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Genetic Clinics

Genetic Clinics is a quarterly newsletter published by the Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow on behalf of Genetics Specialty Chapter of Indian Academy of Pediatrics. The newsletter aims to provide a forum that enhances the practice and education of medical genetics in India. Articles of interest to the medical professionals in the field of medical genetics are welcome. The broad topics include: Genetic bases of diseases, chromosomal disorders, dysmorphic syndromes, malformations, Mendelian disorders, genetics of complex diseases, genetic testing, prenatal diagnosis, perinatal autopsy, teratogenesis, genetic counseling, laboratory practices, professional issues, psychological aspects, social aspects and legal aspects in the practice of medical genetics. The articles undergo limited peer-review at present and editing of content and style.

The categories of article include:

DeNoVo	Original articles with new findings and development in the field of medical genetics are considered. Word limit is 2000. Restrict the number of references to 15.
GeNeViSTA	Review articles, approach to common genetic problems and opinions from experts in the field are considered. Word limit is 1500-2500. Number of references should not exceed 10.
Clinical Vignettes	Brief case reports not exceeding 1000 words. Limit the number of references to 5.
GeNeXprESS	This is intended to serve as a guide to further reading. Articles of interest to clinicians published recently in leading journals are covered. One paragraph should describe the article.
PhotoQuiz	Good quality photographs of a typical genetic disease or clinical sign. Three to four sentences should describe the condition followed by a question asking the readers to identify the condition. There should be preferably one answer to the query which is unambiguous. The answer should also be provided with one paragraph giving crisp information on the condition.
gEne Mails	Letters to the editor discussing the contents of previous issues, comments and suggestions to the editorial board are considered. The section does not ask the response of the author to the comments.
GeneQueries	Clinical case scenarios in practice can be posted and the opinions of experts are sought by the editorial team on further management. The query needs to be precise and unambiguous. Both the question and the answer are published in the same issue.
EvEnTs	Conferences, workshops and continuing medical education programs related to the field of medical genetics are published free of cost. They should be as brief as possible. They are subject to editing of content and style.
GeNeToONs	Cartoons, jokes, humor related to the field of medical genetics are welcome.

Style of references: The articles should conform to Vancouver style of referencing. Only one author is listed.

Photographs of patients: It is the responsibility of the authors to take written consent from the patient or guardian for publication of photographs.

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Diagnosis of Index Case: Why and How?

Shubha Phadke

The second issue of 'Genetic clinics' was in your hands well in time and was appreciated by many reader friends and colleagues. I hope it continues to be regularly published with the contents useful and interesting to you all. This will not be possible without the support of you all. I request everybody to send articles, case reports etc., for publication in the newsletter and share your knowledge and experience with all of us. I also request readers to let us know the topics on which they could like articles in the newsletter. Our mail box is always waiting for suggestions from you.

This issue has an article on approach to a case of mental retardation / developmental delay. Mental retardation / developmental delay is a common clinical presentation requiring genetic counseling. It is a complex symptom with heterogeneous etiologies. In spite of increasing availability of battery of tests, the etiology can not be identified in half of the cases. So identifying the cause of mental retardation continues to remain a challenge to clinician and is a major area of research. Whatever is the etiological diagnosis, there is no curative treatment. Hence, search for the cause many a times turns out to be a frustrating venture for the family and the clinician. Many would like to ask 'Why do we need all these costly and cumbersome efforts of investigations for a child with mental retardation?' It is to provide an answer to the questions of the parents. First, 'why did this happen to our child' and the second question is 'Will it happen once again in our family?' These questions can not be answered accurately unless you know the exact cause of mental retardation in the affected individual i.e. the proband in the family.

Like all medical situations, accurate diagnosis is of paramount importance in genetic counseling. Search for etiology of mental retardation is an extremely difficult task as there are no clinical clues in most of the cases. There is no sure shot algorithmic approach or battery of tests. Diagnostic approach has to be individualized based on clinical examination and some preliminary investigations. The investigations are costly and need to be done step by step. This needs the co-operation of the family. This will be possible only if the clinician communicates clearly with the family about the possible results and their implications for the family. Most of the times it is important to inform the family that the results may not be of direct relevance for the management of the family and cure is not possible. Of course, all attempts should be made to guide the family about supportive, rehabilitative care and training facilities for the optimal outcome. This helps them to have realistic expectations from the costly investigations. Otherwise, telling that there is no treatment for the condition, may be a sudden blow to the parents expecting a miracle prescription after the results of the MRI, karyotype, GCMS, etc. This ends the family-clinician interaction on a frustrating and sour note. The family then may not be receptive to counseling regarding the risk of recurrence in the family and prevention. The above situation and facts sadly remain true for many genetic disorders where diagnosis of probands is essential for genetic counseling and may not be of much use for the affected individual in the family.

The clinician should keep these issues in mind while dealing with patients and families with a genetic disorder. This is necessary for the fruitful and satisfactory outcome of the genetic counseling. Good communication and truthfulness helps to build rapport with the family who would be convinced that whatever possible is being done for them with the intention to help them as much as possible.

Genetic counseling and prenatal diagnosis will continue to remain important modalities to prevent genetic disorders and accurate diagnosis of the proband is the first step in genetic counseling.

Shubha Phadke
1st January, 2009

Orchidometers

Locally made orchidometers for testicular volume assessment (see photograph) are available with the pediatric endocrinology group. Those interested in purchasing one may contact Dr Vijayalakshmi Bhatia (vbhatia@sgpgi.ac.in)

Counseling for exposure to antiepileptic drugs in Pregnancy

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INTRODUCTION

An agent that can cause a birth defect by interfering with the normal embryonic or fetal development is known as teratogen. If a drug taken by a mother during pregnancy interferes with the development of the embryo or fetus, it is known as a teratogenic drug. Perhaps the best example of a teratogen is evident from the thalidomide tragedy in 1960's when the drug thalidomide was given to women in early pregnancy for morning sickness. This resulted in abnormal development of limbs (arms and legs). This was later withdrawn from the market for use in pregnancy. However, for a majority of drugs there are no conclusive studies to prove that they are teratogenic in humans or animals. This article addresses the issues that need to be considered while counseling a patient exposed inadvertently to anti-epileptic drugs (AED) during pregnancy. Teratogenic effect of AEDs depends on several factors

- Dosage and Threshold Effects: A teratogen can cause an effect only after a certain level of exposure is reached.
- Timing: The effect of teratogens depends upon the timing of exposure. The first trimester of pregnancy is the critical period of organ and limb development in the fetus. The fetal brain develops throughout pregnancy and can be affected at any time. Exposure to a teratogen during the two weeks following conception is unlikely to cause birth defects.
- Although teratogens may increase the risk of birth defects, they do not necessarily cause problems in all cases.

