestations include joint hypermobility, joint dislocation, winged scapulae, and pesplanus (Badina *et al*., 2011). All patients had short stature, prominent heels, and a distinctive facial appearance with anteverted nares, fleshy tipped nose, frontal bossing, midface hypoplasia, and prominent heels, and were phenotypically indistinguishable from those with 3M syndrome-1.The 3M syndrome is characterized by poor postnatal growth and distinctive facial features, including triangular facies, frontal bossing, fleshy tipped nose, and fleshy lips. Other features may include skeletal anomalies and prominent heels (Hanson *et al*., 2011).

The Marshall-Smith syndrome is given a phenotypic number 602535 by MIM. It is the malformation syndrome characterized by accelerated skeletal maturation, relative failure to thrive, respiratory difficulties, mental retardation, and unusual facies, including prominent forehead, shallow orbits, blue sclerae, depressed nasal bridge, and micrognathia (Adam *et al*., 2005).

The clinical features as obtained from OMIM matched the parameters we entered in POSSUM. Further studies can be done corresponding to the results obtained.

The queries for patient-5 (18yrs/M) resulted in two syndromes corresponding to the phenotypic characterization done by POSSUM. They were del13q and partial dup6p. However, no connection could be made by the results after entering them in MIM search.

The queries entered in patient-6 (19yrs/M) resulted in three syndromes having all the parameters given in POSSUM. They were microdeletion 15q13, dup7(q11,q23), partial dup9p.

OMIM search for microdeletion 15q13: Recurrent microdeletion syndrome characterized by mental retardation, epilepsy, and variable dysmorphism of the face and digits. There was a description of 9 affected individuals, including 6 probands: 2 with de novo deletions, 2 who inherited the deletion from an affected parent, and 2 with unknown inheritance. Features shared among 3 or more individuals included hypertelorism, up slanting palpebral fissures, prominent philtrum with full everted lips, short and/or curved fifth finger, and short fourth metacarpals. Skeletal and/or joint defects of the hand were observed in 7 of the 9 individuals. Seizures or abnormal electroencephalograms were reported in 7 of the 9 individuals. Sharp *et al.,* 2008 recommended that testing for the 15q13.3 deletion syndrome should be considered in individuals with unexplained mental retardation, seizures, and mild dysmorphic features.

OMIM result of clinical features for patient 6 was matching with the present symptomatic condition.

The queries entered for patient-7 (19yrs/M) resulted in two syndromes relating to the phenotypic parameters. It was "Mental Retardation, dysmorphic facies, acromicria hypogonadism" and "X-linked Mental Retardation Hypotonic facies syndrome" and its MIM number was #309580. A number sign (#) is used with this entry because the phenotype is caused by mutation in the ATRX gene(ATP-dependent helicase ATRX, X-linked helicase II).Transcriptional regulator ATRX contains an ATPase / helicase domain, and thus it belongs to the SWI/SNF family of chromatin remodelling proteins. This protein is found to undergo cell cycle-dependent phosphorylation, which regulates its nuclear matrix and chromatin association, and suggests its involvement in the gene regulation at interphase and chromosomal segregation in mitosis. The term 'X-linked mental retardation-hypotonic facies syndrome' comprises several syndromes previously reported separately. These include Juberg-Marsidi, Carpenter-Waziri, Holmes-Gang, and Smith-Fineman-Myers syndromes as well as a family with X-linked mental retardation with spastic paraplegia. All

these syndromes were found to be caused by mutation in the XH2 gene and are characterized primarily by severe mental retardation, dysmorphic facies**,** and a highly skewed X-inactivation pattern in carrier women (Abidi *et al.,* 2005). Other more variable features include hypogonadism**,** deafness, renal anomalies, and mild skeletal defects.

POSSUM and OMIM data base proved to be really helpful for describing the phenotypic characters in medical terms, and cross checking with the database of such patients.