Two to three percent of all babies are born with some type of birth defect. Exposure to a teratogen may increase this risk, but nothing can eliminate the risk.

CASE PRESENTATION

A 30 year old lady on antiepileptic medicines (tablet Sodium Valproate) for last 15 years for generalised seizures was unexpectedly pregnant. The couple wanted to get genetic counselling regarding their first pregnancy. This was an unplanned pregnancy and the excitement was evident at the appointment. Her husband had given the impression that he was happy to become a father but there were doubts regarding the birth of a healthy baby. They were an educated couple with one of their relatives in the medical profession and were very anxious. The couple had decided to discontinue the pregnancy if the risks were high. The mother was undecided on the outcome of the pregnancy and urgently came for advice.

Family history revealed the lady's mother has had five miscarriages. There were no problems of seizures in other family members.

The main issues that came up during the appointment were:

a) To create awareness about the potential teratogenic effect of sodium valproate
b) The information regarding the role of folic acid in reducing the risk of neural tube defect (NTD) and the correct dose recommended in a pregnancy exposed to valproic acid.
c) The risk of epilepsy in her children.
d) The potential ways of monitoring for teratogenic effects.
e) The potential adverse outcome after the baby is delivered (neonatal complications).
f) The issue of her mother's multiple miscarriages.
g) Preparing for the decision to continue with the pregnancy and its outcome.
h) The requirement of support, preparation and counseling required in pregnancy.

Background information – ANTICONVULSANTS / ANTIEPILEPTICS

- Anticonvulsants: Exposure to antiseizure medications is associated with fetal anticonvulsant syndrome. In general, the exposure to antiepileptic drugs during pregnancy is associated with 2 to 3 fold increase in the risk of major congenital malformations. Risk is more with polytherapy than monotherapy. The malformations seen in the fetuses exposed to antiepileptics are similar to those seen in general population. Approximately 10% of children exposed to Dilantin and other seizure medications will have some growth retardation, digit hypoplasia (shortening), and a characteristic face. Use of valproic acid and carbamazepine in early pregnancy are associated with a 1 to 2% risk of spina bifida. The teratogenic effects of valproate is dose dependent, with higher risk at dosage level >1000 mg/day.
- Recently some studies have investigated the possibility that exposure to AEDs in utero may adversely affect the postnatal cognitive development of the offspring and suggested that valproic acid poses a higher risk compared to other AEDs. This is an important observation, but must be interpreted with caution because of the methodological shortcomings of the studies and because adequately powered prospective studies are necessary to draw a firm conclusion.

Carbamazipine, phenytoin sodium, methylphenobarbitone, phenobarbitone, primidone, sodium valproate are considered in the general category B drugs.

DISCUSSION

Sodium valproate (valproic acid) is a commonly used anticonvulsant. Valproic acid is also used to treat bipolar disorder and to prevent migraine headaches in some patients.

If taken in the first trimester of pregnancy, sodium valproate is associated with a one to two percent risk of neural tube defects (especially spina bifida) in the exposed fetus. Women taking sodium valproate who become pregnant should be encouraged to consider detailed mid-trimester morphology ultrasound for prenatal diagnosis of such abnormalities.

The various teratogenic associations linked to valproic acid are as follows:

- **Fetal valproate syndrome:**
  A distinctive pattern of minor anomalies of the face and digits, i.e. "fetal valproate syndrome", has been described in infants born to women treated with valproic acid during pregnancy.[1-3] This syndrome involves postnatal growth retardation, microcephaly, metopic sutural fusion, developmental delay, midface hypoplasia, epicanthic folds, short nose, broad nasal bridge, long flat philtrum, thin vermilian border of upper lip and micrognathia. This represents the severe end of a spectrum of anomalies. The actual incidence of fetal valproate syndrome is not known. In one series, nine (53%) of 17 infants born to epileptic women who had been treated with valproate during pregnancy had features of fetal valproate syndrome.[4]
- **Spina Bifida:**
  An association between the occurrence of spina bifida in infants and maternal use of valproic acid in the first trimester of pregnancy has been observed in a case control study involving 337 affected children[5]. The risk of spina bifida is increased to about 2% among the children of epileptic women treated with valproic acid during the first trimester of pregnancy. The defect observed is usually lumbar or sacral spina bifida; it is often associated with hydrocephalus.[6,7] Anencephaly is rarely seen. In one study, the valproic acid doses and serum levels were significantly higher in five women with epilepsy who had children with spina bifida than in 84 epileptic women who took valproic acid during pregnancy but had unaffected children.[7,8]
- **Neurological Abnormality:**
  The data about effect of anticonvulsants on cognitive function is not conclusive. Some studies have reported high incidence of mental retardation or special needs in school in children exposed prenatally to anticonvulsants.[9, 10] But large scale studies with long term follow up are needed before definite conclusions can be drawn. Behavioral abnormalities, most often hyperactivity, poor concentration, or autistic features are very common among children with fetal valproate syndrome.[3]

  The risk of having an abnormal child as a result of antiepileptic medication is far outweighed by the dangers to the mother and fetus of uncontrolled epilepsy.

Guidelines and Recommendations for women on AEDs during pregnancy:

1. Women on antiepileptic drugs (AEDs) should receive prepregnancy counseling with regard to the risk of fetal abnormalities.
2. AEDs should be continued during pregnancy and monotherapy should be used if possible at the lowest effective dose as risk of abnormality is greater in women taking combined medication.
3. Folic acid supplementation (5mg) should be commenced four weeks prior to and continue for twelve weeks after conception.
4. Pre-pregnancy counseling about increased risk of malformations and prenatal diagnosis including detailed mid-trimester ultrasound should be offered.

Case Related Counselling

Overall risk to the fetus was to be weighed against a seizure episode that can pose to the developing baby before considering withdrawal or reduced dose of the antiepileptic medication. The option of discussing this issue could also be discussed with her neurologist so that she could be at the minimum risk with the maximum benefit.

It was explained to the lady that there was an increased risk of congenital anomaly mainly NTD. Folic acid is useful in periconceptional period and the woman should have been advised to take 4 mg of folic acid every day as she was in reproductive age group.

It was explained that a 4% risk existed for the child to develop epilepsy based on the population studies of children born to epileptic parents.

The karyotype of the mother should only be done if her mother was a known carrier of balanced translocation or she herself has recurrent spontaneous abortions or infertility. Otherwise, she should be offered Down syndrome screening as is done for all pregnant women. She needs to be offered screening for beta thalassemia as a test offered to all.

It was evident after the talk that she was planning to continue the pregnancy and management options were discussed with her to screen for birth defects.

First trimester ultrasonography has to be done at 12 weeks which would detect anencephaly at the earliest. This along with detailed morphological scan at 18 weeks should detect 97% of the neural tube defects. Other major anomalies will be picked up though minor anomalies and functional problems of the brain would not be picked.

OTHER ANTICONVULSANTS / ANTIEPILEPTICS

Antiepileptic drugs have been associated with an increased risk of major congenital malformations, minor anomalies, specific congenital syndromes, and developmental disorders seen in childhood.[11]

Carbamazepine

Spina bifida occurs in about one percent of pregnancies in which carbamazepine is used as monotherapy. Carbamazepine taken during pregnancy also has been

associated with minor craniofacial defect, fingernail hypoplasia and developmental disability. Carbamazepine also can cause coagulation defects with consequent risk of haemorrhage in the fetus and the newborn infant which may be prevented by the prophylactic administration of vitamin K to the mother prior to delivery.

Phenytoin Sodium

This drug taken during pregnancy has been associated with craniofacial defects, fingernail hypoplasia, developmental disability, growth retardation and less frequently, oral clefts and cardiac anomalies. This clinical pattern is sometimes called the 'fetal hydantoin syndrome'. Phenytoin also can cause coagulation defects with consequent risk of haemorrhage in the fetus and the newborn infant which may be preventable by the prophylactic administration of vitamin K to the mother prior to delivery.

Clonazepam

Clonazepam is a benzodiazepine. These drugs may cause hypotonia, respiratory depression and hypothermia in the newborn infant if used in high doses during labour. Withdrawal symptoms in newborn infants have been reported with this class of drugs.

The use in pregnancy of primidone, phenobarbitone or methylphenobarbitone has been associated with minor craniofacial defect, fingernail hypoplasia and developmental disability. Their use in pregnancy alone, or in combination with other anticonvulsants, can cause coagulation defects in the newborn infant which may be preventable by the prophylactic administration of vitamin K to the mother prior to delivery. Insufficient information is available to determine the teratogenic effects of newer anticonvulsants as compared to conventional anticonvulsants. However lamotrigine during pregnancy is associated with an increased risk of neural tube defects in the offspring.

Organization of Teratology Information Specialists (OTIS) (http://www.otispregnancy.org/) provides useful information and fact sheets on a number of drugs used and is regularly updated.

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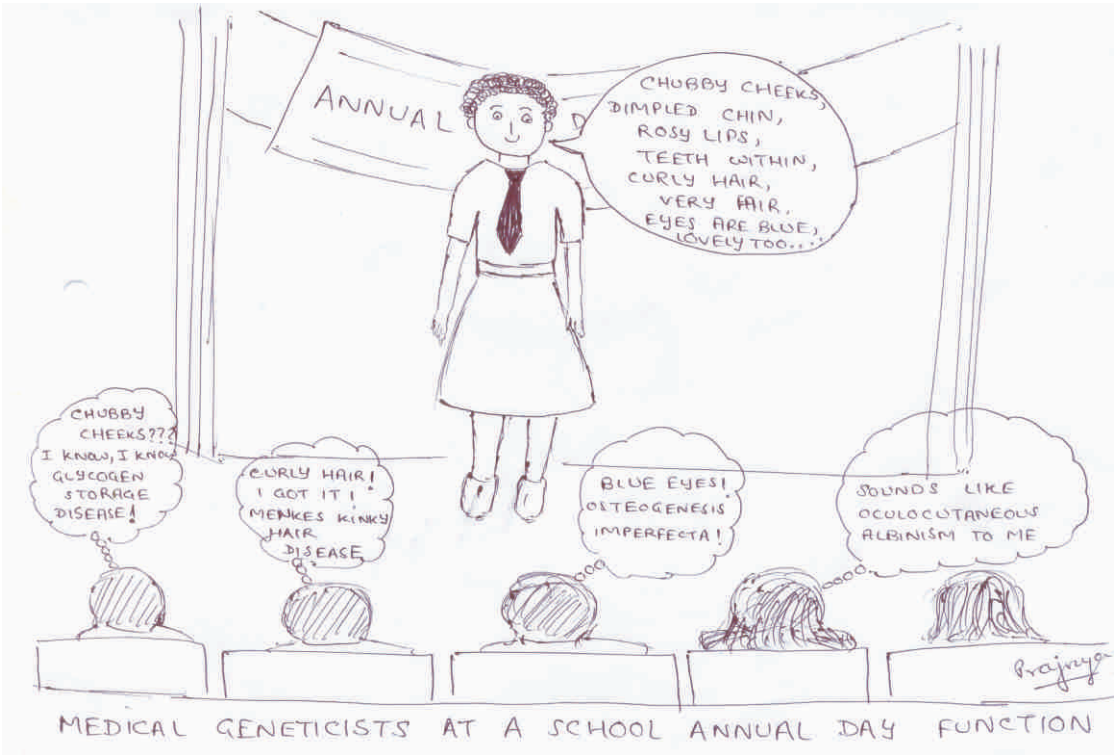
GeNeToONs



Contributed by: Prajnya Ranganath

Approach to a Child with Mental Retardation

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Abstract :

Mental retardation or global developmental delay is an etiologically heterogeneous entity. Having an etiological explanation aids in the development of a specific treatment plan, prognosis and recurrence risk. This paper describes the diagnostic evaluation of the child with developmental delays or mental retardation to assist clinicians in the management of these children.

Mental retardation (often referred to as "intellectual disability" and "cognitive disability"), or global developmental delay is one of the commonest indications for genetic counseling. Early identification and specific etiologic diagnosis has immediate implications with respect to treatment, prognosis and recurrence risks. Recently, a consensus regarding the appropriate laboratory and clinical evaluation of this problem has been reached.[1, 2]

DEFINITIONS

Mental retardation (MR) or developmental delay (DD) is a lifelong disability that presents in infancy or the early childhood years but is difficult to diagnose until the child is older than 5 years, when standardized measures of intelligence become reliable and valid. It is defined as "significant sub-average intellectual function existing concurrently with deficits in adaptive behavior and manifested during the developmental period". The American Association on Mental Retardation defines mental retardation by measures of 3 domains: intelligence (IQ), adaptive behavior, and systems of supports. Thus, one cannot rely solely on the measure of IQ to define mental retardation.[3] Recently, MR has been categorized as "mild' (IQ range 50-70) and "severe" (IQ <50). The term "developmental delay" is usually reserved for younger children (typically younger than 5 years), and the term "mental retardation" is usually applied to older children when IQ testing is valid and reliable.