Although the cause of mental retardation remains unknown in up to 80% of patients and chromosomal imbalances contribute about 29% to mental retardation, with approximately half of the aberrations being cytogenetically visible and the rest being cryptic (Lenhard *et al.,* 2005). There is also wide variation in the category of reported causes of mental retardation: 18.6% to 44.5% of cases have exogenous causes, such as teratogen exposure or infection, and 17.4% to 47.1% have genetic causes. No single approach to the diagnostic process is supported by the literature (John B. Moeschler *et al.,* 2006). Pathogenic chromosomal abnormalities detected through first karyotyping account for 15% of all cases. The second most common cause of MR is clinically recognizable microdeletion syndromes, which accounted for 4.7% of patients with unexplained mental retardation (Anita Rauch *et al.,* 2006). It has also reported that 3.1–3.4% MR us due to microdeletion syndromes in patients with unexplained mental retardation (Van Karnebeek *et al.,* 2002; Devriendt *et al.,* 2003). The detection rate of causative aberrations by Subtelomeric screening in unselected patients with unexplained mental retardation is 2% after thorough clinical and cytogenetic evaluation. The frequency of truly cryptic subtelomere abnormalities is reported to be 2.6% (Yu *et al.,* 2005). The application of banding techniques had made possible not only to detect the chromosome abnormalities but also to delineate the exact points of rearrangements (Tetsuji Kadotani *et al.,* 2003).

All newborns should undergo basic screening for common metabolic disorders shortly after birth. If indicated by neurological signs or regression, MRI and/or extended metabolic workup should be done. The patients should be categorized by use of the DeVries score (De Vries *et al.,* 2001), which is a score to measure the presence of malformations, dysmorphism, growth anomalies, and familial occurrence as frequent signs in chromosomal aberrations. If no laboratory-proven monogenic diagnosis is established, chromosomal analysis should be performed and repeated by GTG-banding with a resolution of at least 450 bands, but can also be 500–550 according to ISCN 2005 (Shaffer and Tommerup, 2005). If

GTG-banding revealed normal results, Subtelomeric screening should be performed in every patient by two-color FISH analysis on metaphase spreads using the optimized BAC/PAC clone set (Knight *et al.,* 2000). Molecular karyotyping should be performed as described by (Rauch *et al.,* 2004).Targeted mutation analyses should be performed by direct sequencing after PCR amplification using exon specific primers and a capillary sequencer. X-inactivation studies should also be performed in mothers of patients as described by (Lau *et al.,* 1997).In order to rule out the presence of any type of chromosomal abnormalities various cytogenetic and molecular cytogenetic techniques should be considered.

The clinical management and research progress of human genetic disorders will be benefitted by teamwork of clinicians, laboratory personnel, researchers, and social workers involved in patient care in addition to the guardians. The current work presents beginning of a small step forward in this direction.

Summary

Mental retardation in humans can be categorized as mild, moderate and severe, with occurrence rate of approximately 2-3% in general population, usually with undiagnosed etiology. Determining whether the disabilities are associated with malformations or multiple congenital anomalies and/or dysmorphic features can be helpful because it can suggest a syndromic clinical diagnosis to a skilled clinician and will guide the selection of diagnostic testing. Genetic abnormalities are the most common identifiable cause of developmental delay and mental retardation. In this study, the GTG banding patterns were studied using phytohemaglutinin-M stimulated lymphocytes cultured from peripheral blood. Seven individuals with uncharacterized mental retardation were investigated in terms of family history, dysmorphic features, multiple malformations, delayed milestones, learning disability and IQ level. Their clinical history and family background were studied to find the etiology of the disorder. Patients with an unexplained cause for mental retardation were analyzed for numerical and structural chromosomal aberration by karyotyping. Also, their phenotypic characterization was done using POSSUM software to get an idea of the underlying condition from phenotypic characters. The chromosomes were studied at 350-400 band level in metaphase stage using Ikaros (Metasystems, Germany). All the seven patients were found to have a normal karyotype with 46,XY chromosome complement and without any numerical and structural abnormality. Using POSSUM results, the possible underlying genetic conditions were indicated and all the patients showed the possibility of submicroscopic mutations which cannot be detected by karyotyping.

Also the presence of mosaicism cannot be ruled out because only one tissue was studied and the tissues from other germ cell layers remained undetected because mosaicism occurs due to cell division error in both meiosis and mitosis, and also from a cell division error after fertilization.

In order to further confirm the etiology of a genetic disorder other advanced cytogenetic tests should be performed to find out the abnormalities that are far from reach of banding technique viz; FISH, high resolution banding, and array-CGH.

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