EXTENT OF PROBLEM

It is generally accepted that MR occurs in 2-3% of the general population. Prevalence of milder MR is seven to ten times more common than severe MR. The male to female ratio ranges from 1.3 to 1.9:1; the higher male incidence is mainly attributed to X-linked MR.

ETIOLOGY OF MENTAL RETARDATION

Cause of severe MR can be determined in 60-70% of cases as compared to mild MR where 35-55% remains idiopathic. Review of literature suggests 6 major categories of mental retardation -

1) Chromosomal abnormalities (Down syndrome - fig 1) (4 – 28%)
2) Recognizable syndromes (Fragile X Syndrome - fig 2, X linked alpha thalassemia - fig 3, Coffin Lowry syndrome, Rett syndrome - fig 4) (3 – 9%)

Fig 1: Down Syndrome
Note the flat facies

Fig 2: Fragile X syndrome
Note the long facies and mild prognathism

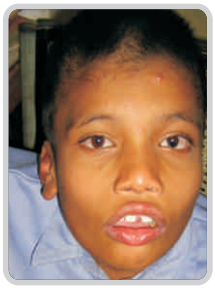

Fig 3: X linked alpha thalassemia
Note the coarse facial features and wide spaced incisors

Fig 4: Rett syndrome
Note the hand stereotypes

3) Structural Central Nervous system malformations (3 – 17%)
4) Acquired disorders like complications of prematurity (2 – 10%), Perinatal conditions (8–13%), Environmental / teratogenes (anticonvulsants, alcohol, infections) (5 – 13%)
5) Metabolic / endocrine causes (1 – 5%)
6) Nonspecific/Unclassified (30-50%)

Down syndrome is the commonest cause of mental retardation whereas Fragile X mental retardation is commonest cause of inherited mental retardation.

APPROACH TO A CHILD WITH MR

The diagnostic approach that should be undertaken for a child with an intellectual disability includes the following:

History-

a) Antenatal period (age at conception, teratogens [drugs and infection] other illnesses, abnormal ultrasound).

b) Perinatal period (prematurity, asphyxia, growth retardation, sepsis, hyperbilirubinemia).

c) Postnatal period (seizures, history of meningitis / encephalitis, abnormal posturing, spasticity, visual and hearing problems, regression of milestones, exposure to lead, behavioral changes, deterioration in school performance).

d) History suggestive of metabolic disorders e.g failure to thrive, recurrent unexplained illness, seizures, ataxia, loss of psychomotor skills, recurrent somnolence/coma, abnormal sexual differentiation, sepsis like manifestations with negative septic screen, poor feeding, lethargy, abnormal odors (urinary / body).

e) Detailed family history including 3 generation pedigree and family history of MR is required to ascertain the pattern of inheritance e.g. autosomal recessive (in case of consanguinity) and X linked mental retardation. Enquire about previous babies with MR or unexplained sib deaths, psychiatric disorders or malformations.

f) Developmental history.

Examination- A necessary component of the evaluation of the child with idiopathic mental retardation is a comprehensive dysmorphologic examination. A syndrome search using various available databases (e.g. London Dysmorphology Database, Neurogenetics database, SYNDROC etc.) is useful in uncertain cases. Apart from routine physical and systemic examination some features merit special mention and should be very carefully looked for (Table I).

Neurologic Examination- Neurological examination may give clues to the diagnosis. e.g. Ataxia, seizure and inappropriate laughter suggest- Angelman Syndrome.

Behavioral phenotype- may be suggestive of some disorder. E.g.-self mutilation in Lesch Nyhan syndrome, Smith Magenis syndrome, autistic features in Fragile X, and duplication 15q, hyperphagia in Prader Willi syndrome, typical hand movement stereotypes and breathing abnormalities in Rett patients. Ophthalmological examination should be done in all cases to look for corneal, lenticular and retinal abnormalities.

Re-evaluation/serial examination increases the rate of diagnosis.

Laboratory approach: The usefulness of baseline investigations for all patients with mental retardation like full blood count, electrolytes, liver function tests, creatinine phosphokinase and urine metabolic screen is debatable. The important category of tests to ascertain causes of MR are as follows and should be done selectively based on the history and physical examination.

1. **Thyroid function tests:** Hypothyroidism must be excluded in all cases of developmental delay especially when clinical features of hypothyroidism are seen and in younger children (< 2 years) where no specific cause could be found. It should also be done in children with Down syndrome.

2. **Cytogenetic studies:** Cytogenetic studies in the evaluation of children with DD/MR are a valuable diagnostic technique in all children with idiopathic DD/MR.[4] The reported frequency of chromosome anomalies detected by high-resolution karyotyping (i.e., 550 -850 bands) in patients evaluated for DD/MR varies between 9% and 36%. Indications for cytogenetic studies are: [i] Mental retardation, [ii]. Failure to thrive, [iii]. Dysmorphism and [iv]. Multiple malformations.

3. **Molecular Cytogenetic techniques** are done to identify smaller chromosome abnormalities associated with MR

a) **Fluorescence in situ hybridization (FISH)** for various known microdeletion syndromes like William syndrome (fig 5), 22qdel syndrome etc.

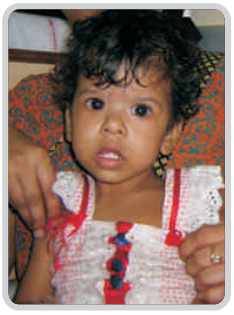

Fig 5: Williams Syndrome

b) **Submicroscopic Subtelomeric Rearrangements** - Approximately, half of all structural chromosomal abnormalities ("segmental aneusomies") include the telomeres of the chromosomes. Usually, deletions /duplications of other subtelomeric regions lead to a phenotype that is not recognized easily, and the deletions often go undetected by routine cytogenetic testing. These are often referred to as "cryptic" subtelomeric chromosome anomalies. Recently, FISH techniques have been applied to examine the subtelomeric regions of each chromosome for abnormalities that are known to cause mental retardation.[5] It has led to the recognition that approximately 7.4% of children with moderate to severe mental retardation have subtelomeric rearrangements.

Subtelomeric FISH investigations are being replaced by newer techniques such as multiple ligation probe amplification (MLPA) and chromosome microarray or comparative genomic hybridization techniques (aCGH).[6] aCGH can detect deletions / duplications present anywhere in the whole genome at a resolution of 1 Mb or smaller. At present, it is a very costly test.

4. **Specific molecular genetic diagnostic testing for single gene disorders:** Molecular genetic diagnostic testing is used to identify mutations when a single gene disorder is suspected clinically. Some monogenic disorders causing MR are fragile X syndrome, Rett Syndrome, alpha thalassemia mental retardation syndrome. The number of genes that have been identified as cause of XLMR syndromes is rapidly growing. Most of the genetic defects that underlie syndromic XLMR are either known or have been mapped to small regions of the X chromosome. The distinction between syndromic XLMR and non-syndromic XLMR is becoming increasingly blurred.

Fragile X syndrome - Fragile X syndrome is seen in approximately 1 to 2% of patients with mental retardation. Higher prevalences are reported in studies in selected groups of patients.[7] A simple PCR based screening test for Fragile X syndrome is being utilized for initial screening. Confirmation is done by Southern blot analysis. Cytogenetic screening for Fragile X has some error rate. The suggestive phenotype of Fragile X syndrome checklist includes long jaw or high forehead, large and/or protuberant ears, hyper extensible joints, soft and velvety palmar skin with redundancy on the dorsum of the hands, testicular enlargement, and behaviors of initial shyness and lack of eye contact followed by friendliness and verbosity.[8] As the clinical features are subtle, Fragile X testing is indicated in all males with idiopathic MR with or without positive family history.

Rett Syndrome - In contrast to fragile X mental retardation, Rett syndrome is a severe, non-progressive neurodevelopmental disorder that exclusively affects females. Clinical diagnosis is suspected on the basis of diagnostic criteria that includes loss of acquired skills, hand skill, hand stereotypes, apparently normal prenatal and perinatal development, autistic like features, deceleration of head growth and, breathing dysfunction. Mutations in MECP2 genes are found in 80% females with Rett syndrome.

5. **Neuroimaging:** Major or minor malformations of the brain are known to be an important finding in patients with DD/MR. Neuro-imaging is a recommended part of the diagnostic evaluation especially in the presence of abnormal findings on examination (microcephaly, macrocephaly, seizures, focal motor findings), and that MRI is preferable to CT. Neuroimaging can be helpful to identify hypoxic ischemic sequelae, vascular insults, neuroectodermal syndromes, structural anomalies, neuronal migration abnormalities. CT provides superiority in the documentation of intracranial calcifications associated with old hemorrhage, tuberous sclerosis complex (fig 6), CMV or toxoplasmosis infection) whereas MRI provides a means of assessing brain with considerable accuracy and sensitivity.

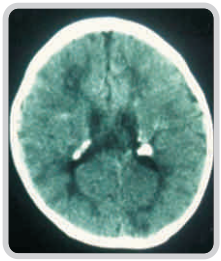

Fig 6: CT scan showing subependymal nodules in tuberous sclerosis

6. **Metabolic work** up is fruitful in situations where there is a strong suspicion. The suspicious symptoms and signs include failure to thrive, recurrent vomiting, intractable seizures, skin manifestations, abnormal odor (urine/body) cataract, etc. Diagnostic yield of metabolic evaluation varies from 0.2%-8.4% in various studies with a mean of about 1%. Consensus states that routine metabolic screening is not recommended. Although tandem mass spectroscopy (TMS) and Gas chromatography-mass spectroscopy (GCMS) can identify various metabolic disorders, their utility for the evaluation of mental retardation is still to be established.

7. **Electrophysiological studies:** Although a specific diagnosis rarely results from an EEG, the high frequency of co-existing seizure disorders or paroxysmal behavior suggests its utility. Visual evoked potentials and brainstem auditory potentials assess the integrity of the visual and auditory pathways.

8. **Other Specific tests** according to the suspected diagnosis should be carried out which may include bone narrow examination, skin biopsies, urine test for mucopolysaccharidoses, enzyme assays, serology for TORCH/HIV, test for HbH inclusion for alpha thalassemia mental retardation syndrome etc. Other ancillary investigations like EMG, NCV, and audiologic examination etc should be performed as per suspected diagnosis.

Treatment and Prognosis: Having an etiological explanation aids in the development of a specific treatment plan; helps families understand prognosis and recurrence risk and at the community level assists in the development of preventive strategies. Treatment is mainly symptomatic and supportive. In most situations when genetic etiology is identified, treatment is not available except in some cases of neurometabolic disorders. Hypothyroidism, if diagnosed as a cause or an associated abnormality, needs to be treated. It is important to remember that all patients should be advised early stimulation and physiotherapy and should always be screened for other handicaps like vision and hearing and special schooling or vocational training may be required. Discourage institutionalization and integrate the children in the society. A multidisciplinary approach is warranted for management. Parental counseling is the mainstay of management and usually more than one session is required. Positive aspects of the disease should be emphasized though truth should not be withheld. One should try and remove myths and guilt from the parents' minds.

Genetic counseling will depend on the etiology. Important points to remember are:

1. **Chromosomal causes of MR** are seldom familial except in situations when one of the parents is a carrier of balanced translocation. Risk of recurrence in de novo chromosomal disorders is low (usually < 1%). In translocation Down syndrome, when one of the parents is a balanced translocation carrier the recurrence risk is variable. It varies from 2.5% if the father carries a 14/21 translocation to 100% if either parent carries a 21/21 translocation.
2. In **single gene disorders,** the risk of recurrence will depend on the mode of inheritance. In an autosomal recessive disorder, the risk of recurrence is 25% for the sibling. In autosomal dominant disorder the risk of recurrence for the sib is 50% if one of the parents is affected. If it is a sporadic case (new mutation), the risk of recurrence in sib is very low but gonadal mosaicism cannot be ruled out. In X-linked recessive

disorders, risk of recurrence for boys is 50% whereas females usually do not manifest.

3. **Environmental causes,** when identified - recurrence is unlikely unless the causative agent keeps on operating e.g. lead exposure, intrauterine exposure to teratogens.
4. In situations where a specific diagnosis is not reached (nonspecific mental retardation) empiric risk figures based on population based studies are used to counsel the family. Table II gives empiric risk figures.[9]

Prenatal diagnosis using Chorionic villus sampling, amniocentesis, cord blood sampling is possible if exact etiology has been established (e.g. previous child affected with chromosomal anomalies, neurometabolic / neurodenenerative disorders).

Conclusion- The evaluation of a child with mental retardation is challenging. Detailed history and analysis of 3 generation family pedigree, dysmorphologic and neurologic examination remain the mainstay of the diagnosis. With the advent of newer techniques, etiological diagnosis can be established in many cases. Etiological diagnosis may not alter the management in all cases, but it is important to counsel the families and explain to them the relevance of investigations. Prenatal diagnosis, if available, plays a major role in the prevention of recurrence in the family.

Table I: Physical Examination in Mental Retardation

FEATURE		EXAMPLE
a)	**Head Size**	
	Microcephaly	Chromosomal disorders (trisomy 13,18), multiple malformation syndromes, autosomal recessive microcephaly, Alpha thalassemia Mental Retardation (ATRX syndrome)
	Macrocephaly	Leukodystrophy, Tay Sachs disease, fragile X, Opitz FG Syndrome, Simpson-Golabi-Behmel Syndrome
b)	**Hair**	Fine (Homocystinuria) Friable and Kinky (Menkes Kkinky hair disease), White patches (Tuberous sclerosis)
c)	**Skin**	
	Cafe au-lait spots	Neurofibromatosis, Tuberous scleorsis
	Depigmented nevi	Tuberous sclerosis
	Pigmentary whorls	Hypomelanosis of Ito
	Eczema	Phenylketonuria
	Photosensitivity	Hartnup
	Dry	Hypothyroidism
	Angiokeratomas	Tuberous sclerosis, fucosidosis
	Icthyosis	Multiple sulfatase deficiency, Sjogren Larson Syndrome
	Photosenstivity	Xeroderma Pigmentosum, Cockayne Syndrome (fig 7)
d)	**Eye abnormalities**	
	Cataracts	Cockayne syndrome, Down syndrome, Galactosemia, Lowe syndrome, Myotonic dystrophy, rubella, cretinism
	Cherry red spot	GM1 gangliosidosis, Mucolipidosis, Niemann Pick disease, Tay Sachs disease
	Dislocated lens	Homocystinuria, Sulfite oxidase deficiency
	Clouding of Cornea	Lowe syndrome, Hunter syndrome, Hurler syndrome
	Glaucoma	Lowe syndrome, Sturge-Weber syndrome
	Chorioretinitis	Rubella, cytomegalic inclusion body disease
e)	**Coarse facies**	Hypothyroidism, Mucopolysaccharidosis
f)	**Hearing defects**	
	Conductive	Mucopolysccharidosis
	Hyperacusis	GM, gangliosidoses Krabbe's disease, Tay Sachs disease
	Sensorineural	Kearns-Sayre, MELAS
g)	**Hepatosplenomegaly**	Storage disorders
h)	**Short stature**	Hypothyroidism, Mucopolysaccharidosis, Cornelia de Lange (Fig 8), Seckel syndrome, Rubinstein Taybi syndrome, Prader Willi syndrome

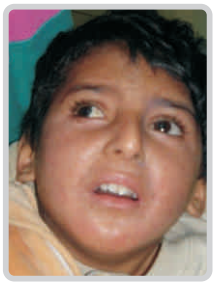

Fig 7: Cockayne syndrome with photosensitivity rash

Fig 8: Cornelia de Lange syndrome with typical facies

Table II: Genetic risks in severe 'non-specific' mental retardation (IQ 50 or less)

AFFECTED	INDIVIDUAL AT RISK	RISK
Isolated case, male or female	Sib (both sexes) Male sib Female sib	1 in 35 1 in 25 1 in 50
Two sibs regardless of sex	Sib of either sex	1 in 4
Isolated case, male or female, parents consanguineous	Sib of either sex	1 in 7
Affected male with affected maternal uncle	Male sib Female sib	1 in 2 Low
One affected parent (either sex)	Sib of either sex	1 in 10
One affected parent & affected child	Sib of either sex	1 in 5
Two affected parents	Sib of either sex	1 in 2

References: (1) Shevell M, et al. Neurology 2003; 60: 367-80. (2) Moeschler JB, Shevell M. Pediatrics 2006; 117: 2304-16. (3) American Association on Mental Retardation. Mental Retardation: Definition, Classification, and Systems of Supports. 10th ed. Washington, DC: American Association on Mental Retardation; 2002. (4) Curry CJ, et al. Am J Med Genet. 1997; 72: 468–77. (5) Flint J, et al. Nat Genet. 1995; 9: 132–40. (6) Shaw -Smith, et al. J Med Genet 2004; 41: 241-8. (7) van Karnebeek CD, et al. Eur J Hum Genet 2005; 13: 6–25. (8) de Vries BB, et al. J Med Genet 1999; 36: 467–70. (9) Harper PS. Disorders of Mental function in Practical Genetic counseling.. 5th edn, Wright Publishers, 1993; 183 – 94.

Hemoglobin D (Iran) masquerading as Hemoglobin E

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We report on a family with thalassemia in which, sequencing of beta globin gene helped in elucidation of a confusing phenotype. This case illustrates the utility and importance of complete molecular analysis in prenatal diagnosis and genetic counseling of patients with genetic diseases.

The family was presented to us with a history of transfusion dependent thalassemia in previous child, and the wife was seen at 13 weeks gestation. Hemoglobin electrophoresis by HPLC in the couple is shown in Table I.

Table I: Hemoglobin electrophoresis by HPLC

	HUSBAND	WIFE
Hemoglobin	11.9 gm%	9.8 gm %
Mean Corpuscular volume (MCV)	57.2 fl	59.1 fl
Mean Corpuscular hemoglobin(MCH)	19.5 pg	19.6 pg
Hemoglobin A	3.2 %	81.5 %
Hemoglobin A2	77.1 %	6.4 %
Hemoglobin F	7.3 %	3 %

The mother was reported as thalassemia carrier and father was reported as HbE homozygous based on high HbA2 levels (Fig1). However the result could not be corroborated with the result in proband, since there was no hemoglobin E in proband.

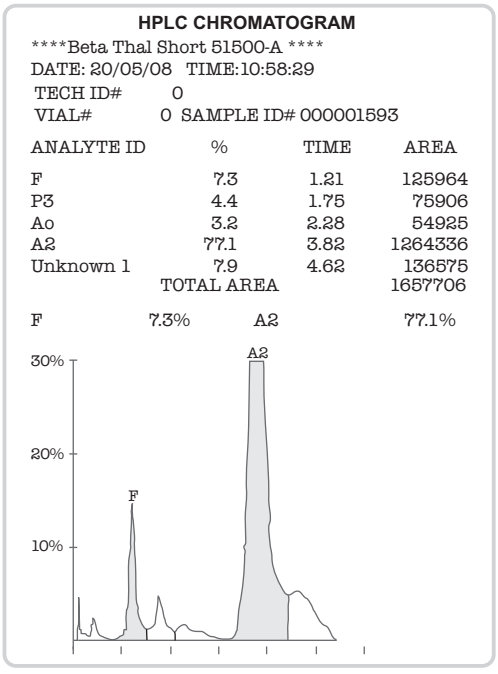


Fig 1: Hemoglobin electrophoresis by HPLC in father

The couple was presented to us for prenatal diagnosis in present pregnancy. The proband was not available for analysis since the child had expired. Since mutation analysis was not done in proband, we planned to do mutation analysis in the parents, so that prenatal diagnosis could be done in the present pregnancy.

We collected blood samples in EDTA and extracted genomic DNA. The first step was to look for common mutations in the South Indian population. Reverse dot blot analysis was done to look for the mutations as shown in Table II.

Table II: Common mutations in south Indian population

S.No.	LOCUS	DESIGNATION	TYPE
1.	IVS 1-5	Substitution	G->C
2.	IVS 1-1	Substitution	G->T
3.	Codon 15	Substitution	G->A
4.	Codon 8/9	Insertion (Frame shift)	+G
5.	Codon 41/42	Deletion (Frame shift)	-CTTT
6.	Codon 26	Substitution (Hb E)	G->A
7.	Codon 6	Substitution (Hb S)	A->T

Reverse dot blot did not show any mutation in both the partners. Further, the mutation of HbE was absent in father, thus proving that he was not HbE homozygous as reported by HPLC analysis.

Sequencing of all the 3 exons of beta globin gene was done to identify the mutations in the couple. Sequencing analysis showed that both the partners were carriers for mutation in codon 5. It was a frameshift mutation caused due to deletion of two nucleotides (-CT) in codon 5. The results are shown in Fig 2. A frameshift mutation leads to jumbled up sequences due to normal sequence on one chromosome and frameshift sequence on other chromosome. This mutation is known to cause beta thalassemia. However, this could not account for the very high HbA2 levels in father. Further analysis of the sequence showed that the father was heterozygous for another mutation in codon 22. This was a substitution mutation (G>C) leading to substitution of glutamic acid by glutamine at codon 22 position. The result is shown in Fig 3. The mutation leads to an abnormal hemoglobin. This variant hemoglobin is referred to as Hemoglobin D (Iran).

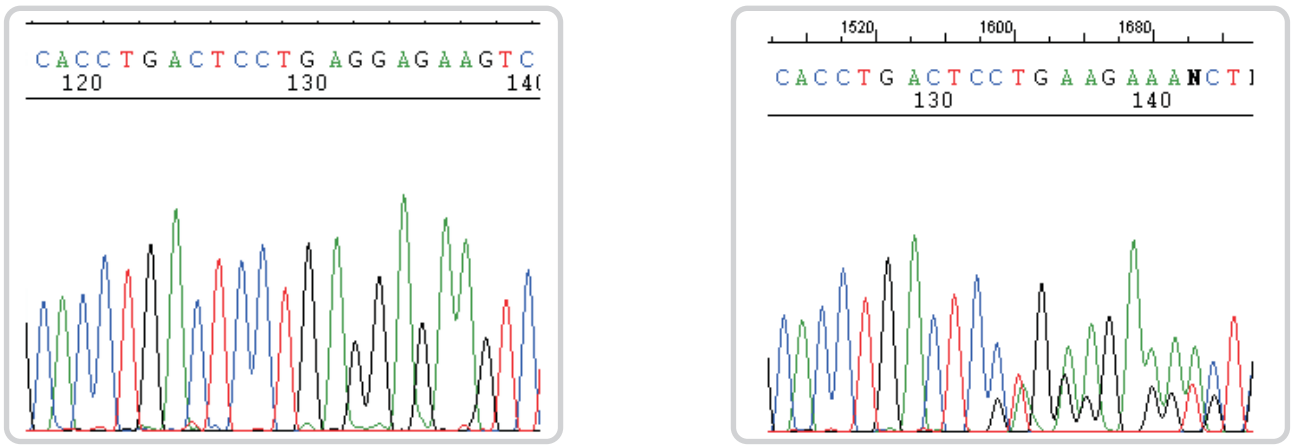
Mutation analysis in the fetus by chorionic villus sampling showed that the fetus had not inherited any allele of the codon 5 mutation from both the parents. However the fetus was heterozygous for Hemoglobin D (Iran) mutation. The couple was counseled that the fetus is unlikely to have thalassemia major, but since the fetus is carrier for Hemoglobin D (Iran)

mutation, the HbA2 levels may be falsely high in the fetus.

In this case, the presence of Hemoglobin D (Iran) mutation in father created a false impression of HbE due to markedly raised HbA2. Hence it is very important to identify the mutations in the proband or the parents before going ahead with prenatal diagnosis. Ideally, it is preferable to identify the mutations before planning a pregnancy so that prenatal diagnosis can be done in stipulated time.

Hemoglobin D (Iran) is a rare hemoglobin variant. It has been reported in few families from Iran, Pakistan and Italy. Since this variant produces a peak in HPLC in the position of HbA2, it can lead to falsely high HbA2 level results.

This case illustrates the importance of complete molecular analysis in proband/parents, before conducting prenatal diagnosis so that accurate prenatal diagnosis and genetic counseling can be done for the family.



Normal Father

Fig 2: Sequencing in parent showing codon 5 di-nucleotide (-CT) deletion

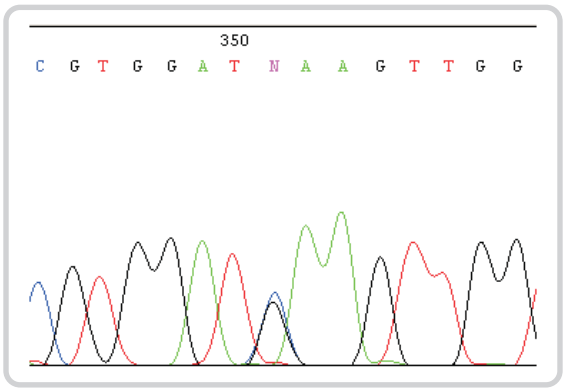


Fig 3: Sequencing in father showing mutation for Hemoglobin D (Iran)

References: (1) Rohe RA, Sharma VS, Ranney HM. Double heterozygosity for beta thalassemia and hemoglobin D Iran (beta 22 glu-to-gln). (Abstract) Meeting Am. Soc. Hemat., Hollywood, Florida , 12/3/1972. (2) Rahbar S. Brit J Haemat 1973; 24: 31-36. (3) Serjeant B, et al. Hemoglobin1982; 6: 57-59.

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http://www.iapindia.org/proforma/IAP_genetics_chapter_application_form.pdf

Contributed by:
Parag M Tamhankar

Population screening for Fragile X: are we going overboard?[1]

Newborn screening has ushered a revolution in early detection and management of genetic diseases like phenylketonuria, congenital hypothyroidism, and various other inherited metabolic disorders. Recently, a pilot study to detect Fragile X syndrome in newborn males was performed by PCR based methods. The detection rate (1:730) was higher than the estimated population prevalence of the disorder. Sex chromosomal aneuploidies (XXY, monosomy X) could also be detected and could pose an ethical dilemma. This approach would help identify cases early on to allow appropriate and timely developmental interventions. Counseling would help carrier mothers make reproductive choices and avail prenatal diagnosis in their future pregnancies. The birth rate of children with this disorder is naturally expected to decline, thereby minimizing the economic burden on families and society. The authors did not screen newborn females. This study is sure to give rise to several questions since the benefits of such a study on the outcome of the condition are not clear. Unlike other diseases accepted for neonatal screening, there is no treatment to prevent mental retardation in fragile X syndrome even after neonatal diagnosis. It is also likely to raise several ethical and social issues.

Not another polymorphism story..[2]

As we flip the pages of bio-medical journals we are bombarded with dozens of studies related to the association of gene polymorphisms with various inherited and multifactorial diseases. Even monogenic diseases are not spared. A recent study by Lettre et al on polymorphisms in modifier genes and clinical variability in sickle cell disease promises to be just not another yarn on polymorphism. They demonstrated that single nucleotide polymorphisms (SNPs) at the BCL11A and HBS1L-MYB loci, in addition to Xmn1 polymorphism, are associated with pain crisis in sickle cell disease patients. These variants modify the clinical picture by regulating HbF levels in these patients. SNPs associated with higher HbF levels lead to decreased frequency and severity of pain crises. However, these associations may need validation from large prospective cohort studies before their clinical value is fully appreciated.

Shotgun sequencing to unravel the fetal genome..[3]

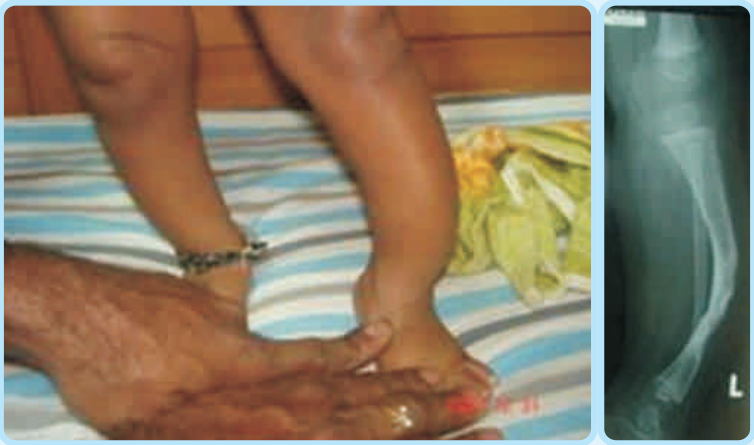
Noninvasive testing for inherited fetal anomalies heralds a paradigm shift in prenatal diagnosis. Fan et al used high throughput shotgun sequencing technology to sequence cell-free DNA from plasma of pregnant women to detect fetal aneuploidies. The previous non-invasive methods to detect fetal aneuploidies such as allelic ratios of placental specific mRNA or imprinted fetal genes or fetal specific alleles in maternal plasma have an inherent drawback. They depend on specific gene polymorphisms that may be unique to populations and hinder the universalization of specific set of markers. This is done away with by shotgun sequencing which involves bombarding isolated fetal DNA with small charged particles producing smaller fragments. These shorter fragments are individually sequenced and the fetal genome sequence can be obtained by software reconstructing the overlapping regions of the fragments. These sequences are then mapped to chromosome loci and are used as "sequence tags". These tags are enumerated and, depending upon over-or-under representation of these tags, the corresponding chromosome aneuploidy can be detected. The sensitivity of detection of fetal aneuploidy of chromosomes 13, 18 and 21 was found to be 100 %. The cost per test was around $ 700. Therefore, it may not be long before we have to change the size of our needles to approach the fetus.

References:
(1) Saul RA, et al. Genet Med 2008; 10: 714-9. (2) Lettre G, et al. Proc Natl Acad Sci USA. 2008; 105: 11869-74. (3) Fan CH, et al. Proc Natl Acad Sci USA. 2008: 105(42): 16266-71.

3

Contributed by: Dr SJ Patil, siddhu_pk@yahoo.com

An 11 month male child has multiple cafe-au-lait spots and unilateral congenital antero-lateral tibial bowing on the left side. Identify the lesion and condition.



The response should be sent to geneticsiap@gmail.com

The names of responders with the correct diagnosis will be published in the next issue.

Answer to the PhotoQuiz 2 *of the previous issue:*

Angiokeratoma corporis diffusum in Fabry disease (OMIM 301500)

They are small 0.1-3 mm red to blue-black papules arising due to vascular lesions and hyperkeratosis of overlying skin. They occur by puberty and occur predominantly over genitalia, hips, groin, buttocks and peri-umbilical area. On microscopy, they show endothelium lined vascular spaces filled with blood. They are seen in Fabry disease caused by deficiency of alpha-galactosidase enzyme.

Correct responses:

Himanshu Goel, Australia

Mohandas Nair, Kerala

Sheetal Sharda, Chandigarh

Saroj A Jalan, Mumbai

Anju Aggarwal, New Delhi

Saminathan D, Trichy

Yesodha Thani, via e-mail

Aditi Dagli, Gainsville, USA

Ravi Goyal, Kota

